THE USE OF CULTURE FLUORESCENCE MONITORING FOR THE CONTROL AND OPTIMIZATION OF BIOLOGICAL NUTRIENT REMOVAL FROM WASTEWATER

by
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ABSTRACT

The objectives of this research were to test the efficacy of continuous real time monitoring of culture fluorescence, associated with intracellular NADH, as an indicator of changes in the biological activity of biological nutrient removal processes, and to determine the usefulness of this tool for the development of control and optimization strategies to improve the efficiency and consistency of biological nutrient removal processes.

For that purpose, intensive studies comparing a number of key process variables with culture fluorescence measurements were done at operating pilot and full scale biological nutrient removal facilities. Results demonstrated several consistent relationships between culture fluorescence and specific key variables. The particular relationships between these variables and fluorescence measurements seemed to be affected by process operating configurations. The gross trends in culture fluorescence showed good correlation with process mixed liquor suspended solids levels in each of the process zones, while the smaller, transient changes in fluorescence related to changes in biological activity. Primary effluent culture fluorescence corresponded to VFA concentrations at all of the plants studied. The consistency of the general fluorescence correlations at each plant allowed general changes in primary effluent VFA content to be predicted on a real time basis from the on-line culture fluorescence data. Similarly, consistent patterns were found between anaerobic zone culture fluorescence and changes in specific anaerobic zone VFA consumption, anaerobic zone mass phosphate release, and anaerobic zone PHA storage. The pattern between anaerobic zone culture fluorescence and specific anaerobic VFA consumption demonstrated the strongest correlation. The consistency of all the patterns should allow changes in anaerobic zone biological activity to be predicted from the changes in measured culture fluorescence. Comparison of the relative differences between anaerobic zone and anoxic zone levels of fluorescence provided an indication of biological denitrification activity. The patterns observed between the various

parameters and culture fluorescence could each be attributed to specific mechanisms and/or metabolic patterns.

The ability to conveniently and rapidly monitor changes in biological activity, using fluorescence monitoring, that altered the process characteristics suggested potential applications for optimizing the biological nutrient removal process through fluorescence-based control. Specifically, these strategies outlined methodology for improving the consistency of effluent phosphorus quality and biological denitrification that involved fluorescence-based regulation of process nutrient load, biological activity, process aeration, and internal recycle control. Preliminary testing at a full scale biological nutrient removal facility demonstrated the ability to achieve continuous, real time nutrient load regulation using culture fluorescence measurements.

From the results obtained with pilot and full scale testing, sludge fluorescence is an indicator of changes in biological activity. Continuous monitoring of this fluorescence is useful, real time technology for the development of control and optimization strategies to improve the performance of biological nutrient removal processes.

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LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
ANA	anaerobic basin/zone
ANO	anoxic basin/zone
AER	aerobic basin/zone
A/O® process	anaerobic/oxic process
ATP	adenosine triphosphate
AVG	average
Bardenpho	Barnard denitrification phosphorus removal process
BEPR	biological excess phosphorus removal
BOD	biochemical oxygen demand
BNR	biological nutrient removal
C	carbon
CIASH	combined ISAH process
CoA	co-enzyme A
COV	coefficient of variance; coefficient of variation
D.O.	dissolved oxygen
EFF	effluent
EMP	Embden-Meyerhof-Parnas pathway
FERM	fermenter
FGR-SGR	combined fixed-growth reactor suspended-growth reactor process
g	gram
GC	gas chromatography, gas chromatographic
HA	hydroxyalkanoate(s)
ISAH	Institute fur Siedlungswasserwirtschaft und Abfalltechnik der Universitat Hannover
NAD^+	nicotinamide adenine dinucleotide (oxidized form)
NADH	nicotinamide adenine dinucleotide (reduced form)
$NADP^+$	nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NFU	normalized fluorescence units
M^+	metallic cation
mg	milligram
MGD	million gallons per day
mL	millilitre
ML/d	megalitres per day
MLSS	mixed liquor suspended solids
MPR	anaerobic zone mass phosphate release (mg P released/g MLSS)
MS	mass spectrometer, mass spectrometry
n	number of observations
nm	nanometers
NO_x	soluble nitrates and nitrites
P	phosphorus, phosphate
PE	primary effluent

PHA	polyhydroxyalkanoate(s)
PHB	polyhydroxybutyric acid
PHV	polyhydroxyvaleric acid
pmf	proton motive force
r^2	correlation coefficient
RAS	return activated sludge
REAER	reaeration basin/zone
SD	standard deviation
TCA	tricarboxylic acid cycle
UCT process	University of Cape Town, South Africa process
VFA	volatile fatty acid(s)
VFA/M	specific VFA consumption (mg VFA consumed/g MLSS)

Note: In this thesis the terms "anaerobic" will refer to the absence of both free oxygen and oxidized nitrogen (nitrite and nitrate), "anoxic" will refer to the absence of free oxygen in the presence of oxidized nitrogen (nitrite and nitrate), and "aerobic" or "oxic" to the presence of free oxygen with or without the presence of oxidized nitrogen (nitrite and nitrate).

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1. INTRODUCTION

1.1. Eutrophication and Phosphate Removal

The eutrophication of natural water systems represents a major surface water quality problem. Eutrophication results from an excessive supply of nutrients which supports the excessive growth of algae and aquatic plants. The abundance of nutrients such as carbon, phosphorus, and nitrogen has been shown to promote eutrophication. Investigation revealed that while much of these nutrients could originate from several non-point sources, point source discharges of wastewater effluents represent major sources of these nutrients. As treatment technologies effectively dealt with the reduction of carbonaceous nutrients in municipal wastewater discharges, attention was turned to the reduction of nitrogen and phosphorus. Because blue-green algae can fix atmospheric nitrogen and introduce it into the aquatic system, phosphorus has been become recognized as the nutrient principally responsible for the excessive eutrophication of natural waters. Consequently, eutrophication control has concentrated on controlling and limiting phosphorus levels in these natural water systems (Manahan, 1984). Limitation of phosphorus loading from point source discharges like municipal wastewater effluents has been the focus of much attention and has proven beneficial for reducing eutrophication (Vallentyne, 1974; WPCF, 1983).

The principal methods of phosphorus removal from wastewater involve physicochemical and biological processes. One common physicochemical phosphorus removal precipitates phosphate as metallic complexes of calcium, iron, and aluminum (Bowker et al., 1987; Wiechers, 1987). Precipitation requires the addition of expensive metallic salts and disposal of the resultant chemical sludge to further increase treatment plant operating costs. Phosphorus removal by other systems, such as alumina adsorption and microfiltration processes, offer promising alternatives to traditional chemical methods (Ames, et al., 1970;

Bult et al., 1993; Yee, 1966), but these processes are still limited.

Biological phosphorus removal was first observed by Srinath et al. (1959) and involves the accumulation and storage of phosphate by sludge biomass in excess of metabolic requirements. Because there is no requirement for chemical addition or chemical sludge disposal, biological excess phosphorus removal (BEPR) processes are often more cost effective than chemical precipitation systems for phosphorus reduction (Canviro Consultants et al., 1986; Morrison, 1988). These observations stimulated intensive research and demonstrated that enhanced phosphate removal results from microbial activity, and that control and optimization of the process requires a thorough understanding of the ecology and physiology of the microbial system (Toerien et al., 1990).

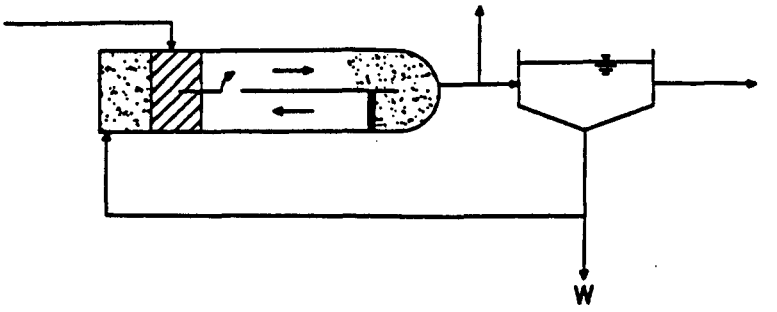
Although biological processes have proven an effective treatment alternative, their inherent biological nature causes variability in process efficiency and effluent quality. The variability of effluent quality and demands of stringent effluent quality criteria highlight the need for better process control and optimization to achieve a more consistent and higher effluent quality. While some researchers have achieved significant results by altering physical aspects of the process (Barnard, 1984; Dold et al., 1984; Oldham, 1985), others have developed biochemical models to provide greater understanding of the process (Comeau et al., 1986; Mino et al., 1987; Wentzel et al., 1986). Despite these advances, the complexity of the microbial population dynamics and the sensitivity of the biomass to changes in conditions have limited the ability to further improve and optimize these systems.

2. LITERATURE REVIEW & BACKGROUND INFORMATION

2.1. Biological Nutrient Removal and Biochemical Models

Biological nutrient removal (BNR) in wastewater can be accomplished by modification of conventional activated sludge processes. In a continuous flow biological nutrient removal process, wastewater is exposed to a variety of conditions as it passes through the process. Typical BNR systems are characterized by anaerobic (no dissolved oxygen or NO_x), anoxic (dissolved NO_x), and aerobic (dissolved oxygen and NO_x) bioreactors in series, with provisions for recycle of mixed liquor and return activated sludge. Some typical BNR suspended growth process configurations are presented in Figure 2.1. Recently, BNR processes incorporating trickling filters or fixed growth reactors (FGR) resulting in a process consisting of a combination of fixed growth and suspended growth reactors (FGR-SGR) have been undergoing intensive research and demonstration as alternatives to traditional BNR configurations (Cooper et al., 1993; Flammino, 1992; Gibb, 1990; Gibb et al., 1993; Gibb, 1994; Goncalves et al., 1992, 1993; Kelly, 1987; Kelly and Gibb, 1989). Despite the many different process configurations used to achieve nutrient removal, the success of these processes rely ultimately on microbial activity. The complex interaction of the various types of organisms that must be cultivated to achieve the combined reduction of biochemical oxygen demand (BOD), nitrogen and phosphorus characteristic of these processes has prompted intensive study and produced descriptive biochemical models.

Nitrogen removal in BNR processes is accomplished by the biological oxidation of ammonia to nitrate followed by the reduction of nitrate to molecular nitrogen. Although a number of microorganisms are capable of oxidizing ammonia under aerated conditions, nitrification is usually attributed to the activity of *Nitrosomonas* and *Nitrobacter* species in wastewater treatment plants (Metcalf and Eddy, 1991; Painter, 1970). The conversion of



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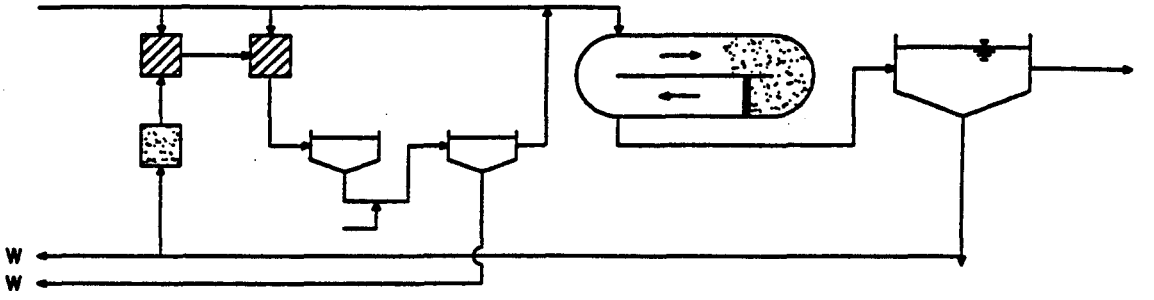

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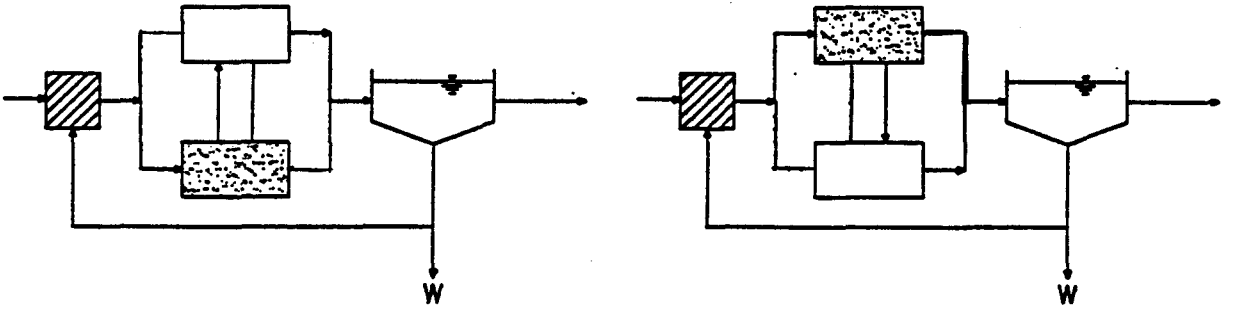


BIO–DENITRO PROCESS

LEGEND:

AEROBIC

ANOXIC

ANAEROBIC

W WASTE SLUDGE

Figure 2.1. Various suspended growth processes for simultaneous nitrogen and phosphorus removal. (Reid Crowther and Partners Ltd., 1993)

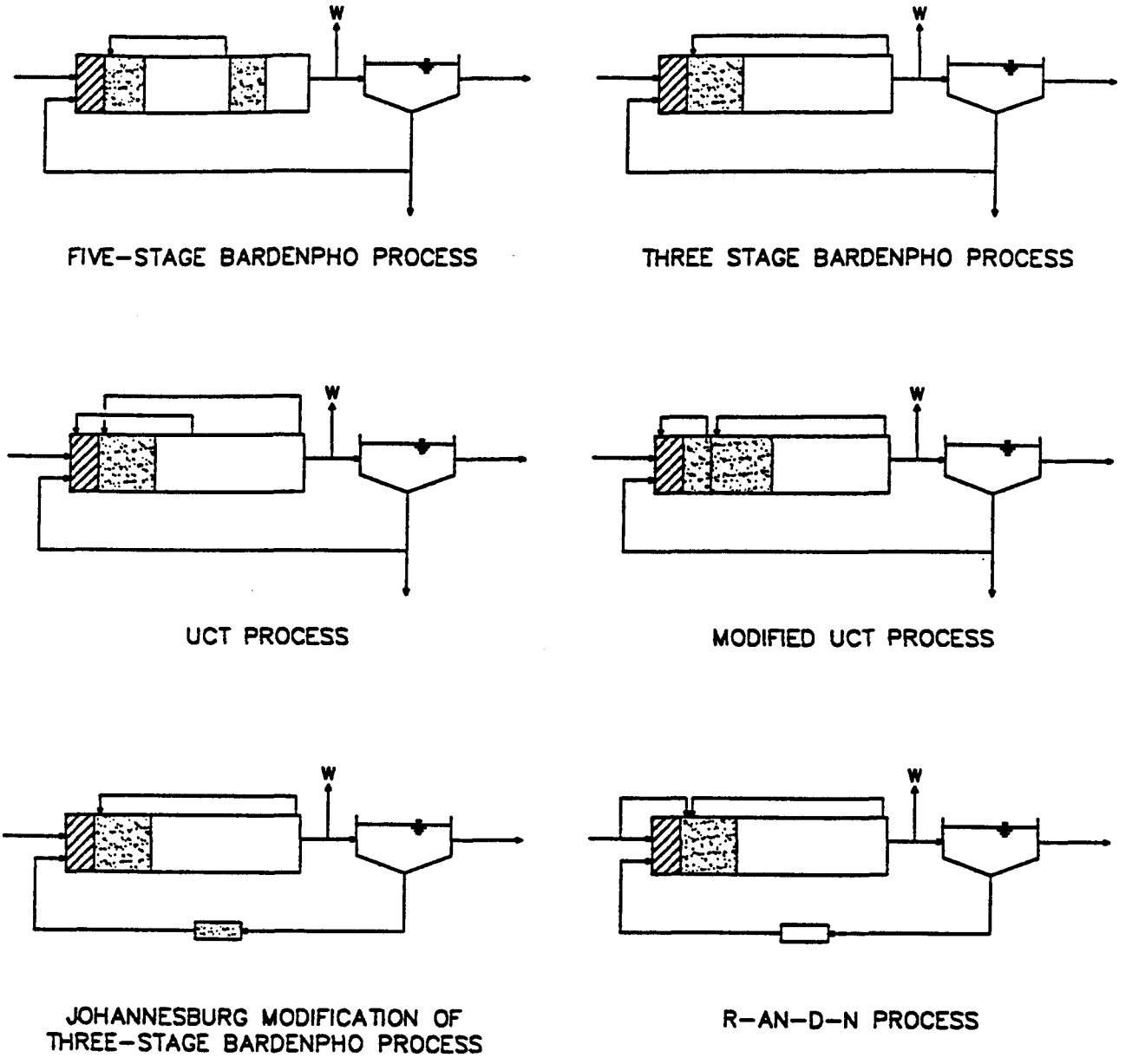


Figure 2.1. Cont'd. Various suspended growth processes for simultaneous nitrogen and phosphorus removal. (Reid Crowther and Partners Ltd., 1993)

nitrate to molecular nitrogen can be accomplished by a wide variety of organisms, and as such has not been attributed to any particular microbial species (Payne, 1981). The discovery of Wuhrmann (1954) that biological denitrification of oxidized nitrogen occurred at low dissolved oxygen levels, prompted the development of activated sludge treatment plant configurations that incorporated zones with very low dissolved oxygen levels (Barnard, 1973; Barth et al., 1968; Ludzak et al., 1962, Wentzel et al., 1991a; Wuhrmann, 1960). These zones became classified as anaerobic and anoxic zones and have become essential components of BNR processes. Many of the important aspects of the biological nitrification/denitrification process are well understood and can be found in most engineering books discussing design and operation of wastewater treatment facilities (Metcalf and Eddy, 1991).

The specific biochemistry of biological excess phosphorus removal (BEPR) is less well understood than biological nitrogen removal although several models have been developed to account for the characteristic behavior of BEPR sludge. Basically, the models suggest that the process relies on the recycling of cultivated biomass between anaerobic and oxic conditions. Under anaerobic conditions, available soluble carbon is taken up by the biomass and converted into specific intracellular carbon storage components, namely polyhydroxyalkanoates (PHA), while polyphosphate reserves are consumed for energy related processes. Later, as cells are cycled to the preferred aerated condition, these carbon stores provide an essential source of carbon for energy generation and growth in conditions where carbon is normally limiting. Some of the energy is stored as polyphosphates, formed from sequestering soluble phosphates, and result in the excess removal of phosphorus observed in these systems. The general models are largely based on and supported by the following observations.

- Anaerobically, soluble VFA are removed from solution (Comeau et al., 1986, 1987; Comeau, 1989; Gerber et al., 1986; Potgieter et al., 1983).
- Increase in soluble phosphate in the anaerobic basin with a decrease in intracellular

polyphosphate reserves (Appeldoorn et al., 1992; Florentz et al., 1984; Haas et al., 1992; Hill et al., 1989; Jing et al., 1992; Mah, 1991; Mino et al., 1987; Murphy et al., 1986).

- Anaerobic increase in intracellular polyhydroxyalkanoates (PHA) (Comeau et al., 1986; Comeau, 1989; Fuhs and Chen, 1975; Mah, 1991; Matsuo et al., 1992, Satoh et al., 1992)
- Aerobic decreases in soluble phosphate and corresponding increases in intracellular polyphosphate reserves (Deinema et al. 1980; Fuhs and Chen, 1975; Hascoet et al., 1985; Hill et al., 1989; Iwema et al., 1985; Mino et al., 1985; Murphy et al., 1986; Ohtake et al., 1985; Suresh et al., 1985; Vasiliadis et al., 1990; Wentzel et al., 1991b).
- Aerobic decrease of intracellular PHA stores (Comeau, 1989; Fukase et al., 1982; Hart, et al., 1982; Lotter et al., 1989; Mah, 1991, Mino et al., 1987).

Currently, there are two leading models, the Mino (Mino et al., 1987) and the Comeau/ Wentzel (Comeau et al., 1986; Wentzel et al., 1986) models, that describe the specific microbial processes that may be responsible for the BEPR process. Both models recognize the importance of anaerobic/oxic recycling of the biomass, the role of short chain fatty acids in the anaerobic selector, and the importance of the PHA as a carbon reserve. Both models recognize the need for reducing power, in the form of NADH (nicotinamide adenine dinucleotide, reduced form) for the synthesis of PHA, but differ in the source of the reducing power. Comeau et al. (1986) and Wentzel et al. (1986) suggest that the required reducing power is supplied by the anaerobic metabolism of the abundant soluble VFA in the TCA or glyoxylate cycles, while Mino et al. (1987) suggest the reducing power is generated from catabolism of carbohydrates, namely intracellular glycogen, in the Embden-Meyerhof-Parnas (EMP) pathway. The Mino model can be criticized for its reliance on glycogen stores since significant glycogen stores could not be detected in some BEPR plants (Comeau, 1989; Nicholls and Osborn, 1979) and were only detected in processes that were glucose enriched (Arun et al., 1988; Fukase et al., 1982; Manoharan, 1988; Somiya et al., 1988; Tsuno et al.,

1987). Despite the fundamental differences between the mechanistic models, the conversion of VFA into PHA compounds, common to both models, not only provides beneficial carbon reserves, but also provides a mechanism for oxidizing NADH under conditions of oxygen limitation. The models suggest that a high cellular NADH/NAD^+ equilibrium initiates PHA storage mechanisms. Mah (1991), however, suggested that the NADH/NAD^+ equilibrium may not be the triggering factor since PHA storage was observed under a variety of equilibria. The utilization of polyphosphate reserves for energy generation and subsequent expulsion of phosphate, in both models, supports the observations of anaerobic phosphate release. A detailed review of these models is provided by Wentzel et al. (1991b) and diagrams outlining these models are presented in Figures 2.2 - 2.5.

2.2. NADH and Microbial Metabolism

Bacterial metabolism fundamentally involves coupled oxidation-reduction reactions that are essential for the breakdown and conversion of substrates, synthesis of new cellular materials, and maintenance of existing cellular biomass. Nicotinamide nucleotides, like NADH (nicotinamide adenine dinucleotide, reduced form), NAD^+ (nicotinamide adenine dinucleotide, oxidized form), NAD(P)H (nicotinamide dinucleotide phosphate, reduced form), and $NAD(P)^+$ (nicotinamide adenine dinucleotide phosphate, oxidized form) drift throughout the cytoplasm and play a central role as electron acceptors and donors in the oxidative-reductive reactions of all organisms. In particular, the NADH/NAD^+ and NAD(P)H/$NAD(P)^+$ redox couples are very important to cellular metabolism (Gel'man et al., 1967). While the two different couples have similar properties and are readily interconverted, the principal functions of each are different. The NADH/NAD^+ pair functions primarily as the electron acceptor in the oxidative breakdown and conversion substrates for cellular processes, while the NAD(P)H/$NAD(P)^+$ pair is used principally as the electron donor in reductive biosynthetic processes (Jones, 1982). Since the functions of each of the different redox couples is not

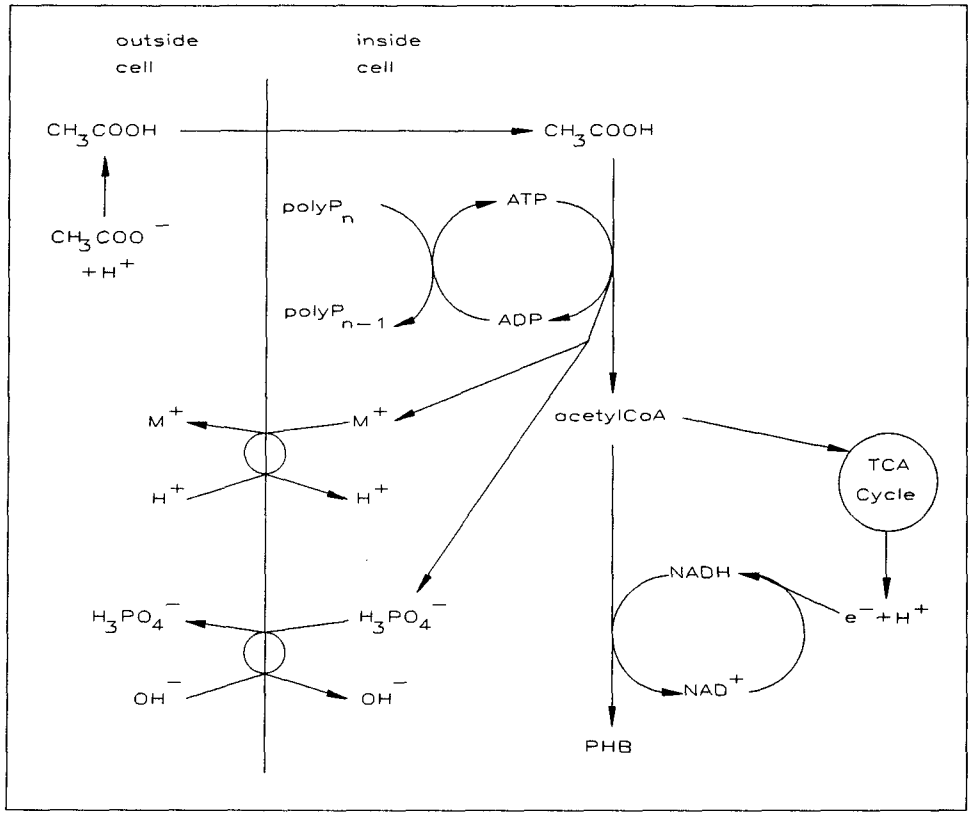


Figure 2.2. Schematic diagram for the behavior proposed by the Comeau/Wentzel biochemical model under anaerobic conditions.(Adapted from Wentzel et al., 1991b).

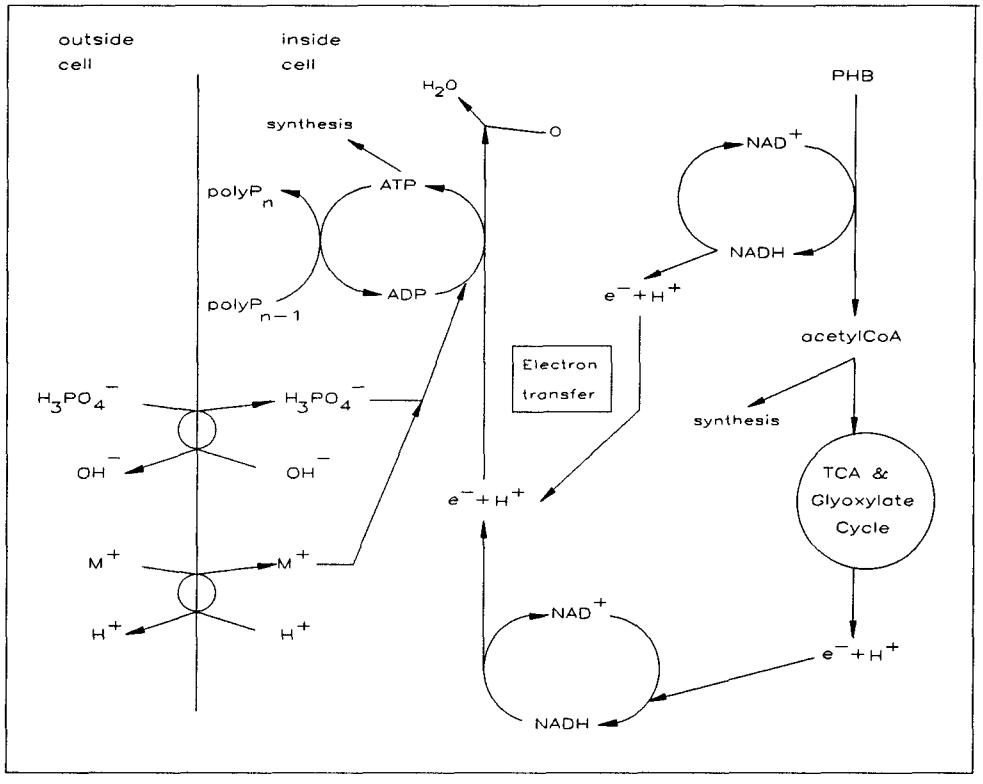


Figure 2.3. Schematic diagram for the behavior proposed by the Comeau/Wentzel biochemical model under aerobic conditions.(Adapted from Wentzel et al., 1991b).

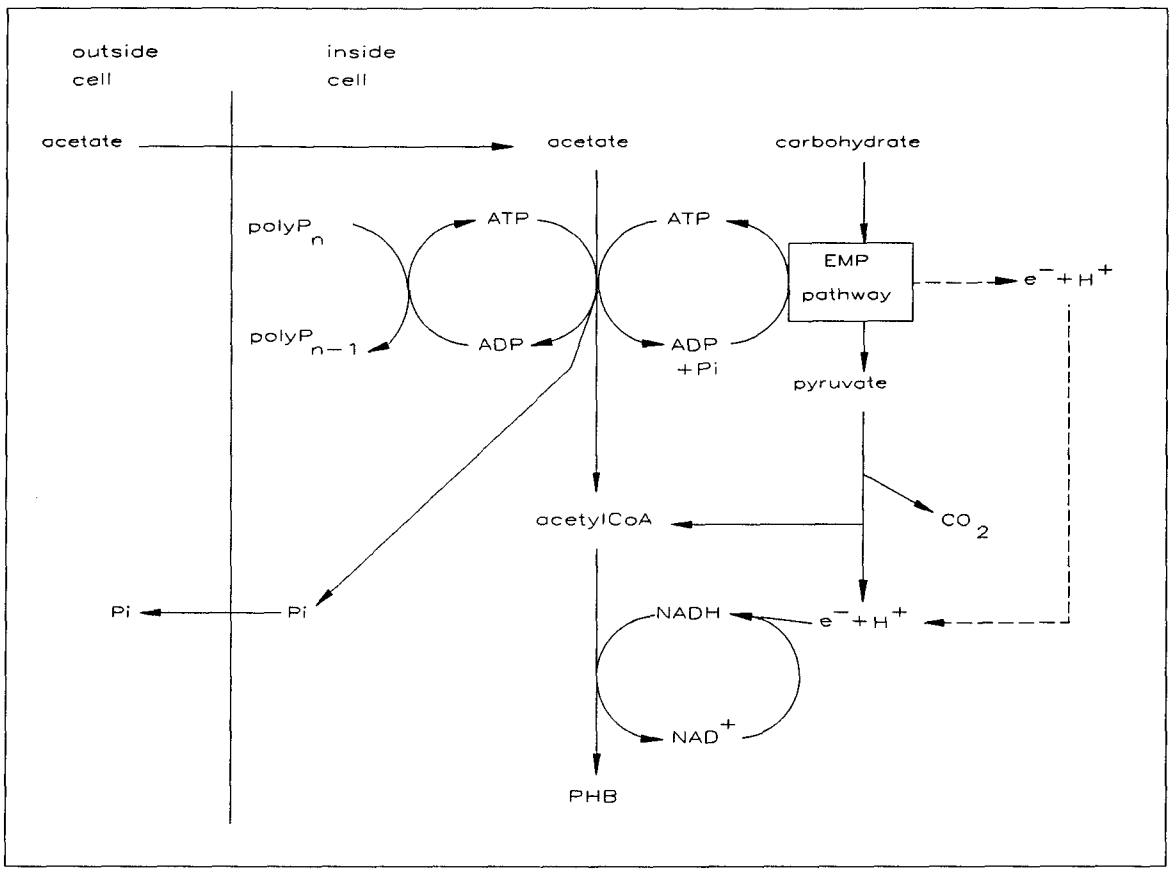


Figure 2.4. Schematic diagram for the behavior proposed by the Mino biochemical model under anaerobic conditions.(Adapted from Wentzel et al., 1991b).

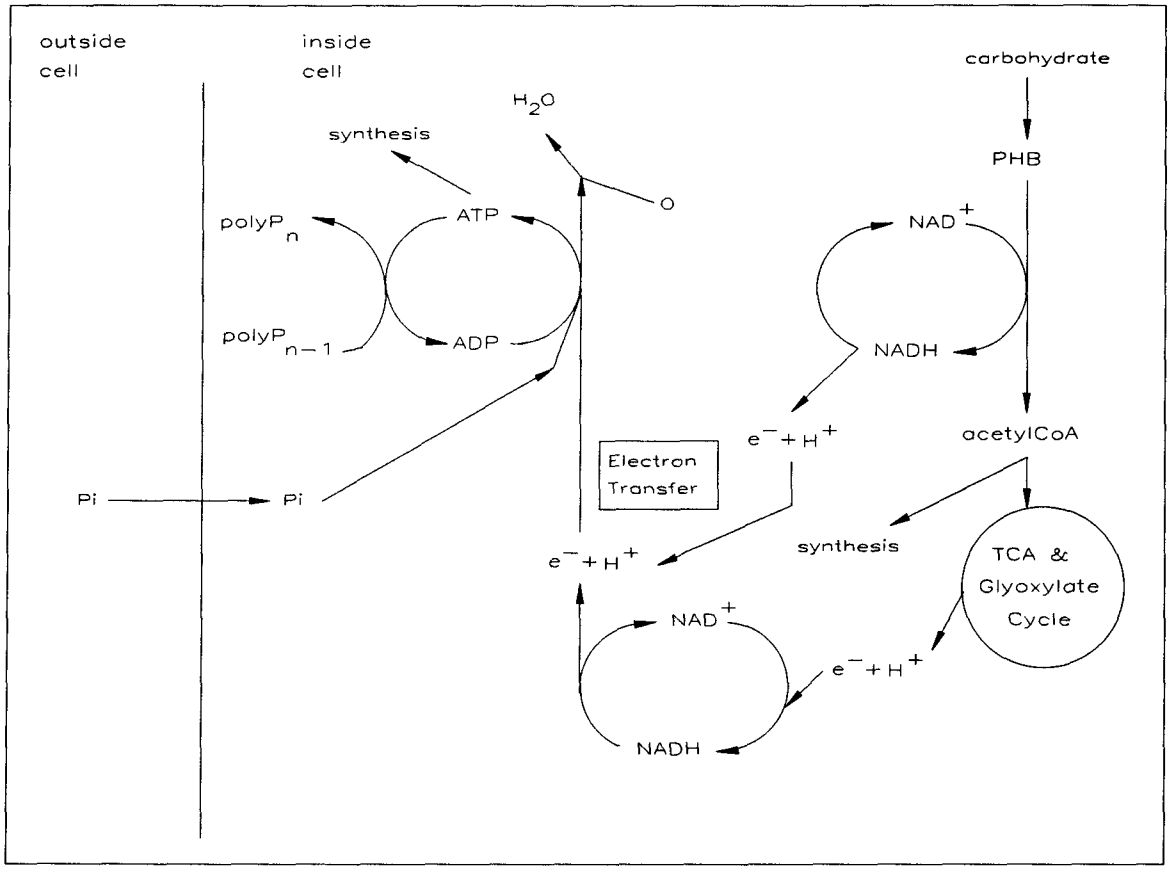


Figure 2.5. Schematic diagram for the behavior proposed by the Mino biochemical model under aerobic conditions.(Adapted from Wentzel et al., 1991b).

absolute, the terms NADH and NAD^+ will be used to represent the reduced and oxidized forms of both nucleotides throughout this thesis.

During metabolism, biochemical reactions generate NADH and NAD^+ that become part of common intracellular pools of NADH and NAD^+. As they are required, NADH or NAD^+ are randomly drawn from the common pools and converted to their oxidized or reduced forms during reaction. Although the total cellular pool of these two compounds is relatively fixed, the constant and rapid interconversion of the two forms results in a dynamically changing NADH/NAD^+ equilibrium that reflects metabolism (London and Knight, 1966; Wimpenney and Firth, 1972).

The principal function of NAD^+ is to provide the oxidative capacity required for the catabolism of substrates. During this process, the NAD^+ is converted to NADH. In order for catabolism to continue, a constant and sufficient pool of NAD^+ must be supplied. To provide a constant supply of oxidative capacity the NADH formed in these reactions must be oxidized to NAD^+. NADH oxidation can be accomplished by a number of mechanisms. The reducing power stored by the NADH can either be used in biosynthetic reactions or transferred to electron transport chains. Ultimately, NADH is oxidized back to NAD^+ so metabolism can continue. Some generalized, reversible reactions of NADH and NAD^+ are depicted in Figures 2.6 and 2.7.

The ability to oxidize NADH and change the relative proportions of NADH and NAD^+ in the total pool is affected by a number of factors, including the availability of electron acceptors. Typical electron acceptors include oxygen, nitrates, and a variety of organic substrates. In most cases the availability of these electron acceptors will affect the rate of NADH oxidation. Many mechanisms are available for oxidizing NADH and include respiratory electron transport chains and fermentative mechanisms. The particular mechanisms used for

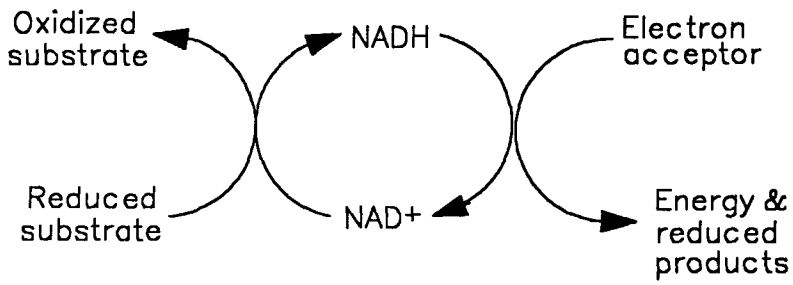


Figure 2.6. Generalized role of NADH in metabolism.

NADH oxidation will be determined, in part, by the energetics of the system. Those mechanisms that produce a higher potential yield are favoured over mechanisms that produce a lower potential yield. Based on the electrical potentials of the coupled half cells (i.e. $1/2O_2/H_2O$, $E^o = 0.84$ V; NOx/N_2, $E^o = 0.74$ V; organic substrate, $E^o << 0.74$ V e.g. fumarate/succinate, $E^o = 0.03$ V; $NADH/NAD^+$, $E^o = -0.32$ V), NADH oxidation by oxygen is energetically the most favourable mechanism, followed next by oxidation by nitrates during the denitrification process, then by fermentative mechanisms that rely on NADH oxidation by organic substrates. Thus, the net $NADH/NAD^+$ equilibrium will be affected by the availability of different electron acceptors. For example, oxygen with a higher oxidation power oxidizes intracellular NADH to a lower level than does nitrate or organic substrates. While general predictions of how changes in environmental conditions will affect the relative proportions of NADH and NAD^+ and the overall equilibrium, the diversity of biochemical reactions that produce and consume these compounds in complex natural environments make quantitative predictions impossible.

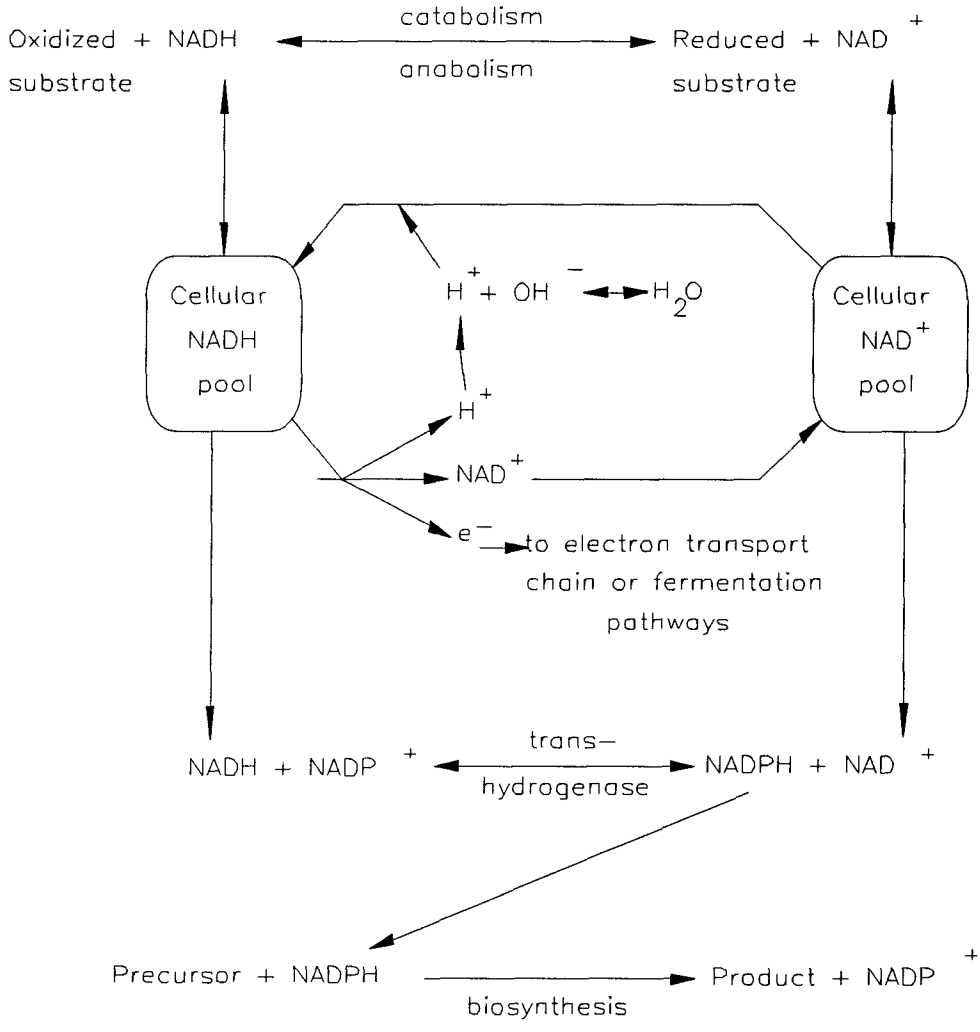


Figure 2.7. Representative reactions of NADH/NAD^+ showing the generalized reactions of metabolism, the existence of cellular NADH and NAD^+ pools, interconversion between the two forms, generalized NADH oxidation by the electron transport chain and fermentation, and the production and use of NADPH.

If the ability to provide adequate cycling of NADH and NAD^+ becomes impaired, the accumulation of NADH or NAD^+ may result in the feedback inhibition of metabolic pathways to prevent the further production and accumulation of NADH or NAD^+ (Lehninger, 1982). While this feedback control will prevent the accumulation of inhibitory levels of NADH or NAD^+, it can also inhibit further metabolism (Lehninger, 1982). This inhibition can persist if the metabolic impairment persists, but will be overridden if the other metabolic needs of the cell become compromised. Since the cell's environmental conditions can strongly influence the cell's metabolic strategies, mechanisms for both NADH oxidation and NAD^+ reduction can be affected by the cell's environmental conditions. Changes in environmental conditions that include changes in the availability of terminal electron acceptors such as oxygen or nitrates/nitrites can significantly alter the intracellular NADH/NAD^+ dynamics. Consequently, the cycling of NADH and NAD^+ required to sustain cellular metabolism is defined and limited by both the metabolic capabilities of the cell and its environmental conditions.

In the presence of air or NO_x, NADH can be readily oxidized to NAD^+ by membrane associated electron transport chains (Figure 2.8; Jones et al., 1982). These chains oxidize NADH by accepting electrons and transferring them to the terminal electron acceptor of respiration, molecular oxygen. The molecular oxygen can come from dissolved oxygen or from nitrates. Respiration results in the rapid oxidation of NADH to NAD^+ with a high yield of energy, largely resulting from the coupling of the exergonic electron transfer reactions to the generation of an electrochemical membrane gradient that is used to synthesize ATP (Boyer et al., 1977; Mitchell, 1961, 1979). NADH oxidation by respiratory electron transport chains serves the dual purpose of providing mechanisms for regenerating the NAD^+ essential for metabolism while generating energy as a by-product. While respiratory electron transport chains provide an efficient mechanism for NADH oxidation and NAD^+ regeneration, NADH

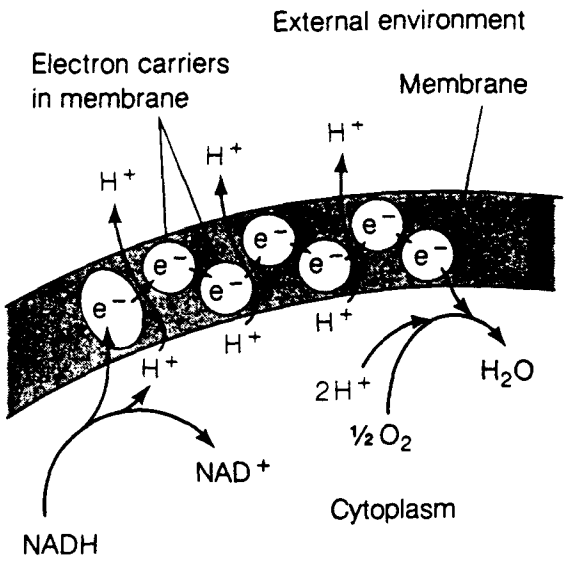


Figure 2.8. Generalized electron transport chain in the bacterial membrane. The terminal electron acceptor, oxygen, can come from dissolved oxygen or from nitrates.

may also be oxidized in biosynthetic reactions (Jones, 1982; Moat and Foster, 1988; Sokatch, 1969).

Electron transport chains are comprised of many components. The functionality and abundance of the individual components change in response to environmental factors. In many organisms, limitations of oxygen or NO_x limit the function of the components and, therefore, limit the ability of these systems to oxidize NADH. Because NADH/NAD^+ cycling is fundamental for metabolism, alternative biochemical strategies are used to oxidize NADH back to NAD^+. NADH oxidation is achieved by transferring the reducing equivalents conserved by NADH during catabolism to one or more endogenous organic oxidants (Jones, 1982). This process is known as fermentation and unlike the more structured and conserved respiratory electron transport systems, is a spatially directionless series of reactions in which chemical group transfer is mediated by soluble cytoplasmic enzymes. In general, the NADH turnover during fermentation is considerably lower compared with respiration. The

comparatively lower turnover may result from a number of factors that include the limitation of suitable organic oxidants for NADH oxidation, the lack of energetically favourable reaction mechanisms, or feedback mechanisms that limit the rate of fermentative reactions, since many fermentations produce toxic wastes. The extent to which microorganisms can utilize fermentative pathways for $NADH/NAD^+$ cycling and metabolism is species dependent.

Despite the environmental conditions and potential limitation in the availability of biochemical mechanisms for NADH oxidation, $NADH/NAD^+$ cycling must be accomplished to sustain a basal level of metabolism. This basal level of metabolism must be maintained to sustain cellular viability and primarily involves the resynthesis of labile compounds which are continually being broken down within the cell (Dietrich and Burris, 1967). In the presence of exogenous nutrients, the $NADH/NAD^+$ cycling and substrates required to maintain this basal metabolism can be supported by respiratory or fermentative catabolism of the exogenous nutrients. In the absence of exogenous substrates, the $NADH/NAD^+$ cycling and substrates may be provided through the respiratory or fermentative catabolism of cellular materials. This process is referred to as endogenous metabolism and can include the catabolism of cellular polysaccharides, lipids, polyphosphates, proteins, ribonucleic acids, free amino acids, and peptides (Goldberg and Dice, 1974; Umbreit, 1976). The endogenous cycle of degradation and resynthesis provides the required cycling of NADH and NAD^+, and the substrates necessary for cellular survival (Moat and Foster, 1988; Sokatch, 1969; Umbreit, 1976). It is uncertain how long endogenous metabolism can sustain cellular viability in the absence of any exogenous substrates. It is also unclear what role endogenous metabolism plays in cellular metabolism when exogenous substrate is present.

At any time, the net steady-state $NADH/NAD^+$ equilibrium provides an indication of the metabolic activity within the cell. Because the total cellular pool of these compounds is relatively fixed, changes in the $NADH/NAD^+$ equilibrium represent changes in the relative

proportions of each species. Although the interconversion of the two species is continuous and affected by numerous reactions, the equilibrium may provide an indication of the cellular metabolic activity (Scheper et al., 1987). Since NADH is rapidly oxidized during respiration, aerobic microbial cultures are characterized by an NADH/NAD^+ equilibrium favouring a majority of NAD^+ (Betz and Chance, 1965; Harrison and Chance, 1970; London and Knight, 1966). In contrast, anaerobic cultures where "favourable" mechanisms for NADH oxidation are comparatively limited are characterized by an NADH/NAD^+ equilibrium favouring a higher proportion of NADH (Betz and Chance, 1965; Harrison and Chance, 1970; London and Knight, 1966). Under all conditions, however, changes in cellular metabolism can cause variability in the equilibrium within these general trends. For example, analysis of the NADH/ NAD^+ equilibrium in a BEPR process showed a higher proportion of NADH relative to NAD^+ in anaerobic conditions compared to aerobic conditions, as expected (Mah, 1991). Under the same anaerobic conditions, however, the relative proportion of NADH could be lowered by inducing reactions that consumed NADH to alter the equilibrium (Mah, 1991). Although the equilibrium changes were minor, the changes illustrate that variability or flux in the distribution of both NADH and NAD^+ species can occur within the conventional definitions of environmental states (i.e. anaerobic, aerobic). In other words, anaerobic or aerobic conditions are not strictly defined by the establishment of specific cellular NADH/NAD^+ equilibria. Instead, each condition encompasses a general range of NADH/NAD^+ equilibria, the extreme limits of which are loosely defined. Thus, by monitoring the intracellular NADH/ NAD^+ equilibrium or changes in the equilibrium, information about the culture metabolic state can be gained.

2.3. NADH Monitoring

Due to the central role that NADH and NAD^+ have in the oxidative reactions of all organisms, Chance and Williams (1956) proposed that monitoring the intracellular redox

potential of these compounds should provide important information regarding patterns of cellular metabolism. Initial attempts to monitor changes in the cellular composition of these compounds employed enzymatic and chemical methods (Cartier, 1968; Takebe and Kitahara, 1963; Wimpenney and Firth, 1972). Most of these methods used extraction techniques for recovering NADH or NAD^+ that were destructive to the cell and too slow to give reliable results because of the rapid turnover rates of these compounds. These problems limited the ability to monitor rapid changes in NADH/NAD^+ and metabolism. Recognition of the spectral and fluorometric properties of NADH and NAD^+ allowed the development of non-destructive methods for the estimation of relative intracellular NADH levels. The observation that NADH is fluorescent with absorption and emission maxima at 340 nm and 460 nm, respectively, while NAD^+ is not, led to the development of numerous techniques for fluorometric determination of relative intracellular NADH levels (Duysens and Amesz, 1957; Estabrook, 1962; Estabrook and Maitra, 1962; Harrison and Chance, 1970; Srinivas and Mutharasan, 1987). Refinement of these techniques and extensive research led to the successful monitoring and control of metabolic activities in biological systems by following the changes in the net NADH/NAD^+ equilibrium indicated by culture fluorescence (Armiger et al., 1986; Betz and Chance, 1965; Beyeler et al., 1981; Beyeler and Meyer, 1984; Chance and Thorell, 1959; Einsele et al., 1978; Einsele et al., 1979; Gschwend et al., 1983; Kwong et al., 1993; Li and Humphrey, 1989; Luong and Carrier, 1986; Maneshin and Arevshatyan, 1972; Meyer et al., 1984; Peck and Chynoweth, 1990; Polakis and Bartley, 1966; Rao and Mutharasan, 1989; Ristoph et al., 1977; Srinivas and Mutharasan, 1987; Watteeuw et al., 1979; Zabriskie, 1979).

