The Biological Sludge Reduction by anaerobic/aerobic cycling

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ABSTRACT

An activated sludge system that incorporates a sidestream anaerobic bioreactor, called the Cannibal process, was the focus of this study. A prior study of this process (Novak et al., 2007) found that this system generated about 60% less solids than conventional activated sludge without any negative effects on the effluent quality. Although that study showed substantial solids reduction, questions remain concerning the specific mechanism(s) that account for the solids loss. In this study, the mechanisms that account for the loss of biological solids was the focus of the investigation.

The first part of this study was conducted to evaluate those effects in terms of the role of iron in the influent wastewater and feeding patterns on the performance of the Cannibal system. It was found that the Cannibal system with high iron in the influent produced less biological solids than the system receiving low iron. The data also showed that the Cannibal system operated under fast feed (high substrate pressure) produced much less solids than the system with slow feed (low substrate pressure). The high substrate pressure was achieved by feeding the influent wastewater to the Cannibal system over a short time period so that the substrate concentration would initially peak and then decline as degradation occurred. This is called “fast feed.” For low substrate pressure, the influent was added slowly so the substrate concentration remained low at all times. This is called “slow feed.” Later, an attempt to increase substrate pressure in the slow feed Cannibal system was conducted by either manipulating the aeration patterns or adding a small reactor in front of the main reactor (selector). It was found that either interrupting aeration in the aerobic reactor or providing a small aerobic reactor in front of the main reactor resulted in an increase in solids reduction.

The second part of this study was to investigate the mechanisms of floc destruction in the fast and the slow feed Cannnibal systems. It was found that higher accumulation of biopolymers (proteins and polysaccharides) occurred in the fast feed system and this was associated with a greater solids reduction in the fast than the slow feed system. In addition, more protein hydrolysis and more Fe(III)-reducing microorganism activity in the fast feed environment were found to be factors in higher solids reduction.

The last part of this study was to investigate the structure of the Cannibal sludge flocs generated under the fast and the slow feed conditions. It was found that the readily biodegradable (1 kDa.) protein is larger in the flocs from the fast feed than the slow feed Cannibal system. This resulted in higher floc destruction in the fast feed condition.

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Chapter 1. Introduction

Activated sludge is an aerobic biological wastewater treatment process that has been widely used to treat both industrial and domestic wastewater for many years. In this process, microorganisms oxidize a portion of the organic matter to carbon dioxide and water by using molecular oxygen as a terminal electron acceptor. Although it has proven to be reliable, efficient, and capable to produce high effluent quality, it also produces significant quantities of excess sludge that require additional processing and disposal. For municipal wastewater, typical amounts of excess sludge production from conventional activated sludge processes may range from 500 to 800 lbs dry weight for each million gallon of wastewater treated (Crites and Tchobanoglous, 1998). The handling and disposal of the sludge are a large fraction (40-50%) of plant operating costs. In general, the solids processing and disposal cost is approximately 315 dollars for a ton of dry sludge (Stensel et al., 2004). For industrial wastewater, sludge may not be processed on-site, but is often hauled to landfills.

Because the goal of wastewater treatment is the degradation of organic matter to carbon dioxide, the ideal process would accomplish mineralization with minimum production of excess sludge. The reduced biomass production can lower total costs for sludge thickening, digestion, dewatering, hauling, and tipping fees. Therefore, many biomass reduction technologies have been proposed and developed during the past decades. The following summary provides a brief description of those technologies in terms of their sludge reduction potential, ease of implement into existing facilities, impacts on plant

operation and effluent quality, reliability and risk associated with the process, and relative capital and operating costs.

Anaerobic Processes

Anaerobic degradation is one of the oldest technologies for stabilizing organics in wastewater. Many reactor configurations have been proposed for anaerobic biotreatment of wastewater. These include upflow anaerobic sludge blanket reactors, anaerobic biofilm reactors, staged anaerobic reactors, and membrane coupled anaerobic reactors. Research on anaerobic methods has demonstrated that this process showed the promise for treatment of moderate strength wastewater at moderate temperatures (Speece, 2008).

The mechanism of biomass reduction for anaerobic bioprocesses is different from aerobic processes in terms of electron acceptors and the products of reactions. Anaerobic processes produce volatile fatty acids and methane from fermentation and methanogenesis respectively. These compounds are highly reduced compared to the production of highly oxidized products (CO_2 and H_2O) from aerobic reactions. The energy per unit substrate available to support cell growth is less for anaerobic microorganisms (Speece, 2008). As a result, the yield is much lower in anaerobic environment. The key issue with the application of anaerobic processes to reduce biomass production is their ability to produce an effluent quality comparable to aerobic process and the treatment efficiency to meet with the discharge standards (Speece, 2008). Secondly, the effectiveness of the system will be

decreased when sulfate reducing microorganisms are present. In addition, undesirable odor can be generated from the process.

Cell Disruption Processes by Mechanical and Thermal Methods

In mechanical and thermal cell disruption processes, physical forces or heat is applied to the activated sludge in a sufficient degree to cause the bacteria cells to rupture and release intracellular components that include polysaccharide, protein, and nucleic acids (Foster, 1992). In activated sludge, cell disruption processes are typically applied to the RAS stream.

The ability to rupture cells in mechanical systems is due to the cavitation caused by the high speed rotating equipment (Foster, 1992). The cavitation causes an ultrasonic resonance which leads to a vibration that can break the cells. For ultrasonic treatment, the ultrasonic waves cause periodical compression of the medium and leading to cavitation. Cavitation creates bubbles that grow in particular size and than collapse within a few microseconds (Wadehra et al., 1999). The rapid collapse creates violent hydromechanical shear forces in the bulk liquid which can disrupt bacteria cells.

High temperature can cause both deactivation and lysis of bacteria cells with the release of cellular material which is dissolved organics. In the process of high temperature and bioaugmentation with thermophilic bacteria, the bacteria were thought to cause greater

solubilization of cell material than by temperature alone (Sakai et al., 2000). This is due to the increase in activity of proteinase enzymes.

A major issue for mechanical and thermal processes is the relationship between the energy input and degree of cell disruption. The performance of processes and energy needs may change as processes become larger. Therefore, research is needed to link plant size with energy requirements so these can be realistically applied to full scale plants.

Cell Disruption by Ozonation

In the ozonation process, a portion of the return activated sludge (RAS) is fed to an ozone reactor where ozone is used to disrupt the bacteria cells and release soluble materials for further degradation in the aeration tank. The demand of ozone is in the range of 0.2-0.4 g O_3 / g TSS destroyed (Yasui et al., 1996). A reduction in sludge mass in the range of 50 to 90% has been found from pilot and full scale wastewater treatment plants (Yasui et al., 1996). This process is shown to be feasible and reliable. However, it is only economical for cases where sludge processing and disposal costs are unusually high. For example, when sludge incineration is needed or hauling distances are in the range of hundreds of miles, this process may be a realistic choice.

