A Respirometric Investigation of the
Activated Sludge Treatment of BKME During Steady State and Transient Operating Conditions

BY

STEVE HELLE

B. Eng., McGill University, 1989
M. Eng., McGill University, 1990

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in The Faculty of Graduate Studies Department of Chemical Engineering

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

APRIL 28TH, 1999

© Steve Helle, 1999
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Chemical Engineering

The University of British Columbia
Vancouver, Canada

Date April 28/99
Abstract

Activated sludge is commonly used to treat bleached kraft pulp mill effluent (BKME). Treatment performance during steady state operation is acceptable, but may be less than adequate during periods of unsteady state operation, such as process spills or temperature changes.

Nineteen batches of BKME were treated in two lab scale activated sludge bioreactors. The effects of the solids retention time (SRT) and the presence of an aerobic selector on the activated sludge treatment of BKME during steady state and transient operating conditions were investigated.

Respirometric methods for the study of activated sludge kinetics were investigated in detail, including the unsteady state nature of the assay, and the presence of multiple substrates. BKME is composed of many different substrates, and the composition was found to be variable from batch to batch. The substrate fractions were classified into two main groups, the readily biodegradable (70%) and the slowly biodegradable (30%). The removal rates of the readily biodegradable substrates were approximately an order of magnitude greater than the removal rates of the slowly biodegradable substrates. The substrate removal rates and the stoichiometry were more dependent upon the wastewater characteristics than the solids retention time or the presence of an aerobic selector.

The pH affects the removal rates of all of the substrates in BKME approximately to the same extent. Below pH 6 and above pH 9, the metabolic activity of the activated sludge was low enough to result in incomplete removal of the slowly biodegradable substrates. Outside of the pH range 5.5 to 9.5, the decay rate of the bacteria increased. Operating at a high SRT (fifteen days) mitigated the negative impact of pH on the biomass.
The optimum temperature for activated sludge operation was related to the steady state operating temperature. For biomass acclimated to 35°C, temperature increases to 45°C resulted in poor treatment performance, due to the increased microbial decay rate. The biomass was capable of partially adapting to the higher operating temperature within a few days. Temperature increases above 42°C resulted in energy spilling during the metabolism of methanol and formic acid, and very low yields on these substrates.
Table of Contents

Abstract .......................................................................................................................... ii
Table of Contents ........................................................................................................ iv
List of Tables ................................................................................................................ viii
List of Figures ............................................................................................................... ix
Dedication ..................................................................................................................... xviii
Chapter 1 Introduction & Objectives ............................................................................. 1
   Introduction ............................................................................................................... 1
   Objectives ............................................................................................................... 3
   Outline ...................................................................................................................... 4
Chapter 2 Literature Review - Activated Sludge Operation .............................................. 7
   2.1 BKME Characteristics ..................................................................................... 7
   2.2 Activated Sludge Treatment of BKME ............................................................. 12
      General BKME Treatment ............................................................................... 12
      Effect of SRT and HRT on Treatment Performance ........................................ 16
      Selectors ............................................................................................................. 20
      Temperature ...................................................................................................... 23
      pH ...................................................................................................................... 25
   2.3 Microbiological Aspects of Wastewater Treatment .......................................... 26
      Substrate Metabolism ..................................................................................... 27
      Variability of Yields and Metabolism ............................................................. 27
      Uncoupled Metabolism .................................................................................... 30
      Endogenous metabolism ................................................................................. 31
   2.3 Effect of Transient Operating Conditions on Activated Sludge Performance ........ 33
      BOD .................................................................................................................. 33
      Temperature and pH Shocks ........................................................................... 34
      Toxic Compounds ........................................................................................... 34
      Operating Conditions ....................................................................................... 38
   2.4 Summary .......................................................................................................... 39
Chapter 3 Literature Review - Activated Sludge Models ............................................... 41
   3.1 Introduction ...................................................................................................... 41
   3.2 Monod .............................................................................................................. 43
      Alternatives to Monod ..................................................................................... 45
   3.3 Multiple Substrates ....................................................................................... 52
      Substrate Interactions ....................................................................................... 53
      Multicomponent Kinetics ............................................................................... 55
      IAWQ Model ................................................................................................... 59
   3.4 Mass Transfer ................................................................................................. 63
      Adsorption ........................................................................................................ 64
      External & Internal Diffusion ......................................................................... 67
      Oxygen Transfer .............................................................................................. 70
   3.5 Temperature .................................................................................................... 71
   3.6 pH .................................................................................................................. 72
Chapter 4 Literature Review - Model Parameter Measurements

4.1 Introduction ............................................................. 75
4.2 Continuous/Pilot Plant Setups ........................................ 78
4.3 Fed Batch Test ........................................................... 79
4.4 Batch Tests ............................................................... 80
4.5 Respirometry  ............................................................ 81
   Introduction .............................................................. 81
   Low F/M Respirometric Methods ..................................... 86
      AOUR and similar assay techniques .............................. 86
      IAWQ and similar assays .......................................... 92
      Measurement of adsorbed substrate ............................. 96
      Toxicity assessment ................................................ 98
      Online respirometry ............................................... 100
   High F/M Respirometric Methods .................................... 101
      Biochemical oxygen demand ...................................... 101
      Maximum growth rate measurements ............................ 104
      Correlation between OUR and viability ........................ 105
4.6 Yield ........................................................................ 107
4.7 Decay ...................................................................... 108
4.8 Summary .................................................................. 109

Chapter 5 Materials & Methods ........................................... 111
5.1 Lab Scale Activated Sludge Units .................................... 111
5.2 Standard Tests .......................................................... 115
5.3 Batch Tests .............................................................. 115
   Wastewater Characteristics ............................................. 115
   Infinite Dilution .......................................................... 116
   Decay .................................................................. 117
   Temperature and pH ..................................................... 117
5.4 Respirometric Method .................................................... 118
   AOUR Procedure ......................................................... 118
   Data Analysis ............................................................. 120

Chapter 6 Respirometry ....................................................... 122
6.1 Respirometric Data Analysis ........................................... 122
   Typical Respirometric Data ............................................. 122
   Curve Fitting ............................................................... 135
   Effect of Biomass Concentration ..................................... 150
   Verification of Models ................................................ 163
   Low Substrate Concentration ......................................... 164
   High Substrate Concentration ......................................... 167
   Summary ................................................................. 171
6.2 Effect of DO Concentration ............................................. 172
6.3 Multi-Substrate Wastewaters .......................................... 179
   Methanol - Acetic Acid Mixtures .................................... 188
   Formic acid - Acetic Acid Mixtures ............................... 197
   Methanol - Formic Acid Mixtures ................................... 202
Chapter 12 Bibliography........................................................................................................385
List of Tables

Table 2.1  BKME Composition and Treatment.........................................................11
Table 4.1  Effect of f/m Ratio .................................................................77
Table 4.2  Respirometric Methods.................................................................84
Table 5.1  Experimental Conditions..............................................................114
Table 6.1  ΔOUR Parameters..............................................................142
Table 6.3  Respirometric Analysis of Substrate Mixtures..........................215
Table 7.1  Reactor Performance..............................................................220
Table 7.2  Summary of Kinetic Constants of the Various Substrate Groups in
              BKME..............................................................237
Table 8.1  Average Activation Energies......................................................349
### List of Figures

**Figure 3.1** Growth rate vs. substrate concentration, Monod, Blackman, and Powell relationships ........................................ 46

**Figure 3.2** Growth rate vs. substrate concentration, Monod and Moser relationships .................................................. 46

**Figure 3.3** Growth rate vs. substrate concentration, Monod, Tessier, and Konak relationships ....................................... 49

**Figure 3.4** Multisubstrate removal during a batch test ................................................................. 49

**Figure 3.5** COD composition of domestic wastewater before and after activated sludge treatment ............................................. 60

**Figure 3.6** a) IAWQ death regeneration model; b) IAWQ endogenous decay model ........................................................... 60

**Figure 3.7** Effect of external mass transfer on the growth rate vs. substrate concentration relationship ............................... 65

**Figure 3.8** Effect of internal mass transfer resistance on the growth rate vs. substrate concentration relationship .................. 65

**Figure 4.1** Interpretation of respirometric data ....................................................................................... 88

**Figure 4.2** IAWQ batch test ................................................................................................................ 95

**Figure 4.3** Growth test ......................................................................................................................... 95

**Figure 6.1.1** Oxygen consumption following methanol injections ................................................................................. 123

**Figure 6.1.2** OUR following methanol injections .................................................................................... 123

**Figure 6.1.3** ΔOUR vs. methanol concentration ......................................................................................... 125

**Figure 6.1.4** ΔOUR vs. methanol concentration ......................................................................................... 125

**Figure 6.1.5** ΔOUR standard deviation vs. methanol concentration ............................................................. 127

**Figure 6.1.6** Oxygen consumption vs. methanol concentration ............................................................................ 127

**Figure 6.1.7** Respirometric yield vs. methanol concentration .............................................................................. 128

**Figure 6.1.8** Respirometric yield vs. methanol concentration .............................................................................. 128

**Figure 6.1.9** OC standard deviation vs. average and respirometric yield standard deviation vs. average ................. 130

**Figure 6.1.10** OUR vs. methanol addition rate ......................................................................................... 130

**Figure 6.1.11** Respirometric yield vs. SUR .............................................................................................. 132

**Figure 6.1.12** SUR vs. methanol concentration ......................................................................................... 134

**Figure 6.1.13** ΔOUR vs. formic acid, acetate, and methanol concentration ...................................................... 134

**Figure 6.1.14** OC vs. formic acid, acetate, and methanol concentration ....................................................... 136

**Figure 6.1.15** SUR vs. formic acid, acetate, and methanol concentration ...................................................... 136

**Figure 6.1.16** ΔOUR vs. methanol concentration with Monod, Powell, and Blackman curve fits .................. 137

**Figure 6.1.17** ΔOUR vs. methanol concentration with Monod, Tessier, Konak, and Moser curve fits .............. 137

**Figure 6.1.18** ΔOUR residual vs. expected value for Monod, Powell, and Blackman curve fits ...................... 139

**Figure 6.1.19** ΔOUR residual vs. expected value for Monod, Moser, Konak, and Tessier curve fits .............. 139

**Figure 6.1.20** ΔOUR residual vs. expected value for all curve fits ............................................................ 141
Figure 6.1.21 Monod parameters 95% confidence intervals .............................................143
Figure 6.1.22 Powell parameters 95% confidence intervals ...........................................144
Figure 6.1.23 $\Delta$OUR vs. methanol concentration, with Monod and Powell curve fits ..........................................................146
Figure 6.1.24 Residuals from Monod and Powell curve fits vs. experiment # ....................146
Figure 6.1.25 $\Delta$OUR vs. acetate concentration, with Monod and Powell curve fits ...............148
Figure 6.1.26 $\Delta$OUR vs. formic acid concentration, with Monod and Powell curve fits ..............148
Figure 6.1.27 $\Delta$OUR vs. substrate concentration for formic acid/methanol/acetate mixtures ..........................................................................................149
Figure 6.1.28 $\Delta$OUR vs. substrate concentration for BKME ............................................149
Figure 6.1.29 $\Delta$OUR vs. methanol concentration, for various MLVSS concentrations, Powell and Monod curve fits .........................................................151
Figure 6.1.30a $\Delta$OUR vs. methanol concentration, for various MLVSS concentrations, a) Powell curve fits; b) Monod curve fits .........................................................151
Figure 6.1.30b $\Delta$OUR vs. methanol concentration, for various MLVSS concentrations, a) Powell curve fits; b) Monod curve fits .........................................................153
Figure 6.1.31 Half saturation constant, maximum $\Delta$OUR vs. MLVSS concentration ...........................................................................................................153
Figure 6.1.32 Monod parameters 95% confidence intervals .............................................154
Figure 6.1.33 Powell coefficients vs. MLVSS concentration ................................................156
Figure 6.1.34 Powell parameters 95% confidence intervals ................................................158
Figure 6.1.35 $\Delta$OUR vs. methanol concentration, for various MLVSS concentrations, external mass transfer curve fits ..........................................................160
Figure 6.1.36 $\Delta$OUR vs. acetate concentration, for various MLVSS concentrations, Powell and Monod curve fits ..........................................................162
Figure 6.1.37 $\Delta$OUR vs. formic acid concentration, for various MLVSS concentrations, Powell and Monod curve fits ..........................................................162
Figure 6.1.38 OUR following methanol injections ..............................................................165
Figure 6.1.39 Methanol concentration and OUR vs. time for a batch test ..........................168
Figure 6.1.40 Respirometric yield vs. time for a batch test ................................................168
Figure 6.1.41 Methanol concentration, acetate concentration, and OUR vs. time during two batch tests ......................................................................................170
Figure 6.1.42 OUR vs. time during growth on methanol and acetate ..................................170
Figure 6.2.1 OUR vs. DO in the presence of excess substrate .............................................174
Figure 6.2.2 OUR vs. DO in the presence of excess methanol .............................................174
Figure 6.2.3 $\Delta$OUR vs. DO and substrate based on the Monod model ................................176
Figure 6.2.4 $\Delta$OUR vs. DO and substrate based on the Powell model ..............................176
Figure 6.2.5 DO vs. time under different methanol feed rates ...........................................177
Figure 6.2.6 OUR vs. flow rate, and OUR vs. DO slope vs. flow rate ..................................177
Figure 6.2.7 OUR vs. DO for replicate methanol injections ...............................................178
Figure 6.2.8 OUR vs. DO for replicate methanol injections ...............................................178
Figure 6.2.9 OUR vs. DO for replicate methanol injections ...............................................180
Figure 6.2.10 OUR vs. DO for one large methanol injection .............................................180
Figure 6.3.1 Simulated OUR vs. time following an injection of two substrates ..... 182
Figure 6.3.2 Simulated SUR and respirometric yield vs. substrate composition ..... 184
Figure 6.3.3 Simulated SUR and respirometric yield vs. substrate composition ..... 184
Figure 6.3.4 OUR vs. time for injections of methanol, acetate and methanol/acetate mixtures ........................................... 187
Figure 6.3.5 OUR vs. time for injections of methanol, acetate and methanol/acetate mixtures ........................................... 187
Figure 6.3.6 ΔOUR vs. substrate concentration for methanol/acetate mixtures ..... 191
Figure 6.3.7 ΔOUR vs. methanol concentration for methanol/acetate mixtures ..... 191
Figure 6.3.8 OUR vs. time for injections of methanol, acetate and methanol/acetate mixtures ........................................... 193
Figure 6.3.9 OC vs. substrate concentration for methanol/acetate mixtures ..... 193
Figure 6.3.10 Measured and expected respirometric yield vs. substrate composition for methanol/acetate mixtures .................... 194
Figure 6.3.11 OUR vs. time for injections of methanol, acetate and methanol/acetate mixtures ........................................... 194
Figure 6.3.12 SUR vs. substrate concentration for methanol/acetate mixtures ..... 196
Figure 6.3.13 SUR, OUR, OC vs. substrate composition for methanol/acetate mixtures ........................................... 196
Figure 6.3.14 OUR vs. time for injections of formate, acetate and formate/acetate mixtures ........................................... 198
Figure 6.3.15 ΔOUR vs. substrate concentration for formate/acetate mixtures ..... 198
Figure 6.3.16 OC vs. substrate concentration for formate/acetate mixtures ..... 199
Figure 6.3.17 SUR vs. substrate concentration for formate/acetate mixtures ..... 199
Figure 6.3.18 OUR vs. time for injections of formate, acetate and formate/acetate mixtures ........................................... 201
Figure 6.3.19 ΔOUR vs. substrate concentration for formate/acetate mixtures ..... 201
Figure 6.3.20 SUR vs. substrate concentration for formate/acetate mixtures ..... 203
Figure 6.3.21 SUR, respirometric yield vs. substrate composition for formate/acetate mixtures ........................................... 203
Figure 6.3.22 ΔOUR vs. substrate concentration for formate/methanol mixtures ........................................... 204
Figure 6.3.23 OC vs. substrate concentration for formate/methanol mixtures ........................................... 204
Figure 6.3.24 SUR vs. substrate concentration for formate/methanol mixtures ..... 206
Figure 6.3.25 OUR vs. time for injections of formate, methanol and formate/methanol mixtures ........................................... 206
Figure 6.3.26 OUR vs. time for injections of formate, methanol and formate/methanol mixtures ........................................... 207
Figure 6.3.27 OUR vs. time for injections of formate, methanol and formate/methanol mixtures ........................................... 207
Figure 6.3.28 ΔOUR vs. substrate concentration for formate/methanol mixtures ........................................... 209
Figure 6.3.29 SUR, respirometric yield vs. substrate composition for formate/methanol mixtures ........................................... 209
Figure 6.3.30 OUR vs. time for injections of formate, methanol, acetate, and formate/methanol/acetate mixtures ........................................... 211
Figure 6.3.31 ΔOUR vs. substrate concentration for formate/methanol/acetate mixtures .................................................................211
Figure 6.3.32 OC vs. substrate concentration for formate/methanol/acetate mixtures .............................................................................212
Figure 6.3.33 ΔOUR vs. substrate concentration for formate/methanol/acetate mixtures .................................................................212
Figure 6.3.34 OC vs. substrate concentration for formate/methanol/acetate mixtures .............................................................................212
Figure 7.1.1 MLVSS vs. time over the course of the project ......................217
Figure 7.1.2 Effluent VSS vs. time over the course of the project ...............217
Figure 7.1.3 SRT vs. time over the course of the project ............................218
Figure 7.2.1 ΔOUR and OC vs. BOD and COD for BKME .........................221
Figure 7.2.2 OUR, BOD and COD vs. time during a batch biodegradation test of BKME .................................................................221
Figure 7.2.3 SUR vs. BOD for BKME measured by three different experimental methods .................................................................223
Figure 7.2.4 OUR vs. time for injections of samples withdrawn during a batch biodegradation test of BKME .................................223
Figure 7.2.5 ΔOUR vs. BOD for BKME samples withdrawn from a batch biodegradation test .................................................................229
Figure 7.2.6 OC vs. BOD for BKME samples withdrawn from a batch biodegradation test .................................................................229
Figure 7.2.7 OUR and BOD vs. time during a batch biodegradation test of BKME .................................................................232
Figure 7.2.8 Composition of BKME ............................................................232
Figure 7.2.9 ΔOUR vs. the corresponding BOD fraction for BKME samples withdrawn from a batch biodegradation test .................................................................234
Figure 7.2.10 Maximum ΔOUR and Monod half saturation constant vs. sample time for BKME samples withdrawn from a batch biodegradation test .................................................................234
Figure 7.2.11 Fractional ΔOUR vs. the corresponding BOD fraction for BKME samples withdrawn from a batch biodegradation test .................................................................236
Figure 7.2.12 Fractional OC vs. the corresponding BOD fraction for BKME samples withdrawn from a batch biodegradation test .................................................................236
Figure 7.2.13 SUR of wastewater fractions vs. the corresponding BOD fraction for BKME .................................................................238
Figure 7.2.14 Treated BKME BOD of the various substrate fractions vs. activated sludge loading .................................................................238
Figure 7.2.15 SUR of the various BKME substrate fractions vs. BOD .........240
Figure 7.2.16 SUR of the various BKME substrate fractions vs. activated sludge loading .................................................................240
Figure 7.2.17 Treated BKME BOD, assuming two substrate fractions, vs. activated sludge loading .................................................................243
Figure 7.2.18 SUR of BKME, assuming two substrate fractions, vs. BOD .....243
Figure 7.2.19 Effect of wastewater composition on treated BKME BOD, assuming two substrate fractions, vs. activated sludge loading .................................................................245
Figure 7.3.1 OUR and BOD vs. time during a batch biodegradation test of BKME ................................................................. 248
Figure 7.3.2 OUR vs. time for injections of samples withdrawn during a batch biodegradation test of BKME ................................................................. 248
Figure 7.3.3 ΔOUR vs. the corresponding BOD fraction for BKME samples withdrawn from a batch biodegradation test ........................................................................ 249
Figure 7.3.4 OC vs. the corresponding BOD fraction for BKME samples withdrawn from a batch biodegradation test ........................................................................ 249
Figure 7.3.5 OUR and BOD vs. time during a batch biodegradation test of BKME ................................................................................... 251
Figure 7.3.6 OUR vs. time for injections of samples withdrawn during a batch biodegradation test of BKME ................................................................................... 251
Figure 7.3.7 ΔOUR vs. readily biodegradable BOD fraction for BKME samples withdrawn from a batch biodegradation test ........................................................................ 252
Figure 7.3.8 OUR and BOD vs. time during a batch biodegradation test of BKME ................................................................................... 252
Figure 7.3.9 OUR vs. time for injections of samples withdrawn during a batch biodegradation test of BKME ................................................................................... 254
Figure 7.3.10 ΔOUR and OC vs. readily biodegradable BOD fraction for BKME samples withdrawn from a batch biodegradation test ........................................................................ 254
Figure 7.3.11 OUR and BOD vs. time during a batch biodegradation test of BKME ................................................................................... 256
Figure 7.3.12 15 day BOD curves for samples withdrawn during a batch biodegradation test of BKME ................................................................................... 256
Figure 7.3.13 BOD_s, BOD_v, OC, COD vs. time during a batch biodegradation test of BKME ................................................................................... 257
Figure 7.3.14 OUR vs. time for injections of samples withdrawn during a batch biodegradation test of BKME ................................................................................... 257
Figure 7.3.15 ΔOUR vs. BOD for BKME samples withdrawn from a batch biodegradation test ................................................................................... 258
Figure 7.3.16 OC vs. the corresponding BOD fraction for BKME samples withdrawn from a batch biodegradation test ........................................................................ 258
Figure 7.3.17 OUR vs. time for a number of batch BKME biodegradation tests ................................................................................... 260
Figure 7.3.18 OUR vs. time for a number of batch BKME biodegradation tests ................................................................................... 260
Figure 7.3.19 BOD vs. time for a number of batch BKME biodegradation tests ................................................................................... 261
Figure 7.3.20 BOD vs. time for a number of batch BKME biodegradation tests ................................................................................... 261
Figure 7.3.21 OUR vs. time for a batch biodegradation test of caustic extraction effluent ................................................................................... 265
Figure 7.3.22 Slowly biodegradable fraction removal rates vs. slowly biodegradable BOD ................................................................................... 265
Figure 7.4.1 Maximum ΔOUR vs. time over the course of the project ................................................................................... 270
Figure 7.4.2 OC vs. time over the course of the project ................................................................................... 270
Figure 7.4.3 Maximum ΔOUR and the Monod half saturation constant vs. wastewater batch over the course of the project .................................................271
Figure 7.4.4 OC and BOD vs. wastewater batch over the course of the project .................................................................................................271
Figure 7.4.5 Respirometric OC vs. wastewater BOD .................................................................................................................................274
Figure 7.4.6 Maximum ΔOUR obtained with methanol, formate, and acetate, vs. time over the course of the project ........................................274
Figure 7.4.7 Respirometric yield obtained with methanol, formate, and acetate, vs. time over the course of the project .........................................275
Figure 7.4.8 Maximum methanol ΔOUR vs. wastewater batch over the course of the project .................................................................275
Figure 7.4.9 Maximum formate ΔOUR vs. wastewater batch over the course of the project .................................................................276
Figure 7.4.10 Maximum acetate ΔOUR vs. wastewater batch over the course of the project .................................................................276
Figure 7.4.11 Ratio of methanol ΔOUR to formate ΔOUR and methanol yield to formate yield vs. time over the course of the project .................278
Figure 7.4.12 Maximum ΔOUR vs. SRT for all of the wastewater batches studied ..........................................................................................278
Figure 7.4.13 Maximum ΔOUR obtained with methanol, formate, and acetate, vs. SRT for all of the wastewater batches studied ..................282
Figure 7.4.14 OUR vs. time for injections of BKME batch L .................................................282
Figure 7.4.15 ΔOUR vs. BOD for BKME batch L ........................................................................285
Figure 7.4.16 OUR vs. time for injections of BKME batch M .................................................285
Figure 7.4.17 OUR vs. time for injections of BKME batch N .................................................286
Figure 7.4.18 OUR vs. time for injections of BKME batch N .................................................286
Figure 7.5.1 Apparent yield vs. SRT ......................................................................................289
Figure 7.5.2 Apparent yield vs. wastewater batch .....................................................................289
Figure 7.5.3 Decay coefficient vs. wastewater batch ................................................................292
Figure 7.5.4 Endogenous OUR and decay coefficient vs. SRT .................................................................................................................292
Figure 7.5.5 Endogenous OUR, ΔOUR obtained with methanol, formate, and acetate, vs. SRT .................................................................................................................295
Figure 7.5.6 Endogenous OUR, ΔOUR obtained with methanol, formate, and acetate, vs. SRT .................................................................................................................295
Figure 7.5.7 OUR, ΔOUR obtained with methanol and formate, vs. time during a batch decay test, semilog graph .................................................299
Figure 7.5.8 OUR, ΔOUR obtained with methanol and formate, and MLVSS vs. time during a batch decay test .................................................299
Figure 7.5.9 OUR, ΔOUR obtained with methanol, formate, and acetate vs. time during a batch decay test, semilog graph .................................................301
Figure 7.5.10 OUR, ΔOUR obtained with methanol, formate, acetate, and MLVSS vs. time during a batch decay test at 35°C .................................................301
Figure 7.5.11 OUR, ΔOUR obtained with methanol, formate, acetate, and MLVSS vs. time during a batch decay test at 20°C .................................................303
Figure 7.5.12 Apparent yield vs. SRT ......................................................................................303
Figure 8.1.1 ΔOUR vs. substrate concentration at different pH's .................................................306
Figure 8.1.2 Maximum ΔOUR and Monod half saturation constant vs. pH ........306
Figure 8.1.3 ΔOUR vs. substrate concentration at different pH's ..............308
Figure 8.1.4 OC vs. substrate concentration at different pH's .................308
Figure 8.1.5 Maximum ΔOUR and Monod half saturation constant vs. pH ........309
Figure 8.1.6 Maximum ΔOUR vs. pH ..................................................309
Figure 8.1.7 Maximum ΔOUR, obtained with methanol, formate, and acetate, vs. pH .................................................................310
Figure 8.1.8 Relative maximum ΔOUR, obtained with methanol, formate, and acetate, vs. pH .................................................................310
Figure 8.1.9 OUR vs. time for injections of methanol at different pH values ....312
Figure 8.1.10 Endogenous OUR vs. pH..................................................312
Figure 8.1.11 OUR vs. time for batch BKME biodegradation tests at pH 8 and at pH 9 .................................................................314
Figure 8.1.12 BOD vs. time for batch BKME biodegradation tests at pH 8 and at pH 9 .................................................................314
Figure 8.1.13 SUR vs. BOD for batch BKME biodegradation tests at pH 8 and at pH 9 .................................................................315
Figure 8.1.14 SUR vs. BOD at pH 8 and at pH 9, measured using the fed batch assay .................................................................315
Figure 8.1.15 ΔOUR vs. substrate concentration obtained with biomass exposed to pH 10 .................................................................317
Figure 8.1.16 Maximum ΔOUR and Monod half saturation constant 10 vs. pH, measured using biomass exposed to pH 10 .................................................................317
Figure 8.1.17 Relative maximum ΔOUR, obtained with methanol, vs. time exposed to various pH changes .................................................................319
Figure 8.1.18 Relative maximum ΔOUR, obtained with formate, vs. time exposed to various pH changes .................................................................319
Figure 8.1.19 Relative maximum ΔOUR, obtained with acetate, vs. time exposed to various pH changes .................................................................320
Figure 8.1.20 Fed batch test data, BOD vs. time after exposure to pH 10 ........320
Figure 8.1.21 First order ΔOUR decay coefficient and maximum ΔOUR vs. pH .................................................................322
Figure 8.1.22 Recovery of relative maximum ΔOUR vs. time after exposure to pH 10 .................................................................322
Figure 8.1.23 Recovery of relative maximum ΔOUR vs. time after exposure to pH 10 .................................................................323
Figure 8.1.24 Relative maximum ΔOUR after exposure to pH 10 vs. biomass concentration .................................................................323
Figure 8.1.25 Recovery of relative maximum ΔOUR obtained with formate vs. time after exposure to pH 10 .................................................................325
Figure 8.1.26 Recovery of relative maximum ΔOUR obtained with methanol vs. time after exposure to pH 10 .................................................................325
Figure 8.1.27 Recovery of relative maximum ΔOUR obtained with acetate vs. time after exposure to pH 10 .................................................................326
Figure 8.2.1 OUR vs. time for injections of methanol following a temperature increase from 34°C to 44.5°C .................................................................329
Figure 8.2.2 OUR vs. time for injections of methanol following a temperature increase from 34°C to 44.5°C, and then a decrease to 34°C .................329
Figure 8.2.3 Maximum ΔOUR, maximum SUR, and respirometric yield, obtained with methanol vs. time from temperature increase from 34°C to 44.5°C .................................................................331
Figure 8.2.4 OUR vs. time for injections of methanol following a temperature decrease from 34°C to 27°C .................................................................331
Figure 8.2.5 OUR vs. time for injections of methanol following a temperature increase from 34°C to 42.4°C .................................................................332
Figure 8.2.6 OUR vs. time for injections of methanol following a temperature increase from 34°C to 49°C .................................................................332
Figure 8.2.7 Relative maximum ΔOUR, obtained with methanol, vs. time from temperature change for various temperature changes ................334
Figure 8.2.8 Respirometric yield, obtained with methanol, vs. time from temperature change for various temperature changes .................334
Figure 8.2.9 Maximum SUR, obtained with methanol, vs. time from temperature change for various temperature changes ..........335
Figure 8.2.10 Maximum SUR, obtained with methanol, vs. time from temperature change for various temperature changes, on a semi-log graph .................................................................335
Figure 8.2.11 Methanol SUR decay coefficients vs. inverse temperature .................................................................337
Figure 8.2.12 Methanol maximum SUR vs. inverse temperature .................................................................337
Figure 8.2.13 Methanol maximum SUR vs. temperature .................................................................338
Figure 8.2.14 Active fraction vs. temperature .................................................................338
Figure 8.2.15 Methanol SUR decay rate and respirometric yield vs. temperature .................................................................339
Figure 8.2.16 Effect of rapid temperature adjustment on maximum ΔOUR, maximum SUR, and respirometric yield .................................................................339
Figure 8.2.17 Maximum methanol ΔOUR vs. temperature .................................................................341
Figure 8.2.18 Relative maximum methanol ΔOUR vs. temperature .................................................................341
Figure 8.2.19 OUR vs. time for injections of formate following a temperature increase from 34°C to 54°C .................................................................342
Figure 8.2.20 Relative maximum ΔOUR, obtained with formate, vs. time from temperature change for various temperature changes ..........342
Figure 8.2.21 Respirometric yield, obtained with formate, vs. time from temperature change for various temperature changes ..........343
Figure 8.2.22 Maximum SUR, obtained with formate, vs. time from temperature change for various temperature changes ..........343
Figure 8.2.23 Formate SUR decay rate and respirometric yield vs. temperature .................................................................345
Figure 8.2.24 Formate maximum ΔOUR vs. temperature .................................................................345
Figure 8.2.25 OUR vs. time for injections of acetate following a temperature increase from 34°C to 45°C .................................................................346
Figure 8.2.26 Maximum ΔOUR, maximum SUR, and respirometric yield, obtained with acetate, vs. time from temperature increase from 34°C to 45°C .................................................................346
Figure 8.2.27 Maximum acetate AOUR vs. temperature ........................................348
Figure 8.2.28 Maximum methanol AOUR, maximum formate AOUR, and maximum acetate AOUR, vs. temperature ........................................348
Figure 8.2.29 Endogenous OUR vs. temperature ........................................350
Figure 8.2.30 Endogenous OUR vs. time from temperature change for various temperature changes ........................................350
Figure 8.2.31 Endogenous OUR, maximum methanol AOUR, and maximum formate AOUR vs. time from temperature change from 34°C to 49°C ........351
Figure 8.2.32 Endogenous OUR, maximum methanol AOUR, and mass transfer limited methanol AOUR vs. time from temperature change from 34°C to 44.5°C ........................................351
Figure 8.2.33 Methanol AOUR vs. substrate concentration at 25°C, 35°C, and 40°C ........................................353
Figure 8.3.1 Maximum AOUR and endogenous OUR vs. time following a temperature increase from 34°C to 44°C ........................................355
Figure 8.3.2 MLVSS and treated effluent BOD vs. time following a temperature increase from 34°C to 44°C ........................................355
Figure 8.3.3 Maximum AOUR measured at 35°C, 45°C, and 25°C, vs. time following a temperature increase from 34°C to 44°C ........................................357
Figure 8.3.4 Maximum AOUR vs. temperature before and after a temperature increase from 34°C to 44°C ........................................357
Figure 8.3.5 MLVSS and apparent yield vs. time in a control reactor, and a temperature shocked reactor ........................................359
Figure 8.3.6 Treated BOD and COD vs. time in a control reactor, and a temperature shocked reactor ........................................359
Figure 8.3.7 Maximum AOUR vs. time in a control reactor, and a temperature shocked reactor ........................................361
Figure 8.3.8 Respirometric yield vs. time in a control reactor, and a temperature shocked reactor ........................................361
Figure 8.3.9 Maximum AOUR vs. temperature using control biomass and biomass exposed to a 10°C temperature increase ........................................363
Figure 8.3.10 Relative maximum AOUR vs. temperature using control biomass and biomass exposed to a 10°C temperature increase ........................................363
Figure 8.3.11 AOUR vs. substrate concentration using control biomass and biomass exposed to a 10°C temperature increase ........................................365
Figure 8.3.12 Maximum AOUR vs. temperature for biomass acclimated to 35°C and biomass acclimated to 27°C ........................................365
Figure 8.3.13 Relative maximum AOUR vs. temperature for biomass acclimated to 35°C and biomass acclimated to 27°C ........................................367
Figure 8.3.14 Respirometric coefficients vs. time during a temperature decrease to 27°C ........................................367
Figure 8.3.15 Apparent yield vs. time during a temperature decrease to 27°C ........................................369
Figure 8.3.16 Optimum temperature vs. operating temperature ........................................369
Figure 8.4.1 ΔOUR vs. pH, at various temperatures ........................................371
Figure 8.4.1a ΔOUR vs. pH, at various temperatures ........................................371
Dedication

This thesis is dedicated to Lucky - who understood the meaning of life.
Chapter 1 Introduction & Objectives

Introduction

Large amounts of water are used in the production of bleached pulp. Due to residual chlorine, it is difficult to recycle the effluent from the bleaching process, and this waste stream, along with others, is discharged to the environment. The combined bleached kraft mill effluent (BKME) contains a large number of organic compounds and toxic compounds. If the wastewater is not treated there is potential for lethal toxic effects on the ecosystem in the immediate vicinity of the outfall. If the wastewater is well mixed and diluted during discharge to be below the acute toxicity threshold in the receiving water, there may still be sublethal toxic effects. In addition, the oxygen demand of the wastewater may lead to eutrophication of the receiving water, especially if nutrients are present. In order to minimise these problems, BKME is treated prior to discharge to the environment.

Many pulp mills have recently installed activated sludge wastewater treatment plants. In the activated sludge process, a consortium of microorganisms grow on the organic compounds in the wastewater. The biomass growth rate is controlled by wasting excess biomass, which must be disposed of. By changing the growth rate (or solids retention time (SRT)), or adding a selector, different microbial populations may be selected. Different populations may lead to different treatment characteristics.

When both the pulp mill and the treatment plant are operating at steady state, the activated sludge process is adequate for removing the easily biodegradable organic compounds and the acute toxicity from BKME. Pulp mills do not always operate at
steady state due to changes in the wood source, and the bleaching process. These changes will have effects on the wastewater composition and subsequently may impact the wastewater treatment.

More severe impacts on the treatment system are possible if process spills occur during the pulping or bleaching operations. For example, if there is a black liquor spill, a large amount of caustic material and organic matter will be sent to the treatment plant. If the spill is large, it may be beyond the buffering capacity of the pH control system, and the activated sludge will be exposed to a pH shock. If the black liquor spill is neutralised before reaching the wastewater treatment system, the activated sludge will still be exposed to an organic shock load.

BKME is typically too hot for biological wastewater treatment, and must be cooled. There can occasionally be problems with a pulp mill's effluent cooling system, especially in the summer months, and the temperature in the activated sludge unit may rapidly increase beyond the optimal, or acceptable, temperature range for the activated sludge biomass.

The goal of this project was to study the effects of rapid temperature and pH changes on the activated sludge treatment of BKME. The tools chosen to quantify the activated sludge performance were the measurement of organic removal rates from the wastewater, measurement of the rate of decay of the biomass, and the measurement of the stoichiometry of the process. A survey of the literature revealed that activated sludge growth rates, and/or organic removal rates, were most often measured using respirometry, so this was the method chosen. As the project progressed, some shortcomings and
invalid assumptions of the respirometric method slowly became evident. This led to a full investigation of respirometry.

Concurrently to the investigation of respirometry, the appropriate models to use to characterise BKME and the activated sludge treatment of BKME were determined. The kinetics of BKME treatment by activated sludge has received limited investigation. The majority of previous studies have used older activated sludge models, ignoring recent findings obtained from municipal wastewater treatment investigations. In this study, these newer models were adapted to apply to the specific case of BKME, which is different from typical domestic sewage.

The activated sludge model and the respirometric method were used to study the effects of pH and temperature on the treatment process. Experiments performed later in the study were done in more detail, as more was known about respirometric methods and the nature of BKME.

**Objectives**

**Hypothesis:** The stoichiometry and rates of substrate metabolism for the activated sludge treatment of BKME are a complex function of: effluent characteristics, environmental conditions (pH, temperature), and operating conditions (SRT, aerobic selector). In order to verify this hypothesis, the following objectives for this project were set:

- To determine the effect of operating conditions and effluent characteristics on the activated sludge treatment of BKME. In particular, to investigate the effects of varying the SRT from 5 to 20 days, adding an aerobic selector, and wastewater composition.
• To evaluate methods for measuring activated sludge organic removal rates, decay rates, and stoichiometry, focusing on respirometric methods.

• To determine the appropriate model to use for the activated sludge treatment of BKME.

• To determine the effects of transient operating conditions on the activated sludge treatment of BKME, such as changing wastewater composition, organic concentration, operating temperature, and operating pH.

Outline

Following is a brief outline of this thesis: chapters 2 through 4 give background information, which is relevant to the topic, and / or the results. Chapters 6 through 8 give the results of this project. Each builds upon the results of the preceding chapters.

Chapter 2 discusses the characteristics of BKME and BKME treatment. In addition, the effect of transient operating conditions on activated sludge (organic concentration, temperature, and pH), and the variability of treatment results are discussed. Since only a small amount of research on the effects of transient operating conditions on BKME treatment is available in the literature, this review will mainly discuss municipal treatment results. This discussion is relevant for chapters 7 (all of chapter 2) and 8 (sections 2.3 and 2.4).

Chapter 3 discusses activated sludge models, the effects of multiple substrates on data interpretation, and the incorporation of mass transfer, pH, and temperature, into activated sludge models. This discussion is relevant for chapters 6 (3.2, 3.4), 7 (3.3), and 8 (3.5, 3.6).
Chapter 4 discusses various assays for measuring activated sludge growth rates and organic removal rates, focusing mainly on respirometric methods. Many different respirometric methods have been developed, however most are very similar, and most make the same assumptions. The discussion of respirometric methods has been divided into two sections - the methods which employ a low food to microorganism ratio, and those which employ a high food to microorganism ratio, (relevant for chapter 6 and 7).

Chapter 5 presents the materials and methods used in obtaining the results presented in chapters 6 through 8.

Chapter 6 is a detailed investigation of the respirometric method, focusing on the measurement of the removal rates of methanol, formic acid, and acetate (known components of BKME). A variety of models are investigated, including ones which contain mass transfer effects. The assumption of pseudo-steady state is discussed. Also investigated were the effects of dissolved oxygen (DO) concentration on respirometry, and interactions among substrates when more than one substrate is present. This last result is important for the interpretation of BKME respirometric data, since BKME is a mixture of many substrates.

Chapter 7 presents the substrate removal rate data obtained using BKME. Major points presented in this chapter are the importance of multiple components in the wastewater for the interpretation of respirometric data, the variability of the removal rates, the variability of the wastewater composition, the effect of operating conditions on the treatment performance, and the adaptation of the biomass to different batches of wastewater. The significance of these findings on activated sludge modeling is discussed. Finally, since results from the measurement of activated sludge yield and
decay do not agree with standard interpretations, possible alternate interpretations of the data are presented.

Chapter 8 discusses the effects of pH and temperature on the performance of the activated sludge process. The effects of sudden temperature changes and sudden pH changes on biomass acclimated to pH 8 and 35°C were investigated. Two week long temperature increases were also investigated to observe the adaptation ability of the biomass.

Chapter 9 ties all of the results together, and summarises the project.
Chapter 2 Literature Review - Activated Sludge Operation

In this chapter a review of the aspects of activated sludge relevant to this project will be presented. The first subject will be the nature and treatability of BKME. Next, factors which affect the activated sludge treatment and stoichiometry will be discussed. Among these factors are the solids residence time (SRT), the hydraulic and environmental characteristics of the activated sludge process, shock loads, wastewater variability, and population dynamics.

2.1 BKME Characteristics

Bleached kraft mill effluent (BKME) is a complex mixture, containing simple inorganic salts, over 300 known (with many more unknown) low molecular weight organic compounds, and many high molecular weight (>1kDa) organic constituents (Axegard et al 1993). The high molecular weight components of BKME are generally assumed to be chlorolignins (Sågfors and Starck 1988, Dahlman et al. 1993), which are the highly oxidized degradation products originating from the residual lignin in the unbleached pulp, and carbohydrates from hemicelluloses (Axegard et al. 1993). There are many toxic compounds present in BKME, mainly resin and fatty acids, and chlorinated phenols. There are also unidentified toxic compounds (Heimburger et al 1988a). Due to the vast amount of different compounds present, pulp mill effluent is usually characterised by general parameters, such as BOD (biochemical oxygen demand), COD (chemical oxygen demand), TOC (total organic carbon), colour, pH, TSS (total suspended solids), and AOX (adsorbable organic halide).
The wastewater from the manufacturing of pulp originates during various aspects of the process. During the kraft pulping process, the lignin is removed from the wood fibres by dissolving it in the cooking chemical solution (white liquor: sodium hydroxide and sodium sulfide). Ninety to ninety-five percent of the lignin is removed as well as some of the polysaccharides, especially the hemicelluloses. The cooking liquor is then concentrated by evaporation, and the cooking chemicals are recovered in the kraft recovery furnace.

The main source of pollution from the pulping process is the condensates generated during concentration of the cooking liquors. The condensates are high in methanol (which comprises 80% of the total BOD of the condensates) (Kemeny and Baerjee 1997, Barton et al 1998), and also contains other alcohols (mainly ethanol), ketones, phenolic substances, sulfur compounds and terpenes. The condensates contain approximately one third of a pulp mill's BOD load (Springer 1993b).

Cooked pulp is washed with water to remove the dissolved organic material and residual lignin from the pulp. This process is called brown stock washing and the wash water is sent to the evaporators. The washing results are improved as the amount of wash water increases, but so is the demand on the evaporators. Black liquor carry-over to the bleach plant will affect the bleach plant effluent quality (Tana and Lehtinen 1996). Older mills often have high washing losses to the sewer; this effluent contains phenolic compounds, organic acids, terpenes, and resin acids (Axegard et al 1993, Kemeny and Baerjee 1997).

Another source of BOD from the pulping process comes from process spills, such as black liquor spills (Springer 1993). Black liquor is very rich in BOD (hydroxy acids,
formic acid, acetic acid), and contains high concentrations of wood extractives, which are toxic (Sjöström 1993).

To further reduce the organic load to the bleach plant, most mills use extended delignification or oxygen delignification. The organic matter removed from the pulp prior to bleaching is sent to the kraft recovery furnace and not to the treatment plant.

The residual lignin in the pulp after the pulping stage is removed in the bleaching process. The bleaching process consists of a chlorination stage (usually with 100% chlorine dioxide substitution), followed by extraction of the chlorinated lignins in alkali, then more bleaching with chlorine dioxide and extraction. It is the first 2 stages of the bleaching process, the chlorination stage (Cl) and the extraction stage (El) which contain most of the pollutants originating in the bleach plant.

The effluent from the extraction stage contains organic compounds with a higher molecular weight than the organic compounds in the chlorination stage effluent. The majority of the AOX in spent chlorination liquor has a molecular weight below 10 000 (80%), with 30% being below 1000. Fifty-five percent of the spent alkali liquor has a molecular weight greater than 25 000, with only 5% being below 1000 (Kringstad and Lindström 1984). Another study of the molecular weight distribution of the AOX in BKME found 85% to have a molecular weight less than 1000 when measured with non-aqueous size exclusion chromatography (Jokela and Salkinoja-Salonen1992). HMW COD and AOX are mainly composed of strongly oxidised degradation products originating from the residual lignin in the unbleached pulp (Axegard et al.1993), much of which will degrade to LMW compounds in the environment (Wilson and Holeran 1992, Eriksson et al. 1985, Fitzsimmons and Eriksson 1990).
The majority of the BOD in the chlorination stage effluent is due to methanol, while formic acid is the major contributor to the extraction stage BOD. LMW acidic compounds identified in BKME (when the pulp is bleached with chlorine) in quantity are acetic acid, glyceric acid, oxalic acid, malonic acid, succinic acid, and malic acid. Next to methanol, the greatest components of the LMW neutral compounds are the various hemicelluloses. Also present are a wide variety of phenolic compounds, many of which are chlorinated. Many of these compounds are biodegradable, but at slow rates (Kringstad and Lindström 1984). When modern bleaching sequences are used, (100% chlorine dioxide substitution, enhanced extraction) methanol is still a major component of the total mill effluent, and alkaline extraction effluent (Kemeny and Banerjee 1997), and both methanol and formic acid are present in significant quantities in the first chlorine dioxide stage effluent (Dahl et al 1998). Totally chlorine free bleach plant effluents also contain significant amounts of monocarboxylic acids, mainly formic acid, glycolic acid, and 3-hydroxypropanoic acid (Ristolainen and Alén 1998). These compounds are formed under both alkaline and acidic bleaching conditions.

Reduction in the amount of pollution generated during bleaching is achieved by substituting $\text{ClO}_2$ for $\text{Cl}_2$ in the first chlorination stage, as long as the level of substitution is greater than 50%. High chlorine dioxide substitution and oxygen delignification decrease COD, BOD, AOX, toxicity, colour, and makes the waste water easier to treat by biological methods. Modern bleaching sequences (extended delignification, oxygen delignification, and chlorine dioxide substitution) produce significantly less organic material (measured by BOD, COD, TOC, AOX, toxicity, and colour), and material of lower MW than the older bleaching processes (Axegard et al 1993, Graves et al. 1993,
New bleaching and cooking methods produce less organic pollution, but the ratio of BOD to COD in the effluent remains approximately the same (Graves et al. 1993, Heimburger 1988a, 1988b, Saunamäki 1995).

Typical characteristics, and treatment efficiency for BKME are shown in table 2.1. The content of BKME depends on many factors, including the type of tree being pulped, the age of the wood chips and the specifics of the process (Graves et al. 1993, Servizi and Gordon 1973, Heimburger 1988a, 1988b). Effluent from production of hardwood pulp contains organic material of lower MW, and less chlorinated phenolics, than the effluent from production of the corresponding softwood pulps (Axegard et al. 1993, Heimburger et al. 1988a). More than twice as much AOX, BOD, and COD is generated during the bleaching of softwood pulp than hardwood pulp (Cook 1990, Heimburger et al. 1988b). The spread in removal efficiencies is due to the differences in the content of BKME, as well as the differences in the wastewater treatment plants used.

Table 2.1 BKME Composition and Treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Amount per ton of wood pulped</th>
<th>Typical Concentration</th>
<th>Removal Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD</td>
<td>10 - 30</td>
<td></td>
<td>80 - 95%</td>
</tr>
<tr>
<td>COD</td>
<td>30 - 120</td>
<td></td>
<td>30 - 70%</td>
</tr>
<tr>
<td>TOC</td>
<td></td>
<td></td>
<td>25 - 50%</td>
</tr>
<tr>
<td>AOX</td>
<td>0.2 - 7</td>
<td></td>
<td>15 - 70%</td>
</tr>
<tr>
<td>Colour</td>
<td>20 - 220</td>
<td></td>
<td>~ 0</td>
</tr>
<tr>
<td>Acute toxicity</td>
<td>200 - 400</td>
<td></td>
<td>90 - 100%</td>
</tr>
</tbody>
</table>

1 Wilson and Holloran 1992
2 Leach 1976
3 Axegard et al. 1993
4 Jokela et al. 1993
5 Heimburger et al. 1988a
The characteristics of BKME from any given mill are highly variable, with variations on the order of 100%, as can be seen in the literature (Simpura and Pakarinen 1993, Lo et al. 1994a). These variations were measured with global parameters. The variation of specific compounds in the wastewater is probably even greater. These variations may be due to changing operating conditions, different species of trees being pulped, or differences in the age of the wood chips used. In a study of a TMP (thermomechanical pulping) mill, resin acid discharge was greater in the winter months than in the summer months, presumably due to greater resin acid degradation during wood chip storage in the warmer summer months. Variation in BKME BOD from ~50 mg/l to greater than 400 mg/l was observed in an eight month study, with large differences observed from day to day. The large increases in BOD corresponded to black liquor spills. Black liquor spills, and the pulping of green chips, also resulted in effluent high in toxicity (Servizi and Gordon 1973). This variability in effluent quality and strength will be expected to influence the wastewater treatment performance.

2.2 Activated Sludge Treatment of BKME

General BKME Treatment

The preferred method of BKME treatment is primary clarification followed by secondary biological wastewater treatment. The most widely used form of biological wastewater treatment for pulp and paper effluents is the aerated stabilisation basin. The advantages of aerated stabilisation basins over activated sludge are much simpler operation, lower costs, resistance to shock loads, and minimal sludge production (Folke and Guerra 1992). The disadvantages are the large land area required and the high
suspended solids content in the treated effluent. The minimum detention time is 4 days, with most mills operating at 10 to 15 days. The high COD to BOD ratio of BKME (table 2.1) implies that it will not be possible to remove all of the organic matter from the wastewater using biological wastewater treatment.

Many of the new treatment plants are activated sludge designs, used to meet today's stricter environmental regulations. The activated sludge process can produce an effluent very low in BOD and SS, and the treatment efficiency is less variable than that obtained with aerated lagoons (Melcer et al. 1995). Detention times are around 3 to 8 hours, making activated sludge very attractive when there is minimal area available for a treatment plant. Operating conditions are chosen to select for floc forming microorganisms with good settling characteristics. This allows for mixed liquor suspended solids (MLSS) concentrations on the order of 2000 to 5000 mg/l and also results in the production of large amounts of excess sludge, 0.5 to 0.75 kg/kg BOD removed. If pure oxygen is used, the MLSS may be in the range of 5000 to 7000 mg/l, allowing for shorter hydraulic detention times. However there is little difference in performance between air and oxygen activated sludge plants (Springer 1993).

One of the pollution parameters of concern in BKME is AOX, although there is no clear evidence linking AOX to environmental effects (Heimburger et al. 1988b, Axegard et al. 1993). In lagoons, AOX removal is hypothesised to occur by sorption onto the biomass and subsequent dechlorination under anaerobic conditions in the benthic layer (Bryant et al. 1987, Bryant and Amy 1989, Chernysh et al. 1992). Activated sludge units are usually reported to achieve greater AOX removal than aerobic lagoons. In the activated sludge process the excess sludge is wasted, and any AOX adsorbed onto the
sludge will also be wasted. (Boman et al. 1988). An alternate explanation is that the higher solids retention time of activated sludge compared to lagoons allows for the growth of biomass which can degrade some of the high molecular weight AOX and COD compounds (Bomann et al. 1988, and Wilson and Holloran 1992). A study of a full scale treatment plant found the main removal mechanism of chlorinated phenols to be sorption into the sludge (Leuenberger et al. 1985). Others have observed minimal adsorption of the AOX onto the biomass (Hall and Randle 1992, Nevalainen et al. 1991, Rempel et al. 1992). Most, if not all, studies have found that conventional aerobic biological treatment cannot remove all of the AOX in the wastewater. To achieve zero AOX goals, chlorine must be eliminated from the bleaching sequence, or the bleach effluent must be recycled.

The other pollution parameters of concern in BKME are BOD, COD, and toxicity. Secondary treatment removes the majority of the BOD and the acute toxicity from BKME. Approximately half of the COD is removed. The remaining COD is a measure of the recalcitrant organic compounds in the wastewater (this is demonstrated by the very low BOD to COD ratio of the treated effluent). [Recalcitrant, as used in this study, refers to those compounds which are not degraded during wastewater treatment, and does not mean that these compounds will not be degraded in the environment.]

More information on the nature of the compounds that are in the wastewater may be obtained by measuring the molecular weight (MW) distribution. In several studies of the molecular weight distribution of the TOC before and after biological treatment, there was greater removal of the low MW organics, and no significant reduction of the high MW organics (Graves et al. 1993, Lindström and Mohamed 1988, Yu 1993). Studies of full scale activated sludge plants treating BKME found removal of all size classes of
AOX from BKME, with a slightly greater removal of the lower MW compounds (Jokela et al. 1993, Stuthridge et al. 1991). Other full scale studies have found limited removal of high MW AOX (Strang 1992) and high MW TOC (>300 daltons) (Sonnenberg et al. 1995). There is a slight increase in the high molecular weight organics during aerobic treatment, due to microbial product formation (Sonnenberg et al. 1995, Bryant and Amy 1989).

Effluent colour is associated with the carbon which is not removed during biological treatment, and is probably composed of lignin residuals (Kemeny and Baerjee 1997).

The biological treatment efficiency of BKME depends upon the pulping and bleaching sequence used at the mill, as well as the wood species being pulped. Oxygen delignification appears to make the effluent more amenable to biological treatment, as well as reducing the total amount of pollutants. Nevalainen et al. (1991) found 40% COD removal, and 22% AOX removal, during activated sludge treatment of conventional effluent. This increased to 50% COD reduction, and 40% AOX reduction, during the treatment of oxygen bleached pulp. There was also a slight increase in BOD removal. In another study, COD removal in the waste treatment plant increased from 27% to 32% with ClO₂ substitution, and to 45% with oxygen delignification. BOD removal decreased with ClO₂ substitution, from 90 to 87%, and decreased to 80% with oxygen delignification, although the overall amount of BOD in the effluent greatly decreased (by 50%) (Graves et al. 1993).
Effect of SRT and HRT on Treatment Performance

The ability to recycle the suspended solids in the activated sludge process allows the solids retention time (SRT) to be controlled independently from the hydraulic residence time (HRT). The SRT is a very important variable, for many reasons. The most obvious, and probably the most important, is that the mixed liquor volatile suspended solids (MLVSS) concentration is directly related to the SRT.

Sludge settleability is a very important treatment parameter for the activated sludge process, and is influenced by the SRT and other factors. If the SRT is too low, the growth rate of the biomass will be above that required for flocculation, and the sludge will not settle. Operation at high SRT may result in sludge with poor settling qualities. Most activated sludge plants designed for the treatment of BKME are operated at SRTs between 5 and 10 days. This allows for removal of some of the more slowly biodegradable compounds, while maintaining a sludge with good settling qualities.

The growth rate (and consequently the SRT) may have an effect on the biomass characteristics, such as cell morphology and the enzymes expressed (Harder and Dijkhuizen 1983). For some bacteria, at high growth rates enzyme systems with low affinity but high capacity for the carbon source are expressed, while at low growth rates enzyme systems with high affinity, low capacity, are expressed.