Most fluorophores like NADH possess characteristic, but often not unique excitation and fluorescence spectra. Spectral fluorescence analysis of any fluorophore with excitation by light of a particular wavelength might produce a fluorescence emission spectrum dissimilar to that produced by the same fluorophore when irradiated with excitation light of a different wavelength. Since it is possible for a single fluorophore to possess different fluorescence

spectra when irradiated with different wavelengths of excitation light, it is possible to identify an excitation wavelength and/or excitation wavelengths that produce distinguishable peaks, or maxima, in the resulting fluorescence spectra. When targeting a specific fluorophore for fluorescence monitoring, it is desirable to identify the excitation wavelength(s) that produce the distinguishable fluorescence maxima so as to enable excitation with one of these excitation wavelengths and detect the resulting fluorescence at the wavelength corresponding to the resulting maxima. In practice, it is not always possible to limit the wavelength of the excitation source to a specific single wavelength, nor limit the wavelength of fluorescence detection to a specific single wavelength. Therefore, once the optimal wavelengths for the spectral analysis of a specific fluorophore are identified, spectral analysis is conducted by limiting the ranges of wavelengths used for excitation and detection of fluorescence to those that form an envelope that encompass the desired wavelengths of excitation and detection. Despite the ability to restrict the wavelength ranges of excitation and fluorescence detection with the intent of targeting the measurement of a specific fluorophore, the diversity of fluorophores that could be present and their multiplicity of fluorescence spectra, complicates the ability to precisely determine the fluorescence spectra of a single fluorophore. Except in the situation where a pure solution containing only a single specific fluorophore is being fluorometrically analyzed, the overlapping fluorescence spectra of different fluorophores prevent precise determination or analysis of a single fluorophore. Consequently, fluorescence measurements of a non-homogenous solution can be considered to be comprised of two different measurements 1) the fluorescence comprised of the fluorescence emitted from the targeted fluorophore, and 2) fluorescence emitted from the other fluorophores in solution with similar excitation and fluorescence emission spectra. The latter component of the total measured fluorescence signal can be called "background" fluorescence.

Recognizing that other fluorophores exist in solution that possess the same spectral properties as NADH means that not all of the fluorescence measured within the particular

excitation and emission ranges used is directly related to NADH (BioChem Technology Inc., 1987; Maneshin et al., 1991). Some people have criticized the use of fluorescence measurements, within the excitation and emission spectra of NADH, as an direct indicator of changes in cellular NADH content or biological activity (Hottiger and Bailey, 1991) after finding poor correlations between cellular NADH levels and measured fluorescence. These researchers, however, did not identify the nature or origin of the materials contributing to their fluorescence measurements, some of which may have been indirectly related to changes in biological activity. Despite criticism from Hottiger and Bailey, the abundance of studies correlating changes in measured fluorescence with changes in intracellular NADH levels and metabolic patterns clearly establishes a strong correlation between culture fluorescence, intracellular NADH levels, and changes in the metabolic state and/or condition of biological mixtures (Armiger et al., 1986; Betz and Chance, 1965; Beyeler et al., 1981; Beyeler and Meyer, 1984; Chance and Thorell, 1959; Einsele et al., 1978; Einsele et al., 1979; Gschwend et al., 1983; Kwong et al., 1993; Li and Humphrey, 1989; Luong and Carrier, 1986; Maneshin and Arevshatyan, 1972; Meyer et al., 1984; Peck and Chynoweth, 1990; Polakis and Bartley, 1966; Rao and Mutharasan, 1989; Ristoph et al., 1977; Srinivas and Mutharasan, 1987; Watteeuw et al., 1979; Zabriskie, 1979). These studies conclusively demonstrated the ability to conveniently monitor and control changes in biological activity using NAD(P)H related fluorescence measurements in a number of pure culture systems on a real time basis. The application of fluorescence monitoring in mixed culture systems has not been studied as extensively. Some preliminary results have demonstrated the potential of continuous culture fluorescence monitoring in mixed sludge systems (Armiger et al., 1990; Isaacs and Henze, 1994; Mah, 1991; Mah et al., 1994, 1995; Schwegler et al., 1995).

3. RESEARCH RATIONALE OBJECTIVES, AND APPROACH

3.1. Rationale

Experience has demonstrated that while biological nutrient removal systems can be successful for achieving an average high effluent quality, these systems are often subject to variability of effluent quality due to the complex interaction of the mixture of various types of organisms that must be cultivated to achieve the combined reduction of biochemical oxygen demand (BOD), nitrogen, and phosphorus characteristic of these systems. As criteria for point source phosphorus discharges from municipal wastewaters become increasingly stringent, it will become more important to reduce the diurnal and weekly excursions that occur in effluent phosphorus concentrations due to changes in hydraulic and nutrient loading. The only way to achieve such reductions is by control and optimization of process performance through feedback regulation of dissolved oxygen (DO) concentrations, process carbon loading, and recycle flows. Traditionally, attempts to control and optimize BNR process performance have been limited by control technologies that rely on indirect indicators of the biological activity directly responsible for nutrient removal. Although concentrations of soluble chemical species can provide useful information, the acquisition, analysis, and interpretation of the data are time consuming and do not provide direct or predictive information that allows timely control actions to be made. The development of equipment for the automated analysis of soluble chemical species like phosphate, nitrate, and ammonia has decreased the time associated with the acquisition and analysis of these chemical species, but the information provided by this equipment still only provides an indirect indication of the biological activity fundamental to the process. While the development of on-line monitoring systems like those for the regulation of dissolved oxygen levels and cycle times in batch or alternating aerobic-anoxic processes have shown some promise for real time control (Charpentier et al., 1989; Jenkins and Mavinic, 1989; Koch and Oldham, 1985; Lotter and Pitman, 1993; Sasaki et al.,

1993; Thornberg et al., 1993; Wareham et al., 1993, 1994), monitoring changes in bulk solution pH, oxidation-reduction potential, or dissolved oxygen still only provides an indirect measurement of biological activity. Because these systems rely on the measurement of bulk solution parameters, it is not always possible to differentiate between changes resulting from true variation in biological activity and changes due to variation in the chemical characteristics of the influent or mixed liquor. Control strategies that can incorporate continuous and direct determination of biological activity under dynamic hydraulic and nutrient conditions will be much more likely to succeed in the regulation and optimization of BNR process performance than those strategies that rely on indirect measures of biological activity, like changes in the characteristics of the bulk liquid, that may not always provide a true indication of biological activity.

Considering the facts of the leading biochemical models, it is postulated that changes in the levels and patterns of specific metabolism in the BNR sludge biomass, namely anaerobic VFA consumption and PHA storage, will be directly reflected in changes in measured culture fluorescence related to changes in the net NADH/NAD^+ equilibrium. It is also postulated that these changes can be correlated to specific changes in the character of the biological mass (e.g. PHA levels, aerobic phosphate removal capacity) and/or key process parameters (anaerobic phosphate release, soluble phosphate inventory). Furthermore, these changes will impact on the efficiency and consistency of nutrient removal, and the different patterns of measured fluorescence and biological activity should correspond to different process efficiencies.

For example, metabolism of volatile fatty acids and polyhydroxyalkanoates (PHA) are key parameters expected to impact both the cellular NADH dynamics and process performance. The direct consumption of NADH in the formation of PHA from VFA (Figure 2.2) is expected to rapidly decrease steady state NADH levels and thus the related measured

fluorescence. Specifically, it is predicted that as VFA consumption and PHA storage increase, the steady state equilibrium level of NADH and its related fluorescence will decrease. Furthermore, since it is predicted that the availability of PHA impacts downstream phosphate removal, changes in VFA and PHA metabolism can affect aerobic phosphate removal capacity and overall effluent phosphate quality. Documenting and correlating the different patterns of fluorescence with the differences in anaerobic VFA and PHA metabolism and their effect on anaerobic phosphate release and aerobic phosphate removal, it should be possible to predict changes in process performance directly from fluorescence data.

Definition of the relationships between various patterns of measured fluorescence, specific biological activities (e.g. VFA consumption, PHA synthesis, phosphate release), and process performance will provide information to control these metabolic reactions fundamental to the process, and lead to the development of real time optimization strategies. Furthermore, the ability to directly recognize and control patterns of metabolism will provide greater confidence in formulating design and operating strategies to maintain more optimal and consistent process performance.

3.2. Objectives and Approach

The principal objectives of this research were to; 1) test the use of continuous, "real time" monitoring of fluorescence, related to intracellular NADH, as an indicator of changes in biological activity, and 2) evaluate the usefulness of "real time" fluorescence monitoring as a tool for the development of novel on-line control and optimization strategies designed to improve BNR process performance.

The experimental testing involved extensive pilot scale testing as well as field studies at full scale BNR plants. Testing involved the intensive monitoring of a number of key

variables under fluctuating and constant carbon (VFA) loading conditions during the hydraulically balanced operation of the pilot plant, and monitoring routinely operating full scale plants. The process monitoring included continuous fluorescence measurements and periodic determination of intracellular polyhydroxyalkanoates (PHA), phosphorus content of the sludge biomass, mixed liquor suspended solids concentrations, and soluble volatile fatty acid, ammonia, nitrate, and phosphate concentrations throughout the various process zones. Analysis included comparing changes in the key parameters and process performance with changes in measured fluorescence to determine the existence of any consistent patterns that would allow changes in the character of the process or its performance to be predicted from fluorescence data. Once specific patterns were identified from the pilot scale testing, these patterns were compared with the patterns obtained from full scale BNR plants. The potential of the patterns was then considered for application in process control and optimization strategies. A fluorescence based control strategy was developed and used to demonstrate the capabilities of culture fluorescence monitoring at a full scale BNR plant.

4. MATERIALS AND METHODS

4.1. Biological Nutrient Removal Treatment Plants Studied

Experiments were conducted at a number of biological nutrient removal facilities treating municipal wastewater. General descriptions of each of the plants studied are provided below.

4.1.1. FGR-SGR pilot plant

The majority of experiments were conducted at a pilot scale FGR-SGR (fixed growth reactor-suspended growth reactor) process operated to achieve biological nutrient removal. Owned and operated by British Columbia Research Inc., the plant is located on the University of British Columbia campus and treats domestic wastewater collected from residential complexes. The entire process consists of two parallel trains housed in a large trailer. The process configuration of one train of the FGR-SGR pilot plant is presented in Figure 4.1. Influent flow is constant at 4.8 L/min and comes from two large stirred storage tanks that are filled twice daily from a municipal sewage collection system. The total process liquid volume, excluding the fixed growth reactor (trickling filter) collection basins is 1950 L, with the anaerobic, anoxic, and reaeration basins accounting for 380 L, 630 L, and 940 L, respectively. To achieve enhanced VFA production, primary sludge is pumped to a mixed fermenter. Fermenter operation provides for partial clarification of the fermenter sludge, and recirculation of the partially clarified VFA-rich fermentate to the primary influent. A more thorough description of the pilot process is provided by Gibb et al. (1993).

During pilot plant experimentation, plant performance was evaluated under constant and variable VFA load conditions. Because of the presence of the storage tanks, fermenter

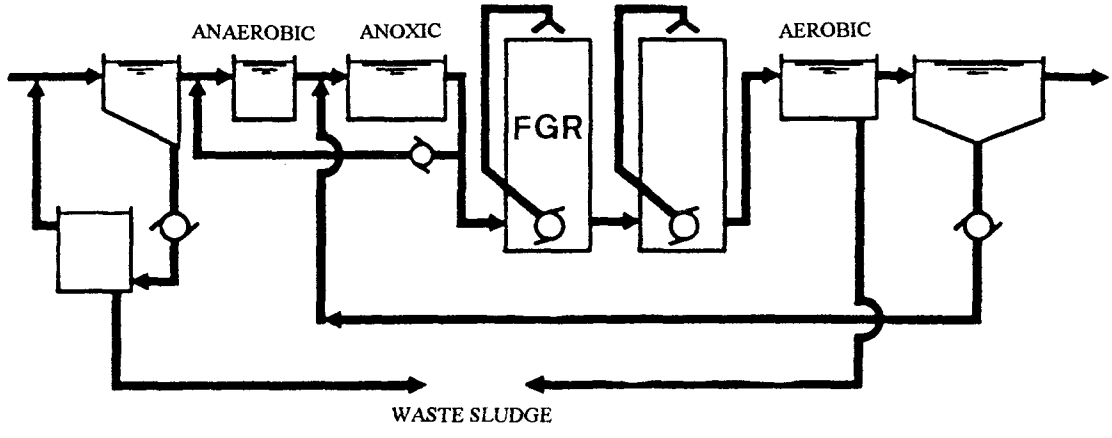


Figure 4.1. Schematic of the pilot scale FGR-SGR process. (Gibb et al., 1993).

VFA production, and constant hydraulic load, the nutrient load to the plant was reasonably constant unless operator adjustments were made. Conditions of variable nutrient loading were achieved by regulating the fermenter feed rate to control fermentate addition to the primary influent. Alternately, typical VFA load variations were simulated by the controlled addition of a sodium acetate solution (Sigma Chemical Co., St. Louis, MO.) using a computer controlled peristaltic pump (Model 420-C Dual Channel Peristaltic Pump, BioChem Technology Inc., King of Prussia, PA.). The pump was operated such that the pumping rate of a freshly prepared stock solution of sodium acetate was ramped from 0 mL/h to 1000 mL/h and back to 0 mL/h over a period ranging from one to three hours. The concentration of the stock solution was controlled to achieve an increase in soluble acetate in the anaerobic bioreactor of approximately 20 mg/L when added at the maximum pumping rate.

4.1.2. Kelowna Pollution Control Centre

Originally designed as a five stage Bardenpho process, this plant located in Kelowna, British Columbia was operated as a 40 ML/d modified three-stage Bardenpho process during the period of this study. The process modifications included changes to allow a portion of the primary effluent stream to be directly discharged into the last anoxic basin, and to allow the discharge of VFA-rich supernatant from a primary sludge thickener, used as a static fermenter, directly into the anaerobic bioreactor. A detailed schematic of the wastewater treatment plant is provided in by Oldham and Stevens (1984). Each bioreactor module is composed of twenty-one 450 m^3 cells, with a combined total volume of 9450 m^3. During the period of this study, only 14 of the individual cells were used. A representative schematic of one of the main process bioreactor modules, as it was operated during this study, is provided in Figure 4.2.

4.1.3. Oaks, PA A/O® Wastewater Treatment Plant

The process located at Oaks, Pennsylvania consists of two parallel trains with a combined capacity of 21 ML/d. A schematic of one train of the A/O® process is presented in Figure 4.3. The main bioreactor of each process train consists of three anaerobic, three anoxic, and four oxic basins. The total volumes of the anaerobic and anoxic zones are 382 m^3 each, with the oxic basins occupying a total of 2544 m^3. Unlike the other processes studied that have on-site facilities for generating volatile fatty acids, the only source of VFA at this facility comes from the generation of volatile fatty acids in wastewater collection system, primary clarifiers, and the anaerobic zone of the main bioreactor.

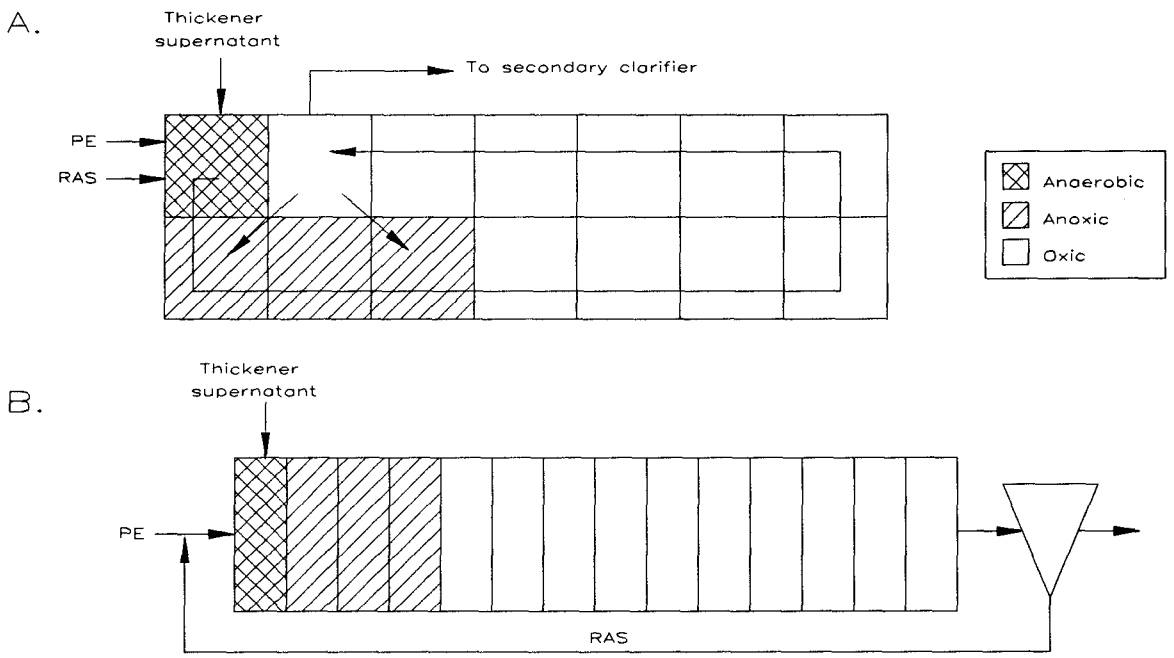


Figure 4.2. Process configuration of the Kelowna Pollution Control Centre. (A) Bioreactor module configuration; (B) Linear configuration of a module.

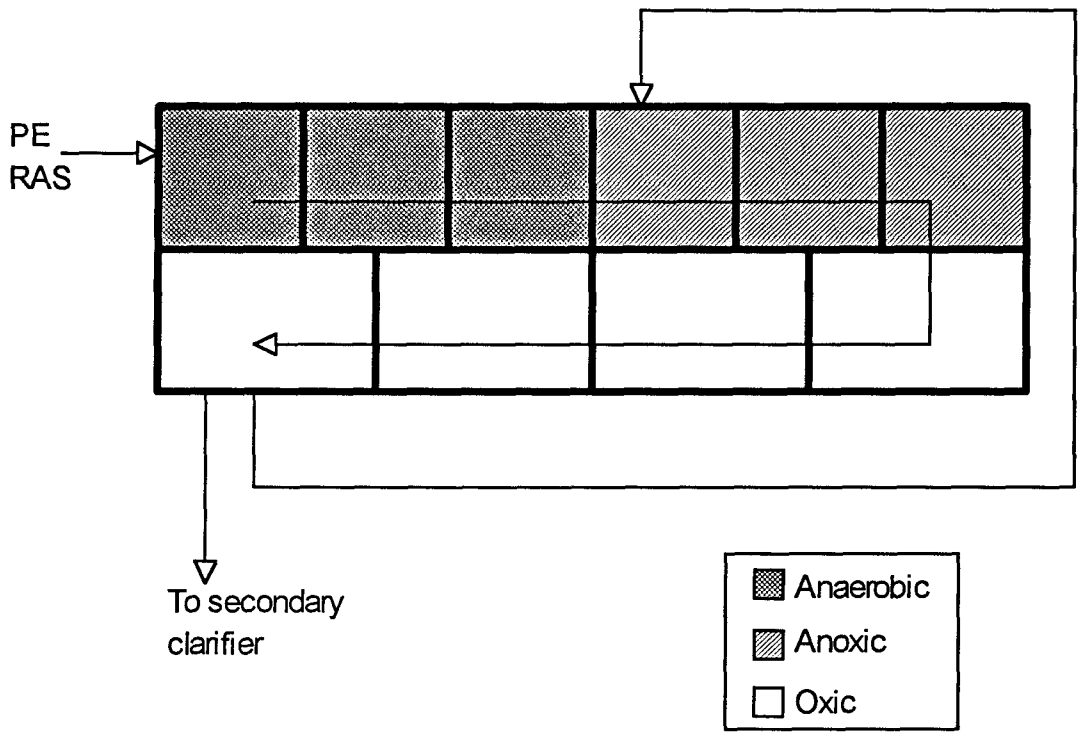


Figure 4.3. Process configuration of the Oaks, PA A/O® wastewater treatment plant.

4.1.4. Penticton Advanced Wastewater Treatment Plant

The advanced wastewater treatment plant located at Penticton, British Columbia is a 18.0 ML/d modified UCT (University of Cape Town, S. Africa) process. Volatile fatty acids are generated on-site by fermentation of primary sludge in complete mix fermenters. The mixed contents of the fermenters are discharged back to the primary clarifiers. Process flows are balanced with the equalization facilities shown in the process schematic (Figure 4.4). The process consists of two parallel trains, each composed of 11 individual cells with a total combined volume 4852 m^3 train. During the period of this study, only 9 of the 11 cells were used in the train studied. The process train was comprised of two anaerobic cells, four anoxic cells, and three oxic cells, with combined volumes of 388 m^3, 1067 m^3 and 1941 m^3, respectively. Specific real time control strategies were tested at the Penticton plant. The details of these tests will be outlined in Section 5.

4.2. Process Sampling

Testing for relationships between key process variables, biological activity, and process performance required intensive sampling for the determination and quantification of key process variables. Table I outlines the general sampling program followed during experimentation at the FGR-SGR pilot plant. Sampling of individual parameters and individual bioreactors was modified at each of the full scale plants studied to accommodate the availability of analytical equipment at each location. In most cases, all of the parameters listed in Table I, except sludge %P and PHA, were measured. Typically, pilot plant grab samples were collected for analysis at thirty or sixty minute intervals over a period ranging from six to twelve hours. All sample collection and preparation was done by the researcher to minimize the introduction of experimental errors from inconsistent sample collection, handling, and

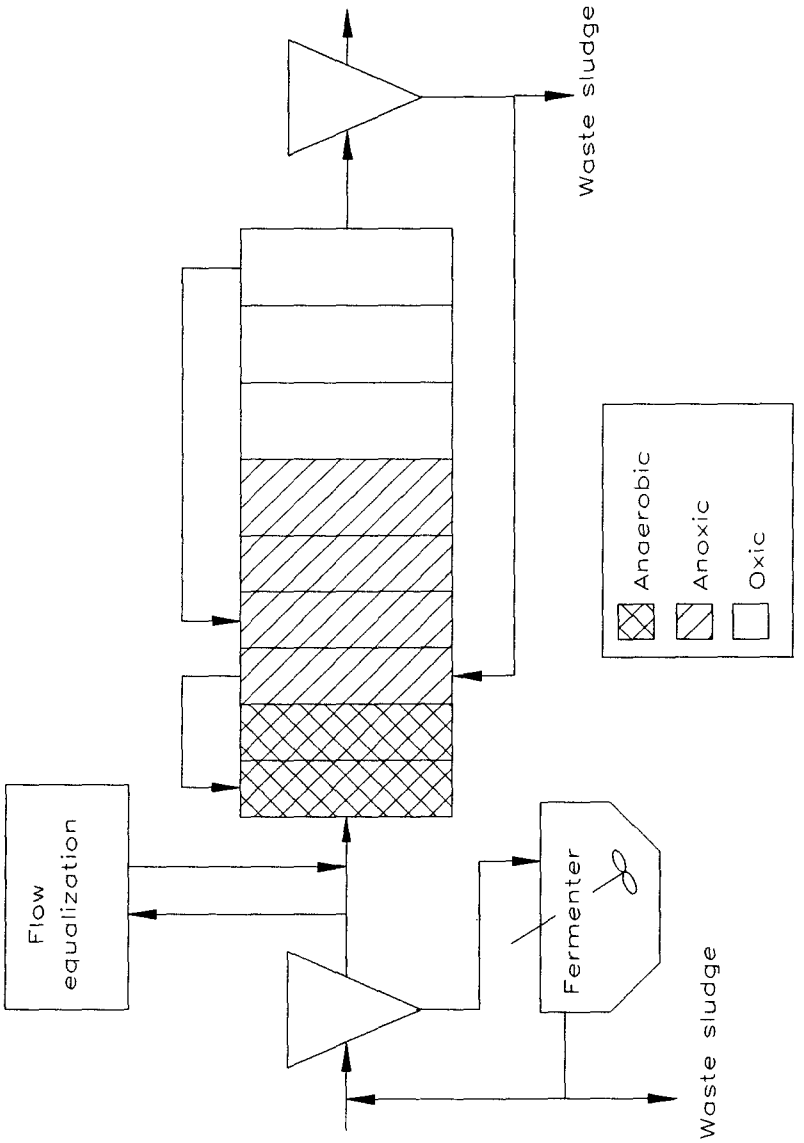


Figure 4.4. Process configuration of the Penticton Advanced Wastewater treatment plant.

Table I. General experimental sampling program for the FGR-SGR pilot plant.

	PE	FERM	ANA	ANO	REAER	RAS	EFF
	X		X	X	X		
VFA:acetic	X	X	X	X			
propionic	X	X	X	X			
butyric	X	X	X	X			
iso-butyric	X	X	X	X			
Soluble:Phosphate	X		X	X	X	X	X
Nitrate	X		X	X	X	X	X
Ammonia	X		X	X	X	X	X
PHA:PHB			X	X	X		
PHV			X	X	X		
Sludge%P			X	X	X		
MLSS	X		X	X	X	X	X

Note: Culture fluorescence measurements were not regularly taken from the reaeration basis.

preparation. During experimentation at the full scale plants, grab samples were collected for analysis at one to two hour intervals for periods ranging over twelve to twenty-six hours.

4.3. Analytical Methods

4.3.1. Culture fluorescence measurements

Continuous fluorescence measurements were made using the BioGuide® system developed by BioChem Technology Incorporated (King of Prussia, Pennsylvania). The system consists of a series of immersible probes interfaced to a computer for data acquisition and processing. The *in situ* probes allow changes in intracellular NADH to be monitored by continuously detecting changes in intracellular fluorescence at the wavelength 445 nm (+/-15nm) caused by excitation at 345 nm (+/-20nm). The information collected from the probes is processed by proprietary software and reports the measured fluorescence in normalized fluorescence units (NFU) on a real time basis. The system has the capability to process and report fluorescence data at one second intervals. Fluorescence measurements were made continuously in the primary effluent, continuously in the anaerobic and anoxic basins of the FGR-SGR pilot plant, and periodically in the reaeration basin. The specific locations and frequencies of fluorescence measurements at the full scale treatment plants were determined by the availability of monitoring equipment and the ease of installation.

4.3.2. Volatile fatty acid (VFA) analysis

Samples for volatile fatty acid analysis were collected and immediately filtered (Whatman filter paper, #4, Whatman Laboratory Division, Maidstone, KY). One millilitre of the filtrate was transferred to an autosampler vial containing 100μL of a 2% phosphoric acid solution, for acidification and preservation, and sealed with an aluminum crimp cap.

Analysis was accomplished by gas chromatography on a Hewlett Packard Model 5880A chromatograph equipped with a flame ionization detector, integrator, and autosampler. A 1μL aliquot of the acidified sample was analyzed using a four foot long, 2 mm I.D. glass column packed with 60/80 Carbopack C/0.3% Carbowax column packing (Supelco Canada Ltd., Oakville, Ontario) for analysis of C1 to C5 compounds. Gas chromatographic analysis was conducted under isothermal conditions with an oven temperature of 120°C and inlet and detector temperatures of 200°C, using helium gas (20 mL/min) as carrier and can detect concentrations within the range between 2 to 100 mg/L of the C1 to C5 compounds and reports concentrations to two decimal places.

4.3.3. Polyhydroxyalkanoate (PHA) determination

Samples for polyhydroxyalkanoate analysis were collected in 30 mL polyallomer tubes and immediately centrifuged. After centrifugation, the supernatant was decanted and the separated solids were rapidly frozen using an ethanol-dry ice bath (95% ethanol and dry ice). Polyhydroxyalkanoates, specifically polyhydroxybutyric acid and polyhydroxyvaleric acid, were extracted from the cells and analyzed using gas chromatography according to the techniques of Comeau et al. (1988). Quantification was accomplished using standards of α-hydroxybutyric acid, β-hydroxybutyric acid, and α-hydroxyvaleric acid (Sigma Chemical Co., St. Louis, MO.). Gas chromatographic analysis was done using a megabore column (30 m, 0.53 mm I.D.) coated with 1 mm of DB-Wax (J&W Scientific, Folsom, CA) and a Hewlett Packard Model 5890 Series II gas chromatograph equipped with a flame ionization detector, Hewlett Packard HP3396 Series II integrator, and autosampler. The gas chromatograph was operated with inlet and detector temperatures of 200°C and 250°C, respectively using helium gas as carrier (30 mL/min). The oven temperature was programmed to enhance the separation and detection of compounds. The initial oven temperature was held at 50°C for 3 minutes then increased to 100°C at a rate of 10°C/min where it was held for 1 minute.

Following this, temperature was increased (3°C/min) to 130°C and held for 1 minute, then increased (30°C /min) to 200°C and held for 5 minutes.

4.3.4. Soluble phosphate, nitrate, and ammonia

Samples for analysis of soluble components were collected, immediately filtered (Whatman filter paper, #4, Whatman Laboratory Division, Maidstone, KY.), and stabilized by acidification (100μL 1N H_2SO_4) or addition of a phenyl-mercuric acetate solution (100μL of 0.1 g phenyl-mercuric acetate in 100 mL acetone). Samples collected from the pilot plant were analyzed using the automated procedures for determination of soluble ortho-phosphate (Quick-Chem Method 10-115-01-1-Z, Latchat Quick-Chem, Milwaukee, WI), nitrate (Quick-Chem Method 10-107-04-1-Z, Latchat Quick-Chem, Milwaukee, WI), and ammonia (Quick-Chem Method 10-107-06-1-Z, Latchat Quick-Chem, Milwaukee, WI) used by the Latchat Quick Chem AE Model 23000-000 analyzer (Latchat Quick-Chem, Milwaukee, WI).

During the studies at full scale facilities, analysis of soluble chemical species was accomplished by a variety of methods, depending on the availability of analytical equipment at each of the plants. Analysis of soluble phosphate was accomplished by either the ascorbic acid method (Method 4500-P-E, APHA, 1992) or by automated analysis using Method 94-70W of the Technicon AutoAnalyser II (Technicon Instruments Corp., Tarrytown, NY). Soluble nitrate was assayed using either the nitrate electrode method (Method 4500-NO3-D, APHA, 1992) using a Hach Model 44560 probe (Hach Co., Loveland, CO.), or the automated procedure of the Technicon AutoAnalyser II (Method 100-70W, Technicon Instruments Corp., Tarrytown, NY). Ammonia analysis was done using the ammonia-selective electrode method (Method 4500-NH3-F, APHA, 1992) using an Orion Model 95-12 probe (Orion Instruments, Cambridge, MA), or Method 98-70W of the Technicon AutoAnalyser II (Technicon Instruments Corp., Tarrytown, NY). Typically these analytical techniques

allowed detection of the assayed species between the detection ranges of 0 to 50 mg/L and reported results with 0.01 mg/L precision.

4.3.5. Phosphorus content of the sludge biomass (%P)

The phosphorus content of the sludge biomass (%P) was determined indirectly using total phosphorus, soluble phosphorus, and mixed liquor suspended solids data. Total phosphorus determination was accomplished by digesting a mixed liquor sample in a sulphuric acid solution (sulphuric acid, potassium sulphate; Technicon Industrial Method 329-74W, Technicon Instruments Corp., Tarrytown, NY) followed by automated analysis on the Latchat instrument using Quick-Chem Method 10-115-01-1-I for total phosphorus analysis (Latchat Quick-Chem, Milwaukee, WI). The phosphorus content of the sludge was determined as the difference between the total phosphorus content of the mixed liquor sample and the soluble phosphorus concentration of the mixed liquor sample.

4.3.6. Mixed liquor suspended solids

Mixed liquor suspended solids levels were determined by collecting a 10 mL mixed liquor sample using a wide mouth automatic pipettor (Brinkman Transpettor) and filtering it through a pre-weighed 5.5 cm glass fibre filter (Whatman 934-AH, Whatman Laboratory Division, Maidstone, KY.). The filter was dried in a 103°C oven for two hours and the suspended solids content determined by comparison with the initial weight of the filter.

4.4. Statistical Techniques

Computerized analysis of data averages, standard deviations, coefficients of variation and linear regression were accomplished using Axum® (release 3.0) produced by TriMetrix Incorporated (Seattle, WA).

5. RESULTS

Results describing the quantification and reproducibility of the individual assays for determining the various process parameters will be presented before consideration of the results from the pilot and full scale process testing. Data describing the results of the evaluation of fluorescence monitoring as a process control tool will be and followed by data from preliminary full scale testing of on-line control using biological activity monitoring.

5.1. Parameter Quantification and Reproducibility

5.1.1. Phosphate, nitrate, and ammonia measurements

Automated analysis of replicate samples of sludge filtrate from the FGR-SGR pilot plant, using the automated Latchat instrument and techniques, determined coefficients of variation of <1%, 14.0%, and 1.2% of the measured concentrations for phosphate (n=6), nitrate (n=6), and ammonia (n=6), respectively over the range of 0-20 mg/L.

5.1.2. Total phosphorus in the sludge

Fourteen replicate samples, each of two FGR-SGR pilot plant sludge samples were digested and analyzed to determine the reproducibility of measurement of the total phosphorus content of the sludge. Analysis determined a coefficient of variation of 9.2% of the calculated concentration of total phosphorus measured.

5.1.3. Mixed liquor suspended solids

The error associated with the modified method for determining mixed liquor suspended solids was estimated by measuring two sets of ten replicates from two different FGR-SGR

pilot plant mixed liquor samples. Analysis revealed a coefficient of variation of 0.5% for each of the replicate sets. The solids concentrations of the two samples used were representative of the typical minimum and maximum mixed liquor suspended solids concentrations expected during normal pilot plant operation.

5.1.4. Culture fluorescence measurements

Culture fluorescence measurements are potentially subject to error resulting from differences in the initial calibration of fluorescence probes and drifting of the calibration over time and from the interference of "background" fluorescence. To minimize errors resulting from instrumentation calibration, an initial calibration was done by placing all of the fluorescence probes in close proximity in the same process zone and calibrating their specific responses with each other. Adjustments to instrument calibration was accomplished by changing specific calibration values in the software controlling the operation of the probes. To minimize error associated with potential calibration drift over time, the probes were recalibrated at least once every two weeks by placing them together in the same bioreactor, observing the response of each over several hours, and adjusting software specific calibration values to ensure consistent response between all probes. During many of the recalibrations, it was noted that little (2 to 3 NFU) or no drift had occurred between the probes.

As mentioned, the sewage has a high intrinsic background fluorescence (Section 2.3), indicating the presence of dissolved materials with fluorescent properties similar to those of NADH. As a result, the characteristics of these components had to be investigated to verify that the fluorescence readings taken in the bioreactors and observed correlations could indeed be related to biological changes and not invalidated by the background components. Because of the complexity of this problem and the dynamic composition of the

sewage, investigation was limited to reviewing previous work and conducting a few simple experiments to demonstrate the biological nature of the measurements.

There are many compounds in the wastewater that possess fluorescent properties similar to those of NADH. Although some of these compounds have been identified (BioChem Technology Inc., 1987; Maneshin et al., 1990a and 1990b), most have eluded identification despite intensive research. To demonstrate the validity of the fluorescence measurements recorded and the patterns observed are indicative of changes in biological activity, three lines of inquiry were followed: 1) does the "background" fluorescence change significantly under the environmental conditions anticipated in this experimentation, 2) does the "background" fluorescence change significantly over short periods of time, and 3) can changes in measured culture fluorescence be directly correlated with measurable changes in biological activity.

To address the first two issues and determine whether the "background" fluorescence was relatively constant, sewage (FGR-SGR pilot plant and Oaks plant) was filtered using 0.2 μm filters and the fluorescence of the filtrate measured under different conditions using the fluorescence probes. Results showed that no significant changes in fluorescence were observed in the filtrate over short periods of time, or during the transition between non-aerated and aerated conditions (Figure 5.1). Comparison of filtrates from samples collected from different days did show, however, that the levels of the "background" fluorescence were not constant between samples. Changes in the levels of background fluorescence recorded using primary effluent filtrate samples showed that "background" fluorescence levels varied a maximum of 200 normalized fluorescence units (NFU) throughout the study period.

A number of experiments demonstrated the response of measured fluorescence to changes in biological metabolism. Several metabolically active compounds can be used to

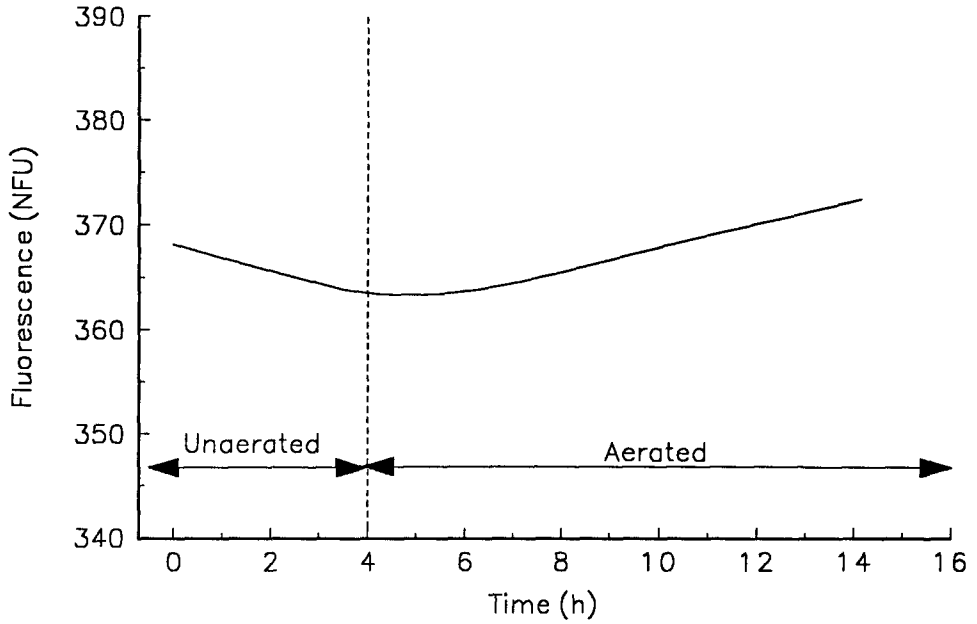


Figure 5.1. Fluorescence of filtered primary effluent under aerated and unaerated conditions.

alter the net cellular NADH/NAD^+ equilibrium (Betz and Chance, 1965). One of these compounds, cyanide, prevents NADH oxidation by oxygen and should result in a net accumulation of NADH (Lehninger, 1982). This accumulation of NADH should result in elevated culture fluorescence. Testing demonstrated that the addition of cyanide (0.5 μM), which does not fluoresce within the excitation and emission spectra of NADH, to FGR-SGR sludge samples immediately increased observed fluorescence, providing evidence for a biological or NADH-related component to the total fluorescent signal (Figure 5.2; Mah, 1991).

Other studies involving the addition of nitrate to anaerobic sludge confirmed the biological nature of the fluorescent signal by demonstrating direct relationships between changes in measured fluorescence and biological denitrification. In these tests, the addition of nitrate was expected to result in a rapid and transient change in the relative amounts of

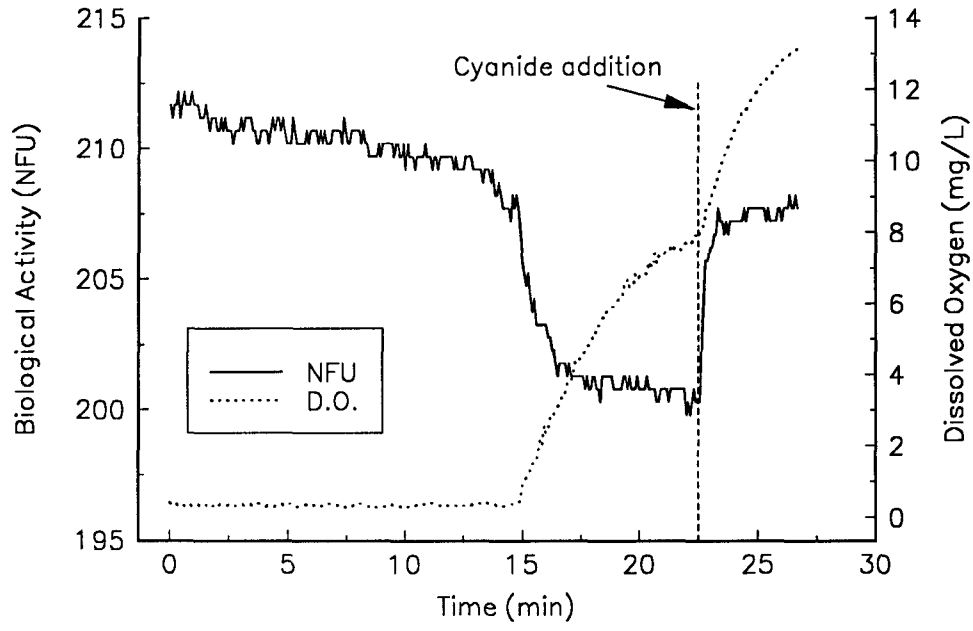


Figure 5.2. Response of fluorescence to a transition from an anaerobic condition to an aerobic condition and the effect of cyanide (0.5 μm sodium cyanide) under aerobic conditions. From "0" minutes to "15" minutes, the sample was maintained in an anaerobic condition. At "15" minutes, the sample was aerated. At "22.5" minutes, cyanide was added to the sample. The transition from anaerobic to aerobic conditions was induced to demonstrate the biological response of NADH and measured fluorescence during the transition.

NADH and NAD^+, resulting from denitrification activity and rapid oxidation of NADH by nitrate. The rapid oxidation of NADH, during denitrification, decreases the net cellular NADH pool and changes the relative proportions of NADH and NAD^+. When denitrification is complete and insignificant levels of nitrate are available, the pools of NADH and NAD^+ should establish themselves at the previous anaerobic equilibrium and fluorescence levels should return to their pre-denitrification level. This test and others showed that the period of time the fluorescent signal remained at the depressed level was proportional to the amount of nitrates added, and that transitions between anoxic and anaerobic conditions could be easily documented with fluorescence measurements and confirmed with soluble nitrate data (Figure 5.46; Armiger et al., 1986; Armiger et al., 1993; Ju and Trivedi, 1992; Mah, 1991). The observed changes in culture fluorescence in these studies were expected and consistent with

other studies that measured the actual changes in the concentrations of NADH and NAD^+ during transitions between metabolic states in various organisms (Betz and Chance, 1965; Harrison and Chance, 1970; Siano and Mutharasan, 1989). Based on all of the information and results of the inquiry followed, it was assumed that the "background" fluorescence did not invalidate any of the observed short term trends in fluorescence.

5.1.5. Volatile fatty acid determination

Quantification of volatile fatty acid concentrations in samples was accomplished using the external standard calibration function of the Hewlett Packard 3380A integrator. Reproducibility estimated using ten replicates of a single filtered primary effluent sample showed good reproducibility with coefficients of variation for the concentrations of acetic and propionic acid less than 3.0%. Comparison of the calibration curves generated from replicate samples of standards, demonstrated a coefficient of variance of 5.0% for this GC method over the detection range of 1 to 50 mg VFA/L.

5.1.6. Polyhydroxyalkanoate quantification

A typical GC chromatograph obtained from a sludge sample is shown in Figure 5.3. The identities of the compounds were obtained by comparison of relative retention times with retention times obtained from available standards and from GC/MS analysis. Only two major carbon storage products, polyhydroxybutyric and polyhydroxyvaleric acid, were routinely analyzed. Hydroxybutyric acid response from the gas chromatograph was calibrated directly using β-hydroxybutyric acid sodium salt as a standard. Standards of β-hydroxyvaleric acid were not available for calibration of hydroxyvaleric acid response. The relative response factors of α-hydroxybutyric and α-hydroxyvaleric acid should be similar to the relative response factors for β-hydroxybutyric and β-hydroxyvaleric acid in the gas chromatograph, based on

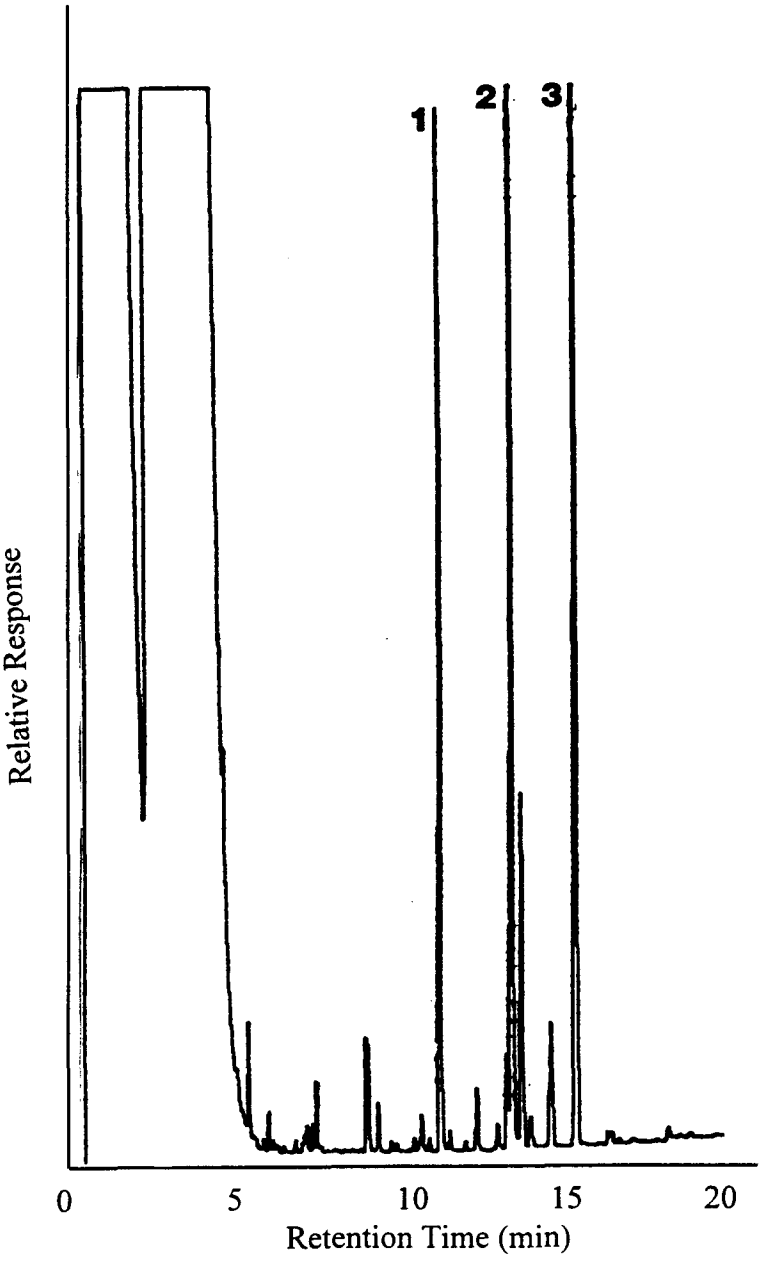


Figure 5.3. Chromatograph of methyl-ester derivatives extracted from sludge. Components identified by comparison with available standards or by GC/MS were methyl esters of β-hydroxybutyric acid (peak 1; retention time, 11.5 minutes), β-hydroxyvaleric (peak 2; retention time, 13.5 minutes), and benzoic acid (peak 3; retention time 15.4 minutes) which was used an internal standard.

the number of carbon atoms per mass of each compound (Willard et al., 1988). Consequently, standards of α-hydroxybutyric and α-hydroxyvaleric acid were obtained and extracted such that triplicate samples of 12.5, 25, 37.5, and 50 μg/ml each of hydroxybutyric and hydroxyvaleric acid were analyzed. The response factors for each concentration were averaged and compared to obtain a response factor for quantification of β-hydroxyvaleric acid. The observed ratio of response factors for a-hydroxyvaleric acid to α-hydroxybutyric acid was calculated as 1.296 and used for quantification of b-hydroxyvaleric acid. This ratio is slightly greater than the ratio of 1.211 (Comeau, 1989) calculated by using standards of valerate and butyrate.

Reproducibility of the quantification procedure was estimated by comparison of ten standard calibration curves prepared from samples of 12.5, 25, 37.5, and 50 mg/ml of hydroxybutyric acid. The observed coefficient of variance determined by comparison of the calibration curves was 6.5%. The accuracy of the measurements was estimated by comparing the determined concentrations of PHA in each of 6 sub-samples of mixed liquor. The coefficient of variation calculated from the determined concentrations of PHA in mg PHA/g dried sludge was 4.0%.

5.2. Process Testing

To test for consistent relationships between biological activity, indicated by fluorescence, and various process parameters, numerous experiments were conducted at several BNR facilities. The terms "biological activity" and "culture and/or measured fluorescence" will both be used to represent "metabolism" in this thesis. In theory, culture fluorescence should provide an indication of metabolic activity. The results from intensive studies of the pilot and full scale processes will be presented by considering data relating

overall general trends in biological activity, indicated by measured fluorescence, to general changes in the character of the process, and by relating specific changes in activity, indicated by measured fluorescence, with specific process variables. Some of these specific relationships include correlations between changes in primary effluent fluorescence and volatile fatty acid concentration, anaerobic zone patterns of fluorescence and VFA consumption, phosphate release, and PHA storage, the relationships of some of the process parameters with each other and with the process efficiency, and the relative patterns of fluorescence and process performance between various process zones.

5.2.1. Gross trends in measured fluorescence

Fluorescence patterns over a one month period demonstrated both short (daily) and long term (monthly) patterns (Figure 5.4). While the daily patterns could be attributed to a number of influences including changes in VFA loading and consumption, the longer term general trends in fluorescence showed good correlation with changes in process mixed liquor suspended solids levels in the FGR-SGR pilot plant process zones. The general pattern showed an inverse relationship between the average level of fluorescence and average daily mixed liquor suspended solids concentrations. This general pattern resulted from differences in the optical characteristics of the mixed liquor and fluorescence detection caused by the differences in mixed liquor suspended solids levels. These differences and the effects of varying solids levels will be discussed later. Correlation coefficient (r2) values relating average daily mixed liquor suspended solids with corresponding average daily fluorescence levels over a three month period were 0.89, 0.88, and 0.81 for the anaerobic, anoxic, and reaeration bioreactors, respectively (Figures 5.5 through 5.7).

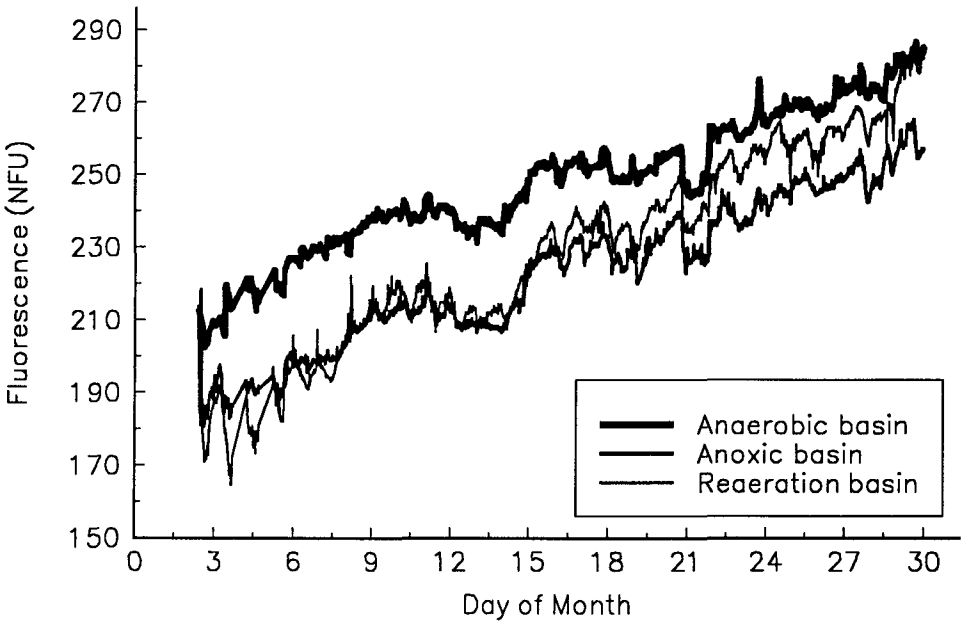


Figure 5.4. Trends in culture fluorescence over a one month period at the FGR-SGR pilot plant (June 1993).