Chemical Uncoupling

Chemicals can be added to biological processes to interfere with the energy capture from biological reactions that occur inside the cell. Chemical uncoupling can cause leaking of the proton gradient across the cell membrane. This proton gradient is responsible for ATP synthesis. As a result, there is less energy production. When the energy is less available for bacterial cells, there is insufficient energy for new cell growth and little sludge production occurs. A number of chemicals including dinitrophenol and chlorinated phenols have been used for chemical uncoupling. The reduction of yield in the range of 50-60% has been found from bench-scale tests (Strand et al., 1999). The major disadvantage for this process is that the uncouplers are toxic chemicals and result in the presence of toxic chemicals in the effluent. Therefore, this sludge reduction process is not practical unless this problem can be solved.

Predator Enhancement Process

The predator enhancement process is the process that uses protozoa to consume dispersed bacteria because the protozoa have a much lower yield compared to bacteria. This process requires a separate first stage in which rapid consumption of soluble substrate can occur

and lead to high growth rate of dispersed bacteria that has not developed sufficient extracellular polymeric substance (EPS) for good flocculation. Then, protozoa can consume the bacteria. The first stage bioreactor is followed by a conventional activated sludge for final treatment. This process has been successfully operated in industrial wastewater plant and an average sludge reduction of 50% has been reported (Lee et al., 1996b). The process is less successful for municipal wastewater treatment which the soluble COD is much lower than industrial wastewater. The process requires a skilled operator to monitor and manage the more complex biological system.

Extreme Solid Retention Time (SRT) Process

The extreme SRT process is an activated sludge system with a mean cell residence time greater than 50 days that can achieve significant sludge reduction by using long term endogenous respiration. The results of research have suggested that other mechanisms such as predator activity may play an important role in sludge reduction and 50-60% of sludge reduction has been reported (Gaudy et al., 1971). The higher mean cell residence time that requires a large volume of aeration tank compared to conventional activated sludge makes the process less feasible for full-scale application.

It can be seen that the idea behind those technologies is to try to produce less sludge from the treatment process. However, most of these sludge reduction technologies either add more plant operating costs or are inappropriate in practical terms.

In this research, we investigated the degradation of sludge generated from a conventional activated sludge system (CAS) by incorporating a sidestream anaerobic bioreactor into the system. This system is called the "Cannibal process". A previous study by Novak et al (2007) demonstrated that the Cannibal process generated about 60% less solids than conventional activated sludge system without any negative effects on the effluent quality. That study was conducted under a specific operation in which the return sludge was retained in an anaerobic bioreactor for 10 days and the interchange rate (the exchange of sludge between anaerobic and aerobic bioreactor) was 10% per day by mass.

Novak et al. (2007) proposed that flocs consist of two important biopolymer fractions, which are divalent cation-bound biopolymer and Fe-associated biopolymers (and perhaps Al^{3+} associated EPS). Therefore, those forms of flocs are expected to be presented in the sidestream anaerobic bioreactor. Park et al. (2006) proposed that the major mechanism for sludge degradation during anaerobic digestion was the reduction of iron with the release of iron associated organic matter, primarily protein, which is easily degraded. This mechanism is thought to apply to the Cannibal process. That is, when settled sludge is cycled to an anaerobic bioreactor, iron is reduced, and organic matter is released and solubilized. The released materials and sludge are returned to the CAS and are rapidly degraded before the released materials can be reflocculated. However, recent research in our lab has shown that up to 70 % of the solids loss that occurs in the Cannibal

process can occur in the anaerobic bioreactor. That is, while the mechanism proposed by Novak et al (2007) accounts for some of the solids loss, the solids loss across anaerobic bioreactor is still unexplained.

In this research, a laboratory study has been conducted to evaluate the Cannibal process and describe the mechanisms that are essential for the reduction of biological solid. The characteristics of sludge produced by low and high biomass yield systems were also determined. In order to gain comprehensive insight in system performance and solids reduction mechanisms, the research was divided into five different sections as shown in chapter 3 to 7.

Chapter 3 is the study of the effect of influent wastewater iron concentration to determine the role of iron on the reduction in the mass of biological sludge generated by the Cannibal process. In addition, the study of reactor operation on system performance was conducted to evaluate the role of substrate pressure on solids reduction.

Chapter 4 is a study to determine if solids reduction of the slow feed Cannibal system could be enhanced by increasing the transient substrate concentration by either shutting the air off for a time or by adding an aerobic selector.

Chapter 5 is a study to thoroughly investigate the mechanisms of solids loss across the anaerobic bioreactor in the Cannibal system. These studies included characterization of activity in the anaerobic bioreactor such as the release of protein and polysaccharide, volatile fatty acid production in the interchange reactor (VFA), gas production, and cations and anions in solution.

Chapter 6 focused on the role of microbially reducible iron, and extracellular protease activity on solids reduction in the Cannibal system.

Chapter 7 is a study to elucidate the nature of the sludge flocs generated under two different circumstances, high and low substrate pressure (fast and slow feeding). Two different extraction methods, cation exchange resin (CER) and sulfide extraction, as well as a digestibility study of the two different sludges under anaerobic and aerobic environments were utilized to examine the floc structure.

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Chapter 2. Literature Review

The Cannibal System

The Cannibal process consists of an activated sludge system integrated with a sidestream anaerobic reactor (interchange reactor) and a recycle circuit (Figure 2-1). The operation involves the interchange of a portion of sludge between the aerobic and the anaerobic zone on a daily basis. The biomass in the aerobic zone is allowed to settle before a portion of it is transferred to the anaerobic bioreactor. Then, the sludge is held in the interchange reactor for the specified detention time before it is recycled back to aerobic reactor. The hydraulic retention time (HRT) of the sidestream anaerobic bioreactor is in the range of 5-10 days and is fed by 10% of mass of settled sludge from the aeration tank. In the laboratory system, the volume of returned sludge from anaerobic interchange reactors to the main reactors is the same as the feed volume. The feed volume is varied based on the settling characteristics of the sludge. Basically, 1/10 of the settled volume is transferred to the interchange reactor. The SBR is operated at 4 cycles per day with a react time of 5 hours and a settle time of 40 minutes in each cycle. However, sludge is cycled to the interchange reactor only once per day. No wastage is provided for the Cannibal system.

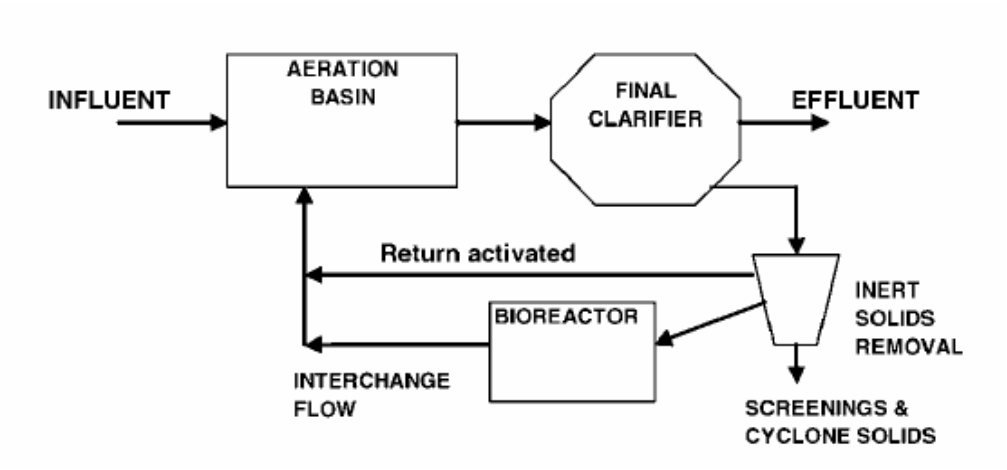

Figure 2-1. Flow scheme for the Cannibal process.