The microbial population present in the wastewater treatment plant depends on the SRT. As the SRT decreases, bacteria with relatively slow growth rates will wash out of the system, resulting in a lower microbial diversity than if the SRT were greater. High SRTs are often necessary for the removal of the more recalcitrant compounds in the wastewater, which are thought to be removed by microorganisms with low growth rates.
The common reasoning for this assumption is that if the more recalcitrant compounds degrade at low rates, then the bacteria which utilise these substrates grow at low rates. An additional explanation is offered by the fact that as the SRT increases, the specific loading of all of the organic compounds decreases. If the recalcitrant compounds are inhibitory, or if they are only metabolised when other preferential substrates are already utilised, then increasing the SRT will increase the removal rates of these compounds.

For difficult to degrade compounds, such as pentachlorophenol, degradation occurs best at high sludge ages (greater than eight days), suggesting catabolic degradation by slow growing specific degraders (Melcer and Bedford 1988, Nyholm et al. 1992, Ettala et al. 1992). Other compounds, such as lindane, have higher removal efficiencies at intermediate sludge ages and high loading rates, implying removal by co-oxidation (Nyholm et al. 1992). In a study of phenol biodegradation, at a range of SRTs (3 to 14 days), the specific phenol loading rate was found to be more important than the SRT in determining the phenol breakthrough (Nakhla et al. 1994). The specific loading and the SRT are related.

The improved removal of compounds with slow biodegradation rates with increasing SRT may be offset by the increase in soluble microbial product (SMP) formation. SMP formation is directly related to sludge age (Rittman et al. 1987, Pribyl et al. 1997). SMPs are also directly related to the influent substrate concentration (Chudoba 1985).

In the treatment of BKME, increasing the SRT, and/or the HRT, is often observed to result in increased COD or AOX removal (Yu 1993, Rempel et al. 1992, Liu et al. 1993, Bomann et al. 1988, Wilson and Holloran 1992, Hall and Randle 1992,
Simpura and Pakarinen 1993, Cook 1990, Strehler and Welander 1994). For example, decreasing the HRT from 20 hours to 2 hours in lab scale activated sludge treatment of bleach plant effluent resulted in the COD removal decreasing from 50% to 40%, and AOX removal decreasing from 63% to 47%. A further reduction in HRT below 2 hours resulted in process deterioration. The mixed liquor solids level was kept constant during these experiments, so the SRT would have decreased with the HRT (Yu 1993). In another study of activated sludge treatment of BKME where the SRT was kept constant, the soluble organic carbon and AOX removal decreased with increasing HRT. The explanation offered was that the increased biomass at lower HRTs resulted in greater adsorption of the organic material onto the flocs. When the SRT was increased, greater AOX and soluble organic carbon removal was observed. The BOD was equally removed at all of the SRTs and HRTs studied (Oleszkiewicz et al. 1992). In a comparison of lagoon to activated sludge treatment systems, the long HRT of the lagoons was found to be more important than the long SRT of the activated sludge units for increasing AOX removal. Only very high SRTs (30 days) achieved equal AOX reduction compared to the long HRT lagoons (Hall and Randle 1992). As the SRT was increased from 10 days to 30 days, COD reduction was found to increase from 44% to 64%, with minimal improvement upon a further increase in SRT to 40 days (Simpura and Pakarinen 1993). Rempel et al. (1992) found a closer correlation between AOX reduction and SRT than between HRT and AOX reduction. An SRT of 10 days or greater gave the best removal of resin acids and chlorinated phenolics (Rempel et al. 1992). Increasing the SRT from 2.5 to 5 days also results in better removal of chlorinated phenolics (Lindström and Mohamed 1988). BOD removal is not usually affected within the treatment parameters.
tested (i.e. the BOD is completely removed at all test conditions which give good sludge settling characteristics). Unlike the BOD assay, AOX and COD measurements encompass compounds whether or not they are easily degraded.

Increasing the HRT provides longer contact between the biomass and the wastewater resulting in increased removal of the more recalcitrant compounds across the treatment system. Conversely, decreasing the HRTs may not allow enough time for degradation of the various compounds in the wastewater. When the removal mechanism of a recalcitrant compound is adsorption onto the sludge, low SRTs will result in greater removal compared to higher SRTs, where biodegradation will be more important (Nyholm et al. 1992). In practical applications the HRT and the SRT are closely related. Increasing the SRT may require increasing the HRT to avoid excessive MLVSS concentrations, making it difficult to determine which parameter is more important.

The effect of HRT on BKME treatment becomes more evident when plug flow systems, or aeration tanks in series, are studied. There is a rapid removal of BOD at the start of the treatment unit, and a slow steady removal of COD across the whole treatment unit (Simpura and Pakarinen 1993, Servizi and Gordon 1973, Oleszkiewicz et al. 1992, Fein 1992, Saunamäki et al. 1991). For example, in an activated sludge unit with a selector, followed by two aeration tanks in series, 96% of the BOD was removed in the selector, with minimal removal in the following aeration tanks. In contrast, 56% of the COD removed was removed in the selector, 22% in the first aeration tank, and the remaining 20% in the second aeration tank (Simpura 1993).
Selectors

Similar to the SRT and the HRT, the aeration tank hydraulic pattern may also have an important impact on the treatment process. Biomass in a plug flow system is exposed to different environmental conditions compared to biomass in a completely mixed system. These environmental differences may affect the microbial kinetics, the stoichiometry, and the microbial population.

Often, biomass from completely mixed (CSTR) systems exhibit poor settling characteristics, such as sludge bulking, pin flocs, or dispersed growth. Sludge bulking is one of the main operating problems of activated sludge. Some causes of sludge bulking are low nutrient levels, low dissolved oxygen levels, low pH, and a low food to microorganism ratio (f/m). The f/m in the aeration tank is the ratio of the substrate concentration in the aeration tank to the biomass concentration. A CSTR activated sludge unit will have a lower f/m than a plug flow activated sludge unit. In a plug flow reactor the f/m will be high initially, and decrease as substrate is removed. For a CSTR, the f/m may be increased by the addition of a selector to the activated sludge process, to provide conditions of relatively high substrate concentrations at the start of the treatment process.

Filamentous microorganisms have a lower growth rate than floc forming bacteria at high substrate concentrations, but a higher growth rate at lower substrate concentrations. The selector insures that the substrate concentration is high, selecting for floc forming bacteria (Chudoba et al 1973). An alternate explanation is that the selector selects for floc formers with higher growth rates than filaments at all substrate concentrations. The selector selects for fast growing organisms simply by providing a
high initial f/m (Ekama and Marais 1986). A number of studies have compared the substrate utilisation rates of biomass from CSTRs with the biomass from systems with selectors. The biomass from units with selectors was found to have faster substrate utilisation rates (Ekama and Marais 1986, Smets et al 1994, Cech et al 1984). A third explanation for the success of selectors is the greater storage capacity of floc formers compared to filaments, and their greater ability to survive starvation periods. Storage was found to be the main removal mechanism for acetate using biomass from an intermittently fed bioreactor. The storage polymers were then utilised in the subsequent starvation period. The formation of storage polymers resulted in increased growth yield, as the substrate was utilised more efficiently (Majone et al 1996, van Loosdrecht et al 1997). A respirometric study of an aerobic selector utilised for the treatment of pulp mill wastewater suggested that the substrate was assimilated in the selector and not stored (Wessberg et al 1993).

In dealing with industrial wastewater, completely mixed activated sludge systems are often used in order to ensure maximum dilution and minimal impact of toxic compounds on the biomass. A synthetic wastewater containing phenol, a potentially toxic compound was treated in a selector, which resulted in inhibition of the bacteria. Better results were obtained using a compartmentalised system, which had better settling sludge than a CSTR treating the same wastewater. In the compartmentalised system, the phenol was removed in the first compartment, with the rest of the compartments serving for endogenous metabolism, which aided in the selection of floc forming bacteria. The maximum specific phenol removal rate was higher for the biomass from the compartmentalised system, as was the yield (obtained respirometrically) (Chudoba et al
degradate compounds, to ensure a loading suitable for acclimation of the biomass.

Selectors do not have to be aerobic, and are often anoxic or anaerobic. A number
of pulp and paper mills use an anaerobic or anoxic selector to prevent sludge bulking
studies found improved settling with the use of a selector. The use of an aerobic selector
also worked in improving sludge settling when treating pulp mill wastewater (Wessberg
et al 1993).

The addition of an anaerobic stage may improve the degradation of certain
compounds in addition to improving sludge settling. Anaerobic processes are efficient in
degrading and dechlorinating low molecular mass molecules, although the rate of
anaerobic dechlorination appears to be too slow to be of practical use in wastewater
treatment (Fitzsimons and Eriksson 1990). In lagoons, both high and low MW AOX
adsorb to aerobic biomass, which settles to the anaerobic zone, where anaerobic
dehalogenation takes place (Wilson and Holloran 1992). One study has greater removal
of AOX across an anaerobic/aerobic lagoon compared to activated sludge, but these were
full scale treatment plants treating different wastes. The anaerobic aerobic system also
removed a greater percentage of the higher MW compounds (Jokela et al 1993). Another
study (lab-scale) found that the anaerobic zone does not increase AOX removal (Hall and
Randle 1992). A study involving the profiling of the microbial community in activated
sludge found that the community structure appears to be affected more by the nature of
the wastewater than the presence of an anaerobic stage (Hiraishi 1998).
It has been shown that activated sludge operated with anaerobic stabilisation of the sludge can lead to synchronisation of the biomass. Synchronisation means that most of the cells are in the same physiological and metabolic state at the same time (Chudoba et al 1991). The same environment has been shown to lead to increased uncoupling between catabolism and anabolism, and hence lower yield (Chudoba et al 1992b).

Temperature

The microbial growth rate increases exponentially with temperature, when the temperature is below the optimum. As the temperature approaches the optimum, the increase in growth rate with temperature decreases. When the temperature passes the optimum, the microbial growth rate decreases rapidly with further increases in temperature. The optimum temperature for wastewater treatment plants is often reported to be around 35°C, with an acceptable range of 15 to 40°C for mesophilic bacteria (Bailey and Ollis 1986). The effluent from a pulp mill is usually warmer than this, so the effluent must be cooled prior to activated sludge treatment. If the wastewater is treated in a lagoon, then the large surface area and residence time may be enough to allow the effluent to cool to the desirable operating range. In the winter months, lagoon performance may decrease due to low temperatures. ASB performance also decreases at temperatures greater than 40°C (Springer 1993).

In a study of aerated lagoons treating BKME, reduced removal efficiencies of chlorinated phenolic compounds, AOX, and toxicity were observed at 10°C compared to 25°C (Melcer et al 1995). The optimal temperature for the treatment of CTMP (chemi-thermomechanical pulping) effluent was at 20°C. Below this temperature, treatment efficiency decreased. Temperature increases up to 40°C brought about a slight decrease
in removal efficiency, while treatment was poor at 50°C. The MLSS concentration was low at 50°C (Liu et al 1993). Increasing the temperature (to 40°C) resulted in lower resin and fatty acid removal due to sludge adsorption, which is an important treatment mechanism when the treatment time is short. Resin and fatty acid biodegradation rates increased with temperatures up to 40°C (Liu et al 1993b). Decreased removal of AOX at higher temperatures (35°C compared to 32°C) across an aerated lagoon was also attributed to decreased sorption onto the biomass at the higher temperatures (Bryant and Amy 1989). Under aerobic conditions, AOX removal was found to increase from 47% at 22°C to 70% at 30°C, while TOC removal was not affected by the rise in temperature (Chernysh et al 1992). A study using an aerobic suspended biofilm found better AOX removal at 50°C compared to 37°C, which was attributed to increased abiotic removal at the elevated temperature (Strehler and Welander 1994). Activated sludge operation at 50°C resulted in increased COD removal compared to operation at 35°C (Barr et al 1996). The maximum growth rate of nitrifying bacteria was found to increase monotonically from 15°C to 25°C (Antoniou et al 1990).

Over a temperature range from 10°C to 35°C, the Monod half saturation constant for nitrification was found to be lowest at 15°C. The nitrification was most efficient at 15°C, which is different from the typical optimum temperature for carbon assimilation (Charley 1980). The optimal temperature for sludge yield is not necessarily the optimum temperature for the growth rate, or substrate utilisation rate (Coultate and Sundaram 1975). Depending on the substrate, and the environmental conditions (aerobic / anaerobic), the Monod half-saturation coefficient has been found to both increase and decrease with increasing temperature (Esener et al 1983, Muck and Grady 1975).
There is greater sludge production at lower operating temperatures, 0.22 kg SS/kg BOD at 29°C compared to 0.33 lb SS/lb BOD at 18°C, during the treatment of pulp mill wastewater (Springer 1993). The lower yield at higher temperatures may be due to the microbial decay. The decay rate of bacteria increases with increasing temperature. A general rule of thumb is that the "decay rate constant is influenced by temperature in a manner similar to the maximum specific growth rate constant" (Muck and Grady 1975). The relationship between microbial yield and temperature sometimes passes through a maximum at approximately 20°C. Specific oxygen demand is much greater at high (50°C) operating temperatures, compared to 35°C (Carter et al 1975).

A further effect of temperature is to decrease the sludge settling velocity and increase the sludge volume index (SVI). The change in SVI is due to changes in the floc structure, and the nature of the exocellular material that the bacteria secrete to flocculate. A temperature range of 10°C to 35°C was studied (Cetin 1990).

pH

For most enzymatic systems, the enzymes are active within a certain pH range, usually 4 to 5 pH units. Inside this pH range, the activity versus pH has a bell shaped profile (often with a maximum around pH 7.4). Outside of this pH range, the proteins denature as they are protonated or deprotonated (Copeland 1996). Similar relationships between activity and pH are found for bacterial cultures as are found with pure enzymes (Rosso et al 1995, Lallai et al 1988).

In a study of CTMP aerobic wastewater treatment, BOD and resin and fatty acid removal was the same at operating pHs from 5 to 8. COD removal was greatest at pH 7 (80%), decreasing to 70% at pH 5, and to 74% at pH 8 (Liu et al 1993). Glucose uptake
rates, using biomass from a waste stabilisation pond, containing algae, were optimal from pH 6 to 8. Below pH 6, there was a slight decrease in the glucose uptake rates, while above pH 9, there was a large decrease. The decrease in glucose consumption above pH 9 was attributed to the algae (Mayo and Noike 1996). For growth on phenol, the microbial yield was found to be maximal at pH 6, decreasing drastically below this pH, and decreasing gradually above this pH (Lallai et al 1988).

An increase in the pH caused an increase in sludge settling velocity, and a decrease in the SVI (a pH range of 6 to 10 was studied). As the pH increased, larger flocs were formed due to an increased number of reactive sites on the cell surfaces and exocellular polymers (Cetin 1990).

The effects of pH and temperature on bacteria growth rate are usually found to be independent (Rosso et al 1995).

**2.3 Microbiological Aspects of Wastewater Treatment**

In this section a brief review of the basic microbiological aspects pertinent to the data interpretation in subsequent chapters will be presented. The majority of bacteria present in activated sludge treatment systems treating BKME are heterotrophs. For heterotrophic bacteria, the carbon source in the wastewater serves two important functions: energy generation and biomass formation. Among the carbon and energy sources for heterotrophic growth in BKME are sugars, organic acids, (including acetate and formate), as well as alcohols, mainly methanol. Activated sludge systems may also contain autotrophic bacteria which obtain their energy from the oxidation of substrates in the wastewater (such as ammonium) and their carbon from carbon dioxide.
Substrate Metabolism

The generation of energy from sugars may be demonstrated using glucose as an example. In many bacteria, the glucose is oxidised to two molecules of pyruvate via the Embden-Meyer-Oscar pathway. This results in the generation of ATP. In aerobic bacteria, pyruvate is oxidised to carbon dioxide via the citric acid cycle. This results in the reduction of NAD and FAD to NADH₂ and FADH₂ respectively. NADH₂ and FADH₂ transfer their electrons to the electron transfer chain in the cellular membrane. Under aerobic conditions, the terminal electron acceptor is oxygen. The electron transfer chain serves to pump hydrogen ions outside of the cellular membrane (which is impermeable to hydrogen and hydroxyl ions), creating a potential across the membrane. This potential is used for the generation of ATP. There are many variations of this pathway, depending on the bacterial species and environmental conditions. When glucose is the growth substrate, cellular building blocks are obtained from many points along the dissimilation pathway.

The dissimilation of acetate may follow a pathway similar to that of glucose. Acetate is oxidised via the citric acid cycle. Electrons from acetate are transferred to NAD and FAD, and then to the electron transfer chain for the generation of membrane potential and ATP. When acetate is the carbon source, an additional pathway is required, which is the glyoxylate cycle. This allows for the synthesis of three and four carbon cellular building blocks. If the glyoxylate cycle was not present, the bacteria would not be able to regenerate the constituents of the citric acid cycle as they are removed to build cellular components.
The dissimilation of one-carbon substrates is different from the metabolism of sugars and acetate. Several different pathways of methanol metabolism have been found in bacteria. The first step involves oxidation of methanol to formaldehyde catalysed by methanol dehydrogenase. This reaction occurs outside the bacterial membrane, in the cytoplasm (in gram negative bacteria). The other substrates in BKME must first be transported across the cell membrane before being dissimilated. Methanol dehydrogenase interacts directly with the electron transfer chain. Methanol is oxidised to formaldehyde. Formaldehyde is transported into the cell and further oxidised to formic acid and carbon dioxide by a number of different pathways. If methanol is to serve as the carbon source, pathways must be present to build cellular material from one-carbon compounds. The common building block for these pathways is formaldehyde. There are several such pathways in a class of bacteria known as methylotrophs.

Variability of Yield and Metabolism

The microbial yield is an important parameter for a waste water treatment process. The yield determines how much sludge must be wasted which may be a large operating expense.

The microbial yield depends upon the relationship between anabolism and catabolism. Microbial cells do not have the ability to exert fine control over the uptake and catabolism of carbon substrate, and can readily dissociate catabolism from anabolism. The anabolic enzymes are adjusted to the growth rate, but the catabolic enzymes are maintained in excess. As a consequence, if the carbon source is added as a pulse to a bacterial culture, catabolism will be higher than anabolism, and microbial yield will be lower than if the carbon source had been added continuously. This has been
observed using bacteria during active growth, as well as washed suspensions of bacteria under conditions where biosynthesis is severely impeded (Tempest and Neijssel 1980, Brooks and Meyers 1973, Stouthamer 1979, Russell and Cook 1995).

One of the main factors which determines the microbial yield is the nature of the substrate. The yields on organic acids are lower than the yields on carbohydrates. This is partly due to organic acids being more oxidised than carbohydrates, and therefore provide less energy (Narang 1997). Different substrates are utilised by different pathways, which has an effect on the amount of carbon assimilated, and hence the yield (Gommers et al. 1988).

If multiple substrates are present, the nature of the substrate mixture will also influence the yield. Substrates such as formate and methanol may not be assimilated when other substrates are present; the enzymes required for methanol and formate assimilation may not even be present. Instead, the methanol or formate will be utilised solely as an energy source. This allows for a greater percentage of the other substrates to be assimilated, increasing the microbial yield. This depends on the particular substrates, the ratio of the substrates, the growth rate, and the bacterial species. Lower ratios of formate or methanol to the other carbon source, and higher growth rates, tend to suppress assimilation of the formate or methanol, and lead to higher yields (due to greater assimilation of the other carbon source) (Harder and Dijhuizen 1982, Gommers et al 1988).

The true microbial yield depends on the substrate, the transport system, cell composition, and metabolic pathway (including the formation of storage polymers). The apparent yield, which is the measured yield, will be lower than the true yield due to
expenditure of energy for non-growth processes. The apparent yield depends upon the true yield, decay rate and endogenous metabolism. These processes are influenced by the f/m, growth rate, substrate composition, population dynamics (predation by protozoa), and feeding patterns (which may trigger energy spilling, select for floc formers over filaments, etc.). The yield also depends upon other environmental factors, such as the presence of metals (Gökçay and Yetis 1996), temperature, and the pH. Even if the true yield was constant, the apparent yield for a wastewater treatment process is influenced by many factors, and will be quite variable (Gaudy and Ramanathan 1971).

**Uncoupled Metabolism**

Anabolism and catabolism are not always perfectly coupled, not all of the energy obtained from substrate oxidation is utilised by the cell. The uncoupling between anabolism and catabolism may be a response to nutrient limitation, and appears to be a common characteristic of growth with an excess of energy (Rusell and Cook 1995). Under nutrient limitation, bacteria will increase the rate of transport of substrate into the cell, and increase the initial metabolism of the substrate. This is in order to maintain the driving force for accumulation of substrate, and also to allow the cells to increase their processes of cell synthesis without being limited by a lack of available energy, if the limiting nutrient were to suddenly increase. These goals may be achieved either through a greater amount of metabolic enzymes, or synthesis of high affinity enzymes (Harder and Dijkhuizen 1983, Neijssel and Tempest 1976, Tempest et al 1985). In chemostat studies at varying growth rates, the maximal carbon uptake rate was found to be constant, independent of the growth rate. The excess catabolic activity of the bacteria decreased as the growth rate increased.
Metabolic uncoupling is necessary to maintain growth potential. If bacteria are adapted for scavenging small molecules from dilute aqueous environments, a sudden increase in substrate would result in accumulation of too much substrate (Koch 1979). This in turn will result in an excess of energy, which must be spilled. Mechanisms for energy spilling include: production of storage compounds, excretion of catabolic products, deletion of sites of oxidative phosphorylation, branching of the respiratory chain, dissipation of the energised membrane, and wastage of ATP (Stouthamer 1979, Russell and Cook 1995). All of these mechanisms result in a decreased yield.

**Endogenous Metabolism, Maintenance Energy and Microbial Decay**

As the SRT increases, the MLVSS concentration also increases. For a given HRT and wastewater, the biomass concentration in an activated sludge unit is directly proportional to the SRT times the net yield. It is commonly observed that the net yield decreases with increasing SRT. This decrease in net yield is attributed to microbial decay and/or endogenous metabolism. These processes become important in activated sludge operation due to the low microbial growth rates typical in wastewater treatment plants. In rapidly growing cultures there is much less of an impact of bacterial decay on the net growth rate.

Maintenance energy is energy expended by the bacteria for maintenance functions. These functions include maintaining ion gradients across cell membranes, protein and RNA turnover, overflow metabolism, metabolic shift, futile cycles, modification of the respiratory chain, and metabolic uncoupling (Tempest and Neijssel 1984, Russell and Cook 1995). When no exogenous substrate is present, the bacteria will obtain the energy required for maintenance from endogenous substrates, and this is
referred to as endogenous metabolism. Maintenance energy and endogenous metabolism are often assumed to be the same, but this is not necessarily the case. When no substrate is present, the amount of maintenance energy required to keep the cell functioning may be lower than when exogenous substrate is present (Russell and Cook 1995).

The maintenance energy is not a constant, but has been observed to be a function of the growth rate (Tempest and Neijssel 1980). Maintenance energy also depends upon the substrate, the limiting nutrients, the temperature, the dissolved oxygen concentration, the substrate addition pattern, the particular bacteria, and the physiological state of the bacteria. All of these factors can change with changing growth rate, causing an increase in maintenance requirements at lower growth rates (Stouthamer 1990).

Under starvation conditions, during endogenous metabolism, bacteria can maintain catabolic enzymes, and immediately shift up metabolism after exposure to substrate. Prolonged starvation results in decreased yield after substrate addition (Koch 1979), presumably due to greater energy wasting.

As the growth rate decreases, the fraction of viable biomass also decreases. A significant fraction of biomass from chemostats operated at low growth rates may be able to degrade substrate, but unable to multiply, and hence, are not viable. In the study of activated sludge it is difficult to count the numbers of viable biomass, but indirect results indicate that activated sludge viability decreases with decreasing growth rate (increasing SRT) (Huang 1982). For activated sludge operated at an SRT of 10 days, the viability of the MLVSS may be as low as 20%. The explanation for the apparent low percentage of viable microorganism in activated sludge is provided by the concept of microbial decay.
Microbial decay is usually attributed to the death of microorganisms and the subsequent utilisation of the cellular material by the remaining microbes. If some of the cellular material released by the decay process is recalcitrant, this material will stay in the system (due to the recycle of sludge in the activated sludge process), and increase with increasing SRT. The viable fraction of the MLVSS will decrease with increasing SRT.

2.4 Effect of Transient Operating Conditions on Activated Sludge Performance

BOD

Wastewater treatment plants may be exposed to organic shock loads - sudden increases in the amount of substrate concentration in the wastewater. The activated sludge response to organic shock loads is varied, and depends upon the prior operating conditions. Increased substrate concentration may result in increased microbial growth rate, changes in the substrate utilisation pattern, changes in the floc structure, and changes in stoichiometry.

A common response to increased substrate is an immediate utilisation of the substrate at an increased rate, but a lag period prior to an increase in the growth rate of the biomass (Rozich and Gaudy 1985). Activated sludge systems are commonly observed to have the capability to absorb shock loads (up to three fold increases in substrate concentration (Krishnan and Gaudy 1976)). This is thought to be a property of biomass cultivated at low growth rates. The biomass maintains a capability to metabolise substrate concentrations greater than in the steady state environment. As a result the
yield during the transient period is usually lower than the yield during steady state. This may be explained by the concept of metabolic uncoupling described above.

Increasing the SRT increases the biomass in the system, which increases the capability to handle shock loads. One study found increasing the SRT from 5 to 10 days resulted in no difference in activated sludge performance. This was hypothesised to be due to the higher percentage of viable cells at the low SRT, smaller flocs, and less slime (reduced mass transfer resistance) (Selna and Schroeder 1978). Another study found biomass at high growth rate had a slow response to substrate increases. Biomass at low growth rates also had a slow response (in growth rate), and the growth rate and oxidation were uncoupled. Biomass at a medium growth rate had the most rapid response to substrate increases (Daigger and Grady 1982a).

Response to changes in substrate concentration involves physiological shifts. During unsteady state operation, as during shock loads, the microbial kinetics depend upon the past culture history, and will change as the environmental conditions change (Mona et al 1979, Manickam and Gaudy 1985, Storer and Gaudy 1969, Selna and Schroeder 1978).

Sludge characteristics may change following large organic shock loads. The increased growth rate of the biomass may result in the formation of pin flocs, which have poor settling characteristics.

Temperature and pH Shocks

Biomass acclimated to 35°C could tolerate shock temperatures up to 45°C, but above this temperature the system was inhibited. Biomass acclimated to 50°C could tolerate temperature drops to 45°C, but not 35°C (Carter 1975). For biomass acclimated
to 25°C, temperature increases to 47°C and 57.5°C resulted in a loss of biomass, and
deterioration of treatment. Within two to four days, the biomass started to recover
(treatment performance recovered with the biomass), although the morphology was
different than prior to the shock. A temperature decrease to 8°C (from 25°C) resulted in
loss of biomass and poor treatment. Lower growth rates, and higher biomass
concentrations, were found to improve the accommodation to the shock temperature
(George and Gaudy 1973). Anaerobic biomass acclimated to 39°C could tolerate
temperature increases to 45°C. Above this temperature, the microbial decay increased,
and the activity of the population decreased (van Lier et al 1990). A 10°C temperature
increase to 35°C resulted in increased substrate removal, and increased biomass growth
rate. There was a lag period of a few hours after the increase in substrate uptake rate
before the growth rate increased (Topiwala and Sinclair 1971).

The response of CSTRs, with and without cell recycle, to pH shocks was studied.
Sudden decreases in pH caused a sudden decrease in biomass concentration, but once the
system had time to acclimate to the new pH, the biomass concentration increased to
levels higher than the pre-shock value. Acclimation consisted of the growth of fungi,
tolerant to low pH. In the system with no cell recycle, recovery of the system following a
pH shock did not depend on the magnitude of the shock, but on the time required for a
new population to develop (approximately five days). In the system with cell recycle,
less change in population was observed; the original population adapted to the new
conditions before the fungi took over (George and Gaudy 1973b).

Uncoupled growth results not only from the presence of an excess energy source
as described above, but also from inhibitory compounds, unfavorable temperatures,
minimal media, and transient periods. (Stouthamer 1979, Coultate and Sundaram 1975). When yeast was grown in chemostats at superoptimal temperatures, the yield was decreased due to increased maintenance by viable cells, and increased energy dissipation by non-viable cells (Van Uden and Madeira-Lopez 1976). For some mesophilic bacteria, decreases in growth yield (due to increased maintenance energy expenditures and uncoupled metabolism) occur at temperatures equal to or greater than the optimum temperature for growth rate (Farmer and Jones 1976, Forrest 1967, Esener et al 1983). Other bacteria exhibit complementary behaviour where uncoupling increases below the optimum temperature for growth (Forrest 1967).

**Toxic compounds**

Activated sludge biomass must acclimate to the compounds present in the wastewater before degradation can occur. For acclimation, the environmental conditions are important. The acclimation period for the biodegradation of nitrilotriacetic acid (NTA) is much shorter in laboratory studies than in full scale systems, presumably due to the prevailing optimum operating conditions in the laboratory (Stephenson et al 1984). For rapid acclimation, the inoculum should contain a large amount of active cells, and the initial substrate concentration should be low enough to avoid substrate inhibition effects, but not too low or acclimation may not occur (Kim and Maier 1986). Adaptation is affected by the presence of other substrates (Tokuz 1991), depends upon the specific compounds, and is influenced by the presence of predatory protozoa (Wiggins et al 1987). Lag periods before acclimation occurs are often on the order of days, but in some cases can be several months (Nyholm et al 1992). Acclimation periods have been explained as the time necessary for microbial populations to grow to sufficient size to
achieve detectable bioconversion rates, induce new enzymes, undergo genetic changes, and to exhaust preferential substrates (Linkfield et al 1989). For the case of phenol, adaptation of the activated sludge was found to involve a shift in the microbial population to a greater percentage of gram negative bacteria, and a less diverse population of protozoa (Lewandowski 1990).

Once the compound of interest is removed from the wastewater feed to the activated sludge plant, the biomass may de-adapt. The course of de-adaptation appears to depend on the specific case. If special enzymes are required, and these enzymes are only produced when the compound of interest is present, then de-adaptation will be related to the SRT (Senthilnathan and Ganczarczyk 1989). In a study using parachlorophenol, the bacteria de-adapted faster than could be explained by the SRT (Arbuckle and Kennedy 1989). Similar results were obtained using 3,5-dichlorophenol, where the biomass de-adapted when 3,5-dichlorophenol was removed from the wastewater for four days (Broecker and Zahn 1977).

Acclimation of the activated sludge process to a particular organic compound will improve the response of the system to a shock load by the same compound. Such acclimation does not necessarily prevent system failure (Tokuz 1991). Activated sludge units operated at longer SRTs appear to be more resistant to toxic shock loads than activated sludge units operated at shorter SRTs (Santiago and Grady 1990, Rozich and Gaudy 1985). Increasing the sludge age also increases the biomass concentration. This results in a decrease in the toxicant to microorganism ratio, which can help to mitigate the inhibitory effects of toxic compounds (Lange et al 1989). Fast growing cells have been
found to be more sensitive to toxic compounds, due to greater permeability into the cells (Bull and Brown 1979).

When multiple phenol shocks were applied to activated sludge biomass, there was a decrease in the specific substrate removal rate with each consecutive pulse (Okaygun et al 1992). Other studies have found increased biodegradation after exposure to a toxic compound, and further increases in biodegradation of toxic compounds during shock loads once the cultures are acclimated (Lewandowski 1990).

Traditionally, CSTRs are thought to provide the best protection against inhibitory shock loads, due to the large dilution factor. Simulation studies indicate that plug flow reactors are better at mitigating the effect of shock loads than CSTRs unless there is severe substrate inhibition (Santiago and Grady 1990). Plug flow reactors should provide a sludge with better settling properties than CSTRs.

Operating Conditions

The SRT affects the growth rate of the microorganism, which affects the physiological properties. SRT also exerts a selection pressure on the population, based on growth rate. Changes in the SRT will cause the biomass properties to change. Approximately three times the difference between the old SRT and the new SRT must pass before a new steady state is achieved (Zaloum 1992).

Other factors which affect treatment are the temperature, pH, f/m ratio, wastewater composition and wastewater strength. All of these factors may be variable. For a mixed microbial population treating a mixture of substrates, steady state may never be achieved. The population dynamics between the various bacterial species, and the predator prey relationship between the protozoa and the bacteria will lead to oscillations
in the populations (Bazin 1990). Shifts in microbial populations were observed after changes in wastewater strength (Okaygun et al 1992). Low dissolved oxygen concentration, or low nutrient levels, may lead to predominance of filamentous bacteria. If the microbial population is changing following changes in environmental conditions, the treatment parameters may be changing as well. Due to these difficulties, a general rule of thumb is to wait 3 to 10 sludge ages after process changes for the biomass to adapt to the new conditions. Adaptation to temperature changes requires from 10 to 20 days to reach new equilibrium conditions (Chou and O'Brien 1987).

2.5 Summary

BKME is a complex mixture of many organic compounds. The composition of BKME depends on the operating conditions of the pulp mill, and can be highly variable. Common constituents are methanol, formate, acetate, sugars, and many toxic compounds such as phenols and resin acids.

BKME treatment by biological processes is very effective, especially for BOD and acute toxicity removal. Treatment efficiency increases with SRT and HRT. This is due to the large amount of recalcitrant substrates in the wastewater which need long contact times (high HRT), or slow growing microorganisms (high SRT), for biodegradation.

The activated sludge operating conditions affect the bacterial population. Decreasing the SRT, or adding a selector, has been observed to result in bacteria with greater growth rates. The population may also be affected by the composition of the wastewater. For example, when grown on methanol as a sole substrate bacteria will synthesise the necessary enzymes for growth on a C1 substrate. If another substrate is
present as a carbon source, the bacteria may not synthesise the enzymes required to use methanol as a carbon source, and use methanol solely as an energy source. Many different bacterial populations and metabolic pathways are possible.

The response of an activated sludge unit to an increase in substrate loading can be complex. The growth rate of activated sludge bacteria is usually low. An excess of dissimilatory enzymes are maintained to scavenge low substrate concentration environments, and to accommodate sudden increases in substrate concentration. This allows the bacteria to handle large increases in substrate concentration, however the energy generated by substrate dissimilation is wasted until anabolic enzymes can be synthesised for an increase in the microbial growth rate. A lag period between the substrate concentration increase and increased growth rate is often observed. The increase in substrate removal rate following an increase in substrate concentration is often immediate.

Bacteria at low growth rates are able to handle changing conditions (substrate, temperature, pH) better than bacteria at high growth rates. Increasing the SRT often results in a greater capacity to handle shock loads, due to the lower growth rate and increased biomass concentration.
Chapter 3 Literature Review - Activated Sludge Models

3.1 Introduction

Many activated sludge models have been developed over the years. The majority of models are based upon the Monod equation. The Monod model is an empirical relationship which relates biomass growth of a pure culture on a single substrate to the substrate concentration, and is similar to the Michaelis-Menton model for enzyme kinetics. In this chapter the models based on the Monod equation, as well as some alternative models, will be discussed.

The treatment of wastewater by the activated sludge process consists of a complicated series of reactions. The bacteria in activated sludge usually grow in flocs, which are an amalgamation of live, dead, and non-viable bacteria held together by exocellular materials excreted by the biomass under the growth conditions typical in wastewater treatment plants. During wastewater treatment, the many different substrates in the wastewater (soluble, colloidal, and particulate), all with different degradation rates, must first be transported into the floc to come into contact with the mixed culture. The substrates are then degraded by the bacteria. Depending on the substrates, the environmental conditions, and the species of bacteria present, the substrates may be used for growth, converted to storage products for later use, or only partially metabolised and returned to the wastewater.

During steady state operation of wastewater treatment plants, the growth rate of the biomass is balanced by the decay rate and sludge wasting. Due to the low substrate concentrations in completely mixed activated sludge reactors, and the corresponding low
growth rates, the decay rate will be a significant factor. The decay of bacteria produces soluble and particulate organics, some of which are biodegradable and will be metabolised by the remaining bacteria, and some of which are recalcitrant (during the residence time of an activated sludge plant).

The Monod model, without modifications, rarely fits experimental data from activated sludge units, due to the complexities discussed above. Many different modifications have been made to improve the model. These include: removing the assumption that the biomass and substrate are homogenous, adding mass transfer steps, either adsorption into the floc and conversion to storage products, or diffusion through the floc before the bacteria can metabolise the substrates, or accounting for soluble microbial product formation. The two most popular modifications in the activated sludge literature seem to be: 1) to divide the substrate into various components with different kinetic coefficients, and 2) to divide the removal of the substrate into various processes, such as an adsorption step followed by a metabolism step. All of these modifications seem to add enough flexibility to allow the models to fit the data. Since almost all activated sludge units are operated at conditions which achieve complete removal of the influent substrate, it is very difficult to compare all of the different models to determine which one is the most appropriate (Orhon et al 1989). The most applicable model probably depends upon both the wastewater and biomass characteristics, as the relative importance of all of the different processes discussed above may change (Sheffer et al 1984).
3.2 Monod

Models of microbial growth relate biomass growth rate with either biomass concentration, substrate concentration, or both. Although biomass concentration can indirectly affect the growth rate through such mechanisms as changing the pH, changing the dissolved oxygen concentration, etc., the growth rate is intrinsically independent of population density (Powell 1972). The Monod equation (equation 3.1, figure 3.1) is the standard way of correlating the microbial growth rate with substrate concentration, and will be discussed before alternatives and modifications are presented. In the absence of endogenous metabolism:

\[ \mu = \mu_{\text{MAX}} \frac{S}{K_M + S} \]  

(3.1)

where \( \mu \) is the specific growth rate and is defined as follows:

\[ \mu = \frac{dX}{dt} \frac{1}{X} \]  

(3.2)

If the yield is defined as:

\[ Y = \frac{dX}{dt} / \frac{dS}{dt} = \frac{\mu}{q} \]  

(3.3)

then, the rate of consumption of substrate is (assuming a constant yield):

\[ q = \frac{dS}{dt} \frac{1}{X} = \mu_{\text{MAX}} \frac{S}{Y K_M + S} = q_{\text{MAX}} \frac{S}{K_M + S} \]  

(3.4)

The apparent microbial yield is not constant. The apparent yield at the low growth rates common in activated sludge units is often lower than the apparent yield of biomass growing at higher rates. It is assumed that the true yield is constant, but the yield appears to decrease because of the increase in the percentage of energy going towards cell maintenance and other non-growth processes at low growth rates. This may
be accounted for in the Monod model by adding a term for microbial decay, usually modeled as a first order reaction (also purely empirical). When the substrate concentration is equal to 0, the change in biomass concentration is due solely to microbial decay as follows:

\[
\frac{dX}{dt} = -k_d X
\]  

(3.5)

The growth rate now becomes:

\[
\mu = \mu_{MAX} \frac{S}{K_M + S} - k_d
\]

(3.6)

This modification allows the Monod model to be applied to continuous activated sludge units. Using mass balances, and assuming that there is no biomass in the influent, and no separation of substrate in the clarifier, the following equations are obtained for substrate, biomass, and yield (Orhon and Artan 1994, pp207-211):

\[
S = \frac{K_M (1 + k_d \theta X)}{\mu_{MAX} \theta X - (1 + k_d \theta X)}
\]

(3.7)

\[
X = \frac{Y(S - S) \theta h}{(1 + k_d \theta X) \theta h}
\]

(3.8)

\[
Y_{obs} = \frac{Y}{1 + k_d \theta X}
\]

(3.9)

According to equation 3.7, the substrate concentration leaving a continuous activated sludge treatment system does not depend on the influent substrate concentration, but only upon the SRT (Grady and Williams 1975). At low substrate concentrations \(S \ll K_M\), the Monod equation simplifies to a first order equation:

\[
q = \frac{q_{MAX}}{K_M} S = k_S
\]

(3.10)
At high substrate concentrations \((S \gg K_M)\), the Monod equation becomes zero order \((q = q_{\text{MAX}})\).

**Alternatives to Monod**

The Monod equation is purely empirical in nature, although there is a similarity to Michaelis-Menten kinetics. It has been widely observed in the literature that the Monod equation approaches its asymptotic value too slowly to accurately model experimental data (Powell 1972, Bader 1978) and a number of alternate models have been proposed (Powell 1972, Bailey and Ollis 1986 p391).

The Blackman model (equation 3.11, figure 3.1) often fits experimental data better than Monod, but due to the discontinuity it is not as easy to use (Bader 1978).

\[
\mu = \frac{S}{A} \quad \text{when } S < A \mu_{\text{MAX}} \\
\mu = \mu_{\text{MAX}} \quad \text{when } S > A \mu_{\text{MAX}}
\]  

According to the Blackman equation, the maximum growth rate is not just some fictional condition approached asymptotically at high substrate concentrations, but is attained at a finite substrate concentration \((S = A \mu_{\text{MAX}})\).

Powell (1972) modified the Monod model by assuming that the substrate must first diffuse into the cell through a membrane according to a first order reaction (which takes place inside the cell membrane), then the substrate is metabolised inside the cell according to Monod kinetics. If steady state is assumed, then the following equation is derived:

\[
S = \frac{\mu}{YF} + \frac{\mu K_M}{\mu_{\text{MAX}} - \mu}
\]  

(3.12)
Figure 3.1 Monod kinetics $L/K = 0$ (---), Blackman kinetics $L/K = \infty$ (---), Powell kinetics $L/K = 2$ (---), Powell kinetics $L/K = 8$ (---), Powell kinetics $L/K = 38$ (---), Powell kinetics $L/K = 398$ (---).

Figure 3.2 Monod kinetics $n = 1$ (---), Moser kinetics $n = 2$ (---), Moser kinetics $n = 3$ (---), Moser kinetics $n = 5$ (---), Moser kinetics $n = 10$ (---).
where \( F \) is a constant related to the substrate diffusivity. In theory \( F \) is a function of the geometry and physical characteristics of the organisms and their environment, but in equation 3.12 it is used as an average parameter for the whole system, obtained empirically. Introducing \( L = \mu_{\text{MAX}}/(Y F) \) and solving for \( \mu \):

\[
\mu = \frac{\mu_{\text{MAX}}(K + L + S)}{2L} \left[ 1 - \sqrt{1 - \frac{4LS}{(K + L + S)^2}} \right]
\]  

(3.13)

Equation 3.13 is shown graphically in figure 3.1, for a number of values of \( L \). Powell compared equation 3.13 with Monod’s and Tessier’s (equation 3.15) using published data, and found that equation 3.13 gave the best fit in the majority of the cases. If \( L \) is small, the Monod model may be used with an apparent half saturation constant \( H = K_M + L \). Koch and Coffman (1970) presented an independent derivation of equation 3.12. Diffusion into the entire cell was assumed instead of diffusion across a membrane (as in Powell’s derivation). The diffusion was assumed to be rapid, resulting in a constant substrate concentration throughout the cell. In comparing equation 3.12 to Monod’s, Koch (1979) found equation 3.12 to give a better fit to the growth vs. substrate (glucose) data. It was hypothesised that the cell’s transport mechanisms are present in excess and capable of supporting the cell’s need for glucose at an external glucose concentration where uptake is non-saturated and still in the first order phase.

Dabes (Dabes et al 1973) derived equation 3.12 (the Powell equation) by assuming that cell metabolism could be simulated using a series of linked reversible enzymatic reactions. If one reaction is much slower than all of the other reactions, the Monod equation results. If there are two slow reactions which are separated by any number of fast reactions with a large equilibrium constant, the Blackman equation (3.11)
results. Equation 3.11 implies that there are 2 regimes, one where the external substrate concentration limits the growth rate, and one where an internal "pacemaker" enzyme limits growth. If two slow reactions are separated by an equilibrium constant which is not large, then equation 3.12 results. Dabes compared equation 3.11 and 3.12 with the Monod equation for a number of published data sets, and found that either equation 3.11 or 3.12 gave the best fit.

The Blackman and Powell equations are derived based upon the assumption of two or more reactions in series, at least one of which follows Monod kinetics. A number of other equations have been proposed to describe experimental microbial growth data. These equations are more complicated mathematically, are completely empirical, and do not appear to be commonly used. They all have the advantage over Monod's in that they saturate faster with increasing substrate concentration than Monod's equation does.

Some examples of these are:

Moser's (figure 3.2):

\[ \mu = \mu_{MAX} \frac{S^n}{K + S^n} \]  \hspace{1cm} (3.14)

Tessier's (figure 3.3):

\[ \mu = \mu_{MAX} (1 - e^{ks}) \]  \hspace{1cm} (3.15)

and Konak's (1974) (figure 3.3):

\[ \frac{d\mu}{dS} = K(\mu_{MAX} - \mu)^n \]  \hspace{1cm} (3.16)

When \( n=1 \), equation 3.16 reduces to Tessier's equation (3.15), if \( n = 2 \), then Monod's equation results. If \( n-1 \) then equation 3.16 may be integrated to give:

\[ \mu_{MAX}^{(1-n)} - (\mu_{MAX} - \mu)^{(1-n)} = (1-n)KS \]  \hspace{1cm} (3.17)
Figure 3.3 Monod kinetics $n = 2$ (---), Tessier kinetics $n = 1$ (---), Konak kinetics $n = 3$ (---), Konak kinetics $n = 5$ (---), Konak kinetics $n = 7$ (---), Konak kinetics $n = 10$ (---).

Figure 3.4 Example of multisubstrate removal during a batch test following equation 3.22. The overall substrate (thick solid line) removal rate is equal to the sum of the individual substrate removal rates (each individual substrate is represented by a separate dotted line).
The advantage of equations 3.11 to 3.17 over Monod's equation is faster saturation of the growth rate with respect to the substrate concentration, which more often allows for a better fit of experimental data. Another source of difficulty with Monod's model is that $K_M$ does not appear to be a constant, but a function of either the biomass concentration or the initial substrate concentration. In continuous studies, the biomass concentration is related to the initial substrate concentration through the yield coefficient, making it difficult to determine which has a greater effect on $K_M$. The majority of studies where $K_M$ was not found to be a constant were studies of continuous systems. During batch tests $X$ is usually constant so the relationship between $X$ and $K_M$ would not be noticed. Contois (1959) proposed that $K_M$ is directly proportional to biomass concentration, resulting in

$$\mu = \frac{\mu_{MAX}^S}{K_M X + S}$$

(3.18)

The explanation given was that at higher biomass concentrations there will be more inhibitory microbial products, resulting in a larger apparent half saturation constant. Fujimoto (1963) derived a similar equation by assuming that substrate must be adsorbed by the cell before it can be utilised. Equation 3.13 was derived using similar reasoning that is used to derive Monod's equation, but used $S / X$ (adsorbed substrate) instead of $S$ (bulk substrate).

Roques et al (1982) also found that $K_M$ was proportional to biomass concentration and proposed the following:

$$\mu = \mu_{MAX} \frac{S}{S + M + b(S_o - S)}$$

(3.14)
This equation can simplify to Monod’s if $b = 0$, to Powell’s if $S \ll S_0$ (and $L$ in Powell’s equation is small), to Contois’s if $M = 0$ (and using $(S_0 - S) = X/Y$, assuming $Y$ is constant). Chen and Hashimoto (1978) and Elmaleh and Ben Aim (1976) found $K_M$ to be a function of initial substrate concentration. Grady and Williams (1975) used a first order approximation of Monod’s equation and also found $K_M$ to be proportional to $S_0$. The main implication of equation 3.14 and similar (equation 3.13, Chen’s, Elmaleh’s, and Grady’s) is the prediction that the substrate concentration in the treated wastewater of a continuous system is a function of the substrate concentration in the untreated wastewater, as was found in the above studies, and not independent of the untreated wastewater substrate concentration as predicted by equation 3.7. It is interesting to note that most of these studies dealt with mixed substrates measured using global parameters (this subject will be discussed in more detail in the next section). In a study dealing with pure compounds, Cech et al (1984) also found $K_M$ to be proportional to the biomass concentration, but the Monod model was still used to describe the data. Roques equation (3.14) reduces to Monod’s during batch tests where initial rates are measured (because $S = S_0$), or the equation of Contois’ may be used instead.

Most of the equations discussed in this section may be simplified to Monod’s under special conditions. Equations for continuous systems similar to equations 3.6 to 3.8 may be developed by adding the term for microbial decay and using appropriate mass balances. Roels (1983, pp237-239) has shown that under substrate limiting growth, the biomass concentration is not a strong function of the microbial model chosen, but depends mainly on the yield coefficient. The substrate concentration under these conditions can not usually be determined accurately enough to choose the proper model.
The Monod model appears to be adequate when substrate is not limiting (zero order region), and for correlating steady state biomass concentration and growth rate in continuous cultures. Even in cases where reliable substrate data allows the use of alternate models, most researchers use Monod due to its resemblance to Michaelis-Menten enzyme kinetics, and it's ease of use (Bader 1978).

### 3.3 Multiple Substrates

All of the preceding models are based upon the assumption that there is only one substrate used for growth, even though these models are often applied to wastewaters. Wastewaters are not usually composed of single substrates, but a variety of compounds all with different biodegradation rates. If wastewaters are characterised using global parameters, such as BOD, COD, or TOC, and one of the preceding models is used, complications may arise (Hao and Li 1987, El-Rehaili 1994, Jones 1973). The kinetics measured using one parameter will not be applicable to the other parameter, and if COD is used, the non-biodegradable fraction must be accounted for. Jones (1973) pointed out that if the kinetics of a multiple substrate mixture are measured using a global substrate measurement (such as COD), the measured kinetics will be dominated by the substrate component with the greatest degradation rate. There have been a number of attempts at dealing with the multicomponent nature of wastewaters. The approach taken by the IAWQ model involves separating the substrates into different fractions depending upon their kinetics and physical properties. Another approach assumes the presence of a large number of substrates in the wastewater and that the substrate removal rate decreases as individual substrate components are removed (Grau et al 1975). This second approach will be referred to as the multicomponent model.
Substrate Interactions

When dealing with microbial growth on mixed substrates, a number of different substrate interactions are possible. The IAWQ model and the multicomponent model assume that the various substrates in the wastewater do not interact. This assumption is based upon the argument that although in batch cultures sequential substrate removal might occur, simultaneous substrate removal is more likely to be the case in a continuous steady state system. Furthermore, the substrate removal rates are assumed to be independent of the presence of other substrates.

In the case of simultaneous substrate removal, the various substrates may still influence the other substrates removal rates. A few models for simultaneous use of multiple substrates have been proposed, such as the one by Gujer and Kappeler (1992),

\[
\frac{dX}{dt} = \mu X \left[ \frac{S_1}{K_{M1} + S_1 S_2} + \frac{S_2}{K_{M2} + S_1 S_2} \right]
\]

(3.20)

where the growth rates on the individual substrates are multiplied by a correction factor (based upon a simplification of competition kinetics). The correction factor is to temper an unrealistic increase in growth rate that would be obtained by simply adding the growth rate due to the two substrates. No experimental evidence was given for this sort of correction factor.

Orhon has proposed that competitive inhibition enzyme kinetics be used to model multiple substrate utilisation, and presented the following expression (Orhon and Artan 1994, pp 138-140):

\[
\frac{dX}{dt} = \mu_1 \frac{S_1 X}{K_{M1} \left( 1 + \frac{S_1}{K_{M2}} \right) + S_1} + \mu_2 \frac{S_2 X}{K_{M2} \left( 1 + \frac{S_1}{K_{M1}} \right) + S_2}
\]

(3.21)
This model is theoretical and not based upon experimental evidence. Both models (3.20 and 3.21) predict that the presence of a second substrate with a slower degradation rate will markedly decrease the degradation rate of the first substrate.

There have been a few cases of substrate interactions using mixed cultures reported in the literature. Wuhrmann (Discussion of Tischler and Eckenfelder 1968) points out that substrate removal rates may not always be independent. In a study using glucose and galactose as substrates, it was found that the removal rates during a batch test were lower when both substrates were present. Another study (Zollinger et al 1964) found that galactose was only removed from solution once all of the glucose had been removed, and sequential substrate utilisation for these substrates was hypothesised. In a study using continuous reactors (Ghosh and Pohland 1972) it was found that at hydraulic residence times typical for activated sludge units (>4 hours), the presence of galactose had no effect on the glucose removal rate. It was also found that the yield was lower when both substrates were present, and the evidence suggested that the biomass used glucose as a carbon source and galactose as an energy source. In contrast to the studies with mixed cultures, there have been many studies of mixed substrate utilisation using pure cultures (see Harder and Dijkhuizen 1982, 1983, Bull and Brown 1979). Since most of these studies were performed using continuous cultures, the results should be more applicable to continuous wastewater treatment systems than the results obtained from batch tests. A general conclusion from these studies is that at low dilution rates mixed substrates are utilised simultaneously, while at higher dilution rates, one of the substrates is likely to be used preferentially before the other substrates are used. This behaviour may be due to the high levels of ATP generated during high growth conditions, which
leads to metabolic controls such as catabolite repression and inhibition (Philbrook and Grady 1985). In batch tests, if carbohydrate mixtures are used, substrate removal is likely to be sequential. If one of the substrates is an organic acid, simultaneous removal can be expected. The pattern of dual substrate removal may also depend upon the previous culture history (Narang 1997). There are exceptions to these generalities; some mixtures are used simultaneously at all dilution rates, while others are used preferentially at all dilution rates.

From the evidence given above, the most likely response of an acclimatised mixed, continuous culture operating at growth rates typical of wastewater treatment systems appears to be simultaneous utilisation of the substrates. The question which remains to be answered is whether or not the individual substrate removal rates are independent of the other substrates, or a function of the other substrates present. In contrast to the predictions of equations 3.20 and 3.21, the growth rate on a substrate mixture has been found to be greater than the growth rate on either of the individual substrates (Narang 1997). The prediction of possible substrate interactions is further complicated if the bacterial culture is not acclimated to the wastewater, such as during a shock load to a wastewater treatment system.

In addition to substrate removal rates and growth rates, biomass yield is also influenced by substrate mixtures. The yields during mixed substrate growth appear to be additive.

Multicomponent Kinetics

If each substrate is removed independently of the others, then the overall removal rate (as measured by BOD or COD) will be a summation of the individual substrate
removal rates (figure 3.4). Tischler and Eckenfelder (1968) showed theoretically that if the individual components are removed according to zero order kinetics, the overall removal rate can be approximated using first order kinetics (this was found empirically - the authors expressed no preference for a pseudo first order reaction over any other pseudo order reaction). They then showed experimentally that glucose, aniline, glycine, phenol, and acetate removal rates were constant during a batch test (zero order kinetics), and the overall removal rate was approximated as the summation of the individual removal rates and modeled using first order kinetics. This concept was also demonstrated using glucose, phenol, and sulfanilic acid as substrates (Siber and Eckenfelder 1980).

Chudoba (Discussion of Tischler and Eckenfelder 1968) has also found zero order substrate removal rates for a range of substrates. Glucose and benzoate were removed from batch culture at constant rates, which were additive for the mixtures (Zollinger et al 1964). From these studies it appears that the metabolism of individual substrates by mixed microbial communities is not influenced by the metabolism of the other substrates present. All of these studies used batch tests which are more likely to exhibit substrate interactions than continuous cultures due to the high initial substrate concentrations.