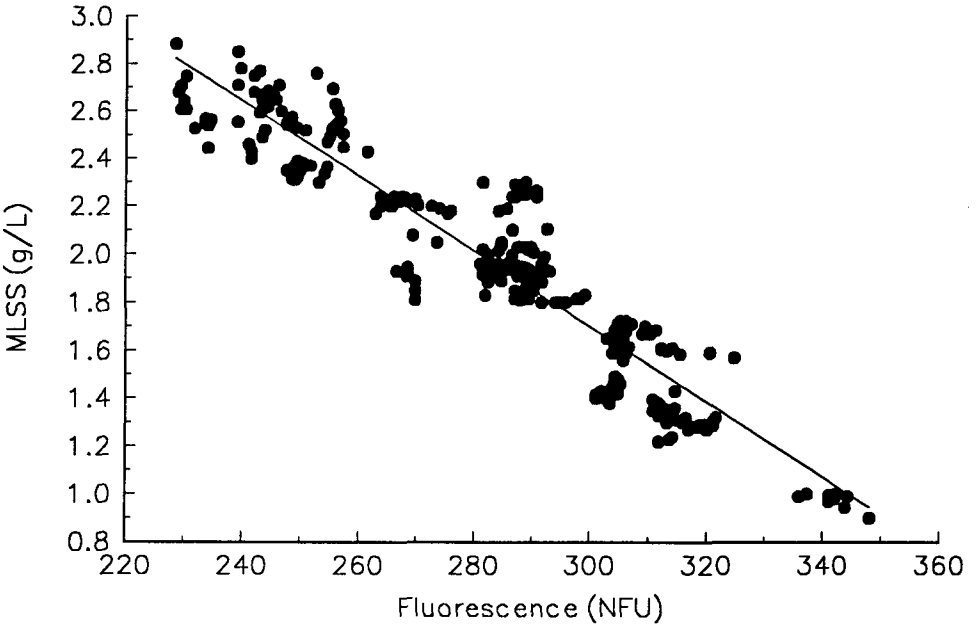


Figure 5.5. Correlation between measured fluorescence and average daily mixed liquor suspended solids concentrations in the anaerobic basin of the FGR-SGR pilot plant over a three month period (July - September 1993).

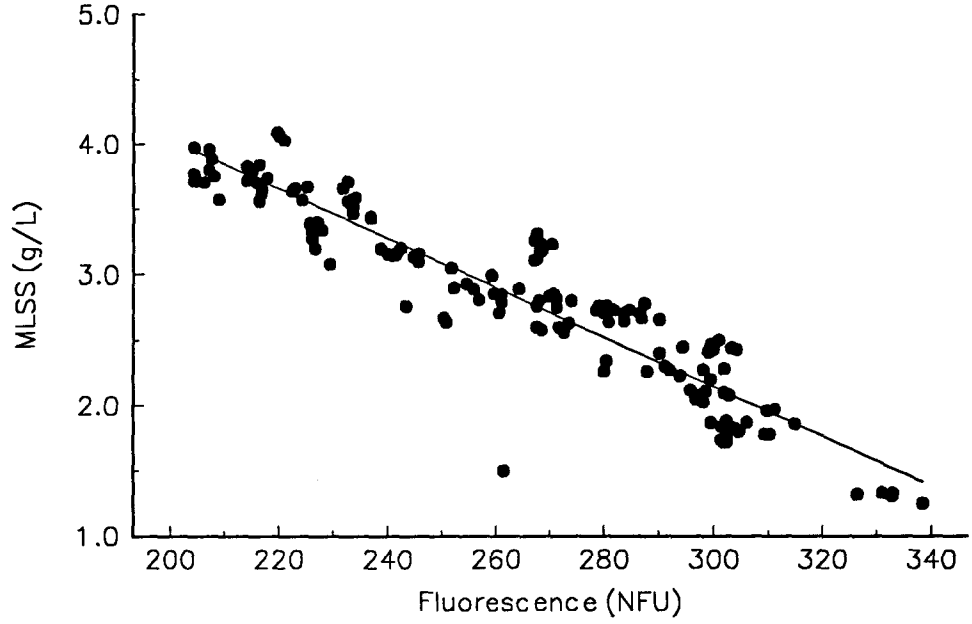


Figure 5.6. Correlation between measured fluorescence and average daily mixed liquor suspended solids concentrations in the anoxic basin of the FGR-SGR pilot plant over a three month period (July - September 1993).

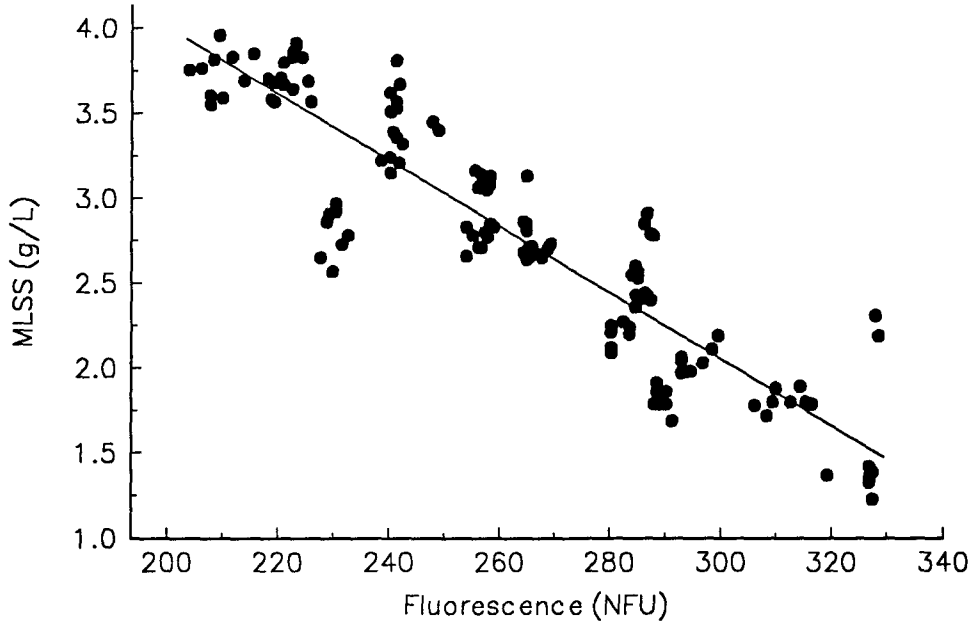


Figure 5.7. Correlation between measured fluorescence and average daily mixed liquor suspended solids concentrations in the reaeration basin of the FGR-SGR pilot plant over a three month period (July - September 1993).

5.2.2. Primary effluent fluorescence and volatile fatty acid concentration

5.2.2.1. FGR-SGR pilot process

To determine whether fluorescence measurements provided useful patterns in the primary effluent, intensive VFA analyses were performed on primary effluent samples. The term "primary effluent" will be used consistently in this thesis to refer to the wastewater consisting of the effluent from the primary clarifiers before discharge into the anaerobic zone of the bioreactor train. Based on the assumption that the net steady state $NADH/NAD^+$ equilibrium can be affected by the production of VFA by fermenting organisms, or the metabolism of VFA by the active bacteria in the primary effluent, some relationship between measured fluorescence and VFA concentration was expected. Once obtained, the VFA data was plotted against recorded fluorescence. The experimental details for all the experiments testing for a relationship between primary effluent fluorescence and VFA concentrations and data are provided in Appendices A and C.

Intensive sampling of the primary effluent was conducted during twenty-two experiments and comprised over 200 individual samples. Data showed a direct correlation between measured fluorescence and volatile fatty acid concentration in the primary effluent stream, in 20 of the 22 experiments. It was discovered after the experiments that the flow cell placed in the primary effluent line to measure fluorescence was plugged with solids, on the two occasions where the direct correlation was not observed. Typical examples of the observed patterns and correlations are presented in Figures 5.8 to 5.11. Simple linear correlation models ($y = mx + b$; where "m" is slope and "b" is the y-intercept) used to determine correlation coefficients (r^2 values) describing the relationship between measured VFA concentrations and fluorescence in those twenty observation days varied from 0.72 to 0.94, with an average of 0.84. Although the calculated correlation coefficients describing

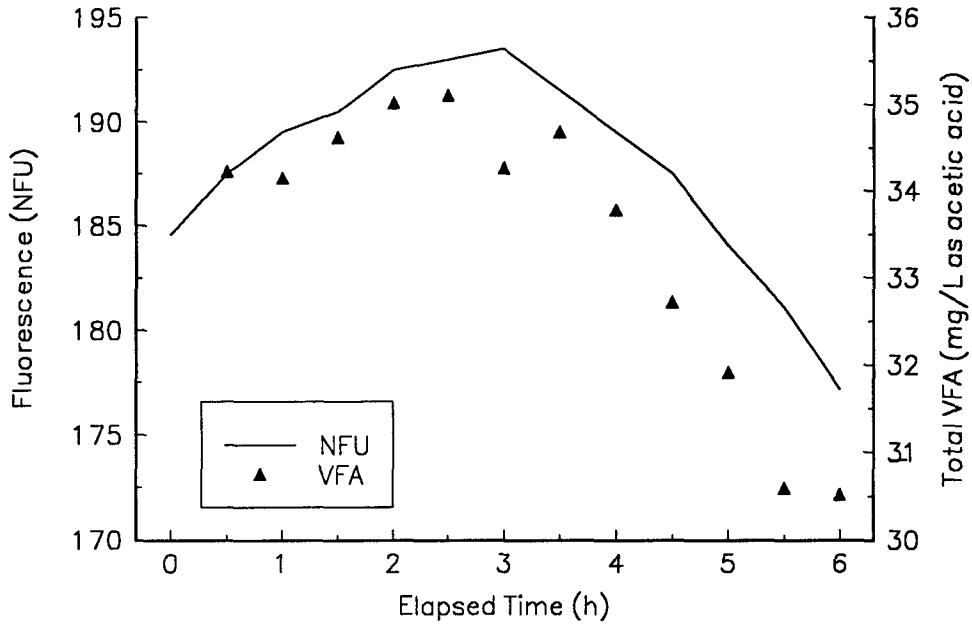


Figure 5.8. Primary effluent fluorescence and VFA concentrations at the FGR-SGR pilot plant. (Experiment F9380-02).

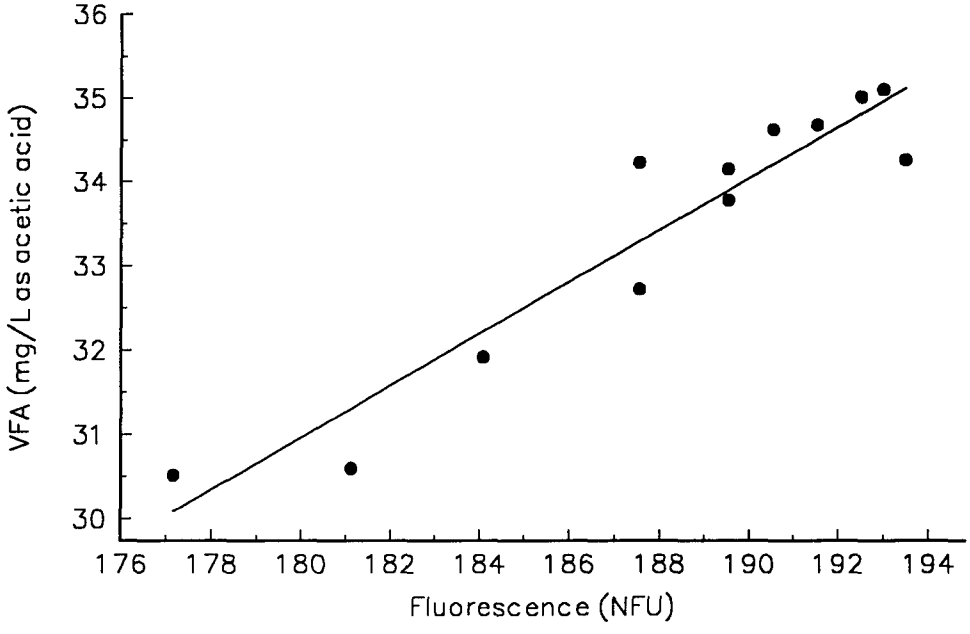


Figure 5.9. Correlation between primary effluent fluorescence and VFA concentrations at the FGR-SGR pilot plant. (r^2= 0.90; Experiment F9380-02).

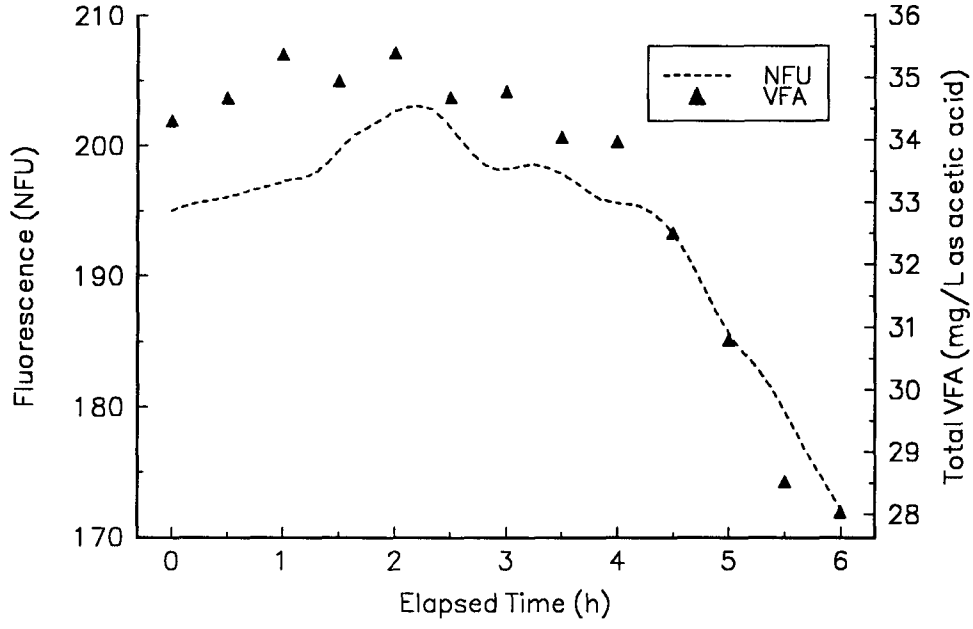


Figure 5.10. Primary effluent fluorescence and VFA concentrations at the FGR-SGR pilot plant. (Experiment F9380-03).

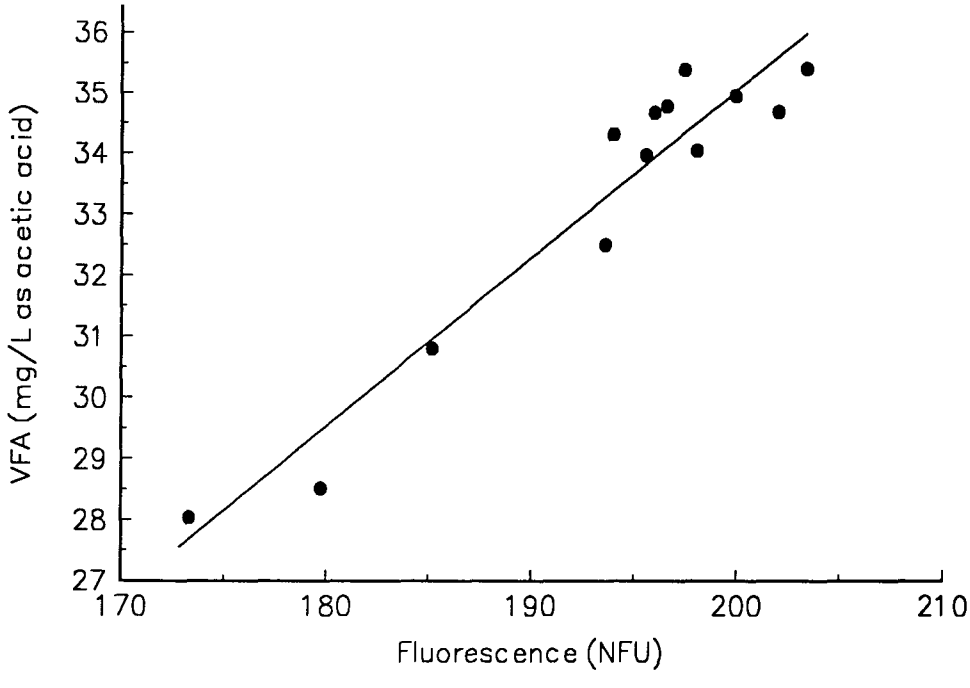


Figure 5.11. Correlation between primary effluent fluorescence and VFA concentrations at the FGR-SGR pilot plant ($r^2 = 0.88$; Experiment F9380-03).

the relationship between fluorescence and VFA concentration from each experiment were high, the specific mathematical descriptions of the regression lines varied considerably between experiments. Slopes of the linear regression lines fitted to the data ranged from 0.20 to 3.79. Table II provides a detailed summary of the correlation coefficients (r^2 values), and mathematical descriptions of the fitted regression lines for each of the 20 experiments. The variations in both the correlation coefficients and the calculated regression coefficients appeared to be random.

The variations of the specific mathematical descriptions of the relationships could be attributed to several factors including changes in the physical condition of the detecting probe that affected the reliability of the fluorescence data, changes in the character of the primary effluent itself (i.e. changes in the level and/or nature of soluble fluorophores), and changes in biological factors (e.g. metabolic capabilities of the biomass affecting the NADH dynamics, population composition). The ability to make precise quantitative predictions of primary effluent VFA concentrations from fluorescence data over the long term (i.e. periods in excess of 24 hours) was limited by the variabilities in the specific mathematical relationships between the two parameters. The consistency of the general pattern, however, provided the capability to make real time, qualitative predictions about the relative level of VFA in primary effluent using the on-line fluorescence measurements. During experimentation, changes in the fluorescence signal were used to predict general changes in the relative VFA content of the primary effluent stream. In all of the cases, measured trends in VFA concentrations followed the predicted trends. Figure 5.12 compares the predicted general trends in primary effluent VFA concentrations generated during experimentation from the fluorescence measurements with the measured trends. These predictions were made directly from the fluorescence data. Analysis of other parameters, including primary effluent phosphate, nitrate, ammonia, or suspended solids levels, showed no correlation with fluorescence.

Table II. Regression analysis of the correlations between primary effluent fluorescence and VFA concentrations at the FGR-SGR pilot plant.

Experiment	Correlation coefficient (r2)	Slope d(VFA)/d(NFU)	Offset (NFU)	Number of observations (n)
F9310-01	0.72	0.20	-30.03	6
F9310-02	0.94	0.27	-46.99	4
F9320-01	0.93	2.09	-372.93	3
F9330-01	0.81	1.94	-326.46	8
F9330-02	0.70	2.71	-479.28	11
F9340-03	0.87	1.51	-212.27	13
F9340-04	0.87	1.07	-123.26	10
F9340-05	0.72	1.70	-171.01	15
F9340-06	0.87	1.68	-244.29	12
F9360-01	0.91	1.29	-154.00	6
F9360-02	0.85	2.57	-326.73	10
F9360-03	0.81	2.00	-671.30	9
F9360-04	0.76	0.22	-29.87	10
F9360-06	0.82	0.59	-61.39	9
F9360-07	0.82	1.50	-232.34	10
F9380-01	0.89	3.31	-528.73	9
F9380-02	0.90	0.28	-18.63	13
F9380-03	0.88	0.28	-21.56	13
F9380-04	0.88	0.82	-87.53	12
F9380-05	0.78	3.79	-602.09	12

Note: Culture fluorescence measurements were used as the independent variable during the regression analysis. Offest refers to the y-intercept value of the linear model.

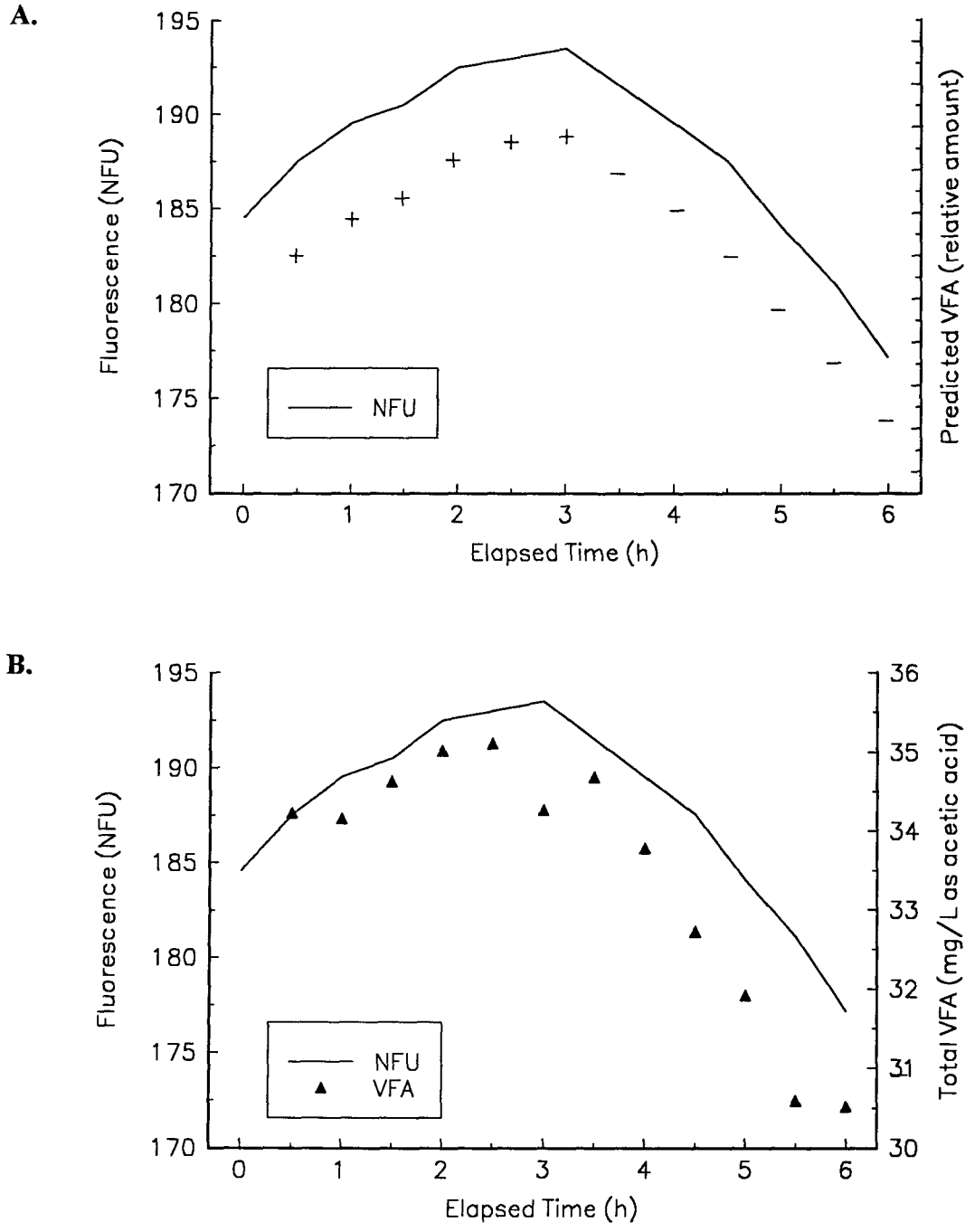


Figure 5.12. A) Primary effluent fluorescence and predicted, levels of VFA. The relative levels of VFA are indicated by the "+" and "-" symbols. The "+" and "-" symbols are used to indicate both the relative amount of VFA and the pattern of change from the previous point. A "+" indicates a relative increase from the previous point, while a "-" indicates a relative decrease from the previous point. B) Primary effluent fluorescence and measured VFA concentration.

5.2.2.2. Kelowna Pollution Control Center

Similar analysis was performed at the Kelowna Pollution Control Center's Three-Stage Bardenpho Treatment Plant. Results from a 14 hour testing period demonstrated the same direct relationship between measured primary effluent volatile fatty acid concentration and fluorescence observed at the FGR-SGR pilot facility. Figure 5.13 shows a direct relationship between measured primary effluent VFA concentration and fluorescence with a linear correlation coefficient of 0.88 (Figure 5.14). The Kelowna plant has a static fermenter for VFA production where the VFA-rich supernatant produced is discharged directly into the anaerobic bioreactor. This configuration accounts for the relatively low VFA concentrations measured in the primary effluent. Unlike the data from the FGR-SGR pilot plant studies that showed daily variations of the specific mathematical definitions describing the relationship between measured fluorescence and VFA concentration, the specific mathematical definitions of the relationship between culture fluorescence and VFA content at this site were much more consistent. The mathematical relationships derived from the data during one experiment (Figures 5.13 and 5.14) were used to predict the primary effluent VFA concentrations in another experiment conducted three days later. Comparison of the predicted and actual measured volatile fatty acid concentrations showed that a reasonable prediction of the VFA concentrations could be made from the previous correlation and the on-line fluorescence measurements (Figure 5.15).

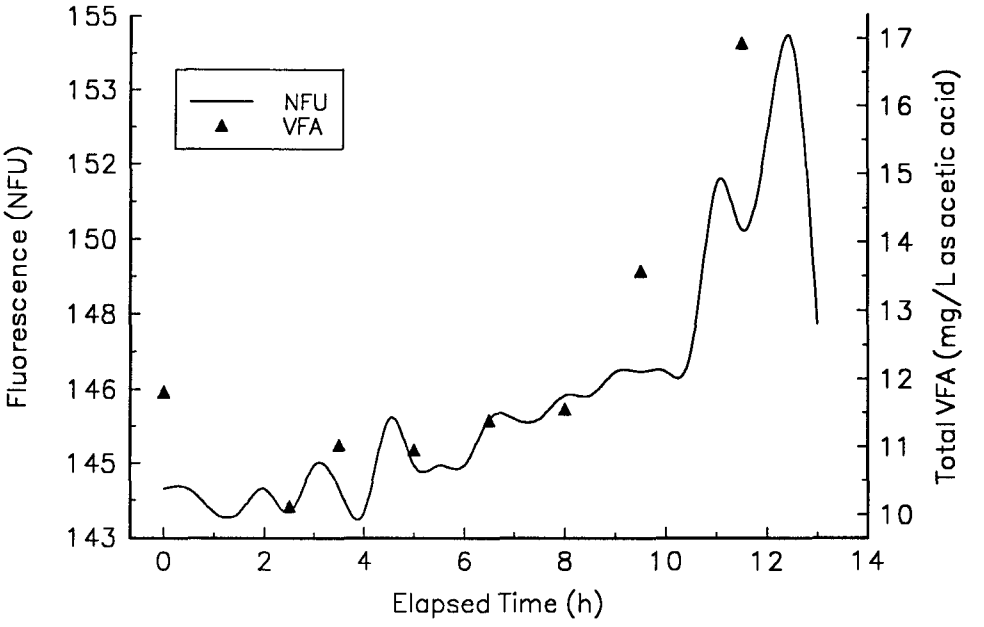


Figure 5.13. Primary effluent fluorescence and VFA concentrations at the Kelowna Pollution Control Centre. (Experiment KL921118).

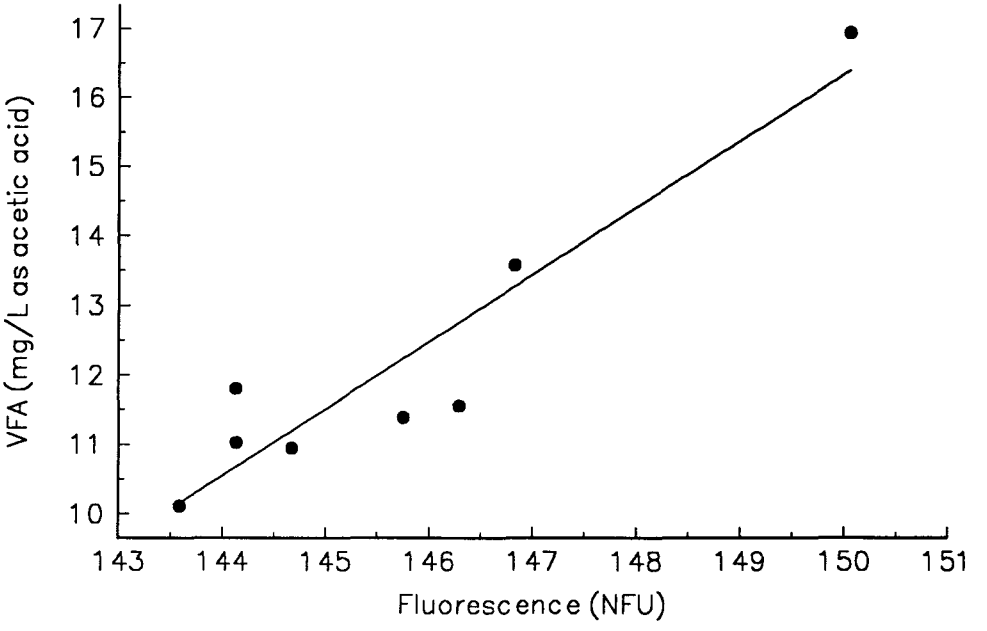


Figure 5.14. Correlation between primary effluent fluorescence and VFA concentrations at the Kelowna Pollution Control Centre.(r^2=0.88; Experiment KL921118).

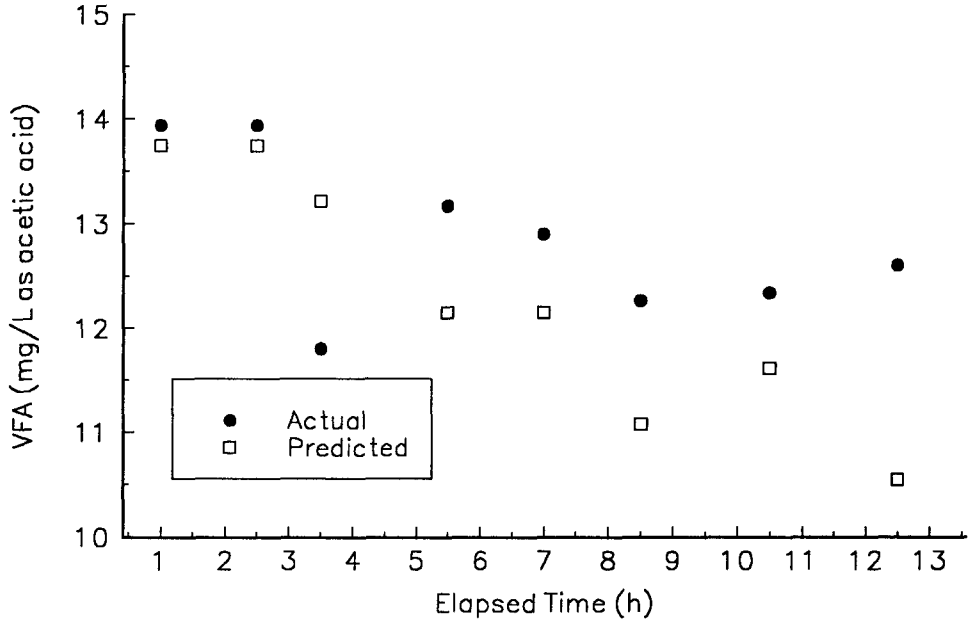


Figure 5.15. Comparison of actual measured VFA concentrations and predicted VFA concentrations in the primary effluent at the Kelowna Pollution Control Centre.(Experiment KL921121).

5.2.2.3. Oaks, PA A/O® Wastewater Treatment Plant

Primary effluent analysis was conducted on two separate days at the Oaks, PA A/O® Wastewater Treatment Plant. Results demonstrated a direct relationship between primary effluent fluorescence and VFA concentration on each day (Figures 5.16 and 5.18) similar to that observed at the other two facilities. Correlation coefficients of 0.87 and 0.79 were calculated for experiments OAK119317 and OAK119319, respectively (Figures 5.17 and 5.19), using the simple linear model. The difference in fluorescence ranges measured between the two days resulted from relocation and cleaning of the fluorescence probe before the second observation day. Consequently, the consistency of the specific mathematical relationship between measured fluorescence and VFA levels could not be evaluated between the two days. The general trends, however, were consistent and did allow qualitative changes in VFA levels to be predicted from the on-line fluorescence data. Like at the pilot plant,

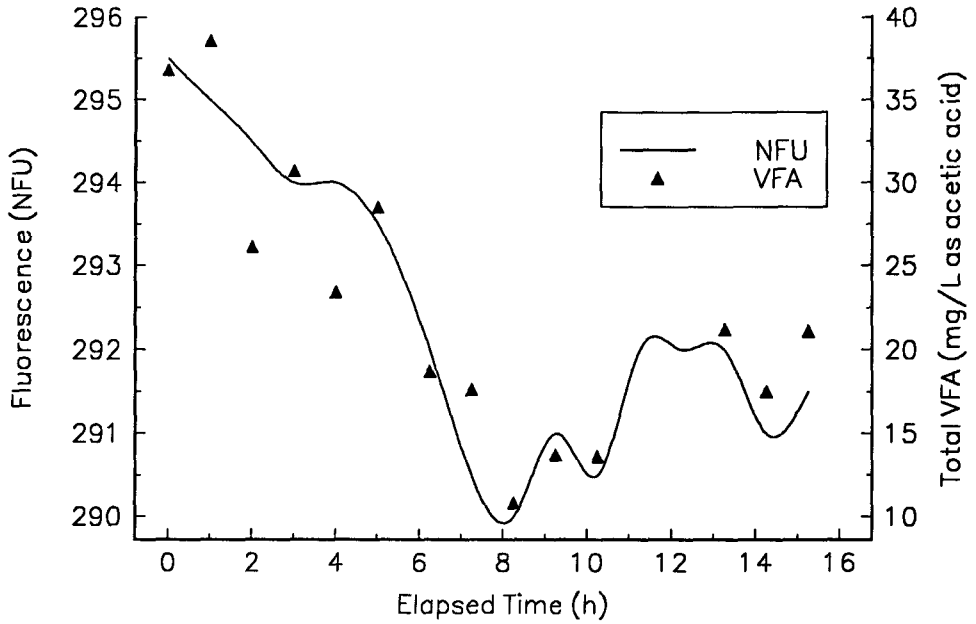


Figure 5.16. Primary effluent fluorescence and VFA concentrations at the Oaks, PA A/O Wastewater Treatment Plant. (Experiment OAK119317).

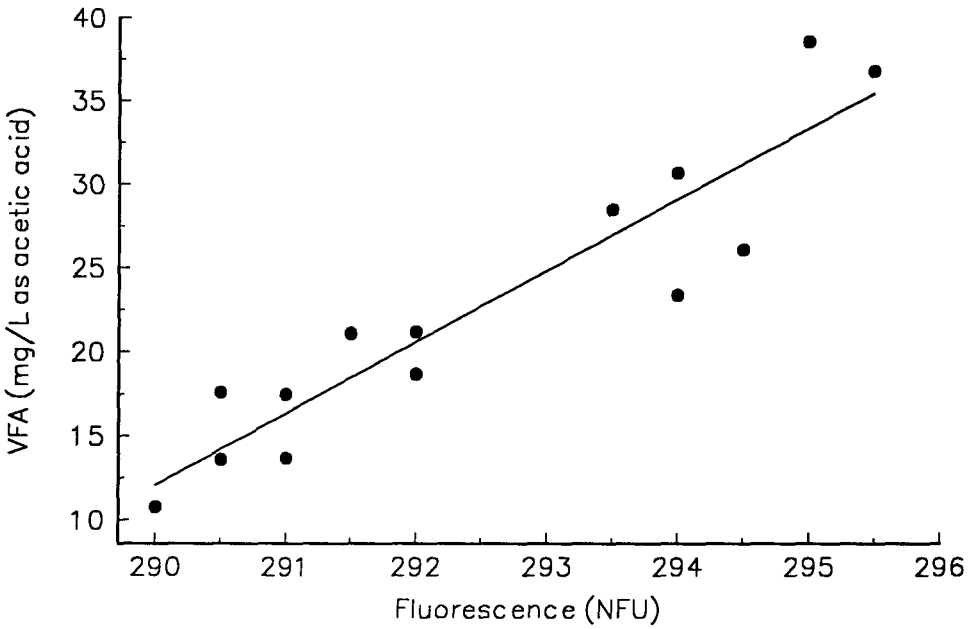


Figure 5.17. Correlation between primary effluent fluorescence and VFA concentrations at the Oaks, PA A/O Wastewater Treatment Plant. (r^2=0.87; Experiment OAK119317).

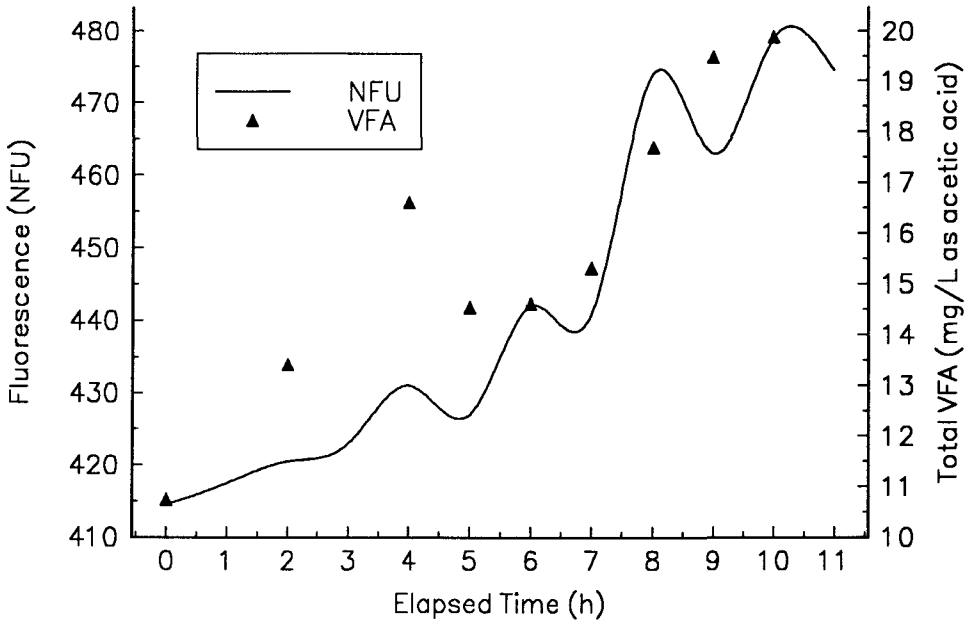


Figure 5.18. Primary effluent fluorescence and VFA concentrations at the Oaks, PA A/O Wastewater Treatment Plant. (Experiment OAK119319).

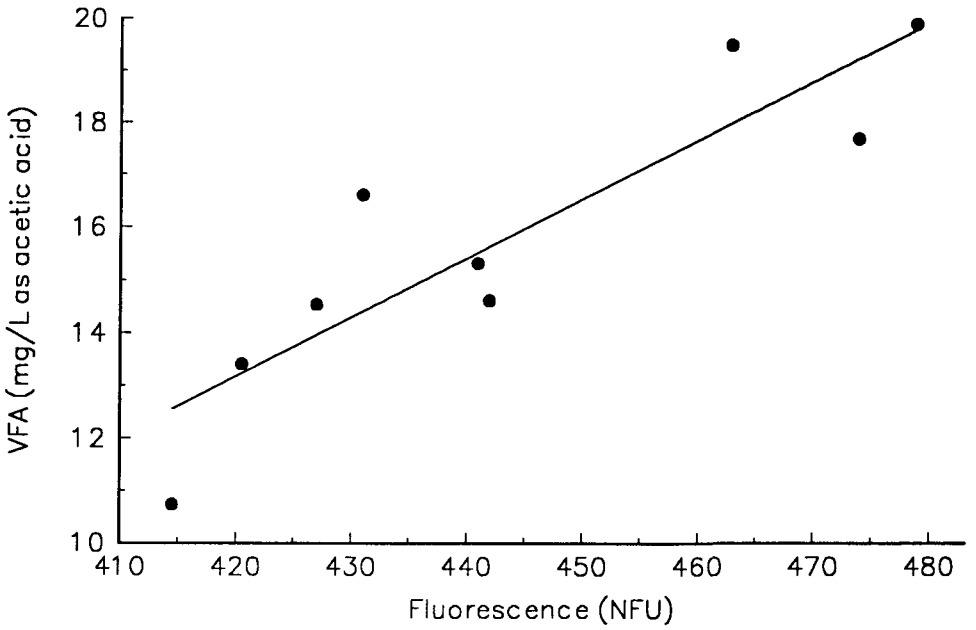


Figure 5.19. Correlation between primary effluent fluorescence and VFA concentrations at the Oaks, PA A/O Wastewater Treatment Plant. (r^2=0.79; Experiment OAK119319).

changes in the measured fluorescence were used to predict general changes in the relative VFA content of the primary effluent during experimentation. Measured trends in VFA concentrations followed the predicted trends (data not shown).

5.2.2.4. Penticton Advanced Wastewater Treatment Plant

The analysis of VFA concentrations and fluorescence in the primary effluent stream showed an inverse relationship between measured fluorescence and VFA concentration. At the Penticton facility, fluorescence and VFA were measured in two different locations in the primary effluent stream. The first measurement point was in the primary effluent stream immediately downstream from the primary clarifiers. The second measurement point was downstream of the equalization basin and immediately upstream of the process train. At this location the primary effluent stream consists of effluent from the primary clarifiers and effluent from the equalization basin and is referred to as the "mix" or "mixed" stream. Comparison of measured primary effluent VFA concentrations and fluorescence, using the simple linear model, showed an inverse relationship between the two parameters (Figure 5.20) with a correlation coefficient of 0.92 (Figure 5.21). Similar comparison of measured VFA concentrations and fluorescence in the "mix" stream also demonstrated an inverse relationship between VFA levels and fluorescence ($r^2 = 0.84$; Figures 5.22 and 5.23), similar to that observed at the first measurement point. Analysis of a number of other measured variables, including suspended solids, soluble phosphate, nitrate, and ammonia, did not provide an obvious explanation for the differences in the pattern observed here with those observed at the other plants. The only apparent difference between this plant and the others is the large amount of fermenter solids in the fermenter recycle stream discharged into the primary clarifiers. At the other facilities studied there is either no side stream fermenter present (Oaks, PA), or partially clarified fermenter supernatant is reintroduced into the primary clarifiers (FGR-SGR pilot plant). At the Kelowna plant, the fermenter supernatant is

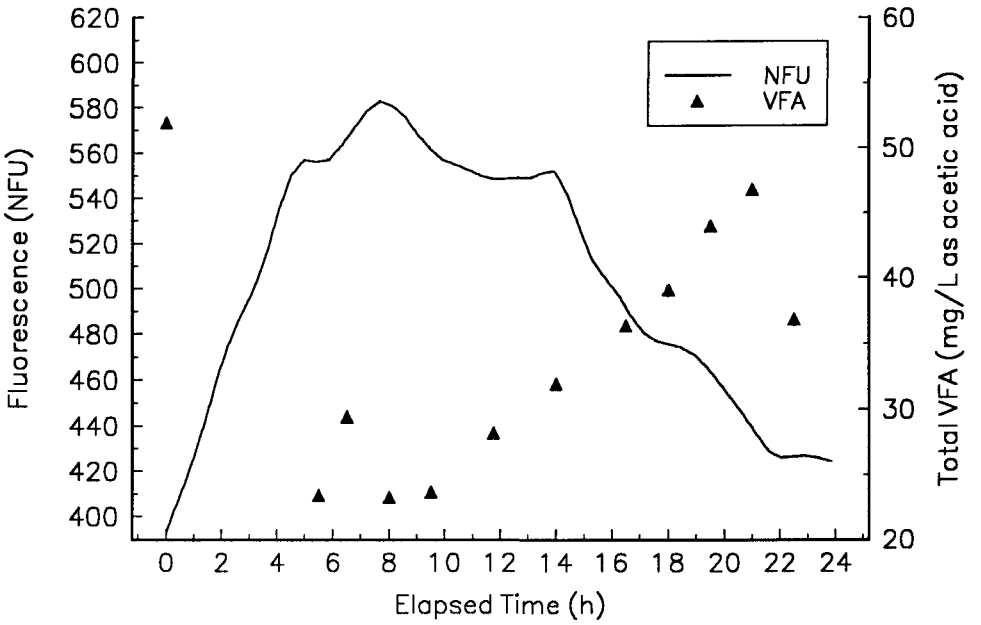


Figure 5.20. Primary effluent fluorescence and VFA concentrations at the Penticton Advanced Wastewater Treatment Plant. (Experiment PT9207).

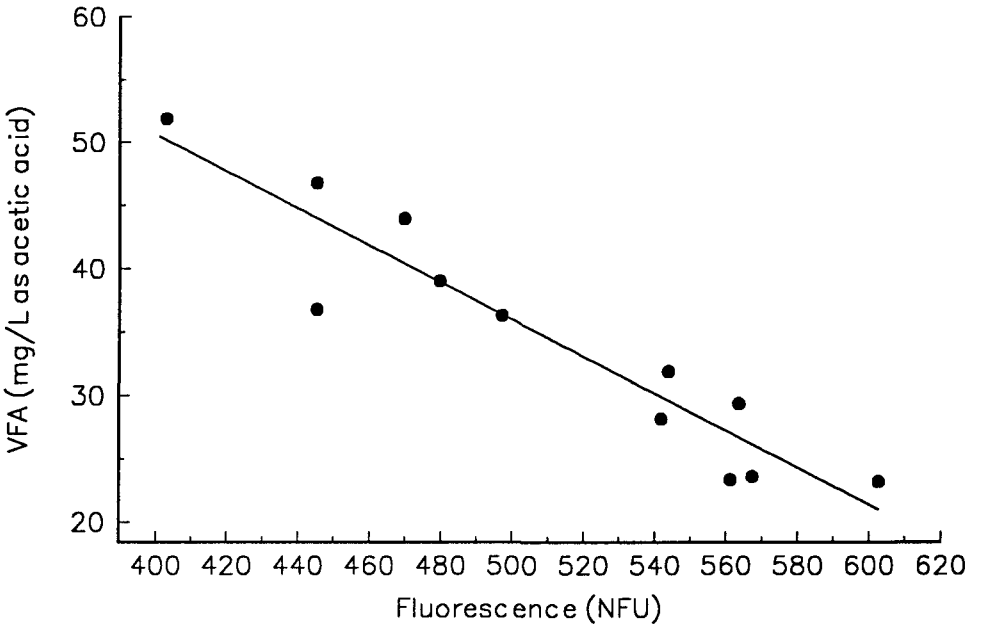


Figure 5.21. Correlation between primary effluent fluorescence and VFA concentrations at the Penticton Advanced Wastewater Treatment Plant. (r^2=0.92; Experiment PT9207).

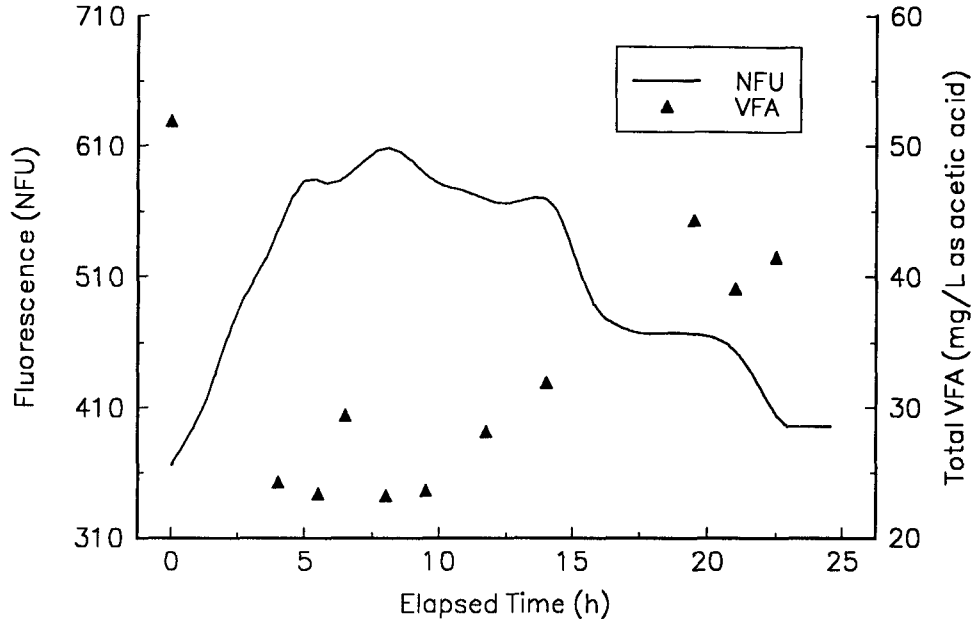


Figure 5.22. Measured fluorescence and VFA concentrations in the "mixed" stream containing flows from the primary effluent and equalization basin at the Penticton Advanced Wastewater Treatment Plant. (Experiment PT9207).

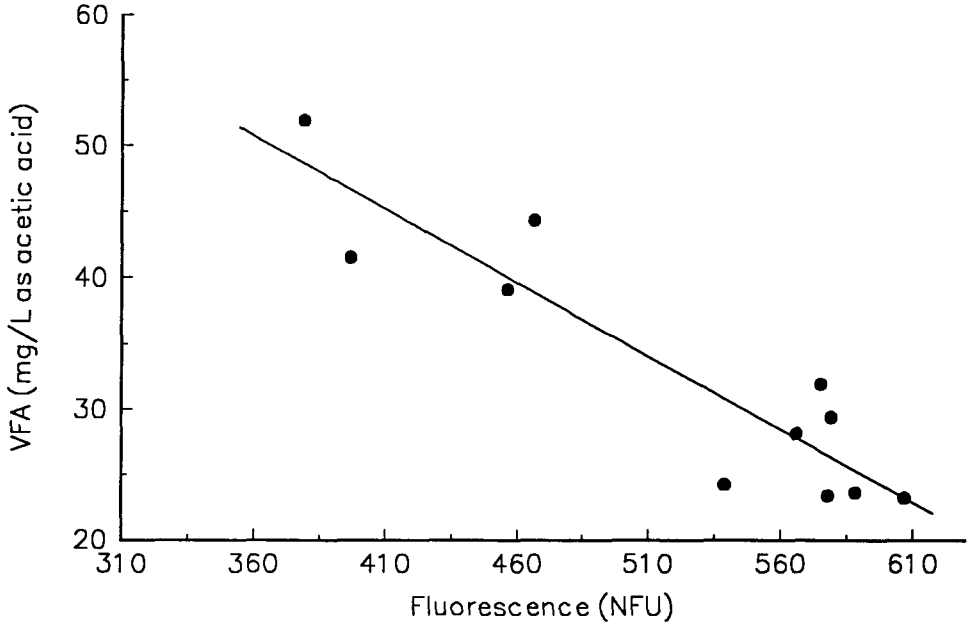


Figure 5.23. Correlation between measured fluorescence and VFA concentrations in the "mixed" stream containing flows from the primary effluent and equalization basin at the Penticton Advanced Wastewater Treatment Plant. (r^2=0.84; Experiment PT9207).

introduced directly into the anaerobic basin. The composition of the primary clarifier contents and primary effluent streams at these plants would contain much less or no fermenter solids.