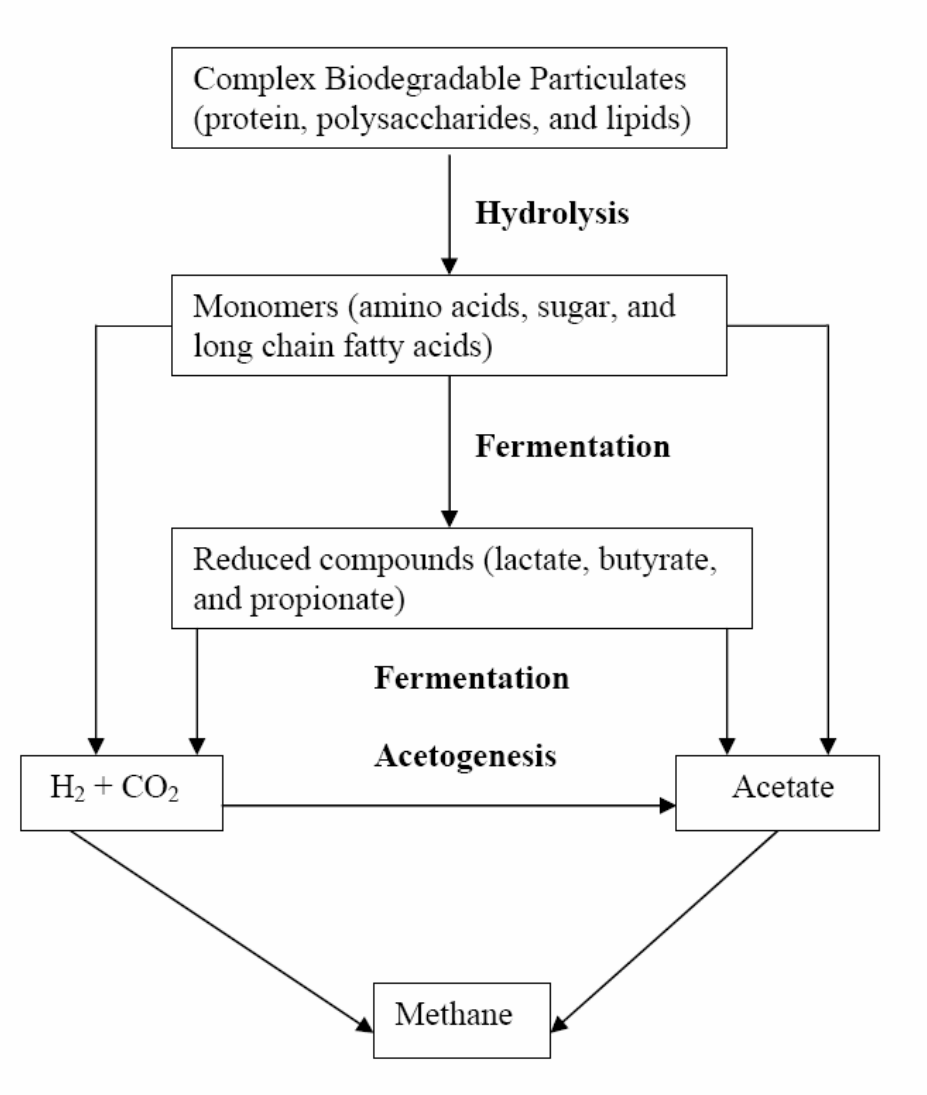

Figure 2-2. Flow scheme for the anaerobic degradation of complex waste across an anaerobic bioreactor.

It appears that the majority of solid loss in the Cannibal system is occurred in the interchange anaerobic reactor. Therefore, it is thought that the mechanism for the loss of the solids is similar to anaerobic digestion, as shown in Figure 2-2.

In anaerobic digestion, the solid loss in the system is thought to occur as shown in Figure 2-2. Similarly, this mechanism might be applicable to the COD loss in anaerobic bioreactor of the Cannibal process. That is, some macromolecules, such as proteins, polysaccharides and lipids are hydrolysed by hydrolytic bacteria. Subsequently, the monomers, amino acids, sugars and fatty acids, are fermented by fermentative bacteria into a range of fermentation products such as acetate, propionate, butyrate, lactate and hydrogen. In the presence of sulfate, sulphate-reducing bacteria consume these fermentation products. When sulphate is absent, hydrogen and acetate, the acetate that has been produced directly by fermentation or indirectly by acetogenesis, are consumed by the methanogens. However, the hydraulic retention time (HRT) and temperature in the digester are higher than those in the interchange reactor. Therefore, the solid loss across anaerobic bioreactor is only a partial portion of the total solid loss in the digester.

In order to better understand the mechanism of solid loss in the Cannibal system and its sludge characteristics, it is essential to perceive the floc structure and model.

The Structure of Activated Sludge Floc

The structural composition of activated sludge flocs can be considered as microorganisms (predominantly floc-forming and filamentous bacteria), organic matter, microbial cells, and cations and anions (Frølund et al., 1996., Higgins et al., 1997). From a biological point of view, bioaggregates such as activated sludge flocs or biofilms consist of cells and organic matter exterior to cells, which has been characterized as extracellular polymeric substances (EPS) or exocellular biopolymers. EPS can be composed of intracellular materials transported to the extracellular environment by active secretion or export, cellular components released by cell rupture, hydrolyzed or digested exocellular substances and materials adsorbed from the environment such as wastewater that has been fed to an activated sludge system ((Nielsen, P. H. et al., 1998). In general, activated sludge EPS consist of lipids, nucleic acids, humic substances, proteins, and polysaccharides (Frølund, B. et al., 1996). Previous studies often indicated that polysaccharides were the most abundant and important EPS compound but a number of recent studies have shown that proteins are more abundant than polysaccharides in activated sludge EPS (Nielsen, P. H. et al, 1996). The viscous properties of EPS in activated sludge floc are responsible for microbial colonies (aggregation) and also bind cells to other particulate materials (cohesion), leading to the flocculent characteristic of activated sludge (Wingender, J., 1999). EPS in activated sludges and biofilms also promote cell-cell recognition and

communication (quorum sensing) and protect cells from severe environmental conditions such as turbulence, dehydration, antibiotics and biocides (Wingender, J., 1999).