Grau et al (1975) developed an empirical equation which models the batch test removal of substrate from a multicomponent wastewater assuming that the overall removal rate is equal to the summation of individual zero-order removal rates:

\[
-\frac{dS}{dt} = kX \left( \frac{S}{S_0} \right)^n
\]  

(3.22)

\( n \) is not limited to integers, but for ease of analysis, \( n \) is usually taken to be either 1 or 2. This equation is different from the limiting case of the Monod equation when \( S \ll K_M \) (equation 3.10), in that the substrate removal rate depends on the ratio of remaining
substrate to original substrate as opposed to simply the substrate concentration. This modification is to account for the decreasing removal rate as components are eliminated from the wastewater. Adams and Eckenfelder (1975) compared equation 3.22 with the first order approximation of the Monod model. Equation 3.22 predicts that the effluent quality will vary with the influent strength and this was supported by the experimental data. The Monod model predicted much less effluent variability than was observed. Equation 3.22, with n=1, developed for multicomponent effluents, is similar to the equation 3.19 (which was also developed to bypass the limitation of the Monod model to relate effluent substrate concentration to influent substrate concentration), if $S_0$ is very large compared to $S$ and $b$ and $M$. If n=1, equation 3.22 is equivalent to the model of Grady and Williams (1975). Equation 3.22 is a generalisation of the empirical first order approximation of the Monod equation, and appears to fit experimental data very well (Grau et al 1975). Unlike equation 3.19, and that of Elmaleh and Ben Aim (1976), equation 3.22 does not predict a maximum substrate uptake rate. Since most wastewater treatment plants operate under low loading conditions, this is unlikely to be a major concern except during shock loads.

An alternate explanation for the success of equation 3.22 is the production of soluble microbial products (SMP) (Rittmann et al 1987). SMP have been found to be produced in proportion to the initial biodegradable organic concentration and also to the concentration of active biomass. When the feed is composed of only one or a few simple compounds, the concentration of organic compounds remaining in the effluent is often found to be proportional to the initial substrate concentration, however the chemical but of a different chemical composition than the original substrate. Chudoba (1985) found
that refractory products may amount to 1-10% of the substrate consumed. Artan et al
(1989) predict that there will be no differences between the performances of completely
mixed and plug flow activated sludge units (assuming substrate removal follows the
Monod model) because they will produce the same amount of microbial products. If
SMP formation is ignored, the Monod equation, as well as equation 3.22, predict that a
plug-flow reactor should have superior treatment efficiency compared to a completely
mixed system. Most studies have found no difference between the two process
configurations.

During plug flow operation, the different substrates may be removed sequentially
instead of simultaneously as assumed by equation 3.22 (Braha and Hafner 1987). The
biomass behavior during plug flow operation should be similar to the biomass behaviour
during the batch tests used to measure the model coefficients. Different microbial
populations will develop under different flow regimes, and the model parameters of plug
flow, completely mixed, and batch reactors may not be comparable.

Argaman (1991) offers a different explanation for the similarity of treatment
efficiencies of plug flow and completely mixed units. If the removal rate is a summation
of zero order reactions, then equation 3.22 should be integrated as a zero order reaction,
even if n is 1 or 2. For a zero order reaction, there will be no difference between batch,
plug flow, or completely mixed continuous reactors. The resulting equations are as
follows. If \( n = 1 \) from batch test data, then

\[
S = S_0 e^{\left(-\frac{1}{n} \frac{S}{S_0}\right)}
\]  

(3.23)

If \( n = 2 \), then
\[ S = \frac{S_0}{1 + \frac{k}{S_0} x_{0h}} \tag{3.24} \]

A common mistake is to use \( k \) obtained from a first order curve fit to batch test data (equation 3.23), and use equation 3.24 to design a continuous stirred tank reactor (which is the first order integration of equation 3.22 for a CSTR). This will result in a major oversizing of the activated sludge system.

Equations 3.23 and 3.24 may be used instead of equation 3.7 in modeling the activated sludge process.

**IAWQ Model**

The multicomponent model accounts for the fact that there are many different substrates present, but still uses global substrate measurements (such as BOD or COD). Greater accuracy would be obtained by measuring each substrate individually. Often, the substrates in wastewaters can be broadly classified into only two different groups based upon removal rates: those rapidly biodegradable, and the slowly biodegradable. Dual substrate models, such as the IAWQ activated sludge model #1, were developed to more realistically model the activated sludge process (see Orhon and Artan 1994 for a thorough treatment of this model). These models divide the organic components of domestic wastewater into various fractions, as shown in figure 3.5 (Henze 1992). The biodegradable component of the wastewater is divided into readily and slowly biodegradable fractions. The division was at first based on the obvious physical property of the wastewater. The readily biodegradable fraction was assumed to be the water-soluble components, while the slowly biodegradable components were assumed to be the particulate matter. It is now realised that the slowly biodegradable component can
**Figure 3.5** Breakdown of COD before and after activated sludge treatment for a typical domestic wastewater. From top to bottom: inert soluble ■, readily biodegradable ■, rapidly hydolysable ■, slowly hydrolysable ■, heterotrophs ■, inert particulates ■.

**Figure 3.6** a) IAWQ model with death - regeneration  
   b) IAWQ model with endogenous decay.  
   S - soluble substrate;  
   $S_p$ - soluble microbial products;  
   $X_S$ - particulate substrate;  
   $X_H$ - biomass;  
   $X_p$ - particulate microbial products.
contain soluble and colloidal matter, as well as particulates. The removal rate of the readily biodegradable component is assumed to follow the Monod equation. The slowly biodegradable fraction is first adsorbed, or enmeshed, by the active biomass, then hydrolysed to readily biodegradable substrates. The hydrolysis step is considered to be the rate limiting step, so the adsorption step is not accounted for in the IAWQ model. Hydrolysis is modeled using a function similar to Monod's, but this is usually simplified to a first order reaction. The readily biodegradable organic substrates produced by hydrolysis may be removed independently of the original readily biodegradable components, as found by Spangers and Vanrolleghem (1995), or there may be substrate interactions as predicted by equation 3.20 (Gujer and Kappeler 1992) or 3.21 (Orhon and Artan 1994). The easiest interpretation is that the readily biodegradable organics produced by hydrolysis are the same as those originally present in the wastewater. The yields of the two fractions are usually considered to be equal, and commonly taken to be 0.67 gCOD/gCOD (Kapeller and Gujer 1992).

The particulate matter in the wastewater is also divided into different components. These are the particulate degradable organics already discussed, the inert particulate matter, and the active biomass. Active biomass is converted to inert particulate matter through microbial decay. There are two ways to model microbial decay (figure 3.6); both give similar results for aerobic systems, and both offer advantages over just using equation 3.5 in that both predict the formation of inert particulate matter from the decay of active mass. This allows the decrease of the viable fraction of biomass with increasing SRT to be predicted, but it also makes it difficult to measure the amount of active
biomass present, since VSS measurements will measure the inert solid matter along with the active biomass.

The first method of modeling microbial decay (figure 3.6b) is by assuming endogenous decay. The biomass decays according to a first order reaction, and a fraction $f_{ex}$ is converted to inert particulate matter while the rest $(1-f_{ex})$ is oxidised during the degradation process.

The second method (figure 3.6a) is the death regeneration approach. In this model the biomass decays according to a first order reaction, with a fraction $f_{px}$ being converted to inert endogenous residue, and the rest $(1-f_{px})$ converted into slowly biodegradable (particulate) substrate. This substrate is then hydrolysed to soluble substrate and converted into active biomass with an assumed yield of 0.67. In the measurement of the decay rates by respirometry, it is assumed that growth on the readily biodegradable substrate, the step which requires oxygen, is the rate limiting step. At low temperatures, the hydrolysis step will be slower than the death or growth step, and microbial decay will be underestimated (Lishman and Murphy 1994).

The IAWQ model accounts for soluble microbial products by assuming that they are produced in relation to the growth and decay rates. Since activated sludge plants are operated at low growth rates, the inert organic compounds produced are usually assumed to be mainly due to microbial decay. The endogenous decay model can easily be modified so that some active biomass is converted into inert particulates, some into soluble products, and the rest is oxidised.
3.4 Mass Transfer

Another complication that arises in the modeling of the activated sludge process results from the three phase nature of the mixed liquor. The substrate might be both soluble and particulate, the biomass tends to grow in flocs, and the oxygen required for metabolism is usually added in gaseous form (air bubbles). A physical mass transfer step may be a rate limiting step in the overall substrate removal process. Mass transfer will probably not interfere with the measurement of $\mu_{\text{MAX}}$, at high substrate concentrations, as the reactions will be kinetically limited. However, at low substrate or low oxygen concentrations (mass transfer may affect both substrate and oxygen availability), the reactions may be mass transfer limited. If this is the case, any measurement of $K_M$ will be affected, and $K_M$ will be overestimated (Shieh 1980, Horvath and Engasser 1974). Sometimes the $K_M$ calculated in the presence of mass transfer resistance is called the apparent $K_M$, but this may lead to confusion when the results are applied to a reactor with different fluid mechanical conditions.

There are several different methods for including mass transfer steps in the activated sludge models. The Powell equation (equation 3.13) accounts for diffusion of the substrate across the cell membrane into the cell with the assumption that the substrate flux across the cell membrane is equal to the substrate utilisation rate. Another approach assumes that the substrate must adsorb onto the floc (which has a finite capacity to hold substrate) before the biomass has access to the substrate. This approach assumes a heterogeneous system. If a homogenous system is assumed, the main mass transfer resistance may be in the bulk solution (external mass transfer resistance) or in the microbial floc (internal mass transfer resistance), and both substrate and oxygen mass
transfer may be rate limiting (Cussler 1997). In this section adsorption and external and internal diffusion will be discussed. The Powell equation was discussed in section 3.2.

Adsorption

The first step in soluble substrate utilisation may be adsorption onto the floc, or the bacteria (Tsezos and Bell 1989). Following adsorption, the substrate is transformed, possibly into storage products, before being metabolised for microbial growth. Biosorption of the substrate in the wastewater prior to metabolism can serve to dampen influent fluctuations of substrate concentration, and help to provide a constant effluent quality (Fujie et al 1997). Many models incorporating a mass transfer step have been developed (Fujie et al 1988, Sheffer et al 1984). The model of Andrews (Busby and Andrews 1975) seems to be the most well developed and accepted. The substrate is treated as one component, but must undergo two reaction steps in order to be utilised by the biomass. The removal of substrate from the wastewater is assumed to be by the pathway shown below.

\[
\begin{array}{c}
S \\
\rightarrow \\
X_S \\
\rightarrow \\
X_A \\
\rightarrow \\
X_I
\end{array}
\]

Substrate  Stored  Active  Inert

The total MLVSS is composed of three fractions: stored mass, active mass, and inert mass. The attachment step is modeled as follows:

\[
\frac{dX_s}{dt} = k_s \left( X_T \frac{f_s S}{S + K_s} - X_s \right)
\]  
(3.25)

The synthesis step is assumed to follow Monod kinetics

\[
\frac{dX_A}{dt} = X_A \mu_{MAX} \frac{X_s}{K_M + X_s}
\]  
(3.26)
Figure 3.7 Effect of external (to the floc) mass transfer on the Monod growth rate vs. substrate concentration relationship.

Figure 3.8 Effect of internal mass transfer resistance on the Monod growth rate vs. substrate concentration relationship.
The formation of inert mass due to microbial decay follows a first order reaction.

\[
\frac{dX_i}{dt} = k_i Y_i X_i
\]  

(3.27)

This model predicts higher substrate removal rates when there is no initial stored mass. This is an alternate explanation for decreasing removal rates during a batch test, with the high initial rate of substrate removal being due to adsorption. As the flocs become saturated, removal occurs by the slower metabolic processes. This predicts a higher ratio of SUR to OUR at the beginning of the batch test than during the rest of the test, which is often found experimentally. This model can explain the increased efficiency of the contact stabilisation activated sludge process. Stored mass is converted into active mass during the stabilisation step, allowing for maximum biosorption of substrate during the contact step.

The amount of biosorption which occurs depends upon the operating conditions of the activated sludge process. Biosorption was found to increase with the SVI and the population of filamentous microorganisms (Pujol and Canler 1992). Adsorption also depends upon the operating temperature (Bell and Tsezos 1987), pH, cell age, and cell viability (Amy et al 1988). Substrate removal by biosorption has been found to increase as the SRT decreases (Jacobsen et al 1993).

For the treatment of BKME by activated sludge, biosorption probably plays a role in the removal of the more difficult to degrade compounds. Biosorption is often related to the octanol water partition coefficient of the compound (Steen and Karickhoff 1981). Increasing hydrophobicity increases biosorption. Adsorption is an important step in the removal of AOX during BKME treatment in a lagoon (Amy et al 1988).
External & Internal Diffusion

Mass transfer effects may be accounted for by using one of two standard chemical engineering mass transfer models. The first assumes that mass transfer is proportional to the concentration difference between the substrate in the bulk fluid and the floc phase. The substrate concentration in the floc is assumed to vary linearly from the center of the floc to the floc-liquid interface. This approach is termed the external diffusion model. In the second method, the solute flux is proportional to the solute gradient. The substrate concentration in the floc will be a minimum in the center of the floc, and close to the bulk concentration at the interface of the floc and bulk solution. This method is termed internal diffusion.

For external diffusion, the following equation may be used:

\[
\frac{dS_B}{dt} = h(S_B - S) - \mu_{MAX}X \frac{S}{K_M + S}
\]  
(3.28)

Equation 3.28 is usually solved by assuming steady state (Horvath and Engasser 1974). This results in an equation similar to Powell's (3.12). The effect of external mass transfer is shown in figure 3.7. When the external mass transfer resistance is large, the reaction rate is independent of the microbial kinetic coefficients. The external mass transfer coefficient, \(h\), is proportional to floc surface area.

For slurries, there is relatively little movement between the suspended particles and the fluid, which may make external mass transfer important. However, it has been shown, both for oxygen and glucose, that external mass transfer has much less of an effect on the overall kinetics than internal mass transfer (Baillod and Boyle 1970, Mueller et al 1966). External mass transfer may be accounted for in the internal mass transfer model by using the appropriate boundary conditions (Ramachandran 1975). The
continuity equation, assuming constant density, constant diffusivity and Monod kinetics, is:

$$\frac{dS}{dt} = -u \nabla S + D \nabla^2 S - \frac{\mu_{MAX} X S}{K_M + S}$$  \hspace{1cm} (3.29)$$

This equation is solved assuming steady state, and the assumption that the advective term is insignificant ($u=0$), i.e. the mass transfer is solely due to molecular diffusion. The flocs are usually assumed to be spherical with a constant size and even distribution of microorganisms. The non-linearity of the Monod expression means that this equation must be solved using numerical methods (Ramachandran 1975). With these assumptions, equation 3.29 becomes:

$$D \frac{\partial^2 S}{\partial r^2} + \frac{2D}{r} \frac{\partial S}{\partial r} = \frac{\mu_{MAX} X S}{K_M + S}$$  \hspace{1cm} (3.30)$$

The effect of internal mass transfer on kinetics is shown in figure 3.8. Unlike the situation for external mass transfer, the reaction rate is always a function of the microbial kinetic parameters (Horvath and Engasser 1974). According to Benefield and Molz (1983) the assumption of pseudo steady state is valid. They calculated that the time required to reach equilibrium in the floc is approximately 2.2 minutes, compared with hydraulic retention times in the order of 4-12 hours for a typical activated sludge plant.

Equation 3.30 predicts that small flocs are better for reducing mass transfer effects. This is in contrast with the large bioflocs required for good settling in the secondary clarifier. Equation 3.30 also predicts that as floc size increases, there will be zero nutrient concentration in the middle of the floc, perhaps leading to loss of viability at the floc centers. If both substrate and oxygen are limiting, more of the floc will be viable than if just one were limiting due to greater nutrient penetration (Benefield and Molz
Benefield and Molz (1983) showed that mass transfer effects can be quite important in determining the response to transient operating conditions. In particular, the microbial kinetics can change from substrate limited to oxygen limited and back to substrate limited during the course of one retention time during a shock load. A more recent study of the effect of internal diffusion on the response to shock loads used the IAWQ model (Tyagi et al 1996). Their model accounted for the natural variability of floc size in a typical activated sludge process. It was found that if mass transfer was ignored, the effect of the shock load would be underestimated. The same kinetic coefficients from the literature were used for both cases. If batch test data are interpreted according to the IAWQ model with mass transfer effects, the coefficients obtained, in particular $K_M$, will be different than if the same data were interpreted according to the IAWQ model without mass transfer effects. Using coefficients obtained from one model in another model in order to simulate shock loads will lead to erroneous results.

Logan and Hunt (1988) have taken the observation that bacteria form flocs at low nutrient concentrations, and proposed that there is greater substrate availability for bacteria in flocs than for free bacteria due to advective mass transfer. If mass transfer is modeled using advection instead of diffusion, similar results are obtained, implying that all of the previous results may also be obtained assuming advective mass transfer is limiting. Comparing free bacteria with bacteria growing in flocs, assuming both are mass transfer limited, it was calculated that the mass transfer to the floc could be greater than the mass transfer to the free bacteria if advective flow was assumed. If the flocs interact with rising air bubbles, this will further increase the mass transfer rate. These results give a competitive advantage to bacteria growing in flocs in low nutrient environments (mass
transfer limited), such as the activated sludge process, and explain why there is no change in cell viability throughout the floc (Wagner et al. 1994).

Similar to adsorption, diffusion depends upon the activated sludge operating conditions. It has been found that the effective diffusivity changes with SVI, suggesting that changes in the diffusivity are due to floc porosity (Eliosov et al. 1996). The importance of floc size on diffusion has been demonstrated by measuring activated sludge before and after disruption of the flocs by blending (Mueller et al. 1966, Baillod and Boyle 1970). The overall reaction rates increased as the floc size decreased. The diffusivity was found to increase with floc diameter.

**Oxygen Transfer**

At dissolved oxygen concentrations greater than 2 mg/l, oxygen should not be rate limiting. Below 2 mg/l, the microbial kinetics may be limited by oxygen. If substrate is already limiting microbial growth, the growth rate will be limited by both substrate and oxygen. Dual limitation may be modeled using the double Monod model or the non-interacting model. The double Monod model (equation 3.31) is most often used, and is supported by experimental evidence (Bae and Rittmann 1996).

\[
\mu = \mu_{\text{MAX}} \left( \frac{S_1}{K_1 + S_1} \right) \left( \frac{S_2}{K_2 + S_2} \right)
\]

(3.31)

If \( S_2 \gg K_2 \) (as is usually the case for oxygen, \( K_{O_2} < 0.2 \) mg/l), then the term for \( S_2 \) (DO) may be ignored, which results in the Monod model.

If oxygen is utilized faster than it can diffuse into the floc, the oxygen concentration in the floc will be lower than the dissolved oxygen concentration in the bulk solution, and oxygen may be a limiting substrate even when the bulk concentration
is greater than 2 mg/l (Beccari et al 1992, Siegrist and Gujer 1987, Mueller et al 1966).

The diffusion of oxygen into the floc is assumed to follow the same rules as the diffusion of substrate into the floc (equation 3.30, with the reaction term replaced by equation 3.31).

The oxygen diffusivity depends upon many factors, such as the type of wastewater, and the properties of the floc. Dissolved oxygen diffusivity through water is temperature dependent, increasing with increasing temperature (Lin et al 1998). This is offset the decreased oxygen solubility at elevated temperatures.

3.5 Temperature

A common rule of thumb is the microbial growth rate doubles for every 10°C increase in temperature. The maximum microbial growth rate can be described as a function of temperature in accordance with the Arrhenius expression

\[ \mu_{\text{MAX}} = Ae^{-(E/RT)} \]  

(3.32)

For most wastewater treatment plants, where the temperature varies in the range of 10°C to no more than 30°C, this equation is usually simplified to

\[ \mu = \mu_0 \theta^{(T-20)} \]  

(3.33)

As the temperature increases above 30°C, the decay rate increases, resulting in a decrease in the net growth rate as the fraction of active enzymes decreases. Enzyme denaturation also follows an exponential relationship with temperature.

\[ k_d = Ae^{-(E/RT)} \]  

(3.34)

If enzyme inactivation is assumed to be irreversible, the following equation is obtained
\[ \mu_{\text{MAX}} = \frac{A e^{-\left(\frac{E_1}{RT}\right)}}{1 + K e^{-\left(\frac{E_2}{RT}\right)}} \]  
(3.35)

This equation accounts for both the increase in reaction rate as the temperature increases, and the subsequent decrease in reaction rate when the temperature rises too high. Equations of the form of 3.35 were developed for analysing enzyme kinetics, and become empirical when applied to microbial growth rates, but nevertheless fit the data. The assumption is that there is one rate controlling reaction, and this reaction is rate controlling at all temperatures.

Many other empirical correlations between growth rate and temperature have been developed. Some of these fit the data better than model 3.34, but at the expense of introducing more parameters. All of the correlations are empirical in nature.

### 3.6 pH

If one enzymatic reaction is considered to be the rate controlling reaction, then the effect of pH on this reaction will also be the effect of pH on the overall growth rate. If the following equilibrium is assumed

\[ E_{\text{inactive}} \overset{\text{OH}^-}{\leftrightarrow} E_{\text{active}} \overset{H^+}{\leftrightarrow} E_{\text{inactive}} \]

the resulting effect of pH on growth rate is

\[ \mu = \frac{c}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} \]  
(3.36)

Alternately, the enzyme substrate complex may be assumed to ionised instead of the enzyme. This results in the following

\[ \mu = \mu_{\text{MAX}} \frac{KH^+}{KK_2 + KH^+ + [H^+]} \]  
(3.37)
Both of these equations predict a bell shaped curve of growth rate vs. pH, which is commonly observed. A number of empirical equations have been proposed as well, but are used less frequently.

Denaturation by pH changes occurs by the enzyme being protonated or deprotonated. The relationship between $k_d$ and the pH may be modeled with the following equation (Laidler and Buntins 1973):

$$ k_d = k \cdot \frac{K_1 + K_2 \cdot pH + K_3 \cdot pH^2}{K_4 + K_5 \cdot pH + K_6 \cdot pH^2} $$

(3.38)

where $K_1, K_2, K_3, K_4, K_5,$ and $K_6$ are related to equilibrium constants for the various sites of pH denaturation, and rate constants for the rate of denaturation.

### 3.7 Summary

Microbial kinetics are usually modeled with Monod’s equation. For a given biomass and a given substrate, the Monod model often saturates too slowly to fit the data. This observation may be accounted for by modeling two reactions in series, with the first reaction usually being a mass transfer step such as diffusion into the floc.

When mixed substrates are used (and measured with a global parameter), the Monod half saturation constant appears to be a function of the initial substrate concentration, and not a constant. This observation may be accounted for by either the production of soluble microbial products, or by the multiple component nature of the wastewater, with each substrate having unique model coefficients.

A common observation of municipal wastewater treatment has been that there are two main processes occurring, one much faster than the other. The model of Busby and
Andrews (1975) accounts for this by dividing the substrate utilisation process into two steps (adsorption and biodegradation), while the IAWQ model divides the substrate into two components (readily and slowly biodegradable). Both of these models help to overcome some major failings of assuming that there is only one substrate, or only one reaction, occurring in an activated sludge unit.

The models may be further refined by accounting for microbial decay, which is responsible for the decrease in yield with increasing SRT. Decay may proceed by endogenous metabolism, or cell lysis, with the resulting liberated organic compounds utilised by the remaining viable bacteria. If microbial decay is assumed to produce non-biodegradable particulate matter, then the active fraction of the sludge may be modeled as a function of SRT.

Most of the models fit the experimental data fairly well. Rarely are the models tested with a secondary set of experiments. For example, if coefficients are obtained in a batch test, they will not necessarily be applied to a continuous system which is treating the same wastewater for verification. There are undoubtedly many different reactions occurring during the activated sludge process, and it is difficult to know which ones are the most important. Most of the models have many parameters and can fit most sets of data, making the choice sometimes seem arbitrary. Assumptions made in interpreting batch test data may not be valid, resulting in errors when the findings are applied to continuous treatment plants.
Chapter 4 Literature Review - Model Parameter Measurements

4.1 Introduction

Microbial model parameters depend upon many factors, including the culture history and the nature of the assay used for their measurement. Two very important aspects of microbial assays, especially when dealing with mixed cultures, are the initial food to microorganism ratio (f/m), and the length of the assay. Results from tests with a short duration and a low initial f/m are highly dependent upon previous culture conditions. For example, pure culture studies, comparing the growth rates of biomass removed from continuous reactors operating at different SRTs, found that $K_M$ decreased and $\mu_{MAX}$ increased with increasing dilution rate (Templeton and Grady 1988). The Monod coefficients were higher when measured by a batch test with a high initial f/m compared to when measured by a fed-batch test with a low initial f/m (Templeton and Grady 1988, Chudoba et al 1992a).

For batch tests, as the initial f/m increases, the growth yield is often observed to decrease. The initial response to a greater substrate concentration than that to which the bacteria are acclimated will be: metabolic uncoupling and a resultant increase in energy spilling (Liu 1996), microbial product formation, or storage. All of these response will result in a lower yield. Once the biomass has adapted to the new conditions by increasing the growth rate, more energy will be expended towards growth than towards non-growth functions, and greater amounts of energy are required for cell growth resulting in a lower yield (Chudoba et al 1992a).
The fact that the measured Monod coefficients and microbial yield depend upon the initial f/m implies that the bacteria are adapting to the new environmental conditions of the batch test. The higher that the initial f/m is, and the longer the assay, the greater the chance that the biomass will adapt. This adaptation may take many forms, such as the selection for species with high growth rates, expression of enzyme systems with higher capacity, or production of a greater amount of anabolic enzymes for reduction of metabolic uncoupling (see section 2.3).

Assay methodologies may be categorised by the initial f/m based, upon the response of the biomass, into those that employ low (f/m < 0.04), intermediate (0.04 < f/m < 2), and high (f/m > 2) f/m ratios (table 4.1). Values of the f/m ratio greater than 0.04 (mg COD/mg VSS) may result in changes in microbial physiology (Grady et al 1996). If the f/m is greater than 2, the microorganisms will multiply, enriching the culture for organisms with the greatest growth rates on the available substrates (Chudoba et al 1992a). Grady et al (1996) have proposed the use of the term extant (and the subscript e) to describe coefficients which are measured without giving the biomass time to adapt to the assay conditions, and the term intrinsic when the biomass is allowed to fully adapt and reach its maximum potential (the growth rate is unrestricted). Extant kinetics represent the capabilities of the culture at the time of sampling.

This section describes various methods for determining activated sludge kinetics, most of which are for measuring extant kinetics. For this project, the actual performance of the bioreactors under real conditions (low substrate, high biomass concentrations), is of more interest than the hypothetical performance under unrestricted growth conditions.
This requires that batch tests employ a low initial f/m, to minimize bacterial growth and metabolic changes (i.e. enzyme induction).

Table 4.1 Effect of f/m ratio

<table>
<thead>
<tr>
<th>F/M</th>
<th>Description</th>
<th>Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/M &lt; 0.04</td>
<td>Measures extant kinetics if short term</td>
<td>ΔOUR, infinite dilution, pilot plants</td>
</tr>
<tr>
<td>0.04 &gt; F/M &lt; 2</td>
<td>Possible changes in microbial physiology</td>
<td>IAWQ test, adsorption tests</td>
</tr>
<tr>
<td>F/M &gt; 2</td>
<td>Measures intrinsic kinetics, growth will occur, possible changes in population</td>
<td>Growth tests</td>
</tr>
</tbody>
</table>

The analysis of batch test data usually involves many assumptions, which may not always be valid depending on the circumstances. One assumption is simultaneous removal of all of the substrates present. The tests start with a high substrate concentration (compared to a continuous unit), so there is a possibility that the substrates (the readily biodegradable and slowly biodegradable) may be removed sequentially instead of simultaneously as assumed.

Microbial growth is assumed to be negligible during the test (for low initial f/m). At the start of the test, the bacteria are taken from a low growth environment (continuous system) and exposed to an excess of substrate (batch test). As the bacteria adapt to the new conditions their growth rate may increase.

The yield is assumed to be constant. As the bacteria adapt to their new environment, it is possible that the yield will change. It is commonly observed that the yield is related to the growth rate, even after accounting for endogenous metabolism. The growth rate increases when the bacteria are exposed to an excess of substrate, so the yield may increase as well.


4.2 Continuous/Pilot Plant Setups

The traditional approach for determining activated sludge model parameters is to operate a series of continuous activated sludge units at different SRTs, measuring the treated effluent substrate concentration at steady state. Unlike the other tests described in this section, these are long term measurements. It is generally assumed that 3 to 10 SRTs are required for a continuous unit to reach steady state (Zaloum 1992), which for a SRT of fifteen days may require 150 days to reach steady state.

The data are interpreted according to the Monod model with microbial decay (equations 3.7 to 3.9). \( Y \) and \( k_d \) may be calculated from equation 4.1, and a graph of \( (S_o - S)/X \) versus \( \theta \). \( \mu_{MAX} \) and \( K_M \) may be calculated from equation 4.2 and a graph of \( 1/S \) versus \( \theta/(1+k_d\theta) \). Many other linearisations of the Monod model are available (Ong 1990), although these two were found to give the best estimates when the error was normally distributed.

\[
\frac{S_o - S}{X} = \frac{k_d \theta_x}{Y} + \frac{1}{Y} \tag{41}
\]

\[
\frac{1}{S} = \frac{\mu_{MAX} \theta_x}{K_M (1 + k_d \theta_x)} - \frac{1}{K_M} \tag{4.2}
\]

The data may also be interpreted assuming a first order reaction (equation 3.10), or using equation 3.22.

A drawback to continuous tests (other than the time involved) is that the biomass will adapt to the different operating conditions, so that each measurement used to calculate the stoichiometric and rate constants will come from a different microbial population. The parameters will be an average of the parameters from each of the different populations. The results will be acceptable for modeling steady state operation,
but extrapolation outside of the test conditions or prediction of microbial behaviour
during shock loads and transient operating conditions may not be reliable.

4.3 Fed Batch Test

In order to avoid the excessive time required for operating continuous systems at
different SRTs, and also the complications arising from changing populations over the
long tests periods, the infinite dilution test was developed. The infinite dilution test
(Williamson and McCarty 1975) involves continuous addition of concentrated feed to a
bioreactor and withdrawal of samples for analysis. Due to the conditions in the
bioreactor it is assumed that steady state with respect to substrate removal is rapidly
reached (less than one hour), that the biomass does not have time to adapt to the batch
test conditions, and no shifts in population will occur. The substrate to be tested is fed to
the bioreactor at different feed rates, from which the substrate uptake rates are calculated.
The steady state substrate concentrations in the bioreactor are measured for each feed
rate, and a curve of substrate uptake versus substrate concentration may be obtained. The
assumption that the biomass does not have time to adapt to the new operating conditions
is validated by work on shock loads to continuous units, where lags in the biomass
growth rate upon an increase of substrate loading were observed (Selna and Schroeder
1978, Mona et al 1979). This is probably due to the low microbial growth rates and the
physiological conditions of the bacteria from activated sludge units (Daigger and Grady

The infinite dilution test has been modified in order to increase the statistical
accuracy of the assay while minimizing the number of experiments (Templeton and
Grady 1988, Philbrook and Grady 1985). The modified test assumes that the Monod
model will fit the data a priori, and involves measurement at only the maximum substrate uptake rate, and one half the maximum substrate uptake rate (which corresponds to $K_M$ for the Monod model). The modified fed batch test will not give any indication if an alternative model to Monod is preferable, or whether there are mass transfer effects.

### 4.4 Batch Tests

Batch tests consist of taking a sample of wastewater, and adding biomass. The substrate concentration, OUR, and biomass concentration are measured to determine the substrate removal rate or the biomass growth rate. If many different starting substrate concentrations are used, the initial rate (growth or substrate uptake) at each substrate concentration may be measured to obtain the relationship between rate and substrate concentration.

A more efficient method involves obtaining the model parameters from a single batch test. If substrate concentration is measured over the duration of the batch test, the coefficients may be calculated by curve fitting the integrated Monod equation to the data (Robinson and Tiedje 1983). The starting conditions are very important for this sort of test, as they will determine if unique parameter estimates may be obtained. If the data follow the Monod model, unique estimates for all of the parameters will not be attainable if the initial substrate concentration is less than $K_M$, or very much greater than $K_M$.

Another possibility for measuring the coefficients from a batch test is to measure the OUR and substrate concentration throughout the batch test, until all of the substrate has been removed. The OUR is used as a measure of the substrate removal rate. A plot of the OUR vs. substrate concentration can be used to obtain the Monod constants (Huang et al 1985).
When substrate is measured by global parameters (such as BOD, COD or TOC) during a batch test involving a multicomponent wastewater, the removal rates will probably not follow the Monod model, but will follow a first or second order model (equation 3.22, Grau et al 1975). If the substrate removal goes through two distinct phases in a batch test (a initial high substrate removal rate followed by a sustained slower removal rate), the IAWQ model, or the model of Busby and Andrews (1975), may be more applicable to the data than the unmodified Monod model.

4.5 Respirometry

Introduction

As applied to wastewater treatment, respirometry is the study of bacterial metabolic rates through the measurement of bacterial respiration rates. In order to measure the biodegradation rates of the organic compounds in the wastewater, an accurate assay must be available to measure the various compounds, especially when low substrate concentrations are used. Analyses for substrate tend to be time consuming, inaccurate, or non-existent, so respirometric methods are often used as a surrogate. The dissolved oxygen concentration can be measured accurately, immediately, and continuously.

If biomass (X), substrate (S), and dissolved oxygen (O) are given in the same basis (i.e. COD), a simple mass balance may be derived (equation 4.3). The change in biomass concentration is equal to the amount of substrate which is removed less the substrate which is oxidised.

\[
\Delta X = \Delta S + \Delta O
\]  
(4.3)
Based upon equation 4.3 and the yield coefficient (equation 3.3), the OUR is proportional to the substrate degradation rate and the biomass growth rate. The equations from section 3.2 can be used to correlate the OUR to the substrate concentration. The relationship based upon the Monod model is shown in equation 4.4.

$$\frac{dO}{dt} = \frac{Y}{1 - Y} \mu_{\text{max}} X S \left( \frac{1}{K_m + S} \right) = \frac{1}{1 - Y} q_{\text{max}} X S \left( \frac{1}{K_m + S} \right) = OUR_{\text{max}} X \frac{S}{K_m + S}$$ (4.4)

Only a limited amount of information may be provided by respirometry. The yield and the initial substrate concentration can not be calculated independently, but require a separate test, either the measurement of the substrate or the yield. Similarly, the active biomass concentration and maximum growth rate are interdependent, and one can not be calculated without prior knowledge of the other (Dochain et al 1995). Most of the respirometric tests are very similar, but different assumptions have been made, allowing for the calculation of different parameters with the same basic data (see appendix for examples).

In most respirometric studies, product formation and energy spilling are assumed to be negligible. The respiration of protozoa and nitrifiers are also assumed to be negligible, as well as their influence on the heterotrophic respiration and growth rate (Mahendraker and Viraraghavan 1995). The OUR due to endogenous metabolism may be accounted for and subtracted from the overall OUR. It is important that the biomass and substrate are measured in consistent units (which accounts for the change in oxidation state as substrate is converted to biomass, as in the measurement of COD). The growth rate and the substrate biodegradation rate are proportional to the OUR (equation 4.4), and the amount of growth and substrate degraded are proportional to the amount of oxygen consumed (equation 4.3), assuming a constant yield coefficient. These
relationships, combined with the ease of use and automation, make respirometry ideal for many different purposes.

Oxygen uptake rates may be measured a number of different ways, including batch tests and continuous methods (table 4.2). The original methods (Warburg) used closed vessels with air in the headspace. The air in the headspace serves to replenish the dissolved oxygen in the liquid phase as it is consumed by the bacteria. The microbial consumption of oxygen is followed by measuring the oxygen decrease in the headspace, often using pressure (the CO₂ produced by the bacteria is removed using a caustic solution).

Air or oxygen may be continuously bubbled through the mixed liquor in a respirometer. In this case, the OUR is measured by following the dissolved oxygen in the liquid phase, or the decrease in oxygen between the inlet and outlet gas streams. The mass transfer of oxygen from the gas phase to the liquid phase must be accounted for in the data analysis. Mass transfer limitations from the bulk liquid to the bacteria are usually assumed to be negligible.

In order to avoid the mass transfer problems and simplify the data analysis, the respirometer may be completely filled with sample and mixed liquor so there is no headspace, with the DO measured using a DO probe. Since oxygen is not added during the test, the oxygen supply for each OUR measurement will be limited to what is initially present in the mixed liquor and sample. Due to the low solubility of oxygen in water this oxygen supply is relatively small. When it is depleted, the OUR measurement must be interrupted and the mixed liquor re-aerated. The OUR is very simply calculated as the slope of the dissolved oxygen concentration versus time.
All respirometric methods require sufficient agitation for mass transfer, regulation of the temperature and pH, and the presence of the necessary nutrients for the microorganisms.

**Table 4.2 Respirometric Methods**

<table>
<thead>
<tr>
<th>Method</th>
<th>Measurements</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headspace</td>
<td>Pressure drop, DO in liquid phase.</td>
<td>Gas to liquid mass transfer.</td>
</tr>
<tr>
<td>Continuous O₂ supply</td>
<td>O₂ in gas inlet/outlet, DO in liquid phase.</td>
<td>Gas to liquid mass transfer.</td>
</tr>
<tr>
<td>Filled</td>
<td>DO in liquid phase.</td>
<td>Limited oxygen supply.</td>
</tr>
</tbody>
</table>

Respirometric methods may be broadly classified into two categories, based upon the initial f/m ratio. The first category of tests employs a small microbial inoculum and a large f/m ratio, and the respiration is followed over a long time period, usually at least one day. These tests are used to:

- determine microbial growth rates,
- measure the biodegradation potential of various substrates,
- measure the inhibition of the growth rate by toxic substances,
- determine the ability of the biomass to adapt to different substrates,
- measure the active fraction of the MLVSS,
- measure the BOD, and
- determine the required treatment time for a given waste.

Significant growth occurs during the high f/m tests, so the microbial composition at the end of the test may not be the same as the composition at the start of test, as discussed in section 4.1. Low initial biomass concentrations are used in an attempt to ensure that the oxygen uptake is a true measure of biomass growth, rather than oxidative assimilation, storage, or maintenance (Gaudy et al 1988). The high f/m also ensures that the biomass
has time to adapt to the new environmental conditions, allowing the bacteria to reach their maximum growth rate regardless of the culture history. If mixed populations are used, as in activated sludge, these kinds of tests will select for the fast growing microorganism, which will have different growth rates and stoichiometry than the slow growers.

The second category of respirometric methods uses large biomass concentrations and smaller f/m ratios, and generally take less than six hours to complete. The f/m ratio ranges from low to medium, and the biomass and substrate concentrations are similar to those found in wastewater treatment plants. The validity of the assumptions made will depend in large part on the initial f/m, as discussed in section 4.1. The greater the initial f/m, the greater the possibility of changes in biomass properties. Theoretically, the biomass is not given sufficient time to adapt to the new conditions. Therefore, the results of these tests will depend strongly on the culture history. It has been argued that these conditions will give growth rate parameters and stoichiometric values closer to those found in an actual wastewater treatment plant than the tests from the first category (Chudoba et al. 1992a). These tests have been used to:

- determine the biomass concentration,
- measure the biodegradation kinetics (with and without inhibition),
- measure the hydrolysis rate of the slowly biodegradable organics,
- measure the substrate adsorption onto the biomass,
- determine the short term BOD,
- determine the readily biodegradable fractions of wastewater, and
- determine the required treatment time.
Under the conditions used in these tests, endogenous respiration and/or maintenance respiration may be a significant fraction of the total OUR and must be taken into account. The energy expended for cell maintenance may either come from available substrate, or from endogenous mass if no substrate is available.

In this literature review, the main focus will be on the methods which employ a low f/m ratio. A few examples will be given of tests using a high initial f/m, specifically those used to determine the maximum growth rate and the active fractions of activated sludge MLVSS. A recent review of respirometric methods, with the emphasis on growth tests, is available (Mahendraker and Viraraghavan 1995).

Low F/M Respirometric Methods

**ΔOUR and Similar Assay Techniques**

Over the years, many respirometric methods have been developed. Most are very similar, and make the same assumptions. The majority of respirometric tests were developed independently rather than by expanding on previously published methods. The most thoroughly developed method for determining the kinetics of concentrated biodegradable compounds which has been used by a number of experimenters is the method of Cech and Chudoba (Cech et al 1984, Chudoba et al 1985). This is the method used in this project, and it shall be referred to as the ΔOUR method and described here briefly. The ΔOUR method consists of a number of batch respirometric tests. The mixed liquor is transferred from a continuous wastewater treatment unit to a respirometer and aerated. When any substrate initially present has been utilised (a constant baseline OUR is attained), the aeration is stopped and a DO probe is inserted into the respirometer. The
endogenous respiration rate is measured. Then, a small amount of substrate is added. This causes a temporary increase in the OUR until all of the added substrate has been metabolised, at which point the OUR returns to the endogenous rate. A typical dissolved oxygen trace for one batch test is shown in figure 4.1a.

The substrate biodegradation rate is assumed to be proportional to the exogenous OUR (or the ΔOUR), which is calculated by subtracting the endogenous OUR from the total OUR after substrate addition. The proportionality constant is $1 - Y$.

$$SUR = \frac{\Delta OUR}{1 - Y} = \frac{\Delta OUR}{OC / S}$$

(4.5)

The yield is calculated according to equation 4.6,

$$Y = \frac{(S - OC)}{S} = 1 - \frac{OC}{S}$$

(4.6)

which is based on the assumption that the substrate which is not oxidised is used for growth, and that all of the added substrate is utilised. OC represents the oxygen consumed due to the added substrate, and does not include the oxygen used for maintenance or endogenous metabolism.

The ΔOUR and the amount of oxygen consumed are calculated by drawing best fit lines through the DO trace. This procedure is repeated using a number of different initial substrate concentrations. Whenever the dissolved oxygen level in the respirometer drops below 2 mg/l, the mixed liquor is reaerated. For data analysis, pseudo steady state is assumed. The bacteria are assumed to immediately reach the metabolic state corresponding to the added substrate concentration. The maximum ΔOUR achieved during the batch test is assumed to be the ΔOUR which corresponds to the initial substrate concentration of that test. If these assumptions are valid, the ΔOUR may be converted to a substrate uptake rate and graphed versus the added substrate concentration.
Figure 4.1 Interpretation of respirometric data.
to obtain the relationship between the substrate uptake rate and the substrate
concentration. The data is almost universally assumed to follow the Monod model,
although any of the equations discussed in chapter 3 may be applied to the data. If there
is substrate inhibition, then an appropriate model must be used.

The curve in figure 4.1a may be transformed into an oxygen consumed (due to
substrate) versus time curve (figure 4.1b) (Smets et al 1996b). This is calculated by
subtracting the DO value at a given time from the initial DO, correcting for the amount of
oxygen consumed due to endogenous metabolism.

\[ OC = DO_0 - DO - t \cdot OUR_{endogenous} \]  

(4.7)

This allows for easier visual inspection of the data, and is similar to the representation of
BOD curves. Another method is to graph the OUR versus time, as is done with the
IAWQ method (figure 4.1c).

The ΔOUR test, or variations thereof, has been used to measure various aspects of
the activated sludge process. The biodegradation rates of wastewater (all substrates
lumped together), and various single substrates, including ammonia and nitrate, have
been measured. The inhibitory effects of selected toxic compounds have been
determined. The substrate removal rates using biomass from different activated sludge
processes were compared on several occasions to determine the effects of different
operating conditions or process configurations. Equation 4.6 has been used both to
measure the microbial yield of single substrates and also to calculate the amount of
biodegradable organics (either as BOD or COD) in unknown samples using an assumed
yield.
One of the variations on the ΔOUR method described above includes using two respirometers. The substrate is added to only one of the respirometers. The DO profile from the respirometer which did not receive substrate is subtracted from the one which did receive substrate, after first correcting for differences between the two respirometers (Smets et al 1994, Ellis et al 1996). The OC and ΔOUR are calculated from the resulting curve as shown in figure 4.1b. The second respirometer is used to correct for changes in the endogenous respiration rate over the course of the test. This will be valid if the change in endogenous OUR is a function of time, but if it is a function of the DO then there will be a slight error introduced since the DO in the respirometer which receives substrate will be lower than the DO in the control respirometer.

Other variations use open respirometers with the mixed liquor aerated throughout the test (Ros and Dular 1992, and Spanjers 1995). The advantage of these methods is the ability to handle large amounts of substrate since there is continuous aeration. In contrast, the ΔOUR method may only be properly used with substrate concentrations that have an oxygen demand of less than 8 mg/l (at 20°C) before reaeration is required. In open respirometers, the gas to liquid mass transfer step must be accounted for in the data analysis. The main resistance to oxygen mass transfer on the liquid side is assumed to be in the liquid phase, and is represented by the liquid side mass transfer coefficient, $K_{La}$. The $K_{La}$ is measured by turning the air flow off and letting the DO level drop (due to endogenous respiration), then following the rate of increase of DO once the air is turned back on. Once the $K_{La}$ is known, the exogenous oxygen uptake rate may be measured by adding known amounts of sample to the respirometer. Before sample addition, the DO should be constant, as a steady state is achieved during which the endogenous OUR will
be equal to the oxygen transferred from the gas to the liquid phase. Upon addition of substrate, the DO will drop as the OUR increases above the endogenous rate. The exogenous oxygen uptake rate is equal to the $K_L a$ times the difference between the DO level before addition of substrate and the minimum DO level after addition of substrate. The oxygen demand, OC, of the added substrate may be calculated by integrating between the DO trace and the endogenous DO. Once the exogenous OUR (or ΔOUR) and the OC are measured, calculations may proceed as with the ΔOUR method.

One criticism of short term batch respirometric methods is the possibility of the loss of enzyme systems during the endogenous respiration period before the start of the test (Grady and Philbrook 1984). This is unlikely to be significant at the SRT's, and corresponding low growth rates, used in most activated sludge units (Cech et al 1984 also see section 2.3). In a further study of the ΔOUR method (Chudoba et al 1986), it was found that the measured Monod constants of activated sludge from a completely mixed unit were approximately constant for the first 10 hours of endogenous respiration, although the endogenous respiration rate decreased during this period. A similar experiment on activated sludge from a contact stabilisation process found that the Monod constants increased during the first 18 hours of endogenous respiration. It was argued that this would be a good method to optimise the contact stabilisation process. Similar results were found in a separate study (Drtil et al 1993a), where it was also found that the yield increased with the duration of the endogenous period before the ΔOUR was measured. The decrease in the endogenous respiration and increase in yield may be due to a decrease in cell maintenance (including energy wasting and spilling) as the bacteria adapt to a higher growth rate caused by the substrate pulses.
The AOUR method has been compared to the infinite dilution test for single substrates (Cech et al 1984). It was found that the respirometric method gave higher $K_M$ values, perhaps due to substrate adsorption (adsorbed substrate would not be measured during the infinite dilution test). An apparent dependence of $K_M$ on the biomass concentration was also noted, although no explanation was offered.

Visual inspection of short term batch respirometric data in the literature reveals that the rate data often saturate faster than allowable by the Monod formula, although the Monod model is used regardless. A possible explanation is that mass transfer effects, which if ignored, may cause the rates to appear to saturate faster than they actually do.

**IAWQ and Similar Assays**

The AOUR test is generally used to determine substrate removal rates, although it may also be used to determine the yield, or the amount of substrate in wastewater. The IAWQ method is used to determine the amount of substrate in wastewater, but it has also been used to determine substrate removal and growth rates. The IAWQ method may be thought of as a AOUR test with only a single large addition of substrate, usually wastewater, with the main goal of measuring the substrate concentration. There are four main differences between the two tests: 1) the endogenous respiration is not accounted for in the IAWQ test, 2) the yield is not measured during the test, but is assumed to be a constant, usually 0.66, 3) the Monod model is assumed, but there is no direct measurement of the half saturation constant, and 4) the test is only performed at one initial substrate concentration. Because of the large amount of substrate present, the mixed liquor requires reaeration many times throughout the test. In order to avoid the
reaeration steps, air may be bubbled continuously through the respirometer and the data analysed as described for the open respirometers in the ΔOUR section.

The standard procedure (Ekama et al 1986) involves adding mixed liquor to wastewater and following the OUR. A typical data set is shown in figure 4.2. The maximum substrate uptake rate for readily biodegradable substrate is calculated from the initial OUR, using an assumed yield, and the amount of readily biodegradable substrate is calculated from the area under the curve, using the same assumed yield. The second OUR plateau is said to be due to hydrolysis of the slowly biodegradable substrate. This portion of the curve cannot easily be used to calculate the maximum hydrolysis rate, since, under the conditions of the batch test the hydrolysis rate will be 60 to 80% of the maximum hydrolysis rate.

The entire OUR vs. time curve may be modeled to calculate the readily biodegradable substrate biodegradation rate coefficients, and the slowly biodegradable substrate hydrolysis rate coefficients. Hydrolysis may be modeled as a first order reaction (Kappeler and Gujer 1992), or by using a relationship similar to the Monod equation (Orhon et al 1995). A more sophisticated approach was used by Spanjers and Vanrolleghem (1995). The IAWQ model was compared to a modified model which assumed that the readily biodegradable component produced by the hydrolysis of particulate matter has its own set of biodegradation coefficients distinct from the original readily biodegradable component. The modified model fitted the batch test data better (this is not surprising since more parameters were introduced). The same study also demonstrated the importance of the initial f/m ratio in obtaining reliable parameter estimates.
Many respirometric tests for measuring substrate removal rates or stoichiometry have been reported on in the literature. Many of these assays do not fit into the classification of the ΔOUR or IAWQ methods, but share many similarities.

One modification of the IAWQ test involves only one OUR measurement so that no re-aeration of the mixed liquor is required (Xu and Hasselblad 1996). This is achieved by diluting the mixed liquor and substrate prior to the start of the test. The breakpoint in the OUR curve is the point at which the readily biodegradable component has been removed. The readily biodegradable substrate is then calculated from the amount of oxygen utilised at this point, using a yield obtained with acetate as the substrate in a separate test.

Another variation involves using a small sludge inoculum, and a sufficient assay time to allow measurable microbial growth (Wentzel et al 1995). The OUR increases as the biomass grows at its maximum rate on the readily biodegradable substrates, and on the hydrolysis products of the slowly biodegradable substrates (figure 4.3). In order to calculate the amount of readily biodegradable organic matter initially present in the wastewater, the amount of oxygen used due to the slowly biodegradable organic matter must be calculated. This procedure involves more manipulation of the data than the procedure of Ekama et al (1986), but does not require activated sludge as the test relies on the biomass initially present in the wastewater. This method employs a high initial f/m and therefore will probably result in changing biomass characteristics throughout the assay, but it is assumed that the stoichiometry will remain constant.

The method used for a detailed investigation of nitrification combined features from the ΔOUR method and the IAWQ test (Ossenbruggen, et al 1996). As in the IAWQ
Figure 4.2 IAWQ batch test for measurement of maximum growth rate and readily biodegradable substrate.

Figure 4.3 Growth test, growth measured using OUR.
test, enough substrate was added to ensure high microbial activity for over an hour. As in the ΔOUR test, the endogenous respiration rate was measured. The OUR, ammonium, and nitrite concentrations were followed throughout the test. The specific OUR was used as the response variable. The kinetics were calculated from the OUR versus ammonium and nitrite concentrations throughout the batch test, not just using the initial OUR and initial substrate concentration as in the ΔOUR test. The test was repeated with five different starting concentrations. The data were modeled using a two step nitrification model.

A combination of the IAWQ method and the ΔOUR method was used to determine the kinetic coefficients of a kraft mill wastewater (Slade and Dare 1993, Slade et al 1991). The amount of readily biodegradable substrate was calculated from the IAWQ method, then the Monod coefficients for readily biodegradable substrate utilisation were measured using the ΔOUR technique.

**Measurement of Adsorbed Substrate**

Respirometric techniques have been used to determine the amount of substrate adsorbed to biomass during batch tests, which is very difficult to measure directly. Using a simple mass balance, the difference between the SUR calculated from the OUR and the measured SUR may be interpreted to be due to substrate adsorption. Major assumptions required are constant rate coefficients and yield throughout the batch test.

In one study, measurement of accumulated substrate during a batch test was made using mass and energy balances (Guiot and Nyns 1986). BKME was used as the substrate. A batch test was performed similar to the IAWQ method, but soluble COD
was measured as well. The OUR profile was similar to the one shown in figure 4.2, and the COD removal appeared to follow the multicomponent rate equation (equation 3.22). It was assumed that the high initial substrate uptake rate (which was greater than the substrate uptake rate calculated from the OUR based on an assumed and constant yield) was due to substrate adsorption. As long as there was adsorbed substrate, the metabolic rate operated at its maximum. The drop in the OUR was interpreted to be the point at which all of the adsorbed substrate had been metabolised, driving the bacteria to metabolise the remaining soluble substrate.

Torrijos et al (1994) also found that the substrate removal rate was greater than the oxygen uptake rate during the first five minutes of a batch test. Assuming a constant yield, this led to the interpretation that a small amount of substrate was adsorbed at the start of the batch test.

Other evidence for substrate adsorption comes from following the change in the yield measured by the ΔOUR method during sludge regeneration. The increase in yield was attributed to the ongoing oxidation of accumulated substrates (Drtil et al 1993a). At the start of sludge regeneration, there was an excess of accumulated substrate, so all of the new substrate added during the respirometric assay was used for growth. The ability of the sludge to adsorb substrate was partially restored after a period of regeneration, thereby increasing the apparent yield (the stored substrate would not be oxidised during the course of the ΔOUR test). There was no attempt at quantifying the amount of adsorbed substrate.

Some studies have indicated that the nature of the wastewater component limits the amount of adsorbed substrate, with only a fraction of all of the organics in the
wastewater being adsorbed. Other studies have concluded (assumed) that the amount of substrate adsorbed is based solely upon the capacity of the biomass, which is greatest at the start of a batch test.

**Toxicity Assessment**

An important use of respirometry is for the determination of wastewater toxicity. Possible applications of toxicity assessment are to track down toxic substreams which may require pretreatment, to determine the maximum concentration of these substreams that a treatment system can tolerate, and to determine the toxicity of different additives used in industrial processes. Respirometric toxicity assays are limited in that they cannot determine the effect of the influent on floc stability and settleability, compaction and effluent clarity, all of which are important treatment parameters of the activated sludge process.

Respirometric toxicity assays are complicated if the toxic compound to be tested is also a food source for the bacteria. If this is the case, at low concentrations the test compound will cause an increase in the OUR, due to increased metabolism. As the concentration is increased, the OUR will start to drop as the toxic effects of the compound are exerted on the bacteria. If just one concentration is tested, it is difficult to tell if the sample is toxic or not. A number of different procedures have been developed to overcome this difficulty.

The first group of methods involve measuring the OUR at many different concentrations of the test compound. If the sample is not a food source, the endogenous OUR will be measured, and any decrease will be an indication of toxicity. If the sample is also a food source for the microorganisms, the OUR in the presence of the sample may
be greater than the endogenous OUR. In this case, the OUR must be measured using various sample concentrations, if the OUR decreases with increasing sample concentration, the sample is toxic (Arthur 1984).

In order to avoid these complications, an excess of substrate may be added to the respirometer, as well as the sample to be tested. This will ensure that the OUR is at its maximum rate regardless of whether the sample being tested is a food source or not. An example of this type of assay is the OECD activated sludge respiration inhibition test, which involves the incubation of activated sludge with synthetic sewage and the test compound. After three hours the OUR is measured and compared to a control sample with synthetic sewage but no test compound. Since the synthetic sewage contains many different substrates in high concentrations, the biomass metabolic rate will be at its maximum and the sample should not cause an increase in the OUR (Klecka et al 1985).