Although the patterns between fluorescence and VFA concentration observed at this site were different from those at the other sites, the consistency of the patterns allowed real time assessment of relative changes in the VFA concentrations in both the primary effluent and "mixed" stream. Previous work conducted at this site also demonstrated similar trends. These patterns were later used to demonstrate the ability to control bioreactor VFA loading in an experiment that will be discussed further.

5.2.3. FGR-SGR plant anaerobic zone fluorescence and process parameters

Intensive study of the pilot scale FGR-SGR process was done to investigate two general questions; 1) is culture fluorescence monitoring in the excitation and emission ranges employed by the fluorescence probe useful as an indicator of changes in biological activity, and 2) how do short term variations in carbon (VFA) load affect characteristic nutrient removal metabolism in the anaerobic zone and, ultimately, effluent quality. First, to evaluate the usefulness of fluorescence monitoring as an indicator of biological activity, several parameters were measured to search for the existence of consistent relationships between measured culture fluorescence and quantifiable measures of biological activity. Second, to determine if short term variations in VFA load impacted on anaerobic zone metabolism and effluent quality, the FGR-SGR process was operated under conditions of variable VFA loading. In this thesis, the term "VFA load" will be consistently used to describe VFA concentration loading, that is, the concentration of VFA in the stream. Testing revealed several consistent patterns between anaerobic zone culture fluorescence and specific anaerobic zone VFA consumption (VFA/M, mg VFA consumed/g MLSS), anaerobic zone mass phosphate release (mg P released/g MLSS), and anaerobic zone PHA storage while revealing that variations in

loading of a key nutrient fraction, VFA, can impact on biological activity and effluent quality. Experimental details outlining the experiments conducted in the anaerobic zones of all the plants studied and the data are provided in Appendices B and C.

5.2.3.1. Anaerobic zone fluorescence and VFA/M

Biochemical models describing biological phosphorus removal identify anaerobic carbon storage as a requirement for excess aerobic phosphorus removal. Additionally, research has demonstrated the importance of suitable carbon substrates, such as volatile fatty acids (VFA), for anaerobic carbon storage (Comeau, et al., 1986; Nichols and Osborn, 1979). Since anaerobic carbon storage is dependent on the availability of "suitable or preferred" nutrients like VFA, the effects of VFA load (i.e. VFA concentration) variation on anaerobic zone VFA consumption (VFA/M) were tested by varying the VFA load to the process. Volatile fatty acid measurements and mass balance equations were used to determine the specific anaerobic VFA consumption (VFA/M), and then compared with recorded fluorescence. Data showed that anaerobic VFA consumption varied directly with VFA loading. Testing under a variety of different VFA loading conditions demonstrated a consistent and inverse relationship between anaerobic zone fluorescence and specific VFA consumption (mg total VFA consumed/ g MLSS) in 15 out of 18 experiments. Representative examples showing the observed pattern are presented in Figures 5.24 and 5.25. Linear regression analysis showed that correlation coefficients relating anaerobic zone fluorescence measurements with VFA consumption averaged 0.80, and ranged from 0.68 to 0.90. The consistency of the general relationship between changes in measured fluorescence and specific VFA consumption allowed relative levels of VFA loading and consumption to be predicted from the fluorescence data. Figure 5.26 compares the predicted trends in anaerobic VFA consumption (VFA/M) with the measured trends. Predictions were made directly from the fluorescence data.

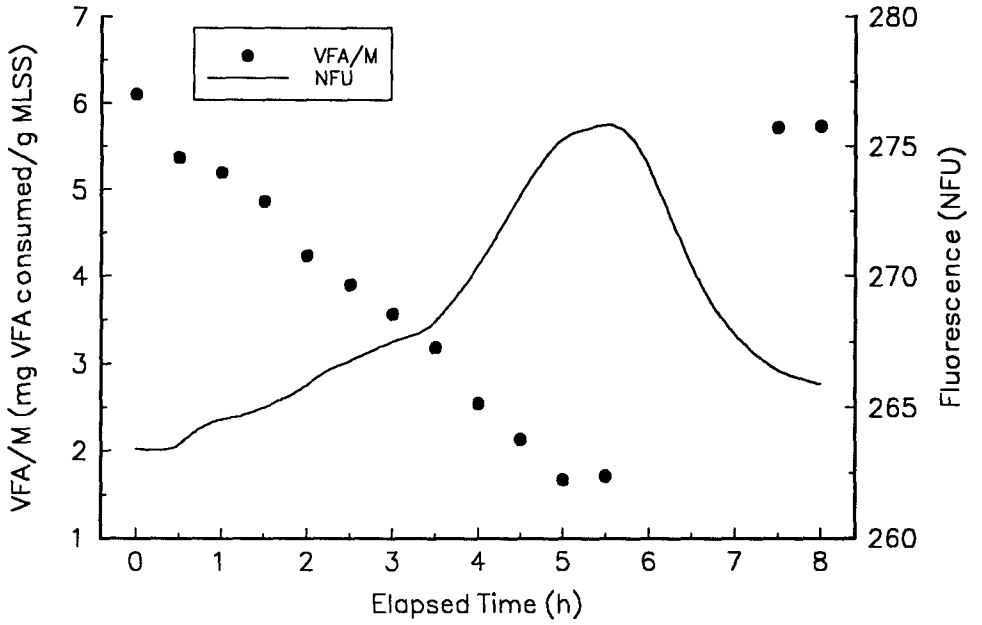


Figure 5.24. Anaerobic zone fluorescence and specific anaerobic zone VFA consumption at the FGR-SGR pilot plant. (Experiment F9340-06).

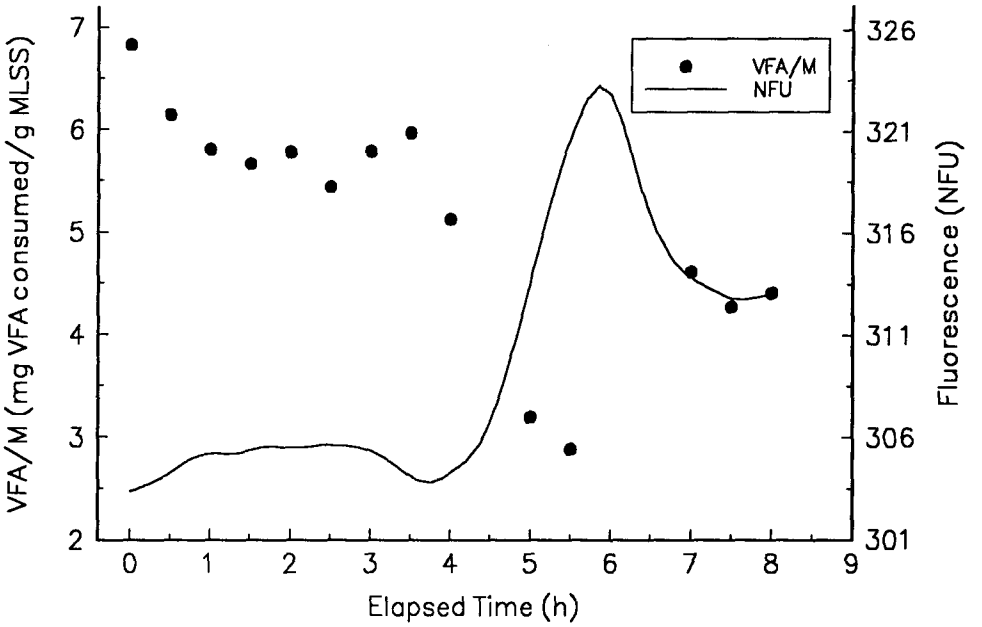
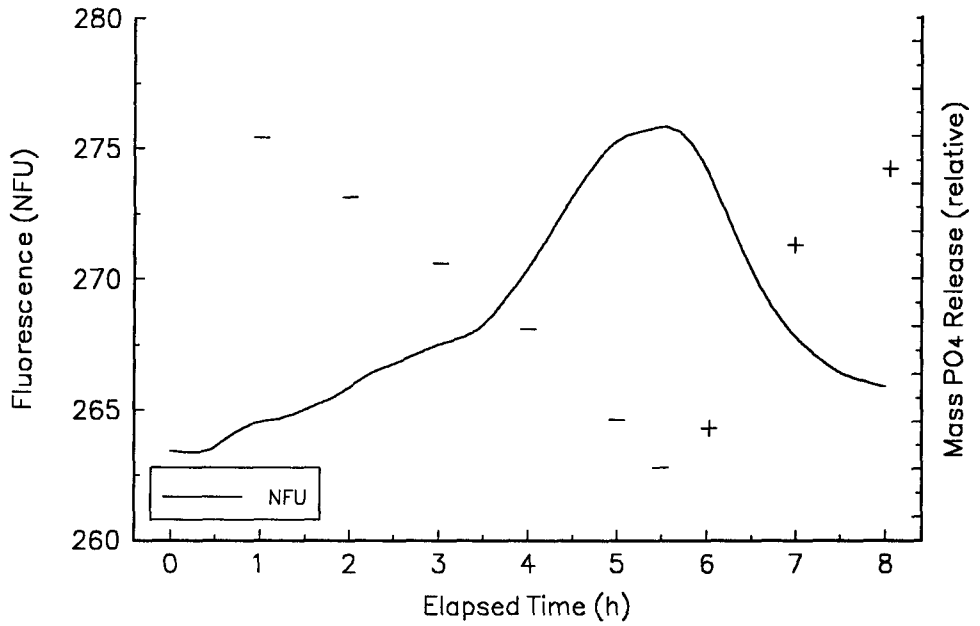


Figure 5.25. Anaerobic zone fluorescence and specific anaerobic zone VFA consumption at the FGR-SGR pilot plant. (Experiment F9360-03).

A.



B.

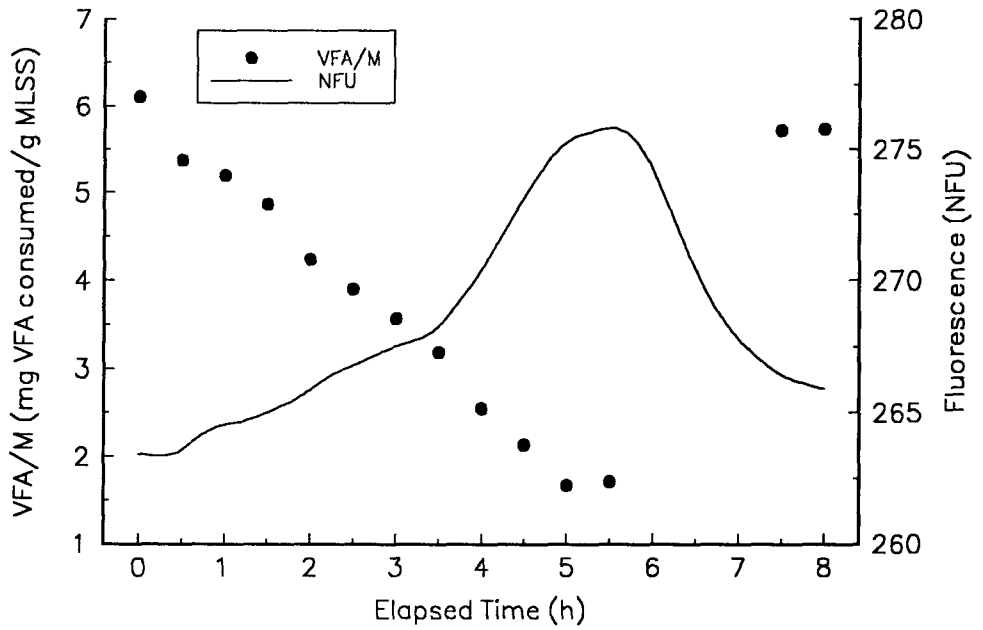


Figure 5.26. A) Anaerobic zone fluorescence relative levels of VFA consumption (VFA/M). The relative levels of VFA consumption are indicated by the "+" and "-" symbols. The "+" and "-" symbols are used to indicate both the relative level of VFA consumption and the pattern of change from the previous point. A "+" indicates a relative increase from the previous point, while a "-" indicates a relative decrease from the previous point. B) Anaerobic zone fluorescence and measured VFA consumption.

Comparison of regression analyses relating the changes in measured fluorescence to changes in specific VFA consumption revealed a high degree of variability in the specific response of fluorescence with changes in specific VFA consumption between experiments (Table III, Appendix C). While this variability limited the ability to predict exact levels of specific VFA consumption and VFA on a real time basis, it did not affect the ability to predict qualitative changes in anaerobic zone VFA loading and consumption (VFA/M) from the on-line fluorescence data.

5.2.3.2. Anaerobic zone VFA consumption, PHA storage, and fluorescence

Based on existing biochemical models that imply relationships between volatile fatty acids, polyhydroxyalkanoates, and NADH/NAD^+, investigation of the relationship between these parameters was done. According to the models, PHA synthesis requires volatile fatty acids and reducing power in the form of NADH. Based on this, it was expected that increases in VFA consumption would result in increases in PHA synthesis and storage. Furthermore, since the PHA synthesis reactions "consume" NADH, it was expected that increases in PHA synthesis and storage would lower the proportion of cellular NADH to NAD^+ and result in a lower level of measured fluorescence than conditions where PHA synthesis was not as significant (Figures 2.2, 2.3, 6.6, 6.7, 6.11). Assuming that the supply of reducing power and the enzymatic mechanisms for PHA synthesis are not rate limiting, the rate of PHA synthesis should be dependent upon the availability of suitable precursors, like VFA. Based on these assumptions, VFA availability and consumption (VFA/M) would directly affect PHA levels, while both VFA/M and PHA levels would be inversely related to NADH utilization and fluorescence.

Data demonstrated that levels of anaerobic PHA storage generally increased as VFA consumption (VFA/M) increased. Because PHA storage and VFA consumption (VFA/M)

Table III. Regression analysis of the correlations between anaerobic culture fluorescence and specific anaerobic VFA consumption at the FGR-SGR pilot plant.

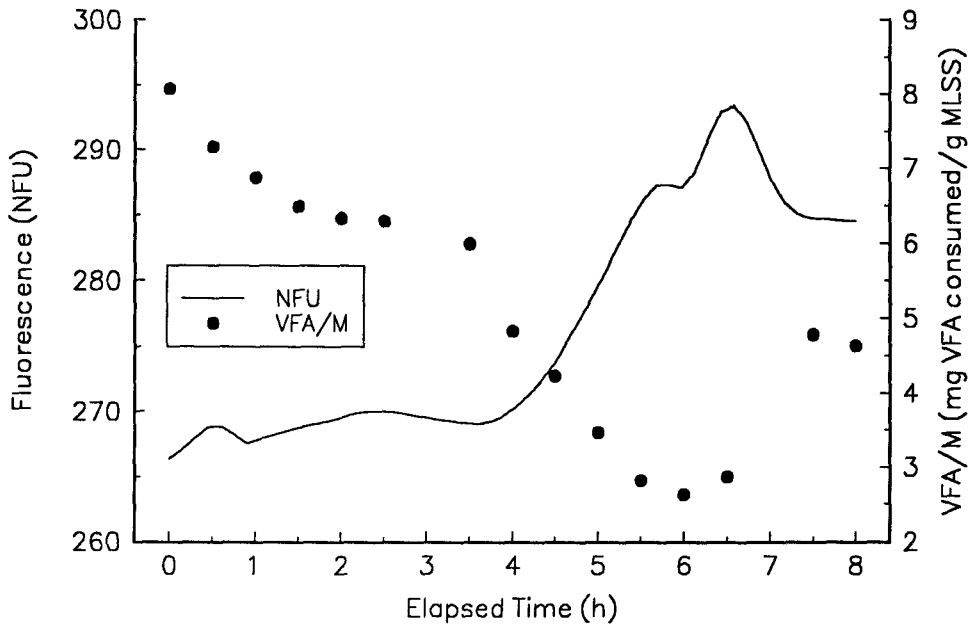
Experiment	Correlation coefficient (r2)	Slope d(VFA/M)/d(NFU)	Offset (NFU)	Number of observations (n)
F9340-01	0.85	-0.45	109.87	10
F9340-02	0.76	-1.22	248.48	16
F9340-03	0.79	-0.08	24.20	9
F9340-05	0.68	-0.14	41.21	8
F9340-06	0.83	-0.34	95.02	14
F9360-01	0.73	-0.19	61.55	9
F9360-02	0.80	-0.20	59.13	14
F9360-03	0.87	-0.21	68.46	14
F9360-04	0.82	-0.77	247.67	11
F9360-06	0.80	-0.22	78.72	9
F9360-07	0.77	-0.98	307.30	8
F9380-01	0.72	-0.41	134.00	10
F9380-02	0.88	-0.22	68.25	11
F9380-03	0.90	-0.35	107.28	9
F9380-04	0.84	-0.28	90.05	8

Note: Culture fluorescence measurements were used as the independent variable during the regression analysis. Offest refers to the y-intercept value of the regression model.

were directly related, and anaerobic VFA/M was shown to be inversely related to measured fluorescence, presumably resulting from changes in the cellular NADH/NAD^+ equilibrium, general changes in PHA storage levels could be predicted from the on-line fluorescence data. Typical examples of the observed patterns are shown in Figures 5.27 and 5.28. If the changes in measured fluorescence result from changes in the intracellular levels of NADH, then the observed inverse relationship between the levels of PHA storage and the NADH/ NAD^+ equilibrium indicated by measured fluorescence, is consistent with the Comeau/Wentzel (Comeau et al., 1986; Wentzel et al., 1986) model that suggests accumulation of PHA consumes NADH.

Comparison of actual PHA concentrations in the sludge biomass were found to be independent of the fluorescence levels between experiments, and linear regression analysis relating the two variables showed a poor mathematical correlation. Calculation of anaerobic PHA yield, determined form mass balance calculations and expressed as millimoles of carbon produced as hydroxyalkanoates (HA) per millimoles of carbon consumed as VFA (mM C as HA/mM C as VFA), consistently showed a yield factor greater than 1.0. Analysis of seventeen experiments showed an average yield factor of 1.73 with a range from 1.34 to 2.43 (Table IV, Appendix D). Comeau (1989) and Mah (1991) also reported high average yield ratios of 1.11 and 1.88, respectively, using the same sewage. This observation suggests that other undetected sources of carbon are present and contributing to PHA synthesis. Mobilization and utilization of these carbon sources would involve metabolic strategies that influence the net NADH levels and subsequent NADH-related fluorescence, complicating the specific mathematical correlations. Despite this, the general responses observed between measured fluorescence, VFA/M, and PHA storage were consistent with biochemical models and allowed on-line, qualitative determination of changes in the intracellular PHA pool.

A.



B.

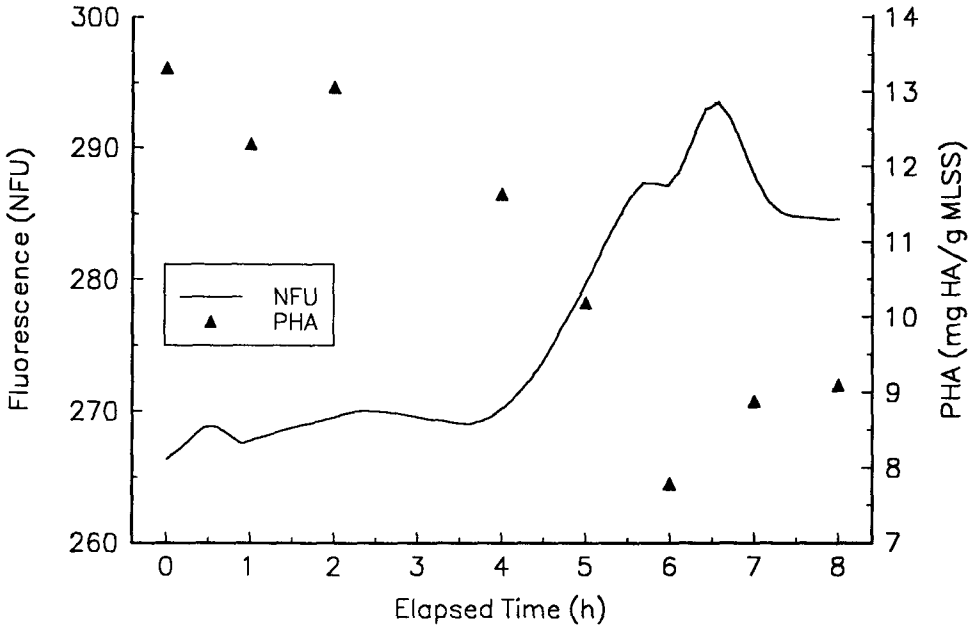
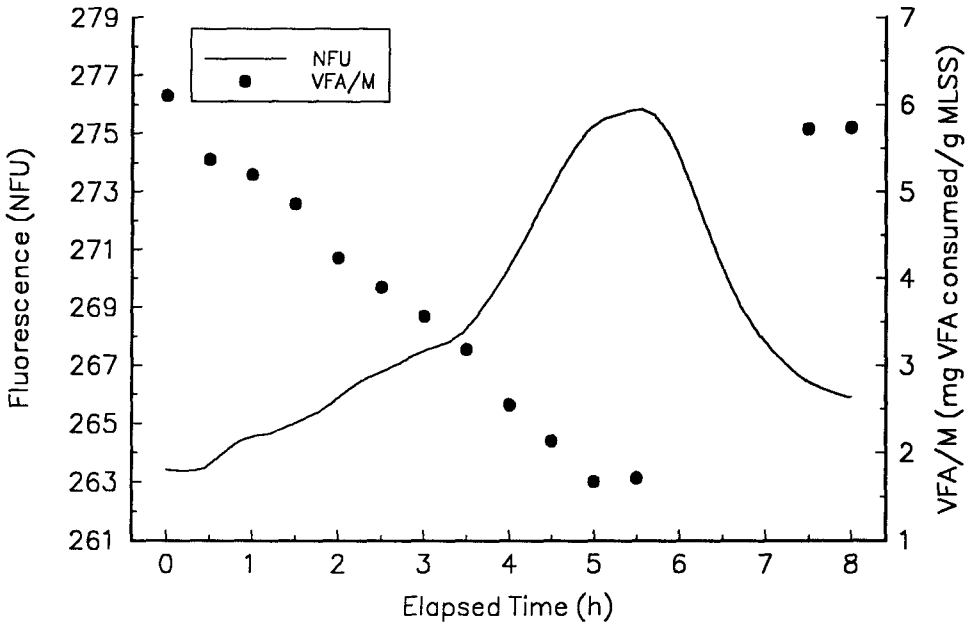


Figure 5.27. Anaerobic zone biological activity, specific anaerobic zone VFA consumption, and PHA storage at the FGR-SGR pilot plant. (A) Measured fluorescence and specific VFA consumption; (B) Measured fluorescence and PHA storage. (Experiment F9360-02).

A.



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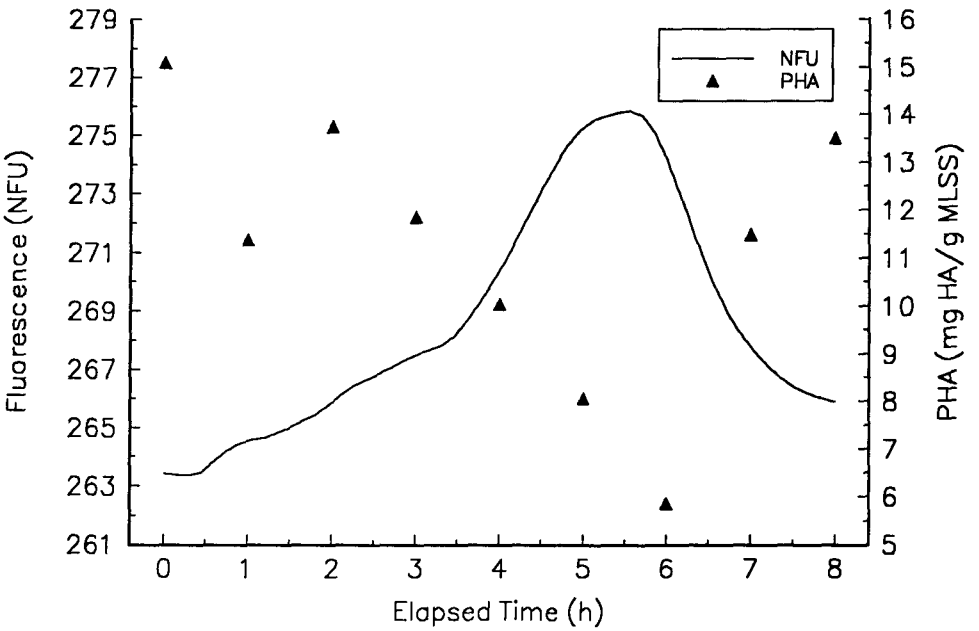


Figure 5.28. Anaerobic zone fluorescence, specific anaerobic zone VFA consumption, and PHA storage at the FGR-SGR pilot plant. (A) Measured fluorescence and specific VFA consumption; (B) Measured fluorescence and PHA storage. (Experiment F9340-06).

Table IV. Average daily molar ratios of PHA yield per VFA consumption at the FGR-SGR pilot plant (mM carbon produced as HA/mM carbon consumed as VFA).

Experiment	Average daily molar ratios of PHA produced per VFA consumed (mM C as HA/mM C as VFA)	Number of observations (n)
F9340-01	2.43	7
F9340-02	1.77	7
F9340-03	1.58	7
F9340-04	1.92	5
F9340-05	1.42	8
F9340-06	1.85	9
F9360-01	1.34	8
F9360-02	1.44	8
F9360-03	1.94	8
F9360-04	1.64	8
F9360-06	1.65	7
F9360-07	1.46	7
F9380-01	1.74	8
F9380-02	1.75	8
F9380-03	1.55	7
F9380-04	1.95	8
F9380-05	2.05	8

5.2.3.3. Anaerobic zone VFA/M, mass phosphate release, and fluorescence

Anaerobic phosphate release results from the utilization of intracellular polyphosphate stores in a variety of metabolic and energetic functions related to VFA uptake and utilization (Figures 2.2, 2.3; Section 6.3.1). Experiments conducted at the FGR-SGR plant revealed consistent patterns between mass phosphate release, specific VFA consumption (VFA/M), and fluorescence measurements in the anaerobic zone. The general pattern observed showed a direct relationship between mass phosphate release and specific VFA consumption, as expected from the biochemical models and widely reported observations. Using mass balance calculations it was shown that the calculated average daily molar ratios of phosphate release per VFA consumed, reported as millimoles P released per millimoles VFA consumed as acetic acid (mM P released/mM VFA consumed), ranged from 0.67 to 2.21, with an average of 1.80 in the pilot plant experiments (Table V). Despite the differences in the specific ratios a plot of mass phosphate release versus specific VFA consumption showed an average correlation coefficient (r^2) of 0.77 (Figure 5.29) over a three month period. The daily correlation values (r^2) ranged from 0.62 to 0.88 during this period.

Data showed an inverse relationship between mass phosphate release and measured fluorescence, similar to the relationship observed between VFA/M and measured fluorescence. Although it is not expected that the mobilization and release of intracellular phosphates from polyphosphate stores directly consumes NADH, a relationship between changes in phosphate release and measured fluorescence (NADH-related fluorescence) is, nevertheless, expected. Due to the inverse relationship observed between VFA/M and fluorescence, and the direct relationship between VFA/M and phosphate release, measured fluorescence and phosphate release are expected to be inversely related. Figure 5.30 shows a typical example of the patterns observed between measured fluorescence and mass phosphate release. Like other correlations between fluorescence and other measured parameters, the correlations and specific

Table V. Average daily molar ratios of anaerobic phosphate released per VFA consumed at the FGR-SGR pilot plant (mM P released/mM VFA consumed as acetic acid).

Experiment	Average daily molar ratios of phosphate released per VFA consumed (mM P released/mM VFA consumed)	Number of observations (n)
F9340-01	2.12	9
F9340-02	2.21	9
F9340-03	2.05	7
F9340-04	2.10	7
F9340-05	1.61	9
F9340-06	1.78	9
F9360-01	0.83	9
F9360-02	2.19	9
F9360-03	1.93	9
F9360-04	1.56	8
F9360-05	0.66	6
F9360-06	1.93	8
F9360-07	1.90	8
F9380-01	1.90	8
F9380-02	1.86	8
F9380-03	1.81	7
F9380-04	1.65	8
F9380-05	2.02	9

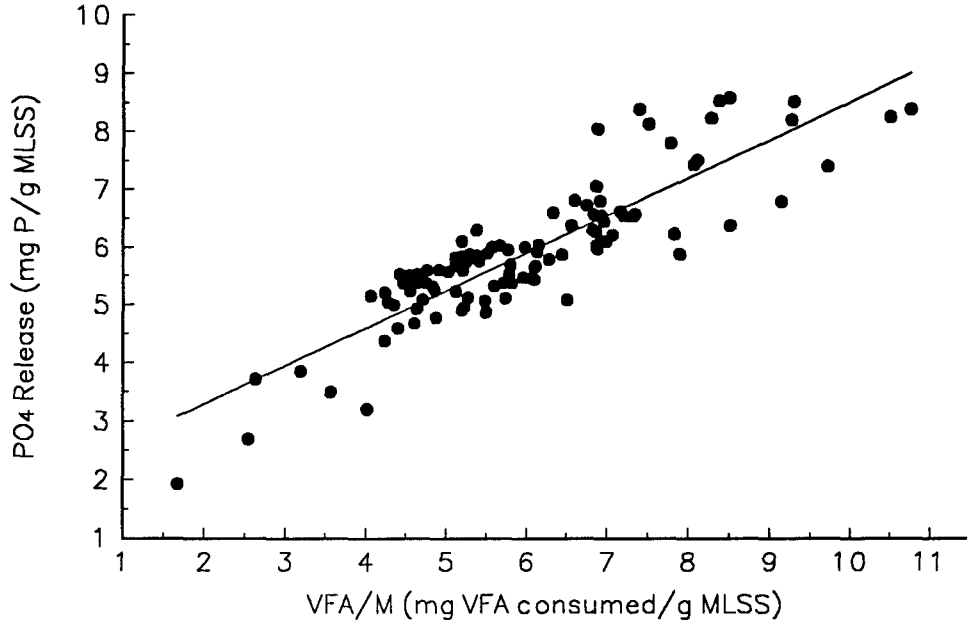


Figure 5.29. Correlation between specific anaerobic VFA consumption and anaerobic mass phosphate release over a three month period at the FGR-SGR pilot plant (July - September 1993; r^2=0.77).

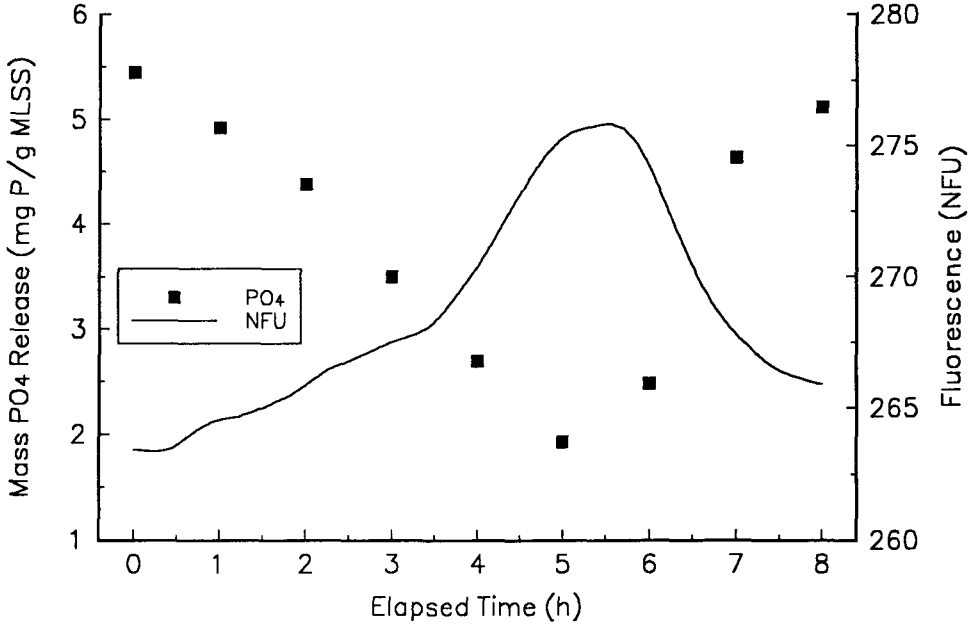


Figure 5.30. Anaerobic zone fluorescence and anaerobic mass phosphate release at the FGR-SGR pilot plant. (Experiment F9340-06).

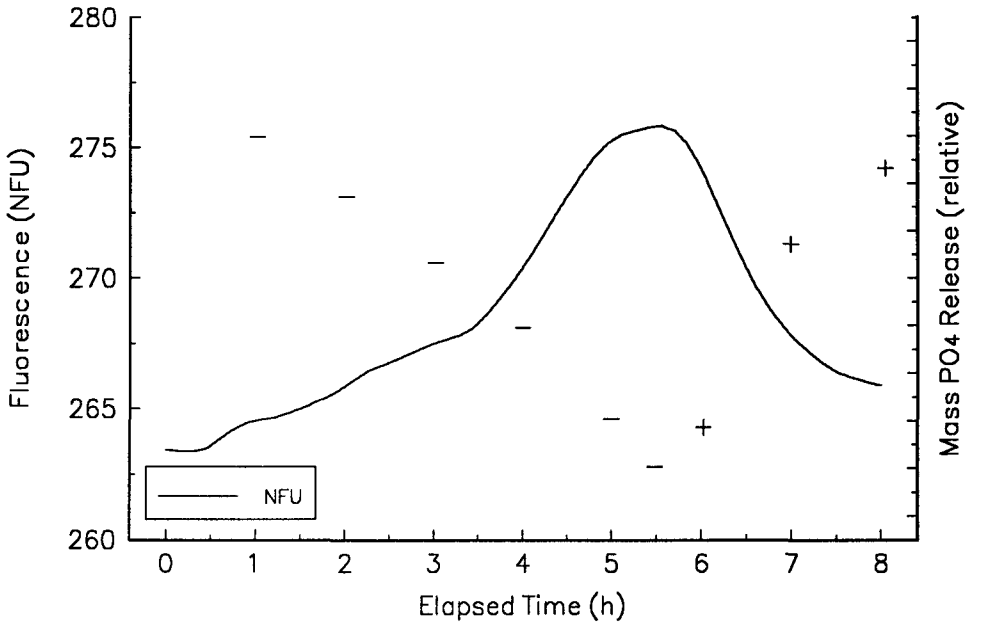
mathematical relationship between fluorescence and mass phosphate release were highly variable between experiments.

Due to the relationships between VFA/M and fluorescence, and between specific VFA consumption and mass phosphate release, changes in the trends of mass phosphate release could be predicted on a real time basis from the fluorescence data. By recognizing the changes in VFA/M indicated by the on-line anaerobic zone fluorescence data, general changes in mass phosphate release could be predicted on a real time basis. Predictions of trends in mass phosphate release, but not actual values of mass phosphate release, that occurred during the experiments from the fluorescence data were supported by laboratory data. Figure 5.31 compares the predicted trends in anaerobic phosphate release generated during experimentation from the fluorescence measurements with the measured trends. The ability to conveniently detect changes in anaerobic mass phosphate release, caused from changes in VFA loading, should provide the capability to control both anaerobic VFA loading and related phosphate release that affects the soluble phosphate inventory in the process.

5.2.3.4. Anaerobic zone VFA/M and soluble phosphate concentrations

Due to the biochemically modeled relationship between specific anaerobic VFA consumption (VFA/M) and mass phosphate release, and the observation that anaerobic phosphate release is the largest contributor to the soluble phosphate pool in the process, the effect of short term variation in VFA load on effluent phosphate concentration was investigated. Short term VFA load variations were introduced by controlling fermenter supernatant addition to the process or by the addition of a sodium acetate solution. Normally, daily variations in VFA loading were determined to be less than 10 mg total VFA/L during the experimentation period. Induced variations in nutrient load resulted in increased normal VFA loading by 20 mg total VFA/L on average. The experimental details and data from this series of experiments

A.



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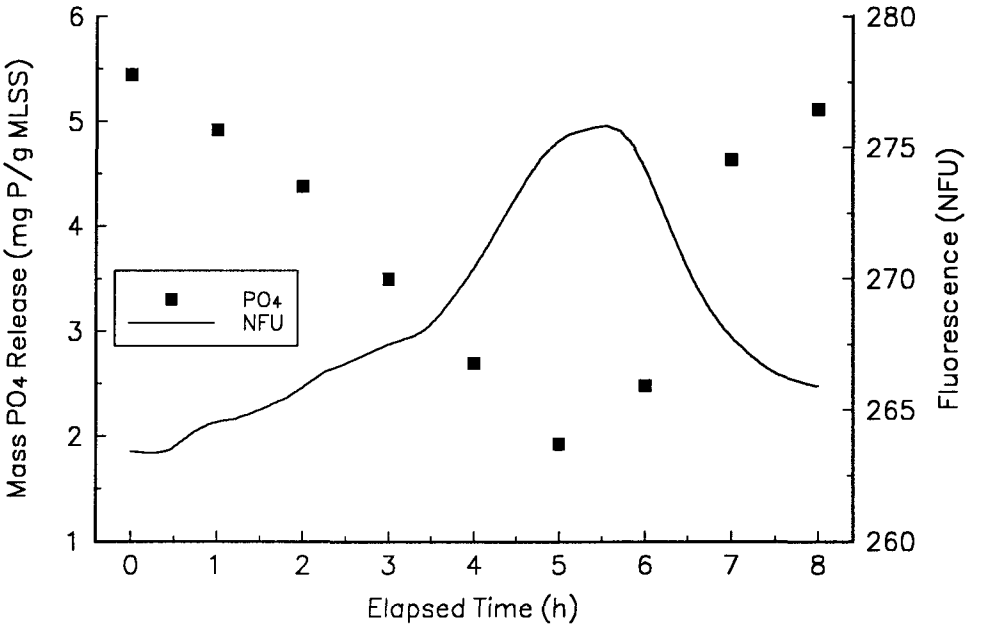

Figure 5.31. A) Anaerobic zone fluorescence and predicted relative levels of mass phosphate release. The relative levels of mass phosphate release are indicated by the "+" and "-" symbols. The "+" and "-" symbols are used to indicate both the relative level of mass phosphate release and the pattern of change from the previous point. A "+" indicates a relative increase from the previous point, while a "-" indicates a relative decrease from the previous point. B) Anaerobic zone fluorescence and measured mass phosphate release.

are provided in Appendices E and F. Data showed that changes in anaerobic phosphate release and effluent phosphate concentrations could be directly attributed to changes in anaerobic zone VFA loading and consumption and phosphate release at the FGR-SGR pilot process. Figure 5.32 illustrates the change in soluble phosphate levels in the anaerobic bioreactor in response to a short term increase in VFA loading. Figure 5.33 follows the progression of the increased soluble phosphate "slug" caused by this transient VFA load variation through the process and into the final effluent. Comparison of the time differential between the peak in soluble phosphate concentration in the anaerobic zone and the final effluent corresponded well with peaks in patterns observed during dye tracer studies done by the addition of a 1 L solution of amino-disulfonic acid (3 g/L) (Figure 5.34).

These results demonstrated the effect that VFA load variation can have on anaerobic phosphate release and effluent quality in systems that seem to have limited aerobic biological phosphate removal capacity. Table VI compares the effects on average effluent phosphate concentrations from induced transient VFA load variations with relatively constant VFA loading. Because the FGR-SGR pilot plant was undergoing a concurrent optimization study during these experiments, the average effluent phosphate concentrations varied considerably between experiments. Despite seemingly high effluent phosphate concentrations, the process was achieving excess biological phosphorus removal that was indicated by the observation that effluent soluble phosphate concentrations were usually below 1.0 mg P/L although soluble phosphorus concentrations leaving the anaerobic bioreactor were typically greater than 25 mg P/L. The experiments with induced VFA load variations also showed that variability in effluent phosphate concentrations about the mean value was significantly greater than the variability observed without induced load variation. Expression of the average coefficient of variation, as a percentage, showed an average value of 51.0% with induced load variability, compared with an average value of 19.3% without induced load variability. This analysis indicated that the measured effluent phosphate concentrations varied just over 2.5 times

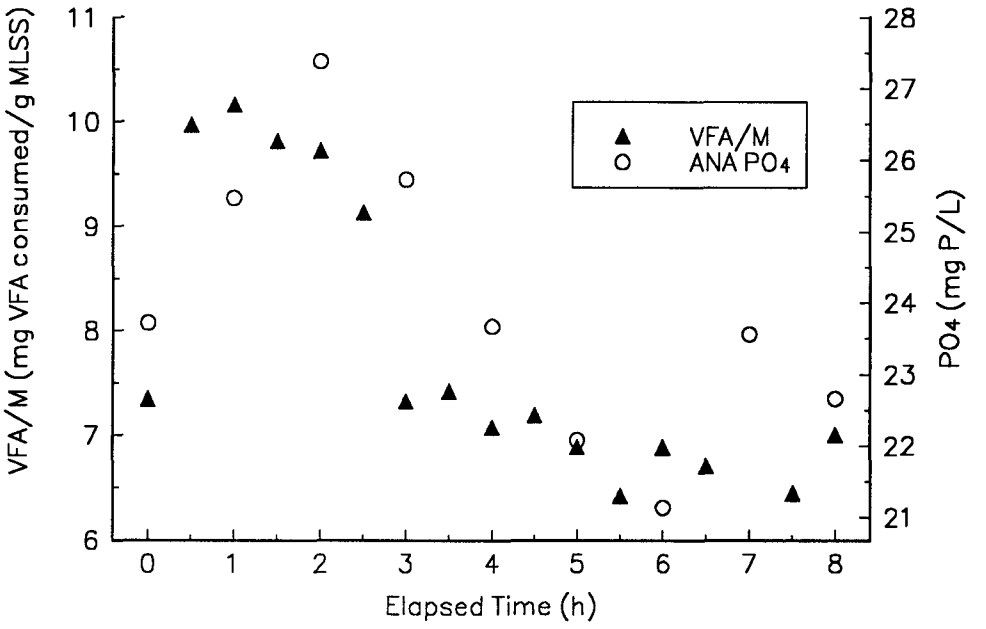

Figure 5.32. Anaerobic zone soluble phosphate concentration in response to nutrient load variation at the FGR-SGR pilot plant.

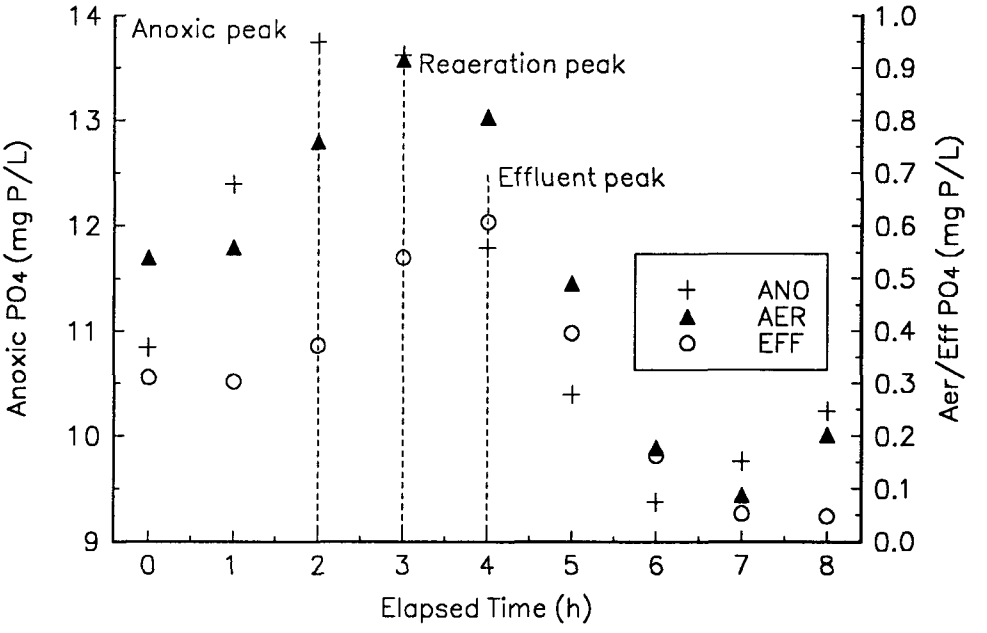

Figure 5.33. Soluble phosphate concentrations throughout the FGR-SGR process in response to nutrient load variation.

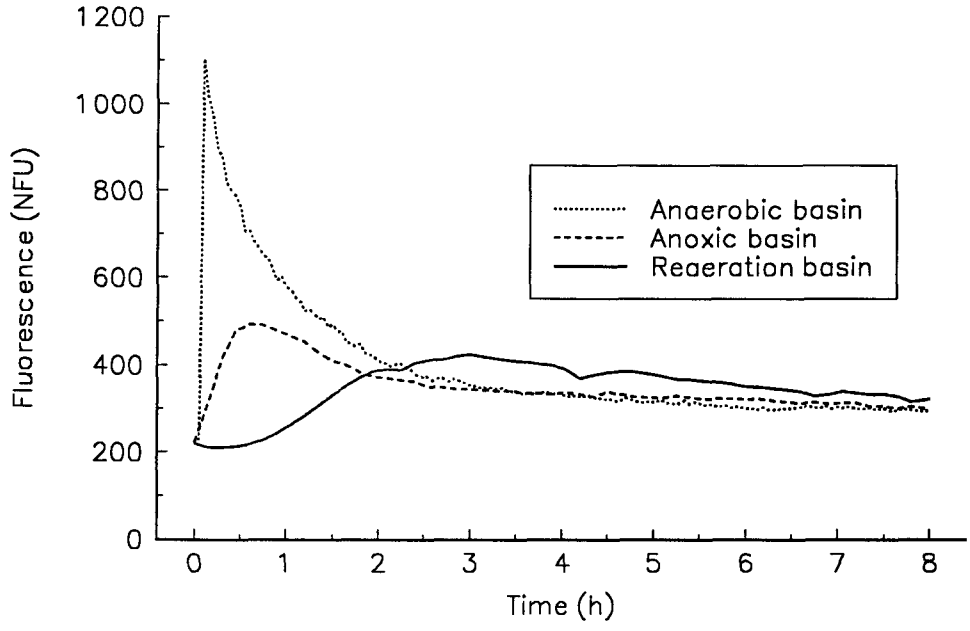

Figure 5.34. Fluorescence patterns showing the hydraulic flow patterns of the FGR-SGR pilot plant during a dye study. (Fluorescent dye added into the primary effluent stream).

more about the mean concentrations when VFA load varied significantly compared to when it was not. This demonstrated that VFA load variability can affect both the concentration and variability of effluent phosphate concentrations over the short term.

5.2.3.5. Other correlations

The ability to monitor changes in anaerobic VFA load and consumption with fluorescence, and the impact of VFA consumption on PHA storage, suggests that VFA load regulation can effect changes in the levels of anaerobic PHA. Biochemical models suggest a relationship between anaerobic carbon storage and aerobic phosphate removal capacity, and imply that changes in the levels of anaerobic PHA storage should alter aerobic phosphate removal capacities. Therefore, the ability to increase anaerobic PHA storage levels by increasing VFA load should result in increased phosphorus removal. To determine the value

Table VI. Comparison of effluent phosphorus quality with and without significant process VFA (nutrient) load variation at the FGR-SGR pilot plant.

Nutrient load condition	Expt	Mean (mg/L)	Min (mg/L)	Max (mg/L)	Range (mg/L)	Std. dev. (SD)	COV (%)	Avg COV (%)
variation	V401	0.52	0.25	0.71	0.46	0.01	2	
variation	V402	0.76	0.17	1.18	1.01	0.34	45	
variation	V405	0.31	0.05	0.61	0.56	0.20	65	
variation	V406	0.20	0.05	0.43	0.39	0.17	85	51
variation	V601	0.57	0.10	1.04	0.94	0.41	72	
variation	V603	3.69	2.52	4.41	1.89	0.74	20	
variation	V605	2.26	1.13	4.90	3.77	1.56	69	
no variation	N403	0.27	0.19	0.39	0.20	0.07	26	
no variation	N404	0.18	0.08	0.33	0.25	0.08	44	
no variation	N602	0.58	0.07	1.06	0.99	0.40	69	
no variation	N604	3.22	2.86	3.66	0.80	0.33	10	
no variation	N606	2.98	2.81	3.10	0.29	0.10	3	
no variation	N607	2.64	2.46	2.82	0.36	0.13	5	19
no variation	N801	2.30	2.18	2.36	0.18	0.07	3	
no variation	N802	1.12	1.06	1.23	0.17	0.06	5	
no variation	N803	1.47	1.36	1.58	0.21	0.07	5	
no variation	N804	0.19	0.10	0.31	0.21	0.04	21	
no variation	N805	0.20	0.16	0.26	0.10	0.04	21	

Note: Conditions where changes in nutrient load that resulted in increases of 20 mg total VFA/L or more were experienced were considered conditions of variable nutrient loading in the table. Conditions where variation in nutrient load was less than 10 mg total VFA/L were considered conditions with no nutrient load variation.

of regulating anaerobic PHA levels, the effect of various levels of PHA storage on overall phosphate removal efficiency was analyzed by comparing levels of anaerobic PHA storage, aerobic phosphate uptake, and overall removal efficiency. Data from the FGR-SGR pilot process studies showed that the amount of anaerobic PHA storage is not directly related to the efficiency or consistency of aerobic phosphate removal. The data did show, however, that aerobic phosphate uptake was related to aerobic PHA consumption (Appendix G). A plot of average specific aerobic phosphate removal versus average PHA consumption did demonstrate a general trend of increasing phosphate removal with increasing PHA consumption (Figure 5.35). Because aerobic PHA consumption did not deplete the cellular PHA pools in these experiments, aerobic phosphate removal did not seem limited by PHA stores. While no direct conclusions can be made from these data regarding the ability to

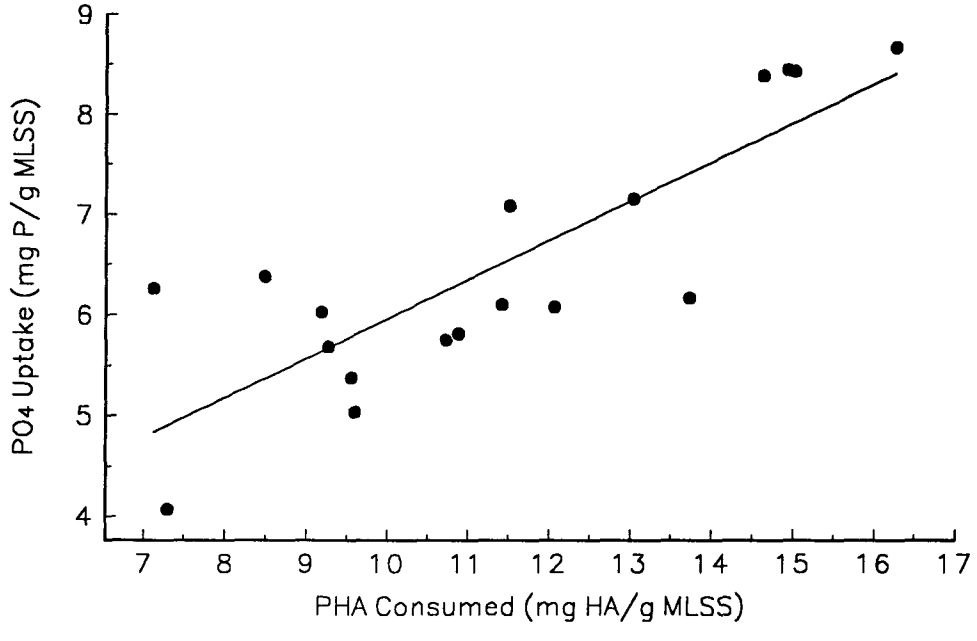

Figure 5.35. Comparison of average specific aerobic phosphate removal and average aerobic PHA consumption at the FGR-SGR pilot plant.

influence anaerobic PHA levels to improve aerobic phosphate removal, some indirect conclusions can be made. Only in conditions where aerobic phosphate uptake is limited by internal PHA stores would the ability to influence internal PHA levels, through VFA load control, be of any benefit.