Lectin Floc Model

Higgins et al. (1997) suggested that extracellular proteins bridged with divalent cations (Ca^{2+} and Mg^{2+}) in activated sludge floc are lectin-like proteins. Lectins are nonenzymatic protein that bind to carbohydrates and are found in a wide variety of organisms including animals, plants and bacteria (Weis et al., 1996). For activated sludge, Higgins et al. (1997) found the presence of a single protein in the exocellular biopolymer extracted from municipal, industrial, and laboratory activated sludge samples. Amino acid analysis and sequencing results suggested that the protein was a lectin-like protein, and binding site inhibition studies demonstrated that the protein had lectin-like activity. The authors further proposed that lectin-like proteins bind to polysaccharides that are cross-linked to adjacent lectin-like proteins. Divalent cations also bridge negatively charged sites on the exocellular biopolymers. They may also be involved in the structural stability and binding activity of the lectin-like protein. Therefore, both cross-linking of polysaccharides and cation bridges act as to stabilize the biopolymer network.

The Aluminum and EPS Complexation

Aluminum is thought to be a good candidate for binding of EPS in bioaggregates due to its high charge valence and low solubility. Park et al. (2006) showed that Al is an efficient scavenger of organic matter in activated sludges and hence improves bioflocculation. The authors investigated eight different WWTPs that none of them received artificial addition of Al salts during the process, and found that higher level of Al in floc corresponding to better quality of plant effluents. However, the floc model for Al and EPS and its degradation is still unknown.

The Role of Iron in Activated Sludge Floc

The oxidation-reduction characteristic of Fe is thought to make a considerably different impact from other cations on activated sludges. It was previously shown that the reduction of Fe in activated sludge either by Fe-reducing bacterium *Shewanella alga* (Caccavo et al., 1996) or by addition of sulfide (Nielsen et al., 1998) led to a significant increase in solution turbidity, consisting of large amount of organic colloids and small amount of free cells, which caused the deterioration of sludge dewaterability. As a result, these studies suggested that Fe^{3+} plays a critical role in floc stability and it might be a more important cation than Ca^{2+} and Mg^{2+}. According to Novak et al. (2003), this iron property is particularly important for anaerobic digestion of activated sludge. They found that deflocculated

activated sludge coupled with Fe reduction undergoes further destruction, leading to volatile solids reduction in anaerobic digestion. Additionally, Park et al. (2006) demonstrated that concentration of Fe in sludge floc was an important factor in determining digestibility of sludge by anaerobic digestion.

Microbial Fe (III) reduction has been widely studied by several researchers. Caccavo et al. (1996) studied the deflocculation of activated sludge by *Shewanella alga* BrY. They found that the turbidity in the solution increased over time with approximately 86% of the total amount of Fe (III) reduction and an increase in Fe (II) concentration. They also found that the maximal Fe (III) reduction rate observed from their experiment corresponded to the Fe (III) reduction rate observed in the anaerobically digested sludge. These authors later isolated the dissimilatory Fe (III)-reducing bacteria from sludge in which Fe (III) reduction is a significant process. Consequently, they hypothesized that Fe (III)-reducing bacterial can have an effect on the sludge floc structure during anaerobic storage period.

Conditioning experiments performed for wastewater sludges also suggested that there is higher affinity of ferric iron for protein aggregation. It was demonstrated that Fe^{3+} salts selectively coagulated solution proteins produced from autothermal thermophilic aerobic digesters (Murthy et al., 2000). Similarly, Park et al. (2006) reported that optimum $FeCl_3$ dose required for conditioning of ten different waste activated sludges was determined by proteins in the sludge solution.

Some researchers have reported that there is an affinity of Fe for sulfur-containing amino acids, and might be related to odor production from anaerobically digested sludges.

According to Subramanian (2004), more Fe in sludge cakes resulted in more sulfur based odor production, suggesting that more Cystein (Cys) and Methionine (Met) was likely associated with higher Fe content in sludge.

In general, floc has different components. Some of them may be susceptible to anaerobic/aerobic cycling, especially iron (Fe). Iron is thought to play important roles for bioflocculation and solid reduction in the Cannibal process. Park et al. (2006) found that as Fe increased, solution biopolymers (proteins and polysaccharides) decreased in the effluent. However, when sludge is deficient of Fe, many organic compounds and biopolymers would remain unflocculated and wash out of the activated sludge system as indicated by deterioration of effluent quality. Those results suggest that higher Fe in activated sludge can improve bioflocculation. In addition, Murthy et al. (2000) demonstrated that Fe(III) salts selectively coagulated protein present in the sludge from autothermal thermophilic aerobic digesters. Therefore, iron is thought to selectively bind to solution protein for bioflocculation in aerobic zone of the Cannibal process.

Protein is the most abundant organic compound in activated sludge EPS (Frølund et al., 1996). Therefore, if solids are to be reduced in the Cannibal system, it is likely that degradation of this type of EPS will be significant. Novak et al. (2003) postulated that the large release of protein from floc during anaerobic digestion was due to iron reduction and subsequent loss of binding between protein and ferric Fe. In addition, Park et al. (2006) proposed that the reduction of iron was a primary mechanism for sludge under anaerobic environment, resulting in the release of iron-associated protein which is easily degradable. Consequently, this type of floc is expected to be degraded and materials released will be

readily biodegradable in anaerobic bioreactor and even in aerobic zone after the sludge is transferred to aerobic reactor.

Microbial Mn(IV) and Fe(III) reduction are important processes in anaerobic environments which organic matter, and/or hydrogen, Mn(IV), and Fe(III) are available. These processes are responsible for anaerobic oxidation of essential amounts of organic matter in aquatic sediments and submerged soil because there are abundant of iron and manganese in most of these environments. Moreover, Mn(IV), and Fe(III) are often quickly regenerated because Mn(II), and Fe(II) are relatively soluble and can diffuse to the oxic/anoxic interface where they are oxidized back to Mn(IV), and Fe(III).

Fe(III) reduction is known to be an important process for the degradation of contaminants in groundwater polluted by petroleum, landfill leachates, municipal sludge, or similar wastes because microbial activity depletes oxygen in such environments and Fe(III) is, in general, the most abundant electron acceptor for organic matter degradation. In addition, Fe(III) reduction is also an important process for bioremediation of uranium in aquifers. The metal reducers can reduce U(VI) which is soluble in oxidized form to U(IV) which is insoluble and precipitates from groundwater. Acetate can be added to subsurface because it can stimulate the growth of *Geobacteraceae* which mainly grow via the reduction of Fe(III) oxides in the subsurface. However, the *Geobacteraceae* will simultaneously reduce U(VI) to U(IV) and effectively remove it from groundwater.

Most organisms that reduce Fe(III) also reduce Mn(IV) and vice versa. Therefore, the process of Mn(IV) and Fe(III) reduction will be referred to as Fe(III) reduction unless when it is important to make a distinction between them.