Toxicity tests are best done using the activated sludge of interest, in order to achieve the most pertinent results. In order to reduce variability and simplify tests, different cultures have been investigated, including *E. coli*, *Vibrio fischeri* (Microtox ©), and Polytox ©. It has been found that the toxicity response depends upon the test culture used. Activated sludge toxicity tests are less sensitive than the Microtox assay and a test using glucose consumption by *E. coli*. This may be due to the large concentration of sludge adsorbing the toxicants and reducing the effective concentration (Dutka and Kwan 1984). The toxicity of 3,5 dichlorophenol was greater when measured by respirometry than when tested in a continuous activated sludge system (Broecker and Zahn 1977).
Growth tests are more sensitive than respiration tests (King 1984). The effect of toxic compounds on microbial growth rates may be measured by using a high f/m respirometric assay.

**OnLine Respirometry**

Respirometry may be used for continuous monitoring and/or control of activated sludge plants. The microbial kinetics, the wastewater strength, the treated wastewater BOD, possible wastewater toxicity, the f/m, and the total or viable biomass may be monitored. These measurements may be used in order to control the activated sludge process (Spanjers et al 1996). On-line respirometers may be batch or continuous. In batch processes, the respirometer is charged, the OUR is measured, then the cycle is repeated. Alternately, flow through respirometers may be used where the DO is measured at the inlet and the outlet of the respirometer and the OUR is calculated from the difference in DO and the flow rates. Open or closed respirometers may be used, and the oxygen may be measured in the gas or liquid phase.

The principles of on-line respirometry are the same as for the respirometric procedures described above. The OUR of samples taken from the start of the treatment process will be proportional to the wastewater strength and biomass viability. The OUR on return sludge samples will give an indication of viable biomass.

On-line respirometry to measure the f/m has been described (Arthur and Arthur 1994). This technique involves two automated manometric respirometers. The first respirometer takes samples from near the inlet to the aeration tank to get a measure of the strength of the wastewater (and check for toxicity), while the other takes samples from the return sludge flow to measure the amount of biomass. In the absence of substrate, the
OUR will be directly proportional to the amount of viable biomass, although the proportionality constant will be different for different sets of biomass. The resulting information may be used to control the recycle rate and the sludge wasting.

A number of respirometers for determination of influent STBOD and toxicity have been developed. Many of these methods use more sophisticated activated sludge models which account for the different fractions of wastewater and biomass. If biomass in the respirometer is limiting the respiration rate (excess substrate), the OUR will be directly proportional to the biomass concentration, and can be used to estimate the viable biomass. If biomass is not limiting, the OUR is proportional to the substrate concentration, and may be used to obtain a rapid measurement (30 minutes) of the BOD$_5$ (Therien 1983).

High F/M Respirometric Methods

**Biochemical Oxygen Demand Measurements**

One common use of batch tests with small initial inocula is to measure the wastewater BOD. In the standard method, diluted wastewater is incubated with a small amount of seed at 20°C, for five days, after which time the dissolved oxygen depletion is measured. The BOD test was devised in 1898 by the British Royal Commision on Sewage Disposal to provide a direct measure of the amount of oxygen depletion in receiving waters (Bailey 1986). The five day period is arbitrary, originally chosen as the residence time of a typical British river. It is often argued that the meaning of the BOD is suspect (Orhon & Artan 1994). Since the substrate degradation often does not go to completion during five days, the BOD$_5$ value will be a function of not just the wastewater
strength, but also the wastewater biodegradability, and the acclimation of the bacterial seed to the sample. The oxygen demand is modeled as a first order process, asymptotically approaching the ultimate BOD value (the value at the completion of the biochemical reactions). The faster the BOD reaction, the greater the percentage of substrate which will be oxidised during the five day test. Usually, wastewater leaving a treatment plant is less biodegradable (smaller first order constant) than the untreated wastewater, so a lower percentage of substrates will be measured in the effluent than in the influent. The ultimate BOD, which is not a function of the oxidation rate, is more meaningful than the intermediate five day value.

The ratio of the $\text{BOD}_5$ to the ultimate BOD is 0.65 to 0.7 for domestic sewage, and anywhere from 0.1 to 0.9 for industrial wastewaters (Orhon & Artan 1994). This ratio depends upon the BOD rate constants. The $\text{BOD}_{20}$ value is often assumed to be equal to the ultimate BOD. The correlation of the $\text{BOD}_5$ with the COD gives an indication of the ratio of biodegradable substrate concentration to total substrate concentration in the sample. If the sample contains only a readily biodegradable substrate, such as glucose, the $\text{BOD}_5$ will be approximately 80% of the COD. The ultimate BOD is usually close to the COD value if the sample contains only readily biodegradable substrates.

Due to a lack of clear meaning of the $\text{BOD}_5$ value, and also to the lengthy time required for measurement, substitute tests to measure wastewater strength are often used. In order to speed up the reactions, many variations of the BOD test use respirometers with headspace, or with the continual addition of air. The greater availability of oxygen means the sample will not have to be diluted as much and a greater microbial seed
concentration may be used than is used in the standard BOD\textsubscript{5} test. This results in faster biochemical reactions and the BOD may be measured in a day or less. One example is the HBOD (headspace BOD) test (Logan and Wagenseiler 1993), which involves the measurement of the dissolved oxygen after one day. Other examples are the many manometric tests. These variations of the standard BOD test are easier to automate than the standard method, so the oxygen demand may be recorded throughout the test, in addition to the BOD\textsubscript{5} the BOD coefficients and the ultimate BOD may be calculated from the data.

The tests described in the previous section (low f/m respirometric methods) for determining short term oxygen demand or readily biodegradable COD may also be used to determine the wastewater strength. These tests use environmental conditions closer to that of the wastewater treatment plant and thus give a more meaningful indication of the wastewater strength. Due to the much higher amount of biomass present, endogenous metabolism must be accounted for or very high oxygen demand values will be measured. Therefore only the oxygen demand due to the initial metabolism of the substrate will be measured. In the BOD test, the oxygen demand due to the growth on the substrate, and also the oxygen demand due to the endogenous decay of the bacteria which have grown on the substrate are measured. Consequently, short term BOD is usually less than the BOD\textsubscript{5}.

If the BOD\textsubscript{5} values are required for regulatory purposes, a correlation may be found between a given respirometric method and a given wastewater, so the respirometric method may be used for more efficient monitoring. The BOD\textsubscript{5} has been successfully correlated with the respiration rate, one hour oxygen demand, and one half hour oxygen
demand (Arthur 1984). Each wastewater, including treated and untreated, requires a separate correlation.

**Maximum Growth Rate Measurements**

Procedures similar to the manometric BOD tests have been used for many purposes, including measuring biodegradability and microbial adaptation to various compounds or wastewaters. However, these results are not directly applicable to activated sludge treatment due to the greatly different environments between the two processes. In this review only a few examples of these tests which have been used to determine activated sludge kinetics shall be described.

Growth tests are performed under different conditions from low f/m tests. A high f/m is used, and the tests usually last longer allowing the bacteria to grow during the course of the respirometric assay. The initial OUR is low due to the small inoculum. As the biomass grows, the OUR increases in direct proportion to the increase in biomass. The endogenous metabolism and decay are assumed to be negligible, and the OUR will increase exponentially until some factor becomes limiting. Using different starting concentrations, the growth rate versus substrate curve can be obtained (Gaudy et al 1988). The Monod constants may also be calculated from just one respirometric growth test using an appropriate initial f/m (Smets et al 1996a). The estimate of the initial biomass concentration will affect parameter determination. Also, if there are insufficient amounts of nutrients present, the calculated yield will be higher than expected. It was recommended that nutrients be present in quantities far in excess of their stoichiometrically required dose in order not to affect the measured kinetic coefficients.
A more efficient way of measuring the maximum growth rate is to measure the change in OUR in the presence of an excess of substrate (Kappeler and Gujer 1992). It can be shown that the slope of the logarithm of the OUR vs. time curve is equal to the maximum growth rate minus the endogenous decay rate (using the IAWQ model). The half saturation constant may be calculated at the point where the OUR drops, under the assumption that the substrate concentration is very low, by applying the IAWQ model to the entire OUR vs. time curve. However, since there is likely to be slowly biodegradable matter still present in the wastewater once the readily biodegradable matter is removed (Wentzel et al 1995), this method will give erroneous results for \( K_M \).

A common critique of methods which involve excessive biomass growth is the changing biomass composition during the tests. At the end of the test, the biomass may not be the same as at the start of the test, and may have different kinetic parameters. In particular, the batch test tends to select organisms with high growth rates (Wentzel et al 1995, Bull and Brown 1979). This has been demonstrated experimentally (Novák et al 1994). The organisms selected based on the maximum growth rate in batch tests do not necessarily play a significant role in the nutrient-limited environment, such as a wastewater treatment plant (Harder and Dijkuizen 1982).

**Correlation Between OUR and Viability**

There have been many attempts to determine the percentage of active biomass in activated sludge using respirometry. One method (Huang et al 1985) involves measuring the maximum OUR using a batch test. The microbial viability is then assumed to be proportional to the OUR. Using cell counts from another paper, it was found that a portion of the OUR was due to non-viable cells. Another method involving OUR
measurements (although it was not clear whether they were endogenous or exogenous rates) also found that a majority of respiration was due to non-viable cells (Walker and Davies 1977). These calculations were based on the assumption that the respiration rate per cell is constant. The OUR was correlated to viable biomass using data obtained from a batch test (Jørgensen et al 1992). Viable biomass in a continuous unit was then calculated by measuring the OUR and using the conversion factor (assuming that the specific OUR of biomass from a continuous activated sludge unit is the same as the specific OUR of a rapidly growing culture).

Another technique involves measuring the maximum specific growth rate using a high f/m batch test, and measuring the volumetric growth rate using a procedure similar to the ΔOUR procedure, then dividing the two numbers to obtain the viable biomass concentration (Blok 1976). Alternately, the initial OUR at the start of the growth test may be used as the measure of the specific growth rate (Orhon et al 1995, Kappelar and Gujer 1992). According to the IAWQ model, under the conditions of a growth test the biomass concentration is proportional (through the yield coefficient) to the OUR times the growth rate. The maximum growth rate is measured during the test, so the initial biomass concentration may be calculated if the yield is known. This method makes the further assumption that, at the start of the growth test, the endogenous metabolism is minimal compared to the overall OUR. If the biomass changes during the growth test (selection for fast growing organisms over bacteria with lower growth rates), the maximum growth rate obtained will be for the fast growing organisms, and not the complete mixture of organisms present at the start of the test. This will result in an overestimation of the initial biomass concentration.
Similar logic has been applied to the determination of the active biomass concentration using the decay test. During a decay test the OUR is proportional to \((1-f_{ex})bHX_H\). The term \(b_H\) is measured during the decay test, \(f_{ex}\) is assumed, therefore using the initial OUR, the initial biomass concentration may be calculated. However the OUR data during a decay test often do not fit the first order model, possibly due to the presence of slowly biodegradable hydrolysable material at the start of the test (Sollfrank et al 1992). If this is the case the active biomass concentration will be overestimated.

The \(\Delta\text{OUR}\) technique in combination with continuous culture techniques (steady state operation at different SRT’s) was used to determine the kinetic constants for the microorganisms responsible for degradation of xenobiotic compounds, rather than to determine the constants for the whole sludge (Chudoba et al 1989a,b). The concentration of responsible microorganisms was calculated from the ratio of the maximum volumetric removal rate calculated from the \(\Delta\text{OUR}\) technique, and the specific removal rate calculated from the continuous reactors. Different populations of bacteria may be present at each of the continuous culture steady states.

4.6 Yield

The accepted method for measuring the yield coefficient is to seed wastewater with a small amount of biomass and measure total and soluble COD. The COD due to biomass is calculated as the difference between the total and soluble COD. In order to calculate the true yield, samples can be taken over a number of days and the apparent yield calculated for each data point. The true yield can be calculated by extrapolating to time = 0 on a graph of \(1/Y\) versus time (Slade et al 1991). This test is very sensitive to the initial values of substrate and biomass due to analytical errors (Orhon and Artan...
1992). The yield may also depend upon the initial conditions (f/m) as discussed earlier, so a more appropriate assay would involve using f/m values closer to f/m values found in continuous systems.

The ΔOUR procedure may be used to calculate bacterial yield if a known amount of substrate is added (equation 4.6). Liebeskind et al (1996) added known amounts of acetate to a respirometer and measured the oxygen consumption. The yield was found to increase from 0.59 to 0.68 as the SRT increased from 1.88 to 24.0 days. This method has also been used by Cech et al (1984), who found a range of yields on various substrates.

Another method of calculating the true yield is to run continuous activated sludge units at different SRT's, and use equation 4.3 to calculate both Y and k_d.

**4.7 Decay**

Microbial decay is measured by removing a sample of mixed liquor and placing it in a batch reactor and measuring the OUR or the biomass concentration for at least 10 days. The method involving OUR measurement was found to be easier and more reliable (Marais and Ekama 1976). The data may be interpreted assuming either the endogenous decay model or the death regeneration model. If the death regeneration model is used, the regeneration step may not be the rate limiting step, especially at low temperatures (Lishman and Murphy 1994). At low temperature hydrolysis may be slower than either death or regeneration. Since the regeneration step is the step which requires oxygen, this may lead to underestimating the decay rate. This argument does not apply to the endogenous decay model, if the assumptions made are correct (oxygen utilisation is by endogenous metabolism and not growth).
When bacteria are exposed to starvation conditions, the metabolism may be slowed and the membrane made more impermeable. This will result in a lower decay rate for starving bacteria than for actively growing bacteria. If this is the case, the decay coefficient measured by batch tests will not be applicable to continuous activated sludge units.

Decay decreases with temperature (Sollfrank et al 1992) and has been found to follow a simplified Arrhenius relationship (Marais and Ekama 1976).

4.8 Summary

Many different respirometric assays for measuring activated sludge substrate removal rates and stoichiometry have been developed. All of these assays may be classified into two broad groups based upon the initial f/m, low initial f/m tests and high initial f/m tests. The results from respirometric batch assays are a function of the assay. Tests which employ a low initial f/m simulate the environmental conditions of the treatment plant to a much greater degree. These tests give an accurate indication of the state and capability of the biomass at the time of sampling.

Respirometry may be used to measure the substrate removal rate, the yield on the substrate, or the substrate concentration if one of these is known a priori. For wastewaters, the substrate concentration and substrate removal rate are usually unknowns. If the yield is known from a separate assay, or assumed, the substrate concentration and removal rate may be calculated using respirometric techniques.

Respirometry may also be used as an indication of the biomass concentration. The maximum OUR is directly proportional to the active fraction of biomass. The proportionality constant is the maximum specific growth rate, which is difficult to
measure under environmental conditions similar to activated sludge (low growth rates).

Changes in the OUR are related to growth or decay of biomass, and may be used to calculate growth rates or decay rates.
Chapter 5 Materials & Methods

5.1 Lab Scale Activated Sludge Units

Two continuous lab-scale activated sludge reactors were operated. Each reactor consisted of a 5-litre cylindrical jacketed Plexiglas aeration tank and a 1.5 litre conical glass clarifier. The working volume of the aeration tank was 4 litres, and the working volume of the clarifier was 1 litre. The aeration tank and the clarifier were connected by a short 1" diameter tube. This tube had to be cleaned periodically to prevent clogging. Reactor temperature was maintained at 35°C by circulating water from a constant temperature bath (Fisher Scientific) through the annular Plexiglas jacket surrounding the aeration tank. The clarifier did not have a water jacket, but was insulated. The temperature of the clarifier varied between 25°C and 30°C depending upon the room temperature.

For one experimental run (run #3), an aerated selector was used ahead of the aeration tank. The selector consisted of a 600 ml jacked glass vessel. Temperature was maintained using the same circulating water bath as the main aeration tank, thereby maintaining the selector temperature very similar to the main aeration tank temperature. The selector volume was 300 ml, 7.5% of the aeration tank volume (which was lowered to 3.7 litres during the operation of the selector). Oxygen was added using an aquarium air pump.

Air was supplied to the aeration tanks using both aquarium air pumps and building air. The redundancy was to ensure a constant supply of air. When the power went down, the building air would (sometimes) stay on providing mixing and air, but
often the building air would be turned off, so a second source was required. The reactor
dissolved oxygen content (DO) was usually between 3 to 5 mg/L, and never fell below 2
mg/l.

Mixing was provided by a magnetic stir bar and a stir plate. The sides of the
aeration tank were cleaned daily in order to prevent wall growth from forming. In order
to reduce biomass attaching to the sides of the clarifier, a pump was set up to mix the
contents of the clarifier every 30 minutes. This did not completely work, so the sides of
the clarifier were wiped clean daily.

Feed, waste sludge, and sludge recycle were pumped using Masterflex peristaltic
pumps (Cole-Parmer, Chicago, IL). Size 14 tubing, either silicone or C-flex, was used
for both the feed line and the recycle line and an influent flow rate of 8 litres/day was
maintained. This gave a hydraulic retention time of 12 hours. The tubing was cleaned of
attached biomass every day, and the flow rate was adjusted as the tubing stretched.
Although biomass grew in the feed line, this had no measurable effect on the effluent
characteristics, COD and BOD. One pump with two pump heads was used for the
recycle of both activated sludge units, but two separate pumps were used for the feed
rates in order to have more precise control. The recycle line clogged repeatedly, but at
the low flowrates desired, it would have been impossible to control using a larger size of
tubing. The recycle ratio was maintained at approximately 1. Sludge waste rate was
regulated using a Chrontrol (San Diego, CA) timer controlling Masterflex peristaltic
pump (size 16 tubing). Approximately 10 millilitres was removed every waste cycle,
which varied from every 20 minutes to every 2 hours depending on the MLVSS levels
and the desired SRT. The sludge wastage rate was measured daily and varied as necessary. The SRT was calculated according to the following formula:

\[ 0_x = \frac{X}{XQ_w + X(Q_O - Q_w)} \]  

(5.1)

Untreated BKME was obtained from Harmac Pacific Ltd. (Nanaimo, BC). Harmac Pacific produces 1135 tonnes per day of bleached kraft pulp from softwood - douglas fir (17%), western hemlock/balsam (55%), and western red cedar (28%). The pulp is bleached using 100% chlorine dioxide substitution (Rodden 1994, Rodden 1998). The effluent was sampled from a sample port before the cooling towers (Harmac Pacific does not have primary effluent clarification). Approximately 50 20 litre buckets were collected on each effluent run. The time to fill all of the buckets was approximately 30 minutes, during which time any variation in the effluent would result in variation in the composition of the individual buckets. The variation between buckets (< 5%) was small compared to the variation between effluent runs (up to 100%). The wastewater was warm when sampled (50°C). Transportation to the fridge took approximately three hours. The effluent was then stored at 4°C until use. Just before use, the pH was adjusted to 7-7.5, (initial pH varied from 4 to 11 depending on the effluent batch) and nutrients were added in the ratio of BOD:N:P = 100:5:1. Nutrients were added in the form of concentrated NH₄OH (Fisher Scientific, Nepean, ON) and H₃PO₄ (BDH, Toronto, ON). Approximately every six weeks a new batch of effluent was obtained from Harmac Pacific.

Over the course of this study, 19 different batches of effluent were obtained from Harmac Pacific Ltd. This effluent was treated over six different runs. Each run was started with fresh sludge from Harmac’s activated sludge unit. The first run involved lab
set up, learning how to operate activated sludge units, and developing experimental methods. The second run investigated the effects of SRT, the third run continued the study of SRT and also looked at the effect of an aerobic selector on the treatment of BKME. The fourth run studied the biomass from Harmac's activated sludge treatment system, before adaptation to the lab scale conditions occurred. During this run, biomass decay rates were measured. During the fifth run, supporting evidence for what was discovered during the third run was sought, however due to low biomass activity, this was only partially successful. During the sixth run, the respirometric method was studied in great detail, and the decay experiment was repeated. Temperature and pH shocks were performed randomly throughout the various runs. The schedule of the effluent batches and operating conditions is presented in table 5.1.

**Table 5.1 Experimental Conditions**

<table>
<thead>
<tr>
<th>Run</th>
<th>Batch</th>
<th>Dates</th>
<th>Reactor #1</th>
<th>Reactor #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>April 21 1994</td>
<td>SRT = 5</td>
<td>SRT = 17</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>June 2 1994</td>
<td>SRT = 5</td>
<td>SRT = 23</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>July 14 1994</td>
<td>SRT = 7</td>
<td>SRT = 26</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>September 2 1994</td>
<td>SRT = 5</td>
<td>SRT = 10</td>
</tr>
<tr>
<td>2</td>
<td>E</td>
<td>November 24 1994</td>
<td>SRT = 5</td>
<td>SRT = 10</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>January 5 1995</td>
<td>SRT = 5</td>
<td>SRT = 11</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>March 1 1995</td>
<td>SRT = 5</td>
<td>SRT = 19</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>April 5 1995</td>
<td>SRT = 5</td>
<td>SRT = 25</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>May 17 1995</td>
<td>SRT = 5</td>
<td>SRT = 17</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>June 29 1995</td>
<td>SRT = 5</td>
<td>SRT = 15</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>August 10 1995</td>
<td>SRT = 7</td>
<td>SRT = 12</td>
</tr>
<tr>
<td>3</td>
<td>L</td>
<td>November 24 1995</td>
<td>SRT = 11</td>
<td>SRT = 11, Aerobic selector</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>January 10 1996</td>
<td>SRT = 10</td>
<td>SRT = 11, Aerobic selector</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>March 8 1996</td>
<td>SRT = 10</td>
<td>SRT = 11, Aerobic selector</td>
</tr>
<tr>
<td>4</td>
<td>O</td>
<td>June 17 1996</td>
<td>Decay experiment</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>P</td>
<td>August 22 1996</td>
<td>SRT = 5</td>
<td>SRT = 19</td>
</tr>
<tr>
<td>6</td>
<td>Q</td>
<td>November 27 1996</td>
<td>SRT = 5</td>
<td>SRT = 23</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>January 18 1997</td>
<td>SRT = 11</td>
<td>SRT = 24</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>March 27 1997</td>
<td>Decay experiment</td>
<td>SRT = 15, Synthetic effluent</td>
</tr>
</tbody>
</table>
5.2 Standard Tests

Mixed liquor suspended solids, BOD$_5$ and COD were determined using standard methods (Greenberg et al., 1992). COD was measured in HACH COD vials, on 2 ml of sample. COD reagents were added to the sample vials using dispensers. The samples were digested in a HACH COD reactor, and absorbance was measured in a HACH 2000 Spectrophotometer at both 600nm and 620 nm. Calibration curves were done for each batch of reagents prepared. BOD was measured in 60 ml Wheaton BOD bottles. The BOD bottles were incubated in a Fisher Scientific incubator at 20°C. Dissolved oxygen was measured using a YSI model 59 DO meter and probe. Seed for the BOD test was obtained from the lab scale reactors. Seed control and samples were done in triplicate when there was enough sample. Acute toxicity was measured using a Microtox 500 analyser.

5.3 Batch Tests

Wastewater Characteristics from Batch Tests

A typical wastewater batch biodegradation test was as follows: 200 ml of concentrated mixed liquor removed from the continuous lab scale unit was added to 800 ml of untreated BKME to provide an initial BOD of 200 mg/l, and an initial MLVSS of 1000 mg/l. A constant temperature was maintained by using glass vessels with water jackets and an external circulating water bath. The mixed liquor was continuously aerated and well mixed using a magnetic stir bar. The OUR was measured by transferring a well mixed sample to a water jacketed BOD vessel maintained at the same temperature as the batch test vessel. The DO concentration in the BOD vessel was
monitored until below 2 mg/l, then the sample was returned to the batch test vessel, and a new sample was transferred to the BOD vessel. Mixing in the BOD vessel was provided by the DO probe and a magnetic stir bar. The OUR was measured for a minimum of six hours. Samples were periodically taken from the main batch test vessel for measuring solids, BOD, COD, and respirometric kinetics (see the ∆OUR procedure). Samples for use in the BOD, COD, and respirometric tests were centrifuged for 15 minutes to remove the solids immediately upon sampling. The solids were discarded (or returned to the continuous unit). BOD and COD tests were either started immediately upon sampling, or the samples were stored at 4°C for a maximum of six hours. Samples for respirometric measurements were either utilised immediately, or frozen overnight to be used the next day. Samples for measuring MLVSS were immediately filtered as per the MLVSS standard method.

Infinite Dilution or Fed-Batch Test

This procedure was based upon the method of Williamson and McCarty (1975). A large volume of mixed liquor (usually one litre) was placed in a water jacketed vessel and fed untreated BKME at a constant rate using a syringe pump. The mixed liquor was aerated and well mixed. The MLVSS concentration was usually adjusted to around 1000 mg/l. Samples were withdrawn for BOD analysis periodically (every 15-30 minutes), centrifuged for 5 minutes to remove the solids, then stored at 4°C until the start of the BOD test. The solids removed from the BOD samples were returned to the test vessel. This ensured that the volume of mixed liquor didn’t vary by more than 5%. The test was run for 2-4 hours. At this point the test was repeated at a new feed rate using fresh sludge. The MLVSS levels were measured at the start and end of the test. The BOD test
on the collected samples was started at the end of the day when all of the samples had been collected.

Batch Decay Test

Decay rates were measured by removing a sample of mixed liquor from the continuous unit aeration tank, placing in a water jacketed vessel, and adjusting the conditions as required (pH and temperature). The sample of mixed liquor was then mixed and aerated at a constant temperature, with no feed, for a number of days or until the biomass activity was very low. At specified time intervals, samples were removed from the decay reactor and placed in the respirometer. Biomass activity was measured as outlined below (the ΔOUR respirometric procedure) after which the sample was discarded. If decay due to extreme temperature or pH was being studied, the sample taken for the measurement of the respirometric activity was adjusted to the temperature or pH the biomass was acclimated to prior to the start of the decay experiment. For example, if the activated sludge was acclimated to a pH of 8, and the decay experiment was carried out at pH 10, then the samples from the decay experiment were adjusted to pH 8 for the respirometric measurements.

Temperature and pH studies

For experiments in which the pH was adjusted, the pH of both the mixed liquor and substrate samples were adjusted to the experimental pH using 10M H₂SO₄ or 10M NaOH. pH adjustment was rapid. Minor adjustments were made with 1M solutions as required.
For all experiments, constant temperature was maintained using water jacketed vessels attached to heated water baths. The response time for small temperature changes was very fast. For large temperature changes, ice water or hot water was added to the water bath to achieve quick temperature adjustment. For experiments at temperatures below room temperature, ice water or cold tap water was added to the water bath as required.

For decay experiments, measurements were taken immediately following the temperature or pH adjustment, until the biomass activity was too low to measure. For steady state experiments, measurements were taken immediately following the pH or temperature change, and continued until the experimental values reached a steady state.

For all experiments where pH and temperature are not specified, the pH and temperature were at the values for which the biomass was acclimated. This is a temperature of 34 +/- 1°C and a pH of 8 +/- 0.5.

5.4 Respirometric Method

ΔOUR Procedure

Activated sludge model parameters were determined through respirometric methods (Cech et al, 1984). Three respirometers were made by Canadian Scientific Glass blowing. Their working volumes were 230, 210, and 205 ml. Temperature was controlled using an external circulating water bath connected to the water jacket on the respirometer. Mixing was provided by both the mixer on the DO probe and a magnetic stir bar. At the start of a test, mixed liquor samples were transferred from the aeration tank of the continuous lab scale system to the respirometer. If a different MLVSS
concentration from the concentration in the continuous unit was desired during the respirometric test, the mixed liquor was diluted with treated effluent, or concentrated by settling and centrifuging. MLVSS was measured either at the start of the test, on a separate sample of mixed liquor from the aeration tank of the continuous lab scale system, or at the end of the test, on the respirometer contents. Upon addition to the respirometer, the mixed liquor was aerated for 15 to 60 minutes at a constant temperature. The duration of the aeration time did not have any noticeable effect on the measured kinetics. After the mixed liquor had been aerated, the air flow was stopped and a DO probe was inserted into the respirometer. Stirring was provided both with a stir plate and from the DO probe itself. The data from the DO meter (YSI model 59, or Orion model) were imported directly into a spreadsheet using Collect. Collect is a software program which takes the data from a RS 232 port. The data are parsed, then sent to the keyboard buffer so any program “thinks” that the data are being typed in from the keyboard. This allowed for real time monitoring of OUR instead of just the DO. The DO and temperature were recorded every second. The probe was allowed time to equilibrate with the sample temperature, and the endogenous respiration rate was measured for at least 2 minutes. Then, a known amount of substrate was added through an injection port using a Becton-Dickson syringe and the change in respiration rate was followed. When the respiration rate returned to the endogenous respiration rate, another sample was added. When the DO level fell below 2 mg/l, the DO probe was removed from the respirometer, and the air flow was started again. Once the mixed liquor sample in the respirometer was saturated with dissolved oxygen, the air flow was stopped and the DO probe was reinserted. More substrate samples were added as before. This procedure was repeated
until a full set of OUR versus substrate concentration data was obtained. When BKME was used as the substrate, varying initial concentrations of BKME in the respirometer were achieved by injecting varying volumes of BKME into the respirometer. The order of injection was arbitrary. When other substrates were investigated, the initial concentration in the respirometer was varied either by injecting varying volumes of a stock solution, or by injecting the same volume of a series of dilutions. The two methods gave similar results. The range of substrate concentrations was chosen to cover the complete range of ΔOUR response, from the initial first order region of the ΔOUR vs. substrate curve, to the zero order region where the biomass are at maximum activity.

Data Analysis

Typical respirometric data obtained using a range of methanol concentrations are shown in figure 4.1a. There are many ways to analyse the data. In the method developed by Cech et al (1984), the DO versus time data is used as is, and has already been described (chapter 4). Alternately, the data may be transformed to oxygen consumption for substrate metabolism versus time as shown in figure 6.1.1. The data may also be transformed by calculating the slope of the curves shown in figure 4.2c by using a smoothing interpolating formula, as was done to obtain the curves shown in figure 6.1.2. In this method of presenting the data, the OUR due to the addition of substrate is equal to the maximum point on the curve minus the OUR before addition of substrate (baseline), this value is referred to as the ΔOUR. The amount of oxygen consumed to metabolise the given substrate is equal to the area between the OUR curve and the baseline extrapolated until it intersects the OUR curve once the substrate has been all metabolised. For fast data analysis in the lab, this was the method which was used with the slope being
calculated at each second using linear regression over 10 data points. When this method was used it became easy to change how many points the slope was calculated over to adjust for the respiration rate.

The three methods of interpreting the data described above all gave the same results for a given data set, with the main difference being in visual presentation. The method used for this project is very similar to the one developed by Cech, et al (1984), but the use of a computer to analyse the data allows for more accuracy (in the original method a strip chart recorder was used). The method used for this project will also be more accurate than the method discussed by Smets et al (1994), which uses two respirometers, as it will be very difficult to get identical conditions (temperature, pH, biomass concentration, etc.) in each respirometer and slight differences can have large effects on data interpretation.
Chapter 6 Respirometry

6.1 Respirometric Data Analysis

Typical Respirometric Data

Even though respirometry has been used extensively in the study of activated sludge kinetics, the majority of the assumptions made in interpreting respirometric data obtained from low f/m batch tests have not been verified. Some of the influences which have not been investigated are the effects of mass transfer, dissolved oxygen concentration, and the assumption of pseudo steady state. Measuring the kinetics of BKME biodegradation is central to the goals of this project. Since methanol is the main readily biodegradable substrate in BKME, and in order to verify the respirometric test using a single substrate before applying the method to a more complex wastewater, the data on methanol kinetics as measured using respirometry will be presented first. Data on formic acid and acetic acid kinetics will be presented as well, since these compounds are also major contributors to BKME BOD.

Typical activated sludge respirometric data for a range of added methanol concentrations are shown in figure 6.1.1 and 6.1.2. In figure 6.1.1, the oxygen consumed due to the addition of methanol has been plotted versus time for many different methanol additions. The OUR increased immediately (< 2 seconds) after the addition of methanol. The more methanol which was added, the more oxygen the biomass consumed. Also, if the region right after the addition of methanol is closely examined, it can be seen that the greater the addition of methanol, the greater the slope of the oxygen consumption versus time curve. This is more evident in the OUR vs. time curves as shown in figure 6.1.2 for
Figure 6.1.1  Oxygen consumption for 8 different methanol injections of varying strength, superimposed for comparison. Initial methanol concentration listed on chart. MLVSS: 3750 mg/l

Figure 6.1.2  Methanol injections, same data as figure 6.1.1, converted to OUR. MLVSS: 3750 mg/l
the same set of data. The ΔOUR is calculated by subtracting the OUR before substrate addition (the endogenous or baseline OUR) from the maximum OUR after substrate addition. The ΔOUR is a measure of the substrate uptake rate and may be plotted versus the amount of methanol added (figure 6.1.3). The ΔOUR followed saturation kinetics, that is, at low substrate concentrations the ΔOUR increased with increasing substrate concentration, until a maximum ΔOUR was reached. The methanol was not inhibitory to the activated sludge at the concentrations used (since the ΔOUR did not decrease at high methanol concentrations). Another set of data, at a lower biomass concentration, is shown in figure 6.1.4. The ΔOUR again followed saturation kinetics but was lower due to less biomass being present. At steady state, the endogenous OUR and the OUR due to available substrate, and consequently the ΔOUR, are directly proportional to the active biomass concentration.

Figure 6.1.5 shows the standard deviation of the ΔOUR data versus the average ΔOUR for each level of methanol addition with more than three replicates. The standard deviation versus methanol concentration is shown in figures 6.1.3 and 6.1.4. The standard deviation does not appear to be a function of the ΔOUR, methanol concentration, or the solids concentration.

Besides the metabolic rates, the yields may also be calculated by respirometry. It is necessary to know the yield in order to convert the oxygen uptake rate into a substrate uptake rate. The oxygen consumption per substrate added is shown in figure 6.1.6 for the 2 data sets already discussed. For each data set the amount of oxygen consumed appeared to be directly proportional to the amount of added substrate, which is expected if the yield is a constant with respect to substrate concentration. If a constant yield is
Figure 6.1.3 ΔOUR (•), average ΔOUR (where more than three replicates) (○), and standard deviation (△) vs methanol concentration. MLVSS 3755 mg/l.

Figure 6.1.4 ΔOUR (•), average ΔOUR (○), and standard deviation (△) vs methanol concentration. MLVSS 1370 mg/l.
assumed, the slope of the oxygen consumed versus methanol concentration is equal to the substrate oxidation coefficient (OC/S). The yields thus obtained (1 - OC/S) from the two sets of data were 0.23 ± 0.01 and 0.36 ± 0.01 (mg COD biomass / mg COD methanol) respectively. Although these two sets of data were collected only a few days apart, the yields were quite different and the 95% confidence intervals did not overlap. The variability of the kinetic and stoichiometric parameters will be discussed in chapter 7.

In the original ΔOUR method (Cech et al. 1984), the yield is calculated for each data point independently (Y = 1 - OC / S). When this was done with the data in figure 6.1.6, it was seen that the yield was not a constant, but increased at low substrate concentrations as shown in figures 6.1.7 and 6.1.8. This was due to the slight curvature of the lines in figure 6.1.6 at low substrate concentration. The ΔOUR data are shown in figures 6.1.7 and 6.1.8 for comparative purposes. The yield appeared to follow an inverse relationship with the metabolic rate at low substrate concentrations, then when the ΔOUR was constant, the yield was also constant. There are three possible explanations for this phenomenon.

The standard deviation of the oxygen consumption data is graphed in figure 6.1.9. The standard deviation increased with increasing oxygen consumption, but the error in oxygen consumption at low methanol concentrations will have a greater impact on the error of the calculated yield. If the error in the OC data is ± OC, then the error in the calculated yield will be +/- OC / COD. At low methanol concentrations, ± OC is approximately 0.001. When the COD is close to this value, the error in the calculated yield will be large. Therefore, the apparent trend in the yield may be just an artifact of the data analysis.
Figure 6.1.5 ΔOUR Standard deviation vs. methanol concentration. MLVSS 3755 mg/l (○), and MLVSS 1370 mg/l (■).

Figure 6.1.6 Oxygen consumption (□), and average oxygen consumption (●), vs methanol concentration, MLVSS 3755 mg/l. Oxygen consumption (▲), and average oxygen consumption (♦), vs methanol concentration, MLVSS 1370 mg/l.
Figure 6.1.7 Respirometric yield (●), average yield (○), and ΔOUR (▲) vs methanol concentration. MLVSS 3755 mg/l.

Figure 6.1.8 Respirometric yield (●), average yield (○), and ΔOUR (▲) vs methanol concentration. MLVSS 1370 mg/l.
A second explanation for greater yield at low substrate concentration may be due to energy spilling as discussed in section 2.4. As the catabolic activity increases, the anabolic capabilities of the bacteria will be surpassed, and the yield will decrease as energy is spilled to avoid the buildup of toxic compounds or ATP. The activated sludge used in the respirometric assay was obtained from continuous activated sludge units. The growth rate of the biomass was low, allowing for energy spilling when the methanol concentration was suddenly increased. If energy spilling is the explanation for the decrease in yield with increasing methanol, the yield may be expected to keep decreasing, instead of leveling off as observed. Also, a greater effect on yield would be expected using the biomass from the activated sludge unit operated at an SRT of 15 days (compared to the biomass from the unit operated at an SRT of 5 days). This was not observed.

A third explanation for the observed behaviour of the yield comes from the non-steady state nature of the method. If at the start of the batch test there is an oxygen reserve in the floc that is not measured by the dissolved oxygen probe, and this reserve gets used up during the test, then more oxygen will be consumed during the metabolism of the substrate than will be measured by the decrease in dissolved oxygen in the bulk mixed liquor. Consequently, the yield will appear to increase at small substrate additions. At larger substrate additions, the oxygen reserve in the floc becomes insignificant compared to the amount utilised from the bulk liquid. Assuming that the reserve of oxygen used in the flocs is a constant, the variation of yield with substrate addition may be modeled (lines in figures 6.1.7 and 6.1.8). The dissolved oxygen reserve in the floc is
Figure 6.1.9 OC standard deviation vs. average OC. MLVSS 1370 mg/l (■), MLVSS 3755 mg/l (●). Respirometric yield standard deviation vs. average yield. MLVSS 1370 mg/l (▲), MLVSS 3755 mg/l (●).

Figure 6.1.10 OUR vs. methanol addition rate. MLVSS: 1500 mg/l
calculated to be 0.005 mgO₂/l mixed liquor, which corresponds to approximately 1.7 mgO₂/l floc (using a MLVSS concentration of 3755 mg/l, and assuming a floc density of 1.25 • 10⁶ mg floc/l floc, (Benefield and Molz 1983)). This value of 1.7 mg/l floc will correspond to the steady state dissolved oxygen concentration in the floc at which the rate of diffusion of oxygen into the floc is equal to the endogenous OUR.

Due to the large error in the calculated yields at low substrate concentrations, it is difficult to determine if the increase in yield is real or not. The yield has been assumed to be constant for a given set of experiments, unless otherwise noted, and has been calculated by linear regression of the oxygen consumption versus substrate addition curves. This method of calculation will put more emphasis on the points at higher substrate concentrations (which agrees with the first and third possibilities outlined above). An alternate method of calculating the yield is shown in figure 6.1.10. Methanol was added continuously to the respirometer, and the OUR was measured. For calculating the substrate uptake rate, the residual methanol was assumed to be negligible, so the substrate uptake rate was equal to the substrate addition rate. The yield is equal to 1 - OUR / SUR, and was 0.41 mg COD/mg COD. The ΔOUR yield calculated using the same biomass was 0.32 to 0.39 mg COD/mg COD, slightly lower than that measured using continuous methanol addition. With the continuous method, the yield decreased with increasing methanol addition rate (figure 6.1.11). As the methanol addition increased, the metabolism increased, which may result in a decrease in the yield as discussed previously.

Using the calculated yield, the substrate uptake rate may be calculated using equation 4.5. The SURs for the two sets of data discussed are presented in figure 6.1.12.
Figure 6.1.11 Respirometric yield vs SUR, continuous addition of methanol to a respirometer. MLVSS: 1500 mg/l
The two sets of data are similar at high substrate concentrations, but quite different at low substrate concentrations. The explanation for the discrepancy at low methanol concentrations will be discussed in the next section. These two data sets were collected just a few days apart, the specific maximum SURs are similar even though the yields are quite different. The amount of enzymes does not appear to have changed, but the amount of the substrate used for energy has. One possible explanation is the presence of slowly biodegradable components present in the mixed liquor during one of the experiments. Another explanation is the presence of compounds or conditions causing stress to the microorganisms, resulting in more substrate being oxidised for maintenance purposes. Yet another possibility is that the substrate utilisation enzyme levels (catabolism) have not changed, but the growth enzymes (anabolism) have changed, resulting in more or less substrate being wasted. The yield on methanol appeared to be more variable than the substrate removal rate.

Different substrates can be expected to have different oxidation kinetics, and different yields. Acetic acid and formic acid are other readily biodegradable substrates present in BKME. A set of ΔOUR data and oxygen consumption data for acetic acid and formic acid is shown in figures 6.1.13 and 6.1.14. For this set of biomass, the ΔOUR due to acetic acid was slightly lower than that due to formic acid, and both were lower than the ΔOUR due to methanol. The oxygen consumption per mg COD formic acid added was similar to the oxygen consumption per mg COD methanol added, implying that the yield on formic acid was similar to the yield on methanol. This is expected since both compounds are one carbon compounds and are probably oxidised by similar pathways. The yield on acetic acid (0.7 mg COD biomass/ mg COD substrate) was much greater
Figure 6.1.12 SUR vs. methanol concentration. MLVSS 1370 mg/l (□), and MLVSS 3755 mg/l (●).

Figure 6.1.13 ΔOUR vs substrate, formic acid (▲), acetate (●), and methanol (■). MLVSS 1810 mg/l
than the yield on methanol and formic acid. The yield on a two-carbon compound is expected to be greater than yield on a one-carbon compound. An important observation from this set of data is that when the OURs are converted to SURs (figure 6.1.15), the SUR of acetic acid was greater than that of formic acid, even though formic acid gave the higher ΔOUR. This was due to the differences in the yields of the two compounds and demonstrates that caution must be exercised when comparing OURs due to the oxidation of different compounds. A higher OUR does not necessarily mean a higher SUR as commonly assumed.

Curve Fitting

The data set of methanol ΔOURs obtained during the treatment of batch S was chosen for identification of the best model since it is the most complete. For the purposes of curve fitting, all data points were treated as equals. More error is expected at low substrate concentrations, where the OUR has to be calculated over a smaller number of data points, but an examination of the standard deviations of the OUR data did not show any trends (figure 6.1.5). Certain linear transformations of nonlinear models, such as the Monod model, may result in unequal weights being applied to the data. In this study, all coefficients have been calculated using non-linear regression.

The Monod, Powell, Moser, Konak, Tessier, and Blackman models were fitted to the experimental data using nonlinear regression techniques (figures 6.1.16 and 6.1.17) and the calculated coefficients are listed in table 6.1. Of the models tested, Monod is the most commonly used in the respirometry literature for non-inhibitory microbial kinetics. Visual inspection of figure 6.1.16 shows that the Monod model does not saturate quickly enough with increasing substrate concentration to adequately describe the data.
Figure 6.1.14 Oxygen consumption vs. substrate, formic acid (▲), acetate (●), and methanol (■). MLVSS 1810 mg/l

Figure 6.1.15 SUR vs substrate, formic acid (▲), acetate (●), and methanol (■). MLVSS 1810 mg/l
Figure 6.1.16 ΔOUR vs. methanol concentration (○), with Monod (—), Powell (—), and Blackman (—), curve fits. MLVSS 3755 mg/l.

Figure 6.1.17 ΔOUR vs. methanol concentration (○), with Monod (—), Tessier (—), Konak (—), and Moser (—), curve fits. MLVSS 3755 mg/l.
Examination of kinetic data in the literature reveals that this phenomenon is not limited to this study, though the Monod model has been assumed in all of these other cases. All of the equations discussed share a common parameter, the maximum ΔOUR, which is the theoretical ΔOUR at infinite substrate concentration. It would be nice to be able to measure this theoretical value, but it is impractical to measure the ΔOUR at infinite substrate concentration. In figure 6.1.16, ΔOUR measurements at various high substrate concentrations (S ≫ K) all had approximately the same value. This value, 3.7 mg O₂ / l minute (0.00098 mg O₂ / mg MLVSS minute), may be called the maximum experimental ΔOUR and be used to compare with the maximum ΔOUR parameter calculated using the various models. For example, the Monod model greatly overpredicts the ΔOUR at high methanol concentration. At 50 mg COD / l, the ΔOUR for the Monod equation (0.00122 mg O₂ / mg MLVSS minute) is 25% greater than the experimental maximum ΔOUR. The experimental ΔOUR did not reach the theoretical maximum ΔOUR as predicted by Monod. Powell’s, Moser’s, Konak’s, and Tessier’s equations all predict similar maximum ΔOUR’s, all of which are slightly higher than the experimental maximum OUR (from 4 to 7% higher), but these are more reasonable values than predicted by Monod. The use of Blackman’s equation results in a maximum ΔOUR coefficient which is slightly (7%) lower than the experimental maximum.

At first glance all of the other equations seem to fit the data better than Monod’s model. An examination of the residuals (expected ΔOUR - actual ΔOUR), shown in figure 6.1.18 and 6.1.19, will help to identify which models are the most appropriate. Any systematic departures from the estimated regression equation (because the model is not adequate), or non constant variance can be detected by studying the residuals.
Figure 6.1.18 Residual vs. expected value for the curve fits presented in figure 6.1.16. Monod (■), Powell (●), and Blackman (▲). MLVSS 3755 mg/l.

Figure 6.1.19 Residual vs. expected value for the curve fits presented in figure 6.1.17. Monod (■), Moser (●), Konak (▲), and Tessier (○). MLVSS 3755 mg/l.
In order to more easily compare the residuals obtained from the different curve fits, they were fitted with polynomial equations, and all of the resulting curves are shown in figure 6.1.20. These curves have no theoretical value, they just make it easier to look visually inspect the residuals. Examination of the residuals (figure 6.1.20) shows that the Monod equation, which has only 2 parameters, has the worst fit with an obvious trend in the residuals. The Blackman equation (2 parameters) also has a poor fit, and residuals seem to follow an opposite trend to the residuals obtained from the Monod curve fit. Both are limiting cases of the Powell equation, which appears to give the best fit to the data (no obvious trends in the residuals). If $L = 0$ in Powell's equation, Monod’s equation results, and if $K = 0$ in Powell’s equation, Blackman’s equation results. Both $L$ and $K$ were found to be significant in Powell's equation ($0$) using the t test. Comparison of the models shows that the third coefficient in Powell’s equation is significant. Tessier’s equation, which has only 2 parameters, fits better than Monod’s and Blackman’s, which have only 2 parameters, and Konak’s equation, which has 3 parameters. Moser’s equation (3 parameters) appears to fit the data fairly well, and the third coefficient, $n$, was found to be significant. Another way to compare regression equations is to compare the pooled variances (Himmelblau 1980). According to this method, the Powell equation gives the best fit to the data, followed by Moser’s equation.
Figure 6.1.20 Residual vs. expected values from figures 6.1.18 and 6.1.19. Monod (—), Powell (—), Moser (—), Konak (—), Tessier (—), and Blackman (—). MLVSS 3755 mg/l.
Table 6.1 ΔOUR Parameters

<table>
<thead>
<tr>
<th>Model</th>
<th>Maximum OUR</th>
<th>K</th>
<th>Other parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monod (3.1)</td>
<td>0.00124</td>
<td>0.774</td>
<td>-</td>
</tr>
<tr>
<td>Powell (3.11)</td>
<td>0.00103</td>
<td>0.0917</td>
<td>L = 0.884</td>
</tr>
<tr>
<td>Moser (3.15)</td>
<td>0.00105</td>
<td>0.358</td>
<td>n = 1.595</td>
</tr>
<tr>
<td>Konak (3.17)</td>
<td>0.00105649</td>
<td>0.990044</td>
<td>n = 1.09735</td>
</tr>
<tr>
<td>Tessier (3.16)</td>
<td>0.00104</td>
<td>1.264</td>
<td>-</td>
</tr>
<tr>
<td>Blackman (3.12)</td>
<td>0.000919</td>
<td>-</td>
<td>A = 3.6159</td>
</tr>
<tr>
<td>Experimental</td>
<td>0.000985?</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In order to investigate parameter correlation, the 95% confidence regions for the Monod and Powell parameters were calculated (figures 6.1.21 and 6.1.22). For the Monod equation, OUR\(_{\text{MAX}}\) and \(K_M\) appear to be correlated, which is indicated by the non-circular nature of the confidence region. Since the Powell equation has three parameters, the confidence regions for 2 parameters are shown on the plane of the third parameter held constant at the value obtained during regression. The confidence regions for the Powell parameters are much smaller than the confidence regions for the OUR\(_{\text{MAX}}\) and \(K_M\) obtained using the Monod equation. The confidence regions for the Powell coefficients may be further reduced if equation 6.1 is used for regression instead of equation 3.13 (figure 6.1.22). Equation 6.1 is obtained by solving equation 3.12 for \(\mu\) before substituting \(L=\frac{\mu_{\text{MAX}}}{(Y \cdot F)}\) for \(F\).

\[
\text{OUR} = \frac{1}{2} \left( K \cdot F + \text{OUR}_{\text{MAX}} + F \cdot S - \sqrt{(K \cdot F + \text{OUR}_{\text{MAX}} + F \cdot S)^2 - 4 \text{OUR}_{\text{MAX}} \cdot F \cdot S} \right) \quad (6.1)
\]

All future calculations of the Powell coefficients were done using equation 6.1, and \(F\) was converted to \(L\) after nonlinear regression. The Powell parameters are also correlated, in particular the parameters \(L\) and \(K\), which is expected since \(L\) is a modifier of \(K\). The Powell half saturation value is equal to \(K+L/2\). \(K\) still has some dependence on the OUR\(_{\text{MAX}}\), but not as much as with Monod’s equation.
Figure 6.1.21 Monod parameters 95% confidence intervals.
Figure 6.1.22 Powell parameters 95% confidence intervals. Outer ellipse (and dashed ellipse in figure 6.1.22c) calculated with equation 3.11; inner ellipse calculated with equation 6.1.
The confidence intervals for the Monod and Powell equations are shown in figure 6.1.23. As expected from the above discussion, the Powell equation has much smaller confidence intervals and approximated the data much better than the Monod equation. At high substrate concentrations, the experimental data do not even fall between the Monod 95% confidence intervals.

The standardised residuals may be used to investigate if the microbial activity is shifting with time. The kinetic assays usually take a few hours per batch of biomass, and may take up to 8 hours if many data points are obtained (as in the data shown in figures 6.1.3 and 6.1.4). During this time period, the biomass is exposed to different environmental conditions than the conditions in the continuous activated sludge unit, from which the biomass was obtained. In particular, the only BOD source is methanol, and the BOD is added discontinuously (once per data point), so that the biomass experiences short bursts of high substrate concentrations, followed by short periods of starvation. There is a possibility that the biomass will adapt to these new environmental conditions, and the OUR response will change over the course of the experiment. Figure 6.1.24 shows the standardised residuals for both the Monod and Powell correlations versus the time that the data point was collected for the experiment shown in figure 6.1.3. The Monod data shows runs in the residuals, probably due to the correlation among the Monod residuals. There is no noticeable trend with time in the residuals from the Powell correlation. The assumption that the biomass does not adapt over the course of the experiment is justified when the Powell equation is used.

The Monod and Powell equations were fitted to acetate oxidation data (figure 6.1.25) and formate oxidation data (figure 6.1.26). For acetate, the Powell equation does
Figure 6.1.23 Methanol ΔOUR (○) vs substrate, Monod (—), and Powell (—), curve fits with 95% confidence intervals (dashed lines). MLVSS 3755 mg/l.

Figure 6.1.24 Residual from Monod curve fit (□), and from Powell curve fit (○) vs. experiment # (each experiment was approximately 2 minutes apart). MLVSS 3755 mg/l.
not give a better fit than the Monod equation (for the data set shown, although the Powell equation resulted in better fits when other data sets were analysed). For formate, the Powell equation gives the best fit. For other formate respirometric data sets, the Monod equation gave the best fit to the data.

BKME is a mixture of methanol, formic acid, acetate, and other compounds. Both the Monod and Powell equations have been developed for application to growth on single substrates. \(\Delta\text{OUR}\) tests were done on a mixture of these compounds to test the applicability of the Powell and Monod models to respirometric data obtained using multicomponent substrate mixtures. \(\Delta\text{OUR}\) curves for methanol - acetate - formic acid mixtures obtained using different biomass samples are presented in figure 6.1.27. For three of the curves, the Monod model gave a better fit than the Powell model. A possible explanation is that these curves were obtained at intermediate MLVSS concentrations (see next section). Another explanation is the presence of formic acid, which often fits the Monod model better than the Powell model. It has been empirically observed that if the respirometric kinetics of one substrate in the mixture are best fit with the Monod model, then the respirometric kinetics of the mixture will also be best fit with the Monod model.

\(\Delta\text{OUR}\) curves for two different samples of BKME also are more closely approximated by the Monod equation (figure 6.1.28). It is very difficult to get a complete \(\Delta\text{OUR}\) curve for untreated wastewater, as the concentrations of the various substrates in effluent are fairly low. Very large injection volumes (up to one half of the volume of the respirometer) are required to approach the maximum OUR. Injections of this size into the respirometer result in a large loss of biomass, which affects the respirometric results.
**Figure 6.1.25** ΔOUR vs. acetate concentration (●), with Monod (solid), and Powell (dotted) curve fits. MLVSS 1810 mg/l.

**Figure 6.1.26** ΔOUR vs. formic acid concentration (▲), with Monod (solid), and Powell (dotted) curve fits. MLVSS 1810 mg/l.
Figure 6.1.27 ΔOUR vs substrate for formic acid/methanol/acetate mixtures. MLVSS values as shown on chart.

Figure 6.1.28 ΔOUR vs substrate for BKME. MLVSS concentrations. 1644 mg/l (■), 1858 mg/l (●).
On a few occasions, sludge was concentrated by centrifugation, then diluted to the original MLVSS concentration with a large volume of fresh effluent to obtain data points at high effluent concentrations. The results obtained with this method are presented in figure 6.1.28. Similar to the methanol - acetate - formic acid mixtures, the oxidation kinetics of effluent were found to follow Monod kinetics. This may be due to the relatively low MLVSS concentrations used for these experiments, as well as to the large number of dilute substrates present in BKME.

In summary, the Powell equation was found to give the best fit to the individual substrate data. As discussed previously, the Powell equation is derived by assuming two or more reactions in series. If just one reaction is dominating, then the Monod equation will result, which was found for certain acetate and formic acid data sets. With substrate mixtures, the Monod equation has been found to give the best representation of the data, and will be considered for future data analysis due to its widespread acceptance in the literature.

Effect of Biomass Concentration

Respirometric data collected using various biomass concentrations with methanol as the substrate are shown in figures 6.1.29 and 6.1.30. Theoretically, the biomass concentration will not have an effect on the Monod parameters, which may be calculated using equation 6.2 if q is expressed on a volumetric basis.

\[ q = q_{\text{MAX}} X \frac{S}{K_m + S} \quad (6.2) \]

where q is the total ΔOUR, and not the specific ΔOUR.
Figure 6.1.29 Methanol ΔOUR vs. substrate, using various MLVSS concentrations. 4440 mg/l (■), 2965 mg/l (▲), 1535 mg/l (●), 755 mg/l (○). The solid curve fits are made with Powell's equation, the dotted curve fits are made with Monod's equation.

