Factors Influencing Dewaterability of Thermophilic Aerobically Digested Biosolids

by

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Abstract

This dissertation reports on research findings from a study investigating factors that influence the dewaterability and other characteristics of thermophilic aerobically digested biosolids. Thermophilic aerobic digestion is a high temperature (50-65°C) sludge treatment process that produces Class A biosolids. Experience from operating full-scale facilities has revealed that dewatering thermophilically digested sludge requires substantially higher dosages of polymers for conditioning, compared to dewatering of mesophilically digested sludge.

The objectives of this research program were to investigate how feed sludge composition, digestion temperature, digestion time, and mixing induced shear affect the dewatering properties of digested sludge. The characteristics of thermophilically digested sludge and mechanisms related to dewatering thermophilically digested sludge were also studied. The experimental work was carried out at laboratory scale, using batch operated aerobic digesters. Dewaterability was measured as specific capillary suction time (SCST).

This research found that feed sludge composition is an important factor affecting dewaterability (measured as SCST) of the digested sludge. Regardless of how the sludge was digested, the measured SCST exponentially correlated to the weight proportion of the secondary sludge contained in the feed. A higher proportion of secondary sludge in the feed resulted in a higher SCST in the sludge. Dewaterability did not correlate to pH, volatile solids, concentrations of ammonia and phosphate in digested sludge, but correlated to concentrations of soluble extracellular proteins and polysaccharides.

Digestion temperature had a significant effect on dewaterability (measured as SCST) of the digested sludge. When the sludge, containing 100% secondary sludge, was digested at 55°C or higher temperatures, digestion resulted in immediate and significant increases in SCST. When the same type of sludge was digested at 40 or 50°C, digestion also resulted in a significant increase in SCST, but the rate of increase in SCST was lower than the rate when the sludge was digested at 55°C or higher. Following the initial surge in SCST, continued digestion at 55°C or higher temperatures for more than 1 d resulted in a reduction in SCST; while continued digestion at 40°C or 50°C did not result in much change in SCST. Mesophilic digestion resulted in a progressive increase in SCST over the entire duration of 10-12 d digestion. Thermophilic
digestion did not result in much change in floc charge, but did cause an immediate reduction in floc size. Digestion at all temperatures resulted in a reduction in solids, changes in pH, conductivity, concentrations of ammonia and phosphate, and phosphorus distribution among the solid and liquid phases of the sludge. However, the deterioration in dewaterability of digested sludge did not correlate to changes in these parameters; instead, it correlated to concentrations of soluble extracellular proteins and polysaccharides in the digested sludge.

The substances affecting dewaterability of thermophilically digested sludge was mainly associated with the liquid phase of the digested sludge. The soluble extracellular proteins had small sizes, with 86% of such materials less than 7,000 Daltons. These proteins could not be effectively stained by Coomassie Brilliant Blue dye, and were not affected by boiling treatment. Protease treatment confirmed that protein played a role in affecting dewaterability.

The deterioration in dewaterability, due to thermophilic digestion, was a physical-chemical phenomenon, not a microbiological phenomenon, although the substances resulting in deterioration of dewaterability (such as extracellular proteins) were originated from bacterial cells. Mechanical shear applied to the digested sludge had a significant effect on dewaterability, regardless of digestion temperatures. The effect of mechanical shear was due to the reductions in floc charge and size.

Digestion and mixing induced shear resulted in changes in distribution of cations among the solid and liquid phases of the digested sludge. However, dewaterability was not associated with changes in ratios of monovalent to divalent cations. Thermophilic digestion and mechanical shear resulted in a reduction in the initial yield and adhesion coefficients of digested sludge. Digested sludge showed non-Newtonian characteristics, in particular, the shear-thinning property. For thermophilically digested sludge, an initial polymer demand needed to be satisfied first, before the SCST could be substantially reduced by polymer conditioning.
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Abs</td>
<td>Absorption</td>
</tr>
<tr>
<td>ATAD</td>
<td>Autothermo thermophilic aerobic digestion</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CST</td>
<td>Capillary suction time/timer</td>
</tr>
<tr>
<td>D.O.</td>
<td>Dissolved oxygen</td>
</tr>
<tr>
<td>MAD</td>
<td>Mesophilic aerobic digestion</td>
</tr>
<tr>
<td>MCRT</td>
<td>Mean cell residence time</td>
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<tr>
<td>MEM</td>
<td>Micro-electrophoretic mobility</td>
</tr>
<tr>
<td>ORP</td>
<td>Oxidation reduction potential</td>
</tr>
<tr>
<td>OUR</td>
<td>Oxygen uptake rate</td>
</tr>
<tr>
<td>SCST</td>
<td>Specific capillary suction time (a measurement of dewaterability)</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulphate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SMD</td>
<td>Surface mean diameter</td>
</tr>
<tr>
<td>SOUR</td>
<td>Specific oxygen uptake rate</td>
</tr>
<tr>
<td>SRF</td>
<td>Specific resistance to filtration</td>
</tr>
<tr>
<td>SRT</td>
<td>Sludge retention time</td>
</tr>
<tr>
<td>TAnD</td>
<td>Thermophilic anaerobic digestion</td>
</tr>
<tr>
<td>TCA</td>
<td>Trichloroacetic acid</td>
</tr>
<tr>
<td>TS</td>
<td>Total solids</td>
</tr>
<tr>
<td>TVS</td>
<td>Total volatile solids</td>
</tr>
<tr>
<td>UBC</td>
<td>University of British Columbia</td>
</tr>
<tr>
<td>USEPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
<tr>
<td>WWTP</td>
<td>Wastewater treatment plant</td>
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</table>
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1.0 Introduction

This dissertation describes a research program that investigated factors influencing the dewaterability of thermophilic aerobically digested biosolids.

The worldwide applications of wastewater treatment contribute significantly to the protection of precious water resources and the improvements of water quality. The operation of a typical municipal wastewater treatment plant produces residual solids, such as primary sludge and secondary sludge (waste activated sludge). In Canada’s Greater Vancouver area, over 20,000 dry tons/year of sludge are produced from wastewater treatment facilities that serve a population of about 1.9 million. Presently, there are about 16,000 publicly owned wastewater treatment facilities in the United States alone, serving 72% of its total population (Laughlin, 1997). The total sludge production from these treatment plants is approximately 6.8 million dry tons/year (Bastian, 1997). With full-scale wastewater treatment facilities, typically, digested sludge are referred to as biosolids, and undigested or partially digested sludge are referred to as sludge. Samples from the laboratory experimental work of this research program are referred to as digested sludge in most cases, and as biosolids occasionally, because the degree of digestion of these sludge samples varied.

Sludge conditioning and dewatering are important steps prior to the final disposal of sludge. The conditioning aims to ensure adequate capture of solids in the dewatering step and to increase the dewatering rate many-fold, through improving the dewatering characteristics of the sludge. The conditioning chemicals most widely used are organic polyelectrolytes, namely polymers. The dewatering is intended to reduce the water content in sludge from about 90-95% to 65-80%, so that the final sludge volume to be stored and transported is substantially reduced. Polymer cost is considered as the most significant cost factor in the operation and maintenance of a sludge handling system (WEF, 1988).

Over the last 20 years, sludge management practice in North American has seen significant changes, moving from landfill disposal to beneficial reuse (e.g. agricultural soil conditioning or forest fertilisation). The increased reuse is due to increased sludge amounts, higher disposal costs, and difficulties in finding appropriate and adequate disposal sites. In 1993, the United States Environmental Protection Agency (USEPA) finalised its technical standards (40 CFR
Part 503) for the use and disposal of biosolids (digested sludge), and introduced the criteria for Class A biosolids. Class A biosolids are essentially pathogen free, meet metal concentrations and vector attraction reduction requirements, and can be beneficially reused without restrictions (WEF, 1995, Bastian, 1997). Wastewater treatment facilities are now actively looking for ways to produce Class A biosolids. Autothermo Thermophilic Aerobic Digestion (ATAD) is a high temperature sludge digestion process that can achieve cost-effective production of Class A biosolids (WEF, 1995). Findings from this research intend to benefit environment managers and consulting engineers in making well-informed decisions in selecting ATAD process, and assist ATAD sludge treatment facilities to reduce the cost of sludge handling operations.

The dissertation is set out in 6 chapters. Chapters 1 and 2 describe the issues of the research, the processes of sludge digestion, conditioning and dewatering, and current knowledge on this subject. Chapter 3 describes the research program, experimental facilities, methodologies and protocols. Chapter 4 presents results and discusses the implications of research findings. Chapter 5 offers conclusions and recommendations. Cited references are listed, following Chapter 5. Details of experimental procedures are in Appendix A.

1.1 Research Needs

The need to carry out research on the dewatering of thermophilically digested biosolids was derived from several factors: biosolids conditioning and dewatering are far from problem free; the efficiencies and effectiveness of conditioning and dewatering need to be improved. WERF (1998) found, from its 1997 Research Need Survey, that WERF subscribers, mostly wastewater treatment utilities and prominent consulting engineers, ranked Biosolids Conditioning & Dewatering as the second priority (the first priority was Automation), and the Biosolids Beneficial Uses as the third priority in its list of 28 items. However, the WERF project on state-of-art biosolids dewatering processes did not address the issue of dewatering thermophilically digested biosolids (Pincince et al., 1997).

Although the ATAD process has been widely used in European countries such as Germany and Great Britain since the 1970s, little information was available on dewatering ATAD biosolids, because European ATAD plants applied digested sludge as a liquid to the land (USEPA, 1990). The ATAD process was introduced into North American in the late 1980s, with 3 full-scale demonstration facilities built in British Columbia of Canada (Kelly, 1990). Since then, over 20
ATAD plants have been constructed and operated in Canada and United States (Table A.1 of Appendix A). Recent experience, from operating full-scale ATAD facilities in Canada and United States, revealed that dewatering ATAD biosolids required much higher dosage of polymers than dewatering mesophilic aerobically digested biosolids.

When compared to that of undigested sludge, the dewaterability of ATAD biosolids was comparable, if the feed contained only primary sludge (Kelly et al., 1993); however, it was worse if the feed consists of secondary sludge (Matsch and Drnevich, 1977, Jewell and Kabrick, 1980, Trim and McGlashan, 1985, Kelly et al., 1993, and Messenger et al., 1993). When compared to mesophilically digested biosolids, ATAD biosolids usually demanded higher dosage of polymers for conditioning, in order to achieve sufficient solids recovery and efficient liquid-solid separation. However, when adequately conditioned, ATAD biosolids could achieve higher cake dryness and satisfactory solids (Table A.1 of Appendix A). Reported polymer dosage to dewater ATAD biosolids at full-scale facilities, as well as typical polymer needs to dewater mesophilic aerobically and anaerobically digested biosolids are summarised in Table A.1 of Appendix A.

Relevant information, regarding dewatering of thermophilic anaerobically digested (TAnD) biosolids, was limited and contradictory. Peddie (2000) reported that polymer needs for conditioning at Greater Vancouver’s two thermophilic anaerobic digestion (TAnD) plants were approximately 12-18 kg/dt TS; cake dryness of dewatered sludge was about 25% from digested mixture of primary and secondary sludges and about 35% for digested primary sludge. Garber et al. (1975) reported that TAnD sludge showed lower polymer dosage (4.5 kg/dt TS vs. mesophilic 6.5 kg/dt TS), higher solids capture, and better cake dryness (30% vs. mesophilic 20%). However, Garber (1982) later reported that TAnD sludge needed higher polymer dosage (5 kg/dt TS vs. mesophilic 2 kg/dt TS); TAnD demonstrated higher solid capture and better cake dryness (30-35%) than mesophilic anaerobically digested sludge (20-25% cake dryness).

In summary, it is apparent that, thermophilic digestion of wastewater sludge becomes more widely accepted as an effective and efficient process to produce Class A biosolids, dewatering thermophilically digested biosolids is substantially more costly than dewatering mesophilically digested biosolids. The knowledge and understanding of dewatering thermophilically digested biosolids are limited and incomplete.
1.2 Research Objectives

The purpose of this research was to seek answers to the following questions:

1. What are important factors that influence the dewaterability of thermophilically digested biosolids?
2. Can the dewaterability of thermophilically digested biosolids be improved through optimizing the digestion operation?
3. What is the mechanism that affects dewatering properties of thermophilically digested biosolids?

To respond to these questions, the basic objectives of this research were:

1. To compare and contrast the characteristics and dewatering properties of thermophilic and mesophilic aerobically digested biosolids.
2. To investigate the effects of feed sludge composition, digestion temperature, digestion time (sludge retention time), and mixing induced shear on dewatering properties of thermophilically aerobically digested biosolids.
3. To investigate the fundamental mechanism that affects the dewatering properties of thermophilically aerobically digested biosolids.
2.0 Literature Review

2.1 Thermophilic and Mesophilic Aerobic Digestion Processes

This section provides a summary of process theories, typical operation and performance characteristics of both thermophilic and mesophilic (conventional) aerobic digestion processes. The information serves as a guide to the design and operation of experimental work for this research program.

2.1.1 Thermophilic Aerobic Digestion

Thermophilic aerobic digestion treats sludge aerobically at thermophilic temperatures (50°C to 80°C). When the digestion can sustain its high temperature operation without supplemental heat beyond what is supplied by mixing energy, such a process is referred to as the Autothermal Thermophilic Aerobic Digestion (ATAD) process, (WEF-ASCE, 1998). In this dissertation, for the convenience and consistence of the presentation, ATAD was used to as an abbreviated term to describe the thermophilic aerobic digestion process, regardless of how the thermophilic digestion temperatures were sustained.

Studies of ATAD process began in 1960s and continued into the 1970s. Example works include those of Woodley (1961), Kambhu and Andrews (1969), Popel and Ohnmacht (1972), Matsch and Drnevich (1977), and Jewell and Kabrick (1980). By the 1980s, about 35 full-scale ATAD facilities were installed and operated in Europe, mostly in Germany (Deeney et al., 1985; USEPA, 1990). ATAD was also investigated for use as the first step in the “thermophilic aerobic pre-treatment - mesophilic anaerobic treatment” process (Trim and MaGlashan, 1985, and Messenger, J. R. et al., 1993). Full-scale ATAD operations were introduced into North America by Kelly (1990) in late 1980s. Since then, ATAD has been applied at full-scale in many places in Canada and United States. This noted research and operation experience led to the development of new knowledge and an understanding of operation and performance of the ATAD process, which includes operating temperature, oxygen supply, mixing energy, sludge feed, volatile solids destruction, and pathogen reduction. Research work conducted at the University of British Columbia advanced the knowledge of volatile fatty acid production (Chu, 1995, and Fothergill, 1996) and digestion of phosphorus-rich sludge in ATAD (Boulanger, 1995, and Cross, 1995). However, the dewatering of ATAD biosolids has never been thoroughly investigated. ATAD process theories, operation and performance are summarised as follows:
Process theory

The ATAD process to thermophilic aerobically biodegrade cell mass can be described by Equation 2.1, where $\text{C}_5\text{H}_7\text{NO}_2$ was used to represent the cell compositions of a microorganism (Matsch and Drnevich, 1977).

\[
\text{C}_5\text{H}_7\text{NO}_2 + 5 \text{ O}_2 \rightarrow 5\text{CO}_2 + 2\text{H}_2\text{O} + \text{NH}_3 + \text{Energy}
\]  

The biological reactions include (1) solubilisation and oxidation of organic matter due to the death of organisms by cell lysis, and (2) Cryptic growth through the use of lysis products by active cells of either the same or different strain (Booth and Tramontini, 1984, and Haner et al., 1994). At thermophilic temperatures, nitrification is inhibited. When sufficient energy is released and retained in the reactor, the temperature in the reactor will rise until a heat balance is achieved.

Thermophilic microorganisms

The classifications include strict thermophiles, having optimum growth temperatures of over 55°C; facultative thermophiles, having an optimum temperature of 50-55°C; and thermo-tolerant thermophiles, having an optimum temperature of 40-50°C (Gaughran, 1947). However, Allen (1953) indicated that the temperature range of the microbial growth was a continuous spectrum; the properties of microbes growing above a certain temperature were not distinctly different from those growing below. Sonnleitner and Fiechter (1983a, and 1983b) found that a microbial populations in a continuous ATAD process are broadly distributed. Temperature variations within this distribution have no lethal effects to the viable population. The temperature range of the isolated bacteria from ATAD is often from 40°C and mostly up to 80°C. The bacterial isolates were all rod-shaped, with cell sizes within the range of 0.5-1 μm times 2-5 μm.

Process feed

This can be either a mixture of primary and secondary sludge, the secondary sludge (waste activated sludge) only, or the primary sludge only. To sustain autothermal operation, the feed must contain a minimum volatile solids content of 25 g/L (2.5%) and 40 g/L COD. A minimum of 3% total solids concentration, with a typical range of 4-6% TS, and 40 g/L COD or greater, is
desirable. A feed of less than 3% solids contains too much water and may not achieve autothermal conditions (WEF-ASCE, 1998).

**Sludge retention time (SRT)**
To achieve sufficient destruction of pathogen and total organic solids, USEPA (1990) recommends a 5 to 6 day SRT. Kelly *et al.* (1993) suggested a 400-500 degree-day (a product of temperature in degree Celsius and total SRT in days, Mavinic and Koers, 1979) is needed for a minimum of 38% volatile solids reduction.

**Aeration and mixing**
These two parameters are related to mechanical mixing devices. Typical operations are designed for a specific power input of 100-250 W/m$^3$, with an air input of 1-4 m$^3$/m$^3$-hour of active reactor volume for feed volatile solids of 2.5-5% (USEPA 1990, and Kelly *et al.* 1993).

**Stages, temperature and pH**
It is usually desirable to operate ATAD as a two stage series semi batch process, to prevent short circuiting of pathogens. The first reactor typically operates at temperatures of 35-50°C and pH near 7.2. The second reactor operates mostly at temperatures of 50-60°C and a pH near 8.0 (USEPA, 1990).

2.1.2 *Mesophilic Aerobic Digestion*
The mesophilic aerobic digestion (MAD), or conventional aerobic digestion, is treat the sludge in the temperature range of 10°C to 40°C. This sludge digestion process has been used for more than 25 years, mostly for design capacity of less than 20,000 m$^3$/d. MAD offers the advantages of low capital costs, simple operation control, less susceptible to toxicity, and the production of stabilized (adequate reduction of volatile solids in sludge) biosolids; however, MAD requires a high cost for oxygen supply (WEF-ASCE, 1998).

**Process theory**
The MAD process to mesophilic aerobically biodegrade cell mass can be described by Equation 2.2, where C$_5$H$_7$NO$_2$ was used to represent the cell composition of a microorganism (WEF-ASCE, 1998).
The biological process involves direct oxidation of biodegradable matter to form extracellular materials and subsequent oxidation of microbial cells by microorganisms. Aerobic digestion is virtually an endogenous respiration process, where substrate is depleted and microorganisms begin to consume their own protoplasm to obtain energy for cell maintenance reactions. Extended aeration at mesophilic temperature promotes nitrification, which will consume alkalinity in the sludge.

**Process feed**
The MAD process is typically used to treat secondary sludge, but has been used for primary and secondary mixtures. The latter one will require substantially increased SRT and oxygen supply, due to additional biodegradable materials.

**Sludge retention time (SRT)**
Treatment objectives govern SRT. In the past, a 10-15 day SRT has been used to achieve 40-45% volatile solid reduction at temperature of approximately 20°C. Jaworski *et al.* (1961) found that beyond digestion period of 15 days, only small increases in the reduction of volatile solids are obtained. However, to meet *40 CFR Part 503* regulations on pathogen reduction, USEPA regulates that SRT has to be 40 days at 20°C, or 60 days at 15°C, in order to meet pathogen reduction criteria, without being required to monitor fecal coliforms periodically (WEF-ASCE, 1998).

**Aeration and mixing**
Aeration demands depend on types of sludge feed and the oxygen supply device. Typically, 0.25-0.33 L/m³-s of digester volume is required for waste activated sludge (WAS), 0.40-0.50 L/m³-s for a mixture of primary and WAS, and 0.33-0.67 L/m³-s for adequate mixing. Mixing power requirements range from 10 to 100 W/m³ of digester volume (WEF-ASCE, 1998).

**Stages, temperature and pH**
MAD is often seen to operate as single stage. Operating temperatures depend on feed temperatures and that of the ambient environment, typically in the range of 15-35°C. When there
is sufficient oxygen and SRT, ammonia will nitrify and form nitrates, resulting in the depletion of alkalinity and a decrease in pH. During long aeration times, pH may drop to pH 5.5 or lower.

In summary, ATAD and MAD are different sludge treatment processes. In this research, ATAD and MAD bioreactors were operated in parallel. The characteristics of digested biosolids from both bioreactors were compared, to identify important factors affecting dewatering properties of ATAD biosolids.

2.2 The Conditioning and Dewatering of Sludge

2.2.1 The Water Distribution in Sludge and Limitation of Dewatering

Understanding water distribution in sludge has the following practical reasons:

1. To acknowledge the limit of dewatering, and assist in selecting appropriate dewatering mechanism and operation. For example, if we recognise that economical water removal has a limit of 70% moisture content, then it is not desirable to pursue further moisture reduction, unless the extra effort and cost can be justified.

2. To optimize sludge conditioning by understanding the fractioning of water in sludge and how much water can be removed by chemical conditioning. For example, if we consider polymer conditioning is primarily to help the removal of water entrapped in the flocs, we may take an alternative approach, such as breaking the floc structure to free water, this may result in the reduction of polymer consumption. We may also accept the limit of water removal by polymer conditioning, and refrain from adding excessive polymer for little increase in water removal.

Vesilind (1994) classified water fractions in the sludge as follows:

1. Free (or bulk) water: this water is not attached to and is not influenced by sludge solids. The free water can be removed by drainage, simple gravitational settling, or application of weak mechanical strains.
2. Interstitial (or floc) water: this water is trapped in interstitial spaces of sludge floc and organisms, and is held within the structure of floc or microbial cells. When the floc or cells are destroyed, this water will be released and become free water. Mechanical energy is needed to destroy and compress floc structure, in order to squeeze out this water.

3. Vicinal water: These are multiple layers of water molecules, which are physically bound to the particle surface by hydrogen bonding. Unlike the interstitial water, this water is associated with a solid surface, and cannot be set free when physical confinement is eliminated. This water cannot be mechanically removed, unless conditioning is applied.

4. Water of hydration: This water is chemically bound to the particles and can only be removed by thermal treatment. For example, thermal conversion of slake lime Ca(OH)\textsubscript{2} to quicklime (CaO) removes water of hydration. Mechanical dewatering cannot remove this water.

Vesilind (1994) considered the historic definition of *bound water* to mean interstitial, vicinal and hydration water. He believed that polymers only influence the interstitial water; mechanical devices can remove free water and much of the interstitial water, but not the vicinal water or hydration water. Dick and Drainville (1995) suggested that polymer conditioning, through particle interactions, could also displace vicinal water and convert it to free water, thereby reducing the vicinal water.

Information on a typical distribution of water fractions and the role of polymer in sludge conditioning is particularly important to the design process. It is recognised that the values of bound water fraction depend on the measurement method (Dick and Drainville, 1995, and Vesilind, 1995).

Katsiris and Kouzeli-Katsiri (1987) measured bound water using differential thermal analysis (DTA). They considered two states of water in the sludge: free water and bound water. The latter form is bound to the solids by (a) sorption on specific sites such as functional groups of proteins and other macromolecules, and/or (b) restricted within pores and capillaries. Their work found that activated sludge of 0.4% DS (total dry solids concentration) has a bound water
content of 9-12 g/g DS (the mass of bound water divided by the mass of total dry solids); that 22
days of aerobic digested sludge with 2.5% DS has a bound water content of 3 g/g DS. They also
observed that the addition of cationic polymer led to the complete depletion of bound water in
the activated sludge sample. They speculated that polymers acted by readily absorbing at solid-
liquid interfaces to displace the molecular water. However, they did not address the role of
polymers in removing interstitial water.

Robinson and Knocke (1992) used the dilatometric method to measure bound water, which was
defined as water that did not freeze at a certain temperatures (e.g. −20°C) below the freezing
point of water. They found that aerobically digested sludge had a bound water of about 5.2 g/g
DS. Polymer conditioning reduced bound water of anaerobically digested sludge by
approximately 50% (from about 3.9 g/g DS to 1.9 g/g DS), and improved dewaterability. The
cake dryness of the dewatered sludge was dictated by the water present in the interstitial spaces,
not those bound to the particle surfaces. Studies on the accuracy and precision of the
dilatometric method were discussed in Smith and Vesilind (1995), Lee (1996), and Wu et al.

In contradiction, Tsang and Vesilind (1990), using a drying apparatus, found that polymer
conditioning of sludge (sludge digestion status was unknown) did not affect the surface
adsorbed and chemically bound water, had a small effect on interstitial water, and primarily
reduced free water.

Chen et al. (1997), and Chu and Lee (1999) attempted to define water-solid bond strength and
correlate this index to the water fractions in sludge. They found that polymers had no significant
effect on the water fraction with high bond strength exceeding 800-1,000 kJ/kg DS, but did
affect the water fraction at an intermediate strength of 40-600 kJ/kg DS, corresponding to the
water that is absorbed to the particle surface. Chu and Lee (1999) postulated that polymer
molecules replaced absorbed surface water, resulting in a decrease in bound water. The
minimum water-solid bond strength occurs when the surface charge is neutralized. Polymer
overdosing results in an increase in bound water, which is due to the absorption of water onto
the polymer segments.
Bound water content cannot be adequately measured by the centrifugal settling method (Lee, 1994) and the capillary suction time method (Chen et al., 1996). Lee (1995) suggested that differential scanning calorimetry (DSC) method could be used to measure bound water content in uniform samples. Snidaro et al. (1998) investigated the binding energy of water molecules in undigested sludge using thermogravimetry, differential scanning microscopy, and microcalorimetry. They found that activated sludge flocs form a gel-like matrix. Conditioning chemicals cannot enter microcolonies that contain between 20 to 30% of water. Vesilind (1979) estimated that a typical activated sludge of 0.5% solids consisted of approximately 75% free water, 20% interstitial (floc) water, and 4.5% vicinal and hydration water.

2.2.2 Theories of Sludge Conditioning and Dewatering

Generally, it is not practical to thicken and dewater sludges of wastewater treatment process without conditioning. Conditioning improves the effectiveness of water removal and the efficiency of dewatering (WEF, 1988). Two widely quoted theories to explain the roles of conditioners are charge neutralisation and bridging of individual particles. A third theory of structure disruption provides a new perspective in understanding on how polymer conditioning affects the structural matrix of the conditioned sludge.

Charge neutralisation

This model is based on the double layer model. A negatively charged particle collects a layer of positively charged ions, which has a loose layer of negative and positive ions around it. Two forces affect ion interactions: one force is the repulsion force due to like charges, and the other is the attraction force called van der Waals force. Conditioning chemicals neutralize and lower the surface charges (zeta potential), so that van der Waals force can agglomerate particles into larger particles, for easier dewatering. This model forms the basis of the application of the streaming current detector for automatic control of polymer dosing in sludge conditioning and dewatering (Dentel and Kingery, 1989, Dentel et al. 1989a, and 1989b, Abu-Orf and Dentel, 1997 and 1998). Charge neutralisation can be monitored using zeta potential measurement.

Particle bridging

This model suggests that conditioning floculants, such as metal hydroxides and organic polyelectrolytes, form long molecular threads or fibres, which attach to several colloids,
bridging the gap, capturing and binding colloids together, and forming strong floc structure, for subsequent efficient dewatering.

**Structure disruption**
This model is used to explain the role of polymers in conditioning gel-like digested sludge. Using scanning electron microscopy (SEM), Poxon (1996) found that digested sludge possessed an extensive gel-like biocolloidal structure that restricts water movement and results in poor sludge dewaterability. The addition of a cationic polymer resulted in a large polymer structure, and the collapse of the original sludge biocolloidal matrix onto the surface of the polymer backbone; this released entrapped water. Snidaro *et al.* (1998) observed a significant amount of water among sludge microcolonies, which provided the evidence that supported such a model.

**2.3 Factors Affecting the Conditioning and Dewatering of Sludge**
Although ATAD biosolids can be dewatered to achieve adequate solid recovery and cake dryness after sufficient polymer is applied for conditioning, the issue is that the excessive amount of polymer consumption needs to be reduced. The solution to this problem is to improve the dewatering properties of ATAD biosolids through optimizing the operation of ATAD process, or to optimize the conditioning process, or a combination of both. Current knowledge on factors that affect dewaterability of digested sludge is described in the following sections.

**2.3.1 Factors Related to Sludge Treatment Process**
The effects of process operation on sludge dewaterability have been investigated on both digested sludge in relation to digestion process, and undigested sludge in relation to biological treatment processes. Lawler *et al.* (1986) studied the effects of anaerobic digestion on sludge dewaterability, and found that dewaterability is more sensitive to digestion variables than the digestion performance itself. The dewaterability (measured as specific resistance) of digested sludge is better than that of the feed sludge (2.0-3.5% TS). Longer detention time did not seem to statistically change the specific resistance of the sludge. No polymer conditioning of the digested sludge was applied in this study.

In studies on the effect of biological treatment process to the settling and dewatering of undigested secondary sludge, Wu (1978) found that chemical flocculation of secondary sludge
was related to the initial concentration of nitrogen or phosphorous in the feed. When these nutrients are limited, sludge organisms grew large capsules and produced a higher surface electric charge per unit of dry weight, thus resulting in a higher chemical demand for separating these capsulated cells from water. Wu et al. (1982) indicated that the activated sludge from a nitrogen limited process always had a higher specific resistance to filtration (SRF), than that from a nitrogen-rich process. Poor filterability of the activated sludge was due to the existence of dispersed and pinpoint flocs and the overproduction of extracellular biopolymers, which were related to the high surface charge around the sludge. Lovett et al. (1983) found that the effectiveness of cationic polymer, in conditioning activated sludge, was dependent on sludge age. They observed that polymer conditioning results in minimum SRF reduction at a sludge age of 5 days, with progressively larger SRF reduction at sludge ages of 7 and 20 days. Knocke and Zentkovitch (1986) found that the mean cell residence time (MCRT) correlated to the dewaterability of the activated sludge through its effect on particle size distribution. Optimum dewatering rate occurred at MCRT values not less than 8 days. Cationic polymer dosages were also a direct function of particle sizes.

2.3.1.1 Feed sludge composition

Experience at full-scale operation indicates that feed sludge types affect the dewaterability of digested biosolids. It appears that sludges containing primary sludge had better dewaterability, following the ATAD digestion. Kelly et al. (1993) found that, following ATAD treatment, the specific capillary suction time (SCST) values of ATAD biosolids at Ladysmith, B.C., containing only primary sludge in the feed, had reduced from 172 to 81.5 seconds/g TS (total solids); at Gibsons, B.C., the feed sludge containing a mixture of primary sludge and trickling filter-solid contact sludge experienced a CST increase from 207 to 417 seconds/g TS; at Salmon Arm, B.C., where sludge contained both primary sludge and high phosphorus content FGR-SGR (fixed growth reactor-suspended growth reactor) sludge, the CST had increased by a factor of 7 times, with an average of up to approximately 20,000 seconds/g TS.

Matsch and Drnevich (1977) found that digested secondary sludge, without primary sludge exhibited a low vacuum filter rate (i.e. poor dewaterability). Jewell and Kabrick (1980) observed that the CST of an ATAD treated mixture of thickened primary and secondary sludge, showed significant deterioration at a hydraulic retention time of 3-8 days. However, dewaterability
showed substantial improvement after approximately 20 days of aeration. Trim and McGlashan (1985) did not see solids-liquid separation of ATAD biosolids by gravity settling. The feed was a 50/50 mixture of primary sludge and waste activated sludge. They reported that unconditioned ATAD biosolids had a CST of over 200,000 seconds, and was impossible to filter through a Buchner filter. Messenger et al. (1993) found that, even after centrifugation, a large mass of fine material remained suspended in samples of ATAD biosolids, and it was very difficult to dewater ATAD biosolids. Lapara and Alleman (1999) indicated that thermophilic aerobic wastewater treatment processes almost always had difficulties in bacterial flocculation and settling.

2.3.1.2 Digestion temperature and thermal pre-treatment of sludge

There was no reported work aiming to control the temperatures of thermophilic digestion for the improvement of dewaterability of the digested sludge.

Thermal pre-treatment of sludge at high temperatures has been used to promote separation of solids from liquids, by releasing cell-bound water. The thermal conditioning process is normally conducted in temperature ranges of 175 to 260°C, for 15 to 30 minutes, under pressures of 250 to 300 psig, without air or 370 to 430 psig with air (WEF, 1988). Such extraordinary treatment will destroy the gel-like structure of sludge and liberate the bound water. Dewatering can then be carried out without chemical conditioning (Swets et al., 1974).

Haug et al. (1978) studied the effect of heat pre-treatment on performance of the anaerobic digestion of a mixture of primary and secondary sludge, also reported the effect of such a thermal pre-treatment on dewaterability (measured the time to collect a certain volume of filtrate) of the treated sludge. They found that dewaterability of about 3%TS waste activated sludge was improved with increased temperatures (from 100 to 225°C) during heat treatment. Although 135°C made some improvements to sludge dewaterability, when the temperature was over 200°C, significant improvement occurred. Subsequent anaerobic treatment had little effect on dewaterability of the sludge that was treated at such high temperatures.

2.3.1.3 Digestion time (sludge retention time)

For a given digestion temperature, longer sludge retention time (SRT) results in higher volatile solid reduction, and more cell lysis into soluble or non-biodegradable waste products. Jewell
and Kabrick (1980) found that the dewaterability of ATAD biosolids deteriorated (measured CST increased from 10 seconds to 1200 seconds), when SRT increased from 3-7 days. Extended aeration improved dewaterability, the CST decreased to 10 seconds following 35 days of aeration. However, the SRT of thermophilic aerobic digestion is typically not more than 10 days, because longer SRT will result in the depletion of volatile solids. Low volatile solids will not be able to support adequate metabolic reactions to generate sufficient heat to sustain thermophilic digestion (Kelly et al. 1995).

2.3.1.4 Mixing-induced shear and power input to digester

Mechanical mixing in the ATAD process is designed to promote sufficient and effective oxygen transfer in the reactor, to keep sludge solids in suspension, to provide a complete mixing environment, and to supplement ATAD with external energy to sustain its thermophilic operating conditions. On the other hand, the shear that is induced by excessive mixing may lead to disintegrated sludge flocs, smaller particles, and higher polymer demands for flocculation. Most full-scale ATAD plants are designed for 100-250 W/m$^3$ power input. Galil et al. (1991) studied the effect of aeration turbulence on the biofloc of activated sludge and dewaterability of this undigested, unconditioned sludge. They found that the biofloc from an activated sludge process had a high sensitivity to the velocity gradient G. The size of the biofloc was reciprocal to the aeration turbulence (expressed in G). The biofloc achieved the lowest values of effluent suspended solids, SVI, and specific resistance at a G of 70.5 second$^{-1}$ (4.9 W/m$^3$).

2.3.1.5 pH, acidity and alkalinity of digestion

pH is a measure of the intensity of acidic or basic conditions of a solution. The pH of neutrality changes with temperature, being 7.5 at 0°C, 7.0 at 25°C, and 6.5 at 60°C (Sawyer et al., 1994). Acidity is a measure of the capacity to neutralize bases. Acidity is of little concern for the ATAD process, since the final pH is often near 8.0 (USEPA, 1990).

Acidity is a measure of the capacity to neutralize bases. Acidity is of little concern for the ATAD process, since the final pH is often near 8.0 (USEPA, 1990).

Alkalinity is a measure of the capacity to neutralize acids. It may consist of hydroxide (OH$^-$), carbonate (CO$_3^{2-}$), bicarbonate (HCO$_3^-$), and ammonia (to form NH$_4^+$), salts of weak acids such as acetic, propionic, as well as sulphide (to form H$_2$S). Kelly (1990) reported that ATAD operation increased the sludge alkalinity to up to 2,000 mg/L as CaCO$_3$, which was mainly
ammonia alkalinity. Ammonia concentrations ranging from 500 to 1,000 mg/L were reported in the ATAD Plant in McMinnville, Oregon. Other ATAD plants reported ammonia concentrations of approximately 300 mg/L. Reported total kjeldahl nitrogen (TKN) of ATAD effluents were approximately 1,000 to 2,000 mg/L (Boulanger, 1995). Chu (1995) and Boulanger (1995) reported the production of up to approximately 1,000 mg/L total volatile fatty acid (VFA, mostly in acetate) under low oxygen aerobic condition in the UBC pilot-scale ATAD reactors. Full-scale ATAD plants reported several times more VFA than those found at UBC (Boulanger, 1995). VFA, if present, will also contribute to the total alkalinity in ATAD biosolids. In contrast, mesophilic aerobic digestion typically decreases sludge's alkalinity and pH, due to nitrification. Anderson (1989) investigated the effect of pH control on aerobic digestion of waste activated sludge in temperature range of 5 to 20°C, reported that pH control could improve volatile solid reduction in certain temperature ranges.