Sources of Substrates (Electron Donors)

In most sediment environments, the source of substrates (electron donors) for Mn(IV) and Fe(III) reduction is the complex of organic matter deposited within the sediments. Microorganisms that are not directly involved in substantial Fe(III) reduction can break down the complex of organic matter to products which are the primary electron donors for Fe(III) reduction. There are Fe(III)-reducing microorganisms that can metabolize the monoaromatic compounds and long-chain fatty acids that are released from complex of organic matter and oxidize them to carbon dioxide by using Fe(III) as the sole electron acceptors. In hydrothermal environment where hydrogen is abundant and hydrogen can serve as the electron donor, it has been apparent that there are hyperthermophilic Fe(III) reducers that can oxidize organic carbon sourced such as acetate, monoaromatic compounds , and long-chain fatty acids (Tor et al., 2001). At the present time, Fe(III) reducing hyperthermophiles are the only organisms available in pure culture to metabolize those compounds in hot environments.

Various Forms of Fe(III) and Mn(IV) in Environments.

Fe(III) and Mn(IV) are highly insoluble at non-acidic pH. The poorly crystalline Fe(III) oxide can be reduced by Fe(III)-reducing microorganisms while the reduction of highly crystalline Fe(III) oxide can be promoted under highly artificial conditions. It has been suggested that microorganisms in rich media can produce reductases that are not synthesized under low nutrient conditions found in many environments. Moreover, the addition of high concentrations of organic acids such as lactate which chelate and make Fe(III) soluble may artificially promote Fe(III) oxide reduction. When conditions that more closely found in sediment environments are performed, the crystalline Fe(III) oxide are not reduced. One reason is that the rate and extent of Fe(III) reduction is correlated to the availability of poorly crystalline Fe(III) oxide. Another possible reason is that the reduction of crystalline Fe(III) is thermodynamically unfavorable at the concentration of acetate and hydrogen found in natural environments.

Soluble Fe(III) that is chelated with organic ligands has been detected in some of anoxic sediment, submerged soils, and in groundwater. The typically low concentration of Fe(III) ,5-50 μM, with the oxidation of electron donors for Fe(III) reduction, such as acetate and hydrogen, available at the concentration of 1 μM and 1nM respectively can potentially yield enough energy to support cell growth (Nevin and Lovley., 2002b). However, this

soluble form of Fe(III) could not be detected in poor organic subsurface environment indicating that this form is not universally available to Fe(III) reducers.

The addition of synthetic Fe(III) chelators can accelerate the metabolism of Fe(III)- reducing microorganisms. The chelators increase the concentration of dissolved Fe(III) which makes more available for microbial reduction. In addition, chelated Fe(III) has a higher redox potential which make Fe(III) reduction more thermodynamically favorable. Therefore, adding chelators is a potential strategy to accelerate Fe(III) reduction for the degradation of organic contaminants in anoxic subsurface environments.

Humic substance is an abundant form of organic matter in many soils and sediment. It may also enhance the reduction of insoluble Fe(III) oxides. Quinone moieties in humic substance can serve as electron acceptors in the respiration of Fe(III)-reducing microorganisms and once reduced to the reduced form, the humics can abiotically transfer electron to Fe(III) oxides, producing Fe(II) form and regenerating the oxidized form of the humic substances. (Navin and Lovley., 2000b).

Electron shuttering via humic acids and other extracellular quinones still leaves the difficulty for Fe(III)-reducing microorganisms to transfer electrons to an extracellular electron acceptors. However, the chelated Fe(III) can make the reduction of these soluble extracellular electron acceptors faster than the reduction of insoluble Fe(III) oxides. Nevertheless, the preliminary studies suggest that direct reduction of insoluble Fe(III) oxides is likely to be the most important mechanisms for Fe(III) oxides reduction (Navin and Lovley., 2000b).

Major Group of Fe(III) AND Mn (IV)-Reducing Microorganisms

Microorganisms that do not conserve energy to support growth from Fe(III) reduction.

Many of these microorganisms reduce Fe(III) as a minor side reaction in their metabolism and do not appear to conserve energy to support growth from this electron transfer.

Two types, for example, of microorganisms that reduce Fe(III), but have not been shown to conserve energy to support growth from Fe(III) reduction are some dissimilatory sulfate-reducing and methanogenic organisms (Bond and Lovley., 2002).

In this case, hydrogen is metabolized to levels that make sulfate reduction or methane production thermodynamically unfavorable. However, this electron flow to Fe(III) by sulfate reducers and methanogens may be an important contributing factor in the inhibition of sulfate reduction and methane production in the presence of Fe(III) in sediments.

Microorganisms that conserve energy to support growth from Fe(III) reduction.

Microorganisms that conserve energy to support the growth from Fe(III) reduction are in both of Bacteria and Archaea. There are three groups of microorganisms that have been studied in significant details.

Geobacteraceae

The first organisms that conserve energy from the oxidation of organic compounds to carbon dioxide with Fe(III) serving as the sole electron acceptor are in the *Geobacteraceae* family. *Geobacteraceae* are often the most abundant microorganisms observed in aquatic sediments, the Fe(III)-reducing zone of aquifer contaminated with petroleum, landfill leachate, as well as in subsurface sediments that electron donors were added to stimulate dissimilatory metal reduction. One of the characteristics that may lead to the predominance of *Geobacteraceae* over other is the ability of many organisms in this family to use acetate as an electron donor. Acetate is a metabolic intermediate in the anaerobic degradation of complex organic matter in a variety of sediments. Moreover, *Geobacter metallireducens* and several other *Geobactor* species also have ability to oxidize various aromatic hydrocarbons to carbon dioxide with Fe(III) serving as an electron acceptors. In addition, many *Geobacteraceae* can use chlorinated compounds as electron acceptors and may also be important in some subsurface environments (Sung et al., 2003).

Shewanella species

Shewanella are facultative organisms, grow rapidly, and are found in a variety of environments. The species in the γ subclass of the *Proteobacteria* have been intensively studied for Fe(III)-reducing microorganisms. However, there is no evidence that these organisms substantially contribute to Fe(III) and Mn(IV) reduction in the environments in which Fe(III) and Mn(IV) reduction are important. The physiology of *Shewanella* species significantly differs from the *Geobacteraceae* that predominate in environments which

Fe(III) and Mn(IV) reduction are important. *Shewanella* species are likely to reduce Fe(III) oxides via mechanisms that are different from the *Geobacteraceae*. Their metabolisms of organic matter are limited. They are not known to utilize acetate. Organic matter that they can use as electron donors are limited to lactate and pyruvate and these substrates are incompletely oxidized to acetate. Thus, *Shewanella* species can transfer less than half of the electrons that could be potentially transferred to Fe(III) by microorganisms that can completely oxidize these substrates with Fe(III) reduction.