Figure 6.1.30a Methanol ΔOUR vs. substrate, using various MLVSS concentrations. 4150 mg/l (▲), 2735 mg/l (●), 1505 mg/l (■), 730 mg/l (○). The curve fits are made with Powell's equation.
The Monod parameters were calculated for the data shown in figure 6.1.29, using all of the data points (curves shown), and separately for each MLVSS concentration (curves not shown). When the parameters were calculated for each biomass level, the specific maximum ΔOUR was constant, regardless of the biomass concentration (figure 6.1.31). However, the half saturation constant was directly proportional to the solids concentration (figure 6.1.31). This is verified by examining the 95% confidence regions for the Monod parameters which show a distinct trend in the half saturation constant (figure 6.1.32). Ideally, the 95% confidence regions for the four data sets would be superimposed, since the same activated sludge was used for the collection of all four sets of data. The 95% confidence regions for the entire data set (dashed ellipse) should be within the regions for the four data subsets, which is clearly not the case.

A consequence of mass transfer effects is an apparent increase in the half saturation constant. To see if the increase in $K_M$ was due to mass transfer effects, the ability of the Powell equation to fit the same data set was investigated. The Powell equation was derived by assuming two reactions in series. At high substrate concentrations, the reaction rate is limited by the number of available enzymes, and the volumetric rate should be directly proportional to the active biomass concentration. At low substrate concentrations, the rate limiting reaction may be either a metabolic reaction or a mass transfer step. If this second rate limiting reaction is a metabolic reaction, then the volumetric rate will be proportional to the biomass concentration (assuming a constant substrate concentration). If the second rate limiting reaction is a mass transfer step, the volumetric rate will also be proportional to the biomass concentration, assuming that changes in biomass concentration are brought about by changes in the number of
Figure 6.1.30b  Methanol ∆OUR vs. substrate, using various MLVSS concentrations.  
4150 mg/l (△), 2735 mg/l (○), 1505 mg/l (■), 730 mg/l (●). The curve fits are made with Monod’s equation.

Figure 6.1.31  Half saturation constant (Monod) vs. MLVSS concentration. Data from  
figure 6.1.28 (■), and figure 6.1.27 (○). Maximum specific ∆OUR, from figure  
6.1.28 (○) and figure 6.1.27 (△).
Figure 6.1.32 Monod parameters 95% confidence intervals, calculated for each MLVSS level (solid circles), and using all of the data (dashed circle).
flocs, and that biomass properties are independent of concentration. In either case, all three kinetic parameters of the Powell equation should be independent of the biomass concentration, when the specific rate is related to the substrate concentration.

Fitting the Powell equation (6.1) to the complete data set, and separately to the four MLVSS subsets, the OUR$_{MAX}$ was found to be a constant regardless of the biomass concentration (figures 6.1.29 and 6.1.30a). Similar to the Monod equation, L, and K, were found to be directly proportional to the MLVSS. (figure 6.1.33). The Powell coefficients, K and L, are closely related to the Monod half saturation constant $K_M$ and appear to follow the same relationship with MLVSS as $K_M$. The Powell equation, similar to the Monod equation, is not suitable for determining the parameters at one MLVSS level, and applying those parameters to predict the results obtained at a different MLVSS concentration.

The Powell equation may be modified to account for the dependence of the coefficients on the biomass concentration if the rate limiting mass transfer step is independent of the biomass concentration (the rate is independent, not the parameter). This situation will arise when the change in biomass is due to a change in floc size and not a change in floc concentration, or if the rate limiting mass transfer step is inside the DO probe and not in the floc or bulk solution. As the biomass changes, the metabolic reaction will change in direct proportion, but the mass transfer rate will remain constant (for a constant substrate concentration). In an equation relating the specific metabolic rate to the substrate concentration, this will have the same effect as making the mass transfer coefficient a function of the biomass concentration. For this scenario, equations 3.11 and 6.1 become:
Figure 6.1.33 Powell kinetic coefficients vs. biomass concentration L (■), K (○), and maximum AOUR (▲).
\[ \mu = \frac{\mu_{\text{MAX}}(K + L_x X + S)}{2L_x} \left[ 1 - \sqrt{1 - \frac{4L_x X S}{(K + L_x X + S)^2}} \right] \] (6.3)

\[ \mu = \frac{1}{2} \left( K F_X + \text{OUR}_{\text{MAX}} X + F_X S - \sqrt{(K F_X + \text{OUR}_{\text{MAX}} X + F_X S)^2 - 4\text{OUR}_{\text{MAX}} X F_X S} \right) \] (6.4)

where L and F have been replaced with L_x and F_x, to demonstrate the dependence of these parameters on the biomass concentration.

Using the modified Powell equation, OUR_{\text{MAX}} and L_x were found to be constant regardless of biomass concentration. This equation gave a better fit to the data than the Monod equation. This was verified by looking at the confidence regions of the parameters for the data set shown in figure 6.1.29. The confidence regions are much smaller for the Powell parameters (figure 6.1.34) than for the Monod parameters (figure 6.1.32). The confidence regions of the modified Powell parameters for the four data subsets are not superimposed, but are scattered about the confidence region for the complete data set with no obvious trends in the parameters.

It must be emphasised that the modified Powell equation is an empirical one, the good fit of the equation to the data does not mean that the assumptions behind the equation are valid. The assumptions made do not make sense for the circumstances used to collect this set of data, but may explain observations in the literature where K_M was found to a function of biomass (see section 3.2, alternatives to Monod). If the change in biomass concentration is due to changing operating conditions, such as the SRT, then the biomass properties will be changing as well, making the mass transfer coefficient a function of biomass concentration. In this study, the biomass properties can be expected to remain constant since all of the data were collected using the same set of sludge. An
Figure 6.1.34 Modified Powell parameters 95% confidence intervals, calculated for each MLVSS level (solid circles), and using all of the data (dashed circles).
alternate explanation for the observed dependence of the kinetic coefficients on the
biomass concentration, which does not require the biomass properties to be a function of
biomass concentration, will be outlined in the following sub-section (verification of
ΔOUR model).

Figure 6.1.35 shows curves calculated using external mass transfer and the
Monod equation (equation 3.28), and external/internal diffusion with the Monod equation
(equation 3.30). The curves were calculated using 2 point collocation techniques. When
diffusion through the bulk solution was ignored, the internal diffusion model did not
approximate the data as well as when external diffusion was included in the model (not
shown). This may be due to the extra parameter when external diffusion is added to the
model. In both cases, the internal diffusion mass transfer coefficient was found to be a
linear function of the biomass concentration. The mass transfer coefficients, similar to
the Monod and Powell coefficients, are supposed to be independent of the biomass
concentration. The dependence of the mass transfer coefficient on the biomass
concentration indicates that equations 3.28 and 3.30 are unsuitable for modeling
respirometric data.

Convective mass transfer into the floc is assumed to be negligible in equations
3.28 and 3.30. This is a common assumption in modeling mass transfer effects in the
activated sludge process. Recent observations indicate that the flocs are more porous
than previously thought, and that the flocs do not contain dead centers as predicted by the
diffusion hypothesis. Assuming that the main mass transfer resistance is due to
convective mass transfer, instead of diffusion, results in the same overall mass transfer
effects as if diffusion is assumed to be the rate limiting step. From the data obtained, it is
Figure 6.1.35 Methanol ΔOUR vs. substrate, using various MLVSS concentrations: 4440 mg/l (•), 2965 mg/l (▲), 1535 mg/l (■), 755 mg/l (○). The solid curve fits are made with Monod's model assuming external mass transfer resistance, the dotted curve fits are made assuming internal and external mass transfer resistance.
difficult to differentiate between the two processes, and both are likely to be important. The situation is further complicated by the heterogeneous nature of the activated sludge flocs, which will result in variable diffusivity.

The modified Powell equation fits the data better than either of the diffusion scenarios. All equations default to the Monod equation when mass transfer becomes non-rate limiting. In the modified Powell equation, if the mass transfer resistance is small, then the equation defaults to the Monod equation with a half saturation constant equal to \( K^+ + \frac{LX}{2} \). This is a function of the biomass concentration, and for the activated sludge used in this study, MLVSS levels below approximately 1000 mg/l resulted in the Monod model giving as good a fit as the Powell model. Above 1000 mg MLVSS/l, the Powell equation usually gave a better fit. Even at low biomass concentrations where the data are fit by the Monod model, \( K \) will still appear to be a function of the biomass concentration.

Respirometric data using acetate as the substrate were collected at several biomass concentrations (figure 6.1.36). The modified Powell equation gave a slightly better fit to the data than the Monod equation. The Monod half saturation constant was found to be a function of the biomass concentration, as when methanol was used as the substrate. The modified Powell coefficients were found to be constant regardless of the biomass concentration.

Respirometric data using formic acid as the substrate, at various biomass concentrations, is presented in figure 6.1.37. For this data, the Powell equation did not give a better fit than the Monod equation, and neither seemed to really fit the data. Neither model accounted for the large increase in \( \Delta \text{OUR} \) observed at high substrate concentrations.
Figure 6.1.36  Acetate ΔOUR vs. substrate, using various MLVSS concentrations: 5060 mg/l (▲), 3560 mg/l (○), 1920 mg/l (■). The solid curve fits are made with Powell’s equation, the dotted curve fits are made with Monod’s equation.

Figure 6.1.37  Formic acid ΔOUR vs. substrate, using various MLVSS concentrations: 5060 mg/l (▲), 3560 mg/l (○), 1920 mg/l (■). The curve fits made with Powell’s equation and with Monod’s equation fell on the same line.
Verification of Models

The Powell equation fit the respirometric data, but the values of L and K were functions of the biomass concentration. One of the major assumptions made in the analysis of respirometric data is that of pseudo steady state. Pseudo steady state implies that as soon as the substrate is added to the respirometer, the bacteria start metabolising the added substrate at a rate equivalent to the steady state rate corresponding to the initial substrate concentration. This immediate change in metabolism causes an immediate change in the oxygen uptake rate which is measured by the DO probe. Under these assumptions, the maximum ΔOUR achieved corresponds to the metabolic rate which corresponds to the added substrate concentration. This assumption may seem less reasonable if the actual process is investigated in more detail. Upon addition of substrate, the substrate mixes throughout the respirometer and diffuses through the bulk solution to the floc. It is possible that the equilibrium constant favors substrate adsorption onto the floc. The substrate must then diffuse into the floc to the bacteria. The next step is transport of the substrate across the cell membrane (except when methanol is the substrate, which is oxidised in the cytoplasm). The substrate is metabolised, a process which involves the transfer of electrons from the substrate to the electron transfer chain. In the case of aerated activated sludge, the terminal electron acceptor is oxygen. The oxygen utilisation rate in the bacteria will increase above the rate during endogenous metabolism. This will cause oxygen from the floc to enter into the bacteria at a greater rate, lowering the oxygen concentration in the floc, causing the oxygen from the bulk solution to diffuse into the floc at a greater rate. Finally, it is the decrease in the bulk oxygen concentration which is measured by the DO probe. In summary, the OUR will
only increase once substrate dissolved oxygen concentration in the floc starts to decrease, which happens after the substrate has diffused into the floc and has been metabolised.

**Low Substrate Concentrations**

The unsteady state nature of the ΔOUR assay can explain the dependence of the parameters \( K_M \) (Monod equation) and \( L \) and \( K \) (Powell equation) on the biomass concentration, if oxygen diffusion is rate limiting. The following discussion assumes that the substrate concentrations is such that the ΔOUR is below the maximum, in the first order region of the ΔOUR vs. substrate curve. If the number of flocs is doubled, the substrate will disappear from the bulk solution twice as fast but each floc will only see half as much substrate (a finite amount of substrate added, evenly distributed to all of the flocs). Consequently, the amount of oxygen consumed per floc will decrease by half, and the driving force for oxygen diffusion from the bulk liquid to the floc will decrease by approximately half. This will result in a decrease in the specific oxygen consumption rate. Since the number of flocs is double, the overall volumetric rate will be very similar to the overall volumetric rate before the number of flocs was doubled.

Different results would be obtained if the substrate concentration in the bulk solution was kept constant. Then, once steady state was attained, the overall rate would be directly proportional to the biomass concentration. In the ΔOUR assay, substrate is added as a pulse, and is removed from the bulk solution before steady state is attained.

This hypothesis is supported by examination of the OUR profiles during the ΔOUR test (figure 6.1.38). The initial OUR following methanol addition was the same regardless of the biomass concentration. As the biomass concentration increased, the maximum volumetric OUR increased, but the rate at which the OUR increased following
Figure 6.1.38 Methanol injections, various methanol concentration. MLVSS 1535 mg/l (---), and MLVSS 4440 mg/l (----).
substrate addition was independent of biomass concentration. The response time of the
\( \Delta \text{OUR} \) assay is mass transfer limited.

This provides insight into the success of the modified Powell equation in fitting
batch test respirometric data. The mass transfer parameter, \( L_X \) or \( F_X \), is probably related
to the oxygen diffusivity, and not the substrate diffusivity, when methanol is used as a
substrate. This observation may not hold for other, larger, substrates. It is the unsteady
state nature of the respirometric method that results in \( L_X \) being a function of the biomass
concentration.

A study using a fluorometer found that the rate limiting step of glucose utilisation
by bakers' yeast was the substrate permease. The mixing time for the glucose to enter the
cells was about four and a half seconds (Einsele et al 1978). This result agrees with the
hypothesis that substrate metabolism will not reach it's maximum rate immediately upon
substrate addition into the bulk liquid.

Given the above discussion, the modified Powell equation may be used to model
respirometric data. The Monod equation may also be modified to account for the
increase in the half-saturation constant with biomass.

\[
q = q_{\text{max}} X \frac{S}{K_X + S}
\]  

(6.5)

If the unmodified Monod equation is used, \( K \) will be overestimated at high biomass
concentrations. If the equation is applied to predict steady state behaviour of activated
sludge plants, the SUR will be underpredicted. Many other factors affect the \( K \) value
(discussed in chapter 7), so the assumption of pseudo-steady state will not result in too
much error when dealing with complex wastewaters.
This discussion supports the hypothesis made earlier that the yield appears to increase at low substrate concentrations due to a small reserve of dissolved oxygen in the floc. The mass of oxygen in the floc appears to depend mainly upon the bulk dissolved oxygen concentration, the oxygen diffusivity, and the floc size.

**High Substrate Concentrations**

When the substrate concentration is high enough to result in the maximum metabolic rate (zero order region) the assumption of pseudo steady state becomes valid. Upon addition of substrate, the OUR will increase until the maximum rate is reached. The OUR will stay at the maximum rate as long as there is enough substrate. Since the rate follows the zero order model at this point (therefore not a function of substrate concentration), the initial concentration is not of critical importance. If the initial concentration were kept constant throughout the test, the rate would be the same as measured with the ΔOUR test.

The maximum SUR calculated from respirometry may be verified using a batch test experiment with the measurement of substrate and OUR. An example of such a batch test is presented in figure 6.1.39. The measured SUR is 0.42 mg BOD/l minute, the SUR calculated form ΔOUR tests performed with the same set of biomass is 0.40 mg BOD/l minute. The ΔOUR method gives a reasonable approximation of the actual SUR, but is much simpler and takes significantly less time. The yield increased as the batch test progressed (figure 6.1.40). Initially, the yield may have been low due to the sudden shock the biomass experienced when the methanol was added. This may have resulted in energy spilling. As the biomass adapted to the new conditions, less energy was spilled, and the yield increased.
**Figure 6.1.39** Batch test, methanol BOD (■) and OUR (○) vs time. Methanol was added at time 0. MLVSS: 2000 mg/l

**Figure 6.1.40** Yield (1-OUR/SUR) calculated for batch test data in figure 6.4.39.
Using a different set of biomass, the batch test was repeated, once using methanol and once using acetate as substrates (figure 6.1.41). For methanol, the measured SUR was 2.2 mg COD/l minute, compared to 2.5 mg COD/l minute calculated by the ΔOUR method. The yield on methanol increased by the end of the batch test, similar to the previous example. For acetate, the measure SUR was 1.3 mg COD/l minute, compared to 1.3 mg COD/l minute calculated by the ΔOUR method. The ΔOUR method gives comparable results to the batch test method. The slight discrepancies are due to the error involved in measuring substrate (the BOD and COD test), and the higher f/m of the batch test. If the SUR were calculated from the OUR over the course of the batch test, the SUR of methanol would appear to decrease. The COD and BOD measurements indicate that the SUR was constant, and that the yield must have been changing.

A standard method for measuring the maximum growth rate is to use a batch test with a high f/m ratio. The maximum growth rate is directly related to the exponential increase in the OUR with time. The maximum growth rate of the activated sludge used in this project was measured on methanol and acetate. There was a long lag phase before the biomass started to grow, and then the growth rate was much higher than predicted by respirometry (figure 6.1.42). The growth calculated for methanol was 0.5 /hour, compared to 0.1 /hour predicted by the ΔOUR assay. The growth rate calculated for acetate was 0.8 /hour, compared to 0.06 predicted by the ΔOUR assay. The probable explanation for the large growth rate is the selection of fast growing microorganisms by the batch test. These organisms were initially present in very small quantities, which explains the long lag phase. In a separate experiment, the maximum growth rate as measured by the ΔOUR assay was estimated before and after a high f/m batch test. The
Figure 6.1.41 COD removal and OUR during two batch tests. Substrate added at time 0. Methanol COD (●) and OUR (---). Acetate COD (△) and OUR (— —). MLVSS: 3100 mg/l.

Figure 6.1.42 OUR vs. time during a batch growth test. Methanol (■), acetate (○ , □), acetate and methanol mixture (△ , ●).
growth rate of the bacteria at the end of the batch test was an order of magnitude greater than the growth rate before the batch test. The high f/m batch test does not appear to be appropriate for the measurement of activated sludge kinetics for the treatment of BKME.

Summary

Respirometric data for methanol, formic acid, and acetate were presented. These substrates were chosen as representative substrates of BKME. By comparing the ΔOUR profiles of these substrates against that of BKME, it is evident that these substrates compose the bulk of the readily biodegradable substrate in BKME, although many other substrates are present as well. Most of the other substrates are other carboxylic acids. When respirometry was performed with sugars (various sugars were used, including xylose), no response was obtained, which implies that these were not readily utilisable substrates. See chapter 7 for further details on the composition of BKME.

The relationship between the OUR and the SUR is dependent on the yield, which is different for each substrate. It is important to note that there is a higher OUR during formic acid metabolism than during acetate metabolism despite the greater removal rate for acetate. This is due to the larger yield on acetate.

The respirometric method appears to be a valid surrogate for measuring the actual SUR. The modified Powell equation was found to give a better fit to the data than the Monod equation. This is hypothesised to be due to the unsteady state nature of the assay. If this is true, care must be taken in applying the batch kinetic constants to continuous systems operating at steady state, particularly K and / or L. The yield, OUR, and SUR calculated at high substrate concentrations are close to the steady state values. This will allow the use of batch test data to calculate the zero order kinetic coefficients. Given the
small saturation coefficients found for methanol, formic acid, and acetate, an assumption of zero order kinetics in dealing with the continuous system should not introduce too much error.

**6.2 Effect of DO Concentration**

The dissolved oxygen concentration may be expected to have a large effect on the oxygen uptake rates. Conventional wisdom states that as long as the DO is above 2 mg/l, it will not affect the metabolic rate of the bacteria, but if the DO falls below this value, then the kinetics will start to become oxygen limited. For the majority of kinetic tests in this study, the DO concentration was kept above 2 mg/l. For a few tests, the DO concentration was allowed to fall to 0 mg/l while the substrate was present in excess (as in figure 6.2.1). For this set of data, it appears that as long as the DO is above 0.5 mg/l, there is little effect on the OUR. Below 0.5 mg/l, the OUR decreases rapidly. The dependence of OUR on the DO concentration appears to be similar to the dependence of the ΔOUR on the substrate concentration. If Monod model is assumed for the dependence of the OUR on DO, the following equation is obtained by using equations 4.2, and 3.31:

\[
OUR = \left( OUR_{baseline} + \Delta OUR_{MAX} \frac{S}{K_M + S} \right) \frac{DO}{K_{DO} + DO}
\]  

(6.6)

Similarly, if Powell kinetics are assumed, equation 6.7 results

\[
OUR = \left( OUR_{baseline} + \frac{1}{2} \left( KF + OUR_{MAX} + FS - \sqrt{(KF + OUR_{MAX} + FS)^2 - 4OUR_{MAX} \cdot FS} \right) \right) \frac{DO}{K_{DO} + DO}
\]  

(6.7)

In figure 6.2.2, the results of two different injections are plotted. Both sets of data were obtained at excess substrate concentration, and the OUR has been divided by
(OUR\textsubscript{baseline}+\Delta\text{OUR\textsubscript{MAX}}) for easier comparison (at high substrate concentrations, the term in brackets in equations 6.6 and 6.7 is equal to (OUR\textsubscript{baseline}+\Delta\text{OUR\textsubscript{MAX}})). For one of the data sets, the OUR appeared to follow Powell kinetics with respect to the DO, while for the other set, the OUR followed Monod kinetics with respect to the DO. The main experimental difference between the two data sets is the MLVSS concentration. These results agree with those discussed in section 6.1, where it was found that the \Delta\text{OUR} followed Monod kinetics at low MLVSS, and Powell kinetics at high MLVSS.

It was hypothesised in section 6.1 that the mass transfer resistance was due to oxygen diffusion and not substrate diffusion (when methanol is the substrate). The coefficient L in the Powell equation is related to the mass transfer resistance. Typical values of L for methanol respirometric data were in the range 0.16 to 0.2 mg/l. Typical values of L for acetate respirometric data were in the range 0.12 to 0.2 mg/l. Values of L for the curves shown in figure 6.2.1 (the relationship between OUR and DO obtained using both methanol and acetate) range from 0.14 to 0.22. The similarity of these three sets of numbers implies that in all three cases the mass transfer resistances are similar. This implies that the main mass transfer resistance is related to the oxygen diffusion into the floc, and not the substrate diffusion. This situation may change when larger substrates are used, but the biomass in this study did not readily metabolise larger substrates (such as carbohydrates), and this was not investigated.

The model constants for DO obtained from the curves in figure 6.2.2, along with the \Delta\text{OUR} model constants for the substrate (methanol), were used to generate figures 6.2.3 and 6.2.4. For simplicity, OUR\textsubscript{baseline} was assumed to be 0. The standard method for measuring the OUR is to place a sample of mixed liquor into a BOD bottle, insert the
Figure 6.2.1 OUR vs DO measured with an excess of substrate. Methanol, MLVSS 2965 mg/l (■); methanol, MLVSS 4440 mg/l (□); acetate, MLVSS 3105 mg/l (▲); acetate, MLVSS 5380 mg/l (△); acetate, MLVSS 1920 mg/l (○); acetate, MLVSS 3560 mg/l (●); acetate, MLVSS 5060 mg/l (▲).

Figure 6.2.2 OUR vs DO measured with an excess of methanol. MLVSS 4440 mg/l (■), MLVSS 1505 mg/l (○).
DO probe and measure the decrease in DO for 15 minutes. Assuming the Powell model for both substrate and oxygen, and assuming that the substrate concentration remains constant, as the DO decreases, the OUR will remain approximately constant until the DO drops below 0.5 mg/l (figure 6.2.4). If the Monod model is assumed, and the substrate concentration is low, the OUR will also be approximately constant as the DO decreases. However, if the substrate concentration is high, the OUR will decrease steadily as the DO drops from 6 to 2 mg/l, and drop even faster below 2 mg/l (figure 6.2.3). If the OUR is a function of the DO, it becomes difficult to measure.

In order to verify this hypothesis, the OUR was measured while feeding methanol continuously to the respirometer at low solids concentration (figure 6.2.5). Under these conditions, the methanol concentration should be approximately constant in the respirometer. The slope of the OUR vs. DO curve was calculated for each methanol concentration and is shown in figure 6.2.6. At low methanol concentrations, the OUR was approximately constant with DO, the slope was 0.01 / minute, compared to 0.07 at high methanol concentrations. As the catabolic rate increased, the dependence of OUR on the DO increased as predicted by figure 6.2.3.

Further verification of the dependence of the OUR on DO was obtained from replicate ΔOUR experiments at different DO concentrations. For small injections, there was no noticeable trend in the ΔOUR as the DO decreased (figure 6.2.7). For larger injections, the ΔOUR decreased slightly as the DO decreased, as expected (figure 6.2.8). The OUR decreased during the injection, and then was slightly lower for the second injection (at a lowered initial DO) during which it also decreased. The only difference between the two injections was the DO concentration. A similar experiment, with a
Figure 6.2.3 ΔOUR vs. DO and substrate based on Monod kinetics for both substrate and DO.

Figure 6.2.4 ΔOUR vs. DO and substrate based on Powell kinetics for both substrate and DO.
Figure 6.2.5 DO vs. time under different continuous methanol feed rates.

Figure 6.2.6 Based on the data in figure 6.2.5. OUR calculated at 2 minutes (■), OUR calculated at 5 minutes (●), Slope of OUR vs. DO plot (▲). MLVSS 1370 mg/l.
Figure 6.2.7 OUR vs. DO for replicate methanol injections, average maximum OUR is graphed with 95% CI. MLVSS 1400 mg/l.

Figure 6.2.8 OUR vs. DO for replicate methanol injections. MLVSS 1400 mg/l.
larger MLVSS concentration is shown in figure 6.2.9. There was no noticeable effect as the DO decreased. This is expected since the kinetics are now described by Powell’s equation. A final example is presented in figure 6.2.10. A very large injection was added to the respirometer and when the DO dropped below 1 mg/l, the DO probe was removed, the mixed liquor aerated, and then the DO probe was replaced. This was repeated until the OUR dropped back to its pre-injection value. The OUR decreased with DO, and the pattern was the same each time the mixed liquor was aerated. This verifies that the decrease in OUR is due to the DO level and not the substrate concentration, as the substrate concentration would be continuously decreasing throughout the test. (Each line in figure 6.2.10 would be lower than the previous one if the decrease in OUR was due to substrate instead of DO).

It appears from the data presented in this section that the dependence of activated sludge kinetics on the DO follows the Powell model. As the diffusional resistance becomes negligible compared to the reaction resistance, which happens at low solids concentrations, the Powell equation simplifies to the Monod equation. Under these conditions, the OUR becomes a function of the DO even at DO values greater than 2 mg/l, which contradicts most assumptions found in the literature. The dependence of the OUR on substrate and oxygen will follow the same form (low MLVSS - both can be modeled with Monod, high MLVSS - both can be modeled with Powell).

6.3 Multi-Substrate Wastewaters

When dealing with wastewaters, the actual composition, sometimes even the major organic source, is often unknown. The biodegradable organic compounds are often treated as a single substrate. In order to apply respirometry to the determination of the
Figure 6.2.9 OUR vs. DO for replicate methanol injections. MLVSS 3750 mg/l.

Figure 6.2.10 OUR vs. DO for one large methanol injection, with re-aeration when the DO reached 1 mg/l. After initial injection (——), after 1st re-aeration (——), after 2nd re-aeration (——), after 3rd re-aeration (——), and after 4th re-aeration (——). MLVSS 2800 mg/l.
kinetics of BKME biodegradation, it is necessary to know the effects that unknown
substrate mixtures have on data interpretation. During the utilisation of multiple
substrates, the metabolism of each substrate will exert an oxygen demand. Since oxygen
is a global parameter, it is difficult to differentiate the oxygen uptake due to the
individual compounds. It is also difficult to differentiate the amount of oxygen consumed
to metabolise each of the individual compounds, and hence difficult to calculate the
individual yields. These effects conspire to make the interpretation of respirometric data
obtained using substrate mixtures difficult.

The discussion in this section will focus on determining the SUR when all
compounds of the mixture are present, i.e. at the start of the batch test. For any given
mixture of substrates, it is likely that the time for complete removal of each substrate will
be different, depending on the initial concentrations and the individual removal rates.
The composition of the mixture will change as the batch test progresses. If the substrates
are removed simultaneously, then the SUR will be high when all of the substrates are
present. As substrates are removed from the mixture, the SUR will decrease as discussed
in the section on n-order kinetics, section 3.3. For a two substrate mixture, initially the
SUR will be equal to the sum of the SUR for the two compounds. When one substrate is
completely removed, the SUR will be equal to the SUR of the remaining substrate. The
best way to deal with this problem is to model the process as a mixture of independent
substrates and calculate the model parameters and stoichiometry for each substrate. This
will be addressed in chapter 7. In this section, the possible error in estimating the
removal rates based upon respirometry and the assumption that the mixture behaves as a
Figure 6.3.1 OUR vs time profile following an injection of two substrates which are used simultaneously.
single substrate is examined. In either case, the possible substrate interactions must still be studied experimentally.

The calculation of the SUR depends on the OUR and the yield. If the yields of the various substrates in a mixture are the same, the calculated yield (equation 4.6) will be the same as the actual yield, and the SUR calculated from the OUR will be correct. If the yields of the various compounds differ, then the yield will appear to change throughout the batch test as substrates are removed. The appropriate yield must be used to convert the OUR to a SUR at each point in the batch test. A further complication is that when more than one substrate is present, the calculated yield will be a weighted average of the yields of the individual substrates, and the calculated SURs may be in error. Consider the case demonstrated in figure 6.3.1. If the two compounds are used simultaneously, the actual SUR is:

\[ SUR_{actual} = SUR_1 + SUR_2 = \frac{OUR_1}{OC_1/S_1} + \frac{OUR_2}{OC_2/S_2} \]

Since the total OUR and the total oxygen consumption are measured, and not OUR_1, OUR_2, OC_1 or OC_2, the measured SUR will be (assuming that the individual OUR's and OC's are additive):

\[ SUR_{measured} = \frac{OUR_{total}}{OC_{total}/S} = \frac{OUR_1 + OUR_2}{(OC_1 + OC_2)/(S_1 + S_2)} \]

\[ (6.9) \]

SUR_{measured} will only be equal to SUR_{actual} if the yields (and thus OC/S) of the two compounds are equal.

Figure 6.3.2 further demonstrates the error introduced by the average yield. We shall assume that there are two substrates, A and B. The yield on substrate A is 0.2 and the yield on substrate B is 0.8. This is an extreme difference, but could be illustrative of
Figure 6.3.2 Actual SUR (horizontal lines), measured Y (weighted average of actual yields,  
---), and SUR calculated using measured yield (-----) for different ratios of a two  
substrate mixture (Y1 = 0.2, Y2 = 0.8, and OUR total = 1).

Figure 6.3.3 Actual SUR (horizontal lines), measured Y (weighted average of actual yields,  
---), and SUR calculated using measured yield (-----) for different ratios of a two  
substrate mixture (Y1 = 0.4, Y2 = 0.6, and OUR total = 1).
a methanol and acetic acid mixture. When both substrates are present in excess, the OUR (and SUR) due to each compound will be at its maximum, and the total OUR (and total SUR) is not a function of the mixture composition. If simultaneous substrate metabolism is assumed, the total OUR will be equal to the sum of the OUR_max's of the individual substrates. The same is true for the actual SUR. However, if the data is analysed assuming there is only one substrate present, then the calculated yield will depend on the wastewater composition, as shown in figure 6.3.2. This is due to the fact that the calculated yield is a weighted average of the individual substrate yields. Therefore, the calculated SUR (which is calculated using the calculated yield) will also vary as the composition of the mixture varies, whereas the actual SUR is independent of the mixture composition. Figure 6.3.2 shows that when there is more of substrate A (Y = 0.2), the SUR is likely to be underpredicted, and when there is more of substrate B (Y = 0.8), the SUR is likely to be overpredicted.

The difference between the calculated SUR and the actual SUR also depends upon the value of the OUR_max for the individual substrates. In the example shown in figure 6.3.2, the total OUR is 1. If the majority of this OUR is due to substrate B (Y = 0.8), then the actual SUR will be 4.25. If the majority of the OUR is due to the metabolism of substrate A (Y = 0.2), then the actual SUR will be 2. In contrast, the calculated SUR is independent of the OUR_max for the individual substrates, in the example shown. The SUR calculated from assuming that the mixture is a single substrate is based on a constant OUR (= 1) and a variable yield, and ranges from 1.5 to > 5.

The example shown in figure 6.3.2 is an extreme case. When the yields of the individual substrates are closer together, the errors in calculating the SUR are not as great
(figure 6.3.3). If the individual yields are the same, there is no error in calculating the SUR. In this case, the calculated yield is independent of the mixture composition, and the actual SUR is independent of the fraction of the OUR due to the metabolism of the various substrates. The OUR may be converted to SUR at any point during the batch test by dividing by the oxidation coefficient.

When dealing with the respirometric analysis of substrate mixtures, it appears to be necessary to determine the yields of the individual compounds independently. If the yields are different, then a method of determining the OUR due to each of the substrates is required. Otherwise, if the mixture is analysed as if it were a single substrate, the calculated SUR will be incorrect.

In order to better understand what is occurring during respirometry and to test the assumptions made in this discussion, substrate mixtures typically found in BKME were investigated. The major assumptions are simultaneous and additive substrate removal, OUR, and OC. It is possible that substrates may be removed sequentially, or that the removal of substrate mixtures may be simultaneous, but follow competition kinetics as proposed by Orhon (Orhon and Artan 1994?). This will further complicate data interpretation. Verification of the difficulties associated with substrates which have different yields on respirometric data interpretation, as predicted by figure 6.3.2 and 6.3.3, was also sought. The mixtures chosen were: methanol - acetic acid, methanol - formic acid, acetic acid - formic acid, and methanol - acetic acid - formic acid. The data obtained using these mixtures was compared to the data obtained using methanol, acetic acid, and formic acid on their own.
Figure 6.3.4 OUR vs time for injections of methanol (2.6 mg COD/l) (—), acetate (1.9 mg/l) (—), and methanol (2.6 mg/l)/acetate (1.9 mg/l) mixture (—). MLVSS: 1550 mg/l.

Figure 6.3.5 OUR vs time for injections of methanol (0.5 mg COD/l) (—), acetate (0.4 mg/l) (—), methanol (0.5 mg/l)/acetate (0.4 mg/l) (—), and expected curve for the methanol/acetate mixture assuming ΔOUR due to methanol and acetate are additive (— — —). MLVSS: 1800 mg/l.
Methanol - Acetic Acid Mixtures

Figure 6.3.4 shows an example of simultaneous substrate utilisation with methanol and acetic acid as the two substrates. The total ΔOUR is equal to the sum of the individual ΔOURs, and the area under the curve is equal to the sum of the area under the individual curves. The acetate is being metabolised at the same rate and with the same yield as when it is the sole substrate. The methanol is also being metabolised at the same rate and with the same yield as when it is the sole substrate. This data is at the maximum uptake rate of both methanol and acetate. If any deviation from simultaneous utilisation is expected, it is most likely to occur when the bacteria are metabolising the substrates at their maximum rates. These results imply that there are two separate biomass components for each of these substrates, as would be expected in biomass taken from a continuous low rate activated sludge unit receiving wastewater that contains both of these substrates. Over four years of activated sludge operation on BKME, this was the response most commonly observed during the oxidation of methanol - acetic acid mixtures.

Although methanol and acetate are removed simultaneously, the difference in the yields of the two compounds will cause difficulties during the interpretation of the respirometric data. For methanol, the ΔOUR is 0.7 mg/l minute, Y is 0.53, and the SUR is 1.5 mg/l minute. For acetate, the ΔOUR is 0.1 mg/l minute, Y is 0.82, and the SUR is 0.56 mg/l minute. For the mixture, the ΔOUR is 0.8 mg/l minute (which is equal to ΔOUR\text{methanol} + ΔOUR\text{acetate}). The measured yield is 0.67 (which is the average of the yield on methanol and acetate). The actual SUR is equal to SUR\text{methanol} + SUR\text{acetate}. 
The additive nature of the individual SURs is verified by examining the OUR profile. The OUR due to methanol oxidation has the same profile whether or not acetate is present. If the methanol SUR were changing in the presence of other substrates, the elevated OUR due to methanol metabolism would either last longer than, or less than, the OUR profile obtained from the oxidation of methanol as the sole substrate. The fact that exactly the same amount of time is required for complete removal from the wastewater whether or not acetate is present implies that the rate of methanol utilisation is not affected by acetate. The same is true for the OUR profile due to acetate oxidation. The calculated SUR (2.4 mg/l minute) is different from the actual SUR (2 mg/l minute due to the calculated yield.

If the yields of the two compounds are known beforehand, the SUR may be calculated from the OUR profile of the mixture, using the following procedure. The OUR profile is used to calculate the ΔOUR due to methanol oxidation and the ΔOUR due to acetate oxidation. These values are divided by the corresponding oxidation coefficients (obtained from the known yields), and added together to calculate the SUR. This method is only possible with certain ratios of substrates. If both substrates are present in quantities which result in their removal from the respirometer at the same time, it will be difficult to determine each substrate’s contribution to the ΔOUR. In this case the SUR of the mixture must be calculated by measuring the SURs of the compounds individually, and then adding them together.

The common response to methanol - acetate mixtures was simultaneous removal, with the OUR, OC, and SUR being additive. On at least one occasion a different response to methanol - acetate mixtures was observed. An example of this response is
shown in figure 6.3.5. The OUR due to the metabolism of the methanol - acetic acid mixture was very close to, but slightly lower than, the OUR due to the metabolism of methanol alone. The two substrates were still used simultaneously, as the time required to metabolise the mixture was the same as the time required to metabolise the individual substrates. The OUR was not additive, the OC was not additive, but the SUR was additive.

The data for a number of methanol - acetic acid mixtures, as well as for methanol and acetic acid, are presented in figure 6.3.6. Regardless of the amount of acetic acid, the maximum ΔOUR on the methanol - acetate mixtures was approximately constant. As the amount of acetic acid increased, the half saturation constant appeared to increase. This was due to the increase in the COD of the solution from the added acetic acid. In figure 6.3.7 the ΔOURs are plotted versus methanol concentration; there is no noticeable difference between any of the curves. This implies that the OUR response was due solely to the methanol. The acetate was being utilised, but was not exerting an oxygen demand when methanol was present. This conclusion is also evident from the OUR profiles. In figure 6.3.5, there was just enough acetic acid to be utilised in the same amount of time as the added methanol, but the OUR profile was similar to the OUR profile of methanol alone. When an excess of acetic acid was added, figure 6.3.8, the acetic acid did not exert an OUR when the methanol was being oxidised. As soon as the methanol was depleted, and the OUR due to methanol oxidation dropped, the OUR then increased to the level it would be at if acetic acid were the sole substrate. The acetic acid was still removed in the same amount of time as if it were the sole substrate even though no oxygen demand was exerted for the metabolism of acetic acid when methanol was
Figure 6.3.6 AOUR vs substrate for a number of methanol/acetate mixtures. Methanol (■), acetate (○), methanol/acetate : 1/2.8 mg COD/mg COD (▲), 1/1.4 (○), 1/0.7 (○), 1/0.35 (●). MLVSS: 1800 mg/l.

Figure 6.3.7 AOUR vs methanol for a number of methanol/acetate mixtures. Methanol (■), methanol/acetate : 1/2.8 mg COD/mg COD (▲), 1/1.4 (○), 1/0.7 (○), 1/0.35 (●). MLVSS: 1800 mg/l.
present. It appears that regardless of how the cell processed the acetate (partially oxidised or not oxidised), the removal rate was constant. The dip in the OUR curve as the catabolism switched from methanol oxidation to acetate oxidation was repeatable (the experiment was repeated four times on two different days), but occurred only for a certain ratio of methanol to acetate, and only for certain strengths of injections.

The yield of acetic acid metabolism must have been changing during the experiment, with the yield being approximately 1 when methanol was present (no acetate being oxidised), and decreasing to 0.76 when no methanol was present, with only a few seconds required for the bacteria to switch between the two modes. The oxygen consumption data for the various mixtures are shown in figure 6.3.9. As discussed previously, if the yields of the two substrates are constant, the measured yield will vary predictably with the mixture composition. For this set of experimental data, the experimental values for oxygen consumption were consistently lower than the predicted values based upon the yield of methanol and acetate. Consequently, the measured yield did not match the predicted values (green curve, figure 6.3.10) which were obtained assuming the actual yields were constant. With the assumption that the acetic acid yield is equal to 1 as long as methanol is present, and equal to 0.76 after the methanol has been completely oxidised, the predicted yield does match the measured yield (red curve, figure 6.3.10). The change in slope of the predicted yield corresponds to the point when the acetic acid and methanol are reduced to zero at the same time. When there was a greater amount of acetic acid added, the methanol was used up before all of the acetic acid was used up, and the OUR profile had a shoulder, which corresponded to the oxidation of the remaining acetate (figure 6.3.11).
Figure 6.3.8 OUR vs time for injections of methanol (0.5 mg COD/l) (---), acetate (1.9 mg/l) (--), acetate (0.75 mg/l), and a methanol (0.5 mg/l)/acetate (1.5 mg/l) mixture (---). MLVSS: 1800 mg/l.

Figure 6.3.9 OC vs substrate for a number of methanol/acetate mixtures. Methanol (■), methanol/acetate: 1/2.8 mg COD/mg COD (▲), 1/1.4 (○), 1/0.7 (□), 1/0.35 (●). Curves for the mixtures were calculated by adding together the OC of methanol and acetate. MLVSS: 1800 mg/l.
Figure 6.3.10 Measured respirometric yield vs substrate (■), expected yield if the yield of methanol and acetate are constant (—), expected yield if the yield of methanol is not constant (—). MLVSS: 1800 mg/l.

Figure 6.3.11 OUR vs time for injections of methanol (—), acetate (—), methanol/acetate mixture 1/2.8 mg COD/mg COD (—), 1/1.4 (—), 1/0.7 (—), 1/0.35 (—). MLVSS: 1800 mg/l.
The ΔOUR may be converted to SUR, but as pointed out earlier, due to the difference in yields of the two compounds ($Y_{\text{methanol}} = 0.2$, $Y_{\text{acetic acid}} = 0.76$), if the measured yields are used, the SUR calculated for the mixtures will be in error (figure 6.3.12). In this particular case, further difficulty is added by the non-constant nature of the yield. A figure similar to 6.3.2 may be constructed (figure 6.3.13). At higher proportions of methanol, the SUR is underpredicted, associated with the lack of OUR due to acetic acid. At lower proportions of methanol, the SUR is overpredicted due to the differences in the yields of the 2 compounds. In this case, it is impossible to calculate the actual SUR of the mixture without measuring the SUR of the compounds individually.

The yield was not constant for methanol - acetate mixtures, the two compounds appeared to be used simultaneously when present as a mixture, and they appeared to be removed at approximately the same rate as they were when present as single substrates. Unlike the first example of the microbial response to methanol - acetate mixtures, in this case the methanol and acetate appeared to be metabolised by the same biomass component.

Continuous culture studies have demonstrated that when bacteria are supplied with two carbon (or greater) compounds, such as acetate, and a one carbon compound, such as methanol or formic acid, they will use the two carbon compound for growth and the one carbon compound for energy. The results presented here show that when methanol is present as an energy source, the bacteria are capable of utilising all of the acetate present without oxidising any of it. There also appears to be a very slight increase in the efficiency of the utilisation of the methanol. Although the bacteria prefer to obtain their energy from the oxidation of the methanol, if there is none present, they are very
Figure 6.3.12  SUR vs substrate for a number of methanol/acetate mixtures. Methanol (■),
acetate (●), methanol/acetate : 1/2.8 mg COD/mg COD (▲), 1/1.4 (○), 1/0.7 (□),
1/0.35 (●). SUR curves for the mixtures were calculated by adding together the SUR
of methanol and acetate (dashed lines). MLVSS 1800 mg/l.

Figure 6.3.13  Measured SUR (▲), OUR (■), and OC (●) vs substrate for a number of
methanol/acetate mixtures. The actual SUR is represented by the dotted line.
MLVSS: 1800 mg/l.
quick to oxidise a portion of the acetate for energy. Methanol provides excess energy because growth on methanol is carbon limited. Growth on acetate is energy limited, and some acetate must be oxidised for energy. If both substrates are present, the bacteria can maximise their yield by using the excess energy obtained from the methanol to metabolise the acetate. This has been found previously in pure culture studies grown on methanol - acetate mixtures.

**Formic acid - Acetic Acid Mixtures**

Formic acid and acetic acid were used simultaneously, similar to methanol - acetic acid mixtures. In figure 6.3.14, the OUR profiles of formic acid, acetic acid, and their mixture, are plotted. At high concentrations, the OUR of the mixture was greater than the OUR of either substrate alone, but less than would be expected if the OURs were additive. At low concentrations, the OUR of the mixture was similar to the OUR due to the formic acid alone, similar to the second methanol - acetate example presented in the previous section. The ΔOUR for a range of substrate concentrations is shown in figure 6.3.15. At all concentrations, the ΔOUR was less than would be predicted by adding together the ΔOUR for the two individual substrates. Since the OUR profiles suggest that the SUR’s are additive, the OC must not be additive. The yield of the mixture was greater than would be expected by assuming the yields of the individual compounds are constant. This is verified by the oxygen consumption data, figure 6.3.16, which demonstrates that oxygen consumption during the oxidation of the mixture was less than expected, hence the yield was greater than expected.

The SUR calculated assuming that the mixture is a single substrate was 2.8 mg/l minute. This is slightly higher than the SUR calculated using the single substrate
Figure 6.3.14 OUR vs time for injections of formic acid (2.2 mg COD/l, 0.4 mg/l) (---), acetate (0.75 mg COD/l, 3.8 mg/l) (--), formic acid/acetate mixture (2.2 mg/l / 3.8 mg/l) (--), (0.4 mg/l / 0.75 mg/l) (---). MLVSS 1800 mg/l.

Figure 6.3.15 ΔOUR vs substrate for a formic acid/acetate mixture. Formic acid (▲), acetate (●), formic acid/acetate (0.57/1 mg COD/mgCOD) (□). OUR curve for the mixture was calculated by adding together the OUR of formic acid and acetate (dashed line). MLVSS: 1800 mg/l.
Figure 6.3.16 OC vs substrate for a formic acid/acetate mixture. Formic acid (▲), acetate (●), formic acid/acetate (0.57/1 mg COD/ mgCOD) (□). OC curve for the mixture was calculated by adding together the OC of formic acid and acetate (dashed line). MLVSS: 1800 mg/l.

Figure 6.3.17 SUR vs substrate for a formic acid/acetate mixture. Formic acid (▲), acetate (●), formic acid/acetate (0.57/1 mg COD/mg COD) (□). SUR curves for the mixture was calculated by adding together the SUR of formic acid and acetate (dashed line). MLVSS: 1800 mg/l.
respirometric data and the assumption of simultaneous substrate utilisation (2.6 mg/l minute, figure 6.3.17). A greater discrepancy between these values would be expected on the basis of the large difference between the yield of acetic acid and that of formic acid (figure 6.3.2). The large difference in yield is partially offset by the increase in the yield of the mixture. If a number of different mixtures had been tested, a relationship similar to the one shown in figures 6.3.10 and 6.3.13 would be expected. In the case of formic acid - acetic acid mixtures, it was not possible to tell which of the substrates was being less oxidised when both are present (compared to when it was the sole substrate), but it was probably the acetic acid. If the formic acid yield is considered to be a constant, then the acetic acid yield changed from 0.76 as a sole substrate to 0.9 in the presence of formic acid. This was not as large a change as with the methanol - acetic acid example, but still significant.

On a separate occasion, with different biomass, three different mixtures of formic acid and acetic acid were studied. Sample data is shown in figure 6.3.18. For the sample data shown, the OUR of the mixture was close to the expected OUR, implying that the yields were constant and the OUR was additive, unlike the previous example of formic acid - acetate mixtures. The data also suggests that the SUR’s of the individual substrates were not influenced by each other, i.e. the SUR was additive as with all of the other examples discussed.

The ΔOUR data is plotted in figure 6.3.19. As with the previous example, the ΔOUR of the mixtures was less than expected by adding together the ΔOURs of the individual substrates, probably due to the yields changing slightly when the two substrates were both present. The calculated yields were slightly greater than expected.
Figure 6.3.18 OUR vs time for injections of formic acid (4.3 mg COD/l) (---), acetate (3.8 mg COD/l) (----), formic acid (4.3 mg COD/l)/acetate (3.8 mg COD/l) mixture (---), expected curve (---) assuming ΔOUR due to formic acid and acetate are additive. MLVSS 2800 mg/l.

Figure 6.3.19 ΔOUR vs substrate for a number of formic acid/acetate mixtures. Formic acid (▲), acetate (●), formic acid/acetate: 1/0.43 mg COD/mg COD (◇), 1/0.9 (△), 1/1.73 (■). MLVSS: 2800 mg/l.
based on the oxidation of the compounds individually (figure 6.3.21). Once again, this is probably due to the increased efficiency of acetate utilisation in the presence of a one carbon energy source (formic acid). As with the previous examples, the SUR calculated by treating the mixture as a single substrate is incorrect (figure 6.3.20) due to the difference in yields of the two compounds. The actual SUR is obtained by adding the SURs of formic and acetic acid together. The relationship between the calculated yield, the calculated SUR, and the actual SUR is shown in figure 6.3.21. The data follows the trends predicted previously. The overprediction of the SUR is somewhat mitigated by the increased yield on the mixture.

**Methanol - Formic Acid Mixtures**

The calculation of SURs of methanol - formic acid mixtures should not be difficult since methanol and formic acid have similar yields. The yield on the mixture will be the same as the yields on the individual substrates. It also has been predicted that the yield on a mixture of two one carbon compounds should not be greater than predicted based upon the yield of the two compounds individually. Therefore, unlike the mixtures involving acetate, the yield on methanol and on formic acid should not change due to the presence of the other substrate.

ΔOUR data is shown in figure 6.3.22, and oxygen consumption data in figure 6.3.23, for a typical response of activated sludge to methanol - formic acid mixtures. The two substrates were utilised simultaneously, and the ΔOUR and oxygen consumption data of the mixture was very close to what we would predict by studying the single substrates. Both the ΔOUR and OC data were additive. As expected, the calculated SUR is very close to the SUR obtained assuming the substrates were removed simultaneously (figure
Figure 6.3.20 SUR vs substrate for a number of formic acid/acetate mixtures. Formic acid (▲), acetate (●), formic acid/acetate : 1/0.43 mg COD/mg COD (□), 1/0.9 (▲), 1/1.73 (●). SUR curves for the mixture were calculated by adding together the SUR of formic acid and acetate (dashed lines). MLVSS: 2800 mg/l.

Figure 6.3.21 Measured SUR (▲), measured yield (■), expected yield if yields are constant (-----), actual SUR (------). MLVSS: 2800 mg/l.
Figure 6.3.22 ΔOUR vs substrate for a formic acid/methanol mixture. Formic acid (▲), methanol (■), formic acid/methanol (0.8/1 mg COD/mg COD) (○). OUR curve for the mixture was calculated by adding together the OUR of formic acid and methanol (dashed line). MLVSS: 2100 mg/l.

Figure 6.3.23 OC vs substrate for a formic acid/methanol mixture. Formic acid (▲), methanol (■), formic acid/methanol (0.8/1 mg COD/mg COD) (○). OC curve for the mixture was calculated by adding together the OC of formic acid and methanol (dashed line). MLVSS: 2100 mg/l.
6.3.24). In this case the mixture behaved as expected based on the analysis of the single substrates. If the mixture were treated as a single substrate, the error in calculating the SUR and the yield would be minimal.

On one occasion, a different response of the activated sludge was observed to methanol - formic acid mixtures. This response was different from all of the other examples discussed in that the SUR's were not additive. Figures 6.3.25, 6.3.26, 6.3.27 show typical OUR profiles from this data set. The oxygen uptake rate of the methanol - formic acid mixture was no greater than the oxygen uptake rate of methanol alone. Unlike the methanol - acetic acid scenario, the amount of oxygen consumed was greater than the amount of oxygen consumed when there was just methanol present. This was especially noticeable at the higher substrate concentrations (figure 6.3.27). The OUR during the oxidation of the mixture remained elevated for longer than the OUR during the oxidation of either methanol or formic acid implying that the SUR was not additive. The substrates were removed at a slower rate when present as a mixture than would be predicted by the data obtained when they were the sole substrates. This data implies that at high substrate concentrations, the substrates were being used sequentially, first the methanol was oxidised, then the formic acid.

ΔOUR data for a number of methanol - formic acid mixtures is presented in figure 6.3.28. The ΔOUR of the mixtures did not increase above that of the ΔOUR due to methanol. If the amount of formic acid present is ignored, and the ΔOUR is plotted versus just the methanol concentration, the ΔOUR increased at low methanol concentrations when there was a large amount of formic acid present. This implies that at low concentrations the methanol and formic acid were used at the same time, but
Figure 6.3.24 SUR vs substrate for a formic acid/methanol mixture. Formic acid (▲), methanol (■), formic acid/methanol (0.8/1 mg COD/mg COD) (○). SUR curve for the mixture were calculated by adding together the SUR of formic acid and methanol (dashed line). MLVSS: 2100 mg/l.

Figure 6.3.25 OUR vs time for injections of formic acid (0.43 mg COD/l) (——), methanol (0.53 mg COD/l) (——), formic acid (0.43 mg COD/l) / methanol (0.53 mg COD/l) mixture (———). MLVSS: 1800 mg/l.
Figure 6.3.26 OUR vs time for injections of formic acid (0.86 mg COD/l) (—), methanol (1.1 mg COD/l) (—), formic acid (0.86 mg COD/l) / methanol (1.1 mg COD/l) mixture (—). MLVSS: 1800 mg/l.

Figure 6.3.27 OUR vs time for injections of formic acid (2.2 mg COD/l) (—), methanol (2.6 mg COD/l) (—), formic acid (2.2 mg COD/l) / methanol (2.6 mg COD/l) mixture (—). MLVSS: 1800 mg/l.
methanol was used preferentially at high concentrations. The same enzyme system must be responsible for the oxidation of formic acid and methanol. This enzyme system will preferentially oxidise the methanol, and then the formic acid. When methanol is oxidised, it is first turned into formic acid, then carbon dioxide. The data can be modeled based upon this sequential oxidation, and the curves in figure 6.3.28 are obtained. They do not match the data perfectly, but are reasonably approximate.

Although the substrates were not used simultaneously, the yields of the methanol-formic acid mixtures can be predicted accurately (figure 6.3.29), implying that the yield of methanol and formic acid were constant even in the presence of each other. This is the expected response of mixtures of two one carbon compounds (yield not changed by the presence of other one-carbon compounds). The calculated SUR of the mixture was very close to the SUR of methanol alone (dashed line, figure 6.3.29). If methanol and formic acid were assumed to be used simultaneously, the SUR would be overpredicted by approximately 25%.

From just the respirometric data of the mixture it is not possible to determine if the two compounds were being used simultaneously or sequentially. Respirometric data of the compounds on their own must be available for comparison. Evidence for sequential utilisation was an increase in $K_M$ but not $OUR_{MAX}$ for the mixture over methanol alone. More evidence was provided by the OUR profiles, where it was shown that the oxidation of the mixture took longer than the oxidation of either of the substrates on their own.
Figure 6.3.28 ΔOUR vs substrate for a number of formic acid/methanol mixtures. Formic acid (○), methanol (■), formic acid/methanol: 1/5 mg COD/mg COD (□), 1/2.5 (●), 1/1.25 (▲), 1/0.63 (●). MLVSS: 1800 mg/l.