5.2.4. Kelowna Pollution Control Center anaerobic zone biological activity

Analysis of the relationships between anaerobic zone fluorescence, specific VFA consumption, and mass phosphate release revealed several consistent relationships. Data from two different experiments demonstrated a consistent, direct relationship between measured anaerobic zone fluorescence and specific VFA consumption. Similarly, mass phosphate release varied directly with both anaerobic zone VFA/M and fluorescence (Figures 5.36 and 5.37). While the relationship between anaerobic zone VFA/M and mass phosphate release was expected from the biochemical models and similar to the pattern observed at the FGR-SGR pilot plant, the direct relationship between anaerobic zone fluorescence and VFA/M was different.

Investigation revealed that the pattern observed at the Kelowna plant might have resulted from different net metabolic patterns than those predicted at the FGR-SGR pilot plant. Differences in process configurations between the Kelowna plant and the FGR-SGR pilot plant may be responsible for the differences in gross metabolic activities and the patterns observed between anaerobic culture fluorescence and specific VFA consumption at each of the plants.

The anaerobic zones of both plants receive a recycle stream of return activated sludge (RAS). The characteristics and origin of these recycle streams, however, are different at each plant. One significant difference between the RAS recycle streams is the levels of nitrates

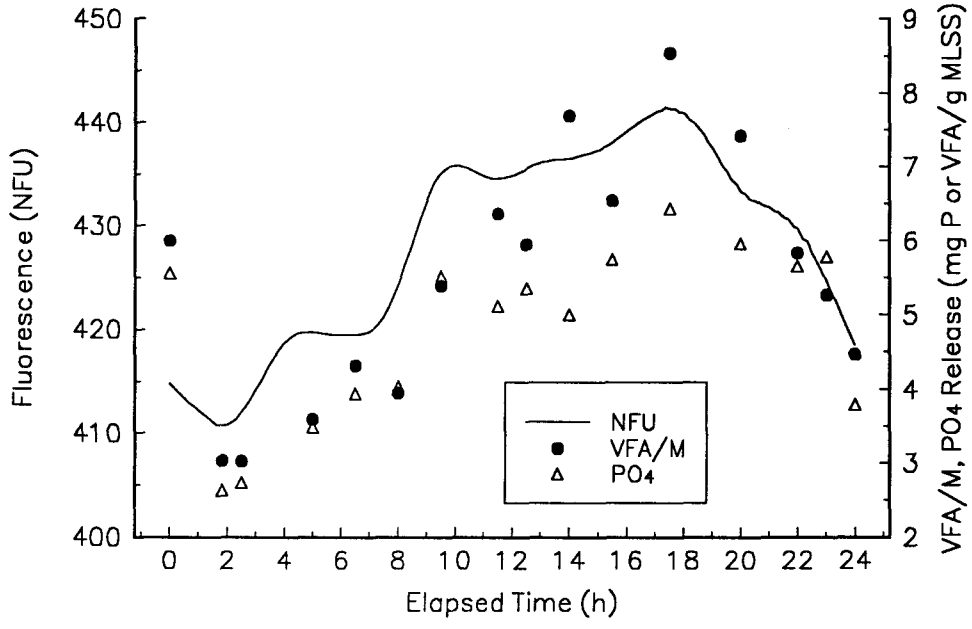

Figure 5.36. Anaerobic zone fluorescence, specific anaerobic VFA consumption, and anaerobic mass phosphate release at the Kelowna Pollution Control Centre. (Experiment KL921118).

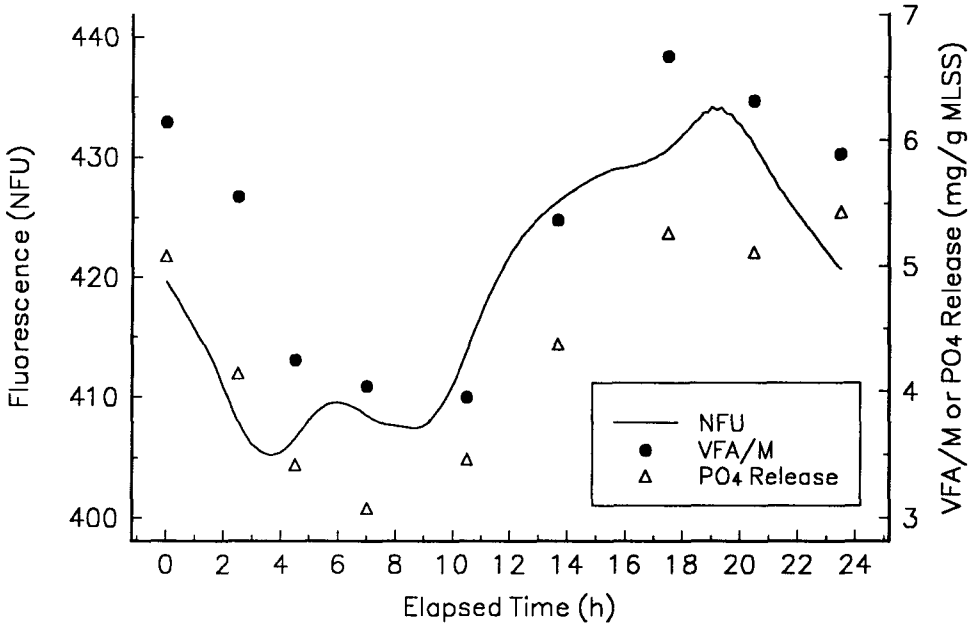

Figure 5.37. Anaerobic zone fluorescence, specific anaerobic VFA consumption, and anaerobic mass phosphate release at the Kelowna Pollution Control Centre. (Experiment KL921121).

in the RAS streams. At Kelowna the RAS discharged into the anaerobic zone originates from the secondary clarifiers and would contain nitrates. At the FGR-SGR pilot plant the RAS discharged into the anaerobic zone originates from the anoxic zone, where denitrification occurs, and would contain insignificant levels of nitrates. Nitrate levels measured at the discharge point of the RAS into the anaerobic zone of the Kelowna plant were plotted against measured fluorescence on each day. Figures 5.38 and 5.39 show an inverse relationship between the measured nitrate concentration and fluorescence. The constant input of nitrates into this zone results in quasi-anaerobic conditions. Since the energetics of NADH oxidation by nitrate are generally more favourable than NADH oxidation by the organic compounds normally associated with anaerobic conditions (Section 2.2), the presence and metabolism of nitrate (i.e. denitrification) are expected to have a significant impact on the $NADH/NAD^+$ equilibrium and NADH-related fluorescence. Therefore, higher levels of nitrate loading are expected to cause higher rates of NADH oxidation that result in an inverse pattern between nitrate levels and measured fluorescence. Consequently, the observed fluorescence patterns might reflect different degrees of anaerobiosis in the zone caused from different levels of nitrate loading and denitrification.

Based on this assumption, it follows that when nitrate loading is lower, conditions would be relatively more anaerobic, and a higher net level of intracellular NADH and NADH-related fluorescence would exist. Assuming that available VFA are partitioned between denitrification and BNR metabolism characteristic of anaerobic conditions (i.e. VFA uptake/ utilization, phosphate release, PHA storage), then as nitrate load and denitrification metabolism decreased, more of the VFA present would be available for those characteristic BNR reactions. As the level of nitrate loading decreased, a greater degree of anaerobiosis would be realized. Thus, greater activity of this characteristic BNR metabolism would be expected to correspond and occur with a higher degree of anaerobiosis. The combination of a lower nitrate load, a greater degree of anaerobiosis, and greater VFA availability for BNR metabolism should

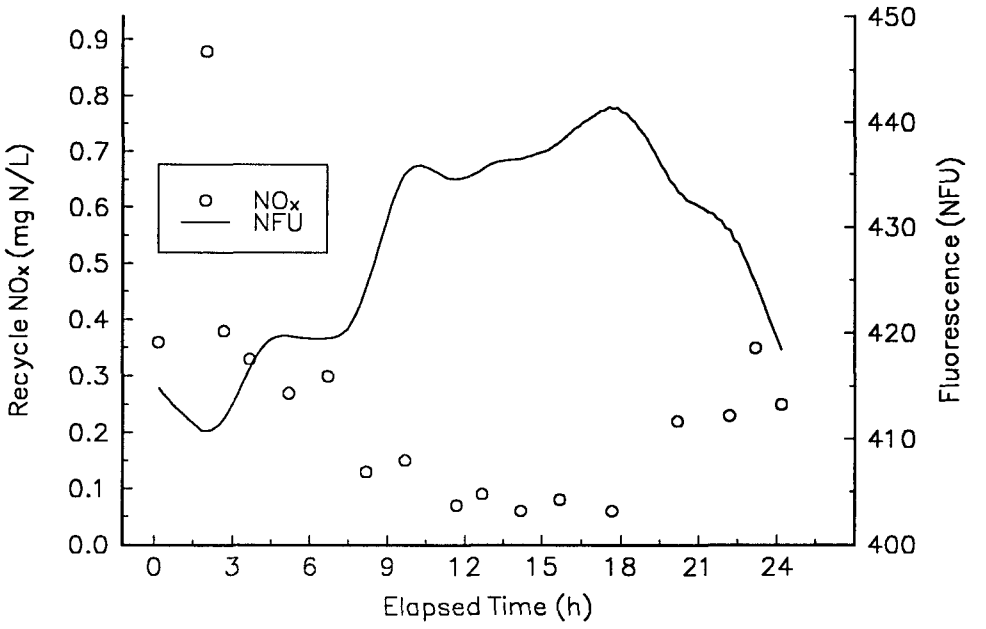

Figure 5.38. Variation in anaerobic zone fluorescence in response to changes in NOx concentration in the return activated sludge stream at the Kelowna Pollution Control Centre. (Experiment KL921118).

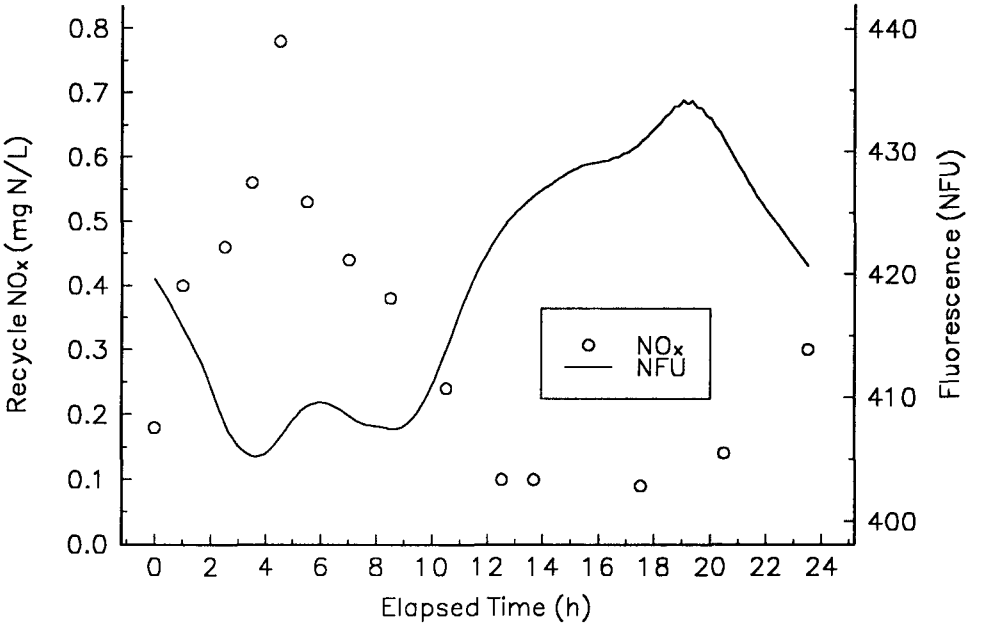

Figure 5.39. Variation in anaerobic zone fluorescence in response to changes in NOx concentration in the return activated sludge stream at the Kelowna Pollution Control Centre. (Experiment KL921121).

result in higher levels of phosphate release. Therefore, an inverse relationship between nitrate load and phosphate release would be expected. Furthermore, a direct relationship between phosphate release and measured fluorescence would be expected, since higher levels of fluorescence correspond with conditions of lower nitrate loading and greater anaerobiosis. Figures 5.40 and 5.41 show the observed patterns between phosphate release and nitrate load observed during the two experiments. More detailed discussion and biochemical modeling to support these theories and patterns are presented in Section 6.3.4.1.

Despite the differences in the general patterns observed in the anaerobic basin at this plant from those observed at the FGR-SGR pilot plant, the consistency of the relationships provides a means for monitoring and predicting relative changes in anaerobic zone VFA/M and phosphate release from fluorescence measurements. This capability has potential for on-line process control and optimization, and could be useful for regulating anaerobic VFA load and mass phosphate release.

5.2.5. Oaks, PA A/O® Wastewater Treatment Plant anaerobic zone biological activity

Data showed consistent patterns between measured fluorescence, specific VFA consumption, and mass phosphate release in the anaerobic zone at the Oaks plant. The general patterns observed were similar to those observed at Kelowna. Specific VFA consumption varied directly with measured fluorescence (Figure 5.42) over the two fifteen hour experimentation periods. As expected from the biochemical models, trends in mass phosphate release followed trends in VFA load and consumption (Figure 5.43) resulting in a general direct relationship between measured fluorescence and mass phosphate release (Figure 5.44).

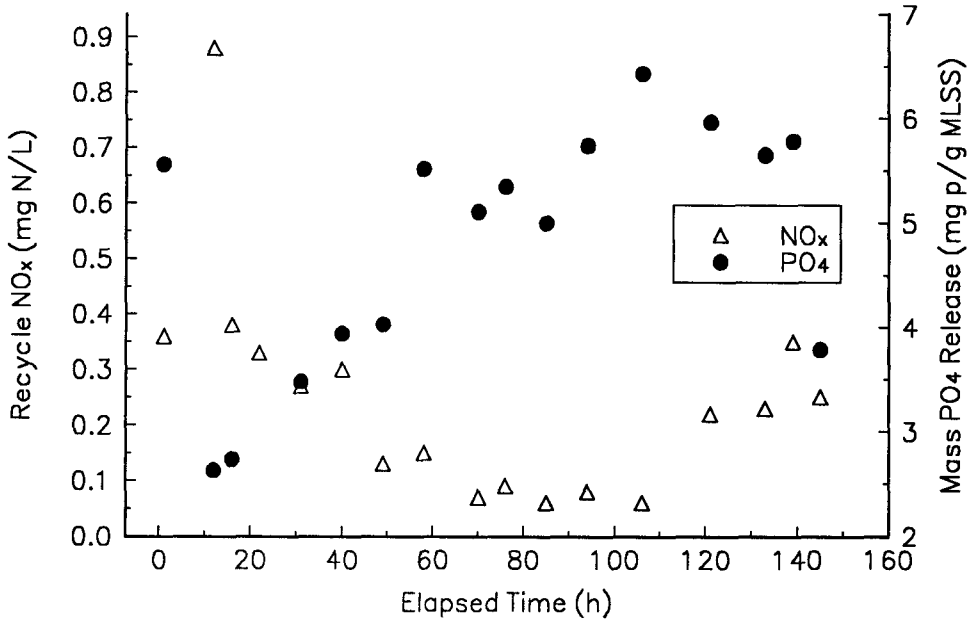

Figure 5.40. Variation in anaerobic mass phosphate release in response to changes in NOx concentration in the return activated sludge stream at the Kelowna Pollution Control Centre. (Experiment KL921118).

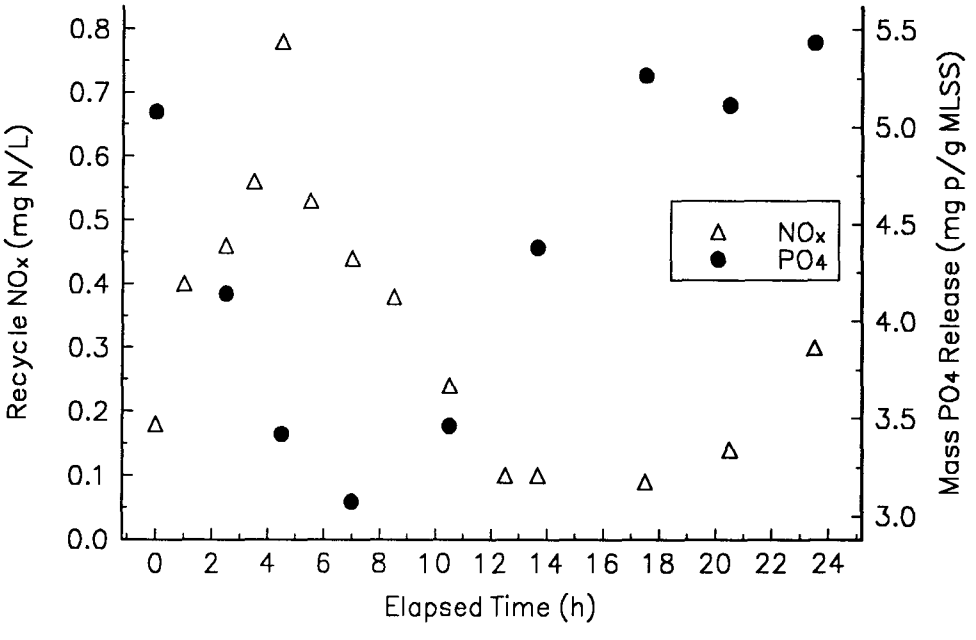

Figure 5.41. Variation in anaerobic mass phosphate release in response to changes in NOx concentration in the return activated sludge stream at the Kelowna Pollution Control Centre. (Experiment KL921121).

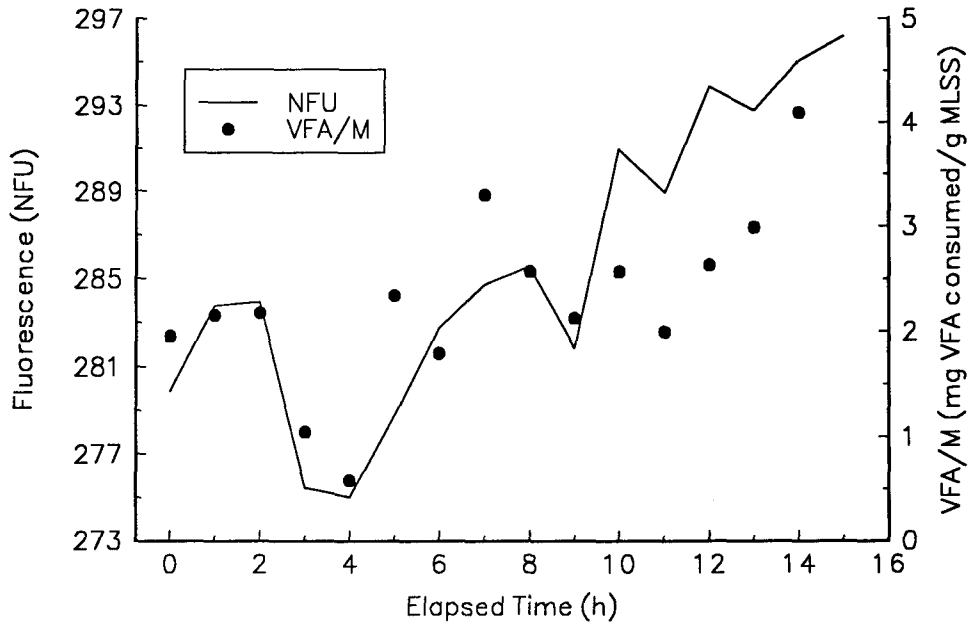

Figure 5.42. Anaerobic zone fluorescence and specific anaerobic zone VFA consumption at the Oaks, PA A/O Wastewater Treatment Plant. (Experiment OAK119317).

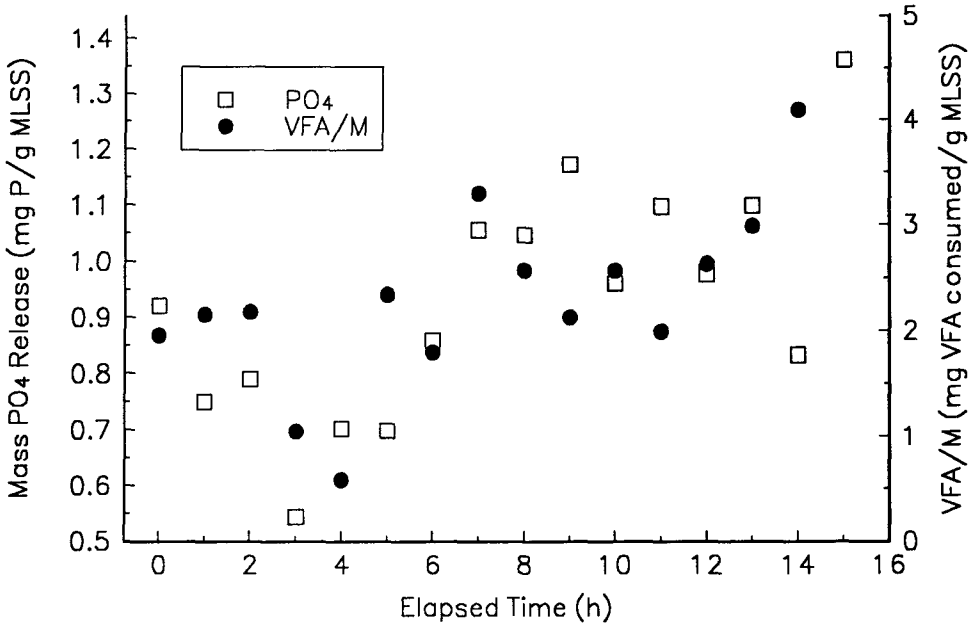

Figure 5.43. Specific anaerobic zone VFA consumption and anaerobic zone mass phosphorus release at the Oaks, PA A/O Wastewater Treatment Plant. (Experiment OAK119317).

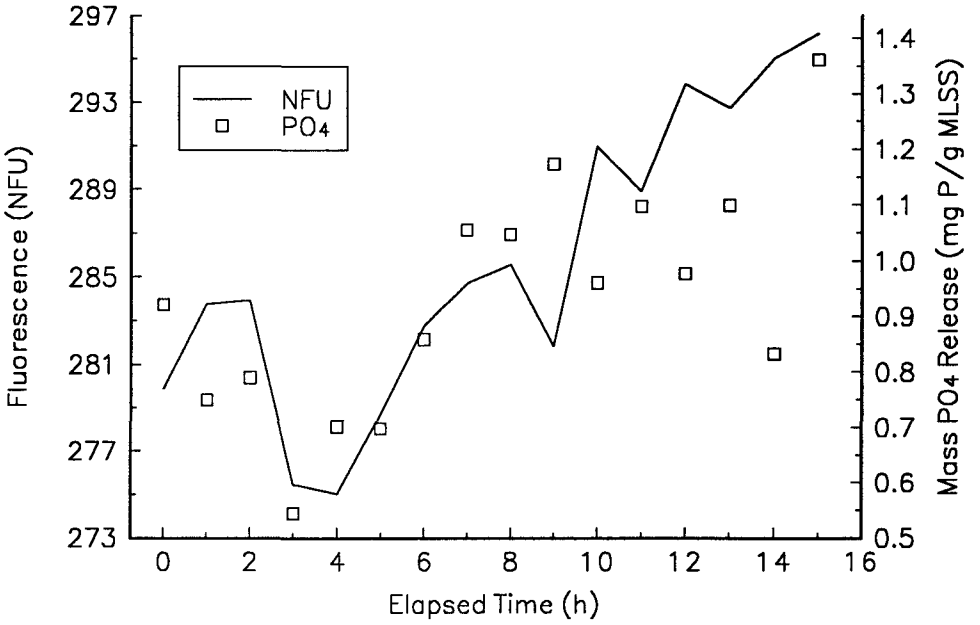

Figure 5.44. Anaerobic zone fluorescence and anaerobic mass phosphorus release at the Oaks, PA A/O Wastewater Treatment Plant. (Experiment OAK119317).

The similarity between the patterns observed at Oaks and Kelowna may be attributed to similarities in net metabolic activity resulting from similarities in the operating configurations of the two processes. Like the Kelowna treatment plant, the anaerobic zone of the Oaks plant receives RAS directly from the secondary clarifiers. The similarity of RAS origin would result in the introduction of nitrates into the anaerobic basin. Only a limited amount of nitrate data was available for analysis from the Oaks experiments. The limited data indicated the same general inverse patterns between nitrate load and VFA consumption, phosphate release, and fluorescence observed at the Kelowna facility. Interestingly, comparison of PHA data with measured fluorescence showed a general direct relationship between the two (Figure 5.45), unlike the patterns expected from biochemical models or those observed at the FGR-SGR pilot plant. Since higher levels of PHA storage are expected with higher VFA availability and greater anaerobiosis, the data supports the previous theory relating observed fluorescence and anaerobic BNR metabolism with changes in nitrate loading. Again, a more

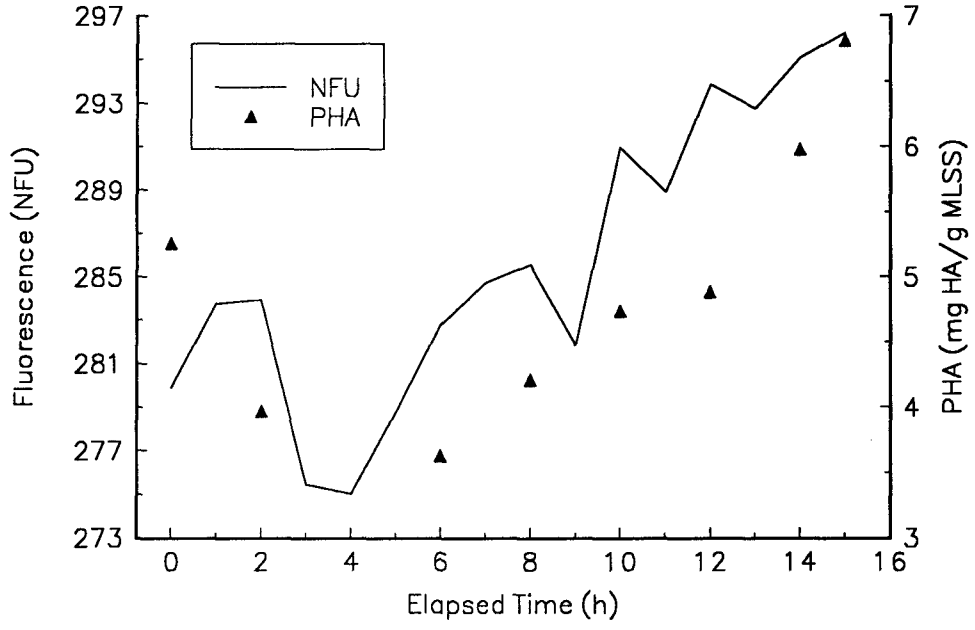

Figure 5.45. Anaerobic zone fluorescence and anaerobic zone PHA storage at the Oaks, PA A/O Wastewater Treatment Plant. (Experiment OAK119317).

thorough discussion supporting the hypothesis and observations can be found in Section 6.3.4.1.

The consistency of the observed relationships between anaerobic zone fluorescence and VFA/M, phosphate release, and PHA storage provides a means to predict relative changes in key anaerobic zone metabolism that has potential uses for process control and optimization. Similarities in the operating configurations and observed patterns between anaerobic zone fluorescence and metabolism of the Oaks and Kelowna plants provide further support for the theories proposed in Section 6.3.4.1., as well as the usefulness of fluorescence monitoring as an on-line indicator of biological activity.

5.3. Relative Fluorescence Patterns

In addition to testing for consistent relationships between measured fluorescence and specific parameters in a single process zone, patterns of fluorescence in different process zones were compared and correlated with changes in process performance at the FGR-SGR pilot plant. Specifically, the relative fluorescence patterns between the anaerobic and anoxic zone were investigated. Previous work demonstrated the sensitivity of the "anaerobic" fluorescence signal to the availability of NADH-oxidizing agents, such as nitrate (Ju and Trivedi, 1992; Isaacs and Henze, 1994; Mah, 1991). Figure 5.46 shows the results of a batch test where anaerobic FGR-SGR sludge was injected with nitrates ($NaNO_3$, Sigma Chemical Co., St. Louis, MO.) to induce transitions between anaerobic and anoxic patterns of metabolism and demonstrate that changes in measured fluorescence could be correlated directly with these transitions. The figure shows that when the nitrate was added to the anaerobic sample, the fluorescent signal dropped immediately to reflect the change in the $NADH/NAD^+$ equilibrium resulting from the rapid oxidation of NADH by nitrate metabolism (Section 2.2) and a shift to a net predominance of NAD^+. This metabolic shift resulted in a lower level of fluorescence. When all of the added nitrate was gone and denitrification was complete, measured fluorescence jumped back up. This jump indicated a different $NADH/NAD^+$ equilibrium with a relatively higher proportion of NADH, characteristic of anaerobic conditions. Previous work had demonstrated that the denitrification time reported by the fluorescence signal, i.e. the duration of time required for the fluorescence signal to recover to an "anaerobic" level after the introduction of nitrate, was proportional to the amount of nitrate removed (Ju and Trivedi, 1992; Mah, 1991; Mah et al, 1995).

Based on the assumption that the bulk of anoxic zone biological activity and measured fluorescence are affected by the presence of nitrate and its metabolism, it follows that the difference in measured fluorescence (ΔNFU) between the anaerobic and anoxic zones should

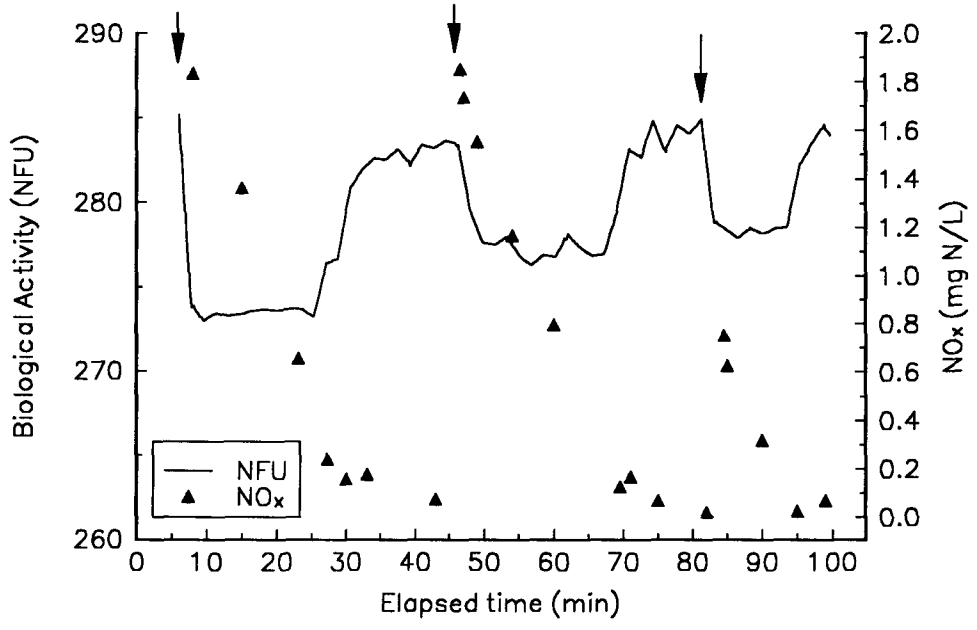

Figure 5.46. Measured fluorescence response during transitions between anaerobic and anoxic conditions. (Anoxic were conditions created by the addition of a nitrate solution. Arrows indicate times of nitrate additions).

reflect changes in bulk metabolism due to biological denitrification. Data collected over a forty day period (Appendix H) at the FGR-SGR pilot plant showed that the average difference (Δ) in fluorescence between the two zones was related to average mass NOx removal in the anoxic basin (Figure 5.47). Additionally, data indicated that as average mass NOx removal decreased, the average level of nitrate leaving the anoxic basin increased (Figure 5.48). Therefore, changes in the difference (Δ) in fluorescence between the two zones could be used as an indicator of process denitrification and suggest the possibility to assess changes in process denitrification conveniently from fluorescence data.

5.4. Fluorescence-based Control Testing

Based on the previous data from this plant and the others indicating that changes in primary effluent VFA concentrations, anaerobic zone VFA consumption, phosphate release

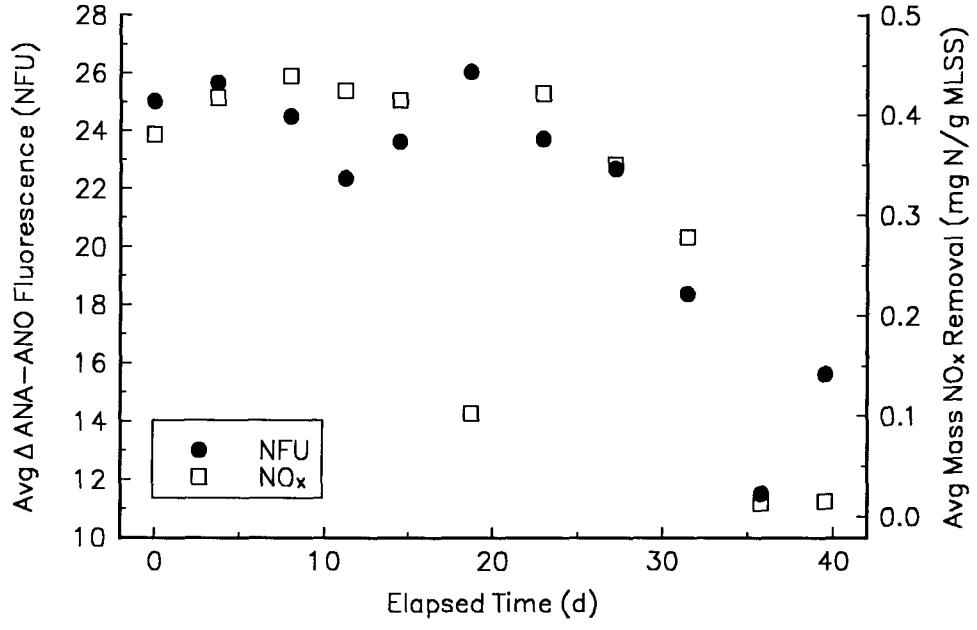

Figure 5.47. Trends in the average differences between anaerobic and anoxic zone fluorescence (ΔNFU) and average mass NOx removal in the anoxic zone at the FGR-SGR pilot plant over a 40 day period.

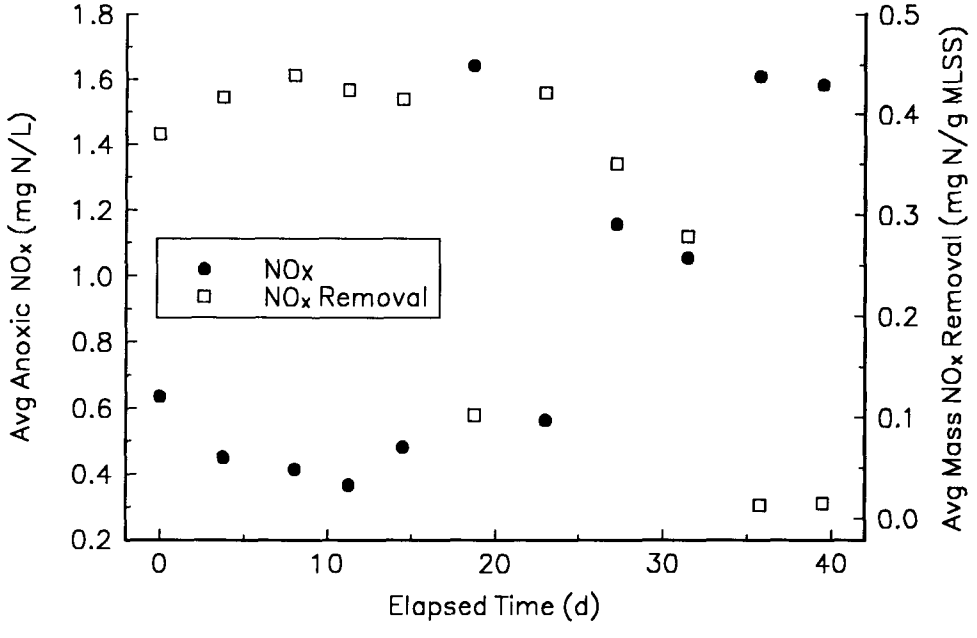

Figure 5.48. Trends in average mass NOx removal in the anoxic zone and average NOx levels leaving the anoxic bioreactor over a 40 day period at the FGR-SGR pilot plant.

and PHA storage could be predicted from fluorescence data, it should be possible to use these capabilities for some useful, real-time process control. Preliminary tests were jointly undertaken with BioChem Technology Inc. at the Penticton Advanced Wastewater Treatment Plant to investigate this possibility. Specifically, tests were designed to demonstrate the ability to balance VFA load and stabilize anaerobic biological activity, namely VFA consumption (VFA/M) and phosphate release, on a real time basis using on-line fluorescence measurements. Because changes in anaerobic culture fluorescence could be correlated to changes in anaerobic nutrient (VFA) load and consumption, phosphate release, and PHA storage at the other plants studied, the same relationships were assumed true at the Penticton plant. Furthermore, it was assumed that the more stable the fluorescence signal measured in the anaerobic zone, the more consistent the anaerobic zone VFA consumption, phosphate release, and PHA storage.

At the Penticton plant, VFA load regulation could be accomplished by manipulating equalization basin operation. Normally, operation was designed to achieve hydraulic balancing to the process train and involved filling the basin during the peak flows and then pumping the stored wastewater to the process during the low flow periods. During the low flow periods, primary effluent bypasses the equalization basin and gets mixed with flows from the equalization basin prior to entering the bioreactors. Essentially, VFA load balancing was accomplished by continuously controlling flows entering and leaving the equalization basin, rather than simply following a sequential fill-then-draw mode of operation. Flows to the equalization basin were controlled by adjusting a gate valve controlling the flow of primary effluent to the equalization basin. Flows leaving the basin were controlled by adjusting the pump discharging liquid back into the primary effluent stream.

Control decisions were based on fluorescence data received from the primary effluent, mix stream, and anaerobic basin. Since the Penticton plant was similar in operating

configuration to the pilot plant with respect to the anaerobic bioreactor, it was assumed that the same type of patterns between anaerobic zone fluorescence and VFA consumption, phosphate release, and PHA storage existed. Specifically, it was assumed that these three parameters all varied inversely with measured fluorescence. As the objective of the test was to control anaerobic VFA consumption (VFA/M) and phosphate release, it was the anaerobic zone fluorescence that was the principal variable when making control decisions. A control region was selected to maintain anaerobic VFA/M within for the experiment. This control region was arbitrarily selected and encompassed about 50% of the previous days anaerobic zone fluorescence and VFA/M profile. Anaerobic zone fluorescence was monitored and when the fluorescence level changed, indicating changes in VFA/M, control action was taken to stabilize anaerobic zone VFA load. If measured fluorescence increased, indicating a decrease in VFA/M and phosphate release, attempts were made to increase the VFA concentration of the stream entering the anaerobic zone to maintain the VFA/M within the control region. If measured fluorescence decreased, indicating an increase in VFA/M and phosphate release, attempts were made to decrease the VFA concentration of the stream entering the anaerobic zone. Based on the previously observed inverse relationships between fluorescence and VFA concentrations in these streams, fluorescence data were collected each minute and used to estimate relative VFA concentrations in the primary effluent and mix stream. From this information, the equalization basin gate valve and pump were controlled to manipulate the VFA concentrations in the stream entering the anaerobic zone. The diagram presented in Figure 5.49 summarizes the logic used to make the required flow control decisions. For example, if the observed VFA/M exceeded an arbitrary set point, control action would be taken to reduce the VFA concentration in the stream. In this case, if the observed VFA concentration of the primary effluent was greater than the VFA concentration of the mix stream, then the flows to and from equalization basin would be increased by opening the gate valve more and increasing pumping to lower the net anaerobic basin VFA load. If, however, the VFA concentration of the primary effluent was less than the VFA concentration of the

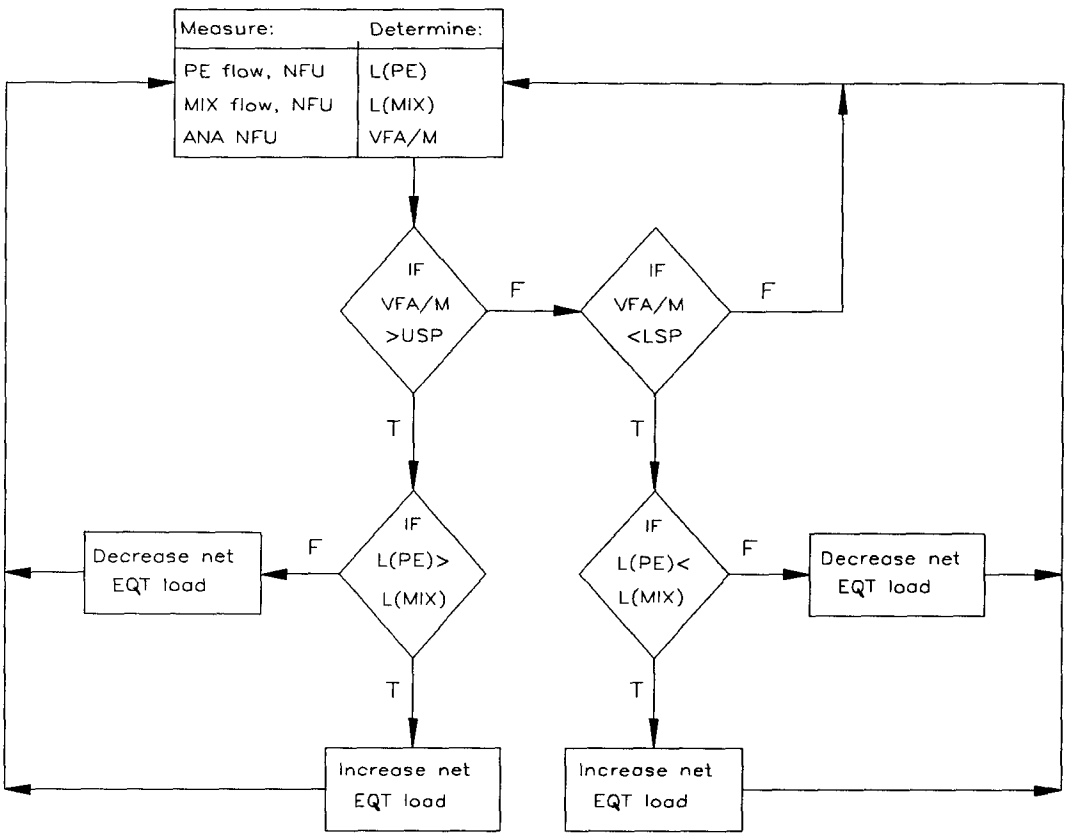

Figure 5.49. Logic diagram used during the VFA control study at the Penticton Advanced Wastewater Treatment Plant. The abbreviations used and their corresponding meanings are as follows: PE, primary effluent; MIX, "mixed" stream containing flows from the primary clarifiers and equalization basin; EQT, equalization basin; ANA, anaerobic zone; L(PE), PE VFA load; L(MIX), MIX VFA load; USP, upper set point; LSP, lower set point; NFU, fluorescence expressed in normalized fluorescence units; VFA/M, specific VFA loading/consumption; T, true; F, false. (Adapted from Armiger et al., 1993).

mix stream, then reducing the net anaerobic basin VFA load would be accomplished by decreasing flow to and from the equalization tank to allow more of the dilute primary effluent to enter the bioreactor. At all times, it was also the objective to attempt to maintain a constant hydraulic load to the process. Figure 5.50 outlines the physical setup of the primary effluent and equalization basin flows and their control mechanisms.

Figure 5.51 shows the results of the experiment demonstrating the ability to regulate VFA load and anaerobic zone VFA/M over a 24 hour period by manipulating equalization tank flows. This figure compares the anaerobic biological activity, indicated by measured fluorescence, for two different twenty-four hour periods, one with VFA (nutrient) load control (NLR) and one without. The two different periods are actually two consecutive days. Except for two occasions when the VFA load was allowed to increase, anaerobic biological activity (NFU) was controlled within an arbitrarily preset control region. During both overloading events, load was increased by decreasing the effective dilution rate of the nutrient stream entering the bioreactor, once intentionally and once by incorrect adjustment of the equalization basin gate valve and pump. On both these occasions the fluorescence signal responded as expected. The previous experimentation suggested that for a plant with this configuration, an increase in the VFA load to the anaerobic basin should cause a decrease in measured fluorescence. After a short time, the dilution rates were increased to demonstrate the ability to regain control of the load.

Using a previous correlation between anaerobic zone fluorescence and VFA/M derived during testing by BioChem Technology, Inc., the data in Figure 5.51 were re-expressed as VFA/M. These converted data are shown in Figure 5.52. Both figures show that combined hydraulic and VFA load control resulted in more stable signal and presumably more stable conditions in the bioreactor. Figure 5.52 shows that when both VFA and hydraulic load were controlled (NLR), biological activity, indicated by measured fluorescence, was maintained

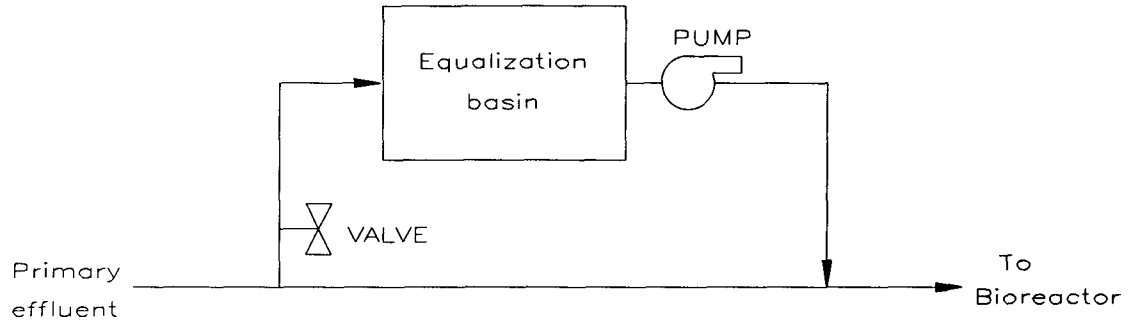

Figure 5.50. Simplified schematic showing primary effluent and equalization basin flows and controls at the Penticton Advanced Wastewater Treatment Plant.

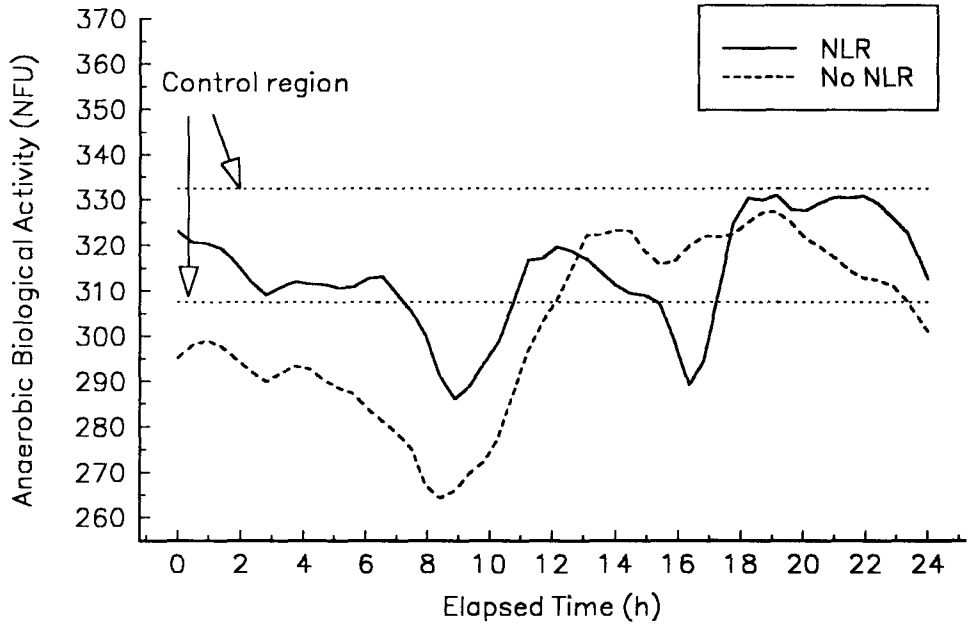

Figure 5.51. Anaerobic zone biological activity profiles, indicated by fluorescence, under conditions of hydraulic load equalization with and without VFA (nutrient) load regulation (NLR). The "test" condition refers to the situation with both hydraulic and load regulation. The "control" refers to the situation with hydraulic regulation only.

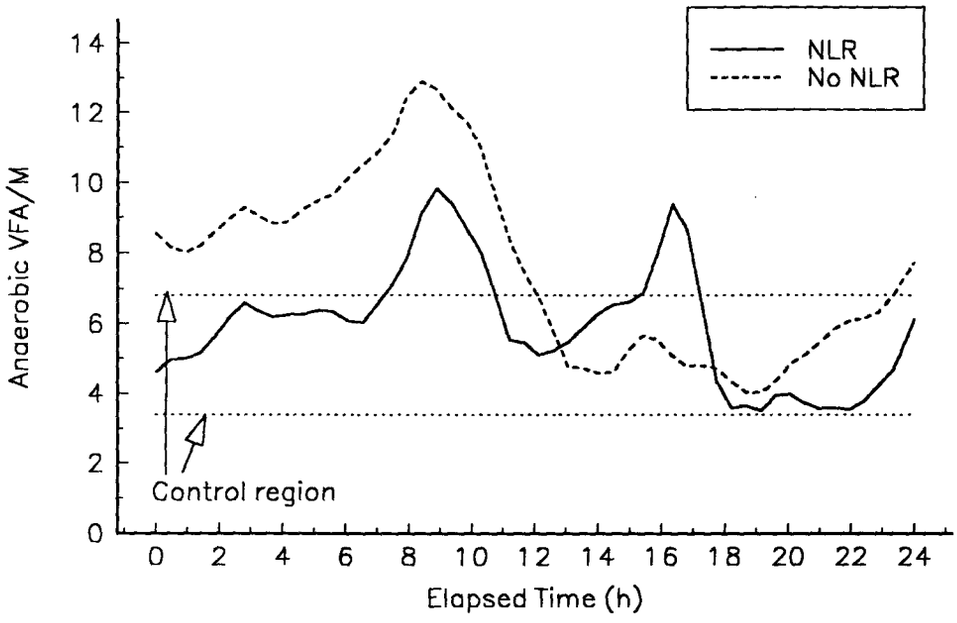

Figure 5.52. Anaerobic zone VFA/M profiles under conditions of hydraulic load equalization with and without nutrient load regulation (NLR). The "test" condition refers to the situation with both hydraulic and load regulation. The "control" refers to the situation with hydraulic regulation only.

within the control region for approximately 85% of the time, except for the two brief excursions. Without VFA load regulation (No NLR), biological activity, indicated by measured fluorescence, remained within the control region only 50% of the time. Furthermore, variation of the activity around the control midpoint was greater when VFA (nutrient) load regulation (NLR) was not employed. Additional data illustrating the equalization gate valve and pump adjustments, primary effluent and mix stream fluorescence, and process influent and effluent flow data can be found in Appendix I.