2.3.1.6 Cations (Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, NH$_4^+$)

Cations affect the characteristics of microorganisms. It was found that, the decreased requirements by thermophilic sporeforming bacteria in growth factors (e.g. nutrients) were associated with the presence of a relatively high concentration of Ca$^{2+}$ and Mg$^{2+}$. Allen (1953) suggested that calcium increases the thermal resistance of *Paramecium* and *E. coli*. Tezuka (1969) found that, when either calcium or magnesium was added to the growth media, both promoted good flocculent growth of *Flavobacterium*, the predominant bacterium of the tested activated sludge. Other mineral salts did not induce similar flocculent growth. However, mineral salts of Na$^+$, K$^+$, NH$_4^+$, Ca$^{2+}$, and Mg$^{2+}$ could all induce good and reversible re-flocculation of disintegrated flocs. Such re-flocculation did not depend on whether the cells were viable or non-viable. Bruss *et al.* (1992) found that Ca$^{2+}$ removal had a negative effects on dewaterability (as measured by SRF) of activated sludge samples, and suggested that sludge floc structure was a three-dimensional, gel-like exopolymer matrix, which was kept together by divalent cations with varied selectivity for the matrix (Cu$^{2+} >$ Ca$^{2+} >$ Mg$^{2+}$).

Several researchers investigated the effect of cations on the settling and dewatering of undigested activated sludges (Higgins and Novak, 1997a, 1997b, Murthy and Novak, 1998, Novak *et al.*, 1998, Novak and Murthy, 1998), and the dewaterability of aerobically digested activated sludge at 20°C (Murthy and Novak, 1999, Novak and Sadler *et al.*, 1999). It was found
that, when the feed concentration of Ca\(^{2+}\) and Mg\(^{2+}\) each was higher than 0.7-2.0 meq/L, the activated sludge's floe strength, settling (measured as sludge volume index) and dewaterability (measured as CST) were improved. When the monovalent to divalent cation ratio in the feed was more than 2, the settling and dewatering (measured as CST) properties deteriorated. The deterioration could be reversed by reducing the cation ratio to less than 2. The direct addition of cations to activated sludge samples improved their settling and dewatering properties, but when the same amount of cations were added in the feed of the wastewater treatment process, greater improvement in the settling and dewatering properties was observed (Higgins and Novak, 1997a).

The addition of calcium to thickened sludge of an industrial sample reduced the optimum dose of conditioning polymer by 30% (Higgins and Novak, 1997b). The addition of Na\(^{+}\) and NH\(_{4}\)\(^{+}\) appears to cause poorer settling and dewatering (measured as CST) of sludge, a modest addition of K\(^{+}\) is beneficial, and excessive addition of K\(^{+}\) was detrimental to the dewatering of sludge (Murthy and Novak, 1998, and Novak and Murthy, 1998). A similar effect of cations on the dewaterability (measured as CST) of aerobically digested sludge was also observed (Murthy and Novak, 1999). Tezuka (1969) and Novak et al. (1998) suggested that these effects are not simply physical-chemical, but may be physiological, and believed that divalent cations help to bind biopolymers and stabilize the matrix of sludge by bridging negatively charged functional groups on biopolymers; an excessive concentration of sodium would prevent the adsorption of divalent cations to the biopolymer, resulting in poor floc properties. Cousin and Ganczarczyk (1999), based on their work of adding up to 100 meq/L Na\(^{+}\) directly to waste activated sludge, suggested that decreased dewaterability (measured as CST) of a sludge could be explained by either a decrease in porosity or an increase in floc size.

Kelly et al. (2000) recognised the significant difference between ATAD microbial characteristics and that of activated sludge or mesophilically digested biosolids, indicated that there was no advantages from adding cations into the feed of wastewater treatment process or into the feed of ATAD. Full-scale work that added pickle liquor (FeCl\(_{2}\)) directly to ATAD digested biosolids at the ATAD plant in Salmon Arm, B.C., achieved reduction in polymer consumption. However, not enough information was revealed from this work on the role of Fe\(^{2+}\) in the reduction of polymer use. Possible explanations include the cation effect, such as those discussed by Novak et al. (1998); the change of pH in the digested sludge (decreased to pH 4.6);
or a simple substitution of the organic polymer with less expensive inorganic pickle liquor as the conditioning chemical.

2.3.1.7 Oxygen supply

Some ATAD facilities reported dissolved oxygen (DO) levels of 0.7-3 mg/L (depending on the height of measurement in the reactor); some others reported DO of 0-0.2 mg/L in ATAD reactors (USEPA, 1990). Boulanger (1995) operated the UBC ATAD facility as fed-batch (daily feed for a SRT of 6 days), and worked with DO ranges of 0 to 5 mg/L. Low DO in the ATAD reactor was associated with a high oxygen demand of the aerobic process at thermophilic temperatures.

Oxygen uptake rate (OUR, expressed as mg/L O₂/hour), and specific oxygen uptake rate (SOUR, expressed as g O₂/day-kgVSS), provides an indication of the intensity of metabolic activities in the reactor. Bomio and Sonnleitner et al. (1989) illustrated an expected OUR profile for a batch operated ATAD system, and indicated that the sludge feed had a significant impact on OUR. Reported SOURs were from 100 to 400 g O₂/day-kgVSS with an average of 287 g O₂/day-kgVSS (USEPA, 1990). Boulanger (1995) reported SOURs of 40 to 316 g O₂/day-kgVS, and noticed that lower SOUR occurred following the feed of each new batch.

An adequate DO level should be maintained in ATAD reactors to ensure sufficient oxygen supply. But it seems to be impractical to pursue accurate DO control in ATAD reactors, due to the high temperatures of digestion. Kalinske (1976) compared the thickening and dewatering properties of oxygen activated sludge with that of air activated sludge, and found no significant difference. The air was used for oxygen supplies in the experimental work presented in this dissertation.

2.3.2 Factors Related to Sludge Characteristics

2.3.2.1 Extracellular biopolymers (proteins and polysaccharides)

Extracellular biopolymers produced by bacteria typically include proteins and polysaccharides (carbohydrate). Extracellular biopolymers occur in two forms, either as capsules that adhere to the outer surface of the cell walls, or as loose slime that is non-adherent to cells and excreted
into the surrounding medium (Wilkinson, 1958). Intracellular biopolymers, such as nucleic acids, are contained inside the cell, but may be released upon cell lysis or cell-wall turnover. Biopolymers are thought to have a number of functional groups such as hydroxyl (-OH) and negatively charged carboxyl (-COO⁻) groups (Sutherland, 1972); these could affect the settling and dewatering properties of sludge floc, through specific protein-polysaccharide interactions, hydrophilic interactions, hydrogen bonding, and ionic interactions (Higgins and Novak, 1997c). Forster (1983) compared biopolymers in activated and digested sludges, and found that polysaccharide and protein are most important constituents in activated sludge, whereas polysaccharides and at times proteins are the most significant surface polymers in digested sludge. Morgan et al. (1990) found that activated sludge produces 70-90 mg biopolymer/g SS, which was electro-negative and carbohydrate dominant. As a comparison, anaerobic UASB granular sludge had 10-20 mg biopolymer/g SS, and is protein dominant.

Most reported research work focused on the effect of biopolymers on biofloculation, settling, and dewatering of undigested activated sludge. Tenney and Stumm (1965) suggested that biopolymers, such as complex polysaccharides and polyamino acids, were excreted or exposed at the surface during the declining-growth and the endogenous respiration phases of activated sludge processes. These biopolymers were of sufficient length to form bridges between microbial particles to promote bioflocculation. Dispersed microorganisms behaved as hydrophilic colloids, which could be flocculated by organic electrolytes.

Busch and Stumm (1968) considered that anionic biopolymers, functioning similar to synthetic polymers, could be specifically adsorbed at microbial surfaces to form bridges with adjacent surfaces that lead to aggregates. Friedman et al. (1969) supported the postulation that the effect of extracellular biopolymer was the same as that of synthetic polymer in the process of bioflocculation. McGregor and Finn (1969) found that the effect of many factors, such as temperature, ionic strength, physiological age, flocculent, bacterial genus, and surface shear, were related to the release of biopolymers by the cell. These biopolymers may increase or decrease the dosage of flocculant required for extracellular aggregation. Ribonucleic acid and extracellular proteins released from washed cells of *Escherichia coli* and *Pseudomonas fluorescens* increased the required amount of flocculant (colloidal alumina and a cationic polymer) for flocculation. A capsular polysaccharide produced from *Lactobacillus delbrueckii* increased flocculant dosage only when attached to the cell surface, but decreased the dosage of
cationic flocculants when released into the suspending media. Forster (1971) indicated that the surfaces in activated sludge were polysaccharide in nature. They were composed of galactose, fucose, mannose, and glucuronic acid. A low SVI (sludge volume index) is associated the dominance of glucuronic acid.

Kato et al. (1971) suggested that extracellular polymers were responsible for the flocculent growth of activated sludge bacteria, because the treatment by enzymes such as cellulase resulted in deflocculation. Pavoni et al. (1972) confirmed that extracellular polymers have a negative charge, and the addition of extracted biopolymer improved the flocculation of kaolinite at a pH of over 4. This effect was attributed to the bridging effect of high molecular weight biopolymers. Tenney and Verhoff (1973) suggested that the microbial autoflocculation in the endogenous growth phase was caused by anionic biopolymers. Gulas et al. (1979) found that the dewatering properties of activated sludge (reported as SRF/TS) was improved with increased specific biopolymer content (total extracellular polymer/MLVSS) in the sludge.

Bowen and Keinath (1985) studied waste activated sludge, primary sludge, and anaerobically digested waste activated sludge, and found that polymer index (PI), a measurement of minimum polymer dose to achieve maximum dewaterability, was statistically related to the contents of carbohydrate, protein, and lipid. To destabilize the sludge, carbohydrate and protein resulted in a lower polymer requirement, but the lipid resulted in a higher polymer requirement. Nielsen et al. (1996) found that when activated sludge was anaerobically stored, the total sludge protein and carbohydrate decreased rapidly within 3 days and correlated to a deterioration of the dewaterability. Novak and Sadler et al. (1999) found that anaerobic and aerobic digestion of waste activated sludge resulted in the release of biopolymers and a deterioration of dewaterability (measured as polymer demands and CST). Anaerobic digestion released much more protein than polysaccharide. Whereas, aerobic digestion mainly released polysaccharides. The protein content in solution appeared to contribute to the increase in the polymer demands of conditioning. Fieldwork conducted at the ATAD facility in College Station, Texas observed increased biopolymers in digested sludge from the ATAD process. These increases corresponded to an increase in the demand for cationic polymer. Higgins and Novak (1997c) suggested that extracellular protein was strongly involved in the aggregation of activated sludge bacteria into flocs.
Apparently, the role of biopolymers in affecting bioflocculation is different from their effects on the dewaterability of the digested sludge. Although biopolymers appear to play a positive role in promoting bioflocculation of activated sludge bacteria, biopolymers in digested sludge seem to correlate to a deterioration in dewatering properties (measured as CST or polymer demands) of the digested biosolids.

2.3.2.2 Floc charge (Zeta potential)

Zeta potential is determined by measuring the electrophoretic mobility of the sludge particles between two electrodes. Higher-charged particles move faster from one electrode to the other. Electrophoretic mobility is related to the density of surface charges, and not the total surface area dependent charges. Eriksson (1987) suggested that, although charge neutralisation is important in sludge conditioning, there is no correlation between electrophoretic mobility (zeta potential) and the optimal dosages of polymer, because of the difference in particle size distribution and surface areas. Cole and Singer (1985) found that dosing to achieve charge neutralisation was not a prerequisite for effective sludge conditioning. They suggested that, although electrophoretic mobility measurements helped to understand the polymer-particle interaction process, it was difficult to predict polymer dosage by electrophoretic measurement results, due to the lack of standard sample preparation procedures. Busch and Stumm (1968) suggested that, because of the hydrophilic surface, the electrostatic repulsive forces between cells might not be the primary reason for the stability of a bacterial dispersion. Reduction of surface potential was not a prerequisite for bioflocculation, as bacterial suspensions could form a stable dispersions even at the iso-electric point (pH 2 to 3 for most bacteria). Pavoni et al. (1972) observed that surface charge reduction was not a necessary precursor for bioflocculation of activated sludge. However, it was noted that dewatering the digested sludge likely followed a different mechanism from promoting the bioflocculation.

2.3.2.3 Floc size and distribution

Particle size distribution affects the total particle surface area and the porosity of cakes formed from these particles, therefore, affecting the required coagulant doses and dewatering properties (WEF-ASCE, 1998). Karr and Keinath (1978a) indicated that solid particles may be classified by their sizes as settleable (over 100 μm), supracolloidal (1-100 μm), true colloidal (0.001-1
μm), and dissolved (smaller than 0.001 μm). They studied the dewaterability of primary, activated, and anaerobically digested sludges having solids concentrations from 0.7 to 1.3%, and found that the effects of pH, biological degradation, mixing, and conditioning might all be explained on the basis of the effects of these factors on particle size distribution. The supracolloidal solids (1-100 μm) had the most significant effect on dewatering properties, due to the blockage of sludge cake and filter medium by these solids. Lawler et al. (1986) found that the anaerobic digestion of mixed primary and activated sludge affects the particle size distribution. Dewaterability (measured as both SRF and CST) was more sensitive to digestion operating conditions rather than the digestion itself. The dewaterability of digested sludges might be improved, when the digestion worked well and the resulting small particles were preferentially removed; or be worsened when digester did not work well, so the large particles were destroyed and small particles were created. Burnett et al. (1997) found that the peaks of particle size of ATAD sludge collected from the ATAD facility in College Station, Texas were similar to that of anaerobically digested sludges from two other sources, all in the range of 5-7.5 μm, but ATAD sludge had relatively fewer particles above 10 μm.

2.3.2.4 Viscosity

Viscosity reflects the characteristics of deformation (strain) under shear. The shear stress is proportional to the shear rate (velocity gradient), and viscosity is the proportional constant. Dick and Ewing (1967) found that activated sludge was a plastic material (non-Newtonian), as well as thixotropic, because solid concentrations affected the yield strength of the activated sludge. They suggested that the thixotropic property of activated sludge meant that some internal structure was broken down with shear. Forster (1981) indicated that sludge viscosity and the particle charge were related, because a reduction of pH resulted in a decrease in particle surface charge and in viscosity. Campbell and Crescuolo (1982) found that it was difficult to apply viscosity to characterize sewage sludge, due to a lack of consistency. Abu-Orf and Dentel (1999) found there was a lack of correlation between the optimum polymer dose and the peak instantaneous viscosity, over a wide range of mixing conditions. Viscosity is temperature dependent, and decreases as temperature of the sludge increases.
2.3.2.5 Other factors

ORP measurement determines the ratio of oxidants to reductants within a solution. It is non-specific with respect to any single compound, but could be used as an indicator to describe the ability to oxidize or reduce given species in the solution. Kelly et al. (1993) reported ORP in ATAD reactors of 100 to −350 mV in Ladysmith, B.C., −50 to −300 mV in Gibsons, B.C., and 0 to −300 mV in Salmon Arm, B.C., but found no correlation between ORP and digestion temperature. ORP may be used in an environment of low dissolved oxygen (e.g. less than 0.5 mg/L D.O.), where D.O. measurements, using a D.O. probe, will be meaningless due to its low level. ORP varies as the pH of the bulk liquid in the ATAD bioreactor changes.

Zita and Hermansson (1994) found that the stability of activated sludge floc was affected by the ionic strength of the medium; increased polymer concentration increased the floc stability. However, when the ionic strength was above 0.1, the floc stability decreased. They suggested that ionic strength is an important factor for the floc stability in wastewater, due to the effect on double-layer thickness.

2.3.3 Factors Related to Sludge Conditioning and Dewatering Process

2.3.3.1 Selection of conditioning chemicals

Sludge conditioning can use either inorganic chemicals such as Al\(^{3+}\) and Fe\(^{3+}\) salts or organic polyelectrolytes (polymers). Wastewater sludge treatments mostly use cationic polymers to condition negatively charged colloidal particles in the sludge. The advantages include reduced volume and weight of thickened and dewatered solids, as well as easier handling and operation (WEF, 1988). Polymer may act as coagulant for charge neutralisation or as flocculent for bridging, or both. Major polymer characteristics include molecular weight, type of charge, charge density, and structure. Cole and Singer (1985) investigated the effect of polymer selection on conditioning of anaerobically digested biosolids (measured as CST). They found that the cationic polymers with a molecular weight of more than 10\(^6\) was more effective in the sludge conditioning. Both high and low charges could achieve effective conditioning. Charge neutralisation was not a prerequisite for effective conditioning, although high charge density cationic polymers did produce charge neutralisation. Tiravanti et al. (1985) observed higher
charge density of polymers at lower pH, suggesting that there was a correlation between the total positive charges added (a product of charge density and polymer dose) and solids concentration, but not between the optimum cationic polymer dose and solid concentration.

Dual-chemical conditionings were studied by Chitikela and Dental (1998) on anaerobically digested biosolids, and Kelly et al. (2000) on ATAD biosolids. Both these studies aimed to substitute the expensive polymer with less expensive chemicals for the reduction of conditioning costs. Chitikela and Dental (1998) tested FeCl₃ and HDTMA (a cationic surfactant) and Percol 757. They found that dual-chemical conditioning resulted in a significant reduction in the dose of individual chemical, compared to the dose needed when each chemical was used by itself. Adding a proportion of the optimum dose of one chemical reduced the requirement of the other chemical by about the same proportion. At least some polymer was required to obtain optimal dewatering, and overall it was not cost effective to use a dual-chemical conditioning system. Kelly et al. (2000) substituted Percol 778FS25 (by Ciba Inc. its properties are described in Table A.2 of Appendix A) with pickle liquor (an industrial liquid waste from the pickling process, mainly ferrous iron). When approximately 45.6 kg FeCl₂/dry ton solids was added, the polymer dose was reduced from an average of 38.5 to 19.7 kg polymer/dry ton solids. The overall cost was reduced by about 50%, due to the low cost of pickle liquor.

2.3.3.2 Mixing intensity and time for flocculation

Flocculation, by mixing for sufficient time, promotes interaction and contact between conditioning chemicals and solid particles in a sludge, as well as the growth of particle aggregates. Werle et al. (1984) found that for undigested primary or activated sludge, a single optimum Gt (the product of mean velocity gradient G and mixing time t) of approximately 7,000 seemed to exist, regardless of the polymer dosage. Tested G was from 250 to 1215 seconds⁻¹, and t was from 8-720 seconds. For activated sludge, G might be traded off for t within a suggested range of G (second⁻¹) to t (in second) ratio of 0.5-60. For primary sludge, high intensity (G of 1,215 second⁻¹) at a polymer dose of 50 mg/L or lower, resulted in the deterioration of particle size. As Gt increased, dewaterability deteriorated (indicated by an increase in the optimum polymer required). This last finding was confirmed by Novak et al. (1988) in a separate study. Novak and Bandak (1989) found that unconditioned anaerobically digested sludge was much more sensitive to shear intensity G (tested G ranging from 260 to
1400 second\(^{-1}\)) than to the mixing duration \(t\) (tested \(t\) ranging from 15 to 600 seconds), and suggested there existed a correlation between CST and \(G^{2.8}\) (rather than \(G_t\)). Lynch and Novak (1991) suggested that extra polymer demand induced by shear, due to the operation of filter press equipment to dewater anaerobically digested sludge, could be simulated at bench scale at a \(G_t\) value of approximately 35,000. WEF (1988) recommended high intensity mixing (\(G\) of 1200 to 1500 s\(^{-1}\) maximum) to be followed by gentler agitation (\(G\) of less than 200 s\(^{-1}\)) for conditioning.

2.3.3.3 pH and alkalinity adjustment

pH affects both the surface charge of sludge particles and properties of the chemical conditioner. Tenney and Stumm (1965), and Busch and Stumm (1968) indicated that most bacteria have an iso-electric point (the pH at which the zeta potential of a solid equals to zero) between pH 2 to 3. Within the range of pH 5 to 9 (as observed in most wastewater and sludge), bacteria carry a net negative charge, resulting from acid-base ionisation of functional groups in the bacterial surface. Tenney \textit{et al.} (1970) found that decreasing the pH from 10 to less than 1 resulted in the change of activated sludge’s micro-electrophoretic mobility (MEM) from \(-2.0\) to \(+1.5\) u/sec/volt/cm. Zero MEM occurred at a pH of about 2.5. They suggested that the pH influences the coiling, degree of ionisation, and charge density of the polymer. Novak and O’Brien (1975) found that in conditioning alum sludge, the cationic polymer becomes much less efficient with higher pH (polymer dose less than 40 mg/L at pH 5, 240 mg/L at pH 10.5). The optimum polymer dose doubled when the pH increased from 7 to 9. They suggested that cationic polymers perform best at neutral to slightly acidic pH. O’Brien and Novak (1977) indicated that to condition chemical sludge from water treatment processes, cationic polymers worked most efficiently at pH values of 7 or below; non-ionic polymer and the anionic polymers with lower percent hydrolysis functioned effectively over a pH range of 6.5-8.5. An increased pH resulted in a decreased polymer effectiveness. Motamedi (1975) improved the dewatering of ferric chloride sludge 2-3 orders of magnitude, by adding HCl and resulting in a decrease in pH from 7.6 to less than 2. Sukenik \textit{et al.} (1977) treated primary, anaerobically digested, and aerobically digested sludges with chlorine (0 to 1,000 mg/L) and sulphuric acid (pH 3 to 7), and found that a decreased pH improved sludge filterability, regardless of whether or not chlorine was applied. Acidity, rather than the chlorine, largely affects the filterability.
Alkalinity describes the capacity of a system to resist pH change. Alkalinity is essential when metal ion chemicals, such as alum and ferric salts, are used for conditioning. These chemicals react with alkalinity to form hydrous metal oxides. Tenney et al. (1970) found that, increasing the alkalinity from 576 mg/L to 3280 mg/L (both as CaCO₃) at pH 7.0 seemed to impair the dewaterability (measured as the time to collect 100 mL of filtrate) of the activated sludge for a given alum concentration; increasing the alkalinity from less than 1000 mg/L to 5000 mg/L (both as CaCO₃) resulted in a slight decrease of the activated sludge in micro-electrophoretic mobility (MEM) from -1.8 to -1.2 μ/sec-volt-cm. Randall et al. (1971) observed that the drainability of activated sludge solids seemed to improve slightly, when the alkalinity increased from 0 mg/L to up to 160 mg/L. The relationship was stronger, when solids concentrations were in the range 1.7% to 2.1% rather than in the range of 2.4% to 3.8%.

2.3.3.4 Cation addition

Cation addition, in the form of inorganic chemicals, had been used to improve the dewaterability of mesophilically digested sludge in many full-scale wastewater treatment plants (WEF 1988). To reduce the conditioning cost of ATAD digested sludge, ferrous chloride, alum, or ferric chloride had been used as substitute to reduce the dosage of polymers (Kelly et al. 2000, Murthy and Novak 2000b).

2.3.3.5 Post digestion cooling and holding

The current practice of dewatering at some full-scale ATAD plants is to cool digested biosolids to about or below 30°C, before conditioning and dewatering. Such ATAD facilities include those at Whistler, BC; Banff, Alberta; Gig Harbour, Washington; Ephrata, Pennsylvania; and Princeton, Indiana. Post digestion holding is currently carried out at facilities in College Station, Texas (Murthy et al. 200a); Ephrata, Pennsylvania; and Princeton, Indiana. However, it is not known whether or not an optimum temperature exists for reducing the polymer dose for conditioning and dewatering.

2.3.3.6 Structure disruption (sonication)

Reported applications of sonication technology in water and waste treatment include ultrasonic pre-treatment of the water or waste aiming to improve physical (e.g. dewatering) properties of

The application of sonication involves the transfer of acoustical energy into liquid and cavitation phenomena. When a high power ultrasound, ranging 20 kHz to 10 MHz (comparing to audible sound frequency of 20 Hz to 20 kHz, Halliday and Resnick, 1988), passes through a liquid, it will induce a series of compression and rarefaction (expansion) cycles in the liquid, causing the formation of micro-bubbles of gas. These bubbles grow and coalesce. If the applied ultrasound energy is high enough that the rarefaction cycle exceeds the molecular attractive forces, or the bubbles reach their resonant size, these bubbles will collapse, creating a powerful shock wave throughout the sample. This process is called cavitation. The energy level to start this process is called “cavitation threshold”.

There are two forms of cavitation: stable and transient cavitation. Stable cavitation occurs at lower acoustic intensities (1 to 3 W/cm². Draman and O’Dette, 1998). Bubbles gradually grow larger over many rarefaction and compression cycles. When bubbles reach a critical size as determined by ultrasound frequency (e.g. 170 µm diameter for 20 kHz), they collapse, and result in structure disruption. Transient cavitation occurs at high acoustic intensities (over 10 W/cm². Draman and O’Dette, 1998). Over one to three cycles, transient bubbles rapidly grow to at least three times their initial size, then violently collapse to form many small bubbles. Transient cavitation may create localized pressures of over 1000 bar and temperatures of over 5000°C. These hot spots have a typical lifetime of less than a microsecond (Clark, 1997, and Draman and O’Dette, 1998). The effectiveness of ultrasound treatment depends on frequency, intensity, external temperature and pressure.

Kowalska et al. (1979) treated undigested sludge at 20 kHz, using a generator of power output of 800 W, and found that sonication (tested from 0 to 300 seconds) increased the sludge’s
specific resistance of filtration (SRF), no matter what polymer was used for conditioning before sonication; however, the water content of the sludge cake was reduced. They observed that sonicated sludge displayed smaller particle sizes. Bien (1988) indicated that the SRF of a sonicated organic sludge (at 20 kHz) increased to a constant value, regardless of the time of sonicating. King and Forster (1990) used a 20 kHz, 0-60 W power output sonicator. They found that, at a power level of 7.5-75 W-min, sonication caused the deterioration of filtration and supernatant quality of the activated sludge, markedly increased the number of smaller particles (1 to 4 μm), and released soluble carbohydrate and protein from the sludge. Morgan and Forster (1992) suggested that an energy input of 20 kHz and 7.5 W sonication seemed to cause charges on surface of the sludge to become more negative. Quarmby et al. (1999) indicated that sonication of 16 to 20 kHz of 100 W/cm², treated at 111 W-min and 356 W-min prior to anaerobic digestion, detrimentally affected the dewatering property of digested sludge (measured as CST).

2.3.3.7 Other factors

Freeze-thaw conditioning of sludge is based on the principles that the ice front of freezing water advances in an orderly fashion, rejects dissolved impurities and pushes these impurities into a more concentrated volume. Knocke and Trahern (1989) found that slow freezing of a solid resulted in the release of internal floc water and the aggregation and densification of the floc structure, this produce excellent dewatering characteristics of the sludge. Vesilind and Martel (1990) suggested that slow freezing rates resulted in an improved dewaterability. Freeze-thaw may be appropriate and cost effective, where natural climatic conditions (e.g. in northern Canada and Alaska, USA) permit such a process to work on its own. The application of freeze-thaw technique on dewatering ATAD biosolids was not investigated in this research work.

2.4 Techniques of Laboratory Assessments of Sludge Properties

2.4.1 Laboratory Techniques to Assess Sludge Dewaterability

The two most used laboratory techniques to quantitatively evaluate sludge dewaterability are capillary suction time (CST) and specific resistance to filtration (SRF). If sludge solids content and filtrate viscosity do not vary among compared samples, SRF can be simplified to time-to-filter (TF).
CST describes how readily a sludge releases its water and records the time for the liquid to travel a specified distance in a chromatography paper by capillary action. It is simple, rapid, and inexpensive to conduct. Vesilind (1988), Tiller et al. (1990), and Unno et al. (1983) provided in-depth theoretical analysis and discussion on CST. Karr and Keinath (1978b) suggested that CST increased dramatically with an increase in the fraction of fragile settleable solids (solids larger than 100 µm, but may pass through 100 µm mesh during filtering due to fragility) even when the total solids concentration was relatively constant. On the other hand, SRF changed little. CST is also more sensitive to the initial particle packing characteristics, because the small tested volumes (approximate 5 mL) result in little sludge solids compaction. Christensen et al. (1993) indicated the CST was not sensitive enough to detect a change in sludge dewaterability within a fairly wide range around the optimal polymer dosage. CST is affected by solids concentration of the sludge and the viscosity of the filtrate, and is normally reported as specific CST (SCST), which is the CST divided by the solids concentration of the sludge sample (APHA et al. 1995).

SRF measures the sample’s filtering rate under vacuum differential through a filter paper in a Buchner funnel. SRF provides greater opportunity for the evaluation of filtration, blinding and compression characteristics of the sludge. SRF has been used to study fundamental principles of sludge filtration and expression (Novak et al., 1988, Heij et al., 1996, Sorensen et al., 1996, Sorensen and Sorensen, 1997, Novak and Agerbaek et al., 1999). Christensen and Dick (1985a, and 1985b) and Vesilind (1979) discussed the methods and procedures of SRF measurements. Typical SRF values of wastewater sludges are 10-100 Tm/kg (Tm = 10^{12} m) for raw waste activated sludge, 100-600 Tm/kg for digested mixed sludges, 1 Tm/kg for adequately conditioned raw primary and waste activated sludge, and less than 1 Tm/kg for well conditioned mixed sludge (Christersen, 1983). A high SRF value suggests the sludge is difficult to dewater. Smollen (1986a and 1986b) found no quantitative relationship between SRF and CST, and suggested that SRF and CST should not be used interchangeably for describing sludge dewatering properties. Karr and Keinath (1978b) indicated that when a sludge contained fines (supracolloids 1 to 10 µm), the passageway through the sludge or the filter medium would be blocked during filtration, in which case SRF was not suitable to adequately quantify the dewatering characteristics of a sludge.
2.4.2 Electron Microscope Imaging

Electron microscopy techniques have been used by many researchers to investigate the structure of activated (Snidaro et al., 1998, Zartarian et al., 1994, 1997, and Jorand et al., 1995), and digested (Poxon, 1996) sludges in order to understand the mechanism of solid-liquid separation of these sludges. The two most widely used techniques are scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM scans the surface of the specimen, using a 2-3 nm spot of electrons, generating secondary electrons that are then detected by a sensor. SEM produces an image of the surface that gives the impression of three dimensions. TEM projects electrons through a very thin slice of specimen to produce a two-dimensional image on a phosphorescent screen. The brightness of a particular area of the projected image is proportional to the number of electrons that are transmitted through the specimen. TEM requires the use of a microtome to create thin slices of the specimen.

2.4.3 Extraction of Soluble and Bound Biopolymers in Sludge

The analysis of extracellular biopolymers in digested biosolids is to determine soluble and bound fractions of protein and polysaccharide content. After a samples is centrifuged at 10,000 ×g for 20 minute, biopolymers in the filtrate are considered as the soluble fraction, and in the remaining sediments are considered as the bound fraction (Higgins and Novak, 1997c, and Novak and Sadler et al., 1999). Novak and Haugan (1981) centrifuged activated sludge at forces up to 18,000×g for 15 minutes, and found that forces higher than 1,000×g did not result in an increased biopolymer recovery. Sanin and Vesilind (1994) used 1,500×g for 5 minutes, to remove soluble biopolymers.

The bound fraction of biopolymers can be extracted, prior to its measurement, or be measured in-situ. Extraction methods include the use of boiling water or organic solvents (i.e. ethanol, Wallen and Davis, 1972); alkali (i.e. NaOH solution, Sato and Ose, 1980, Rudd et al., 1983, and Higgins and Novak, 1997c); heating at temperatures ranging from 60 to 100°C for 1 hour and ethanol/acetone (Clarke and Forster, 1982, Goodwin and Forster, 1985, and Horan and Eccles, 1986); ultrasonication, ion-exchange resin (Rudd et al., 1983, Karapanagiotis et al., 1989, Frolund et al., 1996); high speed centrifugation (Gehr and Henry, 1983, Sanin and Vesilind, 1994). In situ method uses ruthenium red dye adsorption to measure extracellular polysaccharides (Figueroa and Silverstein, 1989, and Poxon and Darby, 1997). Brown and
Lester (1980) compared five extraction methods using activated sludge samples, and found that NaOH treatment resulted in the highest amount of extracted biopolymers. The NaOH method was used for the extraction of bound biopolymers in the experimental work of this dissertation.
3.0 Materials and Methods

3.1 Experimental Facilities

The experimental work was carried out at bench-scale in the Environmental Engineering Laboratory of the University of British Columbia. The experimental set-up is shown in Figure 3.1. Each bioreactor was a stainless steel container, having an inside diameter of 20 cm, a height of 25 cm, and a total volume of 8 L. Sludge was batch digested. The initial volume of the feed sludge in each bioreactor of all Runs was 5 L.

![Figure 3.1. Experimental set-up](image)

When the sludge was digested thermophilically, the bioreactors were placed in a waterbath. A HAAKE E-8 heating unit was used to maintain a constant water temperatures in the waterbath, Therefore, digestion temperature of the sludge in each bioreactor was maintained constant during each run. A VWR-1112 immersion circulator was used in 01Nov27Run and 02Feb17Run, due to the break-down of HAAKE E-8 unit. Several digestion temperatures (40°C, 50°C, 55°C, 60°C, or 70°C) were tested separately in this research program. The water loss, due to evaporation, in each bioreactor was accounted for, and was replaced with distilled water, prior to each sampling. When the sludge was digested mesophilically, bioreactors were placed on the lab bench, and the sludge was digested at ambient room temperatures of approximately 22°C. Temperatures in each bioreactor were verified using a thermometer.

A disc-type air diffuser (5" Deluxe Bubble-disk™ by Penn-Plax Inc.), having an effective diameter of 10 cm, was placed at bottom of each bioreactor. Air from the diffusers provided mixing and fine-bubble aeration. Airflows were regulated using Cole-Parmer 03217-20 airflow meters (glass float material, 1 atm or 14.7 psi inlet pressure). Airflow rates to each bioreactor,
set at 11 volume of air/volume of sludge-hour (00Aug15Run was an exception that used 7 vol./vol.-hr), were identical. In comparison, the design criteria for adequate mixing in a conventional aerobic digesters using diffused air is 1.2 to 2.4 m$^3$/m$^3$-hr (Metcalf & Eddy 2003). As the samplings reduced sludge volumes in the bioreactors, the airflow rates were decreased to maintain the approximately the same air to sludge ratio as the initial setting. Intermittent mixing was manually carried out throughout each run, and immediately before each sampling, to ensure the complete mixing of sludge in each bioreactor. According to Vogelaar et al. (2000), the oxygen transfer rate (OTR) in the thermophilic sludge at 55°C was comparable to OTRs measured in tap water within temperature range of 20-55°C, as the decreased oxygen saturation concentration was completed offset by the increased overall oxygen transfer coefficient ($K_{La}$),

3.2 Sources of Feed Sludge

Feed sludges for the experimental work were taken from the Greater Vancouver Regional District (GVRD) Lulu Island Wastewater Treatment Plant (Lulu WWTP). The Lulu WWTP, currently serving a population of 152,000, handles an annual average wastewater flow of 70 ML/day using a trickling filter-solid contact biological treatment process. The influent to Lulu WWTP is predominantly municipal sewage. The primary sludge used for experiments was taken from the bottom of the primary sludge gravity thickener. The secondary sludge used for these experiments was thickened waste activated sludge (TWAS), taken from the dissolved air flotation (DAF) sludge thickener. Lulu WWTP added polymer (Percol 766 by Ciba Inc.) into pre-thickened secondary sludge, at an average dosage of 1.5-2.0 kg polymer/dry tonne of solids to assist sludge thickening in DAF. According to the operations staff in Lulu WWTP, during the time preceding the dates of sludge collections, no abnormal performance at WWTP was observed.

The collected primary and secondary sludge, each having approximately 4.5% total solids (TS), were stored at 4°C in a cold room, until they were made into feed sludges. Most of storage time was 2 days. Although the storage time of the feed sludge used in 00Aug15Run and 00Nov28Run was relatively long (Table A.3 of Appendix A for details), the trend of thermophilic digestion effect on dewaterability of digested sludge appeared to be comparable to results of other runs of similar digestion conditions (e.g. 100% secondary sludge was digested at 60°C and 22°C in these two runs. The same experimental conditions were repeated in other runs.
such as 01Oct30Run). Therefore, data from 00Aug15Run and 00Nov28Run were accepted and included in subsequent analysis. Prior to the digestion experiments, collected sludges were diluted with tap water to make into feed sludges of approximately 2.5% TS. Bura et al. (1998) studied the effect of storing activated sludge samples at 4°C on concentrations of extracellular biopolymers, and reported that 90-99% proteins and 88-91% carbohydrates remained following one day of storage, 72% of proteins and 61% of carbohydrates remained following 10 days of storage.