Figure 6.3.29 Measured yield (■), expected yield if yields are constant (—). Measured SUR (●), actual SUR (——). MLVSS: 1800 mg/l.
Methanol - Acetic Acid - Formic Acid Mixtures

When three substrates are present, interpretation of respirometric data becomes even more complicated. Two substrates may be utilised simultaneously and the third sequentially, or vice versa. Also, the yields on the substrates may be different when they are in a mixture than when they are on their own.

In the first example of a methanol - acetate - formic acid mixture (figures 6.3.30, 6.3.31, and 6.3.32), the yields on the various substrates did not change when they were present in a mixture. This is evident from figure 6.3.32 where the predicted oxygen consumption of the mixture matches the experimental oxygen consumption of the mixture. If the yield does not change, the ΔOUR can be expected to be additive. This was not the case (figure 6.3.31). The ΔOUR of the three substrates were additive at low substrate concentrations (below saturation of the enzymes), but not at higher concentrations. These results imply that two of the substrates are oxidised by the same biomass component, and as long as they are present below saturating conditions, they will both be oxidised. When the substrates are present in saturating quantities, the biomass will preferentially use one of the substrates first, and only when that substrate has been utilised (or falls below the critical $K_M$ value) will the remaining substrate be utilised. The metabolism of the third substrate is not affected by the presence of the other substrates. Figure 6.3.30 further demonstrates this point. Methanol and acetate, or methanol and formic acid are oxidised first (simultaneously), followed by the remaining substrate (acetate or formic acid). It is difficult to tell if the formic acid or the acetate was oxidised first, as the ΔOUR$_{MAX}$ of the two substrates were similar. Without knowing which substrates are utilised first, the SURs cannot be calculated.
Figure 6.3.30 OUR vs time for injections of formic acid (0.9 mg COD/l) (—), methanol (2.6 mg COD/l) (—), acetate (1.9 mg COD/l) (—), formic acid (0.9 mg COD/l) / methanol (2.6 mg COD/l) / acetate (1.9 mg COD/l) mixture (—). MLVSS: 1500 mg/l.

Figure 6.3.31 ΔOUR vs substrate for a formic acid/methanol/acetate mixture (0.33 / 1 / 0.75 mg COD/mg COD/mg COD). Formic acid (▲), methanol (■) acetate (●), formic acid/methanol/acetate (○). OUR curve for the mixture was calculated by adding together the OUR of formic acid, methanol, and acetate (dashed line). MLVSS: 1500 mg/l.
Figure 6.3.34 OC vs substrate for a formic acid/methanol/acetate mixture (0.33 / 1 / 0.75 mg COD/mg COD/mg COD). Formic acid (▲), methanol (■) acetate (●), formic acid/methanol/acetate (○). OC curve for the mixture was calculated by adding together the OC of formic acid, methanol, and acetate (dashed line). MLVSS: 1800 mg/l.
Figure 6.3.32 OC vs substrate for a formic acid/methanol/acetate mixture (0.33 / 1 / 0.75 mg COD/mg COD/mg COD). Formic acid (▲), methanol (▼) acetate (●), formic acid/methanol/acetate (○). OC curve for the mixture was calculated by adding together the OC of formic acid, methanol, and acetate (dashed line). MLVSS: 1500 mg/l.

Figure 6.3.33 ΔOUR vs substrate for a formic acid/methanol/acetate mixture (0.4 / 1 / 0.75 mg COD/mg COD/mg COD). Formic acid (▲), methanol (▼) acetate (●), formic acid/methanol/acetate (○). OUR curve for the mixture was calculated by adding together the OUR of formic acid, methanol, and acetate (dashed line). MLVSS: 1800 mg/l.
Another example of the utilisation of the three substrates is presented in figures 6.3.33 and 6.3.34. This experiment used the same biomass as a few of the examples discussed above. In particular, the yield on the acetate increased to 1 while methanol was present; and formic acid was used sequentially when methanol was present in saturating concentrations. Here, the yields of the individual substrates changed when they were present in a mixture (figure 6.3.34). The yield of the mixture was greater than expected by calculating from the yields of the individual compounds. This was due to the presence of acetate and two energy sources, methanol and formic acid. The bacteria were able to satisfy their energy requirements from the methanol and formic acid present, and utilised the acetate for growth. Less of the acetate was oxidised than if acetate had been the sole substrate. This resulted in an increase in the yield. These results imply that this sample of activated sludge is carbon limited, and not energy limited. Examination of the ΔOURs showed that the ΔOURs of the individual compounds were additive at low concentrations, but not when the concentration increased. There are two explanations for this. The first is the OUR is lower than expected due to the increased yield on acetate. The second is the formic acid was not oxidised when methanol was saturating.

Summary

If substrate mixtures are composed of substrates with different yields, then the respirometric yield will be a weighted average of the yields of the individual substrates. This makes estimation of the SUR using respirometry very difficult, if not impossible, unless the composition of the mixture is known. In addition to the composition, the individual yields must be known, and the individual OUR's. For unknown mixtures, such as BKME, these problems will present great difficulties.
Data have been presented in this section on the response of activated sludge to various substrate mixtures. The general findings are presented in table 6.3. Sometimes the response was straightforward simultaneous removal of the substrates in the mixture, and sometimes some of the substrates were utilised sequentially. In no case did the removal follow competition kinetics as proposed by various authors. Adding to the difficulty of analysing mixtures where the two compounds have different yields, was the fact that, on occasion, the yields of the substrates were found to be different when other substrates were present. This was the case when acetic acid was present. The easiest mixture to analyse was the methanol - formic acid mixture. The yields of the two compounds are similar, and on most occasions the OUR and OC were additive.

Table 6.3 Respirometric Analysis of Substrate Mixtures

<table>
<thead>
<tr>
<th>Mixture</th>
<th>OUR additive</th>
<th>OC Additive</th>
<th>SUR additive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol - acetic acid</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Formic acid - acetic acid</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Methanol - formic acid</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Methanol - formic acid</td>
<td>No</td>
<td>Yes</td>
<td>no</td>
</tr>
</tbody>
</table>
Chapter 7 BKME Kinetics

7.1 Operating Data

Mixed liquor volatile suspended solids data for the two lab scale reactors are shown in figure 7.1.1. The vertical dashed lines represent changes in wastewater batches. The vertical solid lines represent different experimental runs. Each run was started with fresh activated sludge seed from Harmac Pacific’s wastewater treatment plant. The SRT (figure 7.1.3) in each activated sludge unit was controlled by wasting directly from the aeration tank based on the MLVSS, the VSS in the treated wastewater leaving the clarifier (figure 7.1.2), and the HRT. Unit one was operated at an SRT as close to 5 days as possible, except during the treatment of batches L, M, and N when the SRT was 10 days. The SRT in unit two was maintained as close to 15 to 20 days as possible, except for during the treatment of batches L, M, and N when the SRT was 10 days and an aerobic selector was employed.

The average effluent VSS in reactor one was 32 mg VSS/l and the SRT was not difficult to control. There was day to day variation in the effluent VSS, and also day to day variation in the MLVSS. The MLVSS never achieved a true steady state value even though the SRT was fairly constant for most of unit one’s operation. The biomass concentration seemed to go through cycles where every two weeks it would suddenly drop, then rise again. It is probably close to impossible to achieve true steady state conditions in a biological reactor with mixed populations and mixed substrates, which changes from batch to batch. Predation from protozoa will affect the MLVSS levels, and the populations of the different bacterial species present will probably oscillate. The data
Figure 7.1.1 MLVSS vs. time over the course of the project. Reactor 1 (■), reactor 2 (○). Letters represent wastewater batches, solid vertical lines mark boundaries between experimental runs.

Figure 7.1.2 Effluent VSS vs. time over the course of the project. Reactor 1 (■), reactor 2 (○). Letters represent wastewater batches, solid vertical lines mark boundaries between experimental runs.
Figure 7.1.3 SRT vs. time over the course of the project. Reactor 1 (■), reactor 2 (○). Letters represent wastewater batches, solid vertical lines mark boundaries between experimental runs.
in figure 7.1.1 suggests that a pseudo steady state was achieved with respect to MLVSS in unit one.

The average effluent VSS concentration leaving reactor two was 44 mg/l. Operating at SRTs greater than 15 days often results in sludge with poor settling qualities. This value, combined with an HRT of twelve hours made it difficult to control the SRT as the amount of mixed liquor to waste every day was very small and the fluctuations in the effluent VSS had a large impact on the SRT. One of the causes for the large variation in effluent VSS was the clarifier design. The underflow from the clarifier would occasionally become clogged, allowing the solids to accumulate in the clarifier and overflow with the effluent. Also, the biomass would stick to the clarifier walls, requiring occasional stirring, which then resulted in some biomass overflowing with the effluent before it had a chance to settle. The biomass from unit two usually settled much faster than the biomass from unit one, but stuck to the walls more frequently (either due to a greater amount of polymers present under starvation conditions or to the greater amount of solids present at the higher SRT). Consequently, more biomass from unit two was lost with the treated effluent due to stirring than from unit one. The high levels of effluent VSS made the SRT difficult to control. Figures 7.1.1 and 7.1.3 show that the MLVSS and SRT were highly variable for unit two (except when the SRT was controlled at 10 days). Steady state (with respect to MLVSS concentration) was never attained, but the SRT was significantly higher than the SRT from unit one at all times (except when they were both at 10 days).

The BOD removal, COD removal, and toxicity removal (table 7.1) were the same for both reactors, and did not vary with time. Both units were operated at conditions in
excess of those required for acceptable BKME treatment. Wastewater treatment plants are designed to operate at conditions that give good sludge settling characteristics, these operating conditions help ensure that all of the readily biodegradable organics are removed. The readily biodegradable organics are the source of BOD and acute toxicity in BKME.

Table 7.1 Reactor performance

<table>
<thead>
<tr>
<th></th>
<th>Reactor #1</th>
<th>Reactor #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD Removal</td>
<td>95%</td>
<td>95%</td>
</tr>
<tr>
<td>COD Removal</td>
<td>50%</td>
<td>55%</td>
</tr>
<tr>
<td>Toxicity Removal</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

7.2 Measurement of BKME Kinetics

In this section, various methods for measuring the activated sludge kinetics of BKME treatment are investigated. In order to properly model the kinetics, the multiple substrate nature of BKME must be accounted for. The implications of this finding will be discussed. If the multiple substrate nature is ignored, extrapolation of batch test data to continuous systems will be incorrect.

Discrepancies between kinetic tests

The kinetics of BKME biodegradation by activated sludge may be measured using the respirometric method discussed in chapter 6 (figure 7.2.1). Most studies measuring BKME biodegradation kinetics have ignored the multi-component nature of BKME, and used the BOD or the COD to represent the degradable organics, which was done in this section as well. (To see if this simple approach can be used to adequately model BKME biodegradation kinetics). Various methods of calculating activated sludge
Figure 7.2.1 ΔOUR (■) vs. BOD and COD. OC (○) vs. BOD and COD. Batch L, SRT 5 days.

Figure 7.2.2 OUR (■), BOD (○), and COD (△) vs. time during a BKME batch biodegradation test. Batch L, SRT 5 days.
kinetics were compared. These methods were the ΔOUR test, the batch test, and the infinite dilution fed batch test.

The ΔOUR and oxygen consumption data in figure 7.2.1 is plotted versus the BOD and the COD. The BOD is probably a more accurate description of the biodegradable organics than the COD due to the large amounts of non-biodegradable components in the wastewater. The ΔOUR data follows Monod kinetics, and not Powell kinetics as found for methanol and acetate. This may be due to the multiple substrates present in low concentrations, as well as the difficulty in measuring the kinetics at large concentrations of effluent (due to biomass washout from the respirometer). The oxygen consumption data increases linearly with BOD concentration. Using the substrate oxidation coefficient from figure 7.2.1, the SUR may be calculated from the ΔOUR data (figure 7.2.3). The kinetic coefficients obtained depend on whether BOD or COD is taken as the substrate concentration. The ΔOUR_{MAX} was similar to the values obtained on methanol and formic acid (see chapter 6, and section 7.3). This is as expected since these compounds are a major source of BOD in BKME. The half saturation constant for the metabolism of effluent was much greater than the corresponding values for methanol and formic acid.

Another method of measuring activated sludge kinetics is the batch test (figure 7.2.2). The BOD removal and COD removal were found to follow multicomponent kinetics (equation 3.22). There were three distinct regions in the OUR profile during the batch test. Each region corresponds to the oxidation of a different group of substrates, which will be discussed later. The kinetics of a continuous system may be calculated with batch test data using the method described by Argaman (1991) (figure 7.2.3). This
Figure 7.2.3 Kinetics calculated with: △OUR data (single substrate - Monod) (●), batch test data (n-order kinetics) (◆), and fed-batch test (*)..

Figure 7.2.4 OUR profiles for injections of samples withdrawn during the batch test (figure 7.2.2) Samples from top to bottom (sample time in brackets): sample #1 (0 minutes), 2 (10), 3 (20), 6 (50), 8 (75), 9 (90), 11 (120). Batch L, SRT 5 days.
method assumes that each of the individual compounds is removed following zero order kinetics. This assumption is validated by the small half saturation constants for the typical compounds in BKME found in Chapter 6. The calculated rates were slower than those calculated by the ∆OUR method, which relies on measuring the reaction rate at the start of many separate batch tests.

The maximum SUR during the batch test (figure 7.2.2), which is the rate at the start of the test, was 0.0025 mg BOD/mg MLVSS•minute. This corresponds to the value obtained by respirometry (top curve, figure 7.2.3). If the Monod model is applied to the batch test data, and the maximum SUR is assumed to be 0.0025 mg BOD/mg MLVSS minute, the half saturation constant (the concentration at which the SUR = 1/2 SUR_{MAX}) is approximately 120 mg/l. These coefficients result in a curve which does not fit the data. If the Monod model is fit to the batch test data (figure 7.2.2) using non-linear regression, ridiculously large, and meaningless, coefficients are obtained. It can be concluded that the Monod equation does not adequately describe the experimental batch test data. The data is best represented with the multicomponent model.

A third way of measuring BKME kinetics is the fed batch / infinite dilution method (middle curve, figure 7.2.3). The BOD measurement was used for this experiment since the large amount of non biodegradable COD makes COD measurements impractical. To compare all three methods, the predicted SUR vs. BOD for the ∆OUR method and batch test (modeled with the multicomponent model) are also plotted in figure 7.2.3. Under the flow rates used in the experiment, the ∆OUR results predict BOD values in the fed batch test to be less than 1 mg/l, when they went as high as 25 mg/l. The ∆OUR method greatly overpredicted the total substrate removal rate. On
the other hand, the multicomponent model underestimated the removal rate, and predicted treated BOD values approximately twice the actual values. Neither method (ΔOUR or batch test) accurately predicted the treated BOD values measured during the fed-batch test. The discrepancy between the three kinetic assays is likely due to the multicomponent nature of the wastewater.

The BOD removal rates observed during the fed batch test may be modeled using the Monod model. The maximum substrate uptake rate was 1 mg/l minute. This is much lower than the maximum SUR predicted by the ΔOUR method (3 mg BOD/l minute), and as found in the batch test method (0.5 mg BOD/l minute). Had the flow rates been higher, the data would probably not have followed the Monod model, and the SURs measured would have been higher. Biomass washout prevented higher flow rates from being tested. The half saturation constant calculated based on the fed-batch data was 15 mg/l. This is larger than the half saturation constant obtained by the ΔOUR method, and much larger than the half saturation constants of the pure compounds tested in chapter 6. Although these values of $K_M$ are much larger than the values obtained for single substrates, they are much smaller than most values reported in the literature for wastewater treatment, and for the value obtained by fitting the Monod equation to the batch test data. This high value of $K_M$ is another indication of the multicomponent nature of the wastewater (Jones 1973).

Others have noticed differences between the substrate concentration in the effluent and the values predicted by respirometry, and have attributed the discrepancy to soluble microbial product formation. SMPs would not be expected to form in such large quantities as indicated by the measured BOD, and are not usually measurable as BOD.
Fed-batch tests using methanol, acetate, and formic acid as the substrate, instead of BKME, resulted in negligible BOD in the reactor, thus no SMPs were formed from these compounds. The difference between the fed-batch and ΔOUR methods is also too great to be explained by the phenomena discussed in section 6.3. For example, if sequential substrate utilisation occurred in one of the assays and not the other, the maximum rate where it occurred would be lower, but not sufficiently low to account for the observed differences. Also, sequential utilisation would be more likely under the conditions in the respirometric test due to the higher substrate concentrations employed. The difficulty that the presence of compounds with different yields presents in converting the ΔOUR\textsubscript{MAX} into a SUR\textsubscript{MAX} is also unlikely to be great enough to account for the difference in the two assays. (This is supported by the similarity between the SUR\textsubscript{MAX} obtained from the respirometric test and from the batch test.)

The yield obtained by the ΔOUR method was 0.6 mg COD / mg COD if based on the effluent BOD, and 0.3 mg COD / mg COD if based on the effluent COD. These values are greater than the yield on methanol or formic acid (0.2 to 0.5 mg COD / mg COD). This means that effluent organic compounds with a greater yield than methanol and formic acid are also being oxidised. One possible explanation is the presence of a large quantity of substrates such as acetate with very high yields. As was explained in section 6.3, the yield as measured by respirometry will be an average of the yields of the substrates present. Another explanation, which is certainly valid for the yield calculated based on the effluent COD, is that only a fraction of the organics are being oxidised during the ΔOUR tests, with the other fraction degrading at a slower rate or not at all.
Evidence of Multiple Substrates

The discrepancy between the three methods of measuring the kinetics lies in the multicomponent nature of the wastewater. This is best demonstrated with the batch test data (figure 7.2.2). The BOD and COD removal during the batch test followed the multicomponent model as predicted by Grau et al (1975) for multicomponent wastewaters, with $n = 1.4$. If the different organic components in the wastewater all had the same removal rates, the BOD (or COD) removal would follow the Monod model. If the organics could be divided into two fractions with distinct removal rates, the total biodegradable organics, measured by COD, and the rapidly biodegradable organics, measured by BOD, then the BOD removal would follow the Monod model. Since neither the BOD nor the COD followed the Monod model, the situation is more complicated and there are more than two wastewater components (or the division between components is not the same as the division between BOD and COD).

The OUR profile over the batch test (figure 7.2.2) also suggests the presence of multiple substrates. The OUR starts off high, drops to a plateau after the first 20 minutes, then drops to another plateau after 120 minutes. The standard interpretation of this type of OUR profile is that the initial high OUR is due to the readily biodegradable substrate. When the readily biodegradable substrate is gone, hydrolysis of the slowly biodegradable substrate becomes the rate limiting step. The second OUR plateau is due to the oxidation of the hydrolysis products (the hydrolysis products are metabolised at the same rate as the initial substrate, but the hydrolysis step is too slow for the OUR to be at its maximum). Another interpretation is the adsorption of substrate by the floc. The initial OUR plateau is due to the oxidation of the adsorbed substrate. When all of the adsorbed substrate has
been utilised, the OUR drops to the second plateau while the substrate in the bulk solution is oxidised. Of course, if the remaining substrate has different adsorption characteristics, it will probably also have different kinetics as well. A simpler explanation is suggested by comparison to figure 6.3.30, the OUR profile of the degradation of a three substrate mixture. The first OUR plateau is due to the biodegradation of the first readily biodegradable substrate, the second OUR plateau corresponds to the second substrate, and so on. There are many different biodegradable components in BKME, each with its own removal rate and stoichiometry.

To determine if the OUR and BOD removal behaviour during the batch test is due to the multicomponent nature of the wastewater, samples were withdrawn from the batch test at specified times, and the substrate removal rates of these samples were determined by the respirometric method. The OUR profiles of different batch test samples are shown in figure 7.2.4. The OUR profiles of samples one and two were quite different from samples three to nine, which were different from sample eleven. Note that the BOD of sample eleven is 61.8 mg/l, not much less than sample nine (86.6 mg/l). The ΔOUR vs. substrate curve for each sample of partially degraded wastewater are presented in figure 7.2.5, and the oxygen consumption data is presented in figure 7.2.6. For each sample the Monod model fit the data. As the wastewater was degraded, the removal rates of the samples decreased (the maximum SUR decreased, and the half saturation constant increased), implying that the composition of the wastewater changed throughout the batch test. If the composition did not change throughout the batch test, all of the curves in figure 7.2.5 and 7.2.6 should be superimposed, which is clearly not the case.
Figure 7.2.5 ΔOUR vs. BOD curves of samples withdrawn during the batch test (figure 7.2.2). Batch L, SRT 5 days.

Figure 7.2.6 Respirometric OC of samples withdrawn during the batch test (figure 7.2.2). Sample #1 (■), Sample 2 (●), Sample 3 (▲), Sample 4 (●), Sample 6 (□), Sample 8 (○). Batch L, SRT 5 days.
The respirometric data of the complete wastewater and all of the samples follow the Monod model (figure 7.2.5) even though the batch kinetics are one and a half order (figure 7.2.2) because the respirometric method relies on the ΔOUR at the start of many short batch tests. The initial ΔOUR is due to the summation of the metabolism of all the components present in the particular sample being tested. The mixture of substrates present is always in the same ratio at the start of the respirometric test for a given sample, so it appears to behave as a single compound. As the batch test progresses, substrates are being removed from the wastewater. The first major drop in OUR corresponds to when the first major substrate component has been removed from the wastewater. Samples withdrawn after this time have lower removal rates than the original BKME since they are missing the more readily biodegradable substrates. The second major drop in OUR corresponds to when the second major substrate component has been removed from the wastewater. Samples withdrawn after this time have even lower removal rates than the original BKME since they are missing even more biodegradable substrates.

These results are similar to those reported in continuous systems with tanks in series. In continuous systems, where the substrate concentration is usually low, substrate removal rates are often modeled as following first order kinetics. The first order substrate uptake rate coefficient was found to decrease from the first to the second tank due to the more readily biodegradable substrates being removed in the first tank, leaving a more recalcitrant wastewater to be treated in the second tank (Huang et al 1985).

The differences in the curves in figure 7.2.6 implies that the different components of BKME will have different yields. This is expected from the results in chapter 6.
Wastewater Fractions

The amount of readily biodegradable substrate in wastewater is usually determined by measuring the OUR during a batch test, and measuring the area under the OUR profile, as discussed in Chapter 4. The area under the curve is converted into readily biodegradable substrate using the yield. Usually, a yield of ~ 0.66 is assumed. The assumption of a constant yield is probably incorrect for BKME. The dependence of the yield on wastewater composition was demonstrated in chapter 6. In this chapter it was shown that the wastewater composition changes throughout the batch test, therefore the yield is probably changing throughout the batch test. Initially, the respirometric yield will be a weighted average of the yields of all of the substrates present (probably with an increased yield on the two-carbon and greater compounds due to the presence of one-carbon compounds). As the readily biodegradable substrates are removed one by one, the yield will change to be more representative of the yield of the slowly biodegradable substrates. A variable yield makes it difficult to convert the OUR into a SUR, and also to convert the area under the OUR curve into a substrate concentration.

A more accurate determination of the readily biodegradable component is made from a direct measurement of the substrate. Straight lines may be drawn through the BOD values while the OUR is constant, as through points 3 to 10 (line 2, figure 7.2.7). The first substrate group, represented by the first two BOD points is either made up of a single substrate with a high $K_M$, or more likely a mixture of various substrates (lines 1a and 1b). By point 3, this substrate group is gone, and the bacteria are working on the second substrate group, which is probably methanol. After the methanol has been all used up, acetate is probably the main substrate left (line 3a). When this is gone, the
Figure 7.2.7 BOD (○) and OUR (---) vs time during a batch biodegradation of BKME. Lines drawn represent removal of different wastewater fractions. Batch L, SRT 5 days.

Figure 7.2.8 Composition of BKME. Outer ring represents BOD, inner circle represents COD. Batch L, SRT 5 days.
remaining substrate is a mixture of various compounds with relatively low biodegradation rates (line 3b). If all of the substrates are assumed to be utilised simultaneously, then these lines may be extended to time zero and the initial concentration of each substrate group can be determined. This result is presented in figure 7.2.8 for both BOD and COD fractions. For calculation of the COD fractions, the non-biodegradable COD was assumed to be the amount leaving the continuous reactor at a twelve hour hydraulic retention time.

When estimates are available for the wastewater fractions, the ΔOUR kinetics of each sample may be graphed versus the BOD of the corresponding fraction (figure 7.2.9). For example, the ΔOURs obtained for sample 1 are graphed versus the first readily biodegradable substrate (1a), while the ΔOURs from samples 3 to 10 are graphed versus substrate 2. The results clearly show three distinct ΔOUR vs. substrate curves which correspond to the different OUR segments of the original batch test (figure 7.2.2). The Monod constants for each sample were calculated and are graphed versus the time the corresponding sample was taken during the batch test (figure 7.2.10). This figure confirms the earlier observation that the removal rates were decreasing throughout the batch test. The main reason for this decrease is the disappearance of substrates as the test continues (i.e. the sample taken at 50 minutes (#5) has fewer substrates than the original wastewater (sample #1)).

The ΔOURs of sample 1 (original wastewater) is the summation of the ΔOUR of all of the substrates present. The ΔOUR due to substrate 1a may be estimated by subtracting the ΔOUR due to the rest of the substrates. This procedure starts with the ΔOUR of substrate 3a. Substrate group 3b does not exert a noticeable ΔOUR during the
Figure 7.2.9 \( \Delta \text{OUR} \) of samples withdrawn during the batch test (figure 7.2.2) vs. the corresponding BOD fraction. Sample #1 (■), Sample 2 (●), Sample 3 (▲), Sample 4 (●), Sample 6 (□), Sample 8 (○), Sample 11 (△). Batch L, SRT 5 days.

Figure 7.2.10 Maximum \( \Delta \text{OUR} \) (●) and Monod half saturation constant (▲) of samples withdrawn during the batch test, calculated from the data in figure 7.2.9. Batch L, SRT 5 days.
respirometric test. The ΔOUR due to substrate 2 is calculated by subtracting the ΔOUR due to substrate 3a from the ΔOUR measured on samples 3 to 10. This procedure is repeated for substrate 1b, then 1a. In figure 7.2.11 the estimated ΔOURs of the substrate groups are presented. Group 2 has the greatest removal rate, followed by group 1, and then group 3. The substrate group with the greatest removal rate is not removed first during the batch test because it is present in a large quantity, while there is relatively little of substrate group 1. This observation goes against the common assumption that the compound with the greatest removal rate always disappears first during a batch test.

Similarly, the oxygen consumption for each substrate group may be calculated (figure 7.2.12). Unfortunately, the oxygen consumption due to substrate group #3 could not be measured. The oxygen consumption for groups 1 and 2 are similar. The corresponding yield is 0.44 mg COD/mg COD. This is typical for methanol and formic acid, which are thought to be two of the main constituents of substrate groups 1 and 2. Group 3 is composed of acetate and other 2+ carbon compounds, and the yield will be higher than the yield on the one carbon compounds (chapter 6). A yield of 0.6 was assumed for group 3. This is supported by the yield from the continuous activated sludge units, which is approximately 0.5 (see section 7.5). The yield of the readily biodegradable component is less than the overall yield implying that the yield of the slowly biodegradable component (group 3) must be larger. It would seem that the bacteria preferentially grow on the slowly biodegradable fraction and use the readily biodegradable fraction as an energy source. This has been found in other cases when both one carbon and two carbon compounds are available as substrates. The one carbon compound is used for energy, and the two carbon compound is used for growth. In the
Figure 7.2.11 Fractional ΔOUR of samples withdrawn during the batch test (figure 7.2.2) vs. the corresponding BOD fraction. Sample #1 (■), Sample 2 (●), Sample 3 (▲), Sample 4 (●), Sample 6 (□), Sample 8 (○), Sample 11 (△). Batch L, SRT 5 days.

Figure 7.2.12 Fractional OC of samples withdrawn during the batch test (figure 7.2.2) vs. the corresponding BOD fraction. Sample #1 (■), Sample 2 (●), Sample 3 (▲), Sample 4 (●), Sample 6 (□), Sample 8 (○). Batch L, SRT 5 days.
case of activated sludge treating BKME, the readily biodegradable fraction is assumed to be composed of one carbon compounds, mainly methanol and formic acid.

The SURs of the various components of BKME may now be calculated (figure 7.2.13). The ΔOURs are converted to SURs using the yield factor. Since the ΔOUR of substrate group 3b could not be measured, an alternate method of calculation is required. Based on the SURs calculated for groups 1 and 2, the amount of BOD due to these groups measured during the fed batch test should be negligible. The removal rate of these groups of compounds is great enough for complete removal at the relatively low loadings attained in the fed-batch test. By process of elimination, the substrate being measured during the fed-batch test must belong to group 3, in particular group 3b. If the BOD in the fed-batch test is assumed to be from group 3b, the removal rate of group 3b may be calculated (the feed rate of group 3b is known from figure 7.2.8 and the flow rate) and is presented in figure 7.2.13. This fraction has a large $K_M$ value and is probably composed of many miscellaneous substrates. The Monod constants calculated from the batch test, and from the respirometric tests on the batch test samples are summarised in table 7.2.

Table 7.2 Summary of kinetic constants of the various substrate groups in BKME

<table>
<thead>
<tr>
<th></th>
<th>Overall substrate</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3a</th>
<th>Group 3b</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD</td>
<td>235</td>
<td>46</td>
<td>97</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>$\Delta$OUR$_{MAX}$ batch test</td>
<td>0.0014</td>
<td>0.00067</td>
<td>0.00053</td>
<td>0.0013</td>
<td>-</td>
</tr>
<tr>
<td>$\Delta$OUR$_{MAX}$ respirometry</td>
<td>0.0013</td>
<td>0.00039</td>
<td>0.00094</td>
<td>0.00013</td>
<td>-</td>
</tr>
<tr>
<td>$Y$ batch test</td>
<td>0.66</td>
<td>0.86</td>
<td>0.6</td>
<td>0.56</td>
<td>-</td>
</tr>
<tr>
<td>$Y$ respirometry</td>
<td>0.66</td>
<td>0.44</td>
<td>0.44</td>
<td>0.6*</td>
<td>-</td>
</tr>
<tr>
<td>$\text{SUR}_{MAX}$ batch test</td>
<td>0.0038</td>
<td>0.0030</td>
<td>0.0054</td>
<td>0.00015</td>
<td>-</td>
</tr>
<tr>
<td>$\text{SUR}_{MAX}$ respirometry ($=\Delta$OUR/(1-$Y$))</td>
<td>0.0038</td>
<td>0.00078</td>
<td>0.002</td>
<td>0.00022</td>
<td>0.00013*</td>
</tr>
</tbody>
</table>

* assumed.

* this value was calculated from the fed-batch test.
Figure 7.2.13 SUR of wastewater fractions vs. the corresponding BOD. Group 1a (●), Group 1b (♦), Group 3a (▲), Group 3b (■), Group 2 (○). Batch L, SRT 5 days.

Figure 7.2.14 BOD vs. activated sludge loading for all of the wastewater fractions. Total BOD (—), Group 1a (—), Group 1b (—), Group 3a (—), Group 3b (—), Group 2 (—), based on OUR (—), measured BOD values (○)
The overall kinetics calculated from the batch test data agree with the overall kinetics calculated from respirometry. The kinetics of substrate groups 1 and 2 do not agree. Group one was removed at a greater rate than group 2 during the batch test. Opposite results were obtained by the respirometric method on the partially degraded samples from the batch test. There are two possible explanations for this observation. The first is that the removal rates were actually different between the two tests as they were done on separate days and the removal rates were highly variable (see section 7.3). The second explanation is that at the very high substrate concentrations at the start of the batch test the bacteria behave quite differently than they do at the lower substrate concentrations common in the respirometric method. It is possible that there is a relatively small quantity of substrate group 1 components present and high enough concentrations to saturate the enzyme systems are not attained in the respirometer. The very high yield calculated for group 1 during the batch test is puzzling and implies that not all of the substrate taken up at the start of the test is metabolised, but that some is stored.

Discussion of Significance of Findings

Using the data from figures 7.2.13 (SUR kinetics) and 7.2.8 (BKME composition), the BODs of the treated effluent and the SURs of the various fractions during the fed-batch test may be calculated (figures 7.2.14, 7.2.15, and 7.2.16). At the loadings employed during the fed-batch test, all of the BOD is due to the substrate from group 3 (figure 7.2.14). This will be the case until loadings greater than 4 mg BOD/mg MLVSS day are reached, when incomplete removal of substrates from groups 1 and 2 will occur. This loading is approximately 10 times the typical loading of the continuous
Figure 7.2.15 SUR vs. BOD for all of the wastewater fractions. Total SUR (-----), Group 1a (-----), Group 1b (-----), Group 3a (-----), Group 3b (-----), Group 2 (-----), based on OUR (-----), measured values (O).

Figure 7.2.16 SUR vs. loading for all of the wastewater fractions. Total SUR (-----), Group 1a (-----), Group 1b (-----), Group 3a (-----), Group 3b (-----), Group 2 (-----), based on OUR (-----), measured values (O).
lab scale activated sludge unit. In figures 7.2.15 and 7.2.16 the corresponding SUR's are plotted. The major contribution to the SUR comes from group 2. This is due to the fact that group 2 is the largest contributor to the overall effluent BOD, and has the fastest degradation kinetics.

If the organic compounds in BKME are assumed to be all the same substrate, and the removal rate is obtained from respirometry on the whole effluent (figure 7.2.1), the dashed line in figure 7.2.14 is obtained. No increase in treated effluent BOD is predicted until the loading reaches 4 mg BOD/mg MLVSS day. The BOD in the treated effluent will be underpredicted until very high loadings are reached, and what is coming out of the treatment plant is basically what is going in. Analogously, the SUR will be overpredicted due to the overprediction of the yield \( \text{SUR} = \frac{\text{OUR}}{(1 - Y)} \) (figures 7.2.15 and 7.2.16). The yield is overpredicted during respirometry because substrate group 3 exerts very little oxygen demand even though it comprises a fair portion of the BOD \( Y = 1 - \frac{\text{OC}}{S} \). If the BOD is treated as one compound, the BOD of substrate 3 is used in calculating the yield, and the predicted reaction rates are faster than the actual rates. This explains why the BOD in the treated effluent is underpredicted in figure 7.2.14 if the substrates in the effluent are assumed to have the same removal rates and stoichiometry.

The predicted SUR in figure 7.2.15 (dashed line) follows the Monod form since the calculations were based on a single substrate. However, for the other substrate groups, the SUR vs. total BOD in the treated effluent does not follow the Monod form. If the SURs were plotted against their corresponding substrates, the kinetics would follow the Monod form. The discrepancy is due to the changing nature of the BOD in the treated effluent. As the loading increases, substrate groups 1 and 2 are efficiently
removed, and the concentration of substrate group 3 increases. When the loading increases beyond 4 mg BOD/mg MLVSS day, the concentrations of substrate group 1 and 2 start to increase in the treated effluent. So, initially the BOD is predominately composed of substrate group 3, then ever increasing proportions of groups 1 and 2 are added. For the same reasons the total SUR vs. BOD also does not follow the Monod model. The half saturation constant calculated using the modified infinite dilution test would be approximately 30 mg BOD/l, which is significantly greater than the half saturation constants of the different compounds which make up the BOD.

Noting that since the removal rates of substrate groups 1 and 2 are similar, and greatly different from the removal rate of group 3, the kinetic analysis may be simplified by assuming that the wastewater is composed of just two fractions, the readily biodegradable fraction, groups 1 and 2, and the slowly biodegradable fraction, group 3. The readily biodegradable substrates may be considered those which are completely removed in the continuous activated sludge unit under normal loading conditions. Similarly, the slowly biodegradable ones are the substrates partially removed under the same conditions. There is approximately an order of magnitude difference between the removal rates of the readily and slowly biodegradable fractions.

In order to estimate the removal rates, the following procedure is recommended. First, the readily and slowly biodegradable components of the wastewater may be estimated using a batch test where both OUR and substrate are measured. Second, ΔOUR experiments may be used to measure the removal rate of the readily biodegradable fraction. Due to the strong influence of this fraction on respirometric determinations, this will be a reasonably accurate measurement. Third, the removal rate of the slowly
Continuous feed experiments are at a loading < 2

Figure 7.2.17 BOD vs. loading assuming two wastewater fractions. Arrow marks the loading of the lab scale units.

Figure 7.2.18 SUR vs. loading assuming two wastewater fractions.
biodegradable fraction may be calculated using the fed-batch test and assuming that all of
the measured BOD belongs to the slowly biodegradable fraction. At the flow rates used
in this study, the readily biodegradable substrates make up less than 5% of the BOD
coming out of the fed-batch test (this was verified by measuring the ΔOUR). The third
step will not be as accurate as the ΔOUR measurements in the determination of model
parameters, but these numbers are important for a more accurate determination of
effluent quality. The resulting kinetics are shown in figures 7.2.17 and 7.2.18.

The simplification of BKME to two substrates will not result in too much error
due to the large difference in removal rates between the two groups. As shown in figure
7.2.17, the slowly biodegradable fraction dominates the BOD in the treated effluent under
normal loading conditions. Figure 7.2.18 shows that the metabolic activity of the
biomass measured during the ΔOUR test will be due almost exclusively to the readily
biodegradable substrate. This is especially true in light of the fact that the yield on the
slowly biodegradable fraction is greater than the yield on the readily biodegradable
fraction. Depending on the particular situation (wastewater composition and removal
rates), some intermediate biodegradable organics may behave as readily biodegradable
under low loading conditions, but will start to be incompletely removed at intermediate
loadings. As the loading increases, these substrates will switch from the readily
biodegradable fraction to the slowly biodegradable fraction.

The importance of the ratio of readily biodegradable organic matter to slowly
biodegradable organic matter of a batch of wastewater is shown in figure 7.2.19. The
data in this figure was calculated assuming the maximum substrate uptake rate of the
readily biodegradable component is 0.0018 mg BOD/Img MLVSS minute, with a half
Figure 7.2.19  BOD vs. loading assuming two wastewater fractions, effect of wastewater composition.
saturation constant of 0.9 mg BOD/l, the maximum substrate uptake rate for the slowly biodegradable component is 0.0003 mg BOD/mg MLVSS minute with a half saturation constant of 7.5 mg BOD/l, and a wastewater BOD of 180 mg/l. If there is no slowly biodegradable fraction present, there will have to be a significant increase in the loading (to near 4 mg BOD/mg MLVSS) to the activated sludge unit before an increase in effluent BOD will be noticed. If the wastewater is composed solely of slowly biodegradable material, an increase in effluent BOD will be evident as soon as the loading is increased. For the wastewater studied, with a typical batch being composed of 60 to 80% readily biodegradable matter, an increase in loading will only result in slight increases in effluent BOD, and this BOD will belong mostly (>95%) to the slowly biodegradable component of the organic matter in the wastewater.

Assuming one wastewater fraction when there are two or more with greatly different kinetics will result in error in predicting the performance of continuous activated sludge units based on batch test and ΔOUR kinetic data. However, if the yields of the various substrates are equal, then the SUR and BOD in the continuous system may be accurately determined by measuring the OUR in the continuous system. The SUR is equal to the OUR divided by (1-Y). If the SUR is known, the BOD in the treated effluent may be calculated using a simple mass balance ($S = S_0 - \theta_h \times SUR$). Unfortunately, if the yields of the various compounds are different as with BKME, then the simple relationship between OUR and SUR, and hence between OUR and BOD, is no longer valid. Under these conditions the OUR data become difficult to interpret, and it is best to use the fed-batch data for modeling the wastewater as one substrate. This approach will give adequate predictions of the treated wastewater BOD as long as the loading does not
surpass the removal rate of the readily biodegradable components (i.e. for the data presented here, loading < 4 mg BOD/mg MLVSS).

If the batch data is analysed using the multicomponent model according to the method of Grau et al (1975) and this equation is applied to a continuous system using the method of Argaman (1991), the BOD in the treated wastewater is overpredicted (figure 7.2.3). For BKME, near the end of the batch test, at low substrate concentrations, the remaining substrates have low removal rates. This is reflected in the multicomponent model coefficients. At low loadings the substrate concentration coming out of the continuous system is low, so the batch test predicts low substrate removal rates. This is not the case. A very small portion of the substrate will belong to the readily biodegradable group. This group has a small $K_M$-value and even at low concentrations has a high removal rate. Consequently, the overall removal rate will be greater than that predicted by the multicomponent model. The predictive power of the multicomponent model decreases when there is a large difference in the removal rates of the compounds removed at the start of the batch test and those removed at the end of the batch test. The multicomponent model is dependent on the ratios of the various substrates.

7.3 Variability of Kinetic Coefficients and Wastewater Composition

Batch Tests

Comments

The wastewater batch used to obtain the results discussed in section 7.2 was batch L. Variability in the degradation rates and stoichiometry of different wastewater batches was observed over the course of this project. In this section, respirometric data and batch
Figure 7.3.1 OUR (■) and BOD (●) vs. time during a batch BKME biodegradation test. Batch M, SRT 5 days.

Figure 7.3.2 OUR profiles for injections of samples withdrawn during the batch test (figure 7.3.1). Samples from top to bottom (sample time in brackets): #1 (0 minutes), 2 (10), 4 (30), 6 (50), 10 (120), 14 (300). Batch M, SRT 5 days.
Figure 7.3.3 Batch test samples \( \Delta \text{OUR} \) vs. fraction 1 BOD (■), and vs. fraction 2 BOD (●) (figure 7.3.1). Batch M, SRT 5 days.

Figure 7.3.4 Batch test samples OC vs. fraction 1 BOD (■), and vs. fraction 2 BOD (●) (figure 7.3.1). Batch M, SRT 5 days.
test data for a number of other wastewater batches will be presented in order to demonstrate the variability in the wastewater composition and the microbial kinetics.

Data from wastewater batch M is shown in figures 7.3.1, 7.3.2, 7.3.3, and 7.3.4. The OUR profile in figure 7.3.1 has the same general shape as the one in figure 7.2.2, but the second OUR plateau was lower and lasted longer. The BOD removal during the batch test followed the multicomponent model. The BKME batch appeared to be composed of at least three different groups of substrates. The first substrate was removed during the first 50 minutes of the batch test. As the OUR steadily dropped during this time, the first substrate either had a very large half saturation constant, or was actually composed of many different organic substrates. The second OUR plateau was most likely due to the oxidation of just one substrate, probably methanol. Samples were withdrawn during this batch test and the removal rates of the samples were measured using the ΔOUR method. Examples of OUR profiles of various samples are presented in figure 7.3.2. From these curves, it is evident that the first substrate was removed by the time the sixth sample was taken. This corresponds to the time at which the OUR dropped during the batch test (figure 7.3.1). The ΔOUR data (figure 7.3.3), and the oxygen consumption data (figure 7.3.4), for the two substrates were calculated and support the hypothesis that the first substrate was formic acid and the second was methanol. At the time of this batch test, the yield on formic acid was lower than the yield on methanol, so greater oxygen consumption on formic acid oxidation, as was found for substrate one, is expected. Also, for the set of biomass used in the batch test, the formic acid oxidation rate was significantly greater than the methanol oxidation rate as determined in a separate experiment using the pure substrates. This batch of wastewater had a similar composition
Figure 7.3.5 OUR (■) and BOD (○) vs. time for a batch BKME biodegradation test. Batch N, SRT 15 days.

Figure 7.3.6 OUR profiles for injections of samples withdrawn during the batch test (figure 7.3.5). Samples from top to bottom (sample time in brackets): #1 (0 minutes), 2 (9), 3 (20), 5 (40), 7 (60), 8 (75). Batch N, SRT 15 days.
Figure 7.3.7 Batch test samples $\Delta$OUR vs. BOD (sample time in minutes). Sample #1 (0 minutes) (■), sample 2 (9) (●), sample 3 (20) (▲), sample 4 (30) (●), sample 5 (40) (□), sample 6 (50) (○). Figure 7.3.5, batch N, SRT 15 days.

Figure 7.3.8 OUR (■) and BOD (●) vs. time for a batch BKME biodegradation test. Batch O.
to the batch discussed in section 7.2, but the kinetics were considerably different. Methanol, which is normally removed at a high rate, was removed a relatively slow rate. For this batch, and this set of biomass, methanol behaved as a slowly biodegradable substrate. Consequently, batch M had a large slowly biodegradable fraction, but this was due more to the biomass composition than the wastewater.

The next batch investigated in detail was batch N (figures 7.3.5, 7.3.6, 7.3.7). The OUR during the batch test dropped suddenly four times, meaning that this batch was composed of at least five different substrates (figure 7.3.5). This batch had enough of the different readily biodegradable substrates to see distinct drops in the OUR profile rather than a steady decline as for the first substrate in the previous two batches discussed. The BOD removal followed the multicomponent model as expected for multiple substrate wastewaters. Samples removed during the batch test were analysed using the respirometric method. Example OUR profiles on the samples (figure 7.3.6) show that it is very difficult to differentiate between the different substrates. The starting concentration in the ΔOUR tests on the whole wastewater (sample 1) was much less than the starting concentration during the batch test so the sudden drops in the OUR were not noticeable, and the OUR gradually declined to the baseline. Due to the complexity of this batch, the entire readily biodegradable substrate was treated as one compound with the removal rates as shown in figure 7.3.7. This will be adequate to model the continuous system until very high loadings are attained. The slowly biodegradable fraction of this batch was smaller than the others investigated.

The composition of batch O (figures 7.3.8, 7.3.9, 7.3.10) also appeared to be different from the other batches investigated. All of the readily biodegradable organics
Figure 7.3.9 OUR profiles for injections of samples withdrawn during the batch test (figure 7.3.8). Samples from top to bottom (sample time in brackets): 2 (10 minutes), 3 (20), 4 (30). Batch O.

Figure 7.3.10 Batch test samples ΔOUR (■), and OC (○) vs. BOD. Figure 7.3.8, batch O.
were removed during the first hour of the batch test, and there was only one major drop in the OUR, implying that there was only one major readily biodegradable substrate. The readily biodegradable substrate was probably composed of methanol, formic acid, and other compounds (Kringstad and Lindström 1984), but these compounds were present in just the right ratio to compensate for the different removal rates and therefore to disappear from the batch test at more or less the same time. With the given data it is impossible to separate the readily biodegradable substrate into various fractions. The OUR profiles (figure 7.3.9) from several ΔOUR experiments on different samples from the batch test had a slight shoulder indicating the presence of several substrates which appeared to be removed simultaneously. The readily biodegradable component may be treated as one substrate, with removal rates as presented in figure 7.3.10. In this instance, the ΔOUR was better approximated with the Powell equation than the Monod equation.

The last batch investigated in detail was batch Q (figures 7.3.11, 7.3.12, 7.3.13, 7.3.14, 7.316, 7.3.16). Once again, the substrate removal during the batch test could be approximated with the multicomponent model, and the OUR profile suggests there were at least three different substrates, with a large slowly biodegradable fraction (figure 7.3.11). In order to verify the use of the BOD test as an adequate measure of the substrates during a batch test, the BOD kinetics were measured on a few samples (figure 7.3.12). If the wastewater composition was changing throughout the batch test, BOD may not be an adequate measure of the wastewater strength. It is possible that the BOD test will measure a greater percentage of the substrates in the first sample than in subsequent samples. Figure 7.3.13 demonstrates that the BOD₅, BOD₉, COD, and OC (during the ΔOUR test) all behaved identically. The BOD test measured the same
Figure 7.3.11  OUR (■) and BOD (●) vs. time for a batch BKME biodegradation test. Batch Q, SRT 15 days.

Figure 7.3.12  15 day BOD data for batch test samples (sample time in brackets). Sample 1 (0 minutes) (■), sample 2 (10) (●), sample 6 (50) (▲), sample 10 (105) (●), sample 11 (135) (□), sample 14 (○), BOD out (△). (figure 7.3.11), batch Q, SRT 15 days.
Figure 7.3.13 BKME biodegradation batch test (figure 7.3.11). BOD(5) (■), BOD(u) (●), OC (▲), and COD (◆) vs. time. Batch Q, SRT 15 days.

Figure 7.3.14 OUR profiles for injections of samples withdrawn during the batch test (figure 7.3.11). Samples from top to bottom (sample time in brackets): #1 (0 minutes), 3 (20), 5 (40), 7 (60), 9 (90). Batch Q, SRT 15 days.
Figure 7.3.15 Batch test samples, ΔOUR vs. BOD (sample time in brackets). Sample 1 (0 minutes) ( ), sample 2 (10) (○), sample 3 (20) (△), sample 4 (30) (●), sample 5 (40) ( ), sample 6 (50) (○), sample 7 (60) (△), sample 8 (75) (○), sample 9 (90) (★), sample 10 (105) (▲). (figure 7.3.11), batch Q, SRT 15 days.

Figure 7.3.16 Batch test samples OC vs. fraction 1 BOD (■), and vs. fraction 2 BOD (○) (figure 7.3.11). Batch Q, SRT 15 days.
fraction of substrates for samples throughout the batch test, within the error of the measurements. The \( \text{BOD}_u \) was not measured during the other batch tests, but the \( \text{COD} \) always correlated very closely with the \( \text{BOD} \). Sample OUR profiles from the \( \Delta\text{OUR} \) assay are difficult to interpret (figure 7.3.14), but demonstrate that all the substrates were removed simultaneously. The \( \Delta\text{OUR} \) (figure 7.3.15) reveals the presence of two main substrate groups, with removal rates very similar to what would be expected from examining the OUR profile of the batch test. Once again, the first substrate appeared to be composed of formic acid and a few other compounds, and the second substrate was methanol. This was verified by the yields (figure 7.3.16). Note that for this set of biomass the yield on formic acid was greater than the yield on methanol which is the opposite to the biomass used to test batch M. This was confirmed with independent \( \Delta\text{OUR} \) tests using methanol and formic acid, and may be explained by differences in metabolic pathways. For growth on formic acid and methanol, several different metabolic pathways are possible, and the efficiencies of the different pathways are quite different.

In section 7.2, the removal rates obtained for the wastewater fractions from the batch test, and the removal rates determined by respirometry on the samples from the batch test were not the same (table 7.2). This may have been due to changes in the biomass between the two sets of measurements, or the different initial substrate concentrations. For the wastewater batches discussed in this section, the removal rates obtained from the batch tests and the respirometric method were in much better agreement.
Figure 7.3.17 OUR vs. time for BKME biodegradation tests. Batch A (---), batch B (--), batch J (--), batch K (--), batch L (--).

Figure 7.3.18 OUR vs. time for BKME biodegradation tests. Batch L (---), batch M (--), batch N (--), batch O (--), batch Q (--).
**Figure 7.3.19** BOD vs. time for BKME biodegradation tests. Batch A (■), batch B (○), batch J (●), batch K (□), batch F (▲).

**Figure 7.3.20** BOD vs. time for BKME biodegradation tests. Batch L (■), batch M (○), batch N (▲), batch O (●), batch Q (□).
Other batches were not investigated in as much detail, but serve to illustrate the variability of the wastewater composition and microbial kinetics. The OUR profiles for a number of different batch tests are presented in figures 7.3.17 and 7.3.18 for comparison. The OUR profiles give an indication of the composition of the wastewater, and also of the removal rates of the various substrate fractions. The relative amounts of the various fractions can be estimated by the areas under the different sections of the OUR profile and appeared to vary from batch to batch. Some batches of wastewater behaved as if they contained three distinct fractions (B, N), others as if there were two (A, J, L, M, Q), and others as if there was just one (K, O) readily biodegradable fraction. The BOD measurements corresponding to the OURs are plotted in figures 7.3.19 and 7.3.20, and serve as a verification of the OUR data. The OUR and BOD profiles for the different batch tests were quite different. This is due to both the changing composition of the wastewater from batch to batch, as well as changes in the biomass.

The relative oxidation rates of the different fractions can be compared by comparing the OURs at different points during the batch tests. The initial OUR varied from 1 to 3.8 mg/l minute. This variation is probably due more to the differences in biomass than the differences between the wastewater batches, although at least one batch (K) did not seem to have this fraction of the wastewater. The OUR profile of the first fraction of wastewater sometimes declined gradually to the OUR plateau due to the second substrate group (M). This implies a large $K_M$ value, or more likely, that this fraction is composed of many different substrates. In many of the batches the first fraction to be removed was small, and was removed from the batch test within the first twenty minutes. In these cases, this fraction will dominate the measurement of the
removal rates by batch tests where the initial OUR is measured, and result in erroneously high rate measurements. This first wastewater fraction is probably due to formic acid oxidation. In cases where there appear to be many substrates in this fraction, the other substrates are probably other small molecular weight organic acids. The highest OUR does not necessarily correspond to the largest fraction or the one with the greatest removal rate.

The second OUR plateau in the batch tests usually lasted longer than the first plateau and remained constant until a sudden drop at the end. This second substrate group appears to be the dominant substrate in BKME, in terms of quantity. In most cases this OUR is probably due to methanol oxidation. For one batch (O), there was no second OUR plateau. The organic acids and methanol were present in the right proportion to be removed at the same time during the batch test. The OUR of the second plateau varied greatly from batch to batch, implying that the rate and yield of methanol oxidation varied from batch to batch.

Comparison of the BOD and OUR curves reveals that generally the lower the OUR, the slower the rate of BOD removal, as expected. The batch with the slowest BOD removal had an initial OUR that was comparable to that of the other batches, but the second OUR plateau was very low. All of the BOD removal curves followed the multicomponent model, with $n \sim 1.5$ for most of the batches. For the batch with the lowest BOD removal rate, $n=1$. The BOD remaining after the OUR dropped close to the baseline value gives an indication of the amount of slowly biodegradable substrate in the wastewater. The variability of the multicomponent model coefficients implies that the wastewater composition was changing from batch to batch.
Caustic Extraction Effluent

A batch test with the measurement of OUR was performed using the first caustic extraction stage effluent (figure 7.3.21). This process stream is high in BOD, and one of the major sources of readily biodegradable organics in BKME. The high strength of this wastewater made it possible to determine the various substrate groups and to test the assumption that the readily biodegradable substrates are removed simultaneously during a batch test. In order to avoid adding substrates during the batch test, large samples were removed at two points during the batch test. These samples were then used to resuspend fresh biomass, and large amounts of methanol and formic acid were added.

After the drop in the OUR at 120 minutes, the addition of methanol caused no increase in the OUR, implying that the substrate which was being oxidised at that point was methanol. However, the addition of a large amount of formic acid at this point caused the OUR to increase from 0.8 to 1.2 mg/l minute. Formic acid caused an increase of 0.4 mg/l minute in the OUR in a separate test when no effluent was present. These results imply that the first substrate was formic acid, and was used simultaneously with the second substrate, methanol. The first substrate was mainly formic acid, but formic acid can not account for an OUR as high as 1.9 mg/l minute, so other substrates must be present. These other substrates are probably small molecular weight organic acids. Because the OUR was much higher than can be explained by the oxidation of formic acid, these compounds must be utilised simultaneously. The slow decrease in OUR to the level that is due to formic oxidation implies that there were many different substrates being removed.
Figure 7.3.21 Batch test, OUR vs. time. Caustic extraction effluent.

Figure 7.3.22 Slowly biodegradable BOD removal rates measured by the fed-batch test vs. slowly biodegradable BOD. Batch L (■), batch M (○), batch N (▲), batch O (●).
The OUR drop at -260 minutes is due to the disappearance of methanol. Adding large amounts of methanol after this point caused the OUR to increase back to 0.8 mg/l minute (an increase of 0.4 mg/l minute). However, methanol caused an increase of 0.6 mg/l minute in the OUR in a separate test when no effluent was present. The fact that the addition of methanol to the batch test sample from -260 minutes did not increase the OUR by 0.6 mg/l minute means that either the yield of the remaining substrate and methanol mixture was different than expected (see section 6.3), or the methanol and the remaining substrate were utilised sequentially. If the yield was changing, the remaining substrate was probably acetate. A more likely explanation is that the remaining substrate was being utilised sequentially, since methanol alone causes the OUR to increase from 0.2 to 0.8 mg/l minute. Formaldehyde may be present in the effluent, or it may be a byproduct of the metabolism of other substrates. Methanol and formaldehyde may share a common biodegradation path in the activated sludge used for these tests, so sequential oxidation would be expected, as found for the special case of methanol and formic acid in section 6.3.