Hyperthermophilic microorganisms

Most of the hyperthermophiles that have been evaluated have the ability to oxidize hydrogen with the reduction of Fe(III). Providing Fe(III) as an electron acceptor can expand the metabolisms of some Fe(III) reducers other than what they are with other electron acceptors. For instance, *Thermotoga maritima* was originally characterized as a fermentative microorganism that could divert a small portion of its electron flow to S^o with no increase in cell yield over growth in the absence of S^o. At the presence of Fe(III), *Thermotoga maritime* can grow by using hydrogen as the sole electron donor and Fe(III) as the sole electron acceptor so they are respiratory organisms. Another example is *Ferroglobus placidus.* *F. placidus* is considered as an Fe(II)-oxidizing nitrate reducer and are unable to use organic electron donors to support growth. In the presence of Fe(III), *F. placidus* is able to oxidize acetate and monoaromatic compounds to carbon dioxide with the reduction of Fe(III) to yield energy to support the cell growth. (Tor et al., 2001). *F. placidus* is considered as the first hyperthemophile to oxidize acetate as electron donor

along with *Geoglobus ahangari* (Tor et al., 2001). In addition, *F. placidus* was also the first hyperthermophile to oxidize aromatic compounds (Tor et al., 2001).

Fe(III) appears to be only electron acceptor for some Fe(III)-reducing hyperthermophiles. For example, Strain 121 (*Geogemma barossi*) is organisms found to grow at the temperature as high as 121^{o}C. They were isolated from a hydrothermal vent sample with Fe(III) as the sole electron acceptor.

Electron Acceptors

Oxygen

Most Fe(III) reducers have the ability to use one or more alternative electron acceptors. For example, *Shewanella, Panatoea, Acidiphilum* species are facultative organisms that can grow better in the presence of oxygen (O_2) as electron acceptor than Fe(III). Fe(III) reducers that grow at neutral pH are expected to preferentially reduce oxygen (O_2) over Fe(III) due to the low solubility and redox potential of Fe(III). However, at the low pH when Fe(III) is soluble and at a redox potential similar to oxygen (O_2), oxygen (O_2) and Fe(III) can be reduced simultaneously. Although, Fe(III)-reducing microorganisms in *Geobacteraceae* have previously been classified as strict anaerobes, more detailed investigations of this organism have shown that *G.sulfurreducens* can grow with oxygen (O_2) as sole electron acceptor at concentration as high as 10% oxygen (O_2) in the headspace under the appropriate culturing conditions. The ability to use oxygen (O_2) as an electron

acceptor is an advantage to Fe(III)-reducing microorganisms because Fe(III) will often be most abundant near the oxic-anoxic interface where Fe(III) reducers are likely to be intermittently expose to oxygen (O_2).

Other Metals

Most microorganisms that can reduce Fe(III) can also reduce Mn(IV). Fe(II) produced from Fe(III) reduction can abiotically reduce Mn(IV) to Mn(II) and Fe(II) is converted to Fe(III). As a result, microorganisms that cannot reduce Mn(IV) can indirectly reduce Mn(IV) to Mn(II) via Fe(III) reduction.

In addition to Fe(III) and Mn(IV), many Fe(III) reducers can use other metals as electron acceptors. For example, *G. metallireducens and S. oneidensis* have been reported to grow with U(VI) as the sole electron acceptor. The reduction and precipitation of uranium via microbial U(VI) reduction is effective in removing uranium from contaminated waters in laboratory reactors. The broader application for microbial U(VI) reduction is in the *in situ* treatment of contaminated groundwater. Adding acetate can stimulate the growth of *Geobacteraceae* which can effectively precipitate uranium in the subsurface and prevent its further migration. In the similar manner, *G. metallireducens* can use vanadium as the sole electron acceptor to support growth so vanadium can be removed from groundwater by microbial reduction.

Extracellular Quinones

Some Fe(III)-reducing microorganisms can grow with extracellular quinones as the sole electron acceptor. Humic substance may be abundant source of extracellular quinones for Fe(III) reducers in natural environments. The quinone moieties in these compounds are reduced to hydroquinone state. When Fe(III) is available in environment, the hydroquinone can reduce Fe(III) to Fe(II), regenerating the oxidized form. Therefore, even the concentration of extracellular quinones are low, there can be substantial electron transfer to Fe(III) because each molecule of extracellular quinones can go through multiple cycles of oxidation and reduction. In the laboratory, the humic substances analog anthraquinone-2-6 disulfonate (AQDS) can be used instead of humic substances due to its difficulty to work with and expensive.

Sulfur Compounds

Fe(III) and S^o are often found in the same sediment intervals. Therefore, many Fe(III)-reducing microorganisms have ability to use S^o as an electron acceptor. Sulfide produced in the sulfate reduction zone of sediments can diffuses to Fe(III)-containing sediments and abiotically oxidize to S^o.

Some of sulfate-reducing microorganisms can reduce Fe(III) to Fe(II). However, most of them do not appear to conserve energy to support from Fe(III) reduction. There are two organisms that have been reported to conserve energy during Fe(III) reduction. They are *Desulfotomaculum reducens* and *Desulfobulbus propionicus*.

Nitrate

Nitrate is an alternative electron acceptor for Fe(III)-reducing microorganisms. When nitrate and Fe(III) are available simultaneously, nitrate is generally reduced prior to net Fe(III) reduction. This is not the result of the repression of genes involved in Fe(III) reduction when nitrate is present because nitrate-grown cells can retain the capacity for Fe(III) reduction. It may be because of the preference of electrons diverted to nitrate and/or nitrite reductase in the presence of nitrate. Another reason for the lack of net Fe(III) reduction is that the Fe(II) production is rapidly oxidized to Fe(III) with using nitrate as the electron acceptor.(Finneran et al., 2002c).

Fumarate

Although fumarate is not suppose to be abundant electron acceptor in most sedimentary environments, some Fe(III)-reducing microorganisms are able to use fumarate as the sole electron acceptor to support their growth. For *Geobacter sulfurreducens*, when both Fe(III) and fumarate are provided to the culture that is growing in chemostats, Fe(III) is more reduced. This can be suggested that the transcription of fumarate reductase genes is down regulated in the presence of Fe(III).

Chlorinated Compounds

A number of Fe(III)-reducing microorganisms can also conserve energy to support their growth with chlorinated compounds serving as electron acceptors.

For example, members of the *Geobacteraceae, Desulfuromonas chloroenthenica*, and *Desulfuromonas michiganensis* (Sung et al., 2003) can reduce PCE to *cis*-1,2,dichloroethene (cis-DCE) with acetate as the electron donor. The presence of Fe(III) oxide does not appear to inhibit reductive dechlorination, indicating that Fe(III) reduction and dechlorination may occur simultaneously in contaminated subsurface environments (Sung et al., 2003).

Electrodes

Some Fe(III)-reducing microorganisms can use graphite electrodes as extracellular electron acceptors. They can conserve energy by oxidizing organic compounds such as acetate to carbon dioxide with an electrode serving as the sole electron acceptor.The investigation of this phenomenon showed that the surface of the graphite electrode buried in the anoxic sediments was colonized by microorganisms. In fact, it was primarily occupied by the family of *Geobacteraceae*.