A mixed population of various microorganisms are naturally present in the wastewater sludge. When the sludge was aerated and heated to thermophilic temperatures, thermophilic organisms could develop without seeding in the digested sludge (Bomio et al. 1989). Therefore, there were no inoculation and seeding in all experimental runs. No additional micro-bacteria were added into the digestion bioreactors at the beginning of each run.

3.3 Experimental Program and Design

The experimental program consisted of eleven runs. The information on the duration of each run, the collection and storage of the feed sludge in each run are described in Table A.3 of Appendix A. In each run, 2 to 4 bioreactors were operated simultaneously in batch mode. Full-scale ATAD facilities can be operated in either batch, fill-draw, or continuous flow mode. The selection of batch operation for the experimental work of this research intended to reveal information on the kinetics (i.e. how soon the changes occur) of the effect of thermophilic digestion on the dewaterability and other characteristics of the digested sludge. Bioreactors in each run differed in the operational conditions (e.g. feed sludge compositions, digestion temperatures, mechanical shears). More than half of all experimental runs included duplicates of thermophilic bioreactors (identical conditions of digestion). Each run was identified by the starting date of the run. As an example, “00Aug15Run” indicated that the test started on August 15, 2000. Typical duration of sludge digestion in full-scale ATAD facilities are 5-6 days, therefore, most of the experimental runs were discontinued after approximately 10 days of batch operation (some minor variation in the total digestion time was to suit the schedule of the experimental work). Some longer digestion time (e.g. 30 days in 00Nov28Run) was used in the early stage of the experimental program to assess the general trend of digestion effect on dewaterability of the digested sludge.
3.3.1 *Investigate Factors of Digestion Affecting Dewaterability*

Experimental conditions are summarised in Table 3.1 to 3.3.

Table 3.1. Experimental conditions: effects of feed sludge composition

<table>
<thead>
<tr>
<th>Feed Sludge Composition (wt./wt.)</th>
<th>Thermophilic Digestion</th>
<th>Mesophilic Digestion</th>
<th>Airflow(^b) (v/v-hr)</th>
<th>Run ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary 0%</td>
<td>60°C</td>
<td>22°C</td>
<td>7, 11</td>
<td>Three runs(^c)</td>
</tr>
<tr>
<td>75%</td>
<td>60°C</td>
<td>22°C</td>
<td>11</td>
<td>01Oct30Run</td>
</tr>
<tr>
<td>40%</td>
<td>60°C</td>
<td>22°C</td>
<td>11</td>
<td>00Nov28Run</td>
</tr>
<tr>
<td>0%</td>
<td>60°C</td>
<td>22°C</td>
<td>7</td>
<td>00Aug15Run</td>
</tr>
</tbody>
</table>

\(^a\)Mesophilic digestion was carried out at ambient room temperature of 22°C on average.
\(^b\)Airflow rate is expressed as “volume of air/volume of sludge-hour”.
\(^c\)This condition was tested in three runs: 00Aug15Run (7 v/v-hr), 00Nov28Run and 01Oct30Run (both 11 v/v-hr).

Table 3.2. Experimental conditions: effects of digestion temperatures and duration

<table>
<thead>
<tr>
<th>Thermophilic Digestion</th>
<th>Mesophilic Digestion</th>
<th>Duration (days)</th>
<th>Feed Sludge Composition</th>
<th>Airflow (v/v-hr)</th>
<th>Run ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>40°C</td>
<td>22°C</td>
<td>12</td>
<td>100% secondary</td>
<td>11</td>
<td>01July14Run(^a)</td>
</tr>
<tr>
<td>50°C</td>
<td>22°C</td>
<td>9</td>
<td>100% secondary</td>
<td>11</td>
<td>01June15Run(^a)</td>
</tr>
<tr>
<td>55°C</td>
<td>22°C</td>
<td>10</td>
<td>100% secondary</td>
<td>11</td>
<td>01Nov27Run</td>
</tr>
<tr>
<td>60°C</td>
<td>22°C</td>
<td>20</td>
<td>100% secondary</td>
<td>11</td>
<td>00Aug15Run(^b)</td>
</tr>
<tr>
<td>70°C</td>
<td>22°C</td>
<td>10</td>
<td>100% secondary</td>
<td>11</td>
<td>01July31Run(^a)</td>
</tr>
</tbody>
</table>

\(^a\)Each of these three runs included two thermophilic bioreactors with identical digestion conditions (e.g. feed sludge, digestion temperature, sampling intervals).
\(^b\)Thermophilic digestion at 60°C on 100% secondary sludge feed was also carried out in 00Nov28Run (for 30 days) and 01Oct30Run (for 10 days).
Table 3.3. Experimental conditions: effects of mixing induced shear

<table>
<thead>
<tr>
<th></th>
<th>Thermophilic Digestion</th>
<th>Mesophilic Digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(01Oct30Run)</td>
<td>(01Nov27Run)</td>
</tr>
<tr>
<td>Shear Level</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Mixing Method</td>
<td>Air mixing</td>
<td>Air mixing</td>
</tr>
<tr>
<td>Temperature</td>
<td>60°C</td>
<td>60°C, 20°C</td>
</tr>
<tr>
<td>Feed Sludge</td>
<td>100% secondary</td>
<td>100% secondary</td>
</tr>
<tr>
<td>Airflow (v/v-hr)</td>
<td>11</td>
<td>N/A, 11</td>
</tr>
<tr>
<td>Duration (days)</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

a A mechanical mixer (130 watts, 1550 rpm) was mounted on the open top of a bioreactor to apply high shear to the sludge inside the bioreactor. The mechanical mixing induced air into bioreactors, and to provide oxygen for aerobic digestion. Airflow was not measured. The setup is shown in Figure 3.2.

b The feed sludge to both bioreactors was identical. High power input resulted in the higher temperature in the high shear bioreactor, however, 28°C was still considered as a mesophilic sludge digestion temperature.

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Figure 3.2. The bioreactor with mechanical mixing device
3.3.2 Characterize the Nature of Thermophilic Effect on Dewaterability

This phase of the experimental work was conducted to determine whether the effect of thermophilic digestion on dewaterability was due to changes in physical-chemical compositions of digested sludge, or due to changes in the microbial communities in digested sludge, namely, whether the effect was physical-chemical, or microbiological in nature.

The experimental work consisted of two runs: 01Feb02Run and 01Apr20Run, both used 100% secondary sludge having approximately 2.5%TS as the feed. In each run, thermophilic digestion was carried out at 60°C in duplicates, and one mesophilic digestion was carried out at ambient room temperature of 22°C (as the control). In 01Feb02Run, the 8 L bioreactors (each to contain 5 L feed sludge) were used, samples were taken at 0, 3, 6, 12, 18 hours, 1, 2 days of digestion time. In 01Apr20Run, three 2.8 L glass flasks were used, each to contain 1.5 L feed sludge. Samples were taken at 0, 15, 30 minutes, 1, 2, 3, and 4 hours of digestion time. The smaller initial volume in 01Apr20Run was to facilitate rapid transfer of heat from the waterbath to the sludge, so that the effect of the digestion temperature on dewaterability could be separated from the effect of the digestion time.

3.3.3 Phase (Solid-Liquid) Partition of Substances Affecting Dewaterability

This experimental work was to investigate whether the substances affecting dewaterability were mainly in the liquid phase of the digested sludge, or were mostly associated with the solid phase of the sludge. The experimental work was carried out in two separate tests as replicates. One test used a sample of digested sludge from 01Apr20Run, whereas the second test used a sample of digested sludge from 01Aug23Run. Both tests followed the same testing procedures, as described in the following (using 01Aug23Run as an example):

1. Two bioreactors were operated in parallel (one at 60°C, the second one at room temperature of about 22°C) to produce digested sludge having different dewaterability. The thermophilically digested sludge had poorer dewaterability (higher SCST) than the mesophilically digested sludge.

2. One sample was taken from the thermophilic digester (thermophilic sample, digested at 60°C for 24 hours, having a SCST of 43.5 s L/g). The second sample was taken from the
mesophilic digester (mesophilic sample, digested at 22°C for 6 hours, having a SCST of 11.1 s L/g).

3. Triplicate aliquots of the thermophilic sample and the mesophilic sample were centrifuged at 10,000×g for 20 minutes, then filtered (Fisher G-6 filter paper, 1.2 μm pore size) to become the liquid fraction (filtrate) and the solid fraction (pellet).

4. New samples were reconstituted, according to Table 3.4. The pellet of each of these new samples was re-suspended in and thoroughly mixed with its respective filtrate or distilled water.

5. CST was measured on each of these newly reconstituted samples.

Table 3.4. Composition of reconstituted samples

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Solid Fraction (Pellet)</th>
<th>Liquid Fraction (Filtrate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>T(S)/T(L)</td>
<td>Thermophilic filtrate</td>
</tr>
<tr>
<td>B</td>
<td>T(S)/H₂O</td>
<td>Distilled water</td>
</tr>
<tr>
<td>C</td>
<td>T(S)/M(L)</td>
<td>Mesophilic filtrate</td>
</tr>
<tr>
<td>D</td>
<td>M(S)/M(L)</td>
<td>Mesophilic filtrate</td>
</tr>
<tr>
<td>E</td>
<td>M(S)/H₂O</td>
<td>Distilled water</td>
</tr>
<tr>
<td>F</td>
<td>M(S)/T(L)</td>
<td>Thermophilic filtrate</td>
</tr>
</tbody>
</table>

3.3.4 Characterize Digested Sludge and Relate to Dewaterability

Digestion performance (TS and TVS reduction, pH, conductivity, nitrogen and phosphorus et al.) and properties (cations, viscosity, proteins and polysaccharides, floc charges and sizes et al.) of digested biosolids from various runs were measured and analysed. Relevant data from various runs were grouped and super-imposed for comparison and analysis. These data were correlated to SCST. Results are presented in Chapter 4.

3.4 Sampling and Preservation of Samples

Samples were taken from each batch-operated digester at pre-determined sampling intervals. Sampling was more intensive during the first 2 days of digestion, then became less frequent
during the remaining days of digestion. In most runs, sample collections were discontinued after approximately 10 days of digestion. Prior to each sampling, the contents in each bioreactor were manually mixed thoroughly, the evaporated water was accounted for and was replaced with distilled water. Collected samples were either analysed immediately, or preserved for later analysis, as summarised in Table 3.5. Although, aeration was discontinued during the short sampling periods, Sonneleitner and Fiechter (1983a) reported that short term interruption of aeration was not lethal to the thermophilic microbial population.

Table 3.5. Preservation of samples

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-treatment</th>
<th>Preservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids (TS, TVS)</td>
<td>No</td>
<td>Measured immediately</td>
</tr>
<tr>
<td>CST</td>
<td>No</td>
<td>Measured within 1-2 days</td>
</tr>
<tr>
<td>Soluble proteins, soluble polysaccharides&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Centrifuge/filtration</td>
<td>Freeze at &lt; 0°C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thaw prior to measurements</td>
</tr>
<tr>
<td>Bound proteins, bound polysaccharides&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Remove liquid fraction, then extract from pellet</td>
<td>Freeze at &lt; 0°C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thaw prior to measurements</td>
</tr>
<tr>
<td>pH, conductivity</td>
<td>Centrifuge/filtration</td>
<td>Measured immediately</td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;⁺, NO&lt;sub&gt;x&lt;/sub&gt;, and PO&lt;sub&gt;4&lt;/sub&gt;⁻&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>Centrifuge/filtration</td>
<td>Add H₂SO₄ to pH &lt;2, 4°C</td>
</tr>
<tr>
<td>TKN, TP in filtrate</td>
<td>Centrifuge/filtration, then acid digest the filtrate</td>
<td>Stored at room temperature</td>
</tr>
<tr>
<td>TKN, TP in sludge</td>
<td>Acid digest sludge samples</td>
<td>Stored at room temperature</td>
</tr>
<tr>
<td>Cations (Na&lt;sup&gt;+&lt;/sup&gt;, Ca&lt;sup&gt;2+&lt;/sup&gt; et al.)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Centrifuge/filtration, then acid digest the filtrate</td>
<td>Stored at room temperature (already acidic pH)</td>
</tr>
<tr>
<td>Viscosity of filtrate and sludge</td>
<td>No</td>
<td>Measured within 1-2 days</td>
</tr>
<tr>
<td>SEM Imaging</td>
<td>Fixation of sludge samples&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Fixed samples were placed in a desiccator, room temperature</td>
</tr>
<tr>
<td>Floc size</td>
<td>200 times dilution&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4°C</td>
</tr>
<tr>
<td>Floc charge</td>
<td>Centrifuge/filtration&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4°C</td>
</tr>
</tbody>
</table>
The technique to separate soluble proteins or soluble polysaccharides: the pre-treatment of "centrifugation/filtration", using an IEC Centra-4B high-speed (up to 10,000 \( \times \) g) bench top centrifuge, was done by centrifuging sludge samples at 10,000 \( \times \) g for 20 minutes to obtain the liquid fraction (supernatant), then filtering the supernatant through Fisher G-6 filter paper (1.2 \( \mu \)m pore size) to collect the filtrate. Subsequent measurements were carried out on the filtrate. Measured proteins or polysaccharides in the filtrate were defined as soluble proteins or polysaccharides. Sanin and Vesilind (1994) compared the extracted extracellular polymers (polymeric substances in centrate) of waste activated sludge samples under various centrifugal forces (2,000 to 16,500 \( \times \) g), and reported that 2,000 \( \times \) g for 20 minutes increased the extracted polymers by 6 times, centrifugation speed of higher than 9,000\( \times \)g resulted in only a slight increase in extracted polymers.

The technique to separate bound proteins or bound polysaccharides: centrifuge sludge samples at 10,000 \( \times \) g for 20 minutes, replace the liquid fraction (supernatant) with the same volume of dilute NaOH (10\(^{-3}\) M), re-suspend the pellet in the NaOH solution and thoroughly mix the contents in the test tube by vortexing, centrifuge such newly prepared samples at 10,000\( \times \)g for 20 minutes, collect the supernatant, then filter the supernatant through Fisher G-6 filter paper. The measured proteins or polysaccharides in the filtrate, following such extraction procedures, are defined as bound proteins or polysaccharides.

To measure cations in the feed sludge and the filtrates of all other samples, each sample was acid-digested, following Standard Method 3030F (APHA et al. 1995). The acids used were concentrated H\( \text{NO}_3 \) and 1:1 HCl.

Sludge samples need to be fixed, prior to SEM imaging. The fixation procedures are described in Table A.8 in Appendix A.

Samples for floc size analysis were taken from 00Nov28Run and 01Oct30Run. For 00Nov28Run, sludge samples were first diluted 200 times using tap water, then were stored in cold room (4°C), until the floc size analysis was performed. For 01Oct30Run, sludge samples were stored in the cold room (4°C), then were diluted 200 times using tap water, just prior to floc size analysis.
Floc charges were measured as Zeta potential using the supernatant of sludge samples following the centrifugation pre-treatment.

3.5 Experimental Instruments and Analysis

Where applicable in this dissertation, the Standard Method refers to corresponding methods described in APHA et al. (1995).

3.5.1 Dewaterability and Capillary Suction Time

Dewaterability was measured using a Komline-Sanderson capillary suction timer (CST, shown in Figure 3.3), and was reported as specific CST, namely SCST (CST normalized by TS). Experience of the experimental work revealed that it was impractical to use SRF or time-to-filter (TF) technique to assess the dewaterability of thermophilically digested sludge, because of the filter paper was plugged up in short time, and the time to collect sufficient volume (in several mL) of filtrate for adequate measurement was excessively long. The variations in solids concentrations of collected sludge samples, due to TS reduction through digestion and differences among batches, required solids concentrations be corrected prior to the comparison of measured CST. Therefore, SCST was used to describe the dewaterability of sludge samples throughout this dissertation, except some special experiment (e.g. Figure 4.66, 4.67, and 4.70, where the same sludge sample was used for all aliquots of the experiment).

The CST test unit has a sludge funnel of 1.8 cm in diameter and 2.0 cm in height. The sludge used in each CST test was 5 mL. Whatman No.17 chromatography grade paper was used. The recorded time from the CST device was checked by using a stopwatch. The verification indicated that the timer of the CST device provided reliable and accurate readings. Prior to each use, the CST value of distilled water was tested to confirm that the CST unit worked accurately. Measurements of sludge samples followed Standard Method 2710G.
3.5.2 Floc Charge (Zeta Potential) Measurement

Floc charges were measured as Zeta Potentials on the filtrate of selected thermophilically digested sludge samples, using a Malvern Zetasizer 2000. This instrument measured the electrophoretic mobility of particles in the filtrate, and is capable of measuring the Zeta potentials of particles having sizes of 1 nm to 3 µm. Prior to each use, a standard solution, having a zeta potential of -50 mV, was measured to check the calibration of the instrument.

3.5.3 Floc Size Measurement

Analysis of floc sizes used a Malvern Hydro 2000 M/MU, Mastersizer optical unit (Malvern Instruments Ltd., U.K.). The floc size measurement is based on the principles of Fraunhofer diffraction and Mie light scattering (Standard Method 2560D). This unit can simultaneously measure floc (particle) sizes ranging from 0.02 to 2000 µm. The technique of this measurement requires low concentrations of solids, so that the instrument could recognize and measure sizes of individual particles. Sludge samples were diluted 200 times using tap water, prior to measurements. Although it was desirable to use the filtrate of digested sludge samples for dilution prior to particle size analysis, the 5 L volume of batch digesters could not provide sufficient volumes of filtrate to meet the dilution needs of various samples (each 2.5 mL sludge sample would need 0.5 L of filtrate). A Cole-Parmer MasterFlex pump was connected to the exit end of the Mastersizer, and pulled samples through the Mastersizer for particle size
measurement. A low flow rate of 0.7 mL/min was used to minimize damage to flocs in the process of feeding samples into the Mastersizer.

3.5.4 Viscosity Measurement

Viscosities were measured using a HAAKE VT500 Viscometer and a NV sensor cup for viscosity measurement. Prior to use, the viscometer was calibrated using Brookfield 98.6 cP (25°C) standard solution. The temperatures of tested samples were controlled at 25°C through a water jacket during the viscosity measurement. The system is shown in Figure 3.4.

![Figure 3.4. HAAKE viscometer and NV sensor cup](image)

3.5.5 Protein Analysis and Characterization

3.5.5.1 Lowry assay to measure protein concentrations

The measurement of proteins, either soluble or bound (as defined in Table 3.5), used the Lowry assay (Lowry et al. 1951), with bovine serum albumin (BSA) as the standard. The Lowry assay is a colorimetric method. A HACH DR/2000 spectrophotometer was used for the measurement of protein concentrations at 750 nm wavelength. The standard HACH COD vial of 10 mL size (the vial has a 1 cm diameter) was used in each of measurements. Lowry procedures are adopted from Keleti and Lederer (1974) and are described in Table A.4 in Appendix A. Prior to each set of Lowry measurements, a standard curve was prepared and the coefficient of the standard curve was used to calculate protein concentrations in that set of measured samples. A typical standard
curve is shown in Figure 3.5. Lowry coefficients from various sets of measurements are summarised in Table A.6 in Appendix A. The average of Lowry coefficients of all standard curves is 0.00220, which is comparable with reported Lowry coefficients by others, where BSA was used as the standard: 0.00258 by Raunkjaer et al. (1994) and 0.00291 by Higgins (1995).

![Lowry Assay Standard Curve](image)

Figure 3.5. Typical standard curve for the Lowry Assay (from 01July31Run)

3.5.5.2 SDS-PAGE

SDS-PAGE stands for sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE). This technique uses electrophoretic forces to distribute proteins throughout a gel to produce a protein banding pattern. These patterns reveal information on the size distribution of protein molecules, by referencing to a standard that is composed of known proteins with known molecular weights. Proteins having smaller sizes travel faster, and migrate to the lower part of the gel by the end of the testing process.

The filtrates of selected sludge samples, taken from 01Feb02Run, were used for the SDS-PAGE experimental work. The filtrate samples were pre-treated with trichloroacetic acid (TCA) to obtain concentrated protein solutions, then were applied to a BioRad Mini Protean II mini-nature slab gel apparatus. Another two aliquots of one of above filtrate samples were applied to the BioRad apparatus directly without the TCA pre-treatment. The proteins in the standard ranged from 6,500 Daltons (aprotinin) to 200,000 Daltons (myosin). 1 Dalton is equal to 1 g/mole, or 1 unified atomic mass (e.g. the atomic mass of carbon is 12 Dalton). Further details
on the standard and major reagents used in this SDS-PAGE experimental work are in Table A.7 in Appendix A. Additional information on SDS-PAGE can be found in Ausubel et al. (1999).

3.5.5.3 Dialysis

Dialysis was carried out to characterize the size distribution of proteins. Dialysis bags were made of Spectrum’s Spectra/Por® regenerated cellulose dialysis membrane. Proteins with sizes smaller than pore opening of the membrane migrate to the outside of the bag. The two types of membrane used were: Spectra/Por® 1 having a molecular weight cut off (MWCO) of 6,000-8,000 Daltons, and Spectra/Por® 2 having a MWCO of 12,000-14,000 Daltons. Procedures of dialysis work are described as following.

1. The sample: the filtrate (following centrifugation and filtration) of a thermophilic aerobically digested sample (01Feb02Run, digested for 6 hours) was frozen, until the dialysis test.

2. Sample preparations: the frozen sample was thawed, centrifuged at 10,000×g for 15 minutes, filtered through a 0.45µm membrane filter. The filtrate was then diluted 10 times using distilled water. One aliquot of such prepared sample was placed in a membrane bag. Two bags of different MWCO were used in total.

3. Each membrane bag was sealed, and placed in 800 mL distilled water. The water was then gently stirred.

4. After 17 hours, samples were taken from inside of each of the membrane bags, and from the water surrounding each of the bags.

5. The bags were re-sealed. The water surrounding the bags were removed and replaced with fresh 800 mL distilled water. After an additional 24 hours dialysis (total dialysis time was 41 hours), samples were taken from inside of each of the membrane bags, and from the water surrounding of the bags.
6. The bags were visually inspected. It appeared that no leaks occurred in the bags throughout the dialysis process.

7. The protein concentration of each collected sample was determined, using the Lowry assay. Measured values were corrected for dilution factors, prior to reporting.

3.5.5.4 Trichloroacetic acid (TCA) precipitation of proteins

TCA precipitates most types of proteins, and is often used to concentrate proteins. TCA pre-treatment is the first step in SDS-PAGE procedures. TCA has limited ability to directly precipitate amino acids and peptides. Therefore, analysis on recovery rate of TCA treatment can provide information on characteristics of proteins in a given sample. A total of three samples, as described in Table 3.6, were treated. Each sample was centrifuged at 10,000×g for 15 minutes, then filtered through a 0.45μm membrane filter. Cold 10% TCA was then added into the collected filtrate. The mixture of filtrate and TCA was swirled on ice for 4 minutes, then centrifuged at 10,000×g for 10 minutes. The filtrate was removed. The remaining pellet was re-suspended in 0.1M NaOH. Proteins in both of the filtrate and the re-suspended solution were measured using the Lowry assay.

Table 3.6. Samples in TCA extraction analysis

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sources of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermo</td>
<td>01Feb02Run, digested at 60°C for 12 hours, filtrate</td>
</tr>
<tr>
<td>Meso</td>
<td>01Feb02Run, digested at ~22°C for 12 hours, filtrate</td>
</tr>
<tr>
<td>BSA</td>
<td>2,000 mg/L BSA standard solution</td>
</tr>
</tbody>
</table>

3.5.5.5 Bradford assay to measure protein concentrations

The Bradford assay is an alternative method to measure protein concentrations (Bradford et. al. 1976). The assay is colorimetric based. The Coomassie Brilliant Blue G-250, a dye in Bradford reagent, forms colour with proteins. A detailed description of Coomassie Brilliant Blue G-250 can be found in Osterman (1984). BSA was used as the standard. Four samples from 01Feb02Run were tested: the feed, a thermophilic sample (digested for 12 hr), a mesophilic sample (digested for 12 hr), and a TCA treated sample (thermophilically digested for 3 hr).
Bradford dye reagent was added into the filtrate of each sample, and several BSA solutions of known concentrations. Each prepared sample was vortexed to achieve complete mixing, then was allowed to stand for 10 minutes at the room temperature. Absorbance of each sample was measured at 595 nm wavelength, using a Beckman DU 530 spectrophotometer and a cuvette of 1 cm path length. The Bradford coefficient from this study was 0.00683 ($r^2 = 0.99$, 0-80 mg/L BSA as the standards, absorbance was up to 0.561). The Bradford coefficient appears to be specific to each individual experiment. The Bradford coefficients from other studies, also used BSA as the standard, were reported in a wide range, such as 0.0445 by Raunkjaer et al. (1994) and 0.00154 by Higgins (1995).

3.5.5.6 Heat and protease treatment

Selected samples were treated with boiling or protease to test the role of specific proteins as factors affecting dewaterability. Each of two aliquots of the filtrate from a thermophilic sample (digested at 60°C for 24 hours, taken from 01Aug23Run) was boiled at 100°C for 20 minutes and 60 minutes, respectively. After the treated filtrate cooled down to the room temperature, the pellet from the original sample was re-suspended with its respective treated filtrate and thoroughly mixed by vortexing. The CST of these reconstructed samples was then measured at the room temperature of approximately 22°C.

Three types of protease, all are SIGMA products, were individually tested for their effects:

1. T-7409, a type II-S trypsin from *Porcine pancreas*.
2. P5147, a type XIV bacterial protease from *Streptomyces griseus*, a non-specific protease.
3. P6911, a protease from *Streptomyces griseus*, typically used in nucleic acid isolation procedures.

These protease were supplied as dry powder. Prior to the use, each protease was prepared as a stock solution of 600-700 mg/L. Each of three aliquots of the filtrate (20 mL each) from the thermophilic sample (the same sample as the one that was used in the boiling experiment: digested at 60°C for 24 hours, taken from 01Aug23Run) received one type of protease for a dosage of 50 mg/L. The mixed samples were vortexed, and were placed in a 37°C waterbath for 18.5 hours. After the treated filtrate cooled down to the room temperature of approximately
22°C, the pellet from each of original samples was re-suspended in and thoroughly mixed with its respective treated filtrate. The CST of these reconstructed samples was then measured.

3.5.6 Polysaccharide Analysis and Characterization

3.5.6.1 Dubois assay to measure polysaccharide concentrations

The measurement of polysaccharides, either soluble or bound (as defined in Table 3.5), used the Dubois assay (Dubois et al., 1956), with glucose as the standard. The Dubois assay is a colorimetric method, based on the phenol-sulphuric acid reaction with carbohydrates. A HACH DR/2000 spectrophotometer was used for the measurement of polysaccharide concentrations at 490 nm wavelength. The standard HACH COD vial of 10 mL size (the vial has a 1 cm diameter) was used in each of measurements. The procedures of the Dubois assay were adopted from Keleti and Lederer (1974) and are described in Table A.5 in Appendix A. Prior to each set of Dubois measurements, a standard curve was prepared and the coefficient from the standard curve was used to calculate polysaccharide concentrations in that set of measured samples. A typical curve is shown in Figure 3.6. The coefficients from various sets of measurements are summarised in Table A.6 in Appendix A. The average of Dubois coefficients of all standard curves is 0.0118.

Figure 3.6. Typical standard curve for the Dubois assay (from 01July31Run)
3.5.6.2 Enzyme treatment

Selected samples were treated with enzymes that hydrolyse some forms of polysaccharides to test the role of specific polysaccharides as factors affecting dewaterability. Four types of enzyme, all SIGMA products, were tested individually on their effect on the samples:

1. C-9422, a cellulase, 1,4-[1,3;1.4]-β-D-Glucan 4-glucano-hydrolase from *Trichoderma viride*.
2. H-2125, a hemicellulase from *Aspergillus niger*.
3. A-6380, a Type II-A from Bacillus species, an α-amylase, 1,4-α-D-Glucan-gluconohydrolase.
4. P-4716, a pectinase, polygalacturonase, poly-[1,4-α-D-galacturonide] from *Aspergillus niger*.

These enzymes were made into stock solution of various concentrations (Table 3.7). For each 20 mL aliquot of the filtrate of the thermophilic sample (digested at 60°C for 25 hours, taken from 02Feb17Run), 1 mL of the filtrate was replaced with 1 mL of the stock solution of one type of enzyme. Each filtrate was then vortexed, and was incubated at the appropriate temperatures (Table 3.7) for 24 hours. Following the incubation of the filtrates, the pellet from each of the original samples was re-suspended in and thoroughly mixed with its respective treated filtrate. The CST of these reconstructed samples was then measured at the room temperature.

Table 3.7. Stock concentration and dosage of polysaccharide enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Stock Concentration</th>
<th>Incubation Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>n/a</td>
<td>37°C</td>
</tr>
<tr>
<td>C-9422</td>
<td>3,000 mg/L</td>
<td>37°C</td>
</tr>
<tr>
<td>H-2125</td>
<td>6%</td>
<td>37°C</td>
</tr>
<tr>
<td>Control</td>
<td>n/a</td>
<td>~22°C</td>
</tr>
<tr>
<td>A-6380</td>
<td>1,000 mg/L</td>
<td>~22°C</td>
</tr>
<tr>
<td>P-4716</td>
<td>Original concen. Supplied</td>
<td>~22°C</td>
</tr>
<tr>
<td></td>
<td>as liquid in 5 units/mg proteins</td>
<td></td>
</tr>
</tbody>
</table>
3.5.7 Conventional Analysis

3.5.7.1 pH, temperature, conductivity

pH was measured, using a Beckman G44 pH meter on filtrate of sludge samples. Prior to each use, the pH meter was calibrated, using Fisher pH buffer solutions (either pH 4.0 and 7.0, or pH 7.0 and 10.0 solutions).

The conductivity on filtrates of sludge samples was measured at room temperature, using a Radiometer CDM 3 conductivity meter. Prior to each use, the temperature of the samples was measured manually using a thermometer. The conductivity meter was then calibrated using 0.01 M KCl for the temperature of the samples.

3.5.7.2 Total solids (TS) and Total volatile solids (TVS)

The APHA (1995) Standard Method 2540B (volume based, for Runs carried out from August 2000 to April 2001), or Standard Method 2540G (weight based, for Runs carried out from June 2001 to February 2002) was followed to measure TS. Results from these two methods were within 3.8% difference for a 2.4%TS sample. The TVS analysis followed Standard Method 2540E.

3.5.7.3 Nitrogen (NH\textsubscript{4}+, NOx, TKN) and phosphorus (PO\textsubscript{4}^{3-}, TP)

Ammonia (NH\textsubscript{4}+), nitrite and nitrate (NOx) and phosphate (PO\textsubscript{4}^{3-}) in the filtrates of samples were analysed, using a Lachat Quickchem 8000 Automated Ion Analyser and a Lachat XYZ sampler, according to the QuickChem\textsuperscript{®} methods as the following: NH\textsubscript{4}+-N, QuickChem method No. 10-107-06-1-D (phenolate method, measured in the range of 2.0-100 mg N/L at 630 nm wavelength); nitrite and nitrate (NOx), QuickChem method No. 10-107-04-1-E (cadmium reduction method, measured in the range of 1-100 mg N/L at 520 nm wavelength); and phosphate (PO\textsubscript{4}^{3-}-P), QuickChem method No. 10-115-01-1-Z (ascorbic acid method, measured in the range of 0.002-50.00 mg P/L at 880 nm wavelength).

To measure TKN and TP, 1 mL of each sample was digested using 10 mL of acidic digestion reagent (made of 200 mL H\textsubscript{2}SO\textsubscript{4} and 134 g K\textsubscript{2}SO\textsubscript{4} in 1 L of distilled water) at 140°C for 3.5 hours, and then at 360°C for additional 3.5 hours. Such digested samples were made into a final
volume of 75 mL, then were analysed for NH$_4^+$-N and PO$_4^{3-}$-P, respectively, according to the preceding QuickChem methods.

3.5.7.4 Cations (Ni$^+$, Fe, Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$)

Cations were measured on the sludge and the filtrate of the feed and the digested samples that were taken from 01Oct30Run and 01Nov27Run. Each sample was first acid-digested by following Standard Methods 3030F (nitric acid-hydrochloric acid digestion). Digested samples were then filtered through Whatman 54 hardened filter paper. Cations were measured using a Varian SpectraAA 220 Fast Sequential atomic absorption spectrometer. Analysis results were corrected for dilution factors (required by the needs of acid digestion and measurement) prior to reporting.

The atomic absorption method was used to measure iron (248.3 nm), nickel (232.0 nm), sodium (589.0 nm, KCl was added for a final concentration of 2,000 mg/L of K$^+$ in order to suppress ionization), calcium (422.7 nm, KCl was added for a final concentration of 2,000 mg/L of K$^+$ in order to suppress ionization), and magnesium (202.6 nm).

The atomic flame emission method was used to measured potassium (766.5 nm, CsNO$_3$ was added to a final concentration of 1,000 mg/L of Cs$^+$ in order to suppress ionization).

3.5.8 Scanning Electron Microscope (SEM) Imaging

SEM work included fixation (fixing, drying, and applying conductive coating) and imaging of the samples. The imaging used a Hitachi S-3000N SEM. The sample fixation procedures are detailed in Table A.8 in Appendix A. One sample was a thermophilically digested sludge (approximately 5 d SRT, digested at ~55°C, fed batch operation), taken from the UBC pilot-scale ATAD facility; a second sample was an undigested thickened secondary sludge, having approximately 0.5%TS, taken from the UBC pilot-scale wastewater treatment facility.

3.5.9 pH Adjustment to Condition Digested Biosolids

pH adjustment as a means to improve dewaterability of thermophilically digested biosolids was investigated. One mL of a diluted acid (one of 2%, 10%, 20% H$_2$SO$_4$, or 6%, 30%, 60% HCl,
and 1 mL of distilled water as the control) was added to 20 mL of digested sludge (digested at 60°C for 6 days, taken from 02Feb17Run). The treated samples were vortexed for completed mixing; CST and pH was then measured for each sample.

3.5.10 Polymers to Condition Digested Biosolids

The properties of the two polymers used in this study are summarised in Table A.2 in Appendix A. Polymers were first made into 0.5% stock solution, then were diluted to 0.1% before their addition to selected sludge samples. Test samples included both thermophilically and mesophilically digested sludges, and collected from 00Aug15Run and 00Nov28Run.

3.6 Data Quality Assurance and Control (QA/QC)

The QA/QC program included duplicates in most of runs and replicate runs. For example, two thermophilic bioreactors were operated with identical experimental conditions in 01July31Run (e.g. feed sludge, airflow rates, digestion temperature, mixing, and the sampling technique). SCST of the digested sludge samples were measured separately and compared, acceptable measurements were then averaged for final reporting and analysis. The measurements of most parameters such as proteins, polysaccharides, NH₄⁺, NOₓ, PO₄³⁻, cations included control and sample duplicates.

3.7 Statistical Analysis

Statistical analysis included calculations of the mean, standard deviation and coefficient of variance on measured results of various experiments, or 90% confidence intervals on selected results (e.g. Table 4.3). The student “t test”, by manual analysis, was used to test effects of selected treatments at 90% or 95% confidence (e.g. protease and boiling effects on SCST, Table 4.15).
4.0 Results and Discussions

The experimental results are presented and discussed in this chapter. Sections 4.1 to Section 4.4 describe how major operational factors affect the dewaterability and other characteristics of aerobically digested biosolids, at both thermophilic and mesophilic temperatures. Sections 4.5 to Section 4.15 describe various experiments to investigate the mechanisms of digestion on the dewaterability of digested sludge. The digestion factors that were studied included feed sludge composition (Section 4.1), digestion temperature (Section 4.2), digestion time (Section 4.3), and mixing induced shear that was applied to the sludge during digestion (Section 4.4).

Digestion resulted in changes in the characteristics of digested biosolids. Such changes intrinsically link to the dewatering properties of the digested biosolids. Evaluating the properties of the digested biosolids provides information on the performance of the digestion processes, and helps to identify significant factors that influence dewaterability of the digested biosolids.

4.1 Effects of Feed Sludge Compositions

The feed sludge was made of various proportions of the secondary sludge (waste activated sludge) and the primary sludge, as shown in Table 3.1.

4.1.1 Dewaterability (SCST)

4.1.1.1 Thermophilic aerobic digestion

The net increases in SCST (measured SCST of digested sludge minus SCST of the feed sludge) using the feed containing various proportions of secondary sludge (digested at 60°C), are illustrated in Figure 4.1. The SCST of the feed sludge are shown in Table 4.1. Experimental results revealed that, thermophilic digestion of the feed containing 100% secondary sludge resulted in the largest increase in SCST of 44 s L/g, within one day of digestion. Thermophilic digestion of the feed containing 75% secondary sludge also resulted in a large increase in SCST of 27 s L/g, following 0.75 days of digestion. Continued digestion resulted in a reduction in SCST of the digested sludge using 100% secondary sludge as the feed (the SCST rise in 15-day digested sludge was discussed in the following Section 4.1.1.2), but not much change in SCST of the digested sludge using 75% secondary sludge as the feed (both up to a digestion period of 10 days).
In contrast, for the feed containing 40% (wt.) secondary sludge or 100% (wt.) primary sludge, thermophilic digestion resulted in only a small increase in SCST (an increase of about 3 s L/g in the sludge, using 40% secondary sludge as the feed). The SCST of these two types of sludges remained low (about 5 s L/g) during the period of extended thermophilic digestion. Such low SCSTs were considered readily dewaterable. Results shown in Figure 4.1 suggested that the deterioration in dewaterability, as a result of thermophilic digestion, was mainly due to the presence of the secondary sludge, not the primary sludge.