Figure 5.53 shows fluorescence data from the primary effluent and anaerobic bioreactor under conditions with hydraulic equalization only. The figure shows how anaerobic biological activity (VFA/M and phosphate release), indicated by anaerobic zone fluorescence, is dependent upon the primary effluent VFA load, indicated by primary effluent fluorescence. The trends in anaerobic biological activity appear tightly coupled to trends in primary effluent

load since changes in anaerobic biological activity closely followed changes in the primary effluent. Figure 5.54 shows the situation where combined hydraulic and VFA equalization were used. The figure clearly shows that anaerobic biological activity can be uncoupled from primary effluent loading by manipulating bioreactor loading patterns. These results demonstrated the possibility to regulate anaerobic VFA/M and phosphate release, as indicated by fluorescence, on a real time basis.

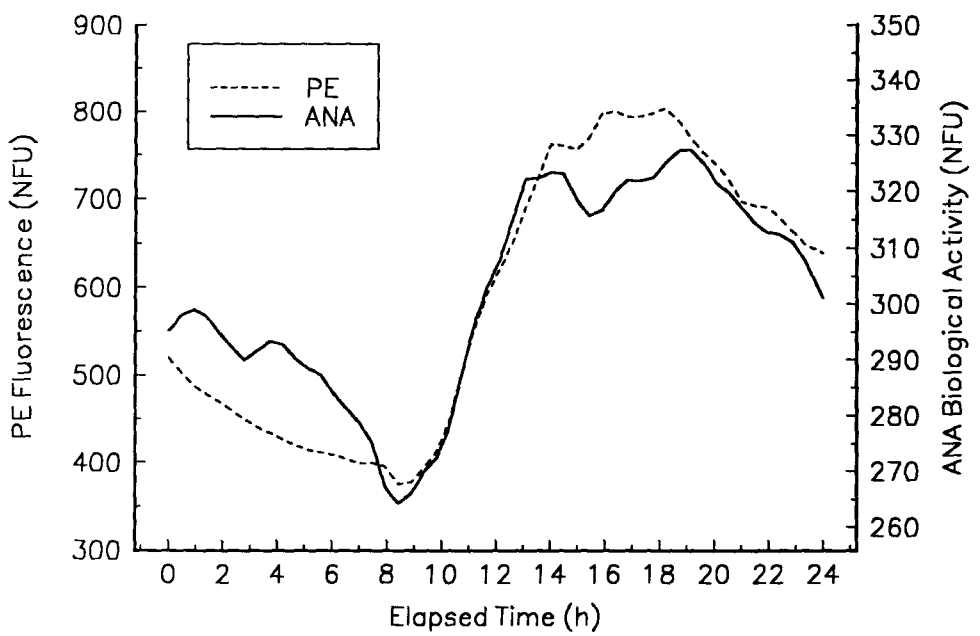

Figure 5.53. Fluorescence and biological activity profiles in the primary effluent and anaerobic basin during normal operation with hydraulic load equalization only.

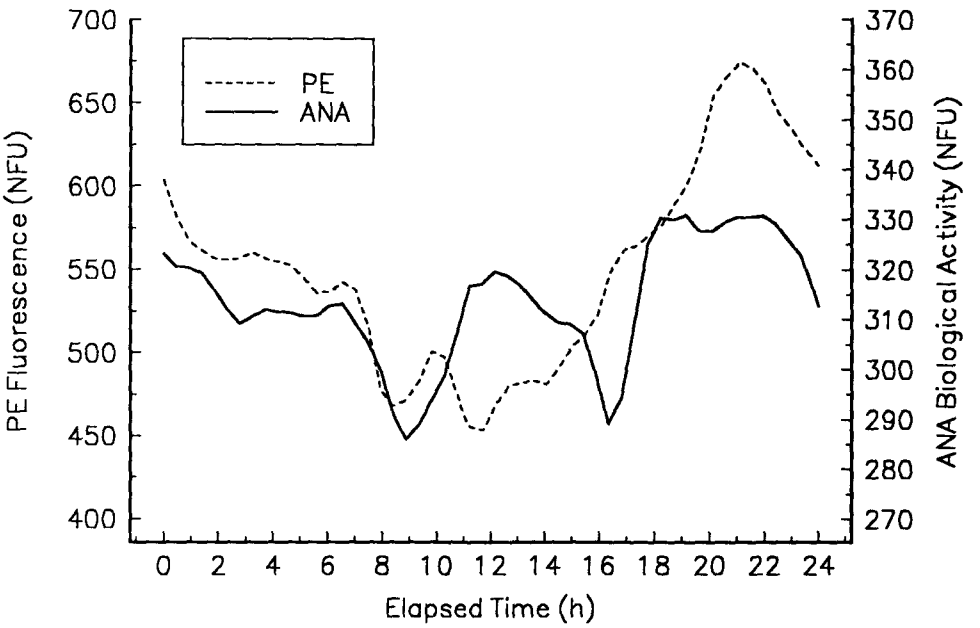

Figure 5.54. Fluorescence and biological activity profiles in the primary effluent and anaerobic basin during operation with both hydraulic and nutrient load regulation.

6. DISCUSSION

Discussion will review the major findings of the experimental results and their potential for process control and optimization strategies. Relationships between the numerous process parameters will be discussed, with focus on those observed in the primary effluent and anaerobic zone. Discussion will attempt to relate the observed patterns to biochemical strategies, but will focus primarily on evaluating on-line culture fluorescence monitoring as a tool for the development of process control and optimization strategies. Variations observed in the specific mathematical relationships between measured fluorescence and the various parameters between experiments will be addressed and the engineering potential for culture fluorescence monitoring considered. The experimental results and data from the preliminary full-scale control testing will be incorporated into proposed control and optimization strategies designed to improve aspects of process performance. The major limitations of the data will be discussed and considered in the proposal of further research.

6.1. Gross Trends in Biological Activity

Data demonstrated that gross trends in culture fluorescence could be correlated with changes in process mixed liquor suspended solids in the various process zones over time. The inverse relationship between process suspended solids levels and observed fluorescence is consistent with, and can be explained by the principles of fluorescence detection used by the probes. Placed directly in the reactor containing the mixture to be monitored, fluorescence measurement is accomplished by illuminating the mixture with the excitation light and measuring the fluorescence emission caused by the excitation light. The suspended solids content of the mixture limits the effective zone of detection by influencing both the penetration of excitation light into the sample as well as the transmission of fluorescence. In a sample free of suspended solids, the penetration of excitation light is limited only by the intensity of

the light source. In this condition, the excitation light emitted from the detecting probe penetrates the surrounding liquid freely to form a field of illumination and emission encompassing the volume of a cone, with the probe tip forming the apex of the cone. Theoretically, the resultant fluorescence can be detected throughout the entire volume defined by the excitation cone. The presence of suspended solids attenuates the penetration of excitation light, such that increasing levels of suspended solids decrease light penetration. As solids levels increase, both the penetration of the excitation light and transmission of emitted fluorescence are reduced, such that detection is only possible for a few millimeters from the end of the probe. Consequently, the region of detection defined by the excitation light penetration range and pattern changes from the larger volume of the cone shape to a smaller volume of a very thin cylinder as the levels of suspended solids increase.

Mixed liquor has a high intrinsic background fluorescence within the excitation and emission ranges employed by the fluorescence probe that is associated with soluble constituents of the mixed liquor. Increasing the suspended solids levels in the mixed liquor has two significant effects on measured fluorescence. First, increasing suspended solids changes the effective detection space by limiting the transmission of excitation light and emitted fluorescence. Secondly, the composition of materials measured within the detection space changes. Since the suspended solids typically have a much lower fluorescence (NADH-related fluorescence) than the soluble background fluorescent components, increasing solids levels affects the fluorescence measurements by decreasing the proportion of highly fluorescent soluble material measured within the detection space. Essentially, increased suspended solids reduces the contribution of the soluble fluorophore to the total measured fluorescence. This results in an inverse relationship between measured fluorescence and suspended solids levels. Increasing solids effectively reduces the volume of the highly fluorescent background material. The relationship defined between fluorescence levels and suspended solids levels represents

a physical effect on the fluorescence measurements rather than changes in the biological activity component of fluorescence detection.

The observed general relationship between fluorescence and mixed liquor suspended solids levels can be attributed to this physical effect. While it may be argued from this explanation that the dependence of fluorescence on suspended solids levels invalidates the usefulness of fluorescence monitoring as an indicator of changes in biological activity, subsequent experimentation clearly demonstrated that the smaller, transient changes in fluorescence could be correlated directly to changes in metabolism independently of suspended solids levels. Although trends in fluorescence levels could provide a method to track long term trends in process mixed liquor solids levels, they offer no real advantage over traditional methods for solids determination since they cannot differentiate between total and volatile solids or offer the accuracy of traditional methods.

6.2. Primary Effluent Fluorescence and VFA Concentration

Data gathered from both pilot and full scale facilities demonstrated consistent patterns between measured fluorescence and volatile fatty acid content in the primary effluent stream. With the exception of the patterns observed at the Penticton Advanced Wastewater Treatment Plant, measured fluorescence varied directly with VFA concentration. Because of the relatively high levels of volatile fatty acids in the primary effluent streams, it is evident that fermentation, either engineered or naturally occurring, is responsible for the high levels of VFA. The direct relationship observed between VFA content and fluorescence can be attributed to several possible theories.

First, the measured fluorescence and resulting pattern might result directly from the changes in the NADH levels of the organisms present in the primary effluent stream. Two

possibilities exist. The first possibility is that the measured fluorescence reflects changes in the steady state NADH levels of fermenting organisms present and responsible for the VFA in the primary effluent stream. Volatile fatty acids are produced from the incomplete oxidation of complex compounds found in sewage, like cellulose, carbohydrates, proteins, and lipids (Metcalf and Eddy Inc., 1991). The catabolism of these compounds is an oxidative process that results in the production and accumulation of reduced pyridine nucleotides, like NADH. Under the fermentative conditions required for volatile fatty acid production, NADH oxidation is limited by a number of factors including the availability of suitable terminal electron acceptors, slower enzymatic rates, and metabolic feedback control mechanisms. Since the rates of NADH oxidation are often slow in fermentative conditions, an imbalance between the rates of NADH production and oxidation might result in a steady state equilibrium with a higher proportion of NADH (Stanbury and Whitaker, 1984). Thus as the rates of fermentation increase, an increase in the steady state level of NADH is expected. Since higher rates of fermentation produce greater amounts of VFA, increases in VFA production should be accompanied by increases in the steady state level of NADH in the fermenting mixture. Assuming that the primary effluent stream contains the organisms responsible for fermentation and VFA production, and that the fluorescence detected reflects the NADH content of these organisms, then the general direct pattern between VFA concentration and fluorescence might be explained. Figure 6.1 illustrates this theory with generalized metabolic cycles.

The second possibility is that the high levels of VFA present cause a net increase in the steady state NADH pool of all of the organisms present in the primary effluent stream. Based on the generalized pathways outlined in Figure 2.3, rapid metabolism of the available VFA in the TCA and/or glyoxylate cycles might result in the rapid production of NADH. Since rates and/or mechanisms for NADH oxidation are comparatively limited, an imbalance between the rates of NADH production and oxidation might result in a net increase in the

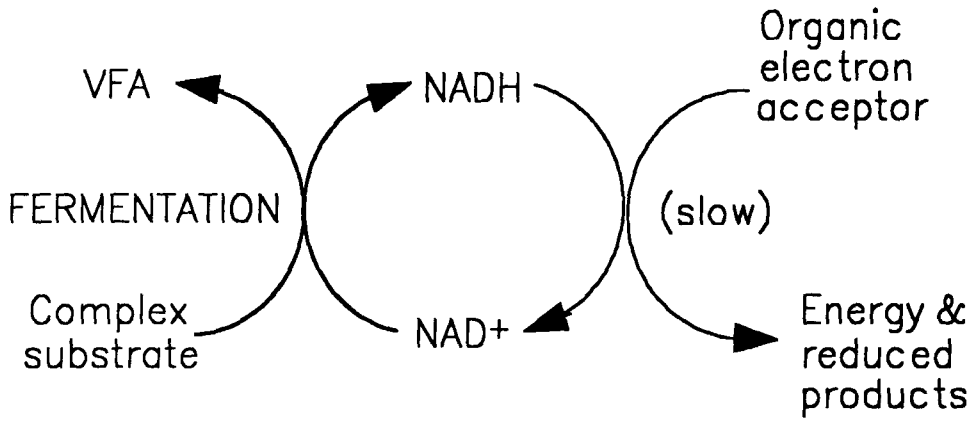

Figure 6.1. Generalized metabolic flows demonstrating the accumulation of NADH during the fermentative production of VFA. Since NADH oxidation is comparatively slower than NADH production by fermentative processes, a net NADH accumulation results.

steady state level of NADH and fluorescence observed. Thus, a direct relationship between VFA concentration and fluorescence might be expected. Figure 6.2 illustrates this theory by showing the generalized metabolic cycles discussed and the limitation of NADH oxidation that could result in the observed pattern.

Although these two possibilities provide explanations for the observed patterns, the level of suspended solids in the primary effluent streams studied (typically less than 100 mg MLSS/L) would be too low to account for the changes observed in the fluorescence signals. Consequently, the measured fluorescence does not solely represent a measure of the NADH level in the biomass.

A third possibility to explain the observed pattern is that the observed fluorescence could result from the naturally occurring fluorophores associated with wastewater. Many

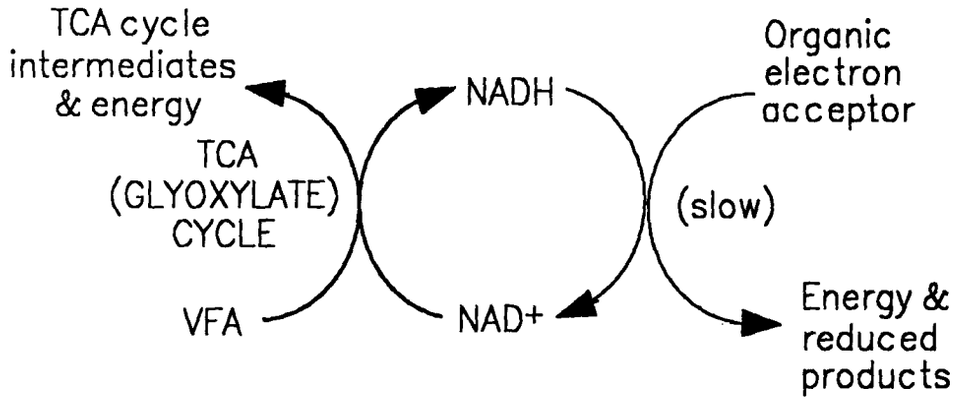

Figure 6.2. Generalized metabolic flows demonstrating the accumulation of NADH resulting from the rapid metabolism of VFA. VFA can be metabolized through the tricarboxylic acid cycle (TCA) and/or the glyoxylate cycle (Comeau et al., 1986), and rapidly produce NADH. Since NADH oxidation by organic substrates is comparatively lower that the production of NADH, a net NADH accumulation results.

constituents of wastewater originating in animal and human wastes (e.g. tryptophan, tyrosine, cofactors, etc.) and or storm water run-off (e.g. polycyclic aromatic hydrocarbons, petroleum products, etc.) are fluorescent compounds with excitation and emission spectra in the same range as NADH. Since wastewater components like human waste contain these fluorophores as well as other compounds that constitute the nutrient load, it might be expected that as the nutrient load of the wastewater increased, the fluorescence of the stream, too, would increase simply from increases in the amounts of these associated fluorophores (Figure 6.3). If it is assumed that a greater supply of substrate (nutrients) supports a greater production of VFA and, therefore, the VFA content increases proportionately to increases in the total nutrient load (Figure 6.3), a direct correlation between VFA content and measured fluorescence could be expected. This too could provide an explanation for the observed pattern.

The last theory indirectly relates the level and/or extent of fermentation activity with observed fluorescence. Findings by Maneshin et al. (1990a, 1990b, 1994 manuscript in

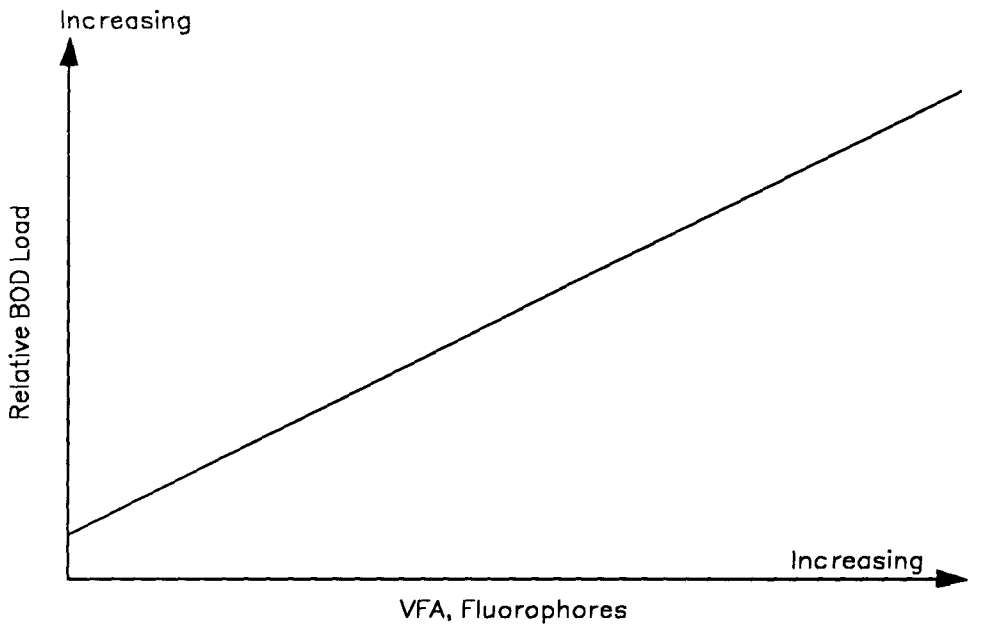

Figure 6.3. Conceptual proposed relationship between organic loading, VFA production and content, and levels of fluorophores in wastewater. The term "fluorophores" refers to fluorescent compounds other than NADH in this figure.

preparation; personal communication) might explain the observed relationship between measured fluorescence and VFA concentration in the primary effluent. Working with both pure (E. Coli K12, ATCC 10798) and mixed culture (sludge) systems, Maneshin showed a direct correlation between fermentation activity and the increase of specific fluorophores into solution. Spectral analyses of the cell free medium identified that folic acid derivatives comprised the majority of the soluble fluorophores responsible for the measured fluorescence. Maneshin found that increases in fermentation activity were accompanied by increases in the presence of these folic acid derivatives in solution. Maneshin suggested that folic acid was released into solution in reduced form as tetrahydrofolic acid. In solution, tetrahydrofolic acid is oxidized to dihydrofolic acid and folic acid which fluoresce within the excitation and emission ranges of NADH. Interestingly, it has been widely recognized that tetrahydrofolic acid possesses a coenzyme activity that has a generalized function in a number of biochemical reactions that may be similar to NADH.

Based on the information provided by Maneshin, it follows that increases in fermentation activity would be characterized by increases in the bulk solution concentrations of both VFA and tetrahydrofolic acid (Figure 6.4). Assuming that the released tetrahydrofolic acid is converted to other fluorescent folic acid derivatives, the fluorescent derivatives would contribute to the direct relationship observed between measured fluorescence and VFA content of the primary effluent streams. Because the folic acid derivatives are soluble components, increases in measured fluorescence could be expected in the absence of any significant biological solids in the stream. Further research would be required to verify this theory. It would be necessary to determine 1) the amount of folic acid derivatives present and their contribution and/or significance to the overall fluorescence signal, 2) whether tetrahydrofolic acid release and conversion is indeed proportional to fermentation activity and VFA production in this system, and 3) to evaluate the stability of these fluorescent compounds in the sewage.

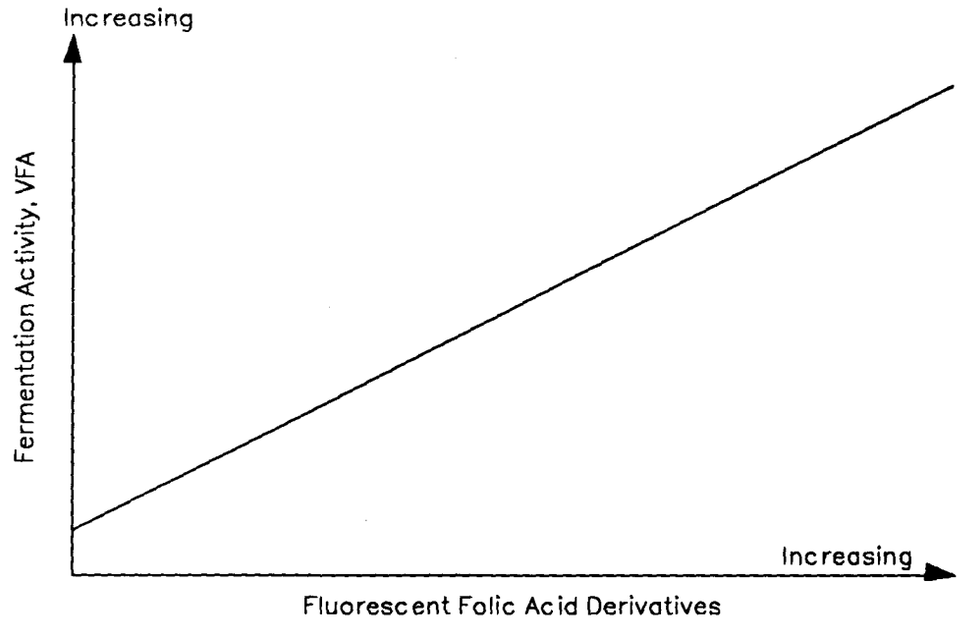

Figure 6.4. Conceptual proposed relationship between fermentation activity, VFA production and content, and fluorescent folic acid derivatives in wastewater. The fluorescent folic acid derivatives referred to in the figure are dihydrofolic and folic acids, both derived from the non-fluorescent tetrahydrofolic acid.

The soluble fluorophore theory provides a viable explanation for the patterns observed in the primary effluent where there is an insignificant amount of biological solids that contribute to the total measured fluorescence. In cases where a significant level of biological solids is present (>1000 mg MLSS/L), the physical and optical conditions of fluorescence detection become significantly altered and limit the usefulness of this explanation for supporting observed patterns, as will be discussed later.

While many explanations can be provided to describe the patterns observed at these plants, none provides a satisfactory explanation for the inverse pattern observed at the Penticton plant. At the Penticton Advanced Wastewater Treatment Plant, measured fluorescence varied inversely with VFA concentrations in the primary effluent and "mix" streams. There are significant differences in the characteristics of the primary effluent stream at the Penticton plant from the others studied. The first difference is the process flow configuration (Figures 4.1 through 4.4). Complete mix side-stream fermenters are employed at the Penticton plant where the well mixed fermenter contents are recycled back to the primary clarifiers. This operating configuration results in the introduction of a significantly larger amount of fermenter solids into the primary clarifiers and primary effluent (150-200 mg MLSS/L) than at the other plants (<100 mg MLSS/L). Although the Penticton primary effluent stream contained higher suspended solids levels, it is possible that the changes in measured fluorescence are not reflective solely of changes in the net cellular NADH level.

Instead, it is likely that the observed fluorescence was related to fluorescence caused by some other fluorophore. If the assumption that changes in primary effluent fluorescence reflect changes in the levels of soluble fluorescent compounds, like the folic acid derivatives found to be a predominant component in other sewage systems, then it might be possible to provide one explanation for the pattern observed at Penticton.

At Penticton, the well mixed contents of the fermenters are discharged directly to the primary clarifiers. Assuming that (1) VFA production in the fermenters is relatively constant, (2) the level of solids in the fermenter is also relatively constant, and (3) that the VFA measured in the primary effluent stream originates from the fermenters (i.e. insignificant contribution of VFA from fermentation activity in the primary clarifiers or the wastewater collection system), then it would be reasonable to assume that higher concentrations of primary effluent VFA occur when influent wastewater flow is lower and dilution of primary clarifier contents was lower. This would result not only in a higher measured VFA concentration, but could also result in more highly reduced conditions in the primary clarifiers and primary effluent stream. Because the conditions maintained in the fermenters are highly reducing, a higher proportion of fermenter contents in the primary clarifiers could result in more highly reducing conditions in the primary clarifiers and primary effluent stream. If it is assumed that tetrahydrofolic acid is released to solution in the fermenters at a rate proportional to fermentation activity and VFA production, and is carried into the primary clarifiers, then more highly reducing conditions in the primary clarifiers might result in a lower proportion of the non-fluorescent tetrahydrofolic acid being oxidized to other fluorescent folic acid derivatives. As a result, an inverse relationship might be expected between measured fluorescence and VFA concentration in the primary effluent stream.

Alternative explanations to explain the unique patterns observed at Penticton include the possibility that the increased solids loading from the fermenter somehow changed the measured fluorescence by changing the net predominant biological activity, net $NADH/NAD^+$ equilibrium, and/or possibly the net composition and characteristics of soluble fluorophores. Clearly, further biochemical and chemical studies, beyond the scope of this research, are required to more confidently determine the specific factors responsible for the observed patterns.

Although the absolute reasons for the observed patterns are uncertain, it is ultimately the consistency of the patterns that is the important feature for determining their usefulness for process monitoring and control. Despite the observation of two different patterns, the consistencies of the general patterns between measured fluorescence and VFA concentrations demonstrated the potential to predict qualitative changes in VFA concentrations in the primary effluent on a real time basis using fluorescence measurements. Recognition of the type of pattern at a particular site should allow general changes in primary effluent VFA content to be predicted from the on-line fluorescence data. Specific quantitative prediction, however, was limited by the variability observed in the specific mathematical relationships between fluorescence and VFA concentration between experiments.

Variability in biological activity from changes in microbial population dynamics, changes in the metabolic capabilities of the biomass, or changes in chemical character of the influent could all contribute to these mathematical inconsistencies. Considering the sensitivity and the complex population dynamics of these systems, it is not unreasonable to expect that biological activity is not constant from day to day. Variability of biological activity can effect changes in observed fluorescence related directly to changes in NADH levels, or related to changes in the release and characteristics of other biogenic fluorophores. Daily variation in the composition of background fluorophores could affect both the general levels of fluorescence observed, and the specific response of the fluorescent probe to changes in the biological components of the signal.

Another factor that may have contributed to the variations observed in the specific mathematical definitions of the relationships between measured fluorescence and VFA concentration at the FGR-SGR pilot plant was the problem of solids accumulation around the detecting probe. At the FGR-SGR pilot plant, primary effluent fluorescence was measured by placing the detecting probe in a small diameter flow cell directly in the primary effluent

line. The low flow velocity and small diameter of this flow cell allowed solids accumulation near the detection window of the probe that could have changed the optical properties of the fluorescence measurements. Although the flow cell was cleaned often (typically once per week), solids did accumulate and could have contributed to some of the variability in specific fluorescent responses and patterns observed.

These factors are likely the principal ones limiting the ability to quantitatively predict VFA levels between experiments. The magnitude of the effect of these variables may, however, be dependent on the scale of the process studied. On a small scale, like that at the pilot plant, the effects of background fluorescence variability and changes in influent character might be more pronounced than at a larger facility where the larger volumes would provide some dilution or dampening effects to either minimize the extent of variability or increase the time required to significantly alter the background components. This may provide some explanation of why the data from the full scale Kelowna plant allowed good quantitative prediction of primary effluent VFA concentrations using fluorescence readings over a three day period. Additionally, the higher flow velocities at the full scale plants significantly reduced the problem of solids accumulation around the detector which could also improve long term consistency. Because of the difference in fluorescence levels between experiments, conclusions regarding the consistency of the mathematical definitions cannot be made from the Oaks, PA data. Long term studies are required at full scale plants to determine the extent that changes in biological activity and background fluorescence have on limiting the quantitative predictive capabilities of fluorescence measurements at large facilities.

6.2.1. General conclusions

The data demonstrated a relatively consistent and direct relationship between measured fluorescence and VFA content in the primary effluent streams studied, with the exception of

Penticton where an inverse relationship was observed. Although several possibilities were presented to explain the biochemical and/or chemical basis for the patterns, further research is required to verify the theories proposed to describe the observed relationships. Despite the existence of two different patterns between changes in measured fluorescence and VFA concentration and the lack of clear explanations for the patterns, the consistency of the general patterns demonstrated that on-line fluorescence measurements are useful for determining trends in the VFA concentrations of the primary effluent streams on a continuous, real time basis. This ability to continuously monitor trends and changes in a key nutrient fraction of the wastewater is a useful capability that directly accomplishes one of the needs identified in a report assessing the research needs for nutrient removal from wastewater (Reid Crowther and Partners Ltd., 1993).

6.3. Anaerobic Zone Biological Activity Patterns

A number of consistent patterns were observed in the anaerobic zones of the plants studied. Consistent relationships between specific VFA consumption, mass phosphate release, and PHA storage were observed. As expected from biochemical models (Figures 2.2, 2.3; Sections 6.3.1 and 6.3.2) and widely reported observations (Section 2.1), both phosphate release and PHA storage were found to be directly correlated with specific VFA consumption. Consistent, but different, relationships between measured fluorescence and these parameters were also observed that seemed to be related to process configurations.

6.3.1. Anaerobic VFA/M and mass phosphate release

At all of the plants where anaerobic zone phosphate release and PHA storage were studied, they were both found to be directly affected by changes in specific VFA consumption in the anaerobic zone. These observations are consistent with leading biochemical models

(Comeau et al., 1986; Wentzel et al., 1986; Mino et al., 1987) that predict direct relationships between VFA utilization, phosphate release, and PHA storage. The direct relationship between anaerobic zone mass phosphate release and specific VFA consumption can be explained by two possibilities.

Anaerobic zone phosphate release may result from cellular mechanisms employed to re-establish a suitable pH gradient across the cell membrane that was disrupted from VFA uptake. Because some simple acids can be transported neutrally across the membrane (Kaback, 1976), uptake and dissociation of VFA could change the pH gradient across the membrane and decrease the proton motive force (*pmf*) that is maintained by the cells within an optimum range (Jones, 1982). Proton expulsion to re-establish the pH gradient and increase the *pmf* could be accomplished by coupling the breakdown of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and proton expulsion by the BF_1 component of the ATPase complex (Figure 6.5; Harold, 1977, 1978; Jones, 1982). As proton expulsion continued and the cellular pool of ortho-phosphate increased from the breakdown of ATP, some phosphate would be released into solution to maintain some cellular equilibrium resulting in increased anaerobic zone soluble phosphate levels. Polyphosphate stores could be used to anaerobically synthesize the ATP (Kornberg, 1957, Kulaev, 1975) needed to provide the energy for proton expulsion and cellular maintenance. Comeau (1989) proposed the possibility of an enzyme similar to ATPase that could couple the hydrolysis of polyphosphate stores with the required proton expulsion.

A second possibility relating anaerobic zone VFA consumption with phosphate release involves mechanisms for the activation of acetic and propionic acids for the synthesis of PHB and PHV, respectively. Both the Comeau/Wentzel (Comeau et al., 1986; Wentzel et al., 1986) and Mino (Mino et al., 1987) models postulate that increases in soluble phosphate can result from the expulsion of phosphate used for the activation of acetate to acetyl-CoA

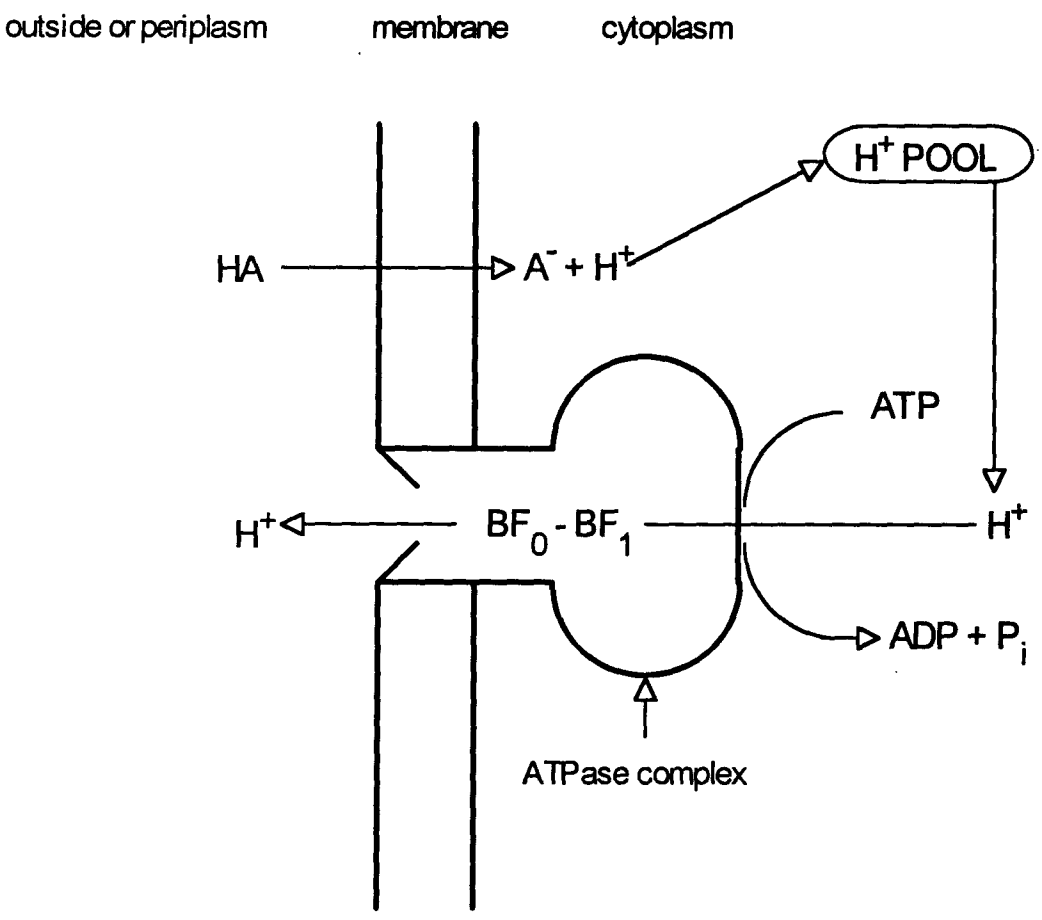

Figure 6.5. Proton expulsion by the ATPase complex.

within the cell. This postulation has been supported by the observations of Doi et al. (1987, 1989). Within the cell, acetate can become phosphorylated. The phosphoryl group of the resulting acetyl-phosphate then becomes exchanged with an CoA to form acetyl-CoA. The phosphate that results from the activation of acetate to acetyl-CoA accumulates in the cell and is released according to some cellular concentration gradient. Because of the similarities in the biosynthetic pathways of PHB and PHV, activation of propionate to propionyl-CoA could also use similar mechanisms that result in the accumulation and expulsion of phosphate. Activation of these acids requires energy that can come from polyphosphate derived ATP or possibly from polyphosphate directly.

The average daily molar ratios of phosphate released/VFA consumed ranged from 0.67 to 2.21 with an overall average of 1.80 in the FGR-SGR studies, compared with other studies that reported ratios ranging from 0.24 to 3.40 (Arvin, 1985; Comeau, 1989; Fukase et al., 1982; Jones et al., 1985; Lotter, 1985; Matsuo et al., 1984; Mino et al., 1987; Potgieter et al., 1983; Somiya et al., 1988; de Vries et al., 1985; Wentzel et al., 1985; Wentzel et al., 1989). The range of reported ratios in the pilot plant studies and the diversity of ratios reported by the other researchers reflects the biological variability both between and within the various systems. Several factors might contribute to the variability in the reported ratios. The variability might result from population composition differences between the various studies or from differences in the biological capabilities of the populations. The different ratios could also reflect differences in the composition and character of the polyphosphate pools. Previous studies have demonstrated that intracellular polyphosphates can exist in several different forms that possess different chemical characteristics affecting both their stability and mobility (Clark et al., 1986; Fuhs and Chen, 1975; Mah, 1991). The differences in the observed and reported ratios might reflect the phosphate release dynamics of different polyphosphate types.

Finally, differences in the reported ratios might also result from the presence of carbon compounds that are produced and rapidly consumed in the anaerobic zone, and hence elude detection. Under the anaerobic conditions in the zone, fermentation of available complex substrates could produce VFA or other usable carbon substrates. If the rates of substrate consumption in the zone were equal or faster than the rate of substrate supply either from anaerobic zone fermentation or from exogenous supplies, then it would be possible for a carbon substrate source and flux to exist that eluded detection and quantification by the limited carbon substrate analysis (VFA analysis) employed in these studies. Since VFA was usually undetectable in the anaerobic zone, and the VFA analysis reports residual, net steady-state VFA concentrations, it indicates that the demand for carbon substrates was usually greater or equal to the supply. Therefore, it is possible that VFA or other usable carbon substrates are produced and utilized in the anaerobic zone that elude detection. Therefore, it would be possible to measure increases in anaerobic zone soluble phosphate levels without actually measuring the total VFA or similar carbon substrates utilized in the process. Depending on the extent of this undetected carbon flux, the calculated ratios of phosphate released per VFA consumed could be highly variable. The possibility of anaerobic zone VFA or carbon substrate production and consumption is also supported by the observation that the amount of carbon stored as PHA was consistently greater than the input of carbon actually measured as VFA.

Despite the variability in the specific average ratios of phosphate released per VFA consumed (VFA/M), the general trends between VFA/M and mass phosphate release were consistent. From the association between the two variables demonstrated in these studies and supported by previous work, the ability to relate general changes in mass phosphate release with general changes in specific VFA consumption should be evident. Thus, with the ability to rapidly monitor one of these parameters (e.g. VFA consumption), information

could be rapidly gained about the other. This ability would be useful for developing process control and optimization strategies.

6.3.2. Anaerobic VFA/M and PHA storage

Since biochemical models and widely reported observations demonstrated a correlation between PHA synthesis and storage and VFA consumption (Figures 2.2, 2.3; Section 2.2), the general direct trend observed between specific VFA consumption and anaerobic PHA storage was expected. At those plants where both PHA and VFA measurements were taken, the observed patterns showed a general direct relationship between the two. Based on the biochemical models, it is predicted that the formation of PHA from VFA serves two useful purposes. The first is the oxidation of NADH in an environment where mechanisms for NADH oxidation are limited and slow. The second purpose is the production of beneficial intracellular carbon stores that affords the cells a selective advantage.

Under all conditions, cells need to maintain a certain level of metabolism for survival. Maintaining this metabolism requires the ability to provide a constant and adequate supply of reducing and oxidizing capacity in the forms of NAD^+ and NADH, while preventing the accumulation of inhibitory levels of either. In order to maintain this balanced condition, cells rely on systems of checks and balances. For example if the cellular NADH pool becomes too large relative to the NAD^+ pool, feedback mechanisms will limit NADH-producing metabolism to prevent its further production and accumulation (Lehninger, 1982). This feedback inhibition of metabolism, although providing a means to maintain tolerable levels of NAD^+ and NADH, can affect cellular viability and survival.

In the anaerobic zone where there is a constant supply of preferred nutrients, VFA, the cells respond by rapidly metabolizing them to extract the energy and materials required

for survival and growth. The rapid metabolism of the VFA results in the rapid production of NADH as a byproduct (Comeau et al., 1986; Section 6.2; Figure 6.2). Because mechanisms for rapidly oxidizing the produced NADH are limited in the anaerobic condition, NADH accumulates. In order to prevent accumulation of NADH to inhibitory levels, the cell must find alternative methods for NADH oxidation to maintain tolerable NADH levels. The PHA synthesis reactions may provide the required mechanisms.

Polyhydroxyalkanoates (PHA), such as polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV), are common microbial storage products (Braunegg et al., 1978). The metabolic pathways for PHB and PHV have been established in various organisms (Doi et al., 1987,1989; Fukui et al., 1976, 1989; Haywood et al., 1988; Oeding and Schlegel, 1973; Peoples and Sinskey, 1989a; Peoples and Sinskey, 1989b; Slater et al., 1988), and a representative diagram outlining PHB metabolism is presented in Figure 6.6. Essentially, PHB and PHV synthesis involves the activation of acetate and propionate to acetyl-CoA and propionyl-CoA, respectively, followed by condensation and reduction of these precursors into either PHB or PHV. Both the relative levels of NADH and acetyl-CoA have been reported to control the activity of various PHA synthesizing enzymes (Jackson and Dawes, 1976; Lotter and Dubery, 1989; Peoples and Sinskey, 1989b), and Wentzel et al. (1986) proposed that PHA synthesis is triggered by a high cellular NADH/NAD^+ ratio. PHA synthesis is believed to be one mechanism used to rapidly readjust the relative proportions of NADH and NAD^+ to prevent feedback inhibition of metabolism by NADH accumulation. Essentially, PHB and PHV assume the roles of alternative electron acceptors reoxidizing NADH to NAD^+, while providing beneficial carbon reserves (Jackson and Dawes, 1976; Page and Knosp, 1989). Ultimately, PHA synthesis allows the cells to regenerate essential NAD^+ through a fermentative mechanism that involves the condensation and reduction of simple available carbon compounds. In response to the environment rich in VFA's and the need to

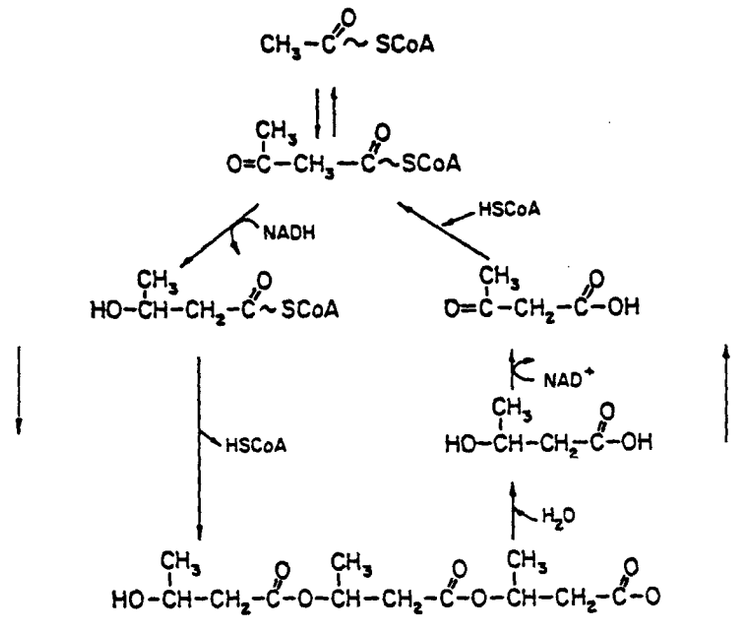

Figure 6.6. Schematic of poly-β-hydroxybutyrate metabolic pathways. (Adapted from Stanier et al., 1976).

oxidize NADH, the cells may undertake PHA synthesis and storage. Thus, as VFA availability and consumption increases, PHA synthesis increases.

Analysis of the experimental data showed a general, direct relationship between VFA consumption and PHA storage. Based on the biochemical link between the two, strong mathematical correlations were expected. Correlations between the two, however, were highly variable and weak. Further analysis revealed a second anomaly that may account for the lack of good correlation. It was found that the carbon yield, the amount of net carbon produced as PHB and PHV per measured carbon consumed as VFA, consistently and significantly exceeded 1.0. This excess suggested that a flux of unmeasured carbon is available for PHA synthesis and storage. Both Mah (1991) and Comeau (1989) reported that anaerobic PHA accumulation could be observed in the absence of any detectable VFA, supporting the theory that some undetected carbon source was available and rapidly consumed for PHA

synthesis and storage. If these carbon substrates were consumed as quickly as they were produced, they would elude detection by the methods used in these studies. Volatile acids produced by fermentative organisms in the anaerobic basin may provide the additional nutrients for the PHA accumulating organisms. Alternatively, the nutrients for PHA storage may be produced by the PHA accumulating organisms themselves. Wallen and Rohwedder (1974) and Schubert et al. (1988) reported that the acids that comprise PHA can be produced from complex higher fatty acids, and Osborn et al. (1986) observed rapid uptake of some long chain fatty acids in the anaerobic basin of a full-scale biological nutrient removal process. Furthermore, Peoples and Sinskey (1989b) and Schubert et al. (1988) have demonstrated that the enzymes responsible for PHA metabolism can also function in fatty acid degradative pathways.

The poor mathematical relationships between VFA consumption and PHA storage and the unusually high calculated yields might be attributed to the production and consumption of an undetected carbon. A more thorough analysis and characterization of the carbon inventory and the diversity and capabilities of the microbial biomass may provide more direct evidence of this phenomenon. If the dynamics of this nutrient pool could be evaluated, recalculation of the yields might produce more realistic values and provide a better correlation between VFA consumption and PHA storage

6.3.3. FGR-SGR pilot plant

The purpose of the experiments conducted at the FGR-SGR pilot plant was to evaluate the usefulness of fluorescence monitoring as a tool for development of on-line control and optimization strategies by searching for consistent patterns between culture fluorescence and key process variables under relatively controlled conditions. Limited attempts were made to establish the metabolic basis for the observed patterns to improve the confidence in

the on-line data. Based on leading biochemical models (Comeau et al., 1986; Wentzel et al., 1986; Mino et al., 1987), several predictions regarding the interrelationships of volatile fatty acid consumption, phosphate release, PHA storage, and net NADH/NAD^+ equilibria were made and tested.

Based on these models and widely reported observations, direct relationships between anaerobic VFA consumption and mass phosphate release, and anaerobic VFA consumption and PHA storage were expected. Results demonstrated several consistent patterns between anaerobic zone fluorescence and these variables that could be correlated to specific changes in biological activity and that could be useful in formulating real time process control and optimization strategies.

6.3.3.1. FGR-SGR anaerobic zone fluorescence

Analysis of the data demonstrated the a good patterns between anaerobic zone culture fluorescence and specific anaerobic VFA consumption (VFA/M). The FGR-SGR pilot plant studies showed that measured fluorescence consistently varied inversely with anaerobic zone specific volatile fatty acid consumption (VFA/M). Because of the population and metabolic diversity of the sludge biomass, precise biological definition of the relationship between VFA/M and measured fluorescence in this complex environment of the sludge is impossible. Several mechanisms, however, may be used to explain the basis and consistency of the observed pattern. These mechanisms include strategies that directly relate the observed changes in measured fluorescence with specific metabolic mechanisms affecting cellular NADH and NADH-related fluorescence, as well as strategies that relate the observed changes in fluorescence to changes in soluble fluorophores, like those presented during discussion of primary effluent fluorescence patterns. Several aspects of these mechanisms and their validity

will be considered in the following discussion. The first of the mechanisms explaining the observed pattern is as follows.

It is clear that at least three major metabolic activities are on-going in the anaerobic basin. First, the production and rapid consumption of usable nutrients by the biomass. Determination of the amount of production or "mobilization" of these nutrients is complicated by their rapid consumption or utilization. Consequently, these nutrients are essentially undetectable. These nutrients are probably derived from the catabolism of available complex compounds like fatty acids, or from other fermentation activity. The breakdown of complex substrates provides "simpler", basic nutrients and cellular building blocks for a variety of uses. These fermentation processes tend to produce NADH and increase the relative proportion of cellular NADH to NAD^+. Second, the requirement for a basal level of metabolism, primarily involving the resynthesis of labile compounds, is common to all organisms. Many of these biosynthetic reactions require reducing power in the form of NADH. Therefore, these reactions tend to consume NADH and cause a drain on the cellular NADH pool. Third, PHA synthesis and storage occur. The specific mechanisms involved with PHA synthesis are predicted to provide a mechanism for NADH/NAD^+ cycling under anaerobic conditions. The PHA synthesis reactions tend to consume NADH and should decrease the relative proportion of cellular NADH to NAD^+ (Comeau et al., 1986; Stanier et al., 1976; Wentzel et al., 1986). These reactions are depicted in simplified form in Figure 6.7 and labeled reactions 1, 2, and 3, respectively. In these reactions, "substrates" may include compounds like fatty acids, carbohydrates, cellulose, and VFA. "Cell building blocks" represents a diversity of compounds that may include simple carbon compounds like VFA.

The biomass requires a certain level of substrates and metabolism for cellular maintenance. When higher levels of exogenous VFA's are available, such as those introduced into the anaerobic zone by the primary effluent, the cells do not have to rely as heavily on the

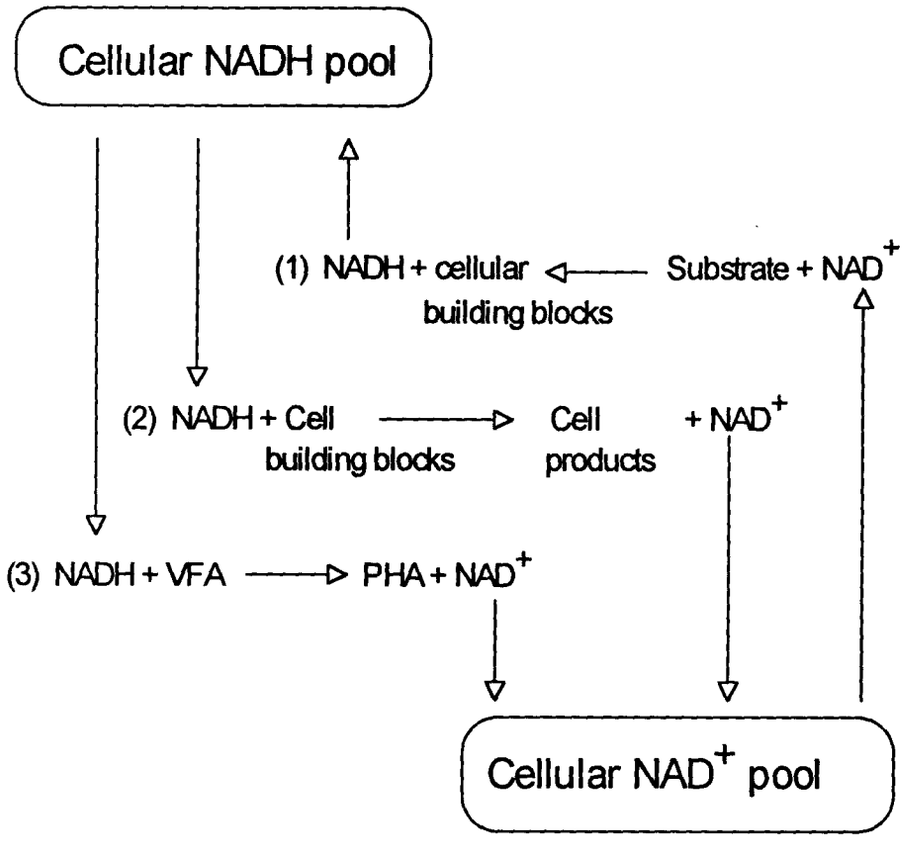

Figure 6.7. Simplified reactions involving NADH/NAD$^+$. Reaction (1) general catabolism and mobilization of nutrients; Reaction (2) general anabolism and biosynthetic reactions; Reaction (3) PHA synthesis reactions. (The reactions shown are generalized, simplified reactions, and as such, not all of the details are shown).

fermentative conversion of complex materials to supply the substrates required to support the basal level of metabolism required for survival. The readily available VFA's can be used for a variety of purposes. It can be assumed that some of the available VFA provide the required substrates for the resynthesis of labile compounds. Once these requirements are met, any excess VFA can be used for other purposes such as PHA storage which provide required $NADH/NAD^+$ cycling and beneficial carbon reserves. In response to their environment with an elevated level of NADH, resulting from the anaerobic conditions, and an excess of readily available VFA, the cells might undertake PHA synthesis. In fact, we observe higher levels of PHA when higher levels of VFA are available. If the exogenous substrates available are sufficient to meet the basal requirements of the cell, then the need to mobilize other more complex nutrients is decreased. In terms of the $NADH/NAD^+$ equilibrium, a net decrease in the cellular NADH pool might be expected. The decreased need to mobilize fermentative mechanisms to supply required substrates would decrease the production of NADH from the fermentative or catabolic reactions for generating cellular "building blocks" (Reaction 1, Figure 6.7), while the increased synthesis of PHA would consume NADH (Reaction 3, Figure 6.7). The excess supply of VFA, which can serve as a source of carbon for biosynthesis, might result in increased anabolism and NADH consumption (Reaction 2, Figure 6.7). The combination of these events may result in a decreased net NADH pool and lower relative fluorescence. The net activity of the three reactions in conditions of excess exogenous VFA is diagrammed in Figure 6.8. The relative thickness of the arrows indicates the relative impact of the reaction on the cellular pools.