The electron transfer to electrodes by Fe(III)-reducing microorganisms have many advantages over the microorganisms that have been described for the development of microbial fuel cells. One major difference is that microorganisms that are not Fe(III) reducers generally require the presence electron-shuttering mediator which can facilitate electron transfer between the cell and the electrode. However, the Fe(III) reducers have the ability to directly transfer electrons to the electrode surface without the need of a mediator. Moreover, the *Geobacteraceae*, *R. ferrireducens*, and *G. fermentans* can transfer approximately 80 % of the electron available in organic substrates to electrodes. In contrast,

other microbial fuel cells can allow the transfer of less than 10% of electron available in organic substrates, even mediator is present. Therefore, most of the electrons remain in the end products. The higher efficiency of Fe(III)-reducing microorganisms and the lack of requirement of electron-shuttering mediators may indicate that they are likely to be a good choice of microorganisms for harvesting electricity from waste organic matter.

Electron Donors

The most common electron donors that most Fe(III) reducer can conserve energy to support their growth are organic acids. For instance, the *Geobacter* species can use acetate as the sole electron donors. Acetate is the key intermediate and likely to be the most important electron donor for Fe(III) reduction in many sedimentary environments.

A unique aspect of the *Geobacter* TCA cycle is the citrate synthase genes. Therefore, *Geobacter* can oxidize acetate via the TCA cycle. The citrate synthase genes in most *Geobacteraceae* that have been evaluated are closely related to citrate synthase genes found in eukaryotes.

It should be noted that *Geobacteraceae* have ability to fix nitrogen in poor nutrient subsurface environments because they contain genes for nitrogen fixation. For example, gene for nitrogen fixation are expressed by *Geobacteraceae* in Fe(III)-reducing petroleum contaminated sediments because the petroleum contamination may provide significant amounts of organic carbon to support microbial Fe(III) reduction but fixed nitrogen is little.

Hydrogen is an electron donor found to support the growth of Fe(III)-reducing microorganisms. Many of these microorganisms including some of *Geobacter* and *Shewanella* species can oxidize hydrogen with the reduction of Fe(III). The ability for hydrogen oxidation coupled to Fe(III) reduction is highly conserved among hyperthermophilic archaea and bacteria. This may be an adaptation to growth near hydrothermal fluids which may be high in hydrogen but have limited organic content. Some of them only grow via this form of respiration.

Some hyperthermophilic Fe(III)-reducing microorganisms are able to oxidize acetate. It is appeared that two hyperthermophiles, *Geoglobus ahangari* and *Ferroglobus placidus,* can conserve energy to support growth from the oxidation of acetate to carbon dioxide with Fe(III) serving as an electron acceptor, indicating that acetate may be anaerobically oxidized in hot environments.

A few Fe(III)-reducing microorganisms in the pure culture can oxidize aromatic compounds. *Geobacter metallireducens* was known that they can oxidize aromatic hydrocarbon in the absence of oxygen (O_2). Their metabolisms can serve as an important process for the removal of these contaminants from polluted groundwater.

The ability of anaerobes to metabolize sugar with the reduction of Fe(III) has been studied. However, the fermentation is a primarily metabolism and they only reduce Fe(III) as a side reaction. Recent studies have indicated that microorganisms can conserve energy through the oxidation of sugars with the reduction of Fe(III). In some cases of studies, sugars are completely oxidized to carbon dioxide with Fe(III) serving as the sole electron acceptor.

Some of Fe(III) reducers can grow with the peptide and/or individual amino acids as the electron donor. However, it has not been determined if the amino acids can be completely oxidized to carbon dioxide with Fe(III) serving as the sole electron acceptor.

In the particular and appropriate conditions, reduced end products of the respiration of Fe(III)-reducing microorganisms can serve as electron donors. For instance, *Acidthiobacillus ferroxidans* is a Fe(III) reducer to grow as an Fe(II) oxidizer at acidic pH. In the reduction of nitrate, the hyperthermophilic Fe(III) reducer *Ferroglobus placidus* use Fe(II) as an electron donor (Tor et al., 2001). *Desulfitobacterium frappieri* which is the Fe(III) reducer can use soluble Fe(II) and the structural Fe(II) in the clay as an electron donor for nitrate reduction. *G. metallireducens* is capable to use Fe(II) as an electron donor to reduce nitrate. However, it is not determined whether this reaction yields energy to support growth.

Some Fe(III)-reducing microorganisms have shown that they can oxidize reduced humic substances and/or AHQDS with nitrate and/or fumarate as the electron acceptor. For example, *Geobacter and Shewanella* are able to conserve energy from the oxidation of AHQDS.

Geobacter species can also utilize the electrode as an electron donor for the reduction of nitrate and fumarate when the electrodes are maintained at a sufficiently negative potential.

Range of Temperature, pH, and Salinity

It has been reported that Fe(III)-reducing microorganisms are capable of growing at temperatures as low as 4^{o}C in pure culture. They can also grow at high temperature (121^{o}C) in a pure culture. *A.ferrooxidans* and *Acidiphilium cryptum* can grow at low pH which Fe(III) is soluble electron acceptor. An arsenate-reducing *Bacillus* species are capable to reduce Fe(III) at pH 9. Fe(III) reduction has been noted in aquifer sediments with a salinity higher than seawater.

Fe(III) and Mn(IV) Reduction Mechanisms

Fe(III) and Mn(IV) are highly insoluble in most environments at circumneutral pH. The common soluble electron acceptor can diffuse into cells to be reduced whereas Fe(III) and Mn(IV) reducers meet the challenge of how to transfer electrons to insoluble electron acceptors. The discovery that exogenous Fe(III) chelators and electron shuttles can stimulate Fe(III) oxide reduction leads to the question if Fe(III) reducers may release Fe(III) chelators or electron shuttles by themselves.

The studies have demonstrated that some Fe(III)-reducing microorganisms release electron shuttling compounds and Fe(III) chelators to promote Fe(III) oxide reduction. For

example, it was observed that *S. oneidensis* released a diffusible compound, though to be a quinone, that might provide electron shuttle for Fe(III) oxide reduction. The studies with closely related *Shewanella algae* demonstrated that it could reduce Fe(III) oxide that it could not directly contact (Nevin and Lovley, 2002b).

Subsequent evidence that is consistent with the reduction of Fe(III) via soluble electron shuttles in *Shewanella* species is the observation that Fe(III) oxides can be reduced at location that are significant away from where cells are attached. In the absence of electron shuttling, Fe(III) reduction would be expected to occur at the point of cell attachment.

In addition to using electron shuttles to reduce the need for cell-Fe(III) oxide contact, *S. algae* also solubilised Fe(III) from Fe(III) oxide, allowing growth on insoluble Fe(III) oxide without direct contact (Nevin and Lovley, 2002b).

Fe(III) oxide reduction in *Geobacter* species have shown that *Geobacter* do not produce electron shuttles or Fe(III) chelators. Subsequent studies have demonstrated that *Geobacter* species have special adaptation to allow them to access insoluble Fe(III) oxides to reduce them.