4.1.1.2 Thermophilic aerobic digestion, repeatability of the observed effect

The experimental results of three replicates of thermophilic aerobically digested 100% secondary sludge are shown in Figure 4.2. Data of the three replicates are from 00Aug15Run (100%SecA), 00Nov28Run (100%SecB), and 01Oct30Run (100%SecC). The feed sludges were collected from various dates during a period of one year. SCST shown in Figure 4.2 are difference between the SCST of the digested sludges and the SCST of the feed sludge. The effects of thermophilic digestion on SCST of the feed containing 100% secondary sludge were, in general, reproducible over the experimental period. The net increase from Run A and B were about identical: both experienced a rapid and large increase in SCST of about 45 s L/g, following about 1 day of digestion. Continued digestion resulted in a decrease in SCST (net gain of SCST was 25 s L/g following 7 days of digestion). The decrease in SCST (indicating an improved dewaterability) agrees with findings in a study on ATAD by Jewell and Kabrick (1980), where the increase in CST during the first 3-8 days of digestion was followed by a substantial decrease in CST after approximately 20 days of digestion. For the Run C in Figure 4.2, although the net increase due to thermophilic digestion is less than the net increase observed in Run A and B, the pattern of measured SCST is similar to that from Run A and B. Such an observation suggested, if the feed contains the same type of sludge, the digestion effect on dewaterability (expressed as net gains in SCST) is not affected by the variations in the feed sludge, due to the difference in the collection date of the feed sludge. The rises in SCST following 12 days (100%SecB) or 15 days (100%SecA) of digestion could be because of a secondary release of the substances that caused the deterioration of dewaterability of digested sludge. Similar rises in mesophilically digested sludge (Figure 4.3, 15-day digested 100%Sec and 20-day digested 40%Sec) were also recorded.
Figure 4.1. Effect of digestion at 60°C on SCST, various compositions in the feed sludge

Figure 4.2. Replicates, effect of digestion at 60°C on SCST of secondary sludge
4.1.1.3 Mesophilic aerobic digestion

The effect of mesophilic aerobic digestion on SCST of digested sludge is shown in Figure 4.3. In general, mesophilic digestion resulted in a progressive increase in SCST, regardless of the types of the feed sludge. However, the rate of increase varied. For the feed containing 100% (wt.) primary sludge (without the secondary sludge), the increase in SCST did not occur until after 6 days of digestion, and the SCST gradually increased by 13.5 s L/g, following 20 days of digestion. For the feed containing 40% (wt.) secondary sludge, there was a progressive increase in SCST with the first 12 days of digestion (increased by 30.5 s L/g), but SCST remained relatively constant at around 30 s L/g, during the additional 18 days of digestion. For the feed containing 75% and 100% (wt.) secondary sludge, a more rapid increase in SCST within the initial days of digestion was observed. After 1 day of digestion, SCST increased by 14.1 s L/g for the 75% feed, and by 17.7 s L/g for the 100% feed. It appears that the rate of increase was greater within the first 1-2 days digestion, than the rate of increase during the additional time of digestion.

![Figure 4.3](image.png)

Figure 4.3. Effect of digestion at 22°C on SCST, various compositions in the feed sludge.
4.1.1.4 Comparison between thermophilic and mesophilic aerobic digestion

The representative data from experimental results shown in Figure 4.1 and Figure 4.3 are summarized in Table 4.1. A positive correlation exists between the presence of the secondary sludge in the feed and the net increase in SCST (or deterioration in dewaterability) of digested biosolids. A higher proportion of the secondary sludge in the feed resulted in a larger increase in SCST, following digestion. Such a finding agrees with reported observations in full-scale thermophilic sludge digestion facilities (Kelly et al. 1993, Matsch and Drnevich 1977, Jewell and Kabrick 1980). It is also noted that, following 6 days of digestion, the net increase in SCST in mesophilic digested sludges was close to or larger than the SCST of thermophilically digested sludges. Visual inspections on both primary and secondary sludges revealed that the undigested primary sludge appeared to be a heterogeneous suspension, containing discrete solids that readily settled out. The undigested secondary sludge was more homogeneous, and showed no distinct tendency for separation between solids and liquid.

4.1.1.5 Overall assessment

The results of this study revealed that, regardless of digestion status of a sludge (undigested or digested, thermophilic or mesophilic), the SCST of a sludge sample correlate exponentially to the weight percentage of secondary sludge contained in the feed sludge (Figure 4.4). Higher SCST corresponded to a higher proportion of secondary sludge in the feed sludge.

4.1.2 Solids Reduction (TS and TVS)

The results of TS and TVS reduction due to thermophilic digestion are shown in Figure 4.5 and Figure 4.6. Both TS and TVS reductions appeared to follow first-order kinetics, which can be described in Equation 4.1 (Metcalf & Eddy, 2003). The calculated endogenous decay rate K of TVS reduction are summarized in Table 4.2 and shown in Figure 4.7. Apparently, when the secondary sludge was a major portion in the feed sludge (e.g. 100%Sec and 75%Sec), the rates of TS and TVS reductions were higher during the initial period of digestion (e.g. K of TVS reduction was 0.033 d\(^{-1}\) from day 2 to day 10 of digestion, using 100%Sec as the feed, as shown in Figure 4.5 and 4.6), and were lower as the digestion continued (e.g. K of TVS reduction was 0.014 d\(^{-1}\) from day 10 to day 20 of digestion, using 100%Sec as the feed, as shown in Figure 4.5 and 4.6). Mavinic and Koers (1981) reported similar observations of higher initial endogenous
decay rate and lower secondary endogenous rate (subsequent to the initial period of digestion) in their studies of mesophilic digestion of waste activated sludge (secondary sludge). Wherever applicable, both initial and secondary decay rates of TVS reduction were reported (Table 4.2 and Figure 4.7; Table 4.4 and Figure 4.22; Table 4.8)

$$\frac{X}{X_0} = -Kt$$

Where:

$X$ and $X_0$: the solids concentrations at time $t$ and time zero, respectively.

$t$: digestion time (day)

$K$: endogenous decay rate (day$^{-1}$)

The rate of TVS reduction from this experiment (e.g. digestion for 10 days at 60°C, or 600 degree-day product, using 75% secondary sludge as the feed, resulted in a 29% TVS reduction) was lower than the rate of TVS reduction typically achieved in full-scale ATAD facilities (more than 38% TVS reduction at 400-500 degree-day product, Kelly et al. 1993). The lower TVS reduction rate was likely due to insufficient adaptation of bacteria to the thermophilic conditions. Among the recorded rates of TVS reduction shown in Figure 4.7, lower rate in TVS reduction generally related to higher proportion of the primary sludge in the feed sludge, which may be because that the primary sludge normally contain more organics but less bacteria than the secondary sludge.

Table 4.1. Effect of feed sludge composition on dewaterability (SCST).

<table>
<thead>
<tr>
<th>Feed Sludge</th>
<th>Undigested Sludge (feed)</th>
<th>Digested Sludge (60°C, 6 d)</th>
<th>Digested Sludge (22°C, 6 d)</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition (wt. /wt.)</td>
<td>(s L/g)</td>
<td>(s L/g)</td>
<td>(s L/g)</td>
<td></td>
</tr>
<tr>
<td>Secondary sludge</td>
<td>Primary sludge</td>
<td>Undigested</td>
<td>Digested</td>
<td>Digested</td>
</tr>
<tr>
<td>100%</td>
<td>0%</td>
<td>17</td>
<td>47</td>
<td>43</td>
</tr>
<tr>
<td>75%</td>
<td>25%</td>
<td>5</td>
<td>32</td>
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<td>40%</td>
<td>60%</td>
<td>3</td>
<td>9</td>
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</tr>
<tr>
<td>0%</td>
<td>100%</td>
<td>1</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure 4.4. Correlation of SCST to proportions of the secondary sludge in the feed sludge
Figure 4.5. Effect of digestion at 60°C on TS, various compositions in the feed sludge

Figure 4.6. Effect of digestion at 60°C on TVS, various compositions in the feed sludge
Table 4.2. TVS decay rates, various feeds, digested at 60°C

<table>
<thead>
<tr>
<th>Feed Sludge Composition</th>
<th>Data Source</th>
<th>Initial K (day)</th>
<th>Secondary K (day)</th>
<th>TVS Avg. daily Red.</th>
<th>TVS red.</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Secondary</td>
<td>00Aug15Run(20)</td>
<td>2-10 (0.033)</td>
<td>10-20 (0.014)</td>
<td>51%</td>
<td>2.6%</td>
</tr>
<tr>
<td>75% Secondary</td>
<td>01Oct30Run(10)</td>
<td>0.25-5 (0.047)</td>
<td>5-10 (0.011)</td>
<td>29%</td>
<td>2.9%</td>
</tr>
<tr>
<td>40% Secondary</td>
<td>00Nov28Run(30)</td>
<td>0-20 (0.010)</td>
<td>20-30 (0.021)</td>
<td>33%</td>
<td>1.1%</td>
</tr>
<tr>
<td>100% Primary</td>
<td>00Aug15Run(20)</td>
<td>0-20 (0.014)</td>
<td>Note (0.014)</td>
<td>23%</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

*TVS reduction rate remained the same through the 20 days of digestion*

![Figure 4.7. Rates of TVS reduction, digestion at 60°C, various feed compositions](image)

4.1.3 pH

The effects of digestion at 60°C on pH were compared in Figure 4.8 (the pH of mesophilically digested sludge are not shown, as such information has been available in many other literature for long time, and is not the interest of this research). The feed containing 100% primary sludge had lower pH than the feed containing 100% secondary sludge. In general, following 5-6 days of thermophilic digestion at 60°C, the pH gradually increased to approximately 7.5, regardless of the composition of the feed. Further digestion from day 6 to day 12 did not result in much
change in pH. Additional digestion from day 12 to day 30 resulted in a decrease in pH. Morgan et al. (1984) digested mixed sludge (primary and humus sludges) at aerobic and thermophilic condition, and reported a consistent increase in pH, regardless of variations in other operation parameters. The pH increased from 5.2-5.7 in the feed sludge, and to 7.4-7.7 in the sludge that was digested for approximately 8 days, at approximate 50°C. The changes in pH shown in Figure 4.8 agrees with the reported pH changes in full-scale facilities, where ATAD digestion resulted in an increase in pH to approximately 8 (USEPA 1990).

Figure 4.8. Effect of digestion at 60°C on pH, various compositions in the feed sludge

4.1.4 Conductivity

Measured conductivities of the filtrate from samples of the digested sludge are shown in Figure 4.9 (a) and (b). As shown in Figure 4.9 (a), thermophilic digestion resulted in similar changes in conductivities in the filtrate of digested sludge, regardless of the varied proportions of the secondary sludge in the feed (40%, 75% and 100% secondary sludge). Digestion resulted in an increase in conductivity within a short time of digestion. Continued digestion resulted in a gradual decrease in conductivity. For example, the conductivity of digested sludge from the feed containing 100% secondary sludge increased from 3.0 mS/cm (feed) to 5.7 mS/cm (4 days digested), then decreased to 1.7 mS/cm (digested for 30 days). The composition of the sludge
affects the measured values of conductivity. When the feed sludge contained a higher fraction of secondary sludge, measured conductivities in the filtrate of the digested sludge were generally higher.

Measured conductivities in the filtrate of mesophilically digested sludge, as shown in Figure 4.9 (b), revealed that, regardless of the feed sludge composition, the mesophilic digestion resulted in a gradual increase in conductivity within the first 9-10 days of digestion. Continued digestion resulted in a slight decrease in conductivity of the filtrate. For example, the conductivity of the filtrate of the digested sludge from the feed containing 100% secondary sludge increased from 3.0 mS/cm (feed) to 5.5 mS/cm (9 days digested), then decreased 4.3 mS/cm after 30 days of digestion.

When the feed contained a higher fraction of the secondary sludge, the measured conductivity in mesophilically digested sludge was higher. Such an observation is similar to what was observed with thermophilically digested sludge. In contrast, the net decreases in conductivities of mesophilically digested sludge are less than the decreases in conductivities of thermophilically digested sludge, during an extended time of digestion.

The data of measured conductivity provided information of charged floc particles in the soluble fraction of the sludge. An increase in conductivity indicated an increase in soluble colloidal particles, which was likely due to the lysis of cells and solublization of complex organics in the sludge as a result of thermophilic treatment.
Figure 4.9. Effect of digestion on conductivity, various compositions in the feed sludge

(a) Digested at 60°C

(b) Digested at ~22°C
4.1.5 Nitrogen (NH$_4^+$)

Feed sludge composition affected the ammonia produced in the digested sludge. Figure 4.10 shows measured ammonia concentrations in the filtrate of sludge that was digested at 60°C from feed of various compositions. The ammonia concentrations in the filtrate of mesophilically digested sludge are not shown, as such information is available in many other literatures. When the feed sludge contained a higher fraction of the secondary sludge, there were higher concentrations of ammonia in the digested sludge. For example, when the feed contained only primary sludge (100% primary sludge), the ammonia concentration was 35 mg/L in the feed, and 56 mg/L in the 2 day digested sludge. When the feed contained 75% secondary sludge, the ammonia concentrations were 405 mg/L in the feed and 762 mg/L in 2 day digested sludge. Continued digestion resulted in a decrease in NH$_4^+$-N in the digested sludge, regardless of the feed sludge composition. For example, for the feed containing 40% secondary sludge, NH$_4^+$-N in the digested sludge increased from 264 mg/L in the feed to 708 mg/L in the 6 days digested sludge, then gradually decreased to 41 mg/L, following 30 days of digestion.

4.1.6 Phosphorus (PO$_4^{3-}$)

Measured PO$_4^{3-}$-P concentration in the digested sludge is shown in Figure 4.11. The digestion temperature was 60°C (the phosphate concentrations in the filtrate of mesophilically digested sludge are not shown, as such information is available in many other literatures. In general, higher PO$_4^{3-}$-P concentrations in the filtrate of the feed sludge or in the digested sludge were related to higher proportions of the secondary sludge in the feed sludge. Regardless of the feed sludge composition, the thermophilic digestion resulted in an increase in PO$_4^{3-}$-P concentration during the first 10 days of digestion. Continued digestion resulted in a decrease in PO$_4^{3-}$-P concentration. For example, the PO$_4^{3-}$-P concentration in the digested sludge increased from 54 mg/L in the feed to 97 mg/L in the 15 day digested sludge, then decreased to 58 mg/L in the 30 days digested sludge, when the 40% secondary sludge was used as the feed.
Figure 4.10. Effect of digestion at 60°C on NH$_4^+$-N, various compositions in the feed sludge

Figure 4.11. Effect of digestion at 60°C on PO$_4^{3-}$-P, various compositions in the feed sludge
4.1.7 Extracellular Proteins

Measurement results are shown in Figure 4.12. The solids concentration was approximately 25 g/L (2.5%TS) in the feed sludge, and 20 g/L (2.0%TS) in the digested sludge. Protein concentrations were measured on filtrate of the feed and digested sludge, and normalized by TS, then reported as a net increase from that in the feed. The digestion temperature was 60°C.

The results in Figure 4.12 revealed that the feed containing a higher fraction of secondary sludge gave a higher concentration of soluble protein in the digested sludge. When the feed sludge contained 100% or 75% secondary sludge, thermophilic digestion resulted in a rapid increase of soluble protein. Following 6-hour of digestion, the protein concentrations increased by 126 mg protein/g TS in the feed containing 100% secondary sludge, and by 93 mg/g in the feed containing 75% secondary sludge. In both cases, continued digestion resulted in a reduction in soluble protein. For the feed containing 40% secondary sludge, or 100% primary sludge (0% secondary sludge), digestion resulted in a gradual increase in soluble protein.

It has been shown, as described in Section 2.3.2.1, that extracellular proteins had significant effect on settling and dewatering properties of sludge. The proteins that adhere to the outer surface of bacteria cells form bridges among bacteria cells and lead to the aggregation of sludge flocs (Busch and Stumm 1968). The positive role of proteins, as part of extracellular biopolymers, in promoting bioflocculation of activated sludge in the secondary clarifier was confirmed in a study by Kato et al. (1971).

Since one of the objectives of sludge dewatering is to remove the water that is entrapped in sludge flocs, the substantial increase of protein concentrations in thermophilically digested secondary sludge would hinder the dewatering of the thermophilic sludge, because these proteins would form a complex matrix in the sludge that would make it difficult for the water to be removed.

4.1.8 Extracellular Polysaccharides

Measured soluble polysaccharides are shown in Figure 4.13. The samples tested are the same as those used for protein measurements (shown in Figure 4.12). The data shown in Figure 4.13 are net increases in polysaccharide concentrations, compared to the values from the feed. They are
reported as normalized concentrations. Digestion effects on concentrations of soluble polysaccharides in digested sludge were similar to the effects on soluble protein. When the 100% or 75% secondary sludge was used as the feed, thermophilic digestion (60°C) resulted in a rapid increase in soluble polysaccharide concentration following a short time of digestion: after 6 hours of digestion, the net increase in soluble polysaccharides in the digested sludge was 40 mg/g (using 100% secondary as the feed) and 31 mg/g (using 75% secondary sludge as the feed). Polysaccharide concentrations decreased slightly during the 6-hr to one day of digestion, then further increased during the one day to 10 days of digestion. When 40% secondary sludge, or 100% primary sludge was used as the feed, the net increase in polysaccharide concentration of the digested sludge was much lower than the concentration from the sludge that used 75% or 100% secondary sludge as the feed. Continued digestion of up to 30 days progressively increased the polysaccharide concentration.

The polysaccharides are considered as part of the extracellular biopolymers, and also contributed to the forming of sludge aggregates. The role of polysaccharides is similar to the role of proteins in affecting the dewaterability of digested sludge, as discussed in the preceding Section 4.1.7.
Figure 4.12. Effect of digestion at 60°C on the amount of soluble protein, various feeds

Figure 4.13. Effect of digestion at 60°C on the amount of soluble polysaccharides, various compositions in feed sludge
4.1.9 Floc Charge (Zeta potential)

Floc charges are shown in Figure 4.14 (a) and (b). Regardless of the feed sludge composition, measured floc charges (as Zeta potentials) were all approximately -15 mV (-15 mV to -18 mV in thermophilically digested sludge, and -12 mV to -15 mV in mesophilically digested sludge). Although there were some variations in measured Zeta potentials, either thermophilic digestion or mesophilic digestion did not result in significant changes in Zeta potential. There has not been other reported work on floc charges of thermophilically digested sludge. The little changes in the floc charges of thermophilically digested sludge suggested that the charge neutralization, as described in Section 2.2.2, is not applicable to explain the deterioration in dewaterability of thermophilically digested sludge.

4.1.10 Floc Size

The effects of digestion on floc size of digested sludge, using various proportion of the secondary sludge as the feed, are shown in Figure 4.15 (a) thermophilic digestion at 60°C, and (b) mesophilic digestion at 22°C. Measured results are expressed as surface mean diameter, or SMD. A lower SMD suggests smaller floc size. The results revealed that, for thermophilic digested sludge, variations in the feed sludge composition did not result in much difference in the SMD of digested sludge (other than some random variations); for mesophilic digested sludge, sludge flocs of digested primary sludge appeared to be larger than those in digested sludge containing 40% or 100% secondary sludge. Thermophilic digestion up to 15 days resulted in a general decrease in the SMD, suggesting a general decrease in floc size. This result indicated that the thermophilic temperature is a dominant factor that determine the floc sizes of digested sludge.
Figure 4.14. Effect of digestion on floc charge, various compositions in the feed sludge

(a) Digested at 60°C

(b) Digested at ~22°C
Figure 4.15. Effect of digestion on floc size, various compositions in the feed sludge

(a) Digested at 60°C

(b) Digested at ~22°C
4.1.11 Feed Sludge Effect on SCST and Characteristics of Digested Sludge

4.1.11.1 100% primary sludge as the feed

When 100% primary sludge was used as the feed sludge, thermophilic digestion at 60°C did not result in much changes in SCST of the digested sludge, during the first 7 days of digestion (Figure 4.1); however, volatile solids reduction occurred from the start of the digestion and continued at a rate of 0.014 day\(^{-1}\) during the 20 days of digestion (Figure 4.6 and Table 4.2). The pH (Figure 4.8) and NH\(_4^+\)-N concentrations (Figure 4.10) also changed. These changes in characteristics of sludge suggested endogenous respiration and continuous biodegradation of microbial organics, and provided evidence of microbial activities.

The measured NH\(_4^+\)-N concentrations in digested sludge was a net result of its production due to biodegradation of organic nitrogen and its depletion due to air stripping at thermophilic temperatures. During the first 7-8 days of digestion, more biodegradable organics were readily available, and the production of NH\(_4^+\)-N exceeded the depletion of NH\(_4^+\)-N, resulting in a net increase in NH\(_4^+\)-N. The decline in NH\(_4^+\)-N concentrations during the period of more than 8 days of digestion indicated that readily biodegradable organics were exhausted, and the newly produced NH\(_4^+\)-N was less than the depleted NH\(_4^+\)-N.

The biodegradation of organics also resulted in the conversion of organic phosphorus to polyphosphorus and eventually ortho-phosphate (PO\(_4^{3-}\)-P). Since the ortho-phosphate remained in the liquid fraction of digested sludge, its concentration is an indicator of organics degradation. The gradual increase in phosphate concentrations during the first 8 days of digestion (Figure 4.11) suggested that the biodegradation of organics in the primary sludge was a gradual process. The timing of such a change in phosphate concentration appeared to be approximately the same as the changes in TVS, and NH\(_4^+\)-N concentrations.

Primary sludge consists of mainly raw organics, instead of bacterial cells. Aerobic digestion would primarily result in the break down of complex organics into simple organics for further metabolic degradation, rather than lysis of cells. Therefore, the changes in the concentration of soluble protein (Figure 4.12) and soluble polysaccharides (Figure 4.13) were relatively small during the initial period (approximate 8 d) of digestion. The decrease in surface mean diameter
(Figure 4.15), suggesting a reduction in floc sizes of digested sludge, indicated the break down of organic aggregates.

Thermophilic digestion did not result in much negative effect on dewatering properties (as measured by SCST) of the digested primary sludge, although, digestion resulted in changes in sludge characteristics. The primary sludge appeared to be a heterogeneous suspension, containing coarse and discrete solids, and possessed a fibre-like structure; this allowed a rapid drainage of water through pores within the sludge. Visual observation of digested primary sludge revealed that the fibre-like structure was not destroyed by thermophilic digestion. Therefore, no significant deterioration of the dewaterability of digested sludge occurred.

4.1.11.2 Mixture of 40% secondary sludge and 60% primary sludge as the feed

When the feed containing 40% secondary sludge and 60% primary sludge was thermophilically digested at 60°C, the net increase in SCST was higher than the increase in SCST of digested 100% primary sludge. Continued reduction of volatile solids (Figure 4.6) and changes in pH (Figure 4.8) throughout the 30 days digestion indicated microbial biodegradation of organics. It is noted that changes in conductivity and NH$_4^+$-N concentrations appeared to follow a similar pattern (Figure 4.9 and Figure 4.10), with both increasing during the initial period of digestion, peaking following 6 days of digestion, then decreasing during the remaining days of continued digestion. Conductivity measures the ability of a solution to carry electric current, and indicates the concentration of ionized substances in the solution. The increase in conductivity during the first 6 days of digestion was likely due to the increase in NH$_4^+$-N concentration, as a result of solubilization and biodegradations of organics in the feed sludge.

The general increase in the concentration of phosphate (Figure 4.11), soluble proteins (Figure 4.12) and soluble polysaccharides (Figure 4.13) indicated the gradual breakdown of complex organics in the sludge. The floc charges showed a slight decrease (Figure 4.14a). The gradual decrease in SMD indicated a reduction in floc size. It appears that, when using 40% secondary sludge as the feed, changes in sludge characteristics had little effect on the dewatering properties of digested sludge. It is likely that the 60% primary sludge contained in the feed had
predominant influence on dewaterability of digested sludge, mostly likely related to the porosity in the primary sludge.

4.1.11.3 Mixture of 75% secondary sludge and 25% primary sludge as the feed

When the feed sludge used 75% secondary sludge, a rapid and significant increase in SCST (Figure 4.1), the concentrations of soluble proteins (Figure 4.12) and polysaccharides (Figure 4.13) occurred following 3 hours of thermophilic digestion at 60°C. The phosphate concentration increased from the start of digestion (Figure 4.11). The secondary sludge mainly consisted of bacterial cells. Typically, protein accounts for 32-41% of TS in untreated secondary sludge (Metcalf & Eddy 2003). Polysaccharide (carbohydrates) also is a major component of cell structure. The substantial increase in protein concentration suggested the release of intracellular proteins, due to cell lysis, and the solubilization of cell proteins to the liquid phase of digested sludge.

In contrast, during the first 6 hours of digestion, volatile solids (Figure 4.6) and pH (Figure 4.8) remained relatively constant, both conductivity (Figure 4.9) and NH₄⁺-N concentrations decreased (Figure 4.10), and floc charges (Figure 4.14a) were also relatively constant. It was after 6 hours of digestion that pH, conductivity, and NH₄⁺-N concentrations increased. It appeared that pH, NH₄⁺-N concentrations, volatile solids, and floc charges were not critical factors influencing SCST.

The initial decrease in pH was likely due to solubilization and acidification of complex organics into simple organics, such as volatile fatty acids (Chu 1995). The initial decrease in NH₄⁺-N concentrations indicated more ammonia was stripped, due to aeration at thermophilic temperatures, than the produced ammonia due to biodegradation of organic nitrogen. The volatile solids reduction occurred concurrently with the increase in NH₄⁺-N concentration, indicating the increased rate of microbial activity, to degrade organics in sludge. However, the reduction in volatile solids was unlikely a fundamental reason for the rapid increase in SCST of thermophilically digested sludge, because TVS reduction occurred several hours after the surge in SCST occurred.
4.1.11.4 100% secondary sludge as the feed

When the proportion of the secondary sludge in the feed sludge increased from 75% to 100%, thermophilic digestion resulted in higher net increases in SCST (Figure 4.1), soluble proteins (Figure 4.12), and polysaccharides (Figure 4.13). Such an increase occurred within short time of thermophilic digestion. Such findings offered additional evidence that substances causing poor dewaterability were mainly associated with the secondary sludge. These substances were released into the sludge, due to cell lysis, shortly after the sludge was subjected to thermophilic treatment. Data shown in Figure 4.2 confirmed that the heat effect on SCST of digested sludge were repeatable phenomenon. The impact of the heat was not affected by the random variations in the secondary sludge. Such variations were due to the differences in collection dates of the feed sludge. The decrease in SMD, indicating a decrease in floc sizes, provided additional evidence on the breakdown of cells.

Volatile solids reduction occurred at a rate of 0.033 day\(^{-1}\) from the start of the digestion, which indicates the microbial biodegradation of readily available organics in the secondary sludge. The increase in phosphate concentration (Figure 4.11) was an additional evidence of organic biodegradation. There was not much change in floc charges (Zeta potentials) throughout the digestion. The decrease in NH\(_4^+\)-N concentrations during the first 6 hours of digestion indicated that more ammonia was stripped than was produced, from the biodegradation process during the initial period of digestion, when bacterial activities was relatively low; this reflected bacterial adaptation to the thermophilic condition.

4.1.11.5 Mesophilic digestion of various feed sludge

Mesophilic digestion resulted in gradual increase in SCST of digested sludge, regardless of types of feed sludge (Figure 4.3). Conductivity (Figure 4.9) and ammonia (Figure 4.10) also increased gradually, which suggested that biodegradation occurred gradually during the course of digestion. The observed difference indicated that impact of thermophilic digestion on dewaterability of digested sludge was primarily heat induced, mostly likely due to cell lysis; this resulted in the release of intracellular materials into the solution.
4.1.11.6 Summary of feed sludge effects on dewaterability

The experimental results indicated that the secondary sludge, not the primary sludge in the feed sludge, was the source of concern, in causing poor dewaterability in the thermophilically digested sludge. The deterioration in dewaterability (as measured by SCST) correlated to increases in concentrations of the proteins and polysaccharides, but was not correlated to changes in pH, volatile solids, ammonia, and phosphate concentrations. Floc charge did not change much as a result of thermophilic digestion.

These findings agreed well with work reported from full-scale studies. When the primary sludge was used as the feed, dewaterability of thermophilically digested sludge was comparable to the dewaterability of the feed sludge (Kelly et al. 1993). When a mixture of the primary and the secondary sludge were used, dewaterability was comparable to (Garber et al. 1975) or poorer than (Matsch and Drnevich 1977, Kelly et al. 1993) the dewaterability of the feed sludge. When a major portion of the feed was the secondary sludge, thermophilically digested sludge was the most difficult to dewater (Kelly et al. 1993). Burnett et al. (1997) reported very high demands in dosage of polymers to dewater thermophilically digested sludge that was produced at the ATAD plant in College Station, Texas, where the thickened secondary sludge was used as the feed. The poor dewaterability of the sludge, when the secondary sludge was present in sufficient amount, was likely due to the gel-like structure formed in the digested secondary sludge (Poxon 1996). Such a gel structure was formed by biocolloids in solution that were extensively hydrated multi-molecular aggregates. These aggregates do not have a distinct particle surface and form a homogeneous sludge.

4.2 Effects of Digestion Temperature

4.2.1 Dewaterability (SCST)

4.2.1.1 Thermophilic aerobic digestion

Findings described in Section 4.1 suggested that the substances causing deterioration in dewaterability are mainly associated with the secondary sludge. Therefore, experimental work to investigate temperature effects on dewaterability of digested biosolids used 100% secondary sludge as the feed. The results are shown in Figure 4.16, which shows the net increases in SCST
of digested biosolids from their respective feeds. Table 4.3 shows the data sources of Figure 4.16.

The temperature of the feed sludge was between 15-20°C. Sludge in the digesters reached targeted temperatures (40°C, 50°C, 55°C, 60°C, or 70°C) within 3 hours, following the start-up of waterbath heating. In general, digestion at 50°C or higher temperatures all resulted in rapid increases in SCST (or deterioration of dewaterability) within the first 2 days, or shorter time of digestion. Higher digestion temperatures resulted in more rapid increases in SCST. Continued digestion of up to 10 or 12 days did not result in a further increase in SCST.

When the sludge was digested at 40°C (typically regarded as pseudo-thermophilic temperature), SCST increased rapidly during the initial 2 days of digestion. An additional 8 days of digestion resulted in a slight increase in SCST. The net increases of SCST were 31.3 s L/g at day 2, and 35.6 s L/g at day 10 of digestion. When the sludge was digested at 50°C, 2 days of digestion rapidly increased SCST by 42.1 s L/g. An additional 7 days of digestion resulted in a slight decrease in SCST.
Figure 4.16. Effect of digestion temperatures on SCST, 100% secondary sludge as the feed
For digestions that were conducted at 55°C, 60°C, and 70°C, similar rapid increases in SCST following short periods of digestion were observed. The increases in SCST are 55.2 s L/g (digested for 0.5 days at 55°C), 44.3 s L/g (digested for 1 day at 60°C), and 41.2 s L/g (digested for 0.5 days at 70°C). However, following the initial surge in SCST, continued digestion resulted in a decrease in SCST at these three digestion temperatures. Higher digestion temperatures eventually resulted in lower SCST for the digested sludge. The net increases in SCST for 70°C digested sludge was 23.8 s L/g after 2 days of digestion, and remained relatively constant (at approximately 22 s L/g) from day 2 to day 7 of digestion. The net increase in SCST for 60°C digested sludge was 31.3 s L/g after 2 days of digestion. It then remained relatively constant (at approximately 30 s L/g) from day 2 to day 6 of digestion. The net increase in SCST for 55°C digested sludge was 35.6 s L/g at 2 days of digestion. The net increase in SCST of two selected digestion times (3-hour and 5 days) for all temperatures are summarised in Table 4.3.

Figure 4.17. Comparing the effects of several digestion temperatures on SCST

The temperature effects on SCST of thermophilic digested secondary sludge are shown in Figure 4.17. The measured SCST from this study agreed well with what Burnett et al. (1997) observed at the ATAD plant in College Station, Texas, where thickened secondary sludge
(~4.5%TS) was thermophilically digested; an increase of CST from 54 seconds (1.3 s L/g) to 1,450 seconds (35 s L/g) was reported.

Table 4.3. Temperature effects on dewaterability (SCST)

<table>
<thead>
<tr>
<th>Digestion Temp. (°C)</th>
<th>70</th>
<th>60</th>
<th>60</th>
<th>50</th>
<th>40</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed CST* (s)</td>
<td>294±40(6)</td>
<td>215±30(5)</td>
<td>484±22(3)</td>
<td>308±83(5)</td>
<td>183±14(6)</td>
<td>294±40(6)</td>
</tr>
<tr>
<td>TS* (g/L)</td>
<td>24.6±0.1(3)</td>
<td>24.9±0.1(4)</td>
<td>25.0±0.2(2)</td>
<td>22.9±0.2(3)</td>
<td>24.5±0.3(3)</td>
<td>24.6±0.1(3)</td>
</tr>
<tr>
<td>SCSTb (s L/g)</td>
<td>12±1</td>
<td>9±1</td>
<td>19±2</td>
<td>13±4</td>
<td>7.5±0.5</td>
<td>12±1</td>
</tr>
<tr>
<td>3-hr CST* (s)</td>
<td>1054±81(4)</td>
<td>865±125(6)</td>
<td>713±149(4)</td>
<td>262±27(4)</td>
<td>341±8(2)</td>
<td></td>
</tr>
<tr>
<td>TS* (g/L)</td>
<td>24.9±0.1(4)</td>
<td>24.0±0.3(4)</td>
<td>23.0±0.6(4)</td>
<td>24.0±0.3(4)</td>
<td>24.7±0.1(2)</td>
<td></td>
</tr>
<tr>
<td>Net SCSTc (s L/g)</td>
<td>30±4</td>
<td>27±4</td>
<td>18±9</td>
<td>3±2</td>
<td>2±7</td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 days CST* (s)</td>
<td>707±41(6)</td>
<td>914±97(3)</td>
<td>832±91(7)</td>
<td>685±75(8)</td>
<td>554±23(3)</td>
<td></td>
</tr>
<tr>
<td>TS* (g/L)</td>
<td>21.3±0.2(4)</td>
<td>21.3±0.2(4)</td>
<td>18.2±0.1(4)</td>
<td>17.6±0.4(4)</td>
<td>20.8±0.3(2)</td>
<td></td>
</tr>
<tr>
<td>Net SCSTc (s L/g)</td>
<td>21±2</td>
<td>24±12</td>
<td>32±5</td>
<td>32±3</td>
<td>15±3</td>
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<tr>
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<td>01Feb02Run</td>
<td>00Nov28Run</td>
<td>01June15Run</td>
<td>01July14Run</td>
<td>01July31Run</td>
</tr>
</tbody>
</table>

aCST and TS are expressed as “average ± one time of standard deviation (number of measurements)”.
bSCST are expressed as “calculated SCST (dividing the average of CST by the average of TS) ± 90% confidence interval”.
cSCST of 3-hr and 5 days digested sludge are expressed as “net increase in SCST (subtract the SCST of the feed from the SCST of the digested sludge) ± 90% confidence interval”.
dData of 55°C were not shown.

4.2.1.2 Mesophilic aerobic digestion

Effects of mesophilic digestion on SCST are shown in Figure 4.18. Data shown are the net increases in SCST of mesophilic digestion, and are from 10 runs (represented by various legends). There are variations in net increase of SCST from different runs of mesophilic digestion. However, SCST of mesophilically digested sludges all showed progressive increases
within the first 12 days of digestion. The net increase of SCST, following 12 days of digestion, was approximately 30 s L/g. Further mesophilic digestion (from 12 to 30 days) did not result in much additional increase in SCST. Bernard and Gray (2000) reported similar effect of aerobic digestion effect on CST. In their work, the feed sludge was 3,000 to 5,000 mg/L mixed liquid activated sludge. Major increase of CST occurred during the first 21 days of digestion.