In summary, the first major substrate in the batch test may have been formic acid (and related compounds), and the oxidation of these substrates is independent of the other substrates present. The second major substrate may have been methanol, and its oxidation appears to delay the oxidation of the third substrate group, probably formaldehyde. These results demonstrate that the assumption of simultaneous removal is appropriate for most of the readily biodegradable compounds, even when the initial BOD is very high. However, a few substrates will be removed sequentially.
Slowly Biodegradable Kinetics

The kinetics of the slowly biodegradable fraction were determined for four of the wastewater batches using the infinite dilution / fed batch test (figure 7.3.22). Due to the difficulty in obtaining the data, and the scatter in the results, the Monod model was assumed for the purposes of data fitting. There is not enough data to attempt to fit more detailed models.

For three of the batches tested (L,N,O), the removal rates of this fraction were very similar. The slowly biodegradable removal rates were approximately an order of magnitude less than the readily biodegradable removal rates. These batches had similar wastewater compositions, dominated by the readily biodegradable fraction, but as pointed out earlier, the organics in the treated effluent (under normal loading) will belong to the slowly biodegradable fraction.

For the fourth batch, M, the removal rate of the slowly biodegradable fraction was twice the removal rate found for the other wastewater batches. For this set of biomass, the removal rate of methanol was low enough for it to show up during the infinite dilution / fed batch test and to be classified as a slowly biodegradable substrate. The low removal rate of methanol during the treatment of this wastewater batch was verified by independent batch assays using methanol as the sole substrate. However, the removal rate of methanol was still larger than the removal rate of the other slowly biodegradable compounds, and thus dominated the measurement of the slowly biodegradable removal rate. If the other slowly biodegradable substrates could be separated from the methanol, the removal rates would probably be similar to the other three batches studied. As a consequence of grouping the methanol into the slowly biodegradable fraction, this
fraction was larger than the readily biodegradable fraction. The readily biodegradable fraction contained only formic acid and similar compounds. The biodegradation rates of this fraction were an order of magnitude greater than the slowly biodegradable removal rates. Figure 7.3.22 indicates that an increase in loading during the treatment of batch M will result in much larger BOD in the treated wastewater than during the treatment of the other batches, emphasizing the importance of the ratios of wastewater fractions (figure 7.2.17).

The slowly biodegradable fraction of BKME is undoubtedly composed of many substrates; the kinetics shown in figure 7.3.22 will be dominated by the substrate with the greatest removal rate. Since the removal rates are low, these substrates may be grouped into one fraction without too much error.

7.4 Operating Conditions & Adaptation

In the previous section, the results of batch tests and fed-batch tests were presented. These results showed the variability of the wastewater composition from batch to batch, as well as the variability of the microbial kinetic coefficients. In this section the results of the ΔOUR tests performed over the course of this project will be presented. The ΔOUR test is comparatively easy to perform, and consequently much more data was collected than from the batch tests. The data also demonstrates the variability of the wastewater, and the variability of the kinetic coefficients. In addition, the adaptation of the bacteria to the different wastewater batches, and the effect of different operating conditions on the microbial kinetic coefficients were investigated.
ΔOUR Coefficient Variability

A summary of the ΔOUR kinetic coefficients measured on the different wastewater batches over the course of this project will be presented. The ΔOUR vs. day of operation is presented in figure 7.4.1; the oxygen consumption data is presented in figure 7.4.2. The maximum rates and stoichiometry of the two lab scale activated sludge reactors followed the same trends over time.

The data is easier to examine when graphed vs. wastewater batch instead of time (figure 7.4.3). The maximum ΔOUR for the biomass from both reactors followed the same trends. The rates were low during the treatment of batch E, and high during the treatment of batch L. The maximum substrate uptake rate of biomass grown on and treating batch L was 1.6 times greater than the biomass grown on and treating batch M. This is a statistically significant difference. The operating conditions of the activated sludge unit were identical throughout the treatment of these two batches (SRT of 10 days, HRT of 12 hours). Inspection of figure 7.4.3 reveals that the wastewater batch being treated seems to have a greater effect on the maximum ΔOUR than the operating conditions (SRT / aerobic selector). The two activated sludge units were fed the same batches of wastewater at the same time, and the maximum ΔOUR of the two different units followed the same trends as the batches of wastewater changed regardless of their different operating conditions. This is due to both the different characteristics of the wastewater batches and to the different populations of microorganisms that are selected by these different batches. The reason for the high maximum ΔOUR during the treatment of batch L is unknown. For the rest of the batches the maximum ΔOUR was approximately the same. It is interesting to note that the ΔOUR test did not indicate the
Figure 7.4.1 Maximum ΔOUR vs. time over the course of the project. Reactor 1 (■), reactor 2 (○). Letters represent wastewater batches, solid vertical lines mark boundaries between experimental runs.

Figure 7.4.2 OC vs. time over the course of the project. Reactor 1 (■), reactor 2 (○). Red line represents BOD. Letters represent wastewater batches, solid vertical lines mark boundaries between experimental runs.
Figure 7.4.3 Maximum ΔOUR and K vs. wastewater batch over the course of the project. Reactor 1 maximum ΔOUR ( ■ ), K ( □ ), reactor 2 maximum ΔOUR ( ● ), K ( ○ ).

Figure 7.4.4 OC ( ▲ ) and BOD (bars) vs. wastewater batch over the course of the project. Shaded area represents slowly biodegradable BOD (where measured).
possibility of poor performance during the treatment of batch M if the loading were to increase (large slowly biodegradable component).

The wastewater composition varied from batch to batch (figure 7.4.4). This variation partially explains the variation in the oxygen consumption data. As the BOD of the wastewater increased, the oxygen consumption during respirometric tests also increased, as expected. Linear regression of the short term ΔOUR oxygen consumption vs. the wastewater BOD (long term oxygen consumption) yields a correlation coefficient of 0.79 (figure 7.4.5). The slope is 0.46. For every milligram of BOD added to the respirometer, 0.46 mg was oxidised. The slowly biodegradable fraction, approximately 30% of the BOD will not be oxidised during the ΔOUR test. 0.24 mg of the readily biodegradable fraction (1 mg - 0.3 mg - 0.46 mg) were not oxidised during the ΔOUR test, which implies an average yield of 0.34 on this fraction (0.24/(0.46+0.24)=0.34). A quick way to estimate the untreated wastewater BOD is to do a ΔOUR test and divide the OC by 0.46. This method is valid for wastewaters which have a similar ratio of slowly to readily biodegradable organics as the wastewater used in this study, and for wastewaters with similar yields.

The strength of the different wastewater batches varied greatly, from 110 to 280 mg BOD/l, but the overall composition did not vary as much. The slowly biodegradable fraction usually made up 25% of the BOD. The large apparent difference in composition of batch M compared to the other batches is due to the methanol component being grouped into the slowly biodegradable fraction for this particular wastewater batch. The actual composition of this batch was similar to the composition of the other batches. The readily biodegradable fraction usually comprised 75% of the wastewater BOD. The data
presented here does not distinguish between the various fractions making up the readily biodegradable component (i.e. methanol, formic acid, and other undefined low molecular weight components), but as discussed in the previous section, the composition appeared to vary from batch to batch.

Variability of Single Substrate Kinetic Coefficients

For the wastewater batches treated in the second half of the study, respirometric tests were performed using methanol, formic acid, and acetic acid as substrates. These are the main components of BKME BOD. The $\Delta$OUR data is presented in figure 7.4.6; the yield data is presented in figure 7.4.7. Unlike the situation when measuring the $\Delta$OUR using BKME, the respirometric yield on methanol, formic acid, or acetic acid, is easily calculated. By providing a constant substrate over time (methanol, formate, or acetate as opposed to BKME which is variable), the variability of the biomass from day to day may be followed.

For clarity, the $\Delta$OUR data is plotted versus wastewater batch for methanol (figure 7.4.8), formic acid (figure 7.4.9), and acetate (figure 7.4.10). The acetate data show very little variation during the treatment of batches L to S. The yield on acetate is also constant during this period (figure 7.4.7). The situation is different for the methanol and formic acid oxidation rates. The removal rate of both substrates increased during the treatment of batch L. For the other batches the removal rates varied, but not as much.

An interesting observation is that although the overall $\Delta$OUR remained constant for most of the wastewater batches, the $\Delta$OUR due to methanol and formic acid fluctuated. For example, during the treatment of batches K, M, and N, the overall $\Delta$OUR was the same. During the treatment of batch K, the majority of this $\Delta$OUR came from
Figure 7.4.5  Respirometric OC vs. wastewater BOD for all of the wastewater batches studied.

Figure 7.4.6  Maximum ΔOUR vs. time over the course of the project. Methanol (■,○), formic acid (▲,▼), acetate (●,○). Reactor 1 filled points, reactor 2 open points. Letters represent wastewater batches, solid vertical lines mark boundaries between experimental runs.
Figure 7.4.7 Respirometric yield vs. time over the course of the project. Methanol (■, □), formic acid (▲, △), acetate (●, ○). Reactor 1 filled points, reactor 2 open points. Letters represent wastewater batches, solid vertical lines mark boundaries between experimental runs.

Figure 7.4.8 Maximum methanol ΔOUR vs. wastewater batch over the course of the project. Reactor 1 (■), reactor 2 (●).
Figure 7.4.9 Maximum formic acid ΔOUR vs. wastewater batch over the course of the project. Reactor 1 (■), reactor 2 (●).

Figure 7.4.10 Maximum acetate ΔOUR vs. wastewater batch over the course of the project. Reactor 1 (■), reactor 2 (●).
methanol oxidation. During the treatment of batch M, the majority of the \( \Delta \text{OUR} \) came from formic acid oxidation (although there was still plenty of methanol in the wastewater). Finally, during the treatment of batch N, the contribution to the overall \( \Delta \text{OUR} \) was equal for methanol and formic acid. This observation becomes evident when the \( \Delta \text{OUR}_{\text{MAX}} \) due to methanol is compared to the \( \Delta \text{OUR}_{\text{MAX}} \) due to formic acid (figure 7.4.11). During the treatment of batch K, this ratio is greater than one, during the treatment of batch M it is less than one, and is approximately one during the treatment of batch N. These results clearly demonstrate the changing microbial populations with time in the lab-scale activated sludge units.

The microbial population appeared to be dependent on the wastewater composition, as it appeared to vary with the wastewater batch, and often followed the same trend in the two units.

The yield on methanol and formic acid also varied from day to day. The ratio of the yield on methanol to the yield on formic acid is presented in figure 7.4.11. The yield on these compounds can change quite rapidly. The ratio of the yields generally followed the ratio of the \( \Delta \text{OURs} \). Low yields on one-carbon compounds are probably due to energy spilling. On days where there are fewer enzymes available for utilising methanol for growth, it can be expected that more of the methanol will be wasted than on days when there are more enzymes available for utilising the methanol for growth. This will result in a lower yield. These results present further evidence for the variability of the microbial population with time.
Figure 7.4.11 Ratio of methanol ΔOUR to formic acid ΔOUR: Reactor 1 (●), reactor 2 (■). Ratio of methanol yield to formic acid yield: Reactor 1 (○), reactor 2 (□). Letters represent wastewater batches, solid vertical lines mark boundaries between experimental runs.

Figure 7.4.12 Maximum ΔOUR vs. SRT for all of the wastewater batches studied. Reactor 1 (■), reactor 2 (●).
Effect of SRT / Selector on Kinetic Coefficients

Figure 7.4.12 shows the relationship between the removal rates of the readily biodegradable component of the wastewater as measured with the ΔOUR assay and the activated sludge operating conditions. These values are averages of the respirometric assays, previously presented in figures 7.4.1 and 7.4.3, for each of the 18 batches treated over the course of this project. As can be seen, the removal rates did not change very much with changing operating conditions. The exceptionally high values at an SRT of 10 days corresponded to the treatment of batch L. For activated sludge operated at SRT's between 5 and 20 days the readily biodegradable kinetics can be considered constant at $q_{\text{MAX}} = 0.0025 \pm 0.0005 \text{ mg BOD/mg biomass minute}$, and $K_M = 1.2 \pm 0.3 \text{ mg BOD/l}$. The removal rates measured on the unit with a selector, at an SRT of 10 days, were also within this range.

The removal rates of the biomass taken directly from the full scale activated sludge plant at Harmac Pacific Ltd. fall within the variation of the values measured in the lab scale units. The wastewater was slightly different between the mill unit and the lab scale unit in that the lab scale unit did not receive foul condensates. The foul condensates wastewater stream is highly concentrated and can account for up to 30% of the overall BOD (mainly methanol) going to the mill treatment system. When the removal rates of the mill's biomass were measured, they were measured without foul condensates present.

The ΔOUR using methanol, formic acid, and acetate as substrates are presented in figure 7.4.13. Similar to the results obtained using wastewater, no dependence on SRT was observed. The addition of a selector had no noticeable effect on the ΔOUR. When using biomass from Harmac Pulp and Paper Ltd.'s activated sludge aeration basin, the
ΔOUR for methanol metabolism was slightly higher than the average value obtained from the lab biomass over the course of the project. The ΔOUR obtained with acetate and formic acid were similar to the values measured using the lab biomass. The yield on acetate was lower for the biomass from Harmac than for the lab biomass. The yields on methanol and formic acid were similar. No noticeable trend in biomass yields as measured by the ΔOUR assay was observed with varying SRT.

Differences in kinetic coefficients at different SRT's were not observed in this study due to the operating conditions of the activated sludge units, most importantly the HRT of 12 hours. This resulted in a low loaded activated sludge unit, and it is possible that the differences between operating at an SRT of 5 or 15 days may only show up at higher loadings. Another explanation is the nature of the respirometric test. This test measures the maximum capability of the bacteria to oxidise substrate. It has already been discussed that when bacteria are grown under starvation conditions, the catabolic and anabolic processes become uncoupled. The bacteria maintain the enzymes for catabolising excess substrate, but will waste the excess energy until they can synthesise the necessary anabolic enzymes. An assay for the catabolic activity might be expected to show less variation with SRT than an assay for anabolic activity. A third explanation for the lack of effect of the SRT on the ΔOUR results is the non-specific nature of the assay. As discussed in the previous section, sometimes when the ΔOUR due to methanol decreased, the ΔOUR due to formic acid increased, maintaining a constant total ΔOUR.

Other studies using respirometric techniques have found large differences between the kinetic coefficients of activated sludge from units with and without selectors. These studies have found that the presence of a selector increased the initial OUR in
batch tests. In this study, no difference was found in the ΔOUR kinetic coefficients whether or not there was a selector. This may be explained by Argaman's (1991) argument that activated sludge removal rates are the same regardless of reactor hydraulics because the rates are actually a summation of many different zero order reactions. The arguments given to explain the similarity of kinetic coefficients at different SRT's also applies to this case.

One major difference when a selector was present was a decrease in the yield. In the unit that was operated with an aerobic selector, there was much less biomass than in the CMAS unit. Most studies on the effect of selectors have found an increase in yield when a selector was employed, but these studies dealt with different wastewaters. In BKME, a large proportion of the biodegradable organic material is methanol and formic acid. These compounds are good energy sources, but poor growth substrates. Under ideal conditions, the bacteria will oxidise the methanol and formic acid for energy, while using the other substrates for growth. When a selector is present, the initial concentration of methanol and formic acid in the selector will be high. If the biomass does not have enough anabolic enzymes present to utilise this energy for growth, this will prompt the biomass to waste a significant proportion of this generated energy. Then, in the main aeration basin, the majority of the energy source has already been removed, forcing the biomass to oxidise a portion of the other substrates for energy. These substrates would have otherwise been used for growth. This results in a lower yield. BOD measurements across the selector revealed that the readily biodegradable substrate was completely removed in the selector.
Figure 7.4.13 Maximum ΔOUR vs. SRT for all of the wastewater batches studied. Methanol (■, □), formic acid (▲, △), acetate (●, ○). Reactor 1 filled points, reactor 2 open points.

Figure 7.4.14 OUR profiles for 5 ml injections of wastewater during biomass acclimation to batch L. Day 0 (——), day 5 (———), day 7 (———), day 16 (———), and day 22 (———).
A second explanation for the lower yield is a greater decay rate in the system with a selector. The majority of the BOD is removed in the selector, consequently the biomass is exposed to a lower substrate loading in the main aeration basin than the biomass in the unit without a selector. These greater starvation conditions will lead to an increased endogenous metabolism and microbial decay.

Adaptation to Different Batches of Wastewater

The variability of the wastewater and the microbial kinetic coefficients have already been discussed. The variability of the parameters is not correlated to the changing operating conditions, but seems to be more related to the specific wastewater batch being treated. Different wastewater batches seemed to select different microbial populations (as evidenced by the changing kinetic parameters for methanol, formic acid, and acetic acid). Upon changing wastewater batches, the biomass took several days to adapt to the new batch, during which time the kinetic parameters gradually changed (a sudden change in the wastewater composition causes a delayed change in the biomass composition). In this section the adaptation of the biomass to various effluent batches (L, M, and N) will be discussed.

During the treatment of batch L, the maximum ΔOUR was initially 2.5 mgO₂/l minute. Within a few days of treating this wastewater batch, the maximum ΔOUR rose to 3.5 mgO₂/l minute and stayed at this level for a month (figure 7.4.14). Towards the end of the treatment of this wastewater batch, the maximum ΔOUR decreased back to 2.5 mgO₂/l minute. This was the wastewater batch with the high ΔOUR. At least part of the ΔOUR response may be attributed to a high ΔOUR on methanol. At the start of the treatment of batch L, the ΔOUR was “normal”, and took a few days to rise to the high
values as the biomass responded to the wastewater. These results seem to indicate that the high ΔOUR was due to changing biomass rather than a different wastewater. The response to this batch of wastewater was similar for both activated sludge units (figure 7.4.1) despite the different operating conditions.

During the treatment of the next wastewater batch (M), the ΔOUR continued to decrease. At the start of batch M, the ΔOUR was the same as the ΔOUR on batch L (figure 7.4.15). This is despite the difference in BOD of the two batches (batch L: BOD = 240 mg/l; batch M: BOD = 210 mg/l). As the biomass adapted to batch M, the ΔOUR decreased to approximately half of the original value. Sample ΔOUR profiles are presented in figure 7.4.16. Initially the ΔOUR rose and then decreased rapidly. Towards the end of the treatment of batch M, the ΔOUR declined gradually to a second plateau. At the start of the treatment of this batch, the methanol and formic acid were removed simultaneously at a high rate. At the end of the treatment of this wastewater batch, the formic acid was still removed at a high rate, but the removal rate of methanol had decreased.

The model parameters continued to change during the treatment of batch N (figure 7.4.17). The maximum ΔOUR increased within a week of treating this batch. The increase may be attributed to the increase in the methanol removal rate, which had decreased during the treatment of the previous batch. The formic acid removal rate decreased as the methanol rate increased. This observation is apparent from examining figure 7.4.11. The biomass in the second activated sludge unit did not follow the same trend during the treatment of batch N (figure 7.4.18). For this set of biomass and wastewater, the removal rate decreased over time.
Figure 7.4.15 ΔOUR vs. BOD. Batch L (○), batch M, day 0 (∗), batch M, day 27 (▲), and batch M, day 57 (□).

Figure 7.4.16 OUR profiles for 3 ml injections of wastewater during biomass acclimation to batch M. Day 9 (-----), day 15, (-- --), day 28 (-- --), day 40 (-----), and day 57 (-----).
Figure 7.4.17 OUR profiles for 3 ml injections of wastewater during biomass acclimation to batch N. Day 1 (--), day 7 (--), day 13 (--), day 18 (--).

Figure 7.4.18 OUR profiles for 5 ml injections of wastewater during biomass acclimation to batch N. Day 7 (--), day 13 (--), day 18 (--), day 26 (--).
In summary, during the treatment of batch L, the maximum ΔOUR increased, then started to decrease. The decrease in ΔOUR continued through the treatment of batch M, and then reversed during the treatment of batch L. The overall trend was similar for both of the lab scale activated sludge units, implying that the variation was not random, but a response to the changing wastewater characteristics. Similar to the overall ΔOUR, the ΔOUR on methanol and formic acid also varied, with the proportion of the ΔOUR due to the two compounds switching. The reasons for the changes in methanol and formic acid metabolism are not known, but are probably related to the relative concentrations in the wastewater.

When measuring the kinetic coefficients of industrial activated sludge plants, provisions must be made for the variations of the wastewater. This study has found that although changing SRT had little effect on the parameters, changing wastewater characteristics caused variations up to 50%. The main cause of variation is due to the different characteristics of different batches of wastewater, but is also due to different microorganisms supported by the different substrates. The biomass from the mill activated sludge system will see more day to day variation in wastewater characteristics than the lab scale activated sludge units, which were fed the same batch of wastewater for periods of up to six weeks. This will probably result in less variation at the mill, as the biomass will not have time to adjust to the fluctuations in the wastewater. The parameters measured using the biomass from the mill were the same as the average values measured on biomass from the lab scale system, again demonstrating the importance of wastewater characteristics over operating conditions. (The full scale operating conditions were quite different from the lab scale conditions.)


7.5 Yield & Decay

Microbial yield

The activated sludge yield on BKME may be calculated from the feed rate, solids wasting rate, and the biomass concentration (figure 7.5.1). As the SRT increases the yield decreases. This result is the expected result, and is a common observation. The decrease in the apparent microbial yield is explained by the concept of microbial decay, and modeled using equation 7.1.

\[ y_{obs} = \frac{Y}{1 + k_d \theta_x} \]  

(7.1)

The “true yield” \( Y \) is assumed to be a constant. Microbial decay is assumed to be directly proportional to the SRT through the decay coefficient \( k_d \), which is also assumed to be constant. The solid line in figure 7.5.1 was calculated using equation 7.1. The true yield was calculated to be 0.52 mg MLVSS/mg BOD, and the decay coefficient, \( k_d \), was 0.028 /day. These values are similar to typical values found in the literature, but the correlation coefficient was only 0.48 due to the large scatter in the data. If the active fraction of the biomass is taken into account, \( k_d \) in equation 7.1 may be replaced with \( b_h \) (Orhon 1994, p295).

Similar to the respirometric coefficients presented earlier in this chapter, the observed yield also varied from batch to batch (figure 7.5.2). Generally, the yield of the biomass from the two lab scale activated sludge units followed the same trend. For most of the batches, the yield in reactor two was lower than the yield in reactor one. This is due to the higher SRT in reactor two, or the presence of a selector. The exceptions were during the treatment of batches C, I, J, K, and R. The yield during the treatment of batch
Figure 7.5.1 Apparent yield vs. SRT. Letters represent wastewater batches, blue letters from unit #1, and pink letters from unit #2. Solid line equation 7.1, dashed line equation 7.9.

Figure 7.5.2 Apparent yield vs. wastewater batch Unit #1 (■), unit #2 (●). Respirometric yield vs. wastewater batch Unit #1 (▲), unit #2 (♦).
C was unusually low in reactor one for unknown reasons. The reasons for the high yields in reactor two during the treatment of batches I, J, K, and R are unknown. One possible explanation for the variability of the yield may be due to the variation of the microbial populations, in particular the variability of the protozoan population. Another explanation is the variability of the wastewater composition, however there is no obvious correlation between the apparent yield and the wastewater composition.

The respirometric yield calculated during the ΔOUR tests is also presented in figure 7.5.2. This yield was calculated using the oxygen consumption data and the readily biodegradable substrate. The respirometric yield is higher than the apparent yield, and similar for the two sets of biomass. The one exception was during the treatment of batch N, when the respirometric yield was lower than the apparent yield. As discussed in section 6.3, the respirometric yield will be an average of the yields on the individual substrates present, and cannot be used to accurately predict the SUR, but should give a good indication of the true yield. The true yield calculated from the data in figure 7.5.1 is also a weighted average of the yields on the various substrates present in BKME, as well as an average over all of the different wastewater batches treated in this study. In theory, the respirometric yield should be the same as the true yield, since endogenous metabolism / decay is accounted for in calculating the respirometric yield. The average respirometric yield over all of the wastewater batches is 0.55 mg COD / mg COD, which is close to the true yield value calculated from the data in figure 7.5.1 (0.52 mg COD/mg COD). The similarity of these results lends some validity to using respirometry to measure the microbial yield, even when many substrates are present.
Decay

In this section, microbial decay will be discussed. Decay is an important aspect of the activated sludge process because of its impact on the yield and sludge production. The decay coefficient was already presented in association with the yield data. These results will be further investigated in this section, with a discussion on the assumptions of 1) a constant decay coefficient and 2) the decrease in active fraction with increasing SRT. Batch test results used to measure decay coefficients will also be presented.

If the respirometric yield is assumed to be the true yield, then the difference between this value and the apparent yield may be used to calculate the decay coefficient (figure 7.5.3) by rearranging equation 7.1:

\[
k_d = \frac{Y - Y_{app}}{Y_{app} \cdot \theta_x}
\]

The decay coefficient \(k_d\) does not appear to be a constant with respect to SRT, but is greater for the biomass from reactor 1, which was operated at a lower SRT. These results disagree with standard activated sludge theory, which assumes that \(k_d\) is a constant. The exception is during the operation of the selector on reactor 2 (batches L, M, and N), during which time this unit had a higher decay coefficient. Possible reasons for the decrease in yield in the unit operated with a selector have already been discussed (section 7.4). The results presented in this section, both the endogenous OUR and \(k_d\), imply that the decay rate increased when a selector was present.

If the specific endogenous OUR is plotted versus SRT, a very similar trend to \(k_d\) is obtained (figure 7.5.4). This is not surprising since the endogenous OUR is a measure of the energy going to cell maintenance and the energy consumed during endogenous
Figure 7.5.3 Decay coefficient calculated from apparent yield and respirometric yield. Unit #1 (■), unit #2 (●).

Figure 7.5.4 Endogenous OUR Unit #1 (■), unit #2 (●) and decay coefficient calculated from apparent yield and respirometric yield. Unit #1 (▲), unit #2 (●).
decay. Both of these processes cause a decrease in the apparent yield, which is what is used to measure $k_d$. The ratio of the endogenous OUR to $k_d$ is approximately 4. This value should be close to 1.42, which is the COD equivalent of activated sludge MLVSS. It should be noted that the endogenous OUR may not be a true measure of the endogenous metabolism since during the OUR test, there may still have been some recalcitrant substrates present. This is unlikely to be the case in this study due to the long HRT used, any remaining substrates will have a very minimal oxygen demand, causing little increase in the overall OUR. However, if this effect were important, it would be more important at lower SRT's.

Others have also found the decay coefficient, $k_d$, to be dependent upon the SRT, increasing with decreasing SRT (Stewart and Ludwig 1962, Giona et al. 1979). The explanation offered by the standard activated sludge theory for the decrease in decay coefficient with increasing SRT is related to the microbial viability. Greater viability at shorter SRT's and a greater percentage of inactive biomass at higher SRT's is predicted. Since only the active biomass will decay, if decay is measured using MLVSS it will appear to be greater at lower SRT's. The decay coefficient used in the IAWQ model, $b_h$, does account for active biomass. The two decay coefficients are related as follows (Orhon 1994):

$$k_d = (1 - f_{ex}) \frac{X_s}{X} b_h$$

(7.3)

According to this relationship, $k_d$ is a function of the active biomass fraction, and hence the SRT. The parameter $b_h$ is a constant, independent of the SRT. The coefficient $f_{ex}$ is the fraction of the microbial mass which decays to particulate matter, and is usually taken to be 0.2. It is this particulate product of the microbial decay which is responsible for the
increase in non-active MLVSS as the SRT increases. Increased viability at lower SRT's also explains the endogenous OUR results (figure 7.5.4).

This study used MLVSS to measure biomass, which does not differentiate between active and non-active biomass. It is very difficult to measure the active fraction and no reliable methods exist. Using indirect measuring techniques, many studies have found the active fraction to vary with the SRT, which agrees with the main activated sludge models that predict a decrease in active fraction with increasing SRT. However, some recent studies have found the active fraction to increase with SRT, using phenol as a substrate (Hobson and Millis 1990). One indirect way of measuring activated sludge activity is by measuring the OUR.

Respirometric results presented previously in this chapter indicate that the active fraction was constant, regardless of the SRT. The maximum specific ΔOURs using effluent, methanol, formic acid, and acetate, were independent of both the SRT and the presence of a selector (when given on a MLVSS basis). If the active fraction was decreasing with SRT, then the maximum specific ΔOUR would have decreased in direct proportion to the active fraction. On occasions where the respirometric activity was different between the two reactors, the biomass from reactor two (longer SRT) had a greater specific activity (figure 7.3.29) which is the opposite of the expected result.

If the OUR and ΔOUR are followed over a few of the experimental runs (figures 7.5.5 and 7.5.6), it is observed that, for the most part, the same trend is followed by all of the measurements. If the endogenous OUR decreased, then the ΔOUR also decreased. If the OUR increased, then the ΔOUR also increased. This result does indicate that both the endogenous OUR and the ΔOUR are related to viability. The fact that the ΔOUR is the
Figure 7.5.5 Endogenous OUR unit #1 (■), unit #2 (○). methanol ΔOUR unit #1 (▲), unit #2 (●), formic acid ΔOUR unit #1 (□), unit #2 (○), acetate ΔOUR unit #1 (△), unit #2 (◊). Data from Run #3.

Figure 7.5.6 Endogenous OUR unit #1 (■), unit #2 (○). methanol ΔOUR unit #1 (▲), formic acid ΔOUR unit #1 (□), acetate ΔOUR unit #1 (△). Data from Run #6.
same between the two activated sludge units while the endogenous OUR is different implies that a factor other than viability is responsible for the difference in endogenous OUR.

If the active fraction is constant, then the decay coefficient ($k_d$) must be a function of the SRT, and not a constant. The net growth rate in activated sludge is related to the SRT by the following equation: $1/$SRT $= \mu - k_d$. The growth rate, $\mu$, is not considered to be constant. There is no logical reason to assume that the decay rate is constant, other than for convenience in modeling the data. At longer SRT's, the microbial growth rate is lower than at shorter SRT's. In studies of pure cultures, the decay rate is often found to be a function of growth rate (chapter 2). In activated sludge, the decay rate is dependent upon the protozoan population, which feeds upon the biomass. The activated sludge unit operated at the shorter SRT probably has a greater percentage of protozoans, and therefore a greater decay coefficient. The decay rate is related empirically to the reciprocal of the SRT by the following equation (curve in figure 7.5.4)

$$k_d = 0.016 + 0.3/\theta_x$$

(7.8)

This may be substituted into equation 7.1 to obtain

$$Y_{app} = \frac{Y}{1 + k_d \theta_x} = \frac{Y}{1.3 + 0.016 \cdot \theta_x}$$

(7.9)

This equation is represented by the dashed line in figure 7.5.1, and the predicted decay coefficients are similar to those predicted by the standard equation (7.1).

Another explanation for the change in decay coefficient is that the fraction of biomass converted into non-biodegradable particulate matter may be changing with the SRT. Increasing the SRT increases the biodegradation of recalcitrant compounds, which
would decrease $f_{ex}$ in equation 7.3. This would probably result in an increase in $k_d$ with SRT, not a decrease as observed.

The variability in the data makes it difficult to determine if the standard yield and decay model (equation 7.1) is the most appropriate, or if the data is better represented by a model with a variable decay coefficient (equation 7.8). In an attempt to further understand activated sludge decay, three long-term decay experiments were carried out. A batch test where the endogenous OUR is measured at various intervals is the standard way for measuring activated sludge decay. The experimental conditions are very different from the conditions in a continuous unit, but interfering processes are eliminated (changing wastewater, growth, etc.)

The first experiment used biomass from Harmac Pacific Ltd.'s activated sludge unit, at 35°C. The other two used biomass obtained from reactor 1, which was operating at steady state and an SRT of 5 days prior to the decay experiment. This biomass was used to initiate two decay experiments, one at room temperature (20°C), and the other at 35°C. In addition to measuring the OUR and MLVSS, the ΔOUR on methanol, formic acid, and acetate was also measured at daily intervals. Since there is no substrate addition, the standard OUR test measures the endogenous metabolism, and as such is a measure of the decay rate of the biomass. The drawback to the standard OUR test is its non-specific nature. The biomass will be utilising oxygen for many purposes, such as: endogenous metabolism, metabolism of extracellular debris from lysed cells, and growth of new biomass on the remains of the old biomass among other uses. The ΔOUR assay will be a more direct estimate of the enzyme activity of the biomass. For example, the ΔOUR on methanol will give an indication of the amount of enzymes present for the
metabolism of methanol. During normal operation, under steady state conditions and
given a constant set of biomass, this is a direct measurement of the amount of active
biomass present.

The data from the first experiment is presented in figures 7.5.7 and 7.5.8. In
figure 7.5.7, the log of the OUR is plotted versus the time of decay. According to the
standard theory, decay is a first order function, therefore the logarithm of the OUR
versus time should yield a straight line. This was not the case. Investigation of data in
the literature reveals that most data does not follow a first order relationship, but that a
straight line is forced through the data anyway.

The ΔOUR on methanol followed a first order decay relationship (figure 7.5.7).
The ΔOUR on formic acid also followed a first order decay relationship for the first ten
days, after which the ΔOUR did not decrease any more (figure 7.5.7). The decay rate
measured using methanol is greater than the decay rate measured using formic acid.
These results seem to indicate that the ΔOUR is a more appropriate method of measuring
the decay rate than OUR measurements, and that there are two separate biomass
components. One of these biomass components is responsible for the metabolism of
methanol and the other is responsible for the metabolism of formic acid, and each
biomass component has a separate decay rate. During a decay experiment it is possible
that enzymes for the metabolism of specific substrates may disappear at a different rate
from the decay of the microorganisms, and from each other.

The standard first order decay model assumes that there is only one biomass
component, with one decay coefficient. In order to model multiple biomass components,
the overall decay coefficient can be assumed to be a weighted average of the individual
**Figure 7.5.7** Batch decay test 1. OUR (■), methanol ΔOUR (●), and formic ΔOUR (▲) vs. time.

**Figure 7.5.8** Batch decay test 1. OUR (●), methanol ΔOUR (■), formic ΔOUR (▲), and MLVSS (△) vs. time.
decay coefficients. This will still result in a first order model, and consequently will not give a better fit to the decay data presented in this section. An alternate approach is to assume that each biomass component decays at a first order rate, and to calculate the overall rate by summing the individual rates together. For the case where there are two biomass components, one which utilises methanol and the other formic acid, equations 7.4 and 7.5 result. The decay coefficients for the two components are obtained from the ΔOUR experiments. The starting concentrations of the two biomass fractions are unknown and must be estimated from the data using equations 7.4 and 7.5. If this is done, the curves shown in figure 7.5.8 are obtained. The starting concentration of biomass responsible for methanol metabolism is ~400 mg/l, and the starting concentration of biomass responsible for formic acid metabolism is 1300 mg/l. There is usually more methanol than formic acid in the effluent, but since the bacteria which grow on methanol have a higher decay rate, there are fewer of them in the aeration basin.

\[
X = X_o + \left( 1 - f \right) \left[ X_{F_o} e^{-b_{F,t}} + X_{M_o} e^{-b_{M,t}} \right]
\]

\[
OUR = (1 - f) \left[ b_F X_{F_o} e^{-b_{F,t}} + b_M X_{M_o} e^{-b_{M,t}} \right]
\]

The data from the second set of decay experiments is presented in figures 7.5.9 to 7.5.11. This set of experiments has very similar results to the experiment just discussed. The decay of OUR did not follow first order kinetics (figure 7.5.9b), but the decay of the ΔOUR due to methanol (figure 7.5.9a), formic acid (figure 7.5.9d) and acetate (figure 7.5.9d) did follow first order kinetics. For this set of biomass, the decay rate was greatest for formic acid, and lowest for acetate. All of the decay rates had a strong dependence on temperature, approximately doubling as the temperature increased from 20°C to 35°C.

Using equations 7.6 and 7.7, with the decay constant obtained from the ΔOUR
Figure 7.5.9 Batch decay test 2; at 20°C (○), and at 34°C (■). A) methanol ΔOUR; B) endogenous OUR; C) acetate ΔOUR; D) formic acid ΔOUR.

Figure 7.5.10 Batch decay test 2 at 35°C. OUR (○), methanol ΔOUR (■), formic ΔOUR (▲), acetate ΔOUR (▲), and MLVSS (◇) vs. time.
measurements, the OUR and MLVSS over the course of the decay experiment may be modeled. The model fits the following equations:

\[ X = X_o - (1 - f) \sum^n_{i=1} \left[X_{no}(1 - e^{-b_i})\right] \quad (7.6) \]

\[ OUR = (1 - f) \sum^n_{i=1} \left[b_i X_{no} e^{-b_i t}\right] \quad (7.7) \]

The results presented in this section indicate that the decay rate depends upon the population of the activated sludge. Since the population varies over time (section 7.4), the decay rates will change over time. It also appears that the decay rates of the different fractions will vary with time, the decay rate will change even if the population remains approximately constant. These results agree with the hypothesis that the decay rate is variable, but do not give an indication of the effect of SRT or selector on the decay rates in continuous systems.

If the first order decay coefficients are combined with the respirometric yields, the apparent yield vs. SRT for methanol, acetate, and formic acid may be calculated (figure 7.5.12). At SRTs greater than 5 days, the net yield on methanol and formic acid is very small. The yield, decay rate, and concentration of these substrates appear to be highly variable. Bacteria which use these compounds as growth substrates will be very susceptible to changes in concentration of these compounds in the wastewater. This was found to be the case from the respirometric data (section 7.4). The higher yield and lower decay on acetate predict that the majority of bacteria in the sludge will use acetate as their growth substrate.

The microbial environment is different between the batch test and the continuous system. The batch test decay experiments demonstrate that microbial decay involves
Figure 7.5.11 Batch decay test 2 at 20°C. endogenous OUR (•), methanol ΔOUR (■), formic ΔOUR (▲), acetate ΔOUR (▲), and MLVSS (○) vs. time.

Figure 7.5.12 Apparent yield vs. SRT calculated from ΔOUR yield and first order decay constant. Methanol yield (—), acetate yield (—), and formic acid yield (—). Solid lines at 35°C, dashed lines at 20°C.
many different reactions and cannot be modeled with a simple first order equation. At the start of the batch test, the decay rate is equal to the decay rate of all of the microbes present, and the overall decay rate may be estimated from the initial endogenous OUR, or perhaps the initial endogenous OUR decay rate. The data presented at the beginning of this section indicates that the decay is variable, and increases with SRT.
Chapter 8 Effect of pH and Temperature Shocks on BKME

Kinetics

In this chapter, the effect of short-term temperature and pH changes on the activated sludge kinetics will be presented. In treating BKME, activated sludge plants may be exposed to various process spills. If the spill is large enough, the ability of the system to regulate the pH may be compromised, and a pH shock sent to the treatment system. Similarly, treatment systems may be exposed to temperature fluctuations. BKME must be cooled before conventional activated sludge treatment. In summer months, there may not be enough cooling available and the temperature in the treatment system may rise. The effects of pH and temperature on microbial kinetics and decay will be presented in this chapter.

8.1 Effect of pH on Kinetics

Readily Biodegradable Kinetics vs. pH

Using the respirometric method, the removal rates of the readily biodegradable substrates in BKME were measured at different pH values. Figure 8.1.1 demonstrates the deleterious effect of pH on the activated sludge kinetics, using biomass from the lab scale unit operated at an SRT of 10 days. BKME was used as the substrate for these tests, and the data was best fitted with the Monod equation. The resulting Monod constants are presented in figure 8.1.2. Both the maximum ΔOUR and the apparent half saturation constant are strong functions of the pH. The optimum value of the maximum ΔOUR is between pH 7 and 8. Below pH 6 and above pH 9, the maximum ΔOUR is less than half
Figure 8.1.1 ΔOUR vs. substrate concentration (BKME) at different pH's, obtained using BKME. pH 5 (■), pH 6 (○), pH 7 (▲), pH 8 (●), pH 9 (◆), pH 10 (◦). Batch D, SRT 10 days.

Figure 8.1.2 Respirometric kinetic coefficients (maximum ΔOUR (■), and Monod half saturation constant (●)) vs. pH, obtained using BKME. Batch D, SRT 10 days.
of its maximum value at pH 7. For this set of biomass, the Monod half saturation constant also varied with pH, decreasing as the pH fell below 7 or rose above 8.

The same experiment was repeated with biomass from the activated sludge unit operated at a 5 day SRT (figure 8.1.3), with very similar results to those obtained with the biomass from the 10 day SRT unit. The oxygen consumption per unit mass of added substrate did not appear to be affected by the pH (figure 8.1.4). These results imply that changes in operating pH will not affect the biomass yield. The maximum ΔOUR (figure 8.1.5) obtained using biomass from the 5 day SRT unit followed the same pattern as the results previously presented. The Monod half saturation constant did not appear to change with changing pH.

ΔOUR data, obtained using different biomass samples and different BKME batches, are presented in figure 8.1.6. All of the data followed the same general trend, with a maximum respirometric activity around pH 7.5 to 8. For some of the biomass samples and batches tested, the biomass was more sensitive to higher pH's than for others. These results are probably related to the composition of the wastewater batches, but are also dependent upon the biomass characteristics. This is demonstrated by the various curves obtained using batch G. The maximum ΔOUR occurs at pH 7.5 when biomass from the 5 day SRT unit was used, whereas the maximum ΔOUR was at pH 8 when biomass from the 20 day SRT unit was used.

Experimental data using methanol, acetate, and formic acid at different pH's were also obtained (figure 8.1.7). For easier comparison, the maximum ΔOUR relative to the ΔOUR at pH 7.5 is plotted in figure 8.1.8. The respirometric rate of formic acid oxidation was less sensitive to higher pH's than the rates of oxidation of methanol and
Figure 8.1.3 ΔOUR vs. substrate concentration at different pH's, obtained using BKME. pH 4 (■), pH 5 (●), pH 6 (∆), pH 7 (●), pH 8 (○), pH 9 (○), pH 10 (∆). Batch B, SRT 5 days.

Figure 8.1.4 OC vs. substrate concentration at different pH's, obtained using BKME. pH 6 (●), pH 7 (∆), pH 8 (●), pH 9 (■). Batch G, SRT 5 days.
Figure 8.1.5 Respirometric kinetic coefficients vs. pH obtained using BKME. Data from batch B: maximum AOUR (○), and Monod half saturation constant (△); Data from batch G: maximum AOUR (●), and Monod half saturation constant (♦). SRT 5 days.

Figure 8.1.6 Maximum AOUR vs. pH obtained using BKME. Data from: batch B, SRT 5 days (■); batch D, SRT 10 days (○); batch G, SRT 5 days (▲); batch G, SRT 20 days (●); batch G, SRT 5 days (▪); batch J, SRT 5 days (●); batch K, SRT 7 days (♦).
Figure 8.1.7 Maximum ΔOUR vs. pH. Data obtained using methanol: batch K, SRT 7 days (■); batch P, SRT 5 day (▲); batch P, SRT 20 days (▲). Data obtained using formic acid: batch K, SRT 7 days (●). Data obtained using acetate: batch P, SRT 5 days (●).

Figure 8.1.8 Relative ΔOUR vs. pH. Data obtained using methanol (■); formic acid (●); and acetate (●).
acetate. Acetate oxidation appeared to be the least sensitive to low pH's. For all three substrates, the respirometric yield did not change with pH. This is demonstrated by the methanol injections plotted in figure 8.1.9. As the pH fell below 7.5, or rose above 8, the OUR dropped. The area between the OUR curve and the endogenous OUR remains the same, and hence the yield is constant. This result means that the conversion factor between the OUR and the SUR is constant regardless of pH, so respirometry can be used to determine the effects of pH on substrate removal in activated sludge units.

If the effect of pH on the endogenous OUR is investigated, a different result from that obtained in the presence of substrates is obtained (figure 8.1.10). The endogenous OUR steadily increases with increasing pH. In the previous chapter, the endogenous OUR was found to be related to microbial decay. This interpretation would lead us to believe that microbial decay is lower at pH 5 than pH 7, which is not the case. Of course, microbial decay due to pH would not be a source of oxygen consumption, so the same interpretation does not apply in this case.

A possible explanation for the data in figure 8.1.10 is provided by the transmembrane protonmotive force which is established across all bacterial cytoplasmic membranes by the action of reversible proton pumps. The protonmotive force is composed of a transmembrane proton gradient and a membrane potential. The pH inside the cell is kept constant in a range from 6.5 to 8.5. When the external pH is low, the transmembrane proton gradient supplies a large fraction of the transmembrane protonmotive force. When the external pH is high, the transmembrane proton gradient is small, and the fraction of the transmembrane protonmotive force supplied by the
**Figure 8.1.9** Methanol injections at different pH's. pH 5.3 (--), pH 5.8 (--), pH 6.3 (--), pH 7.5 (--), pH 8.0 (--), pH 8.7 (--), pH 9.0 (--), pH 9.2 (--), pH 10 (--). SRT 7 days, batch K.

**Figure 8.1.10** Endogenous OUR vs. pH. Data from batch K, SRT 7 days (■), Data from batch P, SRT 5 days (○), and data from batch G, SRT 5 days (▲).
membrane potential increases, resulting in an increase in the endogenous respiration rate with increasing external pH.

**Slowly Biodegradable Kinetics vs. pH**

The respirometric test is a measure of the removal rate of the readily biodegradable substrates in BKME. In order to study the effect that pH changes have on the slowly biodegradable removal rate, batch tests and fed batch tests were performed. pH 9 was chosen since at this pH, the readily biodegradable removal rates are approximately half of what they are at pH 8, so changes in the slowly biodegradable removal rates should be evident. At pH's greater than 9, the removal rates are very low, and would be difficult to measure.

Batch tests, using the same biomass and wastewater batch, were performed at both pH 8 and pH 9. The OUR profiles for these tests are presented in figure 8.1.11. As expected, the OUR was lower at pH 9 than at pH 8. The first OUR plateau corresponded to the oxidation of the first readily biodegradable substrate group, and was slower and took approximately twice as long at pH 9 than pH 8. The BOD data (figure 8.1.12) supports the OUR data, with slower removal rates evident at pH 9. The removal rates for both cases followed the multicomponent model for multicomponent wastewater, with \( n = 1.5 \). If the last 30 mg/l of BOD to be removed in the batch test is considered to be the slowly biodegradable component, then the removal rate of this group of substrates was affected to approximately the same degree as the readily biodegradable component was. The removal rate, calculated from the batch test BOD data, is graphed vs. BOD in figure 8.1.13. The BOD removal rate at pH 8 is approximately double the rate at pH 9 over the entire range of BOD, and hence over the entire range of substrates in this batch of
Figure 8.1.11 OUR profile for batch tests at pH 8 (—) and pH 9 (—). Batch K, SRT 7 days.

Figure 8.1.12 BOD profile for batch tests at pH 9 (■) and pH 8 (○). Batch K, SRT 7 days.
Figure 8.1.13  SUR vs. BOD for batch tests at pH 9 (■) and pH 8 (○). Batch K, SRT 7 days.

Figure 8.1.14  BOD removal kinetics measured using the infinite dilution test at pH 8 (circles) and pH 9 (squares). Biomass from unit treating batch K, SRT 7 days, (○) and (■). Biomass from unit treating batch K, SRT 12 days, (●) and (■).
Fed batch tests also indicate that the slowly biodegradable kinetics are as equally affected by pH changes as the readily biodegradable kinetics (figure 8.1.14). Even at the normal operating conditions, a pH change from 8 to 9 can be expected to have a noticeable impact on the effluent quality. The SUR of the slowly biodegradable substrate is less than half of the value found for other wastewater batches at pH 8 (figure 8.1.14, and also figure 7.3.22). As already discussed in chapter 7, this will have a large effect on the capability of the treatment plant to withstand increases in the slowly biodegradable BOD loading rate. At pulp mills, pH increases of the wastewater may be due to black liquor spills. Black liquor contains a large proportion of slowly biodegradable substrates, so the negative effect of the increase in pH will be compounded by the wastewater characteristics.

Microbial Decay vs. pH

The results presented previously in this section were of microbial kinetics measured at different pH's. While the bacteria were exposed to shock pH's, the bacterial activity remained relatively constant, however, when the pH was adjusted back to its pre-shock value, the activity was often lower than it was before the shock. Short term exposure to shock pH conditions appeared to inactivate some of the microbial activity. In this section, bacterial decay as a function of pH will be presented.

Figures 8.1.15 and 8.1.16 show the results of biomass exposure to pH 10 for up to four hours. Samples were taken from the pH shocked biomass after 0.5, 1, 2, and 4 hours, the pH adjusted back to it's pre-shock value, and then the respirometric kinetics were measured. For this set of biomass, there was a large decrease in the removal rates
Figure 8.1.15 ΔOUR vs. substrate concentration (at pH 8) using biomass exposed to pH 10 for 0 hours (■) 0.5 hours (●), 1 hour (▲) 2 hours (◆) and 4 hours (□). Batch D, SRT 10 days.

Figure 8.1.16 BOD removal kinetics coefficients measured at pH 8, following exposure to pH 10 for the time specified by the x-axis. The kinetics were measured using BKME. Half saturation constant (■) and maximum ΔOUR (●). Batch D, SRT 10 days.
after one half hour exposure to pH 10. Further exposure to pH 10 resulted in a further decrease in the removal rates. While the maximum ΔOUR decreased, the half saturation coefficient remained approximately constant.

The decay of the methanol removal ability of the activated sludge at various pH values is presented in figure 8.1.17. There was very little decay at pH 5.5 and pH 9.5. It was only when the pH rose above 9.5, or fell below 5.5, that accelerated microbial decay seemed to occur. At pH values greater than 10, or less than 5, the microbial decay rate was high. This data was collected at various times, with different biomass samples. While the general effects of pH were the same, some biomass samples seemed more sensitive to shock pH's than others. For example, the sample exposed to pH 4.8 was less severely affected than the sample exposed to pH 5. Similar data, with very similar results were obtained when using formic acid (figure 8.1.18) and acetate (figure 8.1.19) as substrates.

The slowly biodegradable removal rates also decreased after the biomass had been exposed to large pH shocks. Using the fed-batch test at double the normal loading, the removal rate of the slowly biodegradable organic compounds was measured (figure 8.1.20). Before the pH shock, the residual BOD measured was 8 mg/l, after the one hour pH 10 shock, the BOD was 12 mg/l in one case, and 15 mg/l in the other case. The difference between the cases is the source of the biomass, one was from an activated sludge unit operating at an SRT of 7 days, the other from a unit operating at an SRT of 12 days, with a significantly larger concentration of MLVSS. These results correspond to a 30% and 50% decrease in removal rates from the pre shock values for the 12 day and 7
Figure 8.1.17 Respirometric data (obtained at pH 8, using methanol) following exposure to shock pH changes for the time specified by the x-axis. pH change to 3.5 (△), 4.2 (●), 4.8 (■), 5.0 (□), 5.5 (○), 9.5 (▲), 9.9 (●), 10 (●), 10 (○), 10.5 ( ), 10.5 ( ), 11 (○).

Figure 8.1.18 Respirometric data (obtained at pH 8, using formic acid) following exposure to shock pH changes for the time specified by the x-axis. pH change to 3.5 (○), 4.2 (●), 4.8 (■), 5.0 (□), 5.5 (●), 9.5 (▲), 10 (●), 10 (○), 10.5 ( ), 10.5 ( ), 11 (△).
Figure 8.1.19 Respirometric data (obtained at pH 8, using acetate) following exposure to shock pH changes for the time specified by the x-axis. pH change to 3.5 (■), 4.2 (▲), 5.0 (●), 10 (○).

Figure 8.1.20 Fed batch test, BOD vs. time after a one hour shock at pH 10. Biomass from unit treating Batch K, SRT 7 days (■) and SRT 12 days (●).
day SRT biomass respectively. These values are very similar to the results obtained with methanol, formic acid, and acetate (loss of 30% activity after a one hour pH 10 shock).

The decay coefficients of the maximum substrate removal rate of methanol, formic acid, and acetate are plotted in figure 8.1.21. These values were calculated as discussed in section 7.5. It is interesting to note that the decay rates of methanol, formic acid, and acetate activity were all approximately the same, despite the fact that formic acid metabolism was less severely affected by high pH values. These results imply that all of the various types of bacteria are dying, and not just denaturation of specific enzymes responsible for methanol, and formic acid metabolism is occurring.

In some cases, especially with shorter pH shocks, the metabolic activity recovered somewhat within a few hours of the end of the shock load (figures 8.1.22 and 8.1.23). The results presented in figure 8.1.15 to 8.1.19 were measured after the biomass had been returned to the pre-shock pH value, with a minimum of one half hour to acclimate. This should prevent the very short term, large loss of activity from affecting the results. Most likely, many enzymes are denatured before the bacteria are killed, and it takes some time for the enzymes to be re-synthesized or repaired once the pH is returned to a value to which the bacteria are accustomed.

When more biomass was present, the effect of the pH shocks were less severe. In figure 8.1.24, the results of two experiments with very different biomass concentrations are presented. In the first case, biomass from the activated sludge unit operating at an SRT of 23 days was exposed to a 60 minute pH 10 shock. There was a 20% loss of respirometric activity. The same experiment was repeated after diluting the biomass 3 fold. This time a 35% loss of activity was observed. In the second case, biomass from
Figure 8.1.21 First order ΔOUR decay coefficient (circles), and maximum ΔOUR (squares) for methanol (●) (□); acetate(●) (□); and formic acid (●) (□). ΔOUR data using biomass from batch R, SRT 24 days.

Figure 8.1.22 Recovery from pH 10 shocks using different sets of biomass. 10 minute shock, batch G, SRT 5 days (●); 30 minute shock, batch G, SRT 5 days (●); 30 minute shock, batch K, SRT 7 days (▲); 15 minute shock, batch K, SRT 7 days (●); 60 minute shock, batch K, SRT 7 days (○); 60 minute shock, batch N, SRT 10 days (○).
Figure 8.1.23 Recovery from pH 10 shocks using different sets of biomass. 10 minute shock, batch G, SRT 19 days (■); 30 minute shock, batch G, SRT 29 days (●); 60 minute shock, batch N, SRT 11 days (▲).

Figure 8.1.24 Effect of MLVSS on ΔOUR inhibition due to pH exposure. (■) exposed to pH 10 for 60 minutes, biomass from unit treating batch Q, SRT 23 days; (●) exposed to pH 10 for 30 minutes, biomass from unit treating batch K, SRT 7 days.
the activated sludge unit operating at an SRT of 7 days was exposed to a 30 minute pH 10 shock. A 65% loss of activity was observed. When the same biomass was concentrated two fold, only a 60% loss in activity was observed. For a given set of biomass, MLVSS concentration seems to be important in the response of the microorganisms to a pH shock. But the large difference between the two biomass samples implies that biomass characteristics are also very important. The biomass from the unit operating at a longer SRT seems to be more resistant to pH shocks. This may be due to the larger, denser flocs under these operating conditions, which provide more protection against the bulk fluid conditions.