In the situation where there is a relatively lower supply of exogenous VFA, which may not meet the basal requirements, the need to mobilize other nutrient sources and metabolic mechanisms to supply sufficient substrates will be increased. Consequently, the amount of substrate available for PHA synthesis will be reduced. Although there will be an excess of NADH from a combination of the anaerobic conditions and substrate mobilization, the limited

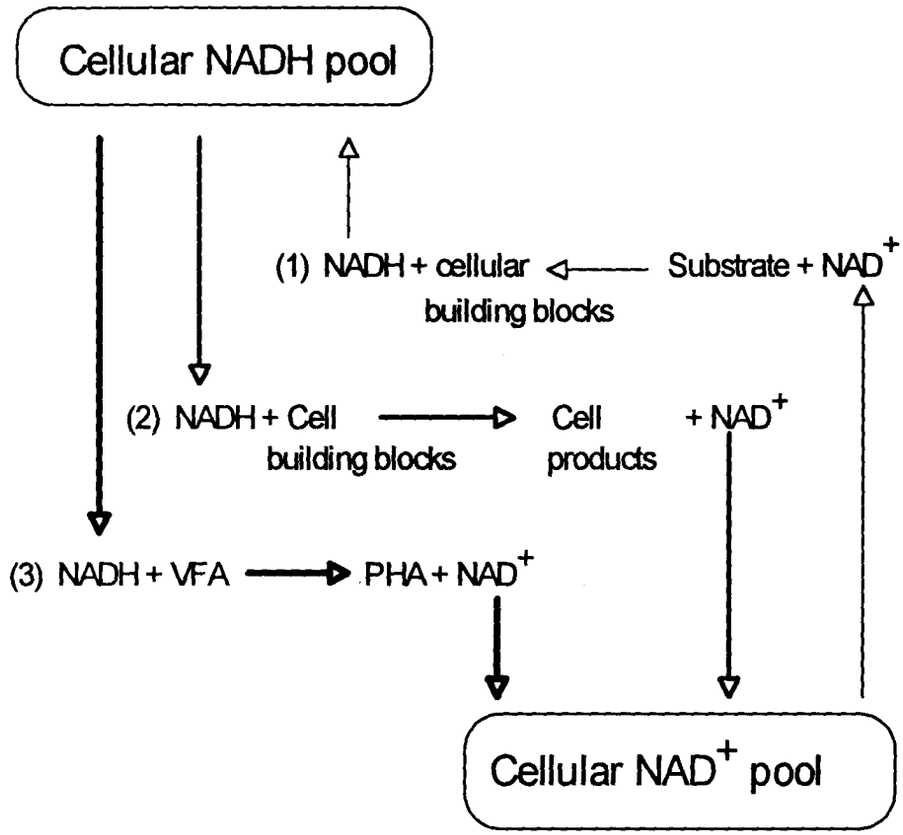

Figure 6.8. Simplified, generalized reactions involving NADH/NAD$^+$ in conditions of high exogenous VFA. Reaction (1) general catabolism and mobilization of nutrients; Reaction (2) general anabolism and biosynthetic reactions; Reaction (3) PHA synthesis reactions. The relative thickness of the arrows indicates the relative impact of the reaction on the cellular NADH/NAD$^+$ pools. (The reactions shown are generalized, simplified reactions, and as such, not all of the details are shown).

availability of VFA for PHA synthesis will result in reduced PHA synthesis. The data demonstrated that lower levels of PHA storage occurred when lower levels of VFA were present. Under these conditions the cells still maintained some PHA synthesis, however, probably to provide necessary NADH/NAD^+ cycling.

Although higher levels of PHA were usually associated with higher levels of measured VFA consumption, analysis of carbon yield, measured as the carbon associated with produced PHA/carbon consumed as VFA, showed two important features. First, the calculated yield factor was consistently and significantly greater than 1.0, as discussed previously (Section 5.2.3.2). Second, the yield factor was highly variable. Analyses revealed that when the measured amount of VFA consumption decreased, the overall level of PHA decreased, but the calculated yield factor increased. Since the yield factor was always greater than one, indicating that more carbon was being produced as PHA than was measured as input carbon from VFA analyses, it implied that a significant pool of carbon substrates, that eluded detection, was being used for PHA synthesis. This further suggests that when supplies of readily available VFA were lower, mechanisms for mobilizing other carbon sources for PHA synthesis and other metabolism were more active.

In terms of the NADH/NAD^+ equilibrium under these conditions, a net increase in the cellular NADH pool might be expected. The increased activity of mechanisms for providing required substrates might result in increased NADH production (Reaction 1, Figure 6.7). Decreased PHA synthesis, due to the lower overall availability of excess VFA, would result in decreased NADH consumption by PHA synthesizing reactions (Reaction 3, Figure 6.7). Similarly, the activity of biosynthetic reactions (Reaction 2, Figure 6.7) might be relatively lower due to the decreased supply of VFA which serve as cellular building blocks. Together, these might result in an increase in the steady state cellular NADH pool and higher relative measured fluorescence. The net activity of the three reactions in conditions of low exogenous

VFA are diagrammed in Figure 6.9. The relative thickness of the arrows indicates the relative impact of the reaction on the cellular pools.

This may provide one metabolic explanation for the observed pattern that showed 1) relatively lower fluorescence levels at higher levels of measured VFA loading, VFA consumption, and PHA; and 2) relatively higher fluorescence when measured VFA loading, VFA consumption, and PHA were lower. The "critical" exogenous VFA level where transition between metabolic patterns that cause a net NADH increase or a net NADH decrease during PHA synthesis could not be determined. Without precise quantification of the "undetected" carbon flux, it is impossible to determine the "critical" point where the exogenous VFA supply is large enough to minimize the fermentative mobilization of carbon substrates and cause the transition in the proposed metabolic patterns between those that result in higher or lower steady state levels of NADH and fluorescence.

A second possible explanation to account for the inverse relationship observed between measured fluorescence and VFA consumption relates changes in fermentation activity and the release of tetrahydrofolic acid with nutrient loading and VFA consumption. In Section 6.2, information was presented that discussed the predominance of folic acid derivatives in fermentation processes and a possible correlation between the release of tetrahydrofolic acid to the bulk solution and fermentation activity. Recalling that tetrahydrofolic acid becomes oxidized to other folic acid derivatives, which fluoresces within the same excitation and emission spectra as NADH, it is possible that fermentation activity in the anaerobic zone results in both the production of VFA and the release of tetrahydrofolic acid and increase of soluble fluorophores. If it is further assumed that the biomass in the anaerobic zone requires a particular, but unknown level of carbon substrates like VFA then when the level of readily available and/or preferred nutrients like VFA was higher, less fermentation of other substrates would be needed to satisfy the cells nutrient requirements. Additionally, the presence of high

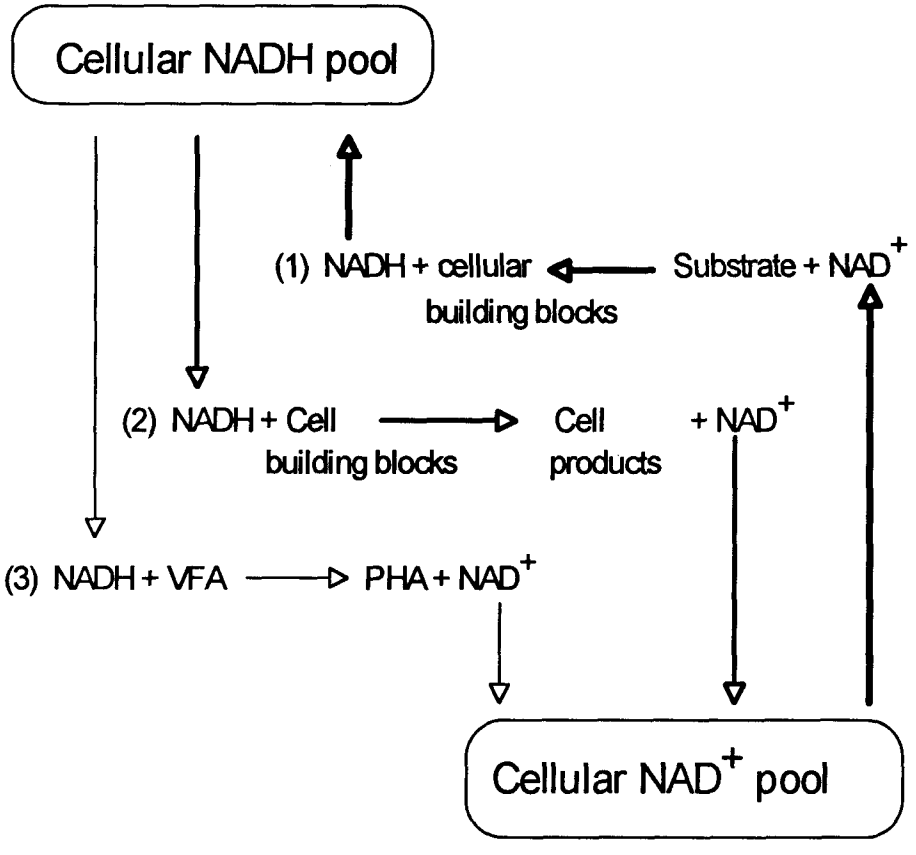

Figure 6.9. Simplified, generalized reactions involving NADH/NAD^+ in conditions of low exogenous VFA. Reaction (1) general catabolism and mobilization of nutrients; Reaction (2) general anabolism and biosynthetic reactions; Reaction (3) PHA synthesis reactions. The relative thickness of the arrows indicates the relative impact of the reaction on the cellular NADH/NAD^+ pools. (The reactions shown are generalized, simplified reactions, and as such, not all of the details are shown).

levels of available nutrients might actually inhibit certain fermentation activity in the anaerobic zone. For example, high levels of soluble VFA could prevent fermentation activity that produces additional VFA or VFA-derived products (Lehninger, 1982). This lower level of fermentation activity would result in a relatively lower release of tetrahydrofolic acid, and relatively lower fluorescent folic acid derivatives in solution than conditions with higher fermentation activity and higher levels of tetrahydrofolic and folic acid in the anaerobic zone. On the other hand, when supplies of VFA were insufficient to meet the required needs, fermentation of other substrates would be required to satisfy the demand for the preferred and/or required nutrients. This would result in a relatively greater level of tetrahydrofolic acid release and fluorescent folic acid derivatives in solution. While it would be expected that some equilibrium would be reached between the fluorescent and non-fluorescent folic acid compounds, the different total concentrations of the two species under different levels of fermentation activity should result in different levels of total measured fluorescence. Based on these conditions, an inverse relationship could be expected between measured VFA consumption, which is indicative of VFA loading, and observed fluorescence. While this explanation could account for the observed pattern, it does not necessarily relate changes in the metabolism of the organisms directly responsible for biological nutrient removal with changes in fluorescence, as the previous explanation did. Furthermore, it is unlikely that the observed pattern could be attributed to changes in the levels of soluble fluorophores, even if their levels were related to changes in biological activity.

While it is entirely possible that the observed patterns resulted solely from changes in the levels of soluble "background" fluorophores, it is unlikely for a number of reasons. First, although some soluble fluorophores could be responsible for the fluorescence patterns observed in the primary effluent streams, the differences in the physical characteristics of the primary effluent streams and the mixed liquor significantly decrease the likelihood that the observed relationships are based solely on changes in the levels of soluble fluorophores.

Essentially, the higher levels of suspended solids in the mixed liquor would reduce the contribution of the soluble fluorophores to the measured level of fluorescence and observed patterns between fluorescence and other variables. The significantly higher levels of suspended solids in the mixed liquor would significantly reduce the contribution from the soluble fluorophore(s) to the observed fluorescence level. Higher suspended solids levels would significantly decrease the proportion of soluble fluorophore(s) excited since a larger portion of the "excited" sample would consist of biological solids that have much different fluorometric characteristics (Section 6.1). Unlike the primary effluent where suspended solids concentrations were very low and the excitation light could penetrate and excite a large volume of liquid containing a high proportion of the soluble fluorophore(s), the excitation light penetrates a much smaller field in the mixed liquor. The higher level of suspended solids not only reduces the effective detection field, it also significantly alters the characteristics of the fluorescence detection. The physical conditions of the optical measurements are significantly different.

Second, if in fact the patterns observed in the mixed liquor were dependent solely on changes in the levels of soluble fluorophore(s), the magnitude of change in the observed fluorescence levels would be much lower. Changes in the level of observed fluorescence would typically range about 20 NFU in the primary effluent stream during the course of an experiment. Changes in the level of observed fluorescence in the mixed liquor would also typically range around 20 NFU during the course of an experiment. Due to the changes in the optical characteristics of the different samples, that is the primary effluent with very low suspended solids and the mixed liquor with significantly higher suspended solids, the magnitude of change in observed fluorescence in the mixed liquor would be expected to be much lower. If the only fluorophores contributing to the observed fluorescence were soluble components, the increased concentration and presence of suspended solids would, undoubtedly, decrease the magnitude of fluorescence readings simply from dilution of the soluble fluorophores and

attenuation of their fluorescence transmission. Therefore, instead of observing a variation in fluorescence of 20 NFU, as was typical of the primary effluent, a range of 5 or 10 NFU might be expected in the mixed liquor if it were only soluble fluorophores responsible for the fluorescence. Based on the trends illustrated in Figures 5.5 - 5.7, it is evident that the level of suspended solids does affect the contribution from soluble fluorophores to the observed fluorescence levels.

Thirdly, assuming that folic acid derivatives might be major soluble fluorophores in the sewage, it is uncertain whether any folic acid derivatives released into solution would be oxidized to their fluorescent form in the highly anaerobic conditions of the reactor. Since it is postulated that the derivatives are released in a non-fluorescent form, oxidation of the derivatives would be required to affect the measured fluorescence.

Based on this discussion and that provided earlier, it is more probable that the observed changes in measured fluorescence observed in the mixed liquor are caused primarily from changes in the levels of NADH-related fluorescence rather than to changes in soluble fluorophores. The coincidence of observing changes in the level of soluble, background fluorophores that demonstrated consistent trends with changes in other variables like VFA consumption or phosphate release would be expected to be small. It is, nevertheless, important to recognize and address the possibility that the patterns might be attributable solely to changes in the levels of soluble fluorophores.

Another explanation relating the pattern between VFA consumption and measured fluorescence with specific biochemistry, involves the requirement for the microorganisms to maintain an appropriate pH gradient across their membranes. In Section 6.3.1.1., phosphate release was attributed, in part, to a cellular strategy designed to re-establish a suitable membrane pH gradient that was disrupted from the uptake and intracellular dissociation of

neutral acids. An alternative mechanism for pH gradient adjustment involves the expulsion of protons from NADH by a membrane-bound dehydrogenase (Harold, 1977). This mechanism could serve the dual purpose of maintaining a suitable pH gradient while regenerating essential NAD^+ from the NADH produced during metabolism. The use of NADH for this purpose may contribute to the observed inverse pattern between specific VFA consumption and fluorescence. Figure 6.10 illustrates this mechanism. While this explanation relates changes in fluorescence measurements to changes in measured specific VFA consumption more directly, it is uncertain whether the level of undissociated acids that existed in the system could account for the pattern.

If we assume that the observed changes in fluorescence were related primarily to changes in NADH-related fluorescence, rather than changes in other soluble fluorophores, then it should be theoretically possible to precisely define the metabolic strategies underlying the changes in fluorescence. Because of the diversity of reactions affecting cellular NADH/ NAD^+ pools, the diversity of microbial species present in the complex system, the diversity of metabolic capabilities of these species, and the inconsistency of the biological composition of the population, precise metabolic definition of the observed relationship between VFA consumption and measured fluorescence in the mixed liquor is impossible. The inability to precisely define the relationship metabolically, however, does not affect the usefulness of the observed relationship for predicting changes in VFA consumption. Instead, it is the consistency of the relationship that determines its usefulness. The consistency of the relationship allowed general changes in VFA load and consumption to be monitored and predicted on a real time basis. Like the relationship between culture fluorescence and VFA levels in the primary effluent, however, precise quantitative predictions could not be made over the long term. Variability in the specific mathematical relationships between anaerobic VFA consumption and culture fluorescence limited the ability to make these precise quantitative predictions.

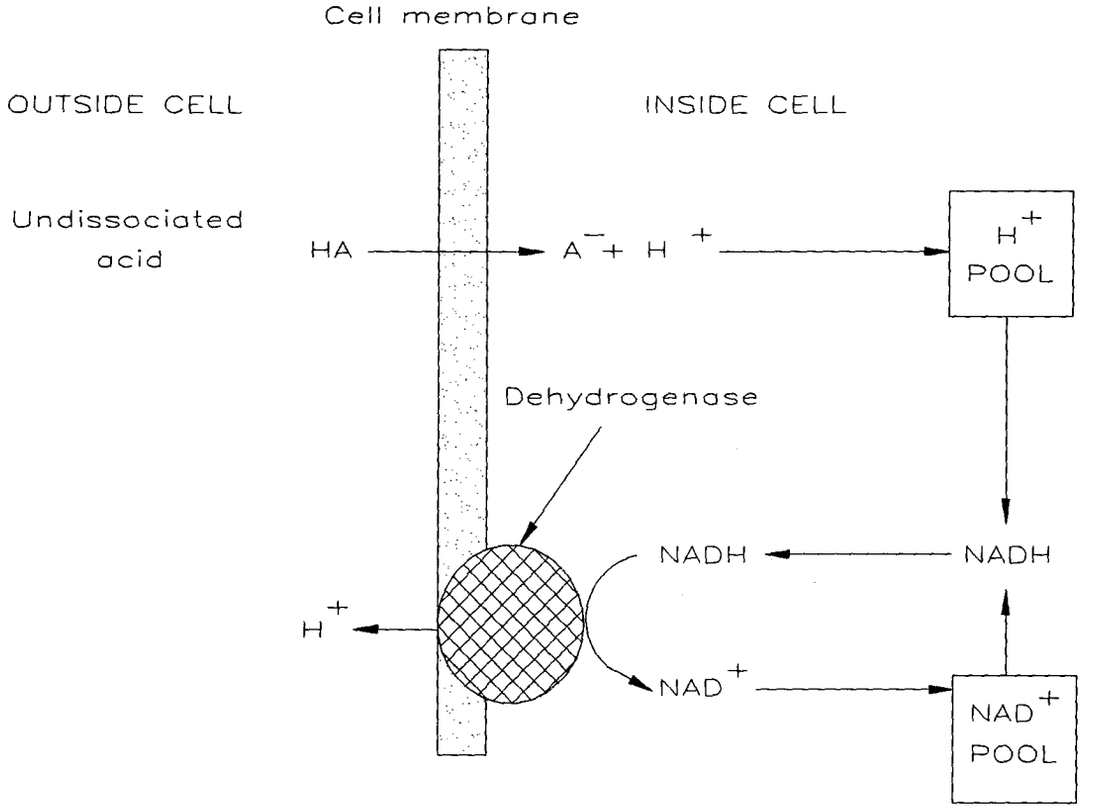

Figure 6.10. Simplified schematic diagram depicting the activity of membrane-bound dehydrogenase. (The reactions shown are generalized, simplified reactions, and as such, not all of the details are shown).

Variability in biological activity due to changes in the population dynamics of the active biomass, changes in the metabolic capabilities of the biomass, or changes in the quality or chemical character of the sewage are significant factors contributing to the mathematical variability. For example, changes in influent character or quality can result in changes in the microbial environment that might cause changes in the distribution of microbial species within the biomass. The size of the $NADH/NAD^+$ pools of different species of organisms are variable (London and Knight, 1966; Wimpenney and Firth, 1972), therefore, any changes in sludge population dynamics might result in changes in the total relative pools of NADH and NAD^+, and the metabolic capabilities of the biomass. Not only could this affect the total range of possible fluorescence measured, it could also influence the magnitude of changes observed within that range. Data also indicated that there is an undetected mobilization and flux of nutrients. This process ultimately requires metabolic strategies that influence the net $NADH/NAD^+$ equilibrium. Without precise definition of these mechanisms, their effect on the net $NADH/NAD^+$ equilibrium and culture fluorescence cannot be assessed. Consequently, these would affect the levels of fluorescence and complicate definition of consistent and specific mathematical relationships.

The ability to use fluorescence data to monitor and predict qualitative changes in specific VFA consumption on a real time basis is useful. Not only could it be used to monitor VFA consumption and loading, it could also be used to predict changes in anaerobic phosphate release. Because of the consistency of the relationship between VFA/M and measured fluorescence and the good correlation between VFA/M and mass phosphate release, changes in mass phosphate release can be monitored and predicted from on-line fluorescence measurements. The usefulness of these capabilities for process control and optimization will be discussed in a later section.

Absolute identification of the specific metabolic mechanisms responsible for the observed pattern between measured anaerobic zone VFA consumption and fluorescent measurements in the crude conditions of the sludge is impossible. The ability to provide limited, plausible, biological explanations for the pattern suggested that changes in measured fluorescence are probably related to changes in the metabolic activity of the biomass. While precise metabolic definition would improve the confidence of fluorescence monitoring as an indicator of biological activity, it is not required. The consistency of the observed patterns, alone, makes them useful. The sensitivity of measured fluorescence to VFA consumption allows changes in nutrient load and VFA consumption to be predicted from on-line fluorescence data. Similarly, the effects of different levels of VFA consumption on phosphate release could be predicted from the fluorescence data. These features are useful and have potential in the development of process control and optimization strategies.

6.3.4. Oaks, PA and Kelowna, B.C.

The purpose of the experiments at the full scale facilities was to compare the results of the somewhat "controlled" pilot plant studies to real situations, to further evaluate the usefulness of fluorescence monitoring for process control and optimization.

6.3.4.1. Anaerobic fluorescence patterns at Oaks, PA and Kelowna, B.C.

The difference in the relationships with anaerobic fluorescence, especially between VFA/M and fluorescence, was inconsistent with the biochemical explanations proposed for the pattern observed at the FGR-SGR pilot plant. As mentioned briefly in the results section, further analyses suggested that the observed pattern might result from differences in the operating configurations of these plants from the pilot plant that cause significant changes in the net metabolism occurring in the anaerobic zones.

Recall that return activated sludge originating from the secondary clarifiers is discharged directly into the anaerobic zones of the Oaks and Kelowna plants (Figures 4.2, 4.3 and 4.4; Sections 5.2.4 and 5.2.5), while return activated sludge is denitrified in the anoxic basin before being recycled to the anaerobic basin of the FGR-SGR pilot plant (Figure 4.1). Since the return activated sludge streams at Oaks and Kelowna were not purposefully denitrified before being discharged into the anaerobic basins, they would contain comparatively higher levels of NO_x than the FGR-FGR pilot plant RAS. The constant input of return activated sludge and NO_x, would result in the establishment of quasi-anaerobic conditions, where a combination of anoxic and anaerobic conditions existed. Because very low NO_x levels were measured at the end of the anaerobic zone despite constant input, it was evident that denitrification was occurring in the anaerobic basin. Mass balance analyses confirmed that NO_x were being removed in the anaerobic basins, presumably by biological denitrification.

Since NADH-oxidizing by nitrate metabolism is more rapid and energetically favourable than NADH oxidation by organic compounds normally associated with anaerobic conditions (Section 2.2), increases in nitrate loading would result in greater and more rapid oxidation of the cellular NADH. Since there is a constant input of nitrates and denitrification is on-going, conditions in the reactor are quasi-anaerobic. Consequently, the observed pattern may reflect an $NADH/NAD^+$ equilibrium under different nitrate and VFA loading scenarios, rather than changes in the equilibrium reflecting fermentative and PHA metabolism typical of a true BNR anaerobic zone. Based on this, increased levels of observed fluorescence would correspond with increased anaerobiosis resulting from decreased nitrate loading and denitrification.

Phosphate release data also supported this theory. Because the extent of phosphate release is related to the extent of anaerobic VFA consumption in biological nutrient removal metabolism, it would be expected that increased levels of phosphate release would correspond

with lower nitrate loading and, hence, more anaerobic conditions. Figures 5.40 and 5.41 demonstrated this predicted trend. Assuming that the available VFA is partitioned between denitrification and biological nutrient removal metabolism it would be expected that as nitrate loading and denitrification activity decreased, more VFA would be available for characteristic biological nutrient removal metabolism (VFA consumption for PHA storage, phosphate release). Assuming that good biological nutrient removal only occurs under anaerobic conditions, and that high nitrate loading compromises the optimal conditions for these reactions, PHA storage and phosphate release would be inversely related to nitrate loading, or directly related to fluorescence as observed (Figures 5.40, 5.41, 5.44, 5.45). Furthermore, if the available VFA were partitioned between denitrification and characteristic BNR metabolism, with a higher propensity for VFA consumption in the denitrification process, then the availability of VFA for characteristic nutrient removal metabolism would also be inversely related to nitrate loading. Thus, the patterns may be explained. Figure 6.11 presents a diagram to illustrate the proposed theory.

Although this theory might relate the changes in observed fluorescence to changes in biological activity, we must consider the case that the changes in fluorescence are related to changes in the levels of soluble fluorophores, like the folic acid derivatives. If we assume that these folic acid derivatives do comprise a major fraction of the soluble fluorophores and if we try to attribute the changes in observed fluorescence to changes in the level of these derivatives, it becomes evident that the observed changes in fluorescence are indeed related to changes in NADH-related fluorescence caused by changes in cellular metabolism. Figures 5.38 and 5.39 clearly show that fluorescence is inversely related to nitrate loading in the anaerobic zone. If it is assumed that tetrahydrofolic acid is released into solution by the biomass and needs to become oxidized to cause fluorescence, we would expect that during periods of high nitrate loading that result in highly oxidizing conditions, measured fluorescence would be highest. The oxidizing conditions should cause a greater amount of the non-

fluorescent folic acid derivatives to become converted to fluorescent forms. Consequently, we would expect a direct relationship between measured fluorescence and nitrate loading. In fact, it is exactly the opposite that is observed. These observations strengthen the earlier discussion that suggests that changes in fluorescence measurements in the mixed liquor are directly related to changes in NADH-related fluorescence and biological activity, rather than changes in soluble fluorophores alone.

Despite the difference in general patterns observed between the different plants, the conclusions regarding the applicability of the fluorescence measurements and related patterns are the same. The additional data provided from these studies provides further evidence to suggest that the specific patterns between measured fluorescence and various biological processes are related to characteristics of process operating configurations. This observation would allow the general relationships (i.e. inverse or direct relationships) between measured fluorescence and various biological processes (e.g. VFA consumption, phosphate release) to be predicted. Once the specific patterns were predicted and/or confirmed, and recognized, the consistencies of the patterns would allow changes in anaerobic zone VFA/M, phosphate release, and PHA storage to be monitored and predicted on a real time basis from on-line fluorescence data. These patterns could prove useful in fluorescence-based control and optimization strategies that relied on controlling these variables.

6.3.5. The difference (ΔNFU) between anaerobic and anoxic zone fluorescence

Investigation of the relative difference between the levels of fluorescence measured in the anaerobic and anoxic zones of the FGR-SGR pilot plant revealed a pattern between the average difference in fluorescence (ΔNFU) between the two zones and average mass NO_x removal in the anoxic zone (Section 5.3). The difference in measured fluorescence

between the zones might be related to differences in the steady state NADH/NAD^+ equilibria caused by the activity of denitrifying organisms.

Biological denitrification involves the conversion of NO_x to nitrogen gas, with the rapid oxidation of NADH (Figures 6.11, 6.12, and 6.13). Generally, the fluorescence level measured in an anoxic zone would be considerably lower than the fluorescence measured in an anaerobic zone, since the relative NADH pool of the anoxic biomass would be comparatively lower than that of the anaerobic biomass. NADH oxidation by nitrate is more rapid and energetically favourable than many of the NADH-oxidizing mechanisms in the anaerobic biomass. Consequently the presence of nitrates will result in metabolism that places a greater drain on the total cellular NADH pool than anaerobic metabolism. Nitrate respiration and

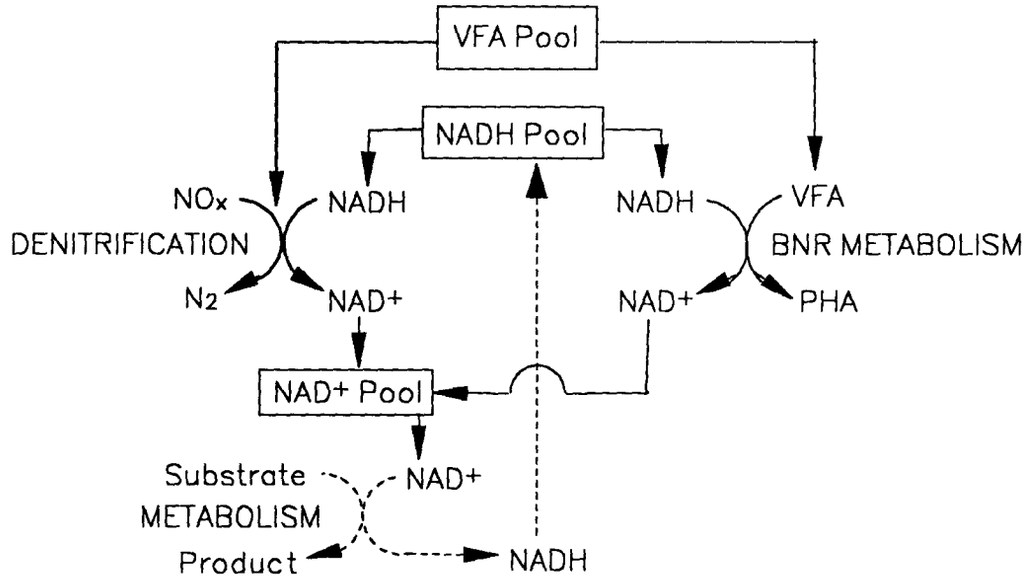

Figure 6.11. Schematic diagram illustrating the theory proposed to describe anaerobic zone patterns at the Kelowna and Oaks plants. The thicker lines of the denitrification reaction indicate that NADH oxidation is comparatively faster than NADH oxidation in the BNR metabolic reactions. Thicker solids lines indicate a greater propensity for the components to be consumed in the reaction. The dotted lines are associated with the production of NADH from general metabolism.

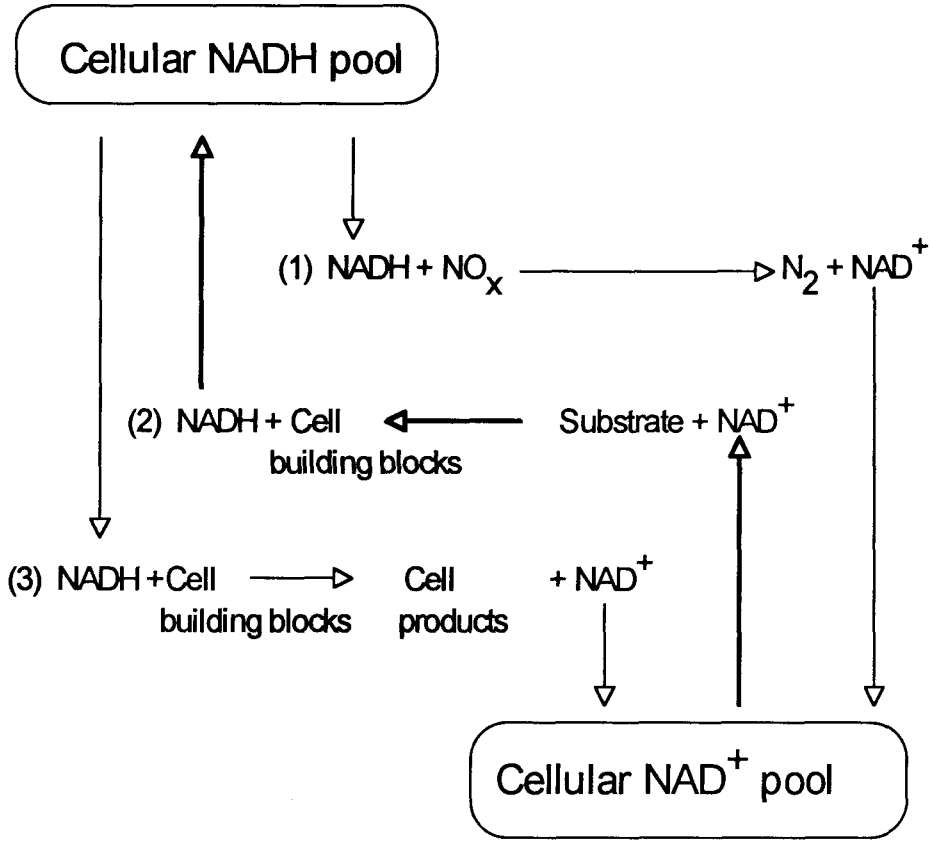

Figure 6.12. Simplified, generalized reactions involving NADH/NAD^+ in the presence of nitrates and low VFA loading. Reaction (1) denitrification reaction; Reaction (2) general catabolism and mobilization of nutrients; Reaction (3) general anabolism and biosynthetic reactions. The relative thickness of the arrows indicates the relative impact of the reaction on the cellular NADH/NAD^+ pools. (The reactions shown are generalized, simplified reactions, and as such, not all of the details are shown).

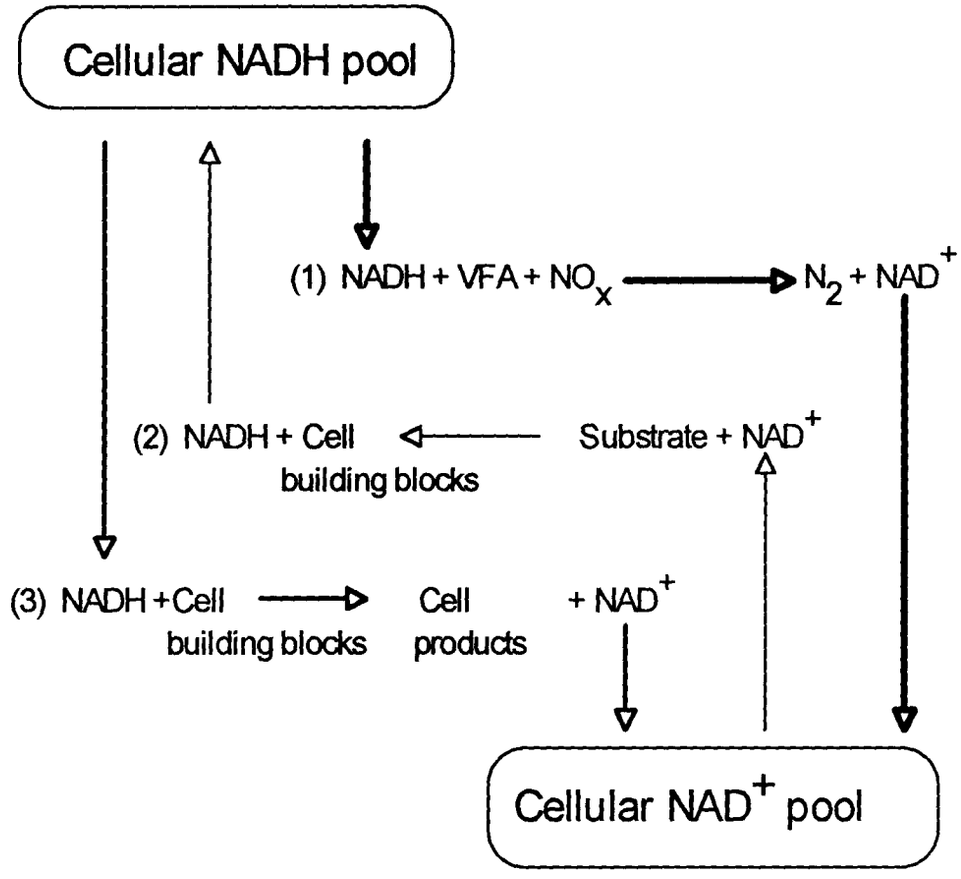

Figure 6.13. Simplified, generalized reactions involving NADH/NAD^+ in the presence of nitrates and high VFA loading. Reaction (1) denitrification reaction; Reaction (2) general catabolism and mobilization of nutrients; Reaction (3) general anabolism and biosynthetic reactions. The relative thickness of the arrows indicates the relative impact of the reaction on the cellular NADH/ NAD^+ pools. (The reactions shown are generalized, simplified reactions, and as such, not all of the details are shown).

denitrification are responsible for causing a lower level of fluorescence in the anoxic zone from the level observed in the anaerobic zone. Therefore, increases in denitrification should result in decreases in the relative proportion of cellular NADH to NAD^+.

A number of reactions affect the level of fluorescence in the anoxic zone. While characteristically lower than anaerobic zone fluorescence, anoxic zone fluorescence can vary while still remaining at the lower comparative level. Denitrification activity can require and be improved in the presence of simple substrates. This observation has led to the addition of methanol or VFA to anoxic zones to improve denitrification rates (Metcalf and Eddy, 1991). Because no soluble VFA's could be detected within or entering the anoxic basin, it can be assumed that metabolic activity within the anoxic biomass is producing the required nutrients. These processes tend to generate NADH (Reaction 2, Figures 6.12 and 6.13). It is also likely that the denitrifying organisms are also doing some biosynthesis during denitrification. These reactions tend to consume NADH (Reaction 3, Figures 6.12 and 6.13). Despite the production of NADH from purposeful nutrient production by the anoxic biomass, it is expected that the net result of denitrification will be a decrease in the relative proportion of NADH to NAD^+. Because NADH is rapidly oxidized in the presence of NO_x, denitrification is expected to quickly deplete the cellular NADH pool. The net effect of denitrification would be a lower proportion of NADH and lower relative fluorescence, compared to conditions without denitrification. Figures 6.12 and 6.13 illustrate the net effects of denitrification under different scenarios using generalized chemical reactions. The relative thickness of the arrows indicates the relative impact of the reaction on the cellular pools.

Based on this explanation which implies that the differences between anaerobic and anoxic fluorescence results from the effects of biological denitrification in the anoxic basin, the relative difference or delta (Δ) between the two levels should provide an indication of the activity of the denitrifying population in the sludge. Data confirmed that the magnitude of

the difference in fluorescence between the two zones was indeed related to the extent of the activity of the denitrifying population (Section 5.3). The relationship between ΔNFU and denitrification activity provides a means to monitor changes in denitrification activity from fluorescence data. By constantly monitoring changes in the difference between anaerobic and anoxic fluorescence levels, changes in denitrification activity might be rapidly detected.

Data from the pilot process and batch tests, which demonstrated the ability to differentiate between anoxic and anaerobic patterns of metabolism using fluorescence data, suggest other benefits that will be considered in detail during discussion of fluorescence-based control and optimization.

6.3.6. General conclusions

Previous discussion pointed out that the observed primary effluent fluorescence patterns had potential for the on-line prediction of changes in primary effluent VFA concentrations. Likewise, the patterns of anaerobic fluorescence could have similar potential for predicting changes in a number of other process variables.

Fluorescence measurements from these studies demonstrated consistent patterns with specific anaerobic VFA consumption, anaerobic mass phosphate release, and anaerobic PHA storage. The consistencies of the patterns observed between VFA/M and measured fluorescence provided a method to monitor changes in anaerobic zone VFA loading and consumption (VFA/M) on a real time basis from on-line fluorescence data. This correlation provides the ability to instantly report changes in the loading and consumption of a key nutrient fraction without the need for time consuming sample collection, preparation, and

analyses. Fluorescence monitoring could provide a fundamental tool for the development of on-line control and optimization strategies that incorporate VFA load regulation.

Due to the specific relationships between anaerobic VFA consumption, phosphate release, and measured fluorescence, monitoring changes in measured fluorescence could provide a means to rapidly and conveniently assess qualitative changes in anaerobic zone phosphate release. The ability to assess changes in this phosphate release can be useful for controlling anaerobic phosphate release specifically through VFA load control strategies. If general control of anaerobic mass phosphate release can be achieved, the process anaerobic zone soluble phosphate inventory could be controlled to eliminate peak phosphorus loading events to the aerobic basins while maintaining the soluble phosphate inventory within aerobic removal capacities.

Analyses of the differences in the levels of measured fluorescence between the anaerobic and anoxic basins at the FGR-SGR pilot plant provided a convenient method for detecting changes in process mass NO_x removal. While analysis of the differences in the relative levels of fluorescence between the anaerobic and anoxic bioreactors cannot differentiate between the possible causes for changes in mass NO_x removal, a decreasing difference can be used to provide an alarm to initiate investigation of the potential problem and take corrective action.

6.3.7. Variability of specific mathematical correlations

The specific mathematical descriptions of the relationships between measured fluorescence and the assayed parameters were highly variable between experiments. This variability limited the ability to use fluorescence monitoring to predict precise quantitative changes in any of the correlated parameters. Some of the possible previously discussed

causes for the variability included the inability to detect and assess the effects of metabolism of undetected substrates on the net cellular NADH/NAD^+ equilibrium. Likewise, the diversity of metabolic reactions that influence the relative proportions of NADH and NAD^+ contributes to the mathematical inconsistencies. Changes in the level of background fluorescence between experiments, discussed earlier, might also contribute to the variability by changing the relative offset (y-intercept) values of the regression lines relating fluorescence to the other variables. Finally, inconsistencies in the biological activity and capability of the complex biomass from day to day are believed to be an important cause of the mathematical variability.

Variability is intrinsic to the observed patterns and result primarily from the biological nature of these systems. Because of the complex nature of the fluorescence signals, the diversity of microbial species, the metabolic capabilities of the different species, and the variability of influent character, determining methods to correct for the inconsistencies and allow quantitative predictions of specific variables to be made from fluorescence data is impossible. While the variability limits the ability to make precise quantitative predictions, it does not affect the capability to assess and predict qualitative changes in key process variables.

6.4. Fluorescence Monitoring for Process Control and Optimization

Experimental results demonstrated the potential for fluorescence monitoring in the development and application of real-time process control and optimization strategies. These strategies might be useful for manipulating certain variables that could be used to control and optimize biological nutrient removal systems. The potential of fluorescence monitoring as a control tool results primarily from the ability to use consistent fluorescence patterns to rapidly assess changes in VFA load, VFA consumption, phosphate release, and PHA storage.

6.4.1. VFA (nutrient) load regulation

The major potential for application of fluorescence monitoring for the control and optimization of biological nutrient removal systems comes from the ability to predict changes in primary effluent VFA concentrations and changes in anaerobic VFA consumption, phosphate release, and PHA storage on a real time basis. The difference in the general patterns observed between fluorescence measurements and the various process parameters demonstrated the configuration and site specificity of the various patterns. This specificity emphasized the importance of preliminary study for pattern recognition. Metabolic definition of the patterns would provide more confidence in the patterns, but is not essential once the particular patterns have been recognized and demonstrated to be consistent.

Application of fluorescence monitoring for predicting changes in primary effluent VFA concentrations and key anaerobic metabolic processes provides the basis for VFA load regulation. While the potential benefits of hydraulic equalization have been recognized for many years (Boon, 1972; Dold et al., 1984; EPA, 1974), the ability to rapidly and reliably assess changes in carbon load has hindered the application of nutrient load balancing (Beck et al., 1986; Edwards et al., 1982). Because measured fluorescence consistently responded in a proportional way to short term changes in VFA loading and consumption and phosphate release at all of the plants studied, the ability to relate changes in measured fluorescence with these key variables has several implications relevant to process control and optimization.

As effluent standards for phosphorus become more stringent, it will become more important to reduce the diurnal excursions that occur in effluent phosphorus concentrations resulting from variations in influent characteristics and biological activity. Such reductions might be achieved by feedback control of equalization basin volumes and recycle flows to

stabilize biological activity through hydraulic and carbon load balancing, especially carbon sources like VFA.

Comparison of experimental data from tests with short term VFA load variation and with stabilized VFA load demonstrated that variation resulted in greater fluctuations in effluent phosphorus concentrations (Section 5.2.3.4., Table VI). Furthermore, data have related these fluctuations directly with increases in anaerobic phosphorus release caused by increased VFA consumption (Section 5.2.3.4). The ability to conveniently and rapidly assess changes in primary effluent VFA concentrations from fluorescence measurement provides the ability to perform VFA load balancing and minimize large fluctuations in VFA loading to the bioreactors, where hydraulic equalization facilities exist. Stabilized VFA load should stabilize anaerobic VFA consumption and phosphate release, minimizing large variations in anaerobic phosphate release and the soluble phosphate inventory in the process. VFA load balancing can be achieved by modifying the traditional modes of hydraulic equalization that simply store primary effluent during high flow periods and feed the stored liquid back during low flow periods. The ability to use fluorescence to rapidly monitor changes in primary effluent VFA concentrations and anaerobic zone VFA consumption and phosphate release provides information that can be used to manipulate equalization tank operation to minimize the fluctuations typically experienced.

The ability to achieve VFA load balancing using this type of approach was demonstrated at the Penticton Advanced Wastewater Treatment Plant (Section 5.4). Using the strategy outlined in Figure 5.49, simultaneous equalization of both hydraulic and VFA load to the bioreactor was achieved over a 24 hour period. The purpose of this study was to demonstrate the ability to balance anaerobic VFA load on a real time basis using on-line fluorescence data. It did not investigate the direct effects of regulated anaerobic VFA loading on the resulting anaerobic phosphate release, changes in the soluble phosphate inventory or

effluent phosphate concentration. Further testing is required to demonstrate the predicted stabilizing effect of VFA load balancing on these variables. It is expected that minimizing the variations in anaerobic VFA load and VFA consumption will eliminate the excursions in effluent phosphorus concentrations associated with fluctuating carbon load and anaerobic phosphate release.

A different control strategy could be used for those systems like Kelowna and Oaks where both VFA load and nitrate levels were predicted to affect anaerobic VFA consumption and phosphate release. Monitoring anaerobic zone VFA/M and phosphate release using fluorescence would allow manipulation of either bioreactor influent quality (carbon concentrations) or return activated sludge recycle rates. If the system did not have the capability to balance hydraulic and carbon loads, anaerobic VFA/M and phosphate release could be stabilized by controlling the return activated sludge recycle rates. Controlling these rates should control the amount of nitrates entering the zone. Because the data showed that changes in phosphate release were related to changes in nitrate loading, stabilizing nitrate loading might minimize large fluctuations in anaerobic metabolic processes and effluent phosphate quality. The relationships between changes in measured fluorescence, denitrification, and phosphate release observed in the anaerobic basin could be used to control the recycle rates. In those systems with the capability to balance hydraulic and nutrient loading, a combination of carbon (VFA) load balancing and return sludge recycle control could be used.

Stabilization of anaerobic zone VFA loading is predicted to have several benefits. Because phosphate originating from anaerobic phosphate release is the largest contributor to the soluble phosphate pool in many BNR processes, controlling the phosphate release through VFA load regulation will allow some control over the soluble phosphate entering the downstream stages of the process. This control could prevent the introduction of soluble

phosphate "slugs" or peaks to the aerobic stage that exceed aerobic biological removal capacities and cause large fluctuations in effluent quality. Simultaneous hydraulic and VFA load balancing will permit the regulation of anaerobic VFA/M and maintain a level of phosphate release and soluble phosphate that do not exceed aerobic removal capacity. This balancing would minimize the short term excursions in effluent phosphate concentrations that can result from transient peaks in anaerobic phosphate release, and prevent the effects of an increasing soluble phosphate inventory (Mah et al., 1994). In those biological phosphate removal facilities that experience large diurnal variations in process carbon loading and must either control the related excursions in effluent phosphate by chemical precipitation or violate discharge criteria, real time fluorescence-based carbon load regulation might result in significant cost savings. By minimizing the frequency or magnitude of these excursions, chemical requirements for phosphorus control could be minimized or eliminated. This chemical reduction would, in turn, reduce the handling and disposal requirements of the resulting chemical sludge.

6.4.2. Optimization of biological denitrification

Another application for process control and optimization using fluorescence monitoring comes from the ability to conveniently differentiate between anaerobic and anoxic patterns of metabolism. Batch and pilot plant studies demonstrated the ability to correlate NO_x removal to differences in the levels of anaerobic and anoxic fluorescence (Sections 5.3 and 6.3.5), thus providing a tool for rapidly and conveniently assessing denitrification performance. Although comparative analysis of oxidation-reduction potential measurements can provide a means to differentiate between different states or conditions (e.g. anoxic vs. anaerobic), comparative analysis of fluorescence provides the means to directly estimate denitrification performance. In theory, oxidation-reduction potential measurements should provide similar information to fluorescence measurements. However, monitoring changes in

bulk oxidation-reduction potential still only provides an indirect measurement of biological activity. Because the measurement of oxidation-reduction potential relies on the measurement of a bulk solution characteristic, it may not always be possible to differentiate changes in measured oxidation-reduction potential caused from changes in biological activity or those caused simply from changes in the chemical characteristics of the bulk solution that have no real effect on biological activity. Furthermore, previous studies demonstrated that metabolism characteristic of a certain conditions (e.g. anaerobic metabolism) could be observed over a wide range of measured oxidation-reduction potential (Mah, 1991). These studies demonstrated that similar levels of denitrification could be observed over a wide range of oxidation-reduction potential measurements. Similarly, the studies showed that PHA synthesis, characteristic of anaerobic conditions could be observed over a wide range of oxidation-reduction potential, that included oxidation-reduction potential values not characteristic of "anaerobic" conditions. These studies suggest that changes in bulk solution parameters do not necessarily reflect true changes in biological activity. Thus, while it may be possible to detect changes in bulk solution parameters, like oxidation-reduction potential, it is not always possible to correlate these changes with true changes in the biological system. This finding could complicate the use of oxidation-reduction potential for determining either the predominant type and/or extent of metabolic activity. Since fluorescence measurements provide an indication of cellular NADH levels that are directly influenced by changes in biological activity, fluorescence measurements should provide a more direct measure of biological activity than other methods that estimate changes in activity from changes in the characteristics of the bulk liquid. Based on the theoretical considerations, fluorescence monitoring has some advantages over oxidation-reduction potential measurement for evaluating biological activity.