4.2.1.3 Comparison between thermophilic and mesophilic aerobic digestion

The SCST of thermophilically digested biosolids are compared with SCST of mesophilically digested biosolids in Figure 4.19. The maximum difference in SCST of thermophilically and mesophilically digested biosolids is close to 45 sec-g/L. This difference occurred following 0.5 days of digestion (digested at 55°C). Further digestion resulted in a smaller difference in SCST between the thermophilically and mesophilically digested sludge; the difference was about 20-30 sec-g/L following one day of digestion; between 10-20 sec-g/L following 2-4 days of digestion; and 5-12 sec-g/L following 6 days of digestion. It appears that if the digestion is longer than 6 days, there is no significant difference in dewaterability (SCST) among thermophilic and mesophilic digested sludges.

4.2.2 Solids Reduction (TS and TVS)

4.2.2.1 Thermophilic aerobic digestion

Sludge digestion is intended to reduce volatile solids in the sludge through endogenous respiration under aerobic or anaerobic conditions. Adequate thermophilic aerobic digestion requires a minimum of 38% volatile solids reduction (USEPA 1990). Total solids and total volatile solids were monitored in each of batch digestion experiments to assess the performance of digestion process. Results of measured TS and TVS from various runs (all used air for mixing and oxygen supplies) are shown in Figure 4.20 and 4.21. The TVS reduction decay rates K are summarized in Table 4.4., and are shown in Figure 4.22.
Figure 4.18. Effect of mesophilic digestion on SCST, 100% secondary sludge as the feed

Table 4.4. TVS decay rates, several temperatures, 100% secondary sludge as feed

<table>
<thead>
<tr>
<th>Digestion Temp.</th>
<th>Data Source (digest. days)</th>
<th>Initial K (day)</th>
<th>Secondary K (d⁻¹)</th>
<th>TVS Red.</th>
<th>TVS RED.</th>
</tr>
</thead>
<tbody>
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<td>40°C</td>
<td>01July14Run (12)</td>
<td>0.04-2</td>
<td>2-10</td>
<td>0.013</td>
<td>44%</td>
</tr>
<tr>
<td>50°C</td>
<td>01June15Run (9)</td>
<td>0.5-2</td>
<td>2-9</td>
<td>0.006</td>
<td>30%</td>
</tr>
<tr>
<td>55°C</td>
<td>01Nov27Run (10)</td>
<td>1-5</td>
<td>5-10</td>
<td>0.012</td>
<td>27%</td>
</tr>
<tr>
<td>60°C</td>
<td>01Oct30Run (10)</td>
<td>0.25-5</td>
<td>5-10</td>
<td>0.000</td>
<td>30%</td>
</tr>
<tr>
<td>70°C</td>
<td>01July31Run (10)</td>
<td>0-10</td>
<td>Note a</td>
<td>0.032</td>
<td>26%</td>
</tr>
</tbody>
</table>

*aTVS reduction rate remained the same through the 10 days of digestion*
Figure 4.19. SCST of thermophilic sludge minus SCST of mesophilic sludge

(a) Digested at □ 70°C, ■ 60°C, and ◊ 55°C

(b) Digested at ○ 50°C and ● 40°C
Figure 4.20. Effect of digestion temperatures on TS, 100% secondary sludge as the feed

(a) Digested at □ 70°C, ■ 60°C, and ◇ 55°C

(b) Digested at ○ 50°C and • 40°C
Figure 4.21. Effect of digestion temperatures on TVS, 100% secondary sludge as the feed

(a) Digested at □ 70°C, ■ 60°C, and ◇ 55°C

(b) Digested at ◆ 50°C and ⬤ 40°C
Figure 4.22. TVS decay rates, several digestion temperatures, 100% secondary sludge as feed

The experimental results revealed that, when the sludge was digested at 40°C or 50°C, TVS reduction occurred at higher rate (0.19 day\(^{-1}\) and 0.23 day\(^{-1}\)) for shorter time (2 days of log TVS reduction) than when the sludge was digested at 55°C or 60°C, where TVS reduction occurred at lower rate (0.051 day\(^{-1}\) and 0.066 day\(^{-1}\)), but for longer time (5 days of log TVS reduction). For the sludge that was digested at 40-60°C, the TVS reduction rates, subsequent to the lag phase, are much lower (0.013 day\(^{-1}\) or less). When the sludge was digested at 70°C, the rate of TVS reduction is 0.032 day\(^{-1}\). Although, the rate was lower than the rates from 40-60°C digestion, the TVS reduction continued at that rate throughout the 10 days of digestion at 70°C. The changes in reduction of total suspended solids during a period of 3 days aerobic thermophilic digestion, reported by Haner et al. (1994), were similar to above findings.

It appears that readily available organics were first biodegraded, resulting in a higher rate of TS and TVS reduction during the initial days of digestion. As digestion continued, readily available organics were depleted, and it was more difficult to biodegrade the remaining biomass; therefore, the rates of TS and TVS reduction were lower. In comparison, Mavinic and Koers (1979) reported an initial endogenous decay rate of 0.055 to 0.076 d\(^{-1}\) in aerobic digestion of
waste activated sludge (digestion at 25°C) from a laboratory study. Metcalf & Eddy (2003) suggested that representative reaction rate \( K \) of waste activated sludge ranged from 0.05 \( \text{d}^{-1} \) at 15°C to 0.14 \( \text{d}^{-1} \) at 25°C, and indicated that the values of reaction rates are affected by the sludge type, temperature, and solids concentrations, which need to be confirmed by bench-scale or pilot-scale studies.

The changes in TS and TVS reduction rates also likely related to increased activities of thermophilic micro-organisms throughout digestion processes. Bomio and Sonnleitner et al. (1989) fed-batch cultivated a mixture of primary and secondary sludge for 14 hours, to test activities (reported as calculated OUR, expressed as \( \text{mg O}_2/\text{L-h} \)) of thermophilic micro-organisms (65°C). They found that activities of thermophilic micro-organism went through five phases during digestion:

1. A decline of OUR (0-1 hr) indicating the inactivation of non-thermopiles due to the heat.
2. Unchanged OUR (1-8 hr) at very low level indicating a lag phase of the thermopiles activities.
3. Exponential increase of OUR (8-12 hr) indicating rapid growth of the thermopiles and the activities.
4. Relatively constant of OUR (12-15 hr) indicating oxygen-limiting phase.
5. A decline of OUR (15-19 hr and beyond) indicating a decrease in thermophilic activities, due to carbon-limiting conditions.

Bernard and Gray (2000) studied aerobically digested secondary sludge (at 16.5 to 20°C, batch operated), and reported that the measured specific oxygen uptake rates (SOUR, expressed as \( \text{mg O}_2/\text{g VSS-h} \)) were relatively constant during the first 3 days of digestion; it then declined rapidly from day 3 to day 10 of digestion, then showed a much slower decrease from day 10 to day 35 of digestion. SOUR is an indicator of microbial activities in the digested sludge. Lower SOUR suggested a decreased rate in biodegradation of sludge.

4.2.2.2 Digestion effects on fraction of volatile solids in sludge (TVS/TS ratio)

The ratios of total volatile solids/total solids are illustrated in Figure 4.23 (a) and (b). As a result of batch operation and reduction in volatile solids, the fraction of volatile solids in sludge decreased throughout digestion at all tested digestion temperatures, as expected. When the
sludge was digested at 40°C and 50°C, the TVS fraction decreased rapidly from about 85% to 78% within 2 days of digestion; additional digestion (up to 10 days) did not result in much reduction in TVS reduction. When the sludge was digested at 55°C and 60°C, TVS reductions occurred during the first 5 days of digestion. The TVS in 70°C digested sludge showed continued reduction in the VS fraction of solids.

4.2.2.3 Mesophilic aerobic digestion

TVS reduction from various tests of mesophilic aerobic digestion (approximate 22°C) are summarised in Table 4.5. The data is Table 4.5 can be compared with the data shown in the last column of Table 4.4, as the feed sludges were the same in each run. The daily average of TVS reduction from the mesophilic aerobic digestion (2.1% daily) was about 50% lower than that from the thermophilic aerobic digestion (3.1% daily). Such a finding agrees with the general understanding that, for a given period of digestion time, thermophilic digestion results in higher reduction in the volatile solids than mesophilic digestion.

Table 4.5. TVS reduction, mesophilic aerobic digestion, various runs

<table>
<thead>
<tr>
<th>Data sources</th>
<th>TVS reduction (%)</th>
<th>Total days of digestion</th>
<th>Avg. daily TVS reduction (at ~ 22°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00Aug15Run</td>
<td>45%</td>
<td>20</td>
<td>2.3%</td>
</tr>
<tr>
<td>00Nov28Run</td>
<td>34%</td>
<td>25</td>
<td>1.4%</td>
</tr>
<tr>
<td>01June15Run</td>
<td>20%</td>
<td>9</td>
<td>2.2%</td>
</tr>
<tr>
<td>01July14Run</td>
<td>33%</td>
<td>12</td>
<td>2.8%</td>
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<td>27%</td>
<td>10</td>
<td>2.7%</td>
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<tr>
<td>01Nov27Run</td>
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<td>10</td>
<td>1.1%</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>2.1%</td>
</tr>
</tbody>
</table>
Figure 4.23. Effect of digestion temperatures on TVS/TS ratio

(a) Digested at □ 70°C, ■ 60°C, and ◇ 55°C, 100% secondary sludge as the feed

(b) Digested at ○ 50°C and ● 40°C, 100% secondary sludge as the feed
4.2.3 pH

Figure 4.24 illustrates digestion effects on pH at various digestion temperatures. All tests used 100% secondary sludge as the feed sludge. In general, digestion at temperatures of 40°C to 70°C resulted in a decrease in pH, within the initial short period of digestion. The time of the initial period varied from 3 hours (50°C) to 48 hours (70°C). The pH changed from approximately pH 7 to pH 6.5 or less. During the period of continued digestion (up to 5 days for 40-60°C, or 7 days for 70°C), pH recovered and progressively increased to pH 7.5 to 8.0. The pH of the 70°C digested sludge is lower than the sludge that was digested at all other temperatures. The drop in pH during the initial period of thermophilic digestion agreed well with what Cheunbarn and Pagilla (1999) reported, where at all of four tested temperatures (55°C to 65°C), the pH decreased from 6.7 to 6.4 during the first 1.5 days of digestion. Such a pH decrease indicated that organics in the sludge were hydrolysed liberating acidic compounds. Continued thermophilic digestion resulted in an increase in pH agrees with what Matsch and Drnevich (1977) reported, where pH of thermophilic digested sludge (> 45°C) increased with sludge digestion time.

Data shown in Figure 4.24 (c) revealed that, in general, mesophilic digestion resulted in progressive increase in pH, regardless of variations in the feed sludge. pH increased from approximately 7 to 7.5, following 10-12 days of digestion. It appears thermophilic digestion resulted in higher net increase in pH than mesophilic digestion.

The larger net increase in pH due to thermophilic digestion likely contributed to higher polymer demand to dewater thermophilically digested sludge. In a study by Novak and O'Brien (1975) on polymer conditioning of chemical sludge from water treatment plants, it was found that, when the pH of conditioned sludge increased from 7 to 9, the dose of cationic polymer required approximately doubled. Such an increase in polymer dosage was likely because cationic polymers were most effective in the neutral and slightly acidic pH range, not alkali pH range.
(a) Digested at □ 70°C, ■ 60°C, and ◀ 55°C

(b) Digested at ◀ 50°C and ● 40°C

(c) Digested at ~22°C, various runs

Figure 4.24. Effect of digestion temperatures on pH, 100% secondary sludge as the feed
4.2.4 Conductivity

Effects of digestion temperatures on conductivity in the filtrate of the digested sludge, using the feed containing 100% secondary sludge, are shown in Figure 4.25. The conductivity were measured at room temperature of approximately 22°C, and the conductivity meter were calibrated for the measurement temperature.

When the sludges were digested at 40°C or 55°C, rapid increases in conductivities occurred within a short time of digestion. For example, the conductivity in the filtrate of 40°C digested sludge increased from 2.2 mS/cm (the feed) to 6.86 mS/cm, following one day of digestion. The conductivity in the filtrate of 40°C or 55°C digested sludge remained relatively constant during the additional 8 days of digestion.

When the sludge was digested at 60°C, the conductivity in the filtrate of the digested sludge increased from 4.0 mS/cm (in the feed sludge) to 6.6 mS/cm (in the 2 days digested). Additional digestion resulted in a decrease in conductivity, to 5.8 mS/cm (in 10 day digested sludge).

Digestion at 70°C affected conductivity in the filtrate of the digested sludge differently. The conductivity of digested sludge increased progressively throughout the entire period of digestion processes, and increased from 3.0 mS/cm in the feed sludge to 5.4 mS/cm, in the 10 days digested sludge.

The digestion effects on conductivity in the filtrate of mesophilically digested biosolids from various runs are shown in Figure 4.25 (c). All the feed sludges used in each run consisted of 100% secondary sludge, but were collected on various dates. Measured conductivities revealed that mesophilic digestion resulted in a progressive increase in conductivity. The increase in conductivity of the filtrate for the feed sludges that was collected on various dates is generally comparable.
Figure 4.25. Effect of digestion temperatures on conductivity, 100% secondary sludge as the feed.

(a) Digested at □ 70°C and ■ 60°C

(b) Digested at ◇ 55°C and ● 40°C

(c) Digested at ~22°C, various runs
The major difference in measured conductivity between thermophilically digested sludge and mesophilic digested sludge is that digestion resulted in a rapid increase in thermophilically digested sludge, but only a gradual increase in mesophilically digested sludge. Such a difference is likely because thermophilic temperature resulted in lysis of cells within a short time, therefore, resulting increased amount of sludge colloidal particles, although, the charges of theses sludge flocs did not changed substantially; whereas mesophilic digestion resulted in gradual breakdown of cells over the course of digestion, therefore the increased in conductivity is also gradual.

4.2.5 Nitrogen (NH$_4^+$, NO$_x$, TKN)

4.2.5.1 NH$_4^+$-N concentrations

Nitrogen is an integral element of biomass. TKN measures the amount of organic N and ammonia in a sample. When the biomass was oxidized, organic N was converted to ammonia and is reported as NH$_4^+$-N. If nitrification occurs, NH$_4^+$-N will be converted to NO$_2^-$ then to NO$_3^-$, which is reported as NOx.

The digestion effects on ammonia concentration in biosolids digested at several temperatures are shown in Figure 4.26. 100% secondary sludge was used as the feed in each run. Reported ammonia concentrations in Figure 4.26 were the net increases in the concentrations (the measured concentrations in digested sludge subtracted the concentration in the feed sludge). For thermophilic digestion, the net increases at temperatures of 40°C and 50°C are comparable (e.g. both increased by about 760 mg/L after 2 days of digestion). Continued digestion resulted in a decrease in NH$_4^+$-N (612 mg/L in 12 day digested sludge).

For the same time period of digestion, when the digestion temperatures were higher, the net increases in NH$_4^+$-N concentrations were lower. Following 2 days of digestion, the net increases of NH$_4^+$-N in digested sludge were 668 mg/L (55°C digested sludge), 380 mg/L (in 60°C digested sludge), and 71 mg/L (in 70°C digested sludge). When the sludge was digested at 55°C and 60°C, the continued digestion resulted in an increase (55°C in first 5 days of digestion, 60°C in first 7 days of digestion), then a decrease in NH$_4^+$-N concentrations (up to 10 days of digestion). When the sludge was digested at 70°C, there was a progressive and continued increase in NH$_4^+$-N concentration. It increased by 282 mg/L after 10 days of digestion.
However, the net increase of the NH$_4^+$-N concentration in 70°C digested sludge was much lower than the net increases from the sludges that were digested at any of the other temperatures.

The ammonia concentrations at the ATAD plant in College Station, Texas were 76 mg/L (5.4 mM) in the feed, 448 mg/L (32 mM) in 2.3 days digested sludge, and 631 mg/L (45.1 mM) in the 6.9 days digested sludge. The ATAD plant in Princeton, Indiana reported similar ammonia concentrations and changes as a result of thermophilic digestion (Murthy et al. 2000a). From this study, the net increase in ammonia concentrations are the highest following approximately 3 days of digestion; the net increase was lower as digestion continued to beyond 5-6 days. The results shown in Figure 4.26(a) also agreed with findings by Mason et al. (1987).

Mesophilic digestion resulted in a gradual increase in ammonia concentration, as shown in Figure 4.26 (c). The net increase in NH$_4^+$-N from the 4 replicates of mesophilically digested sludge (feed were all 100% secondary sludge, but were collected on different dates) were all approximately 600 mg/L after 10 days of digestion. This increase was higher than the net increase of NH$_4^+$-N in sludge that was digested at 55°C, 60°C, or 70°C.

4.2.5.2 NO$_x$-N concentrations

Measured NOx concentrations (sampled at the identical time as the NH$_4^+$-N) were less than 1.0 mg/L for all runs (either thermophilic or mesophilic digestion). These low NOx concentrations indicated that nitrification did not occur during digestion, and explained the gradual build-up of NH$_4^+$-N concentrations in mesophilically digested sludges. Nitrification bacteria (Nitrosomonas and Nitrobacter) could not proliferate at thermophilic temperatures. It was likely that there were insufficient amount of nitrification bacteria in the feed sludge. Therefore, nitrification did not occur in the up to 30 days of mesophilic digestion.
(a) Digested at □ 70°C, ■ 60°C, and ◆ 55°C, 100% secondary sludge as the feed

(b) Digested at ◆ 50°C and ■ 40°C, 100% secondary sludge as the feed

(c) Digested at ~22°C, various runs, 100% secondary sludge as the feed

Figure 4.26. Effect of digestion temperatures on concentration of ammonia
4.2.5.3 Digestion effect on TKN and N distribution among solid-liquid phases

The nitrogen distribution is illustrated in Figure 4.27. The feed used 100% secondary sludge. In Figure 4.27, "TKN-total" measured TKN of the sludge samples that were taken throughout the duration of digestion. "TKN-filtrate" measured the TKN of the filtrate in the same sludge samples that as above. "NH₄⁺-N-filtrate" measured only NH₄⁺-N in the filtrate of above samples.

Under the thermophilic condition (60°C), the digestion resulted in a progressive decrease in "TKN-total" (changed from 1,604 mg/L in the feed sludge to 805 mg/L in 20 day digested sludge), indicating progressive breakdown of organic nitrogen as a result of biodegradation. The "TKN-filtrate" increased from 448 mg/L in the feed to 1,110 mg/L following one day of digestion, then it remained relatively constant at approximately 1,100 mg/L from day 1 to day 7 of digestion. Thereafter, the "TKN-filtrate" decreased to 389 mg/L at the end of 20 days of digestion.

It is noted that the organic nitrogen in the liquid fraction of the sludge (namely the difference between "TKN-filtrate" and "NH₄⁺-N-filtrate") increased substantially following one day of digestion. The large amount of organic nitrogen in the one day digested sludge (at 60°C) suggested that thermophilic digestion resulted in a solublization of organics first, prior to the biodegradation conversion of organics to end product such as NH₄⁺-N. Such a finding agrees with the general understanding that proteins is to be hydrolysed to become amino acids, prior or the nitrogen's ammonification.

As the digestion progressed from day 2 to day 20, the organic nitrogen was smaller and remained relatively constant. The changes in concentrations of the "NH₄⁺-N-filtrate" were similar to that of the "TKN-filtrate". The "NH₄⁺-N-filtrate" increased from 362 mg/L in the feed to 1,027 mg/L in the 3 days digested sludge. Thereafter, it steadily decreased to 150 mg/L by the end of 20 days of digestion.

In contrast, soluble organic nitrogen from mesophilic digestion remained relatively constant throughout 20 days of digestion. Under mesophilic conditions (22°C), the digestion resulted in gradual decrease in "TKN-total". The "TKN-filtrate" increased from 438 mg/L in the feed to 705 mg/L in 2 day digested sludge, and then remained relatively constant at approximately 600
mg/L, during the next 18 days of digestion. "NH₄⁺-N-filtrate" in the filtrate increased from 370 mg/L in the feed, to 606 mg/L after one day of digestion, then gradually decreased to approximately 520 mg/L over the rest of a total of 20 days of digestion. The changes of "NH₄⁺-N-filtrate" were similar to the changes of "TKN-filtrate". The results shown in Figure 4.27 revealed that in filtrate of both thermophilically and mesophilically digested sludge, NH₄⁺-N accounted for a major proportion of measured nitrogen. The results shown in Figure 4.27 provided additional evidence that thermophilic digestion resulted in rapid lysis of cells and the solubilisation of organics. Such an effect occurred with a short time of thermophilic digestion, and is primarily associated with the temperature, rather than the digestion time.

4.2.6 Phosphorus (PO₄³⁻, TP)

4.2.6.1 Phosphate concentrations

Phosphorus exists in sludge in various forms, as phosphate, poly-P, or organic P. Digestion that degrades organics will result in the release of P as phosphate in the liquid phase of the sludge. The analysis of P in various forms assisted in understanding of biomass degradation and revealed information on the effect of digestion on biomass transformation.

Measured soluble phosphate in the 100% secondary sludges that were digested in several temperatures are shown in Figure 4.28. A rapid increase in phosphate occurred within a short period during thermophilic digestion (mostly less than 3 hours). Continued digestion did not result in much additional increase in phosphate concentrations.

In contrast, mesophilic digestion resulted in a gradual increase in PO₄³⁻-P concentration. The results, reported as actual concentrations and shown in Figure 4.28 (c), include the observations for sludges that were collected on various dates. All contained 100% secondary sludge. When the PO₄³⁻-P concentration in the feed was subtracted from all measured concentrations, the net increases in PO₄³⁻-P concentrations were very close at the corresponding time in all the mesophilically digested sludges.
Figure 4.27. Effect of digestion on nitrogen distribution, 100% secondary sludge as the feed.
Figure 4.28. Effect of digestion temperatures on phosphate concentration, 100% secondary sludge as feed.
4.2.6.2 Phosphorus distribution among various phases

Phosphorus in various forms and in various phases of the sludge were analysed with samples taken from 00Aug15Run. The results are shown in Figure 4.29. The non-soluble TP was calculated by subtracting the measured TP in the filtrate of digested sludge samples from the measured TP in the corresponding samples of digested sludge. The Soluble non-PO$_4^{3-}$ was calculated by subtracting the measured soluble PO$_4^{3-}$ from the measured TP in corresponding samples of filtrate of digested sludge. Results revealed that, the majority of P was associated with the solids phase (pellet) of the sludge (81% for the feed sludge, 56-68% for the thermophilically digested sludge, 61-82% for the mesophilically digested sludge, all by weight and was out of total P in various forms/phases). Among the P that was associated with the liquid phase (filtrate), a larger fraction of P was in the soluble form as PO$_4^{3-}$-P (15% for the feed sludge, 23-31% for the thermophilically digested sludge, 13-24% for the mesophilically digested sludge, all by weight and was out of total P in various forms/phases). The remaining P that was associated with colloidal particles in the filtrate might have existed as non-soluble P or poly-P. The PO$_4^{3-}$-P concentrations in the filtrate increased rapidly within the first 2 days of digestion, but were relatively unchanged during the remaining days of digestion, as the solubilization of P to the liquid phase during thermophilic digestion was completed within a short period of digestion.

The increase in the phosphorus excluding PO$_4^{3-}$-P (present in the liquid phase of digested sludge) also occurred during the first 2 days of digestion, and did not change much during the remaining period of 20 days of digestion. The changes in PO$_4^{3-}$-P concentrations indicated the rapid solubilization and cell breakdowns due to thermophilic digestion. Such a finding agreed well with thermophilic effects on concentrations of proteins and polysaccharides. In contrast, the increase in PO$_4^{3-}$-P concentrations during mesophilic digestion occurred over the entire duration of the digestion.
Figure 4.29. Effect of digestion on P distribution, 100% secondary sludge as the feed

(a) Digested at 60°C (00Aug15Run), all measured as P

(b) Digested at −22°C (00Aug15Run), all measured as P
4.2.7 Extracellular Proteins

4.2.7.1 Thermophilic aerobic digestion

The digestion effects on soluble proteins, at several digestion temperatures, are shown in Figure 4.30. In the sludge that was digested at 40°C, soluble proteins increased to 60 mg/g, following one day of digestion, and remained at approximately 60 mg/g from day-1 to day-7 of digestion. Further digestion of up to 12 days resulted in an increase in soluble proteins to 80 mg/g. Sludges that were digested at 50°C, 55°C, or 60°C showed a rapid increase in soluble proteins within a short time of digestion (increased by 95 mg/g in the sludge that was digested at 50°C for 6 hours, and by 144 mg/g in the sludge that was digested at 55°C for 12 hours). Continued digestion of up to 12 days resulted in a decrease in soluble proteins. For the sludge that was digested at 70°C, the soluble proteins increased by 119 mg/g following 6-hour of digestion. Continued digestion resulted in further increase in soluble proteins, which increased by 156 mg/g in total after 7 days of digestion.

In comparison, Goodwin and Forster (1985) reported that, when the municipal activated sludge was placed in a water bath and was heated for 1 hour, increasing the temperature from 50°C to 100°C resulted in a significant increase in the concentrations of the total organic carbon (TOC) in the liquid fraction of sludge (approximately 700 mg/L at 50°C, 1,700 mg/L at 70°C), and an increase in normalized protein concentrations (proteins/TOC) as well. They suggested that the autolysis, due to thermal extraction, increased the abundance of soluble organics and biopolymers.

According to Murthy et al. (2000a), protein concentrations in the digested sludge taken from the ATAD plant in College Station, Texas were 410 mg/L in the feed (~3.7%TS), and 2,080 mg/L following 6.9 days of total digestion (the last tank was 59°C). At the ATAD plant in Princeton, Indiana, protein concentrations in the digested sludge were 240 mg/L in the feed, and 2,790 mg/L following 7.4 days of digestion at 52°C. It appears that measured protein concentrations from this study agreed well with what were reported in full-scale ATAD plants elsewhere.
Figure 4.30. Effect of digestion temperatures on soluble extracellular protein

(a) Digested at ◊ 70°C, ■ 60°C, and □ 55°C, 100% secondary sludge as the feed

(b) Digested at ○ 50°C and ● 40°C, 100% secondary sludge as the feed
4.2.7.2 Mesophilic aerobic digestion, various runs

The concentrations of normalized proteins in mesophilically digested sludge are shown in Figure 4.31, and are reported as the net increase from the feed. Feed sludge was collected on various dates. Mesophilic digestion at approximately 22°C resulted in a gradual increase in soluble proteins (increased by 30 mg/g at the end of 12 days digestion). The changes in soluble proteins, due to mesophilic digestion are approximately the same, even though the feed sludge was collected on various dates. The net increases in concentrations of soluble proteins, due to mesophilic digestion, were much lower than that due to thermophilic digestion. After 5 days of digestion, the net increase of soluble proteins in the mesophilically digested sludge is 12-18 mg/g, but was 121 mg/g in the sludge that was digested at 50°C.

4.2.7.3 Correlation of dewaterability with concentrations of soluble proteins

Dewaterability, expressed as SCST, showed a strong correlation with normalized soluble proteins in the digested sludge, as shown in Figure 4.32. Because it took approximately 1-3 hours for the temperature of the sludge in bioreactors to rise from room temperature of approximately 22°C to 40°C or higher digestion temperatures, data in Figure 4.32 (40°C or higher) included only measured results from samples that were taken after the sludge was digested for 3 hours or longer. At lower digestion temperatures (e.g. 22°C and 40°C), SCST showed a linear correlation with normalized concentrations of soluble protein. At higher digestion temperatures (e.g. 60°C and 70°C), after the normalized concentrations of soluble protein exceeded an apparent threshold (approximately 90 mg/g), increased concentration of soluble protein did not result in further increase in SCST.
Figure 4.31. Effect of mesophilic digestion on soluble extracellular proteins, various runs

Figure 4.32. Correlating dewaterability with extracellular soluble proteins
4.2.8 Extracellular Polysaccharides

4.2.8.1 Thermophilic aerobic digestion

The experimental results are shown in Figure 4.33. In general, digestion effects on the concentration of soluble polysaccharide were similar to the digestion effects on the concentration of soluble protein (Figure 4.30). The 40°C digested sludge has net increases in soluble polysaccharide concentration of 11 mg/g following 7 hours of digestion, and continued to increase to 29 mg/g following 12 days of digestion. For the sludges that were digested at 50°C, 55°C, or 60°C, the digestion resulted in a rapid increase in soluble polysaccharide concentration (net increase of 34 mg/g after 12 hours digested sludge at 50°C, 40 mg/g in 6 hours digested at 60°C, and 52 mg/g in 6 hours digested at 55°C). Further digestion of up to 1 day at these three temperatures resulted in a decrease in soluble polysaccharide concentration. Continued digestion of up to 9-10 days resulted in progressive increases in soluble polysaccharide concentrations. Digestion at 70°C resulted in a rapid increase in soluble polysaccharide concentration (net increase of 48 mg/g in the 6 hours digested sludge). Such an increase continued up to 2 days of digestion (net increase of 56 mg/g in 2 days digested sludge), thereafter, soluble polysaccharide concentration slightly decreased to 51 mg/g in the 10 days digested sludge.

According to Murthy et al. (2000a), polysaccharides concentrations in ATAD plant at College Station, Texas were 110 mg/L in the feed (~3.7%TS), and 900 mg/L following 6.9 days of total digestion (the last tank was 59°C). In the ATAD plant at Princeton, Indiana, polysaccharides were 94 mg/L in the feed, and 1,690 mg/L following 7.4 days of digestion at 52°C. It appears that measured polysaccharides concentrations from this study agreed well with what were reported in full-scale ATAD plants elsewhere.
Figure 4.33. Effect of digestion temperatures on soluble extracellular polysaccharides

(a) Digested at □ 70°C, ■ 60°C, and ◇ 55°C, 100% secondary sludge as the feed

(b) Digested at ◇ 50°C and ● 40°C, 100% secondary sludge as the feed
4.2.8.2 Mesophilic aerobic digestion, various runs

Soluble polysaccharide concentrations in mesophilically digested sludge, from various runs, are shown in Figure 4.34. There were some variations in measured polysaccharide concentrations following one day of digestion; however, sludge collected on different dates showed similar responses to mesophilic digestion. The soluble polysaccharide concentration increased progressively by approximately 8 mg/g, following 10 days of digestion. The increase in soluble polysaccharide concentration, due to mesophilic digestion, was much lower than the increase due to thermophilic digestion, at 50°C or higher temperatures.

Since the soluble polysaccharides indicate the concentration of carbohydrate in the liquid fraction of the digested sludge, rapid increase in concentrations of soluble polysaccharides indicated the rapid disintegration of bacteria cells due to thermophilic digestion. The same rapid cells breakdown did not occur with mesophilic digestion.

4.2.8.3 Correlation of dewaterability with concentrations of soluble polysaccharides

The dewaterability, expressed as SCST, showed a strong correlation with normalized soluble polysaccharides in the digested sludge, as shown in Figure 4.35. Because it took approximately 1-3 hours for the temperature of the sludge in bioreactors to rise from room temperature of approximately 22°C to 40°C or higher digestion temperatures, data in Figure 4.35 (40°C or higher) included only measured results from samples that were taken after the sludge was digested for 3 hours or longer. At lower digestion temperatures (i.e. 22°C, 40°C, 50°C, and 55°C), the SCST showed a linear correlation with normalized concentrations of soluble polysaccharides (concentrations divided by TS). At higher digestion temperatures (i.e. 60°C and 70°C), after normalized concentrations of soluble polysaccharides exceeded an apparent threshold (approximately 40 mg/g), increased soluble polysaccharides did not result in a further increase in the SCST.
Figure 4.34. Effect of mesophilic digestion on soluble polysaccharides, various runs

Figure 4.35. Correlating dewaterability with extracellular soluble polysaccharides
4.2.9 Floc Charge (Zeta potential)

Figure 4.36 (a) shows the measured Zeta potential on particles in the filtrate of thermophilically digested sludge. There were no major differences among Zeta potentials of sludge samples that were digested at several temperatures. Regardless of digestion temperatures, little change in Zeta potential occurred during the 10 days of digestion.

Measured Zeta potentials of mesophilically digested sludge, from various runs, were compared (the feed sludges were collected on various dates), and are shown in Figure 4.36 (b). Measured Zeta potentials are all approximately -15 mV. Digestion for 10 days resulted in some variations, but not substantial changes in the Zeta potential.

Regardless of digestion temperatures, there were little changes in floc charges. Such a finding, in addition to what were reported in Section 4.1.9 (little changes in Zeta potential, regardless of the composition in the feed sludge) provides additional evidence that the charge neutralization is not a applicable model to describe the dewatering characteristics of thermophilically digested sludge.
Figure 4.36. Effect of digestion on floc charge, 100% secondary sludge as the feed

(a) Digested at □ 70°C, ■ 60°C, ◦ 55°C, • 40°C.

(b) Digested at ~22°C, various runs
4.2.10 Floc Size

The digestion effect on floc size, using 100% secondary as the feed, is shown in Figure 4.37, and is summarised in Table 4.6. The distribution of floc size shown in Figure 4.37 is reported as a number-based distribution, namely, the proportion in the number of particles at each particle size diameter out of the total numbers of measured particles.

The experimental results revealed that thermophilic digestion resulted in an immediate reduction in floc size. The particle size that has the maximum number of particles was smaller in thermophilically digested sludge (0.7 μm) than that in the feed sludge (2.2 μm). Continued thermophilic digestion (up to 10 days) resulted in a slight further reduction in floc size, but the changes were less significant than the changes that occurred in the first day of digestion. Particles of less than 1 μm are in the size range of individual bacteria. The results, shown in Figure 4.37 (a) and Table 4.6, suggests that water in the thermophilically digested sludge was mainly associated with individual bacterial cells, rather than floc aggregates. The absence in floc aggregates leads to a lack of paths to allow water to drain easily from the matrix of the sludge, therefore result in a difficulty in dewatering the digested sludge.

Mesophilic digestion also resulted in a reduction of floc size. Although the measured floc size (2 μm) that has the maximum number of particle size was only slightly lower than that in the feed sludge (2.2 μm), the proportion of smaller particles in the total measured particles increased.

It is apparent that thermophilic digestion resulted in smaller floc size, than mesophilic digestion. The floc size of thermophilically digested and mesophilically digested sludges are compared in Figure 4.38 (floc size distribution) and Figure 4.39 (surface mean diameter). Both samples were taken from 00Aug15Run, using 100% secondary sludge as the feed sludge.

Samples of thermophilically digested sludge from two different runs (00Aug15Run and 00Nov28Run, both used 100% secondary sludge as the feed sludge, and were digested at 60°C for one day) are compared in Figure 4.40. Although there are some slight differences in the floc size profiles, in general, measured floc size distributions are comparable.
Figure 4.37. Effect of digestion on the size of flocs

(a) Digested at 60°C, 100% secondary sludge as the feed (00Aug15Run)

(b) Digested at ~22°C, 100% secondary sludge as the feed (00Aug15Run)
Table 4.6. Effects of digestion on floe size (00Aug15Run)

<table>
<thead>
<tr>
<th>Particle Size (μm)</th>
<th>Proportion of total particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed</td>
<td>2.2</td>
</tr>
<tr>
<td>Thermo, 1 day</td>
<td>0.7</td>
</tr>
<tr>
<td>Thermo, 6 days</td>
<td>0.7</td>
</tr>
<tr>
<td>Thermo, 10 days</td>
<td>0.6</td>
</tr>
<tr>
<td>Meso, 1 day</td>
<td>2.0</td>
</tr>
<tr>
<td>Meso, 6 days</td>
<td>2.0</td>
</tr>
<tr>
<td>Meso, 10 days</td>
<td>2.0</td>
</tr>
</tbody>
</table>

\(^a\)Having maximum number of particles

\(^b\)The total time the sample was digested

Figure 4.38. Comparison of the floe sizes, one day digested sludge at 60°C (thermo) and 22°C (meso), 100% secondary sludge as the feed.
Figure 4.39. Floc sizes, 100% secondary sludge digested at 60°C (thermo) and 22°C (meso)

Figure 4.40. Effect of different runs on size of flocs, both digested at 60°C for one day, 100% secondary sludge as the feed
4.2.11 Temperature Effect on SCST and Characteristics of Digested Sludge

4.2.11.1 Thermophilic digestion of secondary sludge at 70°C

When the secondary sludge was digested at 70°C, following an initial rapid surge, the SCST decreased (Figure 4.16a). Such changes suggested that prolonged treatment at higher temperatures destroyed some of the substances initially causing poor dewaterability, or changed the formation, stability, or relevant functional groups of the substances affecting SCST; as such, the rate of destruction exceeded the rate of production of such substances. Volatile solids reduction occurred throughout the entire duration of 10 days of digestion, indicating microbial activity to degrade organics in the sludge (Figure 4.21a). The pH decreased during the first 2 days of digestion, indicating the hydrolysis of acidic organics and solubilization of organics. The pH recovered and increased during the remaining time of digestion (Figure 4.24a), which correlated to an increase in NH$_4^+$-N concentrations. Both conductivity (Figure 4.25a) and NH$_4^+$-N concentrations (Figure 4.26a) showed gradual and consistent increases throughout the 10 days of digestion. Because floc charge (Zeta potential) did not change much (Figure 4.36a), an increase in conductivity indicated an increased amount of charged particles in solution, due to solubilization of organics.