Figures 8.1.25, 8.1.26, and 8.1.27 provide more examples of recovery of metabolic activity following short term pH shocks. Formic acid activity did not seem to recover following pH shocks (figure 8.1.25). On the other hand, methanol activity, which was more severely affected, recovered up to approximately the same values of the formic acid activity within an hour of the pH being returned to normal (figure 8.1.26). A comparison of the recovery of methanol metabolism, formic acid metabolism, and acetate metabolism, for the same set of sludge, following the same pH shock is presented in figure 8.1.27. The formic acid activity remained constant, the methanol activity, initially the lowest, soon recovered to the same level as the formic acid activity. The acetate metabolism recovered to it's pre-shock level within an hour of the pH being adjusted back to normal.

The results in this section demonstrate the negative effects that pH changes may have on activated sludge. Outside of the pH range 6 to 9, metabolic activity falls low enough for an impact in treatment performance to be noticed. Outside of the pH range
Figure 8.1.25  Recovery of formic acid ΔOUR from pH 10 shocks using different sets of biomass. 15 minute shock, batch K, SRT 7 days (■); 60 minute shock, batch K, SRT 7 days (○); 30 minute shock, batch N, SRT 11 days (▲); 30 minute shock, batch N, SRT 10 days (●); 60 minute shock, batch N, SRT 11 days (●); 60 minute shock, batch N, SRT 10 days (○); 60 minute shock, batch Q, SRT 23 days (△).

Figure 8.1.26  Recovery of methanol ΔOUR from pH 10 shocks using different sets of biomass. 15 minute shock, batch K, SRT 7 days (■); 60 minute shock, batch K, SRT 7 days (○); 30 minute shock, batch N, SRT 11 days (▲); 30 minute shock, batch N, SRT 10 days (●); 60 minute shock, batch N, SRT 11 days (●); 60 minute shock, batch N, SRT 10 days (○); 60 minute shock, batch Q, SRT 23 days (△).
Figure 8.1.27 Recovery of ΔOUR activity from pH 10 shocks using different substrates. 60 minute shock, acetate activity (■); 60 minute shock, formic acid activity (●); 60 minute shock, methanol activity (▲). Biomass from unit treating batch Q, SRT 23 days.
5.5 to 9.5, the bacteria start to die, and when the pH is returned to normal, the metabolic activity will be less than it was before the pH shock. If the pH shock lasts too long, this will also have a large effect on the performance of the activated sludge unit. pH affects the removal rates of all of the substrates in BKME approximately to the same extent. These results also indicate that it is best to operate with higher MLVSS concentrations, and preferably with higher SRT's to mitigate the negative effects of pH.

8.2 Effect of Temperature on Respirometric Kinetics

The effect of sudden temperature changes on activated sludge kinetics was investigated. In this section, the effects of temperature on methanol, formic acid, and acetate kinetics will be presented. In measuring the decay due to pH, the kinetics had to be measured at pH 7 to 8, since the pH values which caused microbial decay also eliminated most microbial activity. The respirometric method could not be used to accurately measure the kinetics at pH values greater than 9.5, or less than 5.5. When measuring decay due to increased temperature, it is possible to measure the decay at the shock temperature. In fact, this is more accurate, since increasing the temperature increases the kinetics.

Effect of temperature on methanol respirometry

Figure 8.2.1 shows OUR traces from methanol injections at 34°C, and after a sudden increase to 44.5°C. Immediately after the temperature increase both the baseline OUR and the ΔOUR increased. Corresponding to this increase in OUR, the SUR also increased. This increase in reaction rate is expected, since metabolic reactions are
chemical reactions, and can be expected to vary exponentially with temperature. After 34 minutes exposure to 44.5°C, the ΔOUR had further increased. At this point, however, the SUR had decreased back to its value before the temperature increase. This is evident from the amount of time required to metabolise the added methanol, the amount of time that the OUR stays elevated after methanol addition. After 164 minutes at 44.5°C, the ΔOUR had dropped slightly, and the SUR was lower than the pre-shock SUR. These results are very interesting; the SUR decreases even though the ΔOUR and the OUR increase. The bacteria are responding to the increase in temperature by oxidising more of the methanol, which decreases the yield and increases the ΔOUR. If the changing yield is ignored, the wrong conclusion may be reached - that the SUR increases and stays at an elevated value after an increase in temperature to 44.5°C. Temperature is known to be a trigger for energy spilling by pure cultures. It appears that results obtained with substrate sufficient pure cultures apply equally to activated sludge treating methanol containing wastewaters.

In continuing the experiment, the temperature was returned to 34°C after 200 minutes at 44.5°C (figure 8.2.2). Immediately following the temperature decrease, the OUR and the ΔOUR dropped to approximately what they were before the temperature increase. The SUR also dropped, but unlike the ΔOUR, the SUR dropped to a value much lower than before the temperature rise. The yield was still low. As the biomass had a chance to readjust to 34.5°C, the yield increased, the SUR increased slightly, and the OUR decreased slightly, but these values did not return to their pre-shock values over the course of the experiment. Some of the enzymes must have been permanently denatured by the temperature shock, or some of the biomass killed. The decrease in SUR
Figure 8.2.1 Response of OUR to methanol injections following a temperature increase from 34°C to 44.5°C. Before temperature increase (---), 7 minutes after increase (--), 34 minutes after (--), 164 minutes after (--).

Figure 8.2.2 Response of OUR to methanol injections following a temperature increase from 34°C to 44.5°C, followed by a temperature decrease back to 34°C after 200 minutes. Before temperature increase (---), 164 minutes after increase (---), 6 minutes after decrease (---), 115 minutes after decrease (---), 196 minutes after decrease (---).
which occurred at 44.5°C seems to be permanent.

This experiment is summarised in figure 8.2.3. The response in ∆OUR and SUR was immediate with the temperature change. The response in yield was slower, taking a few hours to reach a new value after the sudden temperature change. The change in ∆OUR seems to be fairly stable, at least over the duration of this experiment. In contrast, the SUR change was not stable. The SUR initially increased following the increase in temperature, then slowly declined as the yield declined. Opposite behaviour was observed when the temperature was returned to its pre-shock value, 34.5°C.

Given this interesting response to temperature changes, more temperature shocks were applied to fresh samples of biomass. Figure 8.2.4 has the results of an 8°C temperature drop, to 27°C, on the kinetics and stoichiometry of methanol metabolism. Immediately following the temperature drop, the OUR, ∆OUR, and SUR dropped. The yield, represented by the area under the ∆OUR curve, remained constant, indicating no increase in energy spilling. Prolonged exposure to the low temperature resulted in a further slight decrease in the ∆OUR and SUR. These results are exactly as expected. A decrease in temperature will result in a decrease in metabolic reaction rates, and energy spilling is not expected following temperature drops.

A temperature increase from 34°C to 42.4°C (figure 8.2.5) resulted in an increase in the OUR, ∆OUR, and SUR. With prolonged exposure to this shock temperature, all of these values decreased, with the SUR soon returning to the same value it was before the shock. Similar to the temperature jump to 44.5°C, an increase to 42.4°C also resulted in energy spilling, though not to the same extent.

With a large temperature increase, from 34°C to 49°C, the OUR increased, but the
Figure 8.2.3 Methanol respirometric coefficients following a temperature increase from 34°C to 44.5°C, followed by a decrease back to 34.5°C. SUR (○), OUR (■), and yield (▲).

Figure 8.2.4 Response of OUR to methanol injections following a temperature decrease from 34°C to 27°C. Before temperature decrease (—), 30 minutes after decrease (— —), 240 minutes after decrease (— — —).
Figure 8.2.5 Response of OUR to methanol injections following a temperature increase from 34°C to 42.4°C. Before temperature increase (— — — — —), 15 minutes after increase ( ), 103 minutes after ( ), 178 minutes after ( ), 276 minutes after (— — — — —).

Figure 8.2.6 Response of OUR to methanol injections following a temperature increase from 34°C to 49°C. Before temperature increase (— — — — —), 19 minutes after increase ( — — — — —), 38 minutes after ( — — — — —), 58 minutes after ( — — — — —), 99 minutes after ( — — — — —), 147 minutes after ( — — — — —), 203 minutes after ( — — — — —).
SUR and the yield decreased immediately (figure 8.2.6). The microbial activity decreased rapidly upon exposure to 49°C. After a few hours exposure to 49°C, the SUR had decreased to a third of its value before the temperature increase. Large temperature increases appear to greatly increase the microbial decay rate.

The ΔOUR data, the respirometric yield, and the SUR (calculated from the previous two values), for a number of sudden temperature shocks are presented in figures 8.2.7, 8.2.8, and 8.2.9 respectively. The ΔOUR responds to temperature changes immediately. All temperature increases, except the largest, caused an immediate increase in the ΔOUR. Temperature increases above 41°C caused a noticeable increase in microbial decay. Temperature increases above 40°C caused the respirometric yield to decrease. The higher the temperature, the faster the decrease in yield. The yield did not decrease to below 0.1 mg COD/mg COD. Temperature increases to 47°C caused an increase in methanol SUR, followed by gradual decrease as the bacteria decayed. Temperature rises above 49°C caused an immediate decrease in methanol SUR. These results are not immediately evident from the ΔOUR results due to the changing yield.

The SUR vs. time exposed to a temperature shock data may be used to calculate first order decay coefficients. For illustrative purposes, the data from figure 8.2.9 is reproduced in figure 8.2.10 on a semi-log plot. The data seems to follow the first order model, and decay coefficients were calculated for each temperature tested. The relationship between the decay coefficient and temperature can be used to calculate the activation energy for the microbial decay reaction (figure 8.2.11). Similarly, the activation energy for methanol metabolism may be calculated using the methanol SUR and temperature data (figure 8.2.12). As the temperature increased above 40°C, the SUR
Figure 8.2.7 Relative methanol ΔOUR following various temperature changes. Initial temperature was 34°C for all cases. Temperature change to 27.0°C (▲), 36.8°C (▼), 41.2°C (■), 42.4°C (●), 43.5°C (▲), 44.5°C (●), 46.5°C (○), 49.2°C (★), 53.5°C (♯). All data normalised to an initial ΔOUR of 1 mg/l minute.

Figure 8.2.8 Respirometric yield on methanol following various temperature changes. Initial temperature was 34°C for all cases. Temperature change to 27.0°C (▲), 36.8°C (▼), 41.2°C (■), 42.4°C (●), 43.5°C (▲), 44.5°C (●), 46.5°C (○), 49.2°C (★), 53.5°C (♯).
Figure 8.2.9 Maximum SUR obtained using respirometry, with methanol as substrate, following various temperature changes. Initial temperature was 34°C in all cases. Temperature change to 27.0°C (●), 36.8°C (▼), 41.2°C (■), 42.4°C (▲), 43.5°C (▲), 44.5°C (○), 46.5 (○), 49.2°C (★), 53.5°C (#).

Figure 8.2.10 Log(SUR) obtained using respirometry with methanol as substrate, following various temperature changes. Initial temperature was 34°C in all cases. Temperature change to 41.2°C (■), 36.8°C (▼), 43.5°C (▲), 42.4°C (▲), 44.5°C (○), 46.5 (○), 49.2°C (★), 53.5°C (#), 27.0°C (●).
started to drop due to the increased microbial decay, so these points were not used in calculating the activation energy. The relationship between the SUR and temperature depends on two competing reactions. The first is the metabolism of methanol, and the second is the denaturation of the enzymes which metabolise methanol. At high temperatures, the second reaction becomes more important than the first, and the rate of methanol metabolism decreases. Using the activation energies calculated from figures 8.2.11 and 8.2.12, the relationship between SUR and temperature may be calculated over the entire temperature range tested (figure 8.2.13). The equation appears to fit the data. The good fit of the model is not surprising even though the reactions being modeled are probably complex. The Arrhenius equation often fits global reaction rates.

If the SUR vs. temperature model is correct, it may be used to calculate the fraction of active enzymes at a given temperature (figure 8.2.14). At 30°C, the majority of enzymes were active, while above this value, the percent of active enzymes decreased rapidly. At 40°C, the majority of enzymes had been inactivated.

When the decay coefficient and the yield are plotted on the same graph, it becomes apparent that above 42°C the biomass was severely affected by the temperature (figure 8.2.15). Above this temperature the respirometric yield decreased drastically, and the first order decay coefficient started to increase at a faster rate with respect to temperature than before. Given the above information, it can be expected that the initial OUR will be quite high as the temperature increases (low yield) even though the SUR does not increase too much, but will rapidly decrease due to the large microbial decay. Figure 8.2.16 shows the initial ΔOUR, SUR, and yield following a temperature change, and also the ΔOUR, SUR, and yield after a few hours at the specified temperature.
Figure 8.2.11 Variation in first order methanol SUR decay coefficients (from figure 8.2.10) as a function of temperature.

Figure 8.2.12 Variation in final methanol maximum SUR (from figure 8.2.9) as a function of temperature. Data points obtained at a temperature > 41°C (□) were excluded from the curve fit.
Figure 8.2.13 Methanol maximum SUR vs. temperature.

Figure 8.2.14 Active fraction (based on methanol metabolism) vs. temperature.
Figure 8.2.15 Methanol SUR decay rate (■) and respirometric yield (●) vs. temperature.

Figure 8.2.16 Effect of temperature adjustment on: maximum methanol DOUR immediately following temperature adjustment (■), and after a few hours of acclimation (●); maximum methanol SUR immediately following temperature adjustment (▲), and after a few hours of acclimation (●); methanol respirometric yield immediately following temperature adjustment (□), and after a few hours of acclimation (○).
Above 42°C, the final yield was lower than the initial yield, and the final SUR was lower than the initial SUR. Above 45°C, the final ΔOUR was lower than the initial ΔOUR. The temperature which triggers energy spilling (42°C) is not the same as the temperature which causes increased microbial decay (45°C).

A summary of all of the experiments measuring methanol kinetics as a function of temperature is presented in figure 8.2.17. These experiments were done with different samples of biomass from activated sludge units operated at different SRT's (which had different specific methanol removal capacities), and different MLVSS concentrations. All the data appears to follow the same relationship with temperature. In order to more easily compare the data, it was converted to a relative activity by dividing by the maximum ΔOUR at the optimum temperature (figure 8.2.18). There is no obvious dependence of the response on the biomass characteristics or concentration, as there was with pH shocks. The activation energies also appear to be the same for each of the experiments. The maximum activity was at 45°C for these experiments because the initial activity, before biomass decay, was measured.

**Effect of temperature on formic acid respirometry**

Temperature has a very similar effect on formic acid metabolism as it has on methanol metabolism. Following a temperature increase from 34°C to 54°C, the ΔOUR increased, the SUR increased, and the yield decreased (figure 8.2.19). With prolonged exposure to 54°C, the ΔOUR decreased, as did the SUR and the yield.

Summary data for a number of sudden temperature changes are presented in figures 8.2.20 (ΔOUR), 8.2.21 (respirometric yield), and 8.2.22 (SUR). From these figures it is evident that a drop in the temperature caused both the ΔOUR and SUR to
Figure 8.2.17 Maximum methanol ΔOUR vs. temperature.

Figure 8.2.18 Maximum methanol ΔOUR vs. temperature. Data from figure 8.2.17, converted to percent of maximum ΔOUR at 45°C
Figure 8.2.19 Formic acid injections following a temperature increase from 34°C to 54°C. Before temperature increase (—, —), 26 minutes after increase (—); 50 minutes after (—), 82 minutes after (—).

Figure 8.2.20 Relative maximum formic acid ΔOUR following various temperature changes. Initial temperature was 34°C for all cases. Temperature change to 27°C (□), 44.5°C (■), 43.6°C (●), 49°C (▲), 53.5°C (♦). All data normalised to an initial ΔOUR of 1 mg/l minute.
Figure 8.2.21 Respirometric yield on formic acid following various temperature changes.
Initial temperature was 34°C for all cases. Temperature change to 27°C (□), 44.5°C (■), 43.6°C (●), 49°C (▲), 53.5°C (◆).

Figure 8.2.22 Maximum SUR obtained using respirometry, with formic acid as substrate, following various temperature changes. Initial temperature was 34°C in all cases. Temperature change to 27°C (□), 44.5°C (■), 43.6°C (●), 49°C (▲), 53.5°C (◆).
decrease, while having no effect on the yield. Temperature increases caused the ΔOUR and SUR to increase (at least initially), and caused the yield to decrease.

The first order decay coefficients may be calculated as they were for methanol metabolism, and are summarised in figure 8.2.23. The decay coefficient and respirometric yield followed very similar patterns as the values calculated using methanol as the substrate. Formic acid is a relatively similar substrate to methanol, so whatever mechanism is responsible for increased energy spilling during methanol metabolism at high temperatures is probably responsible for the energy spilling during formic acid metabolism.

The relationship between the ΔOUR and the temperature was similar to that found with methanol, but the temperature that corresponds to the highest activity was slightly higher when formic acid was used as a substrate (figure 8.2.24). The enzymes responsible for metabolising formic acid were slightly more resistant to high temperatures than those which metabolise methanol.

**Effect of temperature on acetic acid respirometry**

The effect of temperature on acetate metabolism was also investigated. Less data was collected compared to methanol and formic acid, since the removal rate of acetate was lower, and the yield was higher, making the ΔOUR low, and difficult to measure. An 11°C increase in temperature, from 34°C to 45°C, resulted in increased OUR, decreased ΔOUR, and decreased SUR (figure 8.2.25). Unlike when methanol and formic acid were the substrates, the increase in temperature did not cause a decrease in the respirometric yield. The data for this experiment is summarised in figure 8.2.26. The yield did not drop even after three hours of exposure to 45°C. Other experiments
Figure 8.2.23 Formic acid SUR decay rate (■) and respirometric yield (○) vs. temperature.

Figure 8.2.24 Maximum formic acid ΔOUR vs. temperature.
Figure 8.2.25 Response of OUR to acetate injections following a temperature increase from 34°C to 45°C. Before temperature increase (— , —), 6 minutes after increase (—), 41 minutes after (—), 183 minutes after (——).

Figure 8.2.26 Acetate respirometric coefficients following a temperature increase from 34°C to 45°C. SUR (○), OUR (■), and yield (▲).
indicated that the yield did not drop at even higher temperatures (data not shown). The one-carbon substrates were probably used by the biomass as energy sources, and when conditions are unfavourable for growth, the energy generated by the metabolism of these substrates will be wasted. The bacteria did not appear to be able to regulate the rate of consumption of these substrates, the excess energy generated was wasted. With other substrates, such as acetate, which may be more readily used for growth, there were most likely more complicated metabolic pathways available, and the substrate was not usually used as an energy source, but rather as a carbon source.

The relationship between the ΔOUR and temperature for acetate is very similar to that of methanol and formic acid (figure 8.2.27). The main difference is that the yield appears to be a constant. Similar to the results obtained with methanol, the source of the biomass appears to have little effect on the influence of temperature on the kinetics.

Summary

Figure 8.2.28 compares the maximum SUR of methanol, formic acid, and acetate over the temperature range of 20°C to 55°C. The greatest methanol activity is observed at a temperature slightly below that of the other two substrates. The activation energies for methanol, formic acid, and acetate metabolism and decay appear to be similar (table 8.1). The values for the activation energy of substrate metabolism are similar to values found in the literature, but the activation energies for the decay reaction are much greater than other values published (Muck and Grady 1974).
Figure 8.2.27 Maximum acetate $\Delta$OUR vs. temperature. Data obtained using biomass from 5 day SRT unit (■), and from 15 day SRT unit (●). Also shown is yield (▲) vs. temperature.

Figure 8.2.28 Methanol $\Delta$OUR (■), acetate $\Delta$OUR (●), and formic acid $\Delta$OUR (▲) vs. temperature.
Table 8.1 Average activation energies (kJ/kg mol K)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Activation energy for ΔOUR</th>
<th>Activation energy for k_d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>40 600</td>
<td>263 000</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>45 800</td>
<td>269 200</td>
</tr>
<tr>
<td>Formic acid</td>
<td>40 900</td>
<td>297 000</td>
</tr>
<tr>
<td>Endogenous</td>
<td>37 800</td>
<td>267 000</td>
</tr>
</tbody>
</table>

The endogenous OUR follows a similar trend to the exogenous ΔOUR due to methanol, formic acid, and acetate (figure 8.2.29). The activation energies for the endogenous OUR, and the endogenous OUR decay are similar to those for the metabolism of the substrates tested. The effect of temperature the endogenous OUR is similar to the effect on the catabolism of other tested substrates. Similar to the data for acetate (which was obtained with the same sludge), the biomass from the activated sludge unit running at a longer SRT was slightly more tolerant to high temperatures.

The decay of the endogenous OUR at different temperatures was investigated. This data is similar to the data presented for the ΔOUR data, but the decay rate seems to be different (figure 8.2.30). This becomes more evident when the ΔOUR due to methanol and formic acid are plotted on the same graph as the endogenous OUR (figure 8.2.31).

The data presented in this section were obtained at the maximum SUR, under conditions where mass transfer will not affect the respirometric results. The results of an experiment using methanol concentrations which give a ΔOUR below the maximum are presented in figure 8.2.32. Compared to the data obtained at the maximum ΔOUR, the temperature increase had a much smaller effect on the data collected at the low ΔOUR. These results lend support to the hypothesis that unsteady state mass transfer dominates.
Figure 8.2.29 Effect of temperature on endogenous OUR. Biomass from activated sludge unit operated at a 5 day SRT (■), and a 20 day SRT (○).

Figure 8.2.30 Endogenous OUR following various temperature changes. Initial temperature was 34°C for all cases. Temperature change to 49°C (■), 41°C (○), 37°C (▲), 42.4°C (▲), 44.5°C (□), 53.5°C (○), 27°C (△).
Figure 8.2.31 Endogenous OUR (■), methanol ΔOUR (●); and formic acid ΔOUR (▲) vs. time following a temperature change from 34°C to 49°C.

Figure 8.2.32 OUR (●); methanol ΔOUR, biomass limited (■); and methanol ΔOUR, mass transfer limited (▲) vs. time following a temperature change from 34°C to 44.5°C, and then a temperature drop to 34°C.
the respirometric response at low substrate concentrations presented in section 6.1 (the kinetics should have increased with temperature over the whole range of substrate concentrations). AOUR versus substrate curves, collected at three different temperatures, are presented in figure 8.2.33. The half saturation constant appears to increase with temperature. The response of the AOUR at low substrate concentrations is constant regardless of the temperature, further evidence that these results are mass transfer limited.

8.3 Adaptation to Shock Temperatures

In the previous section, the effects of temperature on the activated sludge readily biodegradable removal rates were presented. These were all short term tests, and it is of some interest to see how the activated sludge units can adapt to longer temperature shocks. Therefore temperature shocks were applied to the continuous units. This also enabled an investigation into the effects of the temperature on the slowly biodegradable removal rates. Over the course of this study, three different temperature shocks were applied to the activated sludge units (acclimated to 34°C). Two shocks were to 44°C, and the third was to room temperature.

Temperature Shock #1

The first temperature shock was on the 5 day SRT activated sludge biomass. OUR, AOUR (on BKME), MLVSS, and BOD were measured. The same batch of wastewater was used throughout the temperature shock, which lasted seven days. This temperature shock was to 44°C. This temperature was chosen for two reasons, it becomes difficult to measure the OUR above this temperature, and from batch tests it is obvious that sudden temperature increases above 44°C will have a very large negative
Figure 8.2.33  ΔOUR as a function of substrate concentration at 25°C (●), 35°C (■), and 40°C (▲). Corresponding yield values are shown in outlined symbols.
effect on the biomass. At the end of the temperature shock, the temperature was dropped to close to room temperature, to see how the biomass would respond to a sudden temperature drop after having been acclimated to 44°C.

Immediately following the temperature increase, the specific AOUR decreased (figure 8.3.1). The specific AOUR activity soon recovered back to the same level it was before the temperature shock, and continued to increase. When the temperature was dropped to 27°C after a week at 44°C, the specific activity of the biomass dropped 4 fold, a much greater decrease in activity than was observed when the temperature was decreased from 34°C to 27°C in previous batch tests.

The biomass concentration in the reactor dropped soon after the temperature increase (figure 8.3.2). There was not enough data to calculate the yield, but the loading did not change from the loading before the shock, so the decrease in MLVSS implies a decrease in the apparent yield. As discussed in section 8.2, an increase in temperature decreases the true yield on both methanol and formic acid (major substrates in BKME), and increases the decay rate.

The AOUR data predicts that there should be no significant decrease in BOD removal efficiency following the temperature increase (if the data is interpreted with the BKME being composed of just a single substrate). This is evidently not the case, as the BOD removal efficiency dropped after the temperature increase (figure 8.3.2). These results are easily explained by taking the multicomponent nature of BKME into account. If the slowly biodegradable kinetics are affected by temperature to the same degree as the readily biodegradable kinetics, then a rise in effluent BOD would be expected following a temperature increase to 44°C. The slowly biodegradable BOD removal efficiency did not
Figure 8.3.1 Maximum ΔOUR (□) and OUR (○) following a temperature increase in the continuous unit from 34°C to 44°C, followed by a decrease back to 27°C. The OURs were measured at the same temperature as the continuous unit.

Figure 8.3.2 MLVSS (■) and treated effluent BOD (○) following a temperature increase in the continuous unit from 34°C to 44°C, followed by a decrease back to 27°C.
recover over the duration of the experiment. The ΔOUR results predict a rise in BOD in
the treated effluent following the temperature decrease to 27°C, so the final rise in BOD
is not unexpected.

In the short duration of this shock load, the biomass adapted to the higher
operating temperature quite quickly (figure 8.3.3). Before the temperature shock, the
maximum activity was at 40°C, with the activity dropping rapidly above this temperature
(figure 8.3.4). The specific activity was higher at 35°C than at 44°C. Within two days of
the shock load, the biomass adapted and the specific activity was higher at 44°C than at
35°C, and the maximum activity was at 45°C. When the maximum ΔOUR versus
temperature profile was measured after the biomass had adapted to the new temperature,
the whole curve had shifted approximately 5° to the right compared to the profile before
the shock. The biomass exposed to the higher temperature prefer the high temperatures,
and will be more sensitive to temperature drops than biomass acclimated to 34°C.

Temperature Shock #2

The second temperature shock was similar to first, a 10°C shock from 34°C to
44°C. This time more was known about the kinetics of activated sludge treating BKME,
and the respirometric kinetics of methanol, formic acid, and acetate were measured
during the temperature shock. More care was taken to observe the effects of temperature
on the apparent yield. The set of biomass used for this experiment was grown at a longer
SRT, and the MLVSS concentration was significantly larger than for the first temperature
shock.

The solids concentration dropped steadily in the temperature shocked reactor, but
remained more or less constant in the control reactor (figure 8.3.5). The yield in the
Figure 8.3.3 Maximum ΔOUR measured at 35°C (■), 45°C (●), and 25°C (▲).

Figure 8.3.4 Maximum ΔOUR vs. temperature before (■) and 6 days after (●) a temperature increase to 44°C.
control reactor was approximately 0.6 mg MLVSS / mg BOD, which was high for operation at an SRT of 15 days (normally the yield is in the range of 0.3 to 0.5). The yield in the temperature shocked reactor was 0.25 mg MLVSS / mg BOD, much less than in the control reactor.

The BOD removal followed a similar pattern as shock #1 (figure 8.3.6). BOD removal efficiency decreased following the temperature increase. This result may be explained by decreased removal efficiency of the slowly biodegradable substrates in BKME. The removal rate of the slowly biodegradable compounds will decrease due to both the high operating temperature and the loss of MLVSS. The profile of the COD remaining in the effluent followed a very similar trend as the BOD. Both the residual BOD and COD remained constant in the control reactor.

The ΔOUR kinetics using effluent, methanol, formic acid, and acetate were measured using biomass from the control reactor and the temperature shocked reactor (figure 8.3.7). For the control reactor, these values remained approximately constant for the duration of the experiment. The ΔOUR on formic acid was greater than that on methanol, and the ΔOUR on acetate was very low. For the temperature shocked reactor, the ΔOUR activity decreased for the first four days following the temperature shock, then recovered somewhat. The initial activity was higher than the initial activity in the control reactor due to the higher temperature. The biomass used in this test was less sensitive to high temperatures than the biomass used for the first shock, and the activity at 44°C was greater than that at 34°C. All of the ΔOUR activities decreased at approximately the same rate. This decrease in specific activity is compounded by the decrease in MLVSS, which explains why the BOD and COD kept rising for the first four days. Measurement
Figure 8.3.5 Response of reactor MLVSS and yield to a temperature shock of 10°C. MLVSS (■) and apparent yield (▲) in temperature shocked reactor (44°C) and MLVSS (●) and apparent yield (□) in control reactor (34°C). SRT = 15 days.

Figure 8.3.6 Treatment performance following a temperature shock. BOD out (■) and COD out (▲) in temperature shocked reactor (44°C) and BOD out (●) and COD out (□) in control reactor (34°C). SRT = 15 days.
of acetate removal rates stopped on the fourth day, as they were too low to measure using the respirometric method. The specific activity of the biomass from the shocked reactor fell below the normal variation of specific activity observed during the rest of this project. The large decay rate caused by the temperature shock resulted in a sludge with a low viability. After the fourth day, the ΔOUR activity started to recover as the biomass adapted to the new operating temperature. Adaptation took longer than for the first temperature shock, which adapted in less than two days. This may be due to the different SRT's of the two sets of biomass. The SRT was 5 days in the first shock, which would allow for faster growing microorganisms and a quicker response to environmental changes.

The MLVSS data demonstrated that the apparent yield was lower in the temperature shocked reactor than the control reactor. Figure 8.3.7 provides some explanation for the decrease in yield, the large microbial decay at 44°C. Further explanation for the decreased yield is provided by the fact that the true yield on methanol and formic acid was also lower at 44°C (figure 8.3.8). Over the course of this temperature shock, the respirometric yield did not recover to the values of the control reactor.

The decay rates during the temperature shock (0.025/hr for methanol, and 0.026/hr for formic acid) were less than the decay rates presented in section 8.2 (0.07/hr and 0.016 for methanol and formic acid respectively). As discussed in section 7.5, the decay is variable; the biomass used for the temperature shock appeared to be more resistant than the biomass used for the previously described batch tests. An additional difference between the batch tests and the temperature shock was the continuous addition
Figure 8.3.7 Maximum ΔOUR vs. time for biomass from the temperature shocked reactor and the control reactor. Shocked reactor: maximum effluent ΔOUR (■); maximum methanol ΔOUR (●); maximum formate ΔOUR (▲); maximum acetate ΔOUR (★). Control reactor: maximum effluent ΔOUR (□); maximum methanol ΔOUR (○); maximum formate ΔOUR (△); maximum acetate ΔOUR (☆). SRT = 15 days.

Figure 8.3.8 Respirometric yield vs. time for biomass from the temperature shocked reactor and the control reactor. Shocked reactor: methanol yield (●), formic acid yield (▲), effluent oxygen demand (■). Control reactor: methanol yield (○), formic acid yield (△), effluent oxygen demand (□).
of feed during the shock load. During the batch test, the biomass was alternately starved and fed (during the measurement of the ΔOUR kinetics).

The ΔOUR profiles with respect to temperature for the control and shocked biomass, measured on the sixth day of the experiment, are presented in figure 8.3.9. That the specific activity of the shocked biomass was very low, combined with the low MLVSS, make this biomass susceptible to shock loads, particularly of slowly biodegradable substrates. In order to compare the two different sources of biomass (control and shocked), the relative ΔOUR is presented in figure 8.3.10. Similar to the previous temperature shock, the biomass adapted to the new operating conditions, and became tolerant to the higher operating temperatures. The whole ΔOUR vs. temperature curve had shifted 5°C to the right.

If the ΔOUR vs. substrate curves for biomass samples taken during the course of the temperature shock are examined (figure 8.3.11), it is observed that the temperature shocked biomass has a higher half saturation constant than the control biomass. As discussed in chapter 6, the half saturation constant measured using the respirometric method is possibly a measure of mass transfer resistance. The biomass from the temperature shocked unit appears to have greater mass transfer resistance than the biomass from the control reactor. Compared to the metabolic reactions, the effect of temperature on mass transfer should be small. The solubility of oxygen decreases with increasing temperature, but this is offset by a greater diffusivity. It is possible that the change in mass transfer resistance is due to a changed property of the floc.
Figure 8.3.9 Maximum ΔOUR vs. temperature for biomass from the temperature shocked reactor (methanol (▲) and formic acid (●)), and the control reactor (methanol (■) and formic acid (○)).

Figure 8.3.10 Relative ΔOUR vs. temperature for biomass from the temperature shocked reactor (methanol (□) and formic acid (◇)) (plotted vs. temperature - 5°C), and the control reactor (methanol (■) and formic acid (●)) (plotted vs. temperature).
Temperature Shock #3

The third temperature shock was a temperature change from 34°C to room temperature. The ability of the biomass to adapt to temperature decreases, compared to temperature increases, was investigated. Biomass acclimated to room temperature for three weeks had a lower specific activity than biomass acclimated to 34°C (figure 8.3.12). This result is unexpected, since at room temperature, microbial decay is slower, and the viability should be greater. If the data in figure 8.3.12 is converted to relative ΔOUR for easier comparison (figure 8.3.13), then it is evident that the temperature curve has shifted slightly to the left. For the other temperature shocks, 10°C temperature increases, the ΔOUR vs. temperature curve had shifted five degrees to the right. For the temperature shock to room temperature, a 10°C temperature drop, the curve shifted only a degree or two to the left of the reference curve (obtained with the same biomass before the temperature drop). This may not be significant, since most of the ΔOUR vs. temperature curves were also shifted a few degrees to the left of the reference curve. If the change in ΔOUR profile during the first two temperature shocks was due to the temperature changing from the mesophilic range to the thermophilic range, then a similar response should not be observed with this shock. A short term temperature shift down should not cause too much stress on the biomass, so it will not be forced to respond quickly. Of course different mesophilic bacteria have different optimum temperatures, and it is expected that long term operation at room temperature would eventually select for microorganisms with a lower optimum temperature.

The ΔOUR measured during operation at room temperature were on the high side of the average range for this project (figure 8.3.14). The ΔOUR before, during, and after
Figure 8.3.11 Effect of elevated operating temperature (34°C to 44°C) on ΔOUR as a function of time after temperature increase. Day 1 (■) day 2 (○) day 3 (●) day 4 (□), and the control reactor (▲).

Figure 8.3.12 Effect of temperature on maximum ΔOUR for biomass acclimated to 27°C (methanol (△) and formic acid (●)), and biomass acclimated to 34°C (methanol (□) and formic acid (○)).
the temperature shock to room temperature were approximately constant (all measurements were done at 35°C to eliminate the effect of temperature on the ΔOUR assay). The ΔOUR yields were in the normal range, and also did not vary during the temperature shock (figure 8.3.14). The apparent yield (calculated using the MLVSS and SRT) was higher during the operation at room temperature, compared to operation at 34°C (figure 8.3.15). After the temperature was returned to 34°C, the apparent yield gradually (after 10 days) returned to normal values. The high apparent yield, combined with the “normal” respirometric yield implies that microbial decay was much lower at room temperature than at 35°C. This is as expected from the results presented in section 8.2 and 7.5.

Summary

Temperature increases of 10°C resulted in increased microbial decay, and decreased yield (due both to energy spilling and to the increased decay rate). The decrease in active biomass caused treatment of the slowly biodegradable fraction of BKME to fall below acceptable levels, and an increase in treated effluent BOD and COD was noticed. The biomass adjusted to the shock temperature in a matter of days. The length of time required to adapt appeared to be directly proportional to the SRT, although not enough data was collected for firm conclusions. Full scale treatment systems will not be exposed to sudden temperature increases as in this study. If the temperature changes can be spread out over days, the biomass may have enough time to adapt, preventing poor wastewater treatment. The removal rate of the slowly biodegradable substrate did not appear to recover within the time frame of the temperature shocks.
Figure 8.3.13 Relative maximum ΔOUR vs. temperature for biomass acclimated to 27°C (methanol (Δ) and formic acid (●)), and biomass acclimated to 34°C (methanol (□) and formic acid (○)).

Figure 8.3.14 Respirometric coefficients vs. time before, during, and after a temperature drop to 27°C (from 34°C). Maximum methanol ΔOUR (■), maximum formate ΔOUR (●), maximum acetate ΔOUR (▲). Methanol yield (○), formate yield (□), acetate yield (○). SRT = 15 days.
A temperature decrease to room temperature did not affect activated sludge performance. There was a noticeable increase in apparent yield, due to the decreased microbial decay. This results in greater MLVSS concentrations, which will result in greater resistance to shock loads.

The temperature at which the maximum ΔOUR is at a maximum appears to be a function of the operating temperature (figure 8.3.16). The biomass acclimated to 44°C is more sensitive to lower temperatures than the biomass acclimated to 34°C. If a full scale activated sludge unit is exposed to a temperature increase, the temperature should be gradually re-adjusted to the pre-shock temperature, if possible.

**8.4 Incorporation of Temperature and pH into the Activated Sludge Model**

Temperature and pH effects on enzyme kinetics are supposed to be independent, and additive. In this project, little work was done to investigate the combined effects of pH and temperature on the activated sludge kinetics. One experiment investigated the effect of pH at 44°C. The relationship between the kinetics and the pH was found to be the same as that at 34°C. Using the pH curves from section 8.1, and the temperature curves from section 8.2, the response of the activated sludge to combined temperature and pH changes was calculated. The two sets of experimental data matched the calculated curves (figure 8.4.1). This verifies that temperature and pH effects are additive and independent, at least in the short term.

The combined effects of temperature and pH on microbial decay and respirometric yield were not investigated, but are probably also additive. The respirometric yield was not affected by pH, and it is not expected that combined temperature and pH changes would affect the yield more than temperature changes alone.
Figure 8.3.15  Apparent yield vs. time. Yield at room temperature (□), yield at 34°C (●). SRT = 15 days.

Figure 8.3.16  Temperature at which metabolic activity is at its maximum versus operating temperature.
The two substrate activated sludge model may be simply represented as follows:

\[
V \frac{dS_1}{dt} = QS_{10} - QS_1 - V \frac{\mu_{\text{MAX1}}(pH,T)X}{Y(T)} \frac{S_1}{K_1 + S_1} \tag{8.1}
\]

\[
V \frac{dS_2}{dt} = QS_{20} - QS_2 - V \frac{\mu_{\text{MAX2}}(pH,T)X}{Y} \frac{S_2}{K_2 + S_2} \tag{8.2}
\]

\[
V \frac{dX}{dt} = V \mu_{\text{MAX1}}(pH,T)X \frac{S_1}{K_1 + S_1} + V \mu_{\text{MAX2}}(pH,T)X \frac{S_2}{K_2 + S_2} - V k_d(pH,T)X \tag{8.3}
\]

S_1 represents the readily biodegradable fraction, and S_2 represents the slowly biodegradable fraction.

The relationship between the growth rate and temperature is:

\[
\mu = Ae^{-E/RT} \tag{8.4}
\]

The coefficients for this expression are obtained from curves similar to the one shown in figure 8.2.12. A similar expression is used to relate k_d with temperature (figure 8.2.11).

The yield on the readily biodegradable fraction may be modeled with a switching function. Below 42°C the yield is 0.5, above 42°C the yield is 0.2. The yield on the slowly biodegradable fraction is probably not a function of temperature.

The relationship between the growth rate and pH is provided by equation 3.33. The relationship between k_d and the pH is provided by equation 3.38, which approximates the data in figure 8.1.21 below pH 6.5, and above pH 9.5. Between pH 6.5 and 9.5, negative values for k_d were obtained. This will require a switching function similar to the dependence of yield on temperature. In the pH range of 6.5 to 9.5, k_d is constant and equal to 0.03/hr. Outside of this pH range, equation 3.38 is used.

These equations may be used to predict the effects of short term shocks on the activated sludge process. If the operating conditions change for prolonged periods of time, the kinetic parameters will change. The composition of the BKME may appear to
Figure 8.4.1 $\Delta$OUR vs pH, at different temperatures predicted from data presented in sections 8.1 and 8.2. $\Delta$OUR vs pH at 45°C (■), $\Delta$OUR vs pH at 34°C (●). (maximum $\Delta$OUR normalised to 1 mg/l minute).

Figure 8.4.1a Three dimensional representation of data from figure 8.4.1. $\Delta$OUR vs pH, at different temperatures predicted from data presented in sections 8.1 and 8.2. $\Delta$OUR vs pH at 45°C (red line), $\Delta$OUR vs pH at 34°C (blue line).
change as well. Substrates that were readily biodegradable may become slowly biodegradable. If the temperature and pH affect all substrate removal kinetics equally as the results in this project imply, this will not be a concern for modeling. More accurate modeling will take each substrate into account, instead of just the two main groups.

Temperature effects upon floc structure and settling properties are difficult to model. So is the adaptation time. The kinetic coefficients will be constantly changing as the biomass adapts to new operating conditions. The only way to account for this is to frequently measure the kinetics using the respirometric method.
Chapter 9 Summary and Conclusions

Respirometry

The biodegradation rates of methanol, formic acid, and acetate were studied using respirometry. At high substrate concentrations the respirometric method accurately predicted the substrate removal rate. At low substrate concentrations, the respirometric test was limited by mass transfer resistance. The model coefficients obtained by respirometry were found to be functions of the biomass concentration. The dependence of the specific metabolic rate on the biomass concentration, and the observed increase in yield at low substrate concentrations, was hypothesised to be due to the unsteady state nature of the respirometric assay and stored oxygen in the floc. There is a lag in the response of the OUR caused by mass transfer resistance. The mass transfer resistance appears to be due to dissolved oxygen diffusion into the floc. The ΔOUR data were modeled using the modified Powell equation, with the assumption that the mass transfer term is related to the oxygen diffusivity. As the diffusional resistance becomes negligible compared to the enzymatic resistance, which happens at low solids concentrations, the Powell equation simplifies to the Monod equation.

The OUR is a function of the dissolved oxygen concentration. At low biomass concentrations, this relationship follows the Monod model, and the OUR will gradually decrease with decreasing DO. At high biomass concentrations, the OUR and DO are related through the Powell equation, and the OUR will remain approximately constant until the DO is below 0.5 mg/l.
The pattern of multiple substrate utilisation by activated sludge acclimated to BKME was studied. For most substrate mixtures, utilisation of multiple substrates was simultaneous and additive, although sequential utilisation was observed in a few batch tests. Competition kinetics were not observed. When the substrate mixture contained acetate (a two-carbon substrate) and a one-carbon substrate (methanol or formate), the yield on the mixture was greater than predicted from the yields on the individual substrates. The metabolism of the one-carbon compound provides excess energy, which the bacteria were able to utilise in the anabolism of the two-carbon substrate.

When multiple substrates are present, the yield calculated using respirometry will be a weighted average of the yields of the individual substrates (assuming the yields are constant). This will cause errors in the conversion of the OUR to an SUR. If all of the substrates have identical yields, then this will not present a problem. Otherwise the composition of the mixture must be known in order to use the proper yield in converting the OUR to an SUR.

**BKME Treatment**

Analysis of BKME was performed using batch tests, with withdrawal of samples for respirometric analysis. BKME was found to be composed of many substrates, which could be divided into two main groups, the readily biodegradable fraction, and the slowly biodegradable fraction.

The metabolic activity of the biomass measured during the ΔOUR test will be due almost exclusively to the readily biodegradable substrate. This is especially true in light of the fact that the yield on the slowly biodegradable fraction is greater than the yield on the readily biodegradable fraction. If the slowly biodegradable fraction is not taken into
account in analysing respirometric data, substrate removal rates, and the capacity of the activated sludge to adsorb shock loads, will be greatly overestimated.

The slowly biodegradable fraction dominates the BOD in the treated effluent under normal loading conditions. An increase in the slowly biodegradable loading to the treatment system will result in an increase in the treated wastewater BOD. This will also be caused by a decrease in the slowly biodegradable removal rate due to a change in temperature or pH.

Depending on the particular situation (wastewater composition and kinetics), some intermediate biodegradable organics may behave as readily biodegradable substrate under low loading conditions, but will start to be incompletely removed at intermediate loadings. As the loading increases, these substrates will switch from the readily biodegradable fraction to the slowly biodegradable fraction. This situation was observed during the treatment of batch M.

The wastewater was found to be highly variable from batch to batch. The SRT and an aerobic selector had no effect on the activated sludge substrate removal rates. Increasing the SRT decreased the apparent yield, due to the increased importance of microbial decay at low growth rates. Adding an aerobic selector also decreased the apparent yield. This may be due to both increased energy spilling (in the selector), and increased microbial decay (in the main aeration tank).

The main result of increasing the SRT is to increase the MLVSS concentration. This results in less sludge to dispose of, but greater aeration costs, and a greater load on the clarifier. Since SRT does not affect the activated sludge substrate removal rates, the
larger MLVSS concentration at higher SRTs will give the activated sludge unit a greater capacity for mitigation of shock loads.

The wastewater composition had a greater effect on the substrate removal rates than the operating conditions. Both activated sludge units, which were treating the same batches of wastewater at the same times, followed the same trends in removal rates despite the different operating conditions. The populations of the various fractions of biomass varied over time, probably due to changing wastewater characteristics from batch to batch.

Microbial decay was found to be a function of the SRT and not a constant. The decay coefficient decreased with increasing SRT. Similar to the wastewater characteristics and the activated sludge substrate removal rates, the decay coefficient was found to be highly variable.

**Response to Transient Operating Conditions**

Changes in pH affect the activated sludge process. Outside of the pH range 6 to 9, metabolic activity falls low enough for an impact in treatment performance to be noticed. Outside of the pH range 5.5 to 9.5, the bacteria start to die, and when the pH is returned to normal, the metabolic activity will be less than it was before the pH shock. If the pH shock lasts too long, this will also have a large effect on the performance of the activated sludge unit. pH affects the removal rates of all of the substrates in BKME approximately to the same extent. The results indicate that it is best to operate with high MLVSS concentrations, and preferably with a high SRT to mitigate the negative effects of pH.
The relationship between activated sludge substrate removal rates and temperature is determined by two competing reactions. At low temperatures, the maximum substrate removal rate increases according to the Arrhenius relationship. As the temperature increases to near the optimum, the effect of bacterial decay becomes important. When the temperature increases past the optimum, microbial decay has a large effect and the activity decreases. The relationships between temperature and the removal rates of the different substrates in BKME were approximately the same.

Temperatures above 42°C resulted in energy spilling during the metabolism of methanol and formic acid, but not during the metabolism of acetate.

Operating conditions (SRT) do not affect the activated sludge response to temperature shocks, but increased biomass gives the activated sludge system a greater buffering capacity to withstand shock loads.

The optimum temperature for substrate removal is related to the operating temperature. The biomass takes only a few days to partially adapt to sudden temperatures changes. The time required for adaptation is proportional to the SRT. Operating at high temperatures results in decreased microbial yield and low biomass concentrations. Operating at low temperatures results in increased yield (due to a decrease in microbial decay) and higher biomass concentrations.

The decrease in yield with a selector, and increased energy spilling at high temperatures suggests a strategy for treating BKME with minimal sludge production. A selector may be operated at 42°C causing the bacteria to waste most of the energy otherwise available from methanol and formic acid metabolism (due to the high temperature and high concentration). The main aeration tank may be operated at 35°C to
allow for removal of the slowly biodegradable substrates, to ensure that the biomass does not adapt to the higher temperature in the selector, and to provide a sludge with good settling qualities. The low f/m in the main aeration tank should increase the removal of the substrates with low biodegradation rates. The decrease in yield will require increased aeration. This setup has not been tested, and may be too stressful for the biomass.

The effects of temperature and pH on activated sludge are independent from one another. The relationships between the rate and stoichiometric parameters and pH and temperature may be incorporated into any standard activated sludge model. This model will be acceptable for predicting short term fluctuations in treatment efficiency, but the kinetic parameters are highly variable and can be expected to change as the biomass adapt to new operating conditions.
Chapter 10 Recommendations for Future Work

Like most research, this study has raised more questions than answers. Areas which I think would be of most interest to study are the following:

Activated Sludge Kinetics and Stoichiometry

The kinetics and stoichiometry of the activated sludge system would be much easier to study using a well defined, constant, synthetic wastewater. This will eliminate variations due to changing qualities of the wastewater and make the data easier to interpret. The following projects: studying a greater range of SRT, studying microbial decay, and studying population dynamics, would be interesting extensions of the project presented here.

In this project, no effect of the SRT was found on the activated sludge substrate removal kinetics. Perhaps SRT will become significant if high rate activated sludge systems are studied, which will involve varying the HRT as well as the SRT. As well, it would be interesting to measure the actual microbial growth rates (with a method other than respirometry), in addition to the substrate removal rates (using respirometry). Combined with the results in this project, this will allow more detailed investigation of the microbial yield, and the effect of SRT and other conditions on the yield. In order to allow for greater control of the SRT, membrane bioreactors are recommended.

Run activated sludge units treating a synthetic wastewater that contains only one substrate, varying the SRT and loading. This will allow for calculation of the decay coefficient which may then be compared to the one obtained by batch tests (as performed in this study). The variation of the decay coefficient with SRT may be investigated. If
the active biomass is measured by independent means, the effect of SRT on the active fraction may also be investigated. These results will allow verification of all of the assumptions in the relationship between the decay coefficient, yield, and active fraction with SRT made in current activated sludge models. The results obtained in this study seem to indicate that the common assumptions are not valid, but not enough data was collected for conclusions to be drawn.

If two substrates are used in the synthetic wastewater, in varying ratios, population dynamics may be followed using the respirometric techniques in this project. This will give an indication if the variability in microbial kinetics is due to changing populations or changing wastewater characteristics. Substrate interactions at different SRT's or loadings can be compared to those presented in this study as measured by respirometry.

**Respirometry**

The results in this study demonstrate the importance of mass transfer on the interpretation of respirometric data, but there still remains much to figure out. For example: the effect of the floc structure on mass transfer, and the importance of substrate characteristics on the mass transfer rate.

The relationship between OUR and dissolved oxygen should be determined, and the dissolved oxygen diffusivity should be measured. This may be achieved by measuring the OUR at constant substrate concentration, constant biomass concentrations, and varying dissolved oxygen concentration (from saturation to 0 mg/l), then repeating at different substrate concentrations and biomass concentrations. Constant substrate concentration may be maintained using a syringe pump. The effect of temperature on
dissolved oxygen diffusivity may be investigated by repeating the above experiments at different temperatures.

Run respirometry experiments with a range of substrates, preferably with different diffusivities. Repeat these experiments using sludge obtained from activated sludge plants operated under different conditions, to give different qualities of sludge, with different mass transfer properties. If different sludge samples are not available, the activated sludge flocs could be disrupted with sonication into different sized particles. These experiments will enable the verification of the assumptions concerning mass transfer made in this study.

If the growth rate can be measured during the respirometric test, then validity of the yield obtained by respirometry may be tested. It would be very interesting to see if the response in microbial growth rate follows the substrate uptake rate, and what the effect of the f/m ratio on the relationship between the substrate uptake rate and the growth rate is.

Shock Loads

A more detailed investigation of the time required to adapt to changing operating conditions, in particular to temperature shifts would be interesting. This study should include the measurement of microbial growth rates (with an independent assay) as well as substrate removal (using the respirometric method). The influence of the pre-shock growth rate, the magnitude of the temperature change, and the rate of the temperature change, on the time required for the biomass to adapt should be investigated. Care should be taken to distinguish between the biomass concentration and the SRT (biomass
growth rate). The response of high rate and low rate activated sludge systems should be compared. The effect of shock loads on sludge settling should be monitored.

Operate an activated sludge unit with a temperature profile. A selector at 42°C, followed by the main aeration tank at 35°C. This should result in a small yield if BKME is the wastewater treated. Additional information may be obtained by varying the selector size.
Chapter 11 Nomenclature

ΔOUR Exogenous oxygen uptake rate
AOX Adsorbable organic halide
ATP Adenosine tri-phosphate
BKME Bleached kraft mill effluent
BOD Biochemical oxygen demand
COD Chemical oxygen demand
CSTR Continuous stirred tank reactor
DO Dissolved oxygen
f/m Food to microorganism ratio
HMW High molecular weight
HRT Hydraulic retention time
IAWQ International Association on Water Quality
LMW Low molecular weight
MLVSS Mixed liquor volatile suspended solids
N Nitrogen
OC Oxygen consumption
OUR Oxygen uptake rate
P Phosphorous
SMP Soluble microbial products
SRT Solids retention time
STBOD Short term BOD
SUR Substrate uptake rate
SVI Sludge volume index
TOC Total organic carbon
TSS Total suspended solids
VSS Volatile suspended solids

Where appropriate, the nomenclature recommended by the Working Group (Grau 1987) has been followed.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Quantity name</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>Volume</td>
</tr>
<tr>
<td>X</td>
<td>Particulate material concentration</td>
</tr>
<tr>
<td>Xs</td>
<td>Stored mass</td>
</tr>
<tr>
<td>Xa</td>
<td>Active mass</td>
</tr>
<tr>
<td>Xi</td>
<td>Inert mass</td>
</tr>
<tr>
<td>S</td>
<td>Substrate concentration</td>
</tr>
<tr>
<td>So</td>
<td>Initial substrate concentration</td>
</tr>
<tr>
<td>SB</td>
<td>Bulk substrate concentration</td>
</tr>
<tr>
<td>t</td>
<td>Chronological or running time</td>
</tr>
<tr>
<td>θh</td>
<td>Hydraulic retention time</td>
</tr>
</tbody>
</table>
θ_X \quad \text{Mean solids residence time}

Q \quad \text{Liquid volumetric flow rate}

Q_W \quad \text{Volumetric flow rate of waste activated sludge}

Q_O \quad \text{Influent volumetric flow rate}

R \quad \text{Recycle ratio}

k \quad \text{First order reaction rate coefficient}

q \quad \text{Substrate utilisation rate per unit biomass}

q_{\text{max}} \quad \text{Maximum substrate utilisation rate per unit biomass}

\mu \quad \text{Specific biomass growth rate}

\mu_{\text{max}} \quad \text{Maximum specific biomass growth rate}

\mu_{\text{obs}} \quad \text{Observed or net specific biomass growth rate}

OUR \quad \text{Oxygen uptake rate}

OUR_{\text{MAX}} \quad \text{Maximum oxygen uptake rate}

\Delta\text{OUR} \quad \text{Exogenous oxygen uptake rate}

\Delta\text{OUR}_{\text{MAX}} \quad \text{Maximum exogenous oxygen uptake rate}

K_M \quad \text{Substrate half saturation coefficient}

K_{\text{DO}} \quad \text{Dissolved oxygen half saturation coefficient}

n \quad \text{Reaction order}

A \quad \text{Blackman kinetic coefficient}

L, K \quad \text{Powell kinetic coefficients}

L_X, K_X \quad \text{Modified Powell kinetic coefficients}

b \quad \text{Endogenous decay coefficient for active biomass}

k_d \quad \text{MLVSS decay coefficient}

Y \quad \text{Biomass yield coefficient}

Y_{\text{obs}} \quad \text{Observed or net biomass yield coefficient}

f_{\text{ex}} \quad \text{Inert fraction of biomass}

f_{px} \quad \text{Inert fraction of endogenous particulate matter}

T \quad \text{Temperature}

R \quad \text{Ideal gas constant}

E \quad \text{Activation energy}

A \quad \text{Pre-exponential factor (SUR, or OUR)}

K \quad \text{Pre-exponential factor (microbial decay)}

\theta \quad \text{Temperature coefficient}

\eta \quad \text{Effectiveness factor}

r \quad \text{Floc radius}

\rho \quad \text{Floc density}

u \quad \text{Velocity}

D \quad \text{Diffusion coefficient}

h \quad \text{Mass transfer coefficient}

K_{L,a} \quad \text{Liquid side mass transfer coefficient (gas to liquid) for oxygen}
Chapter 12 Bibliography