Biological denitrification processes could be optimized using fluorescence monitoring, specifically in processes with several anoxic bioreactors operated in series. In these processes, comparison of fluorescence levels throughout the anoxic series with anaerobic levels of

fluorescence would provide information that would indicate whether the anoxic detention time was excessive. If comparison of the fluorescence levels through the series showed a consistent and significant increase in the measured fluorescence in one of the anoxic bioreactors that resulted in a fluorescence level comparable to the observed anaerobic fluorescence level, it would indicate that complete denitrification has been achieved prior to this reactor. If insignificant levels of nitrate were present in the reactor with the elevated fluorescence level, a significantly higher level of fluorescence would be expected. Since nitrates metabolism places a great drain on the cellular NADH pool from NADH oxidation by nitrate, the absence of nitrates would eliminate the drain and result in a higher level of fluorescing NADH in the biomass. This fluorescence pattern would also suggest that anaerobic conditions prevail in the particular anoxic bioreactor demonstrating the elevated fluorescence level, which could result in undesirable secondary phosphate release and deterioration of the biological health and stability of the system. This trend of fluorescence data would also suggest that a process change be made to decrease anoxic detention time. In this capacity, fluorescence data might be useful for formulating optimization strategies intended to improve biological nitrogen removal. More aggressive optimization could be achieved by continuously adjusting anoxic detention time, according to fluorescence data. This control could be achieved by converting anoxic basins to aerobic basins in those plants with the flexibility to operate the basins with or without introduced air, and make appropriate adjustments to aerobic dissolved oxygen levels. Thus, as the point of complete denitrification migrated between anoxic bioreactors, those bioreactors downstream of the bioreactor where complete denitrification was detected could be operated as aerobic basins. The aeration levels in the aerobic basins could then be adjusted to accommodate the changes in aerobic detention time caused from the modified operation.

Continuous, ongoing optimization of biological denitrification could also be achieved through fluorescence-based control. The batch tests demonstrated the ability to detect the

denitrification time of a given sample from the fluorescence data (Section 5.3). Instrumentation is currently being developed to continuously perform *in situ* batch tests of anoxic mixed liquor based on similar principles as those demonstrated by the batch tests. This instrumentation determines and reports the required time to achieve complete denitrification of the anoxic mixed liquor under the ambient hydraulic and nutrient conditions of the bioreactor. The *in situ* evaluation of required denitrification time eliminates the need for time consuming measurement and interpretation of extracellular parameters such as bulk solution chemical oxygen demand (COD), biochemical oxygen demand (BOD), MLSS, or nitrate levels that would otherwise be required to determine the time required to achieve complete denitrification. This information provides the basis for a denitrification optimization strategy that involves feedback control of internal recycle flows (aerobic to anoxic recycle) to achieve optimum denitrification activity under prevailing bioreactor conditions on a real time basis (Armiger et al., 1994a, 1994b; Mah et al, 1995; Schwegler et al, 1995).

6.4.3. General conclusions

Clearly, the experimental evaluation that has been carried out suggested that measured fluorescence is an indicator of changes in biological activity, and that continuous fluorescence monitoring is useful for formulating real time control and optimization strategies designed to improve the performance of biological nutrient removal systems. The ability to monitor and predict changes in a number of parameters that impact on process performance on a real time basis is fundamental for on-line process control and optimization. The direct assessment of biological activity by fluorescence monitoring provided the means to conveniently measure and predict changes in process VFA load, anaerobic zone VFA consumption, anaerobic zone phosphate release, and denitrification. These activities all impact process performance in various ways.

Strategies to improve process performance using fluorescence-based control were developed and some preliminary tests completed. Specifically, these strategies are intended to improve effluent phosphorus quality and consistency and biological denitrification through fluorescence-based regulation of VFA consumption and phosphate release, and internal recycle rates. Further development and testing should provide conclusive evidence supporting the conclusions presented here.

6.5. Limitations of the Data

The observations and conclusions derived from experimentation at pilot and full scale plants are limited primarily by the complexity of the chemical and biological natures of the systems studied, and the ability to exercise control of these systems. Experiments at the FGR-SGR pilot plant were conducted during the same period of the day in most cases, in an attempt to eliminate sample variations. Due to the intensive sampling regime and the number of samples that had to be processed and analyzed, analysis of process parameters was limited to those expected to provide the most useful information or to have direct impact on process performance. Nutrient analysis was limited to volatile fatty acids, a recognized key nutrient fraction, although data suggested the presence of some other important nutrient fraction. The effects of varying levels of background fluorescence on the overall patterns and specific mathematical descriptions of the patterns were not clearly defined. Finally, the discussions relating the observed patterns of measured fluorescence with specific metabolic strategies are mostly speculative. Because of the difficulty of clearly identifying the metabolic strategies in these complex, mixed culture systems, neither the objectives nor the tests were formulated to achieve precise metabolic definitions. Instead, the tests were designed only to search for consistent and useful patterns relating changes in measured fluorescence to changes in key process variables and biological activity.

Although many of the effects of these problems could have been overcome by conducting experiments in laboratory reactors with defined biomass, artificial sewage, and highly controlled conditions, rather than at operating treatment plants, it was felt that the direct usefulness and applicability of the data and their conclusions would have been compromised. Although laboratory testing under defined conditions might have provided more precise explanations and conclusions, the consistency and applicability of these conclusions to real world situations would be questionable. Therefore, rather than attempt to provide limited conclusions from artificially defined conditions which might be invalid for actual process monitoring and control, it seemed more reasonable to derive conclusions from testing under the less controlled conditions at the operating plants. This testing should provide more direct evidence and relevant conclusions for the applicability and usefulness of fluorescence monitoring for process control and optimization. Although the explanations for the observed patterns and conclusions are more speculative than those that might have been generated from extensive laboratory testing, it must be remembered that it is the consistency of the patterns observed, and not necessarily their precise scientific definition and explanation, that proves the usefulness and applicability of fluorescence monitoring for process monitoring and control. Despite many limitations of the data, the observations and conclusions demonstrated the usefulness of fluorescence monitoring for control and optimization of BNR systems.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

The principal objectives of this research were to; 1) test the use of continuous, "real time" monitoring of fluorescence, related to intracellular NADH, as an indicator of changes in biological activity, and 2) evaluate the usefulness of "real time" fluorescence monitoring as a tool for the development of novel, on-line control and optimization strategies designed to improve the efficiency, stability, and consistency of biological nutrient removal processes. Tests were designed to demonstrate that changes in measured fluorescence could be related to changes in biological activity, and then demonstrate the potential of these consistent relationships for real time process control. This testing was done by searching for consistent relationships between patterns of measured fluorescence and key intracellular and process variables under a variety of experimental conditions at various BNR plants. Experimentation and analyses, including extensive pilot plant research and field studies at three different full scale nutrient removal plants, led to the following conclusions:

1. Fluorescence can be used as an indicator of changes in the VFA content of the primary effluent and specific biological activities including anaerobic zone VFA consumption, anaerobic zone phosphate release, anaerobic zone PHA storage, and denitrification. Continuous, on-line monitoring of fluorescence provides a method for assessing changes in these parameters, related to biological activity, on a real time basis.
2. Gross trends in measured fluorescence demonstrated good correlation with process mixed liquor suspended solids concentrations in the various FGR-SGR pilot plant process zones.
3. The specific patterns between measured fluorescence and primary effluent VFA concentrations, anaerobic zone VFA consumption, phosphate release, and PHA storage were site specific and are related to the operating configuration of the process train. The

different patterns could be explained by differences in metabolic patterns resulting from these configuration differences. Precise definition of the general metabolic patterns in these complex mixed culture systems is practically impossible, but further biochemical studies might provide more evidence to improve confidence in the fluorescence measurements. The usefulness of the patterns between measured fluorescence and key variables is not compromised by this lack of precise metabolic definition, as long as they can be demonstrated to be consistent.

4. Primary effluent fluorescence responded consistently and proportionally to changes in VFA concentrations. With the exception of the Penticton plant, primary effluent fluorescence varied directly with VFA concentration. Studies at the Penticton plant showed an inverse relationship between primary effluent fluorescence and VFA concentrations. Several mechanistic theories were provided to explain the patterns. The consistencies of the observed patterns provided a convenient method for assessing qualitative changes in the VFA content of the primary effluent on a real time basis from fluorescence measurements.
5. Anaerobic mass phosphate release and PHA storage were affected by specific VFA consumption (VFA/M) as expected and predicted by biochemical models. Both phosphate release and PHA storage varied directly with VFA/M. The relationship between VFA/M and mass phosphate release demonstrated good mathematical correlation, while the relationship between VFA/M and PHA storage did not.
6. Measured fluorescence demonstrated consistent patterns with specific VFA consumption (VFA/M), mass phosphate release, and PHA storage in the anaerobic zones. VFA/M, phosphate release, and PHA storage varied inversely with measured fluorescence at the FGR-SGR pilot plant, and varied directly with measured fluorescence at the Kelowna and Oaks plants. The differences in the general patterns at the different plants could be related to differences in operating configurations. Despite these differences, on-line fluorescence data provided the ability to conveniently assess changes in trends anaerobic

zone specific VFA consumption, mass phosphate release, and PHA storage.

7. The capability to conveniently determine general changes in VFA loading, consumption, and anaerobic phosphate release on a real time basis from fluorescence measurements provides the basis for an on-line control strategy to stabilize anaerobic biological activity. One method for stabilizing anaerobic biological activity is through VFA load balancing. Preliminary full scale testing at Penticton, B.C. demonstrated the ability to regulate bioreactor VFA load on a real time basis using continuous fluorescence measurements.
8. Analyses and batch testing showed that differences between anaerobic and anoxic levels of measured fluorescence could be related directly to anoxic mass NO_x removal and denitrification. This relationship provided a method for assessing changes in process denitrification from fluorescence data. Estimation of process denitrification from fluorescence data provides the basis for optimization of biological denitrification through a variety of strategies that involve controlling anoxic zone detention time.
9. The specific mathematical descriptions of the relationships between fluorescence and primary effluent VFA concentrations, anaerobic zone VFA consumption, phosphate release and PHA storage were highly variable between experiments. This variability resulted from a number of factors that included changes in the chemical and biological nature of the process. Although this variability limited the ability to make precise quantitative predictions about these parameters, it did not compromise the potential or usefulness of fluorescence monitoring for making qualitative predictions in primary effluent VFA concentrations, anaerobic zone VFA consumption, phosphate release, or PHA storage for use in control and optimization strategies.

7.2. Recommendations for Further Research

Based on the findings of this research, the following recommendations are made for further research:

1. The characteristics of the background fluorescence caused by dissolved substances in the sewage need to be analyzed. This analysis would involve detailed chemical analysis of the sewage components that exhibit similar spectral properties as NADH. Once characterized, the effect of different levels of these fluorescing components on the specific response of the detector probe to induced biological changes in the NADH/NAD^+ equilibrium should be clarified.
2. More detailed characterization of the sewage should address the problem of the additional and undetected carbon substrates in the sludge. Thorough classification of the carbon inventory in the process and the fate of the carbon pools through the process could identify the possible sources of the substrates. Quantitative classification of these chemicals may improve the consistency of some of the specific mathematical relationships and provide more reasonable carbon yield values.
3. Detailed microbial and biochemical studies should be done to determine how the differences in process configurations generally affects overall population dynamics and metabolism in the various process zones. This knowledge might provide some information regarding the differences in patterns observed between measured fluorescence and specific process variables at plants with different configurations. Detailed population analysis in the various zones could reveal relevant information, but have proven difficult in the past. The complexity of the mixed population dynamics and the diversity of metabolic possibilities would likely prevent precise characterization of the metabolic strategies responsible for the differences in the fluorescence patterns.
4. Extensive testing at both pilot and full scale is required to demonstrate the ability and

benefits of the proposed control and optimization strategies on a continuous basis.

5. A survey of a wider range of full scale treatment plants should be conducted to better determine the effect of different process configurations on the general fluorescence patterns.

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9. Appendix A

The experimental procedures used for testing for relationships between primary effluent VFA concentration and fluorescence adhered to the following;

- fluorescence probes were installed directly in the primary effluent channels at the Oaks, Penticton, and Kelowna plants. At the FGR-SGR pilot plant, the fluorescence probe was installed in a flow cell incorporated into the piping carrying primary effluent to the main process train. In all installations, the probes were installed as close to the primary clarifiers as conveniently possible.
- each experiment involved obtaining samples of primary effluent at intervals ranging from thirty to one hundred and twenty minutes and recording fluorescence at intervals ranging from five to sixty seconds. Samples collected for analysis were obtained using a sampling rod in close proximity to the installed fluorescence probes. At the FGR-SGR pilot plant, samples were withdrawn form a sampling port located immediately upstream of the fluorescence probe.
- at the FGR-SGR pilot plant, experiments were conducted over a three month period. Experiments were conducted on separate, non-contiguous days, typically at intervals of two to seven days. At the FGR-SGR pilot plant, experiments were typically conducted during the same time each experiment day. At the other sites, experiments were conducted on non-contiguous days.
- samples were collected, prepared, and analyzed according to the procedures and methods outlined in Section 4 and Table I.
- once the VFA analysis was complete, VFA and recorded fluorescence data were analyzed using a simple linear regression model to determine the correlation (r^2 values) between the two parameters and the specific variables of the simple linear model, namely the slope and y-intercept of the regression line.

10. Appendix B

The experimental procedures used for testing for relationships between fluorescence and VFA consumption, phosphate release, and PHA storage in the anaerobic zone adhered to the following;

- fluorescence probes were installed directly in the anaerobic bioreactors of the treatment plants studied. In those plants where the anaerobic zone consisted of several individual cells, the fluorescence probes were installed in the first cell.
- each experiment involved obtaining samples of anaerobic mixed liquor for analysis of several parameters listed in Section 4. Anaerobic zone mixed liquor samples were collected at intervals ranging from thirty to one hundred and twenty minutes and fluorescence recorded at intervals ranging from five to sixty seconds. Samples collected for analysis were obtained using a sampling rod in close proximity to the installed fluorescence probes. Samples were also collected from the primary effluent and recycle flows entering the zone for analysis of VFA, soluble phosphate, soluble nitrate, MLSS, total phosphate, and PHA content. In addition, data about all flows entering the anaerobic zone were recorded.
- at the FGR-SGR pilot plant, experiments were conducted over a three month period. Experiments were conducted on separate, non-contiguous days, typically at intervals of two to seven days. At the FGR-SGR pilot plant, experiments were typically conducted during the same time each experiment day. At the other sites, experiments were conducted on non-contiguous days.
- samples were collected, prepared, and analyzed according to the procedures and methods outlined in Section 4 and Table I.
- once the analyses were complete, anaerobic VFA consumption (VFA/M) and mass phosphate release were calculated using simple mass balance equations. These parameters were analyzed along with recorded fluorescence data using a simple linear regression

model to determine the correlation (r^2 values) between the two parameters and the specific variables of the simple linear model, namely the slope and y-intercept of the regression line.

- in those experiments at the FGR-SGR pilot plant where VFA load variations were intentionally caused for experimental purposes. Conditions of variable VFA loading were achieved by regulating the fermenter feed rate to control fermentate addition to the primary influent. Alternately, typical VFA load variations were simulated by the controlled addition of a sodium acetate solution (Sigma Chemical Co., St. Louis, MO.) to the primary effluent stream using a computer controlled peristaltic pump (Model 420-C Dual Channel Peristaltic Pump, BioChem Technology Inc., King of Prussia, PA.). The pump was operated such that the pumping rate of a freshly prepared stock solution of sodium acetate was ramped from 0 mL/h to 1000 mL/h and back to 0 mL/h over a period ranging from one to three hours. The concentration of the stock solution was controlled to achieve an increase in soluble acetate in the anaerobic bioreactor of approximately 20 mg/L when added at the maximum pumping rate.
- during the FGR-SGR pilot plant studies, simulatneous studies were being conducted by another researcher. As such, all tests were done under the current conditions of the process. The ongoing studies should not compromise the conclusions derived form the data presented in this thesis.

11. Appendix C

Table VII. Experimental data from the FGR-SGR pilot plant studies.

Run	ET (h)	PE NFU	PE VFA	ANA NFU	ANA MPR	ANA VFA/M
F9360-01	0.50			284.31		6.49
F9360-01	1.00	144.47	33.25	288.03	6.30	5.38
F9360-01	2.00	143.97	30.70	289.89		4.99
F9360-01	2.50	142.98	29.62	289.43		4.62
F9360-01	3.00	141.99	27.69	290.36	5.01	4.35
F9360-01	4.00	139.02	26.41	291.76	5.10	4.71
F9360-01	4.50	138.03	22.96	295.01		4.08
F9360-01	6.50			298.27		4.04
F9360-02	0.00	141.00	47.40	266.61	7.44	8.08
F9360-02	0.50			268.48		7.29
F9360-02	1.00	143.48	40.37	268.01	7.06	6.88
F9360-02	1.50	143.97	37.72	268.48		6.49
F9360-02	2.00			269.87	6.60	6.34
F9360-02	2.50			269.87		6.30
F9360-02	3.00	141.00	37.67	269.87	6.57	6.84
F9360-02	3.50	140.51	35.46	268.48		5.99
F9360-02	4.00	138.52	30.56	269.41	5.33	4.83
F9360-02	4.50	138.52	26.38	273.60		4.23
F9360-02	5.00	135.55	21.32	281.51	6.07	3.47
F9360-02	5.50	134.56	18.01	286.63		2.82
F9360-02	6.00	132.09	17.47	284.31	3.72	2.63
F9360-02	6.50			292.69		2.87
F9360-03	0.00	348.99	33.37	302.93	6.30	6.83
F9360-03	0.50	349.49	30.47	305.72		6.14
F9360-03	1.00	349.98	29.22	305.26	5.70	5.81
F9360-03	1.50	349.49	27.83	305.72		5.66
F9360-03	2.00	350.48	27.71	306.19	5.96	5.78
F9360-03	2.50	349.49	25.60	306.19		5.44
F9360-03	3.00			305.72	5.53	5.79
F9360-03	3.50			303.86		5.97
F9360-03	4.00			304.33	5.24	5.12
F9360-03	4.50	343.05	18.76			
F9360-03	5.00	343.55	15.23	314.10	3.86	3.19
F9360-03	5.50	343.55	13.57	320.62		2.88
F9360-03	7.00			313.17	4.68	4.61
F9360-03	7.50			312.24		4.27
F9360-03	8.00			312.24	4.60	4.40
F9360-04	0.00			311.78	6.38	8.52

Table VII. Cont'd.

Run	ET (h)	PE NFU	PE VFA	ANA NFU	ANA MPR	ANA VFA/M
F9360-04	0.50	272.23	31.45	314.10		7.74
F9360-04	1.00	271.24	29.33			
F9360-04	1.50	269.26	29.46	313.64		7.33
F9360-04	2.00	267.78	30.54	313.17	6.24	7.84
F9360-04	2.50	265.80	27.30			
F9360-04	3.00	270.25	27.92	314.57	5.09	6.51
F9360-04	3.50					
F9360-04	4.00	282.14	32.22	312.71	5.89	7.90
F9360-04	4.50			314.57		6.87
F9360-04	5.00			314.57	5.68	6.08
F9360-04	5.50	260.84	27.35			
F9360-04	6.00	256.39	27.12	314.10	6.26	7.30
F9360-04	6.50	254.41	25.95	313.64		7.04
F9360-04	7.00			311.78	6.79	9.15
F9360-05	5.00	217.76	17.59			
F9360-05	4.50	218.75	21.58			
F9360-05	1.00	220.24	39.95			
F9360-05	4.00	220.73	26.18			
F9360-05	3.50	221.72	30.01			
F9360-05	3.00	224.69	163.17			
F9360-05	2.50	226.18	154.90			
F9360-05	2.00	227.66	170.12			
F9360-05	1.50	228.16	96.17			
F9360-05	0.00	231.63	31.07			
F9360-05	0.50	232.12	29.93			
F9360-06	0.00			321.55	8.05	6.89
F9360-06	0.50	187.06	50.49			
F9360-06	1.00	187.06	50.03			
F9360-06	1.50			320.16		7.39
F9360-06	2.00	191.02	47.81			
F9360-06	3.00			318.76	8.10	7.97
F9360-06	3.50	189.53	50.37	318.76		7.99
F9360-06	4.00			316.90	8.54	8.39
F9360-06	4.50	184.08	49.19	315.04		8.62
F9360-06	5.00					
F9360-06	5.50	176.66	41.12	313.64		8.63
F9360-06	6.00	172.69	44.87			
F9360-06	6.50	169.72	37.28	318.29		7.23
F9360-06	7.00	168.73	36.02	320.16	8.39	7.41
F9360-07	0.00	179.13	50.03			
F9360-07	0.50			304.79		7.42
F9360-07	1.00	181.61	48.59	304.33	8.28	9.08
F9360-07	1.50	181.61	48.58	303.86		8.87
F9360-07	2.00			303.86	8.75	8.98
F9360-07	2.50	181.61	53.12			
F9360-07	3.00	181.11	54.19	302.46	8.61	10.51
F9360-07	3.50	179.13	50.64	302.46		9.56

Table VII. Cont'd.

Run	ET (h)	PE NFU	PE VFA	ANA NFU	ANA MPR	ANA VFA/M
F9360-07	4.00	178.14	43.55			
F9360-07	4.50	175.67	40.46			
F9360-07	5.00	173.68	38.07			
F9360-07	5.50	172.20	38.44			
F9360-07	6.00			303.40	9.13	8.73
F9360-07	6.50			304.79		7.40
F9380-01	0.00	172.20	44.88	306.19	8.24	8.29
F9380-01	0.50	173.68	44.85			
F9380-01	1.00			304.33	8.52	9.31
F9380-01	1.50	175.17	52.71	305.26		10.02
F9380-01	2.00	176.66	53.83			
F9380-01	2.50			305.72		8.08
F9380-01	3.00	175.67	52.25			
F9380-01	3.50			307.12		7.81
F9380-01	4.00	174.18	46.64	305.26	8.21	9.28
F9380-01	4.50			306.19		7.82
F9380-01	5.00	172.20	36.05			
F9380-01	5.50	171.70	37.05	308.98		7.59
F9380-01	6.00	169.72	33.15	309.45	9.47	6.67
F9380-01	6.50			311.31		6.88
F9380-02	0.50	187.55	34.23			
F9380-02	1.00	189.53	34.16			
F9380-02	1.50	190.52	34.63			
F9380-02	2.00	192.50	35.02	288.03	5.87	6.45
F9380-02	2.50	193.00	35.11	288.03		6.38
F9380-02	3.00	193.49	34.27	288.96	5.93	6.14
F9380-02	3.50	191.51	34.69	287.10		6.25
F9380-02	4.00	189.53	33.79	288.50	5.68	6.12
F9380-02	4.50	187.55	32.72	289.43		5.83
F9380-02	5.00	184.08	31.92	291.29	5.33	5.60
F9380-02	5.50	181.11	30.60	290.82		5.33
F9380-02	6.00	177.15	30.52	293.15	5.13	5.27
F9380-02	6.50	175.17	30.60	292.22		5.26
F9380-02	7.00			291.76	5.07	5.48
F9380-03	0.00	193.99	34.31	289.43	5.38	5.82
F9380-03	0.50	195.97	34.67	289.43		6.02
F9380-03	1.00	197.46	35.38			
F9380-03	1.50	199.93	34.95			
F9380-03	2.00	203.40	35.40			
F9380-03	2.50	202.01	34.68			
F9380-03	3.00	196.56	34.78	282.44	5.39	5.72
F9380-03	3.50	198.05	34.04	281.51		5.85
F9380-03	4.00	195.57	33.97	281.98	5.65	6.10
F9380-03	4.50	193.59	32.50	282.44		5.67
F9380-03	5.00	185.17	30.80	283.38	4.96	5.22
F9380-03	5.50	179.72	28.52	284.31		4.90

Table VII. Cont'd.

Run	ET (h)	PE NFU	PE VFA	ANA NFU	ANA MPR	ANA VFA/M
F9380-03	6.00	173.29	28.04	284.77	4.78	4.88
F9380-04	0.00	179.13	61.20			
F9380-04	0.50	179.63	60.93	289.51		8.75
F9380-04	1.00	180.62	60.25			
F9380-04	1.50	179.63	58.80	289.98		8.47
F9380-04	2.00	181.61	61.01	289.05	7.39	9.11
F9380-04	2.50	180.12	59.85	289.51		8.69
F9380-04	3.00	182.50	58.71	288.03	7.59	9.06
F9380-04	3.50	181.02	58.51			
F9380-04	4.00	178.54	59.21	287.57	7.69	8.94
F9380-04	4.50	176.07	53.63			
F9380-04	5.00	169.13	49.89			
F9380-04	5.50	165.17	47.42			
F9380-04	6.00			291.29	7.13	8.19
F9380-04	6.50			291.76		7.84
F9380-05	1.00	170.71	48.73			
F9380-05	1.50	170.81	48.92			
F9380-05	2.00	171.21	50.15			
F9380-05	2.50	170.71	47.81			
F9380-05	3.00	171.70	47.37			
F9380-05	3.50	170.71	46.75			
F9380-05	4.00	171.21	48.31			
F9380-05	4.50	171.21	40.48			
F9380-05	5.00	169.23	33.88			
F9380-05	5.50	168.24	31.69			
F9380-05	6.00	166.75	30.81			
F9380-05	6.50	166.26	31.63			
F9310-01	12.00	232.69	16.60			
F9310-01	13.00	230.90	15.87			
F9310-01	14.00	231.35	16.00			
F9310-01	17.00	233.14	16.80			
F9310-01	18.00	234.92	16.89			
F9310-01	19.00	235.37	16.66			
F9310-02	7.50	253.68	22.61			
F9310-02	9.50	251.45	22.26			
F9310-02	10.50	251.00	21.93			
F9310-02	11.50	249.22	21.39			
F9320-01	8.00	196.51	37.85			
F9320-01	10.00	196.07	35.88			
F9320-01	12.00	194.73	33.83			
F9330-01	0.35	188.92	33.92			
F9330-01	3.35	187.58	33.27			
F9330-01	3.85	187.13	33.11			
F9330-01	4.35	187.13	31.19			
F9330-01	4.85	187.13	31.44			
F9330-01	5.85	186.69	29.46			

Table VII. Cont'd.

Run	ET (h)	PE NFU	PE VFA	ANA NFU	ANA MPR	ANA VFA/M
F9330-01	6.35	186.24	28.95			
F9330-01	7.35	185.35	27.97			
F9330-02	48.35	188.47	33.23			
F9330-02	48.85	189.81	37.13			
F9330-02	49.35	189.81	40.09			
F9330-02	49.85	190.26	38.77			
F9330-02	50.35	190.71	37.18			
F9330-02	50.85	190.26	32.55			
F9330-02	52.35	189.81	32.11			
F9330-02	54.85	188.03	27.68			
F9330-02	55.35	187.58	27.81			
F9330-02	55.85	186.69	27.38			
F9330-02	56.35	186.69	27.84			
F9340-01	0.50			229.83		6.22
F9340-01	1.00			229.83	6.27	6.87
F9340-01	1.50			229.37		7.15
F9340-01	2.00			230.30	6.38	6.57
F9340-01	2.50			230.30		6.00
F9340-01	4.00			231.70	5.83	5.12
F9340-01	5.00			234.02	5.54	4.64
F9340-01	5.50			233.56		4.59
F9340-01	6.00			234.49	5.52	4.54
F9340-01	6.50			234.49		5.11
F9340-01	7.00			233.56	5.61	4.91
F9340-01	8.00			234.02	5.85	5.20
F9340-02	0.50			239.61		6.68
F9340-02	1.00			239.14	6.12	6.94
F9340-02	1.50			239.14		7.83
F9340-02	2.00			241.47	6.73	6.76
F9340-02	2.50			241.47		5.79
F9340-02	3.00			241.01	6.11	5.20
F9340-02	3.50			243.33		4.78
F9340-02	4.00			243.80	5.61	4.76
F9340-02	4.50			242.87		4.57
F9340-02	5.00			243.33	5.21	4.24
F9340-02	5.50			243.33		4.10
F9340-02	6.00			244.27	5.16	4.06
F9340-02	6.50			243.80		4.35
F9340-02	7.00			243.33	5.38	4.47
F9340-02	7.50			241.94		4.37
F9340-02	8.00			241.94	5.05	4.27
F9340-03	0.00	171.21	47.25	245.20	5.86	5.74
F9340-03	0.50	171.70	45.60	244.73		5.50
F9340-03	1.00	170.71	47.60			
F9340-03	1.50	171.21	45.66	246.13		5.47
F9340-03	2.00	170.71	45.71			

Table VII. Cont'd.

Run	ET (h)	PE NFU	PE VFA	ANA NFU	ANA MPR	ANA VFA/M
F9340-03	2.50	170.71	44.73	244.27		5.55
F9340-03	3.00	169.23	43.02	246.59	5.90	5.52
F9340-03	3.50	169.72	42.34	248.46		5.35
F9340-03	4.00	168.73	41.18	247.53	5.87	5.38
F9340-03	4.50	167.25	40.42			
F9340-03	5.00	166.75	40.58	248.46	5.75	5.25
F9340-03	5.50	167.25	38.82			
F9340-03	6.00	166.26	38.71	250.78	5.69	5.12
F9340-04	0.00	156.85	45.60			
F9340-04	0.50	155.36	43.26			
F9340-04	1.00	155.36	43.82			
F9340-04	1.50	155.36	43.22			
F9340-04	2.00	155.36	40.98			
F9340-04	2.50	153.88	40.03			
F9340-04	3.00	152.89	39.71			
F9340-04	3.50	150.90	37.22			
F9340-04	4.00	149.91	36.13			
F9340-04	4.50	148.43	37.20			
F9340-05	0.00			249.85	6.58	7.35
F9340-05	0.50	137.53	68.04			
F9340-05	1.00	138.52	68.89			
F9340-05	1.50	136.05	66.43			
F9340-05	2.00	137.04	65.66			
F9340-05	2.50	134.07	62.72			
F9340-05	3.00	132.58	51.18			
F9340-05	3.50	134.07	51.62	250.32		7.42
F9340-05	4.00	130.60	49.03	248.92	6.22	7.08
F9340-05	4.50	131.10	49.83	250.78		7.19
F9340-05	5.00	127.13	47.72	251.72	5.98	6.89
F9340-05	5.50	128.62	43.81	254.04		6.42
F9340-05	6.00	124.66	46.27	253.11	6.04	6.88
F9340-05	6.50	126.14	46.34	254.51		6.70
F9340-05	7.00	138.03	62.62			
F9340-05	8.00	135.55	49.74			
F9340-06	0.00	164.77	39.74	262.89	5.45	6.10
F9340-06	0.50	164.28	35.23	263.82		5.37
F9340-06	1.00	165.27	34.94	263.82	4.92	5.20
F9340-06	1.50	165.27	32.41	264.75		4.87
F9340-06	2.00	164.28	27.98	265.68	4.38	4.24
F9340-06	2.50	163.78	25.99	267.08		3.90
F9340-06	3.00	162.79	23.99	267.55	3.50	3.57
F9340-06	3.50	158.33	21.34	268.01		3.18
F9340-06	4.00	156.35	17.04	269.87	2.70	2.55
F9340-06	4.50	153.38	14.09	272.67		2.13
F9340-06	5.00	152.89	10.90	275.46	1.93	1.67
F9340-06	5.50	150.90	11.20	275.93		1.71
F9340-06	7.50			266.61		5.72
F9340-06	8.00			266.15	5.12	5.74
F9360-01	0.00			286.63	6.82	6.61

12. Appendix D

Table VIII. Experimental PHA production and VFA consumption data.

Run	Time	Ratio (PHA/VFA)	Average Ratio
F9340-01	7:00	2.41	2.43
F9340-01	8:00	1.55	
F9340-01	9:00	2.94	
F9340-01	10:00	2.28	
F9340-01	11:00	1.37	
F9340-01	13:00	4.15	
F9340-01	15:00	2.32	
F9340-02	7:00	2.19	1.77
F9340-02	8:00	1.86	
F9340-02	9:00	1.35	
F9340-02	10:00	1.20	
F9340-02	11:00	1.42	
F9340-02	13:00	2.14	
F9340-02	15:00	2.26	
F9340-03	7:00	1.24	1.58
F9340-03	8:00	1.66	
F9340-03	9:00	1.47	
F9340-03	10:00	1.63	
F9340-03	11:00	1.42	
F9340-03	12:00	1.45	
F9340-03	13:00	2.17	
F9340-04	8:00	2.21	1.92
F9340-04	9:00	2.47	
F9340-04	10:00	2.18	
F9340-04	12:00	1.51	
F9340-04	14:00	1.21	
F9340-05	7:00	0.22	1.42
F9340-05	8:00	1.15	
F9340-05	9:00	1.52	
F9340-05	10:00	1.96	
F9340-05	11:00	1.54	
F9340-05	13:00	1.53	
F9340-05	14:00	1.54	
F9340-05	15:00	1.90	
F9340-06	7:00	1.52	1.82
F9340-06	8:00	1.26	
F9340-06	9:00	2.16	
F9340-06	10:00	2.01	
F9340-06	11:00	2.26	
F9340-06	12:00	3.79	
F9340-06	13:00	0.40	
F9340-06	14:00	1.34	
F9340-06	15:00	1.68	
F9360-01	06:30	1.26	1.34
F9360-01	07:30	1.70	
F9360-01	08:30	1.76	

Table VIII. Cont'd.

Run	Time	Ratio (PHA/VFA)	Average Ratio
F9340-01	7:00	2.41	2.43
F9340-01	8:00	1.55	
F9340-01	9:00	2.94	
F9340-01	10:00	2.28	
F9340-01	11:00	1.37	
F9340-01	13:00	4.15	
F9340-01	15:00	2.32	
F9340-02	7:00	2.19	1.77
F9340-02	8:00	1.86	
F9340-02	9:00	1.35	
F9340-02	10:00	1.20	
F9340-02	11:00	1.42	
F9340-02	13:00	2.14	
F9340-02	15:00	2.26	
F9340-03	7:00	1.24	1.58
F9340-03	8:00	1.66	
F9340-03	9:00	1.47	
F9340-03	10:00	1.63	
F9340-03	11:00	1.42	
F9340-03	12:00	1.45	
F9340-03	13:00	2.17	
F9340-04	8:00	2.21	1.92
F9340-04	9:00	2.47	
F9340-04	10:00	2.18	
F9340-04	12:00	1.51	
F9340-04	14:00	1.21	
F9340-05	7:00	0.22	1.42
F9340-05	8:00	1.15	
F9340-05	9:00	1.52	
F9340-05	10:00	1.96	
F9340-05	11:00	1.54	
F9340-05	13:00	1.53	
F9340-05	14:00	1.54	
F9340-05	15:00	1.90	
F9340-06	7:00	1.52	1.82
F9340-06	8:00	1.26	
F9340-06	9:00	2.16	
F9340-06	10:00	2.01	
F9340-06	11:00	2.26	
F9340-06	12:00	3.79	
F9340-06	13:00	0.40	
F9340-06	14:00	1.34	
F9340-06	15:00	1.68	

Table VIII. Cont'd.

Run	Time	Ratio (PHA/VFA)	Average Ratio
F9360-01	06:30	1.26	1.34
F9360-01	07:30	1.70	
F9360-01	08:30	1.76	
F9360-01	10:30	1.60	
F9360-01	11:30	1.01	
F9360-01	12:30	1.23	
F9360-01	13:30	0.97	
F9360-01	14:30	1.19	
F9360-02	06:30	1.05	1.44
F9360-02	07:30	1.02	
F9360-02	08:30	1.49	
F9360-02	10:30	1.65	
F9360-02	11:30	1.74	
F9360-02	12:30	1.89	
F9360-02	13:30	1.16	
F9360-02	14:30	1.49	
F9360-03	06:30	1.40	1.94
F9360-03	07:30	1.92	
F9360-03	08:30	1.63	
F9360-03	10:30	2.06	
F9360-03	11:30	2.72	
F9360-03	12:30	1.45	
F9360-03	13:30	2.06	
F9360-03	14:30	2.25	
F9360-04	06:30	1.51	1.64
F9360-04	07:30	1.88	
F9360-04	08:30	1.78	
F9360-04	09:30	2.05	
F9360-04	10:30	1.78	
F9360-04	11:30	1.18	
F9360-04	12:30	1.58	
F9360-04	13:30	1.32	
F9360-06	06:30	1.87	1.65
F9360-06	07:30	1.21	
F9360-06	09:30	1.83	
F9360-06	10:30	1.55	
F9360-06	11:30	1.76	
F9360-06	12:30	1.39	
F9360-06	13:30	1.94	
F9360-07	06:30	1.58	1.46
F9360-07	07:30	1.67	
F9360-07	08:30	1.41	
F9360-07	09:30	1.33	
F9360-07	10:30	1.40	
F9360-07	12:30	1.40	

Table VIII. Cont'd.

Run	Time	Ratio (PHA/VFA)	Average Ratio
F9360-07	13:30	1.40	
F9380-01	06:30	2.19	1.74
F9380-01	07:30	1.63	
F9380-01	08:30	1.49	
F9380-01	09:30	2.14	
F9380-01	10:30	1.63	
F9380-01	11:30	1.55	
F9380-01	12:30	1.83	
F9380-01	13:30	1.48	
F9380-02	06:30	1.54	1.75
F9380-02	07:30	1.74	
F9380-02	08:30	1.27	
F9380-02	09:30	1.80	
F9380-02	10:30	1.60	
F9380-02	11:30	1.90	
F9380-02	12:30	2.44	
F9380-02	13:30	1.71	
F9380-03	06:30	1.31	1.55
F9380-03	07:30	1.45	
F9380-03	08:30	1.84	
F9380-03	09:30	1.69	
F9380-03	10:30	1.42	
F9380-03	11:30	1.55	
F9380-03	12:30	1.59	
F9380-04	06:30	1.75	1.95
F9380-04	07:30	1.45	
F9380-04	08:30	2.18	
F9380-04	09:30	2.19	
F9380-04	10:30	1.87	
F9380-04	11:30	2.41	
F9380-04	12:30	1.92	
F9380-04	13:30	1.86	
F9380-05	06:30	1.97	2.02
F9380-05	07:30	2.21	
F9380-05	08:30	1.43	
F9380-05	09:30	1.73	
F9380-05	10:30	2.08	
F9380-05	11:30	2.09	
F9380-05	12:30	2.95	
F9380-05	13:30	1.71	

13. Appendix E

The experimental procedures used for testing the effects of transient VFA loading on effluent phosphate concentrations at the FGR-SGR pilot plant adhered to the following;

- VFA load variations were achieved by regulating the fermenter feed rate to control fermentate addition to the primary influent. Alternately, typical VFA load variations were simulated by the controlled addition of a sodium acetate solution (Sigma Chemical Co., St. Louis, MO.) to the primary effluent stream using a computer controlled peristaltic pump (Model 420-C Dual Channel Peristaltic Pump, BioChem Technology Inc., King of Prussia, PA.). The pump was operated such that the pumping rate of a freshly prepared stock solution of sodium acetate was ramped from 0 mL/h to 1000 mL/h and back to 0 mL/h over a period ranging from one to three hours. The concentration of the stock solution was controlled to achieve an increase in soluble acetate in the anaerobic bioreactor of approximately 20 mg/L when added at the maximum pumping rate.
- samples were collected occasionally from the anaerobic zone, and regularly from the effluent stream for analysis of soluble phosphate concentrations. Effluent samples were collected at intervals ranging between thirty and ninety minutes.
- at the expected peak of VFA load, a primary effluent sample was collected and assayed for VFA concentration to confirm that the VFA concentration of the primary effluent stream did, in fact, increase to the desired level.
- analysis of parameters was accomplished by the methods and procedures outlined in Section 4.
- once the analyses were complete, effluent soluble phosphate concentrations were analyzed for determination of mean, minimum, maximum, and range of phosphate concentration, as well as for determination of standard deviations, coefficients of variation and average coefficients of variations for the grouped data.

14. Appendix F

Table IX. Effluent phosphate data.

Expt.	Time	PO_4 (mg P/L)	Average PO_4
V401	07:00	0.49	0.52
V401	08:00	0.46	
V401	09:00	0.54	
V401	10:00	0.65	
V401	11:00	0.66	
V401	12:00	0.71	
V401	13:00	0.57	
V401	14:00	0.35	
V401	15:00	0.25	
V402	07:00	0.87	0.76
V402	08:00	0.88	
V402	09:00	0.86	
V402	10:00	1.15	
V402	11:00	1.18	
V402	12:00	0.81	
V402	13:00	0.58	
V402	14:00	0.34	
V402	15:00	0.17	
N403	07:00	0.35	0.28
N403	08:00	0.29	
N403	09:00	0.25	
N403	10:00	0.39	
N403	11:00	0.19	
N403	12:00	0.24	
N403	13:00	0.22	
N404	08:00	0.23	0.18
N404	09:00	0.33	
N404	10:00	0.14	
N404	11:00	0.19	
N404	12:00	0.18	
N404	13:00	0.12	
N404	14:00	0.08	
V405	07:00	0.31	0.31
V405	08:00	0.30	
V405	09:00	0.37	
V405	10:00	0.54	
V405	11:00	0.61	
V405	12:00	0.40	
V405	13:00	0.16	
V405	14:00	0.05	
V405	15:00	0.05	

Table IX. Cont'd.

Expt.	Time	PO_4 (mg P/L)	Average PO_4
V406	07:00	0.43	0.20
V406	08:00	0.43	
V406	09:00	0.38	
V406	10:00	0.24	
V406	11:00	0.14	
V406	12:00	0.06	
V406	13:00	0.05	
V406	14:00	0.06	
V406	15:00	0.05	
V601	06:30	1.04	0.57
V601	07:30	1.02	
V601	08:30	1.01	
V601	09:30	0.81	
V601	10:30	0.56	
V601	11:30	0.29	
V601	12:30	0.17	
V601	13:30	0.14	
V601	14:30	0.10	
N602	06:30	1.06	0.58
N602	07:30	1.04	
N602	08:30	0.99	
N602	09:30	0.77	
N602	10:30	0.53	
N602	11:30	0.42	
N602	12:30	0.25	
N602	13:30	0.10	
N602	14:30	0.07	
V603	06:30	4.41	3.69
V603	07:30	4.37	
V603	08:30	4.38	
V603	09:30	4.19	
V603	10:30	3.87	
V603	11:30	3.60	
V603	12:30	3.19	
V603	13:30	2.68	
V603	14:30	2.52	
N604	06:30	3.55	3.22
N604	07:30	3.66	
N604	08:30	3.50	
N604	09:30	3.28	
N604	10:30	3.11	
N604	11:30	2.86	
N604	12:30	2.89	
N604	13:30	2.88	

Table IX. Cont'd.

Expt.	Time	PO_4 (mg P/L)	Average PO_4
V605	06:30	1.36	2.26
V605	07:30	1.21	
V605	08:30	1.13	
V605	09:30	1.50	
V605	10:30	3.48	
V605	11:30	4.90	
N606	06:30	3.02	2.98
N606	07:30	2.97	
N606	08:30	3.10	
N606	09:30	3.10	
N606	10:30	2.89	
N606	11:30	2.97	
N606	12:30	2.81	
N606	13:30	2.97	
N607	06:30	2.80	2.64
N607	07:30	2.82	
N607	08:30	2.65	
N607	09:30	2.59	
N607	10:30	2.46	
N607	11:30	2.57	
N607	12:30	2.68	
N607	13:30	2.52	
N801	06:30	2.34	2.30
N801	07:30	2.33	
N801	08:30	2.36	
N801	09:30	2.33	
N801	10:30	2.19	
N801	11:30	2.32	
N801	12:30	2.31	
N801	13:30	2.21	
N802	06:30	1.10	1.12
N802	07:30	1.16	
N802	08:30	1.09	
N802	09:30	1.23	
N802	10:30	1.09	
N802	11:30	1.06	
N802	12:30	1.17	
N802	13:30	1.10	
N803	06:30	1.45	1.47
N803	07:30	1.58	
N803	08:30	1.52	
N803	09:30	1.43	
N803	10:30	1.51	
N803	11:30	1.36	
N803	12:30	1.44	
N804	06:30	0.31	0.19

Table IX. Cont'd.

Expt.	Time	PO_4 (mg P/L)	Average PO_4
N804	07:30	0.23	
N804	08:30	0.20	
N804	09:30	0.17	
N804	10:30	0.16	
N804	11:30	0.17	
N804	12:30	0.15	
N804	13:30	0.10	
N805	06:30	0.16	
N805	07:30	0.17	
N805	08:30	0.19	
N805	09:30	0.19	
N805	10:30	0.20	
N805	11:30	0.23	
N805	12:30	0.26	
N805	13:30	0.17	

15. Appendix G

Table X. Phosphate removal and PHA utilization data.

Test Date	PO4 removed (mg P/L)	MLSS (mg/L)	PO4 removed MLSS (mg P/g MLSS)	PHA used (mg HA/g MLSS)
Aug 9	23.01	3729	6.17	13.73
Aug 12	20.98	3689	5.69	9.27
Aug 16	22.91	3798	6.03	9.19
Aug 19	21.22	3646	5.82	10.89
Aug 23	23.43	3304	7.09	11.52
Aug 25	12.61	3094	4.08	7.30
Sept 1	17.10	2678	6.39	8.49
Sept 6	17.48	2789	6.27	7.12
Sept 13	11.13	2209	5.04	9.60
Sept 15	11.06	1811	6.11	11.42
Sept 20	8.21	1350	6.08	12.07
Sept 27	15.17	1808	8.39	14.64
Sept 29	17.39	2061	8.44	15.03
Oct 4	20.37	2410	8.45	14.94
Oct 7	15.43	2680	5.76	10.73
Oct 9	14.53	2700	5.38	9.56
Oct 11	24.50	2825	8.67	16.28
Oct 13	22.97	3209	7.16	13.05

16. Appendix H

Table XI. Average daily mass nitrate removal and fluorescence data.

Day	Average daily ANA-ANO NFU	Average daily Mass NOx Removal (mg N/g MLSS)	Average daily ANO NOx (mg N/L)
0	25.04	0.38	0.64
4	25.67	0.42	0.45
8	24.51	0.44	0.42
11	22.36	0.42	0.37
15	23.63	0.42	0.48
19	26.00	0.10	1.64
23	5.00	0.42	0.56
28	23.72	0.35	1.16
32	22.69	0.28	1.06
36	18.40	0.01	1.61
40	15.63	0.01	1.58

17. Appendix I

Data from the Penticton real-time control tests.

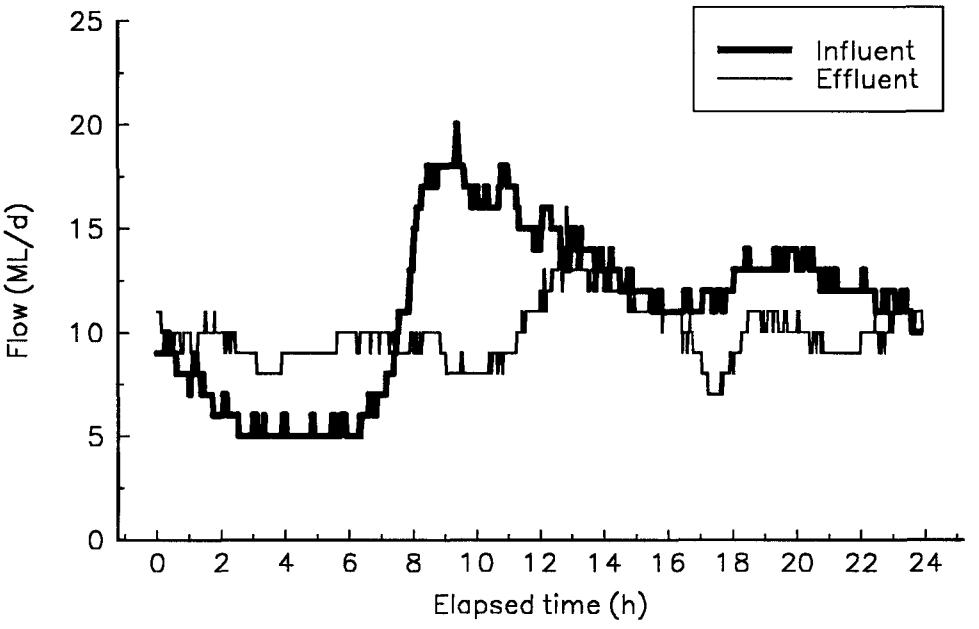

Figure 17.1. Process influent and effluent flow data.

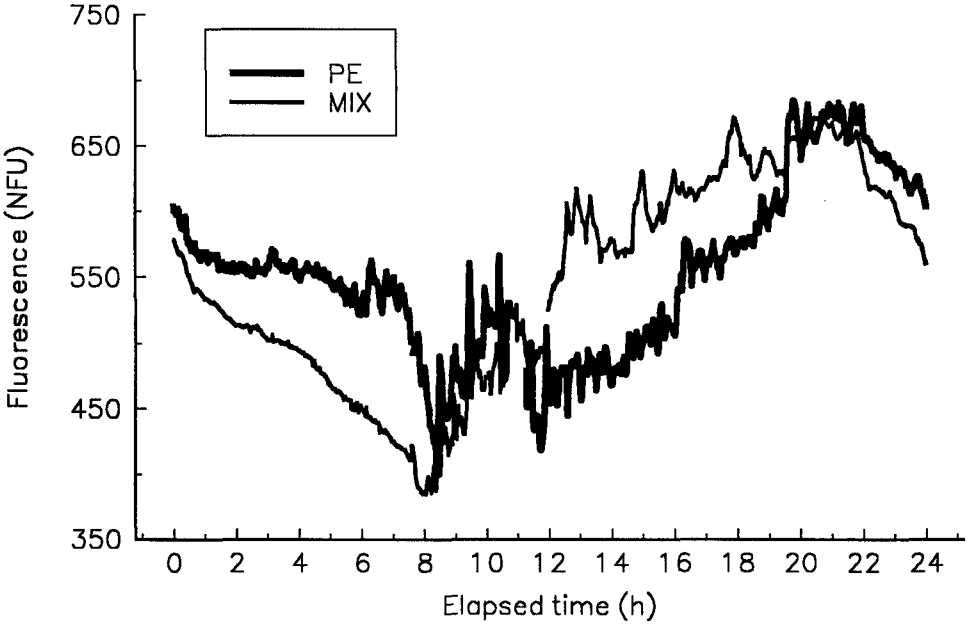

Figure 17.2. Primary effluent and mix stream fluorescence.

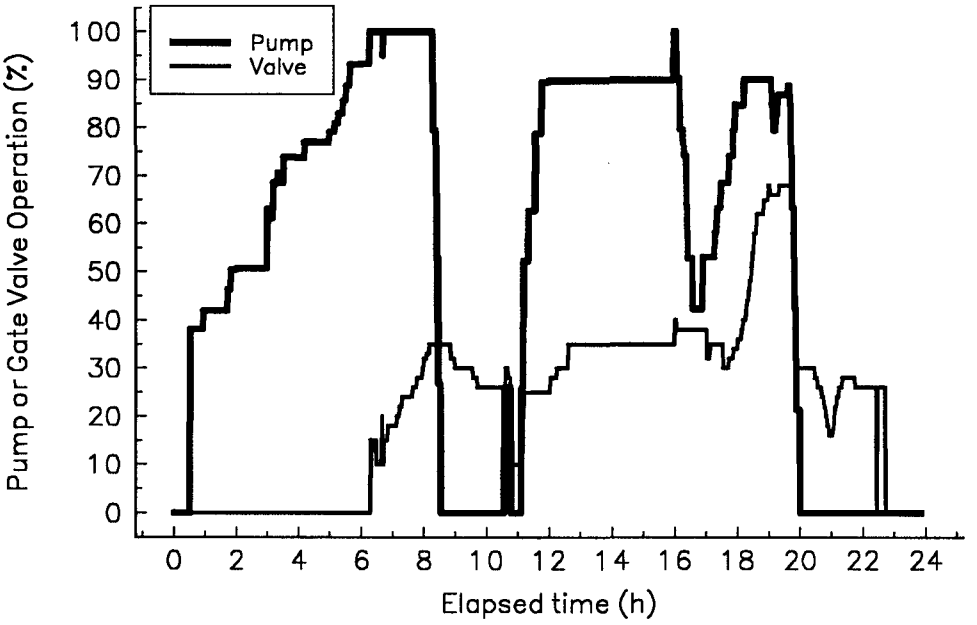

Figure 17.3. Equalization pump and gate valve operation records.