Phosphate (Figure 4.28a), soluble protein (Figure 4.30a), and soluble polysaccharides (Figure 4.33a) showed immediate surges within a short time, since the start of digestion; thus suggested the destruction and lysis of bacterial cells due to the impact of heat treatment at 70°C. However, following the initial surge, the normalized concentrations (divided by total solids concentrations) of proteins and polysaccharides showed slight decrease, but note that the changes were not as significant as the decrease in SCST.

4.2.11.2 Thermophilic digestion of secondary sludge at 55°C and 60°C

The effect of 60°C digestion, on dewaterability, was similar to that of 70°C digestion. Following an initial rapid surge, the SCST also decreased (Figure 4.16a). Volatile solids reduction occurred after a short lag time (6 hours). During the 5 days to follow, the TVS reduction followed first order kinetic. The rate of TVS reduction at 60°C was higher than the rate at 70°C (Figure 4.22). pH (Figure 4.24a), conductivity (Figure 4.25a), and NH$_4^+$-N concentrations (Figure 4.26a) all decreased during the first 6 hours of digestion, then gradually increased throughout the remaining time of digestion. The decrease in pH indicated initial acidification and solubilization.
of organics. The initial decrease in conductivity suggested the depletion of charged particles in solution, and indicated the removal of readily available organic particles due to biodegradation. The initial decrease in \( \text{NH}_4^+ \)-N concentrations was likely a result of ammonia stripping due to aeration at high temperature. Floc charge (Zeta potential) became slightly more negative, but the changes were small (Figure 4.36a). The hypothesis that thermophilic digestion resulted in immediate solubilization and breakdown of complex organics to smaller particles was supported by the finding that one day of digestion at 60°C resulted in an immediate decrease in particle sizes (Figure 4.37a).

The effect of digestion at 55°C on dewaterability was similar to that at 60°C. Following an initial rapid surge, the SCST also decreased (Figure 4.16a). Volatile solids reduction occurred after a lag time of 24 hours. The rate of TVS reduction at 55°C was comparable to the rate at 60°C (Figure 4.21a). TVS reduction followed first order kinetics during the first 5 days of digestion. pH (Figure 4.24a) decreased within the first 24 hours of digestion, and then increased as digestion continued. Conductivity (Figure 4.25b), and \( \text{NH}_4^+ \)-N concentrations (Figure 4.26a) all gradually increased throughout digestion. The decrease in pH indicated initial acidification and solubilization of organics. Digestion at 55°C did not result in much change in floc charge (Figure 4.36a).

The immediate surge in concentrations of phosphate (Figure 4.28a), soluble protein (Figure 4.30a), and soluble polysaccharides (Figure 4.33a) following a short time of digestion suggested the destruction and lysis of bacterial cells due to the impact of heat treatment, at either 55°C or 60°C. Following the initial surge, the normalized concentrations (divided by total solids concentrations) of proteins and polysaccharides decreased, indicating possible destruction of these biopolymers, due to digestion at high temperatures.

4.2.11.3 High temperature digestion of secondary sludge at 40°C and 50°C

For digestion at 40°C and 50°C, SCST initially increased very rapidly, and then flattened during subsequent digestion of up to 10 days. Such a behavior suggests that an equilibrium balance was achieved between production and destruction of substances (or relevant functional groups) causing poor dewaterability. During the first 2 days of digestion that followed a short lag time, TVS reduction followed first order kinetics. The first order TVS reduction lasted shorter time at
40°C and 50°C than at 55°C to 70°C, but the kinetic coefficients K of TVS reduction measured at 40°C and 50°C digestion was approximately 4 times higher than the TVS reduction rate measured from 55-70°C digestion (Table 4.4 and Figure 4.22).

Such an observation deviated from the general understanding that, for every 10°C rise in digestion temperatures, the reaction rate K approximately doubles (Metcalf and Eddy 2003, Kelly et al. 1995). The aerobic digestion in this study was carried out in batch mode. No bacterial seeds of thermopiles were introduced into the feed sludge at the start of digestion. The microbial communities in the feed sludge were originally acclimated to mesophilic temperatures. After the sludge was heated to temperatures of 40°C or up to 70°C, the microorganisms, containing diversified and abundant species, experienced a self selection process in order to adapt to a new environment. It is likely that there would be a reduction in species diversity with a selection for bacteria that can better tolerate high temperatures. TVS reduction offered evidence of microbial activity and indicated the presence of thermopiles, the microorganisms that function at thermophilic temperatures. The rate of TVS reduction was an indicator of activities of the self selected microbial matrix. These bacteria would need to acclimate to the high digestion temperature before their ability to biodegrade organics could be fully materialized. 40°C is a smaller temperature jump from the ambient temperatures of approximate 22°C than 70°C. It is easier for the bacteria to adapt to 40°C than to 70°C. The higher TVS reduction rate at lower digested temperatures (40°C or 50°C) was likely due to microorganisms acclimating better at lower temperatures.

For TVS reduction in full-scale operation, well acclimated bacteria are always present in either fed-batch or continuous flow mode digesters. These bacteria would facilitate immediate destruction of biodegradable organics and do not need acclimatizing to the high temperatures. Therefore, higher TVS reduction was reported at higher digestion temperatures. The lab scale batch digesters in this study did not have acclimated seeds at the beginning of digestion. Higher temperatures, within a short time, resulted in stronger impact on bacterial communities in sludge, and a longer time to complete the acclimatizing process; therefore, the TVS reduction rate would be lower. Regardless of digestion temperatures, rates of TVS reduction decreased significantly following the initial log phase reduction, indicating the exhaustion of readily biodegradable organics (a result of smaller driving forces of reaction).
The pH of digested sludge decreased within the first day of digested at both of 40°C and 50°C, and then increased as digestion progressed (Figure 4.24b). The initial decrease in pH indicated acidification and solubilization of degradable organics in the sludge. At 40°C, ammonia concentrations and conductivity continued to increase throughout the digestion process. The phosphate increased in the first 7 hours of digestion at 40°C, and remained relatively constant thereafter. At 50°C, digestion temperatures, ammonia remained relatively constant during the first 12 hours of digestion, and then increased as digestion continued (Figure 4.26b). Phosphate increased in the first 3 hours of digestion, and remained relatively constant thereafter (Figure 4.28b).

The changes in extracellular proteins and polysaccharides (Figure 4.30b and 4.33b) provided additional information on changes of bacterial cells. At 40°C, the cell lysis occurred gradually during the first 24 hours of digestion, because the increases in protein and polysaccharide concentrations occurred gradually. In contrast, at 50°C digestion temperatures, cell lysis occurred immediately after the digestion started, indicating 50°C or higher temperatures was sufficient to result in an immediate cell lysis.

4.2.11.4 Mesophilic digestion of secondary sludge at 22°C

Digestion at room temperatures (~22°C) resulted in a gradual increase in SCST over 12 days of digestion time (Figure 4.18). The averaged daily TVS reduction was lower than the rate from thermophilic digestion. The gradual increase in SCST of mesophilically digested biosolids (22°C) indicated a gradual build-up of substances affecting SCST. Destruction of these substances during mesophilic digestion appeared to be insignificant.

Mesophilic digestion resulted in slight increase in pH within one hour of digestion. The pH remained relatively constant during the next 6 days of digestion, and then showed a gradual increase during the remaining time of digestion (Figure 4.24c). Conductivity increased gradually within the first 7 days of digestion (Figure 4.25c), suggesting solubilization of organics to form increased amount of charged particles. Continued digestion resulted in a gradual and continued increase in ammonia concentrations (Figure 4.26c) and in phosphate concentrations (Figure 4.28c). Similarly, extracellular proteins (Figure 4.31) and polysaccharides (Figure 4.34) also showed gradual increase over the entire period of digestion. Floc charge (Zeta potential) did not
change much in the 6 days of digestion (Figure 4.36b). These observations indicated that the biodegradation and changes of organics in sludge was a gradual process in the mesophilic digestion process. The SCST increase appeared also associated with the gradual increase in extracellular proteins and polysaccharides.

4.3 Effects of Digestion Time

The effect of digestion time was assessed for two purposes: firstly, to evaluate whether or not there is an opportunity to optimize sludge retention time of the digestion process to improve the dewaterability of the digested sludge; secondly, to investigate the nature of the thermophilic digestion effect on dewaterability. The assessment of effects of digestion time are based on results shown in Figure 4.1 (various feed composition, thermophilic digestion at 60°C), 4.3 (various feed composition, mesophilic digestion), 4.16 (various digestion temperatures, 100% secondary sludge as the feed), and 4.18 (mesophilic digestion from various runs, 100% secondary sludge as the feed).

4.3.1 Characterizing the Nature of Thermophilic Effect on Dewaterability

The purpose of the experiment was to determine whether the effect of thermophilic digestion on dewaterability was a physical-chemical, or microbiological phenomenon. The experimental conditions are summarized in Table 4.7. In the 01Apr20Run, samplings were more intensive, and a smaller initial volume of feed sludge was digested. The smaller volume was to facilitate a faster rise in temperature of sludge to reach the designated digestion temperature of 60°C, so that the effect of digestion time and digestion temperature could be uncoupled.

The experimental results shown in Figure 4.41 revealed that, regardless of the differences (volumes of the feed sludge, and sizes of bioreactors) in the initial experimental set-ups, the effects of thermophilic digestion on the SCST of digested sludge were approximately the same. The most significant increase in the SCST occurred within 6-hour of thermophilic digestion. The remaining period of the 48 hours of thermophilic digestion resulted in a slight decrease in SCST. Furthermore, a large increase in SCST already occurred (by 10 s L/g, 01Apr20Run, shown in Figure 4.42), following one hour of thermophilic digestion. These experimental results suggested that the rapid deterioration was due to “heat shock” and was likely due to a physical-
chemical effect, rather than due to the growth of new microbial communities. The thermophilic temperature caused the death of microorganics and the lysis of cells, releasing proteins and polysaccharides that affect the SCST.

Table 4.7. Experimental conditions: nature of thermophilic effects

<table>
<thead>
<tr>
<th></th>
<th>01Feb02Run</th>
<th>01Apr20Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary sludge in feed</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Feed volume (duplicates)</td>
<td>5 L in 8 L bioreactor</td>
<td>1.5 L in 2.8 L flask</td>
</tr>
<tr>
<td>Thermophilic temperature</td>
<td>60°C</td>
<td>60°C</td>
</tr>
<tr>
<td>Time to reach 60°C</td>
<td>3 hours (1st sample)</td>
<td>1 hour</td>
</tr>
<tr>
<td>Experiment duration (hours)</td>
<td>48</td>
<td>4</td>
</tr>
<tr>
<td>Air supplied (vol./vol.-hr)</td>
<td>11</td>
<td>22</td>
</tr>
</tbody>
</table>

Figure 4.41. Effect of digestion on SCST at 60°C (thermo) and 22°C (meso) from two different runs, initial 48 h, 100% secondary sludge as the feed
4.3.2 Effects of Digestion Time on Dewaterability

For thermophilically digested secondary sludge, a significant deterioration of dewaterability occurred in short time (within one day of digestion at most temperatures) of digestion. Thereafter, sludge retention time (SRT) resulted in lower SCST at digestion temperatures higher than 55°C (Figure 4.16). However, SCST were relatively constant from day 2 to day 9 of thermophilic digestion. For thermophilically digested biosolids using mixed secondary and primary sludge, or primary sludge as the feed, a longer SRT resulted in slightly increased SCST, but the increase was not significant when compared to that using secondary sludge feed (Figure 4.1). In comparison, for mesophilically digested biosolids, increased SRT resulted in progressive and gradual increase in SCST in secondary, mixed, and primary sludge (Figure 4.3 and 4.18). Therefore, SRT appeared to be an important factor affecting dewaterability of mesophilically digested biosolids.

A typical sludge retention time is 5-6 days for thermophilic aerobic digestion, and 15-20 days for mesophilic aerobic digestion. It appeared that the opportunity to optimize SRT of
thermophilic aerobic digestion, to obtain a biosolids with improved dewaterability, was not available, since the effect due to thermophilic digestion occurred within one day of digestion, and there were not many changes in SCST from day 2 to day 9 of digestion. Figure 4.18 suggested that the increase in the SCST occurred within the first 12 days of mesophilic aerobic digestion; therefore, to improve dewaterability (to have a lower SCST) within an acceptable SRT range of mesophilic digestion, cannot be achieved by varying the SRT.

Novak and Bivins (2000) digested 1.5% waste activated sludge anaerobically, reported that the CST of the sludge increased from 15 seconds in the feed to over 700 seconds, following 1.5 days of digestion. Additional digestion up to 3 days resulted in additional increases to a CST of approximately 900 seconds, but the increase was much less significant.

For thermophilic digestion digested sludge (50°C to 70°C), the substantial increase in SCSTs occurred within 3 hours of digestion (Figure 4.41), when the temperatures of the sludge reached thermophilic temperatures. Such an effect could occur as short as one hour (Figure 4.42). Extended digestion resulted in limited reduction in SCST at higher temperatures, but SCST remained high. Therefore, there is little opportunity to optimize digestion temperatures, in order to achieve improved dewaterability properties. In contrast, mesophilic digestion resulted in a gradual increase in SCST. Therefore, a lower SCST was recorded at a shorter digestion time.

The results of this study suggest that, to minimize deterioration in dewaterability of thermophilic biosolids, digestion temperatures of 60°C or higher are desirable. This would then result in a degree-day product of between 300-360, thus the ATAD digesters may be designed for a smaller size and be operated at the higher range (60°C or higher) of thermophilic temperatures.

4.4 Effects of Mixing Induced Shear

4.4.1 Dewaterability (SCST)

The high shear that was applied to sludge in the digesters resulted in a negative effect on dewaterability, in both thermophilically and mesophilically digested sludge (Figure 4.43, reported as net increases in SCST from SCST of the feed sludge). For thermophilically digested sludge, following 2 days of digestion, highly sheared sludge (1,550 rpm) had a SCST of 197 s
L/g (calculated SCST, where SCST of the feed sludge was not subtracted), while the air-mixed sludge had a SCST of 56 s L/g (calculated SCST, where SCST of the feed sludge was not subtracted). The slight decrease in SCST of 1.5-day digested sludge was considered as a random variation, due to the difficulty experienced in controlling the operation of the sheared sludge bioreactor.

In the air-mixed sludge, following 5 days of digestion, the SCST of thermophilically digested biosolids was close to the SCST of mesophilically digested biosolids. In contrast, when high shear was applied to the sludge, thermophilically digested biosolids showed substantially higher SCST than mesophilically digested biosolids. In the first 24 hours of high shear mixing, the SCST of thermophilically and mesophilically digested biosolids was similar. Differences occurred following one day of digestion. Although the SCST declined following 2 days of digestion, there was a consistent difference between thermophilically and mesophilically digested biosolids. Full-scale thermophilic aerobic digestion processes use mechanical mixing or high speed pumping to mix and to supplement the energy required for sustaining auto-heated operation. High shear applied to the sludge would be a major factor contributing to poor dewaterability of thermophilically digested biosolids. The results shown in Figure 4.43 suggested that mechanical shear, that was applied to the digested sludge is likely a more important factor than digestion temperatures, in affecting the dewaterability of the digested sludge.
Figure 4.43. Effect of shear on SCST of digested sludge, 100% secondary sludge as the feed
4.4.2 Solids Reduction (TS and TVS)

The effects of high shear on measured TVS throughout digestion are shown in Figure 4.44 (a) and (b). Experimental results revealed that, for thermophilic digestion (tested at 60°C), TVS reduction in highly sheared sludge was not higher than the TVS reduction in the air-mixed sludge. In contrast, for mesophilic digestion, high shear effectively improved TVS reduction. The TVS reduction coefficients are summarised in Table 4.8, and are shown in Figure 4.45.

The results in Table 4.8 indicated that, for thermophilic digestion at 60°C, the air-mixed sludge had a slightly higher TVS reduction coefficient (0.072 day\(^{-1}\)) than the sheared sludge. Highly sheared sludge, that was digested mesophilically, had a higher TVS reduction coefficient than that of the air-mixed sludge. Results suggest that, for the purpose of TVS reduction, as long as adequate mixing can be maintained, there was no benefit from to apply high shear on sludge during the digestion process.

Table 4.8. TVS decay rates, effect of shear

<table>
<thead>
<tr>
<th>Digestion Temperatures</th>
<th>Duration (days)</th>
<th>K (day(^{-1}))</th>
<th>TVS reduction (%)</th>
<th>Total days of digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheared (thermo, 60°C)</td>
<td>0.25-5</td>
<td>0.066</td>
<td>30%</td>
<td>10</td>
</tr>
<tr>
<td>Air-mixed (thermo, 60°C)</td>
<td>1-7</td>
<td>0.072</td>
<td>36%</td>
<td>10</td>
</tr>
<tr>
<td>Sheared (meso, 22°C)</td>
<td>1-10</td>
<td>0.065</td>
<td>43%</td>
<td>10</td>
</tr>
<tr>
<td>Air-mixed (meso, 22°C)</td>
<td>1-10</td>
<td>0.020</td>
<td>11%</td>
<td>10</td>
</tr>
</tbody>
</table>
Figure 4.44. Effect of shear on TVS of digested sludge, 100% secondary sludge as the feed.
Figure 4.45. Effect of shear on TVS reduction coefficient of digested sludge at 60°C (thermo) and 22°C (meso), 100% secondary sludge as the feed

4.4.3 pH

The shear effects on pH are shown in Figure 4.46 (a) and (b). For thermophilically digested sludge at 60°C, a decrease in pH of both the sheared and the air-mixed sludge occurred after 3 hours of digestion (changed from pH 7 to pH 6.5). The pH remained at pH 6.5 from the period of 3 hours to 6 hours of digestion. Following 1 day of digestion, the pH of the sheared sludge increased to pH 7.9, and then gradually decreased to pH 6.8 at the end of 10 days of digestion. The pH of the air-mixed sludge increased gradually to 7.5 at the end of 10 days of digestion. No rapid increase in pH occurred with the air-mixed sludge.

For mesophilically digested sludge, the pH in the sheared sludge increased from pH 7.1 (the feed) to pH 8.0 (2 days digested). Continued digestion resulted in a decrease of pH to 6.1 (in 10 days digested). For the air-mixed sludge, the pH generally decreased within 5 days of digestion (pH 7.5 in 1-hour digested sludge to pH 6.8 in 5 days digested sludge), but an increase in pH occurred from day 5 to day 10 of digestion.
It is noted that, for the sheared sludge, digestion at thermophilic and mesophilic temperatures resulted in similar effects on the pH of the digested sludge: an increase in pH within 1-2 days of digestion, then a decrease in the period of continued digestion.

4.4.4 Conductivity

For both thermophilically digested and mesophilically digested sludge, the digestion effects on conductivity in the filtrate of the sheared sludge were different from conductivity in the filtrate of the air-mixed sludge, as shown in Figure 4.47 (a) and (b). Thermophilic digestion resulted in an immediate decrease in conductivity in both the sheared and the air-mixed sludge. Such a decrease occurred during the first 6 hours of digestion. For the sheared sludge, continued digestion resulted in a brief increase in conductivity (one day digested), then a progressive decrease in conductivity. In contrast, the conductivity in the air-mixed sludge increased, and then remained relatively constant during continued digestion.

Mesophilic digestion resulted in comparable increases in both the filtrates of the sheared and the air-mixed sludges, during the first 2 days of digestion, as shown in Figure 4.47 (b). When digestion continued (from day 2 to day 10), the conductivity in the filtrate of sheared sludge gradually decreased to 3.5 mS/cm (10 day digested sludge). In contrast, the conductivity in the filtrate of the air-mixed sludge continued to increase to 6.8 mS/cm (10 day digested sludge). It is noted that shear resulted in a gradual decrease in conductivity, when the sludge was either thermophilically or mesophilically digested for more than 2 days.
Figure 4.46. Effect of shear on pH of the digested sludge, 100% secondary sludge as the feed
(a) Digested at 60°C, 100% secondary sludge as the feed

(b) Digested at 22-28°C, 100% secondary sludge as the feed

Figure 4.47. Effect of shear on conductivity of the digested sludge
Conductivity provides a rapid estimation of ionized dissolved solids in a sample. The initial decrease in conductivity indicated the depletion of readily available organics in the sludge due to microbial biodegradation of organic particles. The subsequent increase in conductivity indicated an increase in dissolved solids as a result of cell lysis, and also correlates to the rapid increase in ammonia and phosphate concentration. Extended digestion resulted in a decrease in conductivity indicating the removal of soluble organics, due to biodegradation.

4.4.5 Nitrogen (NH$_4^+$)

The measured NH$_4^+$-N concentrations in digested sludge (reported as actual concentrations, not the net increase from the feed sludge) that were shear mixed, and air-mixed are shown in Figure 4.48 (a) and (b). The feed sludge used 100% secondary sludge. For thermophilically digested sludge, NH$_4^+$-N concentrations in both the sheared and the air-mixed sludge decreased within the first 6 hours of digestion (e.g. 471 mg/L in the feed sludge, and 354 mg/L in 6-hour digested and sheared sludge). Such a decrease in NH$_4^+$-N concentrations was likely due to stripping at the thermophilic condition of 60°C. Thereafter, the NH$_4^+$-N concentration in the sheared sludge increased to 481 mg/L in one day digested sludge, and then progressively decreased to 80 mg/L in the 10 days digested sludge.

In contrast, the NH$_4^+$-N concentration in the air-mixed sludge increased to 851 mg/l following 2 days of digestion. An additional 5 days of digestion resulted in only a slight increase in NH$_4^+$-N concentration to 901 mg/L. Further digestion resulted in a decrease of NH$_4^+$-N to 758 mg/L.

When the sludge was digested mesophilically, the NH$_4^+$-N in the sheared sludge increased to 565 mg/L (in 2 days digested sludge), then decreased to 377 mg/L (in 10 days digested sludge). In contrast, the NH$_4^+$-N concentrations in the air-mixed sludge steadily increased throughout the 10 day of digestion. The measured NH$_4^+$-N was 486 mg/L in the 2 days digested sludge, and 744 mg/L in the 10 days digested sludge.
(a) Digested at 60°C, 100% secondary sludge as the feed

(b) Digested at 22-28°C, 100% secondary sludge as the feed

Figure 4.48. Effect of shear on ammonia production of digested sludge
4.4.6 Phosphorus (PO$_4^{3-}$)

As shown in Figure 4.49 (a), when the sludge was thermophilically digested, the PO$_4^{3-}$-P concentrations in the sheared sludge increased from 62 mg/L in the feed sludge to 170 mg/L in the one day digested sludge and was 126 mg/L in the 10 days digested sludge. In comparison, PO$_4^{3-}$-P concentrations in the air-mixed sludge increased to 96 mg/L after 3 hours of digestion, then reached 117 mg/L in 10 days digested sludge. PO$_4^{3-}$-P concentrations in the air-mixed sludge were significantly lower than that in the sheared sludge.

The results from mesophilically digested sludge are shown in Figure 4.49 (b). The PO$_4^{3-}$-P concentrations in the sheared sludge increased from 29 mg/L in the feed to 124 mg/L in the 2 days digested sludge. Continued digestion resulted in a slightly lower PO$_4^{3-}$-P concentration (113 mg/L in the 10 days digested sample). The PO$_4^{3-}$-P concentrations in the air-mixed sludge progressively increased to 84 mg/L in the 10 days digested sludge, but are much lower than the concentrations in the sheared sludge. Shear resulted in the release of a larger amount of PO$_4^{3-}$-P into the liquid phase (filtrate) of digested sludge.
(a) Digested at 60°C, 100% secondary sludge as the feed

(b) Digested at 22-28°C, 100% secondary sludge as the feed

Figure 4.49. Effect of shear on phosphate of digested sludge
4.4.7 Extracellular Proteins

The shear effect on the concentrations of soluble protein is shown in Figure 4.50. For thermophilically digested sludge, the concentration of soluble protein in both the sheared and the air-mixed sludge increased rapidly, following a short time of digestion. Following 6 hours of digestion, the concentration of soluble protein increased to 126 mg/g in the air-mixed sludge, and to 134 mg/g in the sheared sludge. Between 6 hours to 7 days of digestion, higher concentrations of soluble protein were formed in the sheared sludge than in the air-mixed sludge. From day 1 to day 10 of digestion, the concentrations of soluble protein in the sheared sludge decreased. In 10 days digested sludge, the concentration of soluble protein in the sheared sludge was about the same as in the air-mixed sludge. For mesophilically digested sludge, the concentration of soluble protein in the air-mixed sludge increased gradually during the 10 days of digestion (from 11 mg/g in the feed sludge to 40 mg/g in the 10 days digested sludge). The concentration of soluble protein in the sheared sludge initially increased (to 57 mg/g in 2 days digested sludge), then gradually decreased to 14 mg/g in the 10 days digested sludge.
(a) Digested at 60°C, 100% secondary sludge as the feed

(b) Digested at 22-28°C, 100% secondary sludge as the feed

Figure 4.50. Effect of shear on soluble extracellular protein of digested sludge
4.4.8 Extracellular Polysaccharides

The shear effect on concentrations of soluble polysaccharide is shown in Figure 4.51. The thermophilically digested sludge showed a rapid increase in soluble polysaccharide concentrations, in both the sheared (increase to 48 mg/g after 3 hours of digestion) and the air-mixed (increase to 45 mg/g after 6 hours of digestion) sludges. Thereafter, soluble polysaccharide concentrations decreased slightly in both the sheared and the air-mixed sludge (to 42 mg/g in the sheared and 6 hour digested sludge, and to 40 mg/g in the air-mixed and one day digested sludge). Continued digestion resulted in a gradual increase in the soluble polysaccharide concentration, in both the sheared and the air-mixed sludges. The concentrations of soluble polysaccharide were generally higher in the sheared sludge than in the air-mixed sludge. When the sludge was digested mesophilically, soluble polysaccharide concentrations in the air-mixed sludge increased gradually from 3 mg/g in the feed sludge to 11 mg/g in the one day digested sludge. The measured concentration of soluble polysaccharide in the sheared sludge was substantially higher than the soluble polysaccharide concentrations in the air-mixed sludge (37 mg/g in the sheared sludge vs. 8 mg/g in the air-mixed sludge, both sludges were digested for 5 days). Mesophilic digestion of the sheared sludge for more than 5 days resulted in a decrease in soluble polysaccharide concentration (18 mg/g in 10 days digested sludge).
(a) Digested at 60°C, 100% secondary sludge as the feed

(b) Digested at 22-28°C, 100% secondary sludge as the feed

Figure 4.51. Effect of shear on soluble extracellular polysaccharide of digested sludge
4.4.9 Floc Charge (Zeta potential)

The effects of shear on floc charge (measured as Zeta potential) are shown in Figure 4.52. For the air-mixed sludge, Zeta potentials of thermophilically digested sludge (-15 mV to -20 mV) were slightly lower than that of mesophilically digested sludge (-10 mV to -15 mV). Continued digestion of up to 10 days, at either temperature, did not result in significant changes in the Zeta potential of the digested sludge. Shear applied to sludge had a significant effect on the Zeta potential of the digested sludge. Zeta potentials in thermophilically digested and the sheared sludge decreased from -15 mV to -31 mV, following 2 days of digestion, and further decreased to -34 mV in the 10 days digested sludge. The majority of reductions in Zeta potentials occurred within the first 2 days of digestion. In contrast, when the sludge was air-mixed, thermophilic digestion did not result in much change in Zeta potential. Zeta potentials in the sheared and mesophilically digested sludge also had a large reduction during the 10 days of digestion (decreased to -29.7 mV in the 7 days digested sludge).
(a) Digested at 60°C, 100% secondary sludge as the feed

(b) Digested at 22-28°C, 100% secondary sludge as the feed

Figure 4.52. Effect of shear on floc charges of digested sludge
4.4.10 Floc Size

The effects of shear on the sizes of flocs from thermophilic digestion are shown in Figure 4.53. Figure 4.54 showed changes of the Surface Mean Diameter (SMD) of both the sheared and the air-mixed sludge that was digested over a 10 days period. The results revealed that the sheared sludge have smaller floc sizes than the air-mixed sludge. Digestion did not result in much change in the SMD of the air-mixed sludge; however, the SMD in the sheared sludge decreased significantly from the SMD of the feed sludge, and are much smaller than the SMD of the air-mixed sludge.

4.4.11 Shear Effect on SCST and Characteristics of Digested Sludge

Increased SCST, due to shear, is likely due to (1) increased concentrations of extracellular biopolymers (2) reduced particle sizes (3) more negative floc charges.

Mixing induced shear had a significant effect on dewaterability of digested sludge, either thermophically, or mesophically (Figure 4.43). For thermophilically digested sludge, the SCST in the sheared sludge was over 4 times of the SCST in the air-mixed sludge. The SCST of the sheared sludge that was mesophically digested was also substantially higher than the SCST of the air-mixed sludge that was digested thermophilically (Figure 4.43). Such a finding indicated that mechanical shear applied to digested sludge had a more predominant effect on SCST than the digestion temperature.

When the sludge was digested thermophilically, high shear did not result in higher TVS reduction than in the air-mixed sludge (Figure 4.44a), suggesting that digestion temperature and adaptation of bacteria were the rate limiting factors. When the sludge was digested mesophically, the rate of TVS reduction in the sheared sludge was higher than the rate of TVS reduction in the air-mixed sludge.
Figure 4.53. Effect of shear on floc sizes, 100% secondary sludge digested at 60°C for 3 h

Figure 4.54. Effect of shear on surface mean diameter of all measured particles, digested at 60°C, 100% secondary sludge as the feed
The pH of the sheared or the air-mixed sludge both decreased within the first 6 hours of digestion, then increased as digestion continues (Figure 4.46). The decrease in pH corresponded to the initial decrease in conductivity (Figure 4.47) and ammonia concentrations (Figure 4.48). A reduction in pH indicated acidification and solubilization of organics in the sludge. The reduction in ammonia concentration, likely due to ammonia stripping, had a larger impact than the solubilization of organics on conductivity, resulting in a decrease in conductivity.

The subsequent pH increase in the air-mixed sludge related to an increase in ammonia concentrations and an increase in conductivity. It is noted that, in both the sheared and the air-mixed sludges, the profiles of pH, conductivity, and ammonia changes agreed very well (Figure 4.46, 4.48, and 4.49), which offered additional evidence of intrinsic relations among these three factors. Digestion caused changes in the ammonia concentration (a combined result of aeration stripping and degradation of organic nitrogen). Ammonia is an important component of conductivity measurements, and affects hydrogen ion balance in a solution, therefore affecting the pH in the digested sludge.

The substantially higher phosphate concentrations in the sheared sludge than in the air-mixed sludge, shown in Figure 4.49, indicated that the high shear that was applied to sludge resulted in increased lysis of cells. The higher concentration of extracellular proteins and (Figure 4.50) and polysaccharides (Figure 4.51) in the sheared sludge were also likely due to increased lysis of cells. The mechanisms involved in the effects of shear on SCST of digested sludge appeared to be different from the effects of temperature on SCST. The increases in the amount of extracellular biopolymers were not comparable to the substantial increase in SCST.

The unique effect of shear on digested sludge was the more negative floc charge (Figure 4.52) and immediate decrease in SMD (Figure 4.54). Smaller floc sizes would result in fillings of pore opening and blocking of water passage channels in the sludge, therefore resulting in deterioration in dewaterability.

4.5 Partition of Substances Affecting Dewaterability

Results of partitioning the substances affecting dewaterability between the liquid and solid phases are shown in Figure 4.55. The data were from 01Aug23Run. It appears that substances
affecting dewaterability primarily resided in the liquid fraction of the digested biosolids. The key of samples is in Figure 4.55 and in Table 3.4. Thermophilic biosolids (Sample A, SCST of 48 s L/g) exhibited poorer dewaterability than mesophilic biosolids (Sample D, SCST of 18 s L/g). Replacing the filtrate from Sample A (thermophilic biosolids) with distilled water resulted in a 64% reduction in SCST (18 s L/g in Sample B). Replacing the filtrate from Sample A with the filtrate from Sample D resulted in a 53% reduction of SCST (23 s L/g in Sample C). The preceding effects indicated that, with thermophilic biosolids, a substantial amount of substances causing poor dewaterability resided in the liquid fraction. Replacing the filtrate from Sample D (mesophilic biosolids) with distilled water resulted in only about 22% SCST reduction (14 s L/g in Sample E), suggesting that, with mesophilic biosolids, most of substances affecting dewaterability were from the solid fraction (pellet). In contrast, replacing the filtrate of Sample D (mesophilic) with filtrate of Sample A (thermophilic) resulted in a 209% increase in the SCST (56 s L/g in Sample F). This was an additional indication that the substances causing poor dewaterability in thermophilically digested biosolids are mainly from the contribution of the liquid fraction in thermophilic biosolids. Such a finding supports the observations that SCST correlated to the soluble proteins and polysaccharides in the thermophilically digested sludge.

Table 4.9 presents an analysis to determine the individual contribution of the liquid fraction and the solids fraction to the total CST of both of thermophilically and mesophilically digested sludge. This analysis result revealed that the liquid fraction contributed to 64% to the measured CST in thermophilically digested biosolids, but only 23% to the measured CST of mesophilically digested biosolids.
A = thermo pellet in thermo filtrate T(S)/T(L); B = thermo pellet in distilled water T(S)/H₂O; C = thermo pellet in meso filtrate T(S)/M(L); D = meso pellet in meso filtrate M(S)/M(L); E = meso pellet in distilled water M(S)/H₂O; F = meso pellet in thermo filtrate M(S)/T(L); Key to the samples also in Table 3.4. Error bars represent 90% confidence intervals.

Figure 4.55. Partitioning between the solid and the liquid phases of factors affecting SCST

4.6 Effect of NH₄⁺ on Dewaterability

Because the changes in NH₄⁺ concentrations due to thermophilic digestion occurred simultaneously with the changed in SCST of the digested sludge, the effect of NH₄⁺ concentrations on the dewaterability of sludge was studied by increasing NH₄⁺ concentrations in a feed sludge that contained 100% secondary sludge. The test was conducted at the room temperature and the sludge was not digested. Concentrated NH₄⁺ (10 g/L as NH₄⁺-N) was prepared using NH₄Cl (reagent grade). 0.52 mL, 1.58 mL, or 4.44 mL of the NH₄Cl stock solution was added to one of the three sludge samples. Another three sludge samples were treated with distilled water at the volumes that are equal to the volumes of NH₄Cl that were added into the spiked samples. The CST was measured for each of spiked samples and control samples, and was reported as SCST. Results are reported in Table 4.10, and shown in Figure 4.56.
Table 4.9. Partitioning factors affecting SCST (01Aug23Run)

<table>
<thead>
<tr>
<th>Sample ID (key in Figure 4.55 and Table 3.4)</th>
<th>Measured CST (s)</th>
<th>Calculated CST (s)</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled H₂O</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A T(S)/T(L)</td>
<td>1,125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B T(S)/H₂O</td>
<td>408</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C T(S)/M(L)</td>
<td>525</td>
<td>507</td>
<td>Calculated = 97% of measured</td>
</tr>
<tr>
<td>T(S)</td>
<td>403</td>
<td></td>
<td>T(S)=B-Distilled H₂O=36% of A</td>
</tr>
<tr>
<td>T(L)</td>
<td>722</td>
<td></td>
<td>T(L)=A-T(S)=64% of A</td>
</tr>
<tr>
<td>D M(S)/M(L)</td>
<td>448</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E M(S)/H₂O</td>
<td>349</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F M(S)/T(L)</td>
<td>1,383</td>
<td>1,066</td>
<td>Calculated = 77% of measured</td>
</tr>
<tr>
<td>M(S)</td>
<td>344</td>
<td></td>
<td>M(S)=E-Distilled H₂O=77% of D</td>
</tr>
<tr>
<td>M(L)</td>
<td>104</td>
<td></td>
<td>M(L)=D-M(S)=23% of D</td>
</tr>
</tbody>
</table>

aThe CST of calculated T(S)/M(L) = T(S)+M(L) = 403+104=507 s
bThe CST of calculated M(S)/T(L) = M(S)+T(L) = 344+722=1,066 s

Table 4.10. Spiked NH₄⁺-N effect on dewaterability

<table>
<thead>
<tr>
<th>Sample</th>
<th>Final NH₄⁺-N Concen. (mg/L)</th>
<th>pH¹</th>
<th>Conductivity¹ (mS/cm)</th>
<th>CST±SD (n) (g/L)</th>
<th>TS (s L/g)</th>
<th>SCST (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (not spiked)</td>
<td>112</td>
<td>7.1</td>
<td>1.6</td>
<td>130±11(3)</td>
<td>26.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Spike 1</td>
<td>200</td>
<td>7.1</td>
<td>2.5</td>
<td>114±10(3)</td>
<td>26.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Control of Spike 1</td>
<td>109</td>
<td>7.1</td>
<td>1.5</td>
<td>122±2(3)</td>
<td>26.3</td>
<td>4.6</td>
</tr>
<tr>
<td>Spike 2</td>
<td>375</td>
<td>7.0</td>
<td>4.3</td>
<td>103±10(3)</td>
<td>25.8</td>
<td>4.0</td>
</tr>
<tr>
<td>Control of Spike 2</td>
<td>123</td>
<td>7.2</td>
<td>1.5</td>
<td>108±14(3)</td>
<td>25.8</td>
<td>4.2</td>
</tr>
<tr>
<td>Spike 3</td>
<td>978</td>
<td>7.0</td>
<td>9.9</td>
<td>95±5(3)</td>
<td>24.4</td>
<td>3.9</td>
</tr>
<tr>
<td>Control of Spike 3</td>
<td>122</td>
<td>7.1</td>
<td>1.5</td>
<td>102±11(3)</td>
<td>24.4</td>
<td>4.2</td>
</tr>
</tbody>
</table>

¹Stock NH₄⁺-N solution pH 4.9, conductivity 80 mS/cm.
When the NH$_4^+$-N concentrations in the undigested feed sludge was increased from 112 mg/L to 978 mg/L (a concentration comparable to the concentration in thermophilically digested sludge), only a slight decrease in SCST (decreased by 1.0 s L/g) was observed (Spike 3). Therefore, increased NH$_4^+$-N concentrations were not the factor that significantly increased SCST of thermophilically the sludge.

4.7 Effect of PO$_4^{3-}$ on Dewaterability

Because the changes in PO$_4^{3-}$ concentrations due to thermophilic digestion occurred simultaneously with the changed in SCST of the digested sludge, the effect of PO$_4^{3-}$ concentrations on dewaterability of sludge was studied by increasing PO$_4^{3-}$ concentrations in a feed sludge that contained 100% secondary sludge. The test was conducted at the room temperature, and the sludge was undigested. Concentrated PO$_4^{3-}$ of 4.1 g/L as PO$_4^{3-}$-P and 9.2 g/L as Na$^+$ was prepared using Na$_3$PO$_4$ (reagent grade). 0.3 mL, 0.7 mL, or 1.2 mL of the stock solution was added to one of three sludge samples. Another three sludge samples from the same source were treated with volumes of NaCl (9.2 g/L as Na$^+$) that were equal to the volumes of Na$_3$PO$_4$ that were added into spiked samples. The CST was measured for each spiked sample.
and the corresponding control sample. The SCST results are shown in Figure 4.57, and reported in Table 4.11.

When the $\text{PO}_4^{3-}$-P concentration in undigested feed sludge was increased from 32 mg/L to 92 mg/L (a comparable concentration to the concentration observed in thermophilically digested sludge), only a small change in SCST (1.9 s L/g increase) was observed (Spike C). Such a small change might be due to a slight increase in pH. However, such an increase in SCST is negligible compared to the large increase in SCST observed during thermophilic digestion. Therefore, $\text{PO}_4^{3-}$-P was not a significant factor affecting the dewaterability of the sludge.

Table 4.11. Effect of spiked $\text{PO}_4^{3-}$-P on dewaterability

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\text{PO}_4^{3-}$-P (mg/L)</th>
<th>pH$^a$</th>
<th>Conductivity$^a$ (mS/cm)</th>
<th>CST±SD (n)</th>
<th>TS (g/L)</th>
<th>SCST (s L/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (not spiked)</td>
<td>32</td>
<td>7.1</td>
<td>2.1</td>
<td>144±19(3)</td>
<td>26.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Spike A</td>
<td>44</td>
<td>7.1</td>
<td>2.1</td>
<td>155±8(3)</td>
<td>26.4</td>
<td>5.9</td>
</tr>
<tr>
<td>Control of Spike A</td>
<td>29</td>
<td>7.2</td>
<td>2.4</td>
<td>142±5(3)</td>
<td>26.4</td>
<td>5.4</td>
</tr>
<tr>
<td>Spike B</td>
<td>67</td>
<td>7.3</td>
<td>2.3</td>
<td>164±12(3)</td>
<td>26.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Control of Spike B</td>
<td>30</td>
<td>7.0</td>
<td>2.5</td>
<td>177±8(3)</td>
<td>26.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Spike C</td>
<td>92</td>
<td>7.3</td>
<td>2.5</td>
<td>187±11(3)</td>
<td>26.1</td>
<td>7.2</td>
</tr>
<tr>
<td>Control of Spike C</td>
<td>31</td>
<td>7.1</td>
<td>2.7</td>
<td>132±5(3)</td>
<td>26.1</td>
<td>5.1</td>
</tr>
</tbody>
</table>

$^a$Stock Na$_3$PO$_4$ solution pH 12.0, conductivity 25.2 mS/cm. stock NaCl solution pH 5.8, conductivity 35.8 mS/cm

4.8 Effect of pH on Dewaterability

Results of the conditioning of digested sludge through pH adjustment are shown in Figure 4.58 (on the previous page). Two acids were use in the tests (concentrated H$_2$SO$_4$ and HCl). Both acids effectively improved dewaterability of digested sludge (lower CST). The lowest CST occurred when the pH of tested filtrate was approximately pH 2. Further pH reduction to pH 1 resulted in an increase in CST.
Figure 4.57. Effect of phosphate on SCST of undigested secondary sludge

![Graph showing the effect of phosphate on SCST of undigested secondary sludge.](image)

Legends: • H\textsubscript{2}SO\textsubscript{4} ○ HCl

Figure 4.58. Effect of pH on CST, digested at 60°C

![Graph showing the effect of pH on CST.](image)
The pH affects both the surface charge density of colloidal particles, and the effectiveness of polymeric chemicals (e.g. coiling, degree of ionizations, and charge density). Sukenik et al. (1977) tested the effect of chlorine and acid addition on dewaterability of raw primary sludge, anaerobically digested, and aerobically digested sludge (from two small secondary wastewater plants, mesophilic digestion), and reported that pH was the most significant factor influencing dewaterability (measured as filterability). Tested pH in this study ranged from pH 2 to 8, a lower pH resulted in substantially improved dewaterability. Forster (1968) and Tenney et al. (1970) reported that, when the pH was lowered to approximately 2.5, Zeta potentials of activated sludge (undigested) approached to zero.

4.9 Scanning Electron Microscope (SEM) Analysis

Figure 4.59 (a) shows the SEM photo of a thermophilically digested sludge sample, having a CST of approximately 1000 s. Figure 4.59 (b) shows the SEM photo of an undigested thickened secondary sludge sample, having a CST of approximately 100 s. The SEM photos revealed a gel-like structure in the matrix of the thermophilically digested sludge, but not in the matrix of the readily dewaterable secondary sludge. Such a gel structure appears to be similar to the gelatinous surface feature from a sample of an anaerobically digested sludge, as reported by Poxon (1996). Apparently, it would be difficult for the water to separate from the solids in such a structure, as water and solids form an integrated matrix, and there are no readily available paths to allow the water to drain from the matrix. Since a gel structure is generally formed by extracellular biopolymers, including proteins and polysaccharides, the surface feature, shown in Figure 4.59(a), provided indirect evidence that extracellular biopolymers likely play an important role in affecting the dewaterability of the digested sludge.
Figure 4.59. SEM photos of thermophilically digested sludge and secondary sludge

(a) A sample of thermophilically digested sludge taken from UBC ATAD Pilot Facility

(b) A sample of secondary sludge taken from UBC Wastewater Pilot Plant
4.10 Effects of Extracellular Proteins on Dewaterability

4.10.1 Concentrations of Soluble, Bound, and Total Proteins

The concentrations of soluble and bound proteins are summarised in Table 4.12, and are shown in Figure 4.60 (the feed was 100% secondary sludge) and Figure 4.61 (the feed was 100% primary sludge). The data were from 00Aug15Run, where the sludge was digested for 20 days, and proteins in the samples taken during the first 15 days were measured. Data shown are measured actual concentrations. The techniques to separate soluble and bound proteins were described in Section 3.4.

Table 4.12. Concentrations of bound and soluble proteins (mg/L)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location of proteins</th>
<th>Thermo. (60°C)</th>
<th>Meso. (22°C)</th>
<th>Thermo. (60°C)</th>
<th>Meso. (22°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100% secondary</td>
<td>100% secondary</td>
<td>100% primary</td>
<td>100% primary</td>
</tr>
<tr>
<td>Feed</td>
<td>Bound proteins</td>
<td>1,410</td>
<td>1,410</td>
<td>321</td>
<td>321</td>
</tr>
<tr>
<td>5 days</td>
<td></td>
<td>775</td>
<td>545</td>
<td>183</td>
<td>233</td>
</tr>
<tr>
<td></td>
<td></td>
<td>469</td>
<td>387</td>
<td>273</td>
<td>320</td>
</tr>
<tr>
<td>Feed</td>
<td>Soluble proteins</td>
<td>373</td>
<td>373</td>
<td>119</td>
<td>119</td>
</tr>
<tr>
<td>5 days</td>
<td></td>
<td>1,884</td>
<td>850</td>
<td>488</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,501</td>
<td>570</td>
<td>725</td>
<td>171</td>
</tr>
<tr>
<td>Feed</td>
<td>Proportion of soluble to total proteins</td>
<td>23%</td>
<td>23%</td>
<td>27%</td>
<td>27%</td>
</tr>
<tr>
<td>5 days</td>
<td></td>
<td>71%</td>
<td>61%</td>
<td>73%</td>
<td>39%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>76%</td>
<td>60%</td>
<td>73%</td>
<td>35%</td>
</tr>
</tbody>
</table>

*The duration of the sample digestion.*
(a) Digested at 60°C, 100% secondary sludge as the feed.

(b) Digested at ~22°C, 100% secondary sludge as the feed.

Figure 4.60. Effect of digestion of secondary sludge on concentrations of bound and soluble proteins
For the undigested sludge, only a small portion of total measured proteins existed in soluble form (21% in the 100% secondary sludge, and 27% in the 100% primary sludge). The thermophilic digestion of the sludge, using 100% secondary sludge as the feed, resulted in a decrease in bound proteins, a substantial increase in soluble proteins, and an increase in total protein concentration (bound plus soluble). Continued digestion resulted in a decrease in concentrations of total protein, soluble proteins, and bound proteins. In digested sludge, the concentration of soluble protein accounted for the major portion of the total protein concentrations (e.g. 72% in 10 days digested sludge). Mesophilic digestion of the sludge using 100% secondary sludge as the feed resulted in a progressive decrease in total and bound protein concentration, but a gradual increase in soluble protein concentrations. The proportion of soluble protein in total protein increased from 23% in the feed sludge to 60% in 10 days digested sludge. The thermophilic digestion of the sludge using 100% primary sludge as the feed resulted in a slight decrease in the concentrations of bound proteins, but a substantial increase in the concentration of soluble protein and total protein. Mesophilic digestion of sludge using 100% primary sludge as the feed resulted in a slight decrease in concentration of bound and total proteins, but showed little change in the concentration of soluble protein, within the first 5 days of digestion. The concentration of protein in both portions increased from day 5\textsuperscript{th} to day 10\textsuperscript{th} of digestion.

4.10.2 Characterizing Extracellular Proteins

4.10.2.1 SDS-PAGE experiment

SDS-PAGE test was conducted twice, both used filtrate of digested sludge samples that were taken from 01Feb02Run, which used 100% secondary sludge as the feed. The standards used in both tests included proteins of various molecular weights, ranging from 6,500 Daltons (aprotinin) to 200,000 Daltons (myosin). The bovine serum albumin (66,200 Daltons) was also included in the standard.
Figure 4.61. Effect of digestion of primary sludge on concentrations of bound and soluble extracellular proteins

(a) Digested at 60°C, 100% primary sludge as the feed

(b) Digested at ~22°C, 100% primary sludge as the feed
In the first test, samples included filtrate of the feed, thermophilically (60°C) digested sludge (taken at 3, 6, 18, and 48 hours), and mesophilically digested sludge (taken at 3, 6, 18, and 48 hours). Samples were loaded to the test apparatus as duplicates. No visible protein bands appeared from the samples, although normal protein band from the “standard” sample was identified. Such a finding could be due to two reasons: (1) the proteins in the filtrate of thermophilically digested sludge samples were not extracted by TCA. (2) the same proteins could not be stained by the Coomassie brilliant blue R-250 stain reagent. Experimental results shown in Section 4.10.2.3. and Section 4.10.2.4 (both sections to follow) revealed that both reasons are the causes.

In the second test, two aliquots of one sample from thermophilically digested sludge (3.0 hours) were used. The first aliquot was directly applied to the apparatus, the second aliquot was treated with TCA concentration procedures, and the concentration was adjusted to match the first aliquot, then the sample was applied to the SDS-PAGE apparatus. Again, no visible protein bands were identified from the samples, although a normal protein band, corresponding to the “standard” sample, was identified. The reasons for the no visible bands could include (1) the proteins were too dilute to form a visible band. (2) the same proteins could not be stained by the Coomassie brilliant blue R-250 stain reagent (confirmed in Section 4.10.2.4).

In comparison, Higgins (1995) carried out a SDS-PAGE analysis on extracellular bound proteins of the biomass from three activated sludge systems (one municipal, one industrial, and one laboratory scale), and found that the molecular weight of the proteins were approximately 15,000 to 17,000 Daltons.

4.10.2.2 Dialysis of soluble proteins

The filtrate of a thermophilically digested sludge sample (60°C for 6 hour, 01Feb02Run, 100% secondary sludge as the feed) was dialysed. Results, shown in Figure 4.62, indicated that 86% of the proteins had sizes of less than 7,000 Daltons, and only 9% of the proteins had sizes larger than 13,000 Daltons. Forster (1982) fractionated proteins in a digested sludge with a membrane system (the digester feed was a mixture of primary and humus sludge), and reported that 15.3% of the proteins had a nominal molecular weight cut-off of less than 10,000 Daltons.
4.10.2.3 TCA precipitation of soluble proteins

Trichloroacetic acid (TCA) is commonly used to concentrate proteins in solution. Typical proteins can be precipitated with TCA. This method allows proteins to be extracted into the pellet formed in the treatment, and therefore be concentrated. Three samples were tested, two were taken from the 01Feb02Run (using 100% secondary sludge as the feed sludge): the first one was the filtrate of a sludge that was digested at 60°C for 12 hours (the thermo.), the second one was the filtrate of a sludge that was digested at room temperatures for 12 hours (the meso.). The third sample was bovine serum albumin (BSA) of 2,000 mg/L. Proteins were measured using the Lowry assay; and measurement results are shown in Figure 4.63 and are summarised in Table 4.13.

TCA extraction results revealed that the measured BSA concentration in the control sample agreed well with the anticipated BSA stock concentration (2,113 mg/L vs. 2,000 mg/L, a 5.7% difference). Results of proteins measurements on digested sludge samples (thermo. and meso.) showed good recovery rate (98-103% recovery). Therefore, the values of the measured concentrations using the Lowry assay are valid. The TCA extracted 91% of available protein in the BSA sample. Such a high extraction rate is as expected. However, the extraction rate of soluble protein, by TCA in both thermophilically and mesophilically digested sludge was low.
Only 6-8% of proteins in digested sludge were extracted by TCA. Therefore, soluble materials measured by the Lowry assay in samples of digested sludge possessed a characteristic that is different from that of a typical protein, such as BSA.

![TCA Extraction](image)

Figure 4.63. Measured protein concentrations in TCA extraction experiment

**Table 4.13. TCA proteins extraction**

<table>
<thead>
<tr>
<th>Sample</th>
<th>BSA Stock (mg/L)</th>
<th>Thermophilically</th>
<th>Mesophilically</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (measured, mg/L)</td>
<td>2,000 mg/L</td>
<td>2,113 mg/L</td>
<td>2,407 mg/L</td>
</tr>
<tr>
<td>In supernatant (mg/L)</td>
<td>2,292 mg/L</td>
<td>1913 mg/L</td>
<td>190 mg/L</td>
</tr>
<tr>
<td>In TCA pellet (extracted, mg/L)</td>
<td>1913 mg/L</td>
<td>10 mg/L</td>
<td>10 mg/L</td>
</tr>
<tr>
<td>Rate of recoverya</td>
<td>92%</td>
<td>103%</td>
<td>98%</td>
</tr>
<tr>
<td>Rate of TCA extractedb</td>
<td>91%</td>
<td>8%</td>
<td>6%</td>
</tr>
</tbody>
</table>

*aRate of recovery = (proteins in supernatant + proteins in pellet)/(proteins in control)*

*bRate of TCA extracted = (proteins in pellet)/(proteins in control)*
4.10.2.4 Bradford assay versus Lowry assay for protein measurements

The Bradford assay is an alternative method to measure concentrations of protein. The Bradford assay and the Lowry assay were compared in measurements of soluble protein in sludge samples that were taken from 01Feb02Run. Three samples were tested: the first one was thermophilically digested at 60°C for 12 hours, the second sample was mesophilically digested at the room temperature for 12 hours, and the third was the undigested feed sludge. Measurement results are summarised in Table 4.14, and are showed in Figure 4.64. The results revealed that the measured concentration of protein, using the Bradford assay, were substantially lower than the measured concentration of protein using the Lowry assay (e.g. Bradford to Lowry ratio was 1 to 5.4 for soluble protein in the thermophilically digested sludge). Such findings agreed with the work reported elsewhere (Raunkjaer et al. 1994, Higgins 1995).

Raunkjaer et al. (1994), measuring proteins in an untreated domestic wastewater, found that the measured protein concentrations by the Bradford assay were 7 times (the dissolved proteins, passed through 0.45 μm membrane filter), and 4 times (the total proteins) lower than that by Lowry assay, respectively. Higgins (1995), measuring the soluble (supernatant of the 8,000 × g centrifuged sample) extracellular proteins from the mixed liquor of two municipal activated sludge plants, found that protein concentration from the Bradford method was up to 25 times less than the concentration from the Lowry method.

Table 4.14. Measured concentration using the Bradford assay and the Lowry assay (soluble protein in digested sludge)

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Feed</th>
<th>Thermophilically digested sludge</th>
<th>Mesophilically digested sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>By Bradford assay (mg/L)</td>
<td>21</td>
<td>395</td>
<td>63</td>
</tr>
<tr>
<td>By Lowry assay (mg/L)</td>
<td>129</td>
<td>2,144</td>
<td>175</td>
</tr>
<tr>
<td>Ratio of Bradford to Lowry</td>
<td>1:6.1</td>
<td>1:5.4</td>
<td>1:2.8</td>
</tr>
</tbody>
</table>
Figure 4.64. Concentration of proteins measured in the same sample by the Bradford assay (Brad) and the Lowry assay (Lowry)

4.10.2.5 Overall assessment on characteristics of proteins

Findings from the experimental work of the proteins dialysis, TCA precipitation of proteins, and Bradford assay to measure concentrations of proteins indicated that the soluble proteins, from the liquid fraction of thermophilically digested samples, are low molecular weight. Both SDS-PAGE and Bradford assay are dye-binding methods in principle, using Coomassie Brilliant Blue 250 (CBB-250, described in Osterman 1989) to react primarily with basic and aromatic amino acid residues, especially arginine. However, arginine accounted for only a small fraction (approximately 200 µmol/g or ~20 mg/L protein) of detected amino acids in extracellular proteins of activated sludge samples (Higgins and Novak 1997c). To be bound by CBB-250, proteins must have a macromolecular structure of at least 8-9 peptide bonds (Raunkjaer et al. 1994), and would have a molecular weight of approximately 800-900 Daltons.

Although high concentrations of proteins were detected by Lowry assay, SDS-PAGE assay did not identify protein bands; proteins concentrations measured by the Bradford assay were substantially lower than that by Lowry assay. Therefore, proteins in the tested samples likely do
not possess macromolecular structure, otherwise, the proteins would have been bound to CBB-250, and produced sufficient response to SDS-PAGE and Bradford assay. The lower detection limit of the Bradford method is approximately 3,000 to 5,000 Daltons in molecular weight (Bio-Rad Laboratories, n.d.). If proteins are smaller than the detection limit, the absorbance will be very low, and measured concentrations of protein would be very low. The protein dialysis results revealed that 86% of proteins are less than 7,000 Daltons. TCA was ineffective in precipitating proteins having small molecular sizes, indicating proteins in thermophilically digested sludge have smaller molecular sizes.

Novak and Bivins (2000) reported that the majority of soluble protein from a thermophilic anaerobically digested sludge sample had sizes of less than 1,000 Daltons (using an Amicon YM1 membrane), while the majority of soluble polysaccharide that passed through a 1.5 \( \mu \)m filter paper were larger than 30,000 Daltons (using an Amicon YM30 membrane).

Bomio and Sonnleitner et al. (1989) pulsed thermophilic culture (65°C) with 4 g/L of gelatine and reported an immediate increase in OUR of the thermopiles. Such an observation suggested that proteolytic enzymes effective on gelatine were present in thermophilic microorganisms. The presence of such specific enzymes implies the existence of gelatine-like protein in thermophilic culture, although the gelatine may also be broken down by these enzymes. Bio-Rad Laboratories (n.d.) reported that gelatine protein yielded exceptionally low colour responses to the Bradford assay. Therefore, it is likely the protein in the thermophilically digested sludge possessed characteristics like gelatine.

4.10.3 Role of Extracellular Proteins (Protease Treatment)

Protease refers to enzymes that degrade specific proteins. Protease treatment of the filtrate of thermophilically digested sludge, aiming to destroy the functionality of proteins in the treated samples, resulted in a statistically significant reduction of SCST (95% confidence), as shown in Table 4.15, and Figure 4.65 (the T7409 showed a 16% reduction, the P5147 showed a 13% reduction, the P6911 showed a 19% reduction). However, protease treatment did not result in much changed in concentrations of proteins in the tested samples. In contrast, boiling the filtrate at 100°C for 20 and 60 minutes did not cause a significant reduction in SCST (95% confidence, shown in Table 4.15 and Figure 4.66), although the concentrations of proteins showed a 15-26% reduction. Boiling treatment of 60 minutes would denature typical proteins. Such effects are
mostly due to impacts on functional structure of proteins (e.g. unfolding under boiling temperature). The ineffectiveness of the boiling treatment on SCST suggested that the substances formed during thermophilic digestion were heat stable materials, such as carbohydrates or unusually heat stable proteins. Sonnleitner and Fiechter (1983b) reported that *Bacilli* formed a major fraction of thermophilic microbial composition. Haner et al. (1994) suggested that *Bacilli* produced heat-stable extracellular proteases. Such findings explained the ineffectiveness of boiling to change dewaterability of digested sludge.

Table 4.15. Effects of protease and boiling treatment on dewaterability

<table>
<thead>
<tr>
<th>Condition</th>
<th>CST (s)</th>
<th>t value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Protein concen. (mg/L)</th>
<th>Change from control</th>
<th>Polysacch. concen. (mg/L)</th>
<th>Change from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(37°C)</td>
<td>1111±55(3)</td>
<td>1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,500</td>
<td></td>
<td>584</td>
<td></td>
</tr>
<tr>
<td>Protease T7409</td>
<td>931±51(3)</td>
<td>4.4</td>
<td>2,507 +0.3%</td>
<td>657</td>
<td>+13%</td>
<td></td>
</tr>
<tr>
<td>Protease P5147</td>
<td>972±54(3)</td>
<td>3.2</td>
<td>2,360 -6%</td>
<td>589</td>
<td>+0.9%</td>
<td></td>
</tr>
<tr>
<td>Protease P6911</td>
<td>902±34(3)</td>
<td>5.7</td>
<td>2,555 +2%</td>
<td>559</td>
<td>-4%</td>
<td></td>
</tr>
<tr>
<td>Boiling Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(tested at ~22°C)</td>
<td>1016±68(3)</td>
<td></td>
<td>3,570</td>
<td>1,017</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 min. boiling</td>
<td>979±90(3)</td>
<td>0.6</td>
<td>3,022 -15%</td>
<td>838</td>
<td>-18%</td>
<td></td>
</tr>
<tr>
<td>60 min. boiling</td>
<td>1004±71(3)</td>
<td>0.2</td>
<td>2,632 -26%</td>
<td>733</td>
<td>-28%</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Control of protease test was compared to Control of boiling test.

<sup>b</sup>Three replicates with each sample, 95% and 90% critical values for one tailed "t test" are 2.132 and 1.533, respectively.
Error bars: 90% confidence intervals

Figure 4.65. Effect of protease treatment on dewaterability,

Error bars: 90% confidence intervals

Figure 4.66. Effect of boiling treatment on dewaterability
The 13-19% reduction in SCST, due to protease treatment, confirmed that proteins in the liquid phase played a role in the overall interactions affecting dewaterability of thermophilically digested biosolids. Such a reduction in SCST, although small from the perspective of full-scale applications, suggests that the nature of extracellular proteins effect on dewaterability is complex. Thermophilic digestion may produce many different types of proteins. These proteins may all affect dewatering properties in one way or another. Therefore, denaturing proteins, by using only one type of protease each time, would not be adequate to achieve a substantial reduction of SCST.

The current knowledge on which proteins, or which functional groups of proteins, affect dewaterability is limited. The three types of protease identified in this study may not be the most effective ones. Extracellular proteins residing in the solid phases (entrapped in pellet) of biosolids may also contribute to the observed effect on dewaterability. Possible interacting effects of proteins and polysaccharides may also be significant, in affecting dewaterability.

4.11 Effects of Extracellular Polysaccharides on Dewaterability

4.11.1 Concentrations of Soluble, Bound, and Total Polysaccharides

The concentrations of soluble and bound polysaccharides are summarised in Table 4.16, and shown in Figure 4.67 (the feed used 100% secondary sludge) and Figure 4.68 (the feed used 100% primary sludge). The data were from 00Aug15Run, where the sludge was digested for 20 days, and polysaccharides in the samples taken during the first 15 days were measured. Data shown are measured actual concentrations. The techniques to separate soluble and bound polysaccharides were described in Section 3.4.

In general, digestion effects on the concentration of soluble polysaccharides showed similar trends as the concentration of soluble protein. In undigested sludge, only a small portion of polysaccharides are in soluble form (11% was soluble when the 100% secondary sludge was used as the feed). For thermophilically digested sludge, using 100% secondary sludge as the feed, one day of digestion resulted in a substantial increase in concentration of soluble polysaccharide (increased from 78 mg/L in the feed to 847 mg/L in one day digested sludge), a large reduction in concentration of bound polysaccharide (reduced from 657 mg/L to 258 mg/L
in the one day digested sludge), and a net increase in concentration of total extracellular polysaccharide. Continued digestion resulted in a gradual decrease in concentration of bound polysaccharide. The concentration of soluble polysaccharide was relatively unchanged. For mesophically digested sludge, using 100% secondary sludge as the feed, digestion resulted in a significant reduction in the concentration of bound polysaccharides, a gradual and slight increase in concentration of soluble polysaccharide and a decrease in the concentration of total polysaccharides. When the feed used 100% primary sludge, thermophilic digestion resulted in a decrease in bound polysaccharides, a substantial increase in soluble polysaccharides, and an increase in total polysaccharides. For mesophically digested 100% primary sludge, digestion resulted in a reduction in bound polysaccharides and total polysaccharides, a slight increase in concentrations of soluble polysaccharides during the first 5 days of digestion, and a large increase in concentrations of soluble polysaccharides, during day 5th to day 15th of digestion.

Table 4.16. Concentrations of bound and soluble polysaccharides (mg/L)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location of polysacch.</th>
<th>Thermo. (60°C)</th>
<th>Meso. (22°C)</th>
<th>Thermo. (60°C)</th>
<th>Meso. (22°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100% secondary</td>
<td>100% secondary</td>
<td>100% primary</td>
<td>100% primary</td>
</tr>
<tr>
<td>Feed</td>
<td>Bound polysacch.</td>
<td>657</td>
<td>657</td>
<td>189</td>
<td>189</td>
</tr>
<tr>
<td>5 days</td>
<td></td>
<td>196</td>
<td>82</td>
<td>58</td>
<td>56</td>
</tr>
<tr>
<td>15 days</td>
<td></td>
<td>175</td>
<td>59</td>
<td>87</td>
<td>84 b</td>
</tr>
<tr>
<td>Feed</td>
<td>Soluble polysacch.</td>
<td>78</td>
<td>78</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>5 days</td>
<td></td>
<td>923</td>
<td>217</td>
<td>225</td>
<td>26</td>
</tr>
<tr>
<td>15 days</td>
<td></td>
<td>781</td>
<td>246</td>
<td>247</td>
<td>81 b</td>
</tr>
<tr>
<td>Feed</td>
<td>Proportion of soluble to total polysacch.</td>
<td>11%</td>
<td>11%</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>5 days</td>
<td></td>
<td>82%</td>
<td>73%</td>
<td>80%</td>
<td>32%</td>
</tr>
<tr>
<td>15 days</td>
<td></td>
<td>82%</td>
<td>81%</td>
<td>74%</td>
<td>49% b</td>
</tr>
</tbody>
</table>

a The duration of the sample digestion.

b This sample was digested for 10 days, not 15 days.
(a) Digested at 60°C, 100% secondary sludge as the feed

(b) Digested at ~22°C, 100% secondary sludge as the feed

Figure 4.67. Effect of digestion of secondary sludge on concentrations of bound and soluble polysaccharides
Figure 4.68. Effect of digestion of primary sludge on concentrations of bound and soluble polysaccharides

(a) Digested at 60°C, 100% primary sludge as the feed

(b) Digested at ~22°C, 100% primary sludge as the feed
4.11.2 Role of Extracellular Polysaccharides (Enzyme Treatment)

Several aliquots of a thermophilically digested sludge sample (60°C for 25 hours, 100% secondary sludge as the feed from 02Feb17Run) were treated at a 50 mg/L dosage with various enzymes that hydrolyse polysaccharides. The results are shown in Table 4.17 and Figure 4.69. Enzyme treatment did not result in a significant reduction in the CST; instead, the addition of P4716 resulted in an increase in CST, which corresponded to a 153% increase in the concentration of polysaccharide in the filtrate of treated sample.

Error bars: one time of standard deviation.

Figure 4.69. Effect of enzyme treatments on dewaterability
Table 4.17. Effects of polysaccharide enzyme treatment on dewaterability

<table>
<thead>
<tr>
<th></th>
<th>CST (^c) (s)</th>
<th>Change in CST</th>
<th>Polysacch. concen. (mg/L)</th>
<th>Change from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control A(^a) ((37°C))</td>
<td>677±140(2)</td>
<td></td>
<td>599</td>
<td></td>
</tr>
<tr>
<td>Enzyme C9422</td>
<td>672±116(3)</td>
<td>-1%</td>
<td>510</td>
<td>-15%</td>
</tr>
<tr>
<td>Enzyme H2125</td>
<td>642±25(2)</td>
<td>-5%</td>
<td>693</td>
<td>+16%</td>
</tr>
<tr>
<td>Control B(^b) ((~22°C))</td>
<td>719±63(2)</td>
<td></td>
<td>566</td>
<td></td>
</tr>
<tr>
<td>Enzyme A6380</td>
<td>746±5(2)</td>
<td>+4%</td>
<td>570</td>
<td>+1%</td>
</tr>
<tr>
<td>Enzyme P4716</td>
<td>921±160(3)</td>
<td>+28%</td>
<td>1430</td>
<td>+153%</td>
</tr>
</tbody>
</table>

\(^a\) Control A was the control for samples treated by C9422 (a cellulase), H2125 (a hemicellulase)  
\(^b\) Control B was the control for samples treated by A6380 (α-amylase), P4716 (a pectinase)  
\(^c\) CST is reported as “average ± one time of standard deviation (number of measurements)”

4.12 Cations Analysis

Cations analysis was carried out on selected samples of thermophilically and mesophilically digested sludge. The analysis of nickel is to confirm that no nickel related heavy metals toxicity that may hinder the acclimatization and activities of thermophilic bacteria, as there was reported high concentrations in the influent to the GVRD’s Lulu Island WWTP before. The analysis of Fe, Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\) was to seek for additional information on how thermophilic digestion affect the transformation and changes of bacteria and organics in the feed sludge, and to compare with findings reported in other studies.

4.12.1 Nickel (N\(^{+}\))

The concentrations of nickel in the feed sludge, the filtrate of the feed and digested sludge were measured. Regardless of digestion conditions (thermophilic vs. mesophilic, sheared vs. air-mixed), the nickel concentration in all tested samples were below detection limit (< 0.1 mg/L), and are negligible. Nickel toxicity to biodegradation of sludge, tested in this study, was not a concern.

4.12.2 Iron (Fe)

Iron was measured as total iron, and did not distinguish between ferrous and ferric iron. The iron in the feed sludge, in the filtrate of the feed and the digested sludge were measured. The results are shown in Figure 4.70 and Table 4.18.
Table 4.18. Measured cations in the sludge and the filtrate of the feed sludge

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fe</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Digest. temp.</th>
<th>TS in the feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>01Oct30Run (Thermo)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60°C</td>
<td>23.8 g/L</td>
</tr>
<tr>
<td>In sludge of the feed (mg/L)</td>
<td>419</td>
<td>67</td>
<td>158</td>
<td>282</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In filtrate of the feed (mg/L)</td>
<td>27</td>
<td>66</td>
<td>132</td>
<td>47</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of filtrate/sludge</td>
<td>6%</td>
<td>99%</td>
<td>84%</td>
<td>17%</td>
<td>50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>01Nov27Run (Meso)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22°C</td>
<td>21.0 g/L</td>
</tr>
<tr>
<td>In sludge of the feed (mg/L)</td>
<td>524</td>
<td>82</td>
<td>147</td>
<td>230</td>
<td>81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In filtrate of the feed (mg/L)</td>
<td>15</td>
<td>59</td>
<td>63</td>
<td>28</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of filtrate/sludge</td>
<td>3%</td>
<td>72%</td>
<td>43%</td>
<td>12%</td>
<td>23%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For undigested sludges, the majority of iron was associated with the solid fraction of the sludge. The iron in the liquid phase (filtrate) accounted for only 3%-6% of the total available iron in the feed sludge. When the digested sludge was air-mixed, the concentration of iron in the filtrate of thermophilically digested sludge was close to that in mesophilically digested sludge. When high shear was applied to the digested sludge, the measured iron in the filtrate of both thermophilically and mesophilically digested sludge was much higher than the iron in the air-mixed sludge. The most significant increase in iron concentration occurred in the filtrate of high-sheared and thermophilically digested sludge (increased from 27 mg/L in the filtrate of the feed to 175 mg/L in the 2 days digested sludge). The iron concentration in the high-sheared and mesophilically digested sludge increased from 15 mg/L in the feed, to 69 mg/L in 2 days digested sludge, then further increased to 91 mg/L in the 10 days digested sludge. Mechanical shear, other than digestion temperature, was the most effective factor for the release of iron from the solid phase into the liquid phase of the sludge.
Figure 4.70. Effect of digestion and shear on concentration of Fe in filtrate of sludge, digested at 60°C (thermo) and 22°C (meso), 100% secondary sludge as the feed

4.12.3 Sodium (Na⁺)

The results of measured sodium concentrations in the feed sludge, in the filtrate of the feed sludge and digested sludge are shown in Figure 4.71 and Table 4.18. The majority of Na⁺ (72% - 99%) in the undigested sludge was present in the liquid phase of the sludge. Regardless of digestion temperatures (thermophilic vs. mesophilic), or the shear applied to the digested sludge, the digestion did not result in much change in Na⁺ concentrations in the filtrate. Although, the reported sodium concentrations in the feed sludge varied: 292 mg/L (12.7mM) in the College Station ATAD plant of Texas, and 53 mg/L (2.3 mM) in the Princeton ATAD plant of Indiana, thermophilic digestion did not result in much change in sodium concentrations in solution (Murthy et al. 2000a).
Figure 4.71. Effect of digestion and shear on concentration of Na$^{+}$ in filtrate of sludge, digested at 60°C (thermo) and 22°C (meso), 100% secondary sludge as the feed

4.12.4 Potassium (K$^{+}$)

The results of measured potassium concentrations in the feed sludge, in the filtrate of the feed sludge and digested sludge are shown in Figure 4.72 and Table 4.18. The total K$^{+}$ in the feed sludges that were collected on different dates is comparable (158 mg/L vs. 147 mg/L). The distribution of K$^{+}$ in solid and liquid phases of the sludge varied. 84% of total K$^{+}$ in the feed of 01Oct30Run was in the liquid fraction (filtrate). 43% of total K$^{+}$ in the feed of 01Nov27Run was in the liquid fraction (filtrate). Thermophilic digestion resulted in a slight increase in K$^{+}$ concentrations in the filtrate of digested sludge. The K$^{+}$ concentration in the filtrate of mesophilically digested sludge increased from 63 mg/L in the feed sludge to 109 mg/L in the 2 days digested sludge, and remained relatively unchanged during the additional 8 days of digestion (104 mg/L in the 10 day digested sludge). At either thermophilic or mesophilic digestion condition, K$^{+}$ concentration in the sheared sludge was not much different from the concentrations in the air-mixed sludge. The effects of thermophilic digestion on potassium concentration in solution are similar to what were reported at two full-scale ATAD plants. In the College Station ATAD plant of Texas, K$^{+}$ increased from 55 mg/L (1.4 mM) in the feed to 94 mg/L (2.4 mM) following approximately 2.3 days of thermophilic digestion, and remained
relatively constant during the remaining 4.6 days of thermophilic digestion (Murthy et al. 2000a).

Figure 4.72. Effect of digestion and shear on concentration of K\(^+\) in filtrate of sludge, digested at 60°C (thermo) and 22°C (meso), 100% secondary sludge as the feed

4.12.5 Calcium (Ca\(^{2+}\))

The results of measured calcium concentrations in the feed sludge, in the filtrate of the feed sludge and digested sludge are shown in Figure 4.73 and Table 4.18. The feed sludges that were collected on different dates had comparable total Ca\(^{2+}\) concentrations (282 mg/L vs. 230 mg/L). The Ca\(^{2+}\) in the filtrate (the liquid fraction) accounted for a small fraction of total Ca\(^{2+}\) in the feed sludge (12-17% of total). In the air-mixed sludge, neither thermophilic nor mesophilic digestion result in an increase in Ca\(^{2+}\) concentrations in the filtrate of digested sludge. Instead, the Ca\(^{2+}\) in the filtrate of thermophilically digested sludge decreased from 47 mg/L (in the feed) to 10 mg/L (in the 10 days digested sludge). In the sheared and thermophilically digested sludge, the Ca\(^{2+}\) in the filtrate of digested sludge increased substantially during the first 2 days of digestion at 60°C (from 47 mg/L in the feed to 96 mg/L in the 2 days of digested sludge). Continued digestion did not result in a further increase in Ca\(^{2+}\) concentrations. In mesophilically
digested sludge, the Ca\(^{2+}\) concentrations in the filtrate of the sheared and the air-mixed sludge are approximately the same, during the first 5 days of digestion. After 10 days of digestion, the Ca\(^{2+}\) concentration in the filtrate of the sheared sludge (63 mg/L) was higher than that in the filtrate of the air-mixed sludge (21 mg/L). In the College Station ATAD plant of Texas, calcium concentrations in solution increased from 52 mg/L (1.3 mM) to 96 mg/L (2.4 mM) following 2.3 days of digestion, then decreased to 48 mg/L (1.2 mM) in the remaining 4.6 days of digestion. In contrast, in the Princeton ATAD plant of Indiana, the calcium concentration was 120 mg/L (3.0 mM) in the feed, then decreased to 60 mg/L (1.5 mM), following 14.8 days of thermophilic digestion (Murthy et al. 2000a).

![Graph showing the effect of digestion and shear on concentration of Ca\(^{2+}\) in filtrate of sludge, digested at 60°C (thermo) and 22°C (meso), 100% secondary sludge as the feed]

Figure 4.73. Effect of digestion and shear on concentration of Ca\(^{2+}\) in filtrate of sludge, digested at 60°C (thermo) and 22°C (meso), 100% secondary sludge as the feed

4.12.6 Magnesium (Mg\(^{2+}\))

The results of measured magnesium concentrations in the feed sludge, in the filtrate of the feed sludge and the digested sludge are shown in Figure 4.74 and Table 4.18. The total Mg concentration in the feed sludge that was collected on different dates was comparable (68 mg/L vs. 81 mg/L). The Mg in the filtrate (liquid fraction) of undigested filtrate varied (50% vs. 23%).
The thermophilic digestion of the air-mixed sludge resulted in a slight decrease in filtrate Mg concentrations, as the digestion progressed (changed from 34 mg/L in the feed sludge to 16 mg/L in 10 days digested sludge). Mesophilic digestion of the air-mixed sludge did not result in many changes in Mg concentrations. When the sludge was thermophilically digested, the Mg\(^{2+}\) concentrations in the filtrate of the sheared sludge were higher than that in the filtrate of the air-mixed sludge. Mg\(^{2+}\) in the filtrate of mesophilically digested and the sheared sludge progressively increased throughout the 10 days of digestion (19 mg/L in the feed, 56 mg/L in the 2 days digested sludge, and 85 mg/L in the 10 days digested sludge). The measured magnesium concentration in the feed sludge of full-scale ATAD plant varied: 12 mg/L (0.5 mM) in the College Station ATAD plant of Texas, and 99.6 mg/L (4.1 mM) in the Princeton ATAD plant of Indiana. In the College Station ATAD plant, thermophilic digestion resulted in an initial increase of magnesium to 21.9 mg/L (0.9 mM), following 2.3 days of digestion, then a decrease to 7.3 mg/L (0.3 mM), following 6.9 days of digestion. But in the Princeton ATAD plant, magnesium decreased to 2.4 mg/L (0.1 mM), following 14.8 days of thermophilic digestion (Murthy et al. 2000a).

![Figure 4.74. Effect of digestion and shear on concentration of Mg\(^{2+}\) in filtrate of sludge, digested at 60°C (thermo) and 22°C (meso), 100% secondary sludge as the feed](image-url)
4.12.7 Digestion Effect on Cations Distribution in Digested Sludge

Digestion caused changes in the distribution of cations among liquid and solid phases of the sludge. Experimental results from this study revealed that, following 3 hours of digestion, the ratios of monovalent (Na plus K) to divalent cations (Ca and Mg) were less than 1.5 in the liquid phase of digested sludge (regardless of air-mixed or high-sheared). The mono to divalent ratios (M/D ratio) was less than 0.5 in the solid phase of digested sludge. Murthy et al. (2000a, 2000b) suggested that increased ratios of monovalent cations to divalent cations (M/D), due to thermophilic digestion, contributed to increased polymer demand for conditioning thermophilically digested sludge. Murthy’s work was based on samples taken from a full-scale continuously operated ATAD plant, and included NH₄⁺-N concentrations as part of monovalent cations. Digestion resulted in an increase in ammonia concentration, which subsequently contributed to an increase in M/D ratios. However, the substantial increase in SCST occurred before the changes in M/D ratios occurred. Therefore, the deterioration in dewaterability was unlikely due to the changes in M/D ratios. The Ca and Mg concentrations did not change much due to thermophilic digestion. The increase in Ca and Mg concentrations was likely due to the destruction of sludge aggregates.

4.13 Viscosity of Digested Sludge and Filtrate of Sludge

The viscosities of representative samples from 01Oct30Run were measured to provide additional information for the description the characteristics of thermophilically digested sludge. The feed sludge in this Run contained 100% secondary sludge. The thermophilic digestion temperature was 60°C. Selected samples included both the air-mixed and high-sheared sludge, and filtrate of the sludge. Shear rates of up to 500/sec. were applied to the tested samples. Typical results of measured viscosity are shown in Figure 4.75 (a) and (b). Results of viscosity measurements of other samples (both of sludge and filtrate) had similar profiles to the one in Figure 4.75.
(a) Measured viscosity at various shear rates, digested at 60°C for 1 h, 100% secondary sludge as the feed.

(b) Viscosity and shear relationship, digested at 60°C for 1 h, 100% secondary sludge as the feed.

Figure 4.75. Typical result of viscosity measurement (measured at 25°C)
The results revealed that the sludge (both of undigested and digested) and the filtrate are non-Newtonian. When the shear rate that was applied to the sludge sample increased from zero to 100 sec\(^{-1}\) within 0.5 minute, the viscosities of the digested sludge decreased rapidly from approximate 260 mPaS (cP) to 80 mPaS (cP). Such a reduction in viscosity indicated "shear thinning" properties of the feed sludge. When the shear rate remained at 100 sec\(^{-1}\), the measured viscosity remained relatively constant at approximate 80 mPaS (cP). The sludge appeared not to possess the thixotropy property. When the shear rate increased to 500 sec\(^{-1}\), the viscosity was further reduced to approximate 20 mPaS (cP). When the shear rate remained at 500 sec\(^{-1}\) for 0.5 min, no thixotropy characteristic was observed. When the shear rate was reduced from 500 sec\(^{-1}\) to 100 sec\(^{-1}\), the viscosity recovered to approximately 70 mPaS (cP). This value was 13% lower than the measured viscosity when the shear rate first reached to 100 sec\(^{-1}\). When the shear rate was reduced to zero, the viscosity was approximate 200 mPaS (cP). This value was 23% lower than the viscosity that was measured prior to the test cycle.

The sludge exhibited pseudo-plastic flow behaviour. The relationship between the viscosity and the shear rate appeared to follow an Ostwald model, which is described in Equation 4.2 (HAAKE 1989).

\[
(4.2) \quad Y = a * X^b.
\]

Where:

- \(Y\): the measured viscosity (mPaS, or cP)
- \(X\): the shear rate that is applied to the tested sample (s\(^{-1}\))
- \(a, b\): Ostwald coefficients.

The coefficient "a" provides information on the initial yield to applied shear of the tested sample. The coefficient "b" provides information on the adhesion characteristics of the tested sample.

- \(b<1\) pseudoplastic flow behaviour;
- \(b=1\) Newtonian flow behaviour;
- \(b>1\) dilatant flow behaviour.

Figure 4.75 (b) showed a strong \((r^2=0.91)\) logarithmic correlation between the viscosity and the shear rate. Such a relationship agreed with what Moeller and Torres (1997) reported on the relationship between viscosity and shear rate of aerobically digested sludge. The yield coefficient "a" (equal to 2,998.5 for the digested sludge) provides information on the initial yield value of the tested sludge to the applied shears (where \(X\) equals to 1). It indicates the
power required to break up the large-scale network of sludge (Dentel 1997). Higher values in "a" imply that it is more difficult to disrupt floc structure of the tested sludge. The power coefficient "b" (also named as adhesion coefficient, equal to -0.82 for the feed sludge) provides information on required power to overcome viscous resistance, and to continuously break bonds among various groups of particles in sludge (Dentel 1997); it also indicates the strength of affinity among particles in tested sample. A lower "b" implies lower affinities among particles of tested samples.

The yield coefficient "a" of measured viscosities of the sludge and the filtrate of the sludge (digested at 60°C for 10 days, viscosity measurement temperature was 25°C) is shown in Figure 4.76 (a) and (b). The shear that was applied to the digested sludge affected the yield coefficient of the digested sludge. For the sheared sludge, thermophilic digestion resulted in a decrease in the yield coefficients (rapidly decreased from 3,672 in the feed sludge to 1,654 in 18 hour digested sludge, then gradually decreased to 241 in 10 day digested sludge). In contrast, for the air-mixed sludge, thermophilic digestion resulted in a decrease in yield coefficients in the first 18 hours of digestion (decreased to 1,669 in 18 hour digested sludge), then an increase in yield coefficient to 2,924 in 10 day digested sludge. The yield coefficient of the sheared sludge was significantly lower than that of the air-mixed sludge. The yield coefficient of the filtrate was substantially lower than the yield coefficient of the sludge. Digestion resulted in a rapid decrease in the yield coefficient of the filtrate within 3 hours of digestion. Further digestion up to 10 days did not result in much change in yield coefficients of filtrate (from either the sheared or the air-mixed sludge).
(a) Measured on sludge digested at 60°C. Viscosity was measured at 25°C.

(b) Measured on filtrate of the sludge digested at 60°C. Viscosity was measured at 25°C.

Figure 4.76. Effect of digestion and mixing induced shear on initial yield coefficients
The adhesion coefficients from the tested sludge and the filtrate of the tested sludge are shown in Figure 4.77 (a) and (b). For the sheared sludge, thermophilic digestion resulted in a gradual decrease in adhesion coefficient (from 0.83 in the feed sludge to 0.60 in 10 day digested sludge). For the air-mixed sludge, the thermophilic digestion resulted in a decrease in adhesion coefficients in the first 18 hours of digestion (0.73 in 18 hour digested sludge), then an increase during the next 9 days of digestion (0.89 in 10 day digested sludge). The adhesion coefficients of the sheared sludge were consistently lower than that of the air-mixed sludge during the 10 days of digestion. For digested sludge, the changes in adhesion coefficients due to digestion followed the same trend as changes in yield coefficients. For filtrates of digested sludge, the adhesion coefficients decreased within 3 hour of digestion, and remained relatively constant during the additional time of 7 days of digestion.

Forster (1981) reported that the viscosity of activated sludge was affected by the pH of the sample; lower pH resulted in a decrease in viscosity. Viscosity also positively correlates to CST. Higher viscosity in liquid phase of a sludge sample resulted in a higher CST of the sample (Vesilind 1988).
(a) Measured on sludge digested at 60°C. Viscosity was measured at 25°C.

(b) Measured on filtrate of the sludge digested at 60°C. Viscosity was measured at 25°C.

Figure 4.77. Effect of digestion and mixing induced shear on adhesion coefficients
4.14 Effect of Polymer Conditioning on Dewaterability

4.14.1 Polymer Conditioning of Digested Sludge, Various Feed

A cationic polymer (Percol 757) was applied to digested sludge samples that were taken from 00Aug15Run and 00Nov28Run. Feed sludge included 100% secondary, 40% secondary, and 100% primary sludge. The resulted, as shown in Figure 4.78 (a) and (b), revealed the followings:

1. For the thermophilically digested sludge, when the feed contained higher proportion of secondary sludge, a higher dosage of polymer was needed to achieve the same amount of SCST reduction. Polymer conditioning resulted in a more rapid SCST reduction in digested sludge, when the primary sludge was used as the feed.

2. For the mesophilically digested sludge, the rate of the SCST reduction, following the polymer conditioning, appeared to be approximately the same, regardless of feed sludge compositions. When the 100% secondary sludge was used as the feed, the SCST reduction did not occur until an initial dosage of approximately 3 kg/dt polymer was applied. Trim and McGlashan (1985) reported that approximately 14.3 kg polymer/dry tonne solids reduced the CST of ATAD digested sludge to 15 seconds.

4.14.2 Comparison between Thermophilic and Mesophilic Digestion

Two samples were taken from 01Feb02Run to compare the effect of polymer conditioning on the SCST of digested sludge; one was thermophilically digested at 60°C, and the second was mesophilically digested at room temperature (22°C). Both samples were digested for 24 hours, and used 100% secondary sludge as the feed. The results (from 01Feb02Run), shown in Figure 4.79, revealed that the polymer dosage to achieve comparable SCST reduction in thermophilically (at 60°C for 1 d) digested sludge was higher than that in mesophilically (at 22°C for 1 d) digested sludge. SCST of mesophilically digested sludge decreased immediately, following the addition of polymers. However, an initial dosage of polymer needed to be applied to the thermophilically digested sludge, before the SCST was substantially reduced.
(a) Digested at 60°C, 100%Sec and 40%Sec (00Nov28Run, 6 d), 100%Prim (00Aug15Run, 11 d)

(b) Digested at 22°C, 100%Sec and 40%Sec (00Nov28Run, 6 d), 100%Prim (00Aug15Run, 11 d)

Figure 4.78. Effect of polymer conditioning on SCST, various compositions in feed sludge
4.14.3 Comparison between Two Different Cationic Polymers

Two types of cationic polymers were compared for their effectiveness to reduce SCST of both of thermophilically and mesophilically digested sludge: Percol 757 was a long chain, medium charged polymer; Percol 368 is a short chain, high density polymer. The results are shown in Figure 4.80 (a) and (b). For both thermophilically and mesophilically digested sludge, Percol 757 appears a more effective polymer in reducing the SCST of thermophilically digested sludge. When the polymer dosage in thermophilically digested sludge exceeded 8 kg/dt, Percol 757 resulted in more rapid decrease in the SCST than Percol 368. Figure 4.80 (a) revealed that, for both types of polymers, an initial polymer dosage needed to be satisfied in the thermophilically digested sludge, before the SCST of the sludge can be reduced substantially.
(a) Digested at 60°C for 22 d (00Aug15Run)

(b) Digested at ~22°C for 22 d (00Aug15Run)

Figure 4.80. Comparing conditioning effects of two types polymers on the SCST
The need to satisfy an initial demand in sludge of polymers appeared to relate the higher biopolymers and smaller particles sized in thermophilically digested sludge. As extracellular biopolymers acts as a polymer sink, the polymer increased with an increased concentration of biopolymers.

4.15 General Overview of Research Findings

Digestion, either thermophilically or mesophilically, resulted in a reduction of total solids and total volatile solids. TVS reductions indicated bacterial activities in the sludge. However, solids reduction did not occur, until after the rapid increase in SCST of thermophilically digested sludge occurred. Therefore, the deterioration in dewaterability of thermophilically digested sludge was not caused by the reduction in solids.

Digestion also resulted in changes in pH, conductivity, and ammonia concentrations (NH$_4^+$-N). However, the changes in SCST did not correlate to changes in these three factors. The experiment work of an ammonia spike in undigested sludge (Section 4.6) provided additional evidence that ammonia concentration present in digested sludge was not a cause in affecting SCST. The changes in phosphate correlated well with changes in SCST. Digestion resulted in cell lysis and degradation of organics, therefore resulting in a release of phosphate, which originally was part of the cells. However, the changes in phosphate concentration appeared not to be a cause of the changes in SCST, as evidenced by experiment work presented in Section 4.7. The changes in SCST correlated well with changes in concentrations of extracellular biopolymers, including both of proteins and polysaccharides. When the rapid increase in SCST occurred, the only parameter that also showed a rapid increase was the extracellular biopolymers.

From measured concentrations of bound and soluble extracellular proteins, it was apparent that thermophilic digestion of either 100% secondary sludge, or 100% primary sludge, resulted in an increase in total proteins, indicating new extracellular proteins that were produced as a result of the digestion process. When the 100% secondary sludge was used as the feed, the decrease in bound protein and a simultaneous increase in soluble protein indicated that, additional to the cell lysis, thermophilic digestion also resulted in a detachment of bound protein from the cell surface. In contrast, when 100% primary sludge was used as the feed, bound proteins showed
little changes over the period of 10 days digestion, instead total proteins and soluble protein increased. This observation suggests that it was the cell lysis, not the detachment of bound proteins from the surface of cells, resulted in the increase in soluble extracellular protein. Similarly, it appeared that extracellular polysaccharides from the thermophilically digested secondary sludge originated from cell lysis rather than from detachment from cell surface. In case of polysaccharides, bound polysaccharides decreased, indicating the degradation of polysaccharides.

Digestion did not result in much change in floc charges. However, the shears applied to the digested sludge caused more negative floc charges. The floc size appeared to be an important factor affecting SCST. Thermophilic digestion resulted in an immediate decrease in floc size. Thermophilic digested sludge had smaller particle sizes than mesophilically digested sludge. High shear, applied to the sludge during the digestion, resulted in higher surface area and smaller particle sizes. Water flows through sludge cakes would be plugged more easily by smaller particles, therefore, it would more difficult to dewater sludge having small particles.
5.0 Summary, Conclusions, and Recommendations

The research work presented in this dissertation investigated how feed sludge composition, digestion temperature, digestion time, and mixing induced shear affected the dewatering properties of thermophilic aerobically digested sludge. The characteristics of digested sludge were studied and were correlated to the dewatering properties of the corresponding samples. The mechanisms of thermophilic digestion effects on dewatering properties of digested sludge were explored. Findings from this research program are summarised and conclusions are offered in Section 5.1. Future work to advance the knowledge in this subject area are recommended in Section 5.2

5.1 Summary and Conclusions

5.1.1 Factors Influencing Dewaterability of Thermophilically Digested Sludge

1. Feed sludge composition is an important factor affecting dewaterability (measured as SCST) of digested sludge. Regardless of how the sludge was digested, the measured SCST exponentially correlated to the weight proportion of the secondary sludge contained in the feed. A higher proportion of the secondary sludge in the feed resulted in a higher SCST in the sludge. For the several different compositions of feed sludge that were tested, changes in dewaterability due to digestion did not correlate to the changes in pH, volatile solids, concentrations of ammonia and phosphate in the digested sludge.

Digestion did not result in significant changes in floc charge (measured as Zeta potential), but resulted in a reduction in floc size (measured as surface mean diameter). The dewaterability of the digested sludge correlated to concentrations of soluble extracellular proteins and polysaccharides in the digested sludge.

2. Digestion temperature has a significant effect on dewaterability (measured as SCST) of the digested sludge. When the sludge, using 100% secondary sludge as the feed, was digested at 55°C or higher temperatures, digestion resulted in immediate and significant increases in SCST, indicating a rapid deterioration in dewaterability of the digested sludge. When the same type of sludge was digested at 40°C or 50°C, digestion also resulted in a larger increase in SCST, but the rate of increase in SCST was lower than the rate when the sludge was digested at 55°C or higher temperatures. Following the initial surge in SCST, continued digestion at 55°C or higher temperatures for more than 1 d
resulted in a reduction in SCST; while continued digestion at 40°C or 50°C did not result in much change in SCST. Mesophilic digestion resulted in a progressive increase in SCST during the 10-12 d digestion. Thermophilic digestion did not result in much change in floc charge, but an immediate reduction in floc size. Digestion at all temperatures resulted in a reduction in solids, changes in pH, conductivity, concentrations of ammonia and phosphate, and phosphorus distribution among various phases of the sludge. However, the deterioration in dewaterability (an increase in SCST) of the digested sludge did not correlate to changes in these parameters. The effect of temperature on SCST correlated to concentrations of soluble extracellular proteins and polysaccharides in the digested sludge.

3. The effect of thermophilic digestion on dewaterability occurred within a short time (1 h) after the temperature of the digested sludge reached 60°C, indicating that the deterioration in dewaterability, due to thermophilic digestion, was a physical-chemical phenomenon, not a microbiological phenomenon. The thermophilic temperature caused the lysis of cells, resulted in the spilling and release of intracellular biopolymers to become extracellular biopolymers. Following the initial surge in SCST, continued digestion did not in much change in SCST, indicating that the opportunity to optimize digestion time for improved dewaterability was not available.

4. Mechanical shear, applied to the digested sludge, had a significant effect on dewaterability of both thermophilically and mesophilically digested sludge. When compared to the effect of digestion temperature, shear had a more significant effect on dewaterability of the digested sludge. Shear effects on dewaterability did not correlate to changes in pH, conductivity, concentrations of ammonia and phosphate. Excessive shear did not result in substantially higher concentrations of soluble proteins and polysaccharides in the digested sludge, instead, a decrease in floc charge and floc size, which contributed to deterioration in dewaterability of the digested sludge.

5.1.2 Characterizing Dewatering Properties of Digested Sludge

1. The substance affecting dewaterability of thermophilically digested sludge was mainly associated with the liquid phase of the digested sludge.
2. Neither ammonia concentration nor phosphate concentration was a significant factor in affecting the dewaterability of sludge. Lowering the pH of the digested sludge was effective in improving the dewaterability of digested sludge (a reduction in SCST).

3. The soluble extracellular proteins had small sizes, with 86% of such proteins less than 7,000 Daltons. These proteins could not be effectively stained by Coomassie Brilliant Blue dye, and were not affected by boiling treatment. Protease treatment, using three selected enzymes resulted in a 13-19% reduction in SCST, suggesting that proteins played a role in affecting dewaterability, although the changes in protein concentrations were small. Enzyme treatment on soluble polysaccharides did not result in improvements in dewaterability.

4. Digestion and mixing induced shear resulted in changes in distribution of cations among the solid and liquid phases of the digested sludge. However, dewaterability was not associated with changes in the ratios of monovalent to divalent cations.

5. Thermophilic digestion and mechanical shear resulted in a reduction in initial yield and adhesion coefficients of the digested sludge. Digested sludge showed non-Newtonian characteristics, in particular, the shear-thinning property. For thermophilically digested sludge, an initial polymer demand needed to be satisfied first, before the SCST could be substantially reduced by polymer conditioning.
5.2 Recommendations for Future Work

It is recognised that continued efforts in this subject area are needed to expand the current knowledge and understanding of dewatering properties of thermophilically digested sludge. The followings are recommended:

1. To investigate effective methods to destroy the matrix of soluble extracellular proteins and polysaccharides, therefore, to assist the release of water from the matrix of the digested sludge.

2. To identify the composition of the soluble extracellular proteins (e.g. to isolate and to conduct an amino acid analysis) and soluble polysaccharides (e.g. to isolate and to characterize the carbohydrates) in the matrix of the digested sludge and to investigate the interrelation between these two biopolymers; to search for more in-depth information of the role of biopolymers in affecting dewaterability of the digested sludge.

3. To relate measured SCST with the polymer dosages that will be needed to achieve readily dewaterable products, therefore, to predict polymer dosage from the measured SCST.

4. To carry out pilot or full-scale experimental work to extend the potential for applying findings from this laboratory work in engineering application.


References


USEPA (1990) *Autothermal Thermophilic Aerobic Digestion of Municipal Wastewater Sludges*. U.S. Environmental Protection Agency. EPA/625/10-90/007 September, Cincinnati, OH.


Appendix A. Experimental Procedures and Details
<table>
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<th>Location of ATAD Plants</th>
<th>Wastewater Treatment Processes</th>
<th>Feed Sludge Type</th>
<th>Polymer Dose (kg/dt TS) /Type</th>
<th>Cake Dryness (Total Solids)</th>
<th>Dewatering Equipment</th>
</tr>
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<tbody>
<tr>
<td>Whistler, BC, Canada</td>
<td>TF-SC\textsuperscript{a}. Chemical P removal</td>
<td>P\textsuperscript{b} and WAS\textsuperscript{c}</td>
<td>12 to 15 / Percol 757, 368</td>
<td>28-31%</td>
<td>Belt filter press</td>
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<td>Parksville, BC, Canada</td>
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<td>8 to 12 / Percol 757, 368</td>
<td>28%</td>
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<td>Salmon Arm, BC, Canada</td>
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<td>33 to 44 / Percol 778FS25</td>
<td>30-40%</td>
<td>Centrifuge (Pieralisi FP600)</td>
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<tr>
<td>Avon-Vail, CO, USA</td>
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<td>Princeton, IN, USA</td>
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<td>20-25%</td>
<td>Gravity belt dewatering.</td>
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<tr>
<td>Process Type</td>
<td>WAS Type</td>
<td>Solids (%)</td>
<td>WAS Treatment</td>
<td>Notes</td>
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<tr>
<td>Aerobically digested (conventional)</td>
<td>P and WAS</td>
<td>2-8</td>
<td>18% (typical)</td>
<td>Belt filter press</td>
<td></td>
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<tr>
<td>Anaerobically digested (conventional)</td>
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<td>22% (typical)</td>
<td>Belt filter press</td>
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<tr>
<td>Aerobically digested (conventional)</td>
<td>WAS</td>
<td>1.5-3</td>
<td>8-10%</td>
<td>Centrifuge</td>
<td></td>
</tr>
<tr>
<td>Anaerobically digested (conventional)</td>
<td>P and WAS</td>
<td>2-4</td>
<td>17-21%</td>
<td>Centrifuge</td>
<td></td>
</tr>
</tbody>
</table>

a TF-SC = trickling filter-solid contact process.
b P = primary sludge.
c WAS = waste activated sludge (secondary sludge).
d FGR-SGR = fixed growth reactor-suspended growth reactor.
e CAD = conventional (mesophilic) aerobic digestion.

Table A.2. Conditioning chemicals

<table>
<thead>
<tr>
<th></th>
<th>Percol 757&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Percol 368&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Percol 778FS25&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Ciba Inc. (formerly Allied Colloid Inc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location used</td>
<td>Whistler</td>
<td>Whistler</td>
<td>Salmon Arm</td>
</tr>
<tr>
<td>Charge type</td>
<td>Cationic flocculant</td>
<td>Cationic coagulant</td>
<td>Cationic flocculant</td>
</tr>
<tr>
<td>Backbone monomer</td>
<td>Quaternary acrylate/acrylamide copolymers. Backbone is acrylamide (ACM)</td>
<td>Homopolymer of diallyl-dimethylammonium chloride (DADMAC)</td>
<td>Quaternary acrylate/acrylamide copolymers. Backbone is acrylamide (ACM)</td>
</tr>
<tr>
<td>Charge producing group</td>
<td>The quat group dissociates to produce a (+) charge</td>
<td>The chloride dissociates from the ammonium group to produce a (+) charge</td>
<td>The quat group dissociates to produce a (+) charge</td>
</tr>
<tr>
<td>Charge density (% monomer that produce a charge)</td>
<td>60% weight</td>
<td>100% weight</td>
<td>80% weight</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>10-15 million</td>
<td>0.5-1 million</td>
<td>10-15 million</td>
</tr>
<tr>
<td>Form and % activity</td>
<td>Powder form. May have 3-12% moisture. Approx. 90% active.</td>
<td>Powder form. May have 3-12% moisture. Approx. 90% active.</td>
<td>A dispersion of polymer in mineral oil. Approx. 50% active.</td>
</tr>
</tbody>
</table>

<sup>a</sup>These polymers were used in the experimental work of this research

<sup>b</sup>This polymer was used in the full-scale ATAD plant in Salmon Arm, BC.

The information in Table A.2 was provided by Ciba Inc. in 1999.
Table A.3. Details of sludge collection for the experimental work

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Lab runs ID</th>
<th>Duration of the run (d)</th>
<th>Date of sludge collection(^a)</th>
<th>Day</th>
<th>In storage (d)</th>
<th>Air flow (v/v-hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>00Aug15Run</td>
<td>20</td>
<td>Aug. 8, 2000</td>
<td>Tues.</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>00Nov28Run</td>
<td>30</td>
<td>Nov. 17, 2000</td>
<td>Fri.</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>01Feb02Run(^b)</td>
<td>2</td>
<td>Jan. 31, 2001</td>
<td>Wednes.</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>01Apr20Run(^b)</td>
<td>4 hr.</td>
<td>Apr. 19, 2001</td>
<td>Thurs.</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>01June15Run(^b)</td>
<td>9</td>
<td>June 13, 2001</td>
<td>Wednes.</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>01July14Run(^b)</td>
<td>12</td>
<td>July 12, 2001</td>
<td>Thurs.</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>01July31Run(^b)</td>
<td>10</td>
<td>July 27, 2001</td>
<td>Fri.</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>01Aug23Run(^b)</td>
<td>1</td>
<td>Aug. 21, 2001</td>
<td>Tues.</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>01Oct30Run</td>
<td>10</td>
<td>Oct. 29, 2001</td>
<td>Mon.</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>01Nov27Run</td>
<td>10</td>
<td>Nov. 26, 2001</td>
<td>Mon.</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>11</td>
<td>02Feb17Run</td>
<td>6</td>
<td>Feb. 14, 2002</td>
<td>Thurs.</td>
<td>3</td>
<td>11</td>
</tr>
</tbody>
</table>

\(^a\) All sludges used in the experimental work were collected at GVRD Lulu Island Wastewater Treatment Plant, which is located in Richmond, BC (approximately 40 min. drive from UBC campus). Collection was carried out mostly between 10AM to 2PM. Collected sludge was placed in the cold room (4°C), following the return to the UBC Environmental Engineering Laboratory on the same date.

\(^b\) Duplicate thermophilic digesters, having identical experimental conditions, were operated in these runs.
Table A.4. Procedures of the Lowry assay (to measure concentrations of protein)

Reference: adapted from Keleti and Lederer (1974)
Standard: bovine serum albumin (BSA) 0, 50, 100, 200, 300, and 400 mg/L
Reagents:
   A. 2 g NaOH, 10 g Na₂CO₃, 0.1 g Na-K tartrate per 500 mL distilled water
   B. 0.5 g CuSO₄·5H₂O per 100 mL distilled water
   C. Mix 10 mL solution A + 0.2 mL solution B prior to use (or same ratio for a larger volume)
   D. Mix 1 part of Folin phenol (2 N) with 1 part of distilled water prior to use
Method:
1. Estimate the protein concentrations in the samples. Dilute the samples, as needed, to make the protein concentrations in the diluted samples not more than 400 mg/L.
2. Place 0.6 mL of sample into a COD vial (approximate 9 mL capacity)
3. Add 6 mL reagent C. shake to mix
4. Let stand for 30 minutes
5. Add 0.6 mL reagent D. shake to mix
6. Let stand for a minimum of 20 minutes, but not longer than 2 hours, at room temperature (approximately 22°C)
7. Measure absorbance of all standard and samples at 750 nm wavelength using a spectrophotometer
Table A.5. Procedures of the Dubois assay (to measure concentrations of polysaccharide)

Reference: adapted from Keleti and Lederer (1974)
Standard: dextran (glucose) 0, 10, 20, 40, 60, and 80 mg/L
Reagents: A. 5.5 mL liquid phenol (90%) added to 94.5 mL distilled water (95% final concentration)

Method:
1. Estimate the polysaccharide concentrations in the samples. Dilute the samples, as needed, to make the polysaccharide concentrations in the diluted samples not more than 80 mg/L
2. Place 1 mL of sample into a COD vial (approximate 9 mL capacity)
3. Add 1 mL reagent A. shake to mix
4. Rapidly and carefully add 5 ml concentrated H₂SO₄, shake (carefully to avoid burning yourself with the acid)
5. Let stand at room temperature (approximately 22°C) for 10 minutes
6. Let stand in a 30°C water bath for 20 minutes
7. Measure absorbance of all standard and samples at 490 nm wavelength using a spectrophotometer (the colour is stable at room temperature of approximately 22°C for 2-3 hours)
Table A.6. Coefficients of Lowry assay and Dubois assay, all runs

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Lab runs ID</th>
<th>Lowry coefficient</th>
<th>Dubois coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>00Aug15Run</td>
<td>0.00241</td>
<td>0.0114</td>
</tr>
<tr>
<td>2</td>
<td>00Nov28Run</td>
<td>0.00227</td>
<td>0.0122</td>
</tr>
<tr>
<td>3</td>
<td>01Feb02Run</td>
<td>0.00230</td>
<td>0.0117</td>
</tr>
<tr>
<td>4</td>
<td>01Apr20Run</td>
<td>0.00202</td>
<td>0.0116</td>
</tr>
<tr>
<td>5</td>
<td>01June15Run</td>
<td>0.00207</td>
<td>0.0127</td>
</tr>
<tr>
<td>6</td>
<td>01July14Run</td>
<td>0.00208</td>
<td>0.0111</td>
</tr>
<tr>
<td>7</td>
<td>01July31Run</td>
<td>0.00211</td>
<td>0.0093</td>
</tr>
<tr>
<td>8</td>
<td>01Aug23Run</td>
<td>0.00204</td>
<td>0.0110</td>
</tr>
<tr>
<td>9</td>
<td>01Oct30Run</td>
<td>0.00233</td>
<td>0.0128</td>
</tr>
<tr>
<td>10</td>
<td>01Nov27Run</td>
<td>0.00233</td>
<td>0.0128</td>
</tr>
<tr>
<td>11</td>
<td>02Feb17Run</td>
<td>0.00221</td>
<td>0.0116</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>0.00220</td>
<td>0.0118</td>
</tr>
<tr>
<td>Std. dev.</td>
<td></td>
<td>0.00014</td>
<td>0.0011</td>
</tr>
<tr>
<td>Std. dev./Average</td>
<td></td>
<td>6%</td>
<td>9%</td>
</tr>
</tbody>
</table>
Table A.7. SDS-PAGE procedures

The SDS-PAGE experimental procedures was adapted from the manuals of several laboratory courses on microbiological techniques (MICB 322 and MICB 323, 1999), offered in the Department of Microbiology and Immunology, UBC. Further details are described in Ausubel (1999).

The proteins in the standard included: myosin (200,000 Daltons), galactosidase (116,500 Daltons), phosphorylase B (92,500 Daltons), bovine serum albumin (66,200 Daltons), ovalbumin (45,000 Daltons), carbonic anhydrase (31,000 Daltons), trypsin inhibitor (21,500 Daltons), lysozyme (14,400 Daltons), aprotinin (6,500 Daltons). The bromophenol blue tracking dye is 670 Daltons.

The Coomassie brilliant blue R-250 stain reagent was prepared using 0.25% Coomassie brilliant blue R-250, 50% methanol, 10% acetic acid.

The destaining reagent was prepared using 5% methanol and 7.5% acetic acid.

Deionized water was used for all experimental procedures.
Table A.8. Procedures of sample fixation for SEM imaging.

1. Filter the sample to transport solids in suspension to the filter medium.
2. Warm up 2.5M glutaraldehyde (the fixing solution) in 0.1 M cacodylate buffer of pH 7.0 to 32°C.
3. Place samples in the fixing solution for 30 minutes.
4. Wash samples with 0.1 M cacodylate buffer 3 times for 5 minutes each.
5. Place samples in 0.1 M OsO₄ solution for 30 to 60 minutes (OsO₄ solution is extremely toxic and should be handled strictly in fumehood with gloves on).
6. Rinse 1-2 times with distilled water.
7. Dehydrate the sample with successive ethanol solutions of 30%, 50%, 70%, 85%, 95%, 100%, 100%, 100% (total 8 times). Each wash is 5 minutes in duration.
8. Critical point drying (CPD) samples with liquid CO₂ using a Balzers Union CPD 020 unit.
9. Sputter coat the sample with gold using a Nanotech SEM PrepII Sputter Coater.