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Chapter 3. The Impact of Influent Wastewater and Substrate Pressure on the Cannibal™ Process Performance

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Abstract

A laboratory study of the Cannibal™ process was undertaken to determine the effect of the influent wastewater iron concentration and substrate pressure on the performance of the Cannibal system. In the first phase, three sequencing batch reactors were operated—two using the Cannibal configuration and the other as conventional activated sludge. The influent for the first Cannibal process contained higher iron than that for the second Cannibal system. It was found that the Cannibal process generated up to 80% less solids than the conventional activated sludge system without any negative effect on the effluent quality or the settling characteristics of the activated sludge. The data also showed that the Cannibal system with high iron in the influent produced less biological solids than the system receiving low iron. This suggests that iron plays an important role in solids reduction. In the second phase, two Cannibal processes were operated, one under high substrate pressure (fast feed) and the other under low substrate pressure (slow feed). The

Cannibal system operated under fast feed produced much less solids than the system with slow feed. Oxygen uptake data for the centrate taken from the Cannibal bioreactor showed that a higher concentration of readily biodegradable organic matter was released into solution in the fast feed Cannibal bioreactor than in the slow feed system. It is thought that the Cannibal process with a high substrate pressure produces a floc structure that is more easily biodegraded than that from a system with low substrate pressure system.

Keywords: sludge, biosolids, activated sludge, solids reduction, high substrate pressure, low substrate pressure, fast feed, slow feed.

Introduction

Activated sludge is an aerobic biological wastewater treatment process that has been used for many decades to treat both industrial and domestic wastewater. In this process, microorganisms oxidize a portion of the organic matter to carbon dioxide and water by using molecular oxygen as a terminal electron acceptor. Although it has proven to be reliable, efficient, and capable of producing high effluent quality, it also produces significant quantities of excess biomass that require additional processing and disposal. For municipal wastewater, typical amounts of excess sludge production from conventional activated sludge processes may range from 500 to 800 lbs dry weight for each million gallon of wastewater treated (Crites and Tchobanoglous, 1998). The handling and disposal

of the sludge are a large fraction (40-50%) of plant operating costs (Crites and Tchobanoglous, 1998).

Because the goal of wastewater treatment is the degradation of organic matter to carbon dioxide, the ideal process would accomplish mineralization with a minimum production of excess sludge. The reduced biomass production can lower total costs for sludge thickening, digestion, dewatering, and final disposal. Therefore, many technologies have been proposed and evaluated in terms of their sludge reduction potential, ease of implement into existing facilities, impacts on plant operation and effluent quality, reliability and risk associated with the process, and relative capital and operating costs.

Over the past decades, several technologies have been developed to decrease sludge production from wastewater treatment. However, there are limitations for those technologies. For instance, biomass reduction by cell disruption using ozonation, mechanical shear, or thermal methods has been reported to reduce sludge by about 50-70%, but the cost for plant operation and the effect on effluent quality are of concern (Camacho et al., 2002). Chemical uncoupling is believed to reduce sludge in the range of 50-60%, but the major draw back for this process is the presence of toxic chemicals in the effluent (Strand et al., 1999). Anaerobic processes seem to be the most promising biomass reduction technology and a variety of designs are possible to provide 40-50% sludge reduction. The major concern for these processes is the ability to achieve high quality effluent. Although most technologies are able to reduce sludge production, they still encounter problems with implementation, effluent quality, reliability of the processes, and capital and operating cost (Macarie et al., 2000., Frankin et al., 2001., Lettinga et al., 2001.). In general, most of these

sludge reduction technologies either add significantly more costs or are inappropriate in practical terms.

In this study, we investigated the degradation of sludge generated from a conventional activated sludge system (CAS) by incorporating a sidestream anaerobic bioreactor into the system. This system is called the "Cannibal process". A previous study by Novak et al. (2007) demonstrated that the Cannibal process generated about 60% less solids than a conventional activated sludge system without any negative effects on the effluent quality. That study was conducted under a specific operation in which the return sludge was retained in an anaerobic bioreactor for 10 days and the interchange rate (the exchange of sludge between anaerobic and aerobic bioreactor) was 10% per day by mass.

Novak et al. (2003) investigated the mechanisms of floc destruction under anaerobic and aerobic digestion and proposed that flocs consist of two important biopolymer fractions, a divalent cation-bound biopolymer fraction and an Fe-associated biopolymer fraction. Therefore, those forms of flocs are expected to be present in the sidestream anaerobic bioreactor. Park et al. (2006) proposed that the major mechanism for sludge degradation during anaerobic digestion was the reduction of iron with the release of iron-associated organic matter, primarily protein, which is easily degraded. This mechanism is thought to apply to the Cannibal process. That is, when settled sludge is cycled to an anaerobic bioreactor, iron is reduced, and organic matter is released and solubilized. The released materials and sludge are returned to the aerobic reactor and are rapidly degraded before the released materials can be reflocculated.

Although the previous research by Novak et al. (2007) showed substantial solids reduction by the Cannibal process, that research did not investigate the effect of influent wastewater characteristics and reactor operation on system performance.

To better understand this process, this study was undertaken to

1. Determine the role of iron in the influent on the reduction in the mass of biological sludge generated by the Cannibal process.
2. Determine the role of system operation on the mass of biological solids generated by the Cannibal process. Specifically, the role of substrate pressure on solids reduction was investigated where substrate pressure was defined as the peak concentration of substrate that is seen in the aerobic (activated sludge) reactor over time. To achieve high substrate pressure, the influent was added over a short time period so that the substrate concentration would initially peak and then decline as degradation occurred. This is called "fast feed." For low substrate pressure, the influent was added slowly so the substrate concentration remained low at all times. This is called "slow feed."

Materials and Methods

For the laboratory studies, two operational phases were used. In the first phase, two Cannibal systems and a conventional activated sludge were run, as shown in Figure 3-2.

Each one of the Cannibal systems was operated as a sequencing batch reactor (SBR) with a sidestream anaerobic bioreactor while the control system was run as a sequencing batch reactor without a sidestream reactor (SBR). Sludge was not wasted from the Cannibal system but it was returned to the main reactor from the anaerobic bioreactor. For the control system, concentrated biomass was wasted from the settled mixed liquor suspended solid during the settling phase. The volume in the react tank was 3 L, and the feed was 2 liters/day for all systems. The sidestream anaerobic bioreactors had a 10-day hydraulic retention time (HRT) and were fed 10% of mass of the settled sludge from the SBR. The volume of returned sludge from the anaerobic bioreactors to the main reactors was the same as the feed volume. The HRT for all SBRs was 1.5 days. The SBRs were operated at 4 cycles/day; with a 5 hour react time and a settle time of 40 minutes. All systems were fed with synthetic wastewater with a feed period of 5 minutes.

For the second phase, two Cannibal systems were operated in the same manner as in the first phase without the control system. The first Cannibal system was fed within 5 minutes (fast feed) in order to produce high substrate pressure. The second system was fed over a period of 4 hours (slow feed) to provide low substrate pressure.

A soluble synthetic feed with a chemical oxygen demand (COD) of 400 mg/L was used. The feed composition is shown in Table 3-1. The influent to the SBR reactors was fed to all systems from a single tank. Feed was prepared every other day. For the first phase, 12 mg/L of alum ($Al_2(SO_4)_3.18H_2O$) which contained 1 mg/L aluminum (Al) but no iron was added to the influent of the first Cannibal system while the same concentration of alum and 6 mg/L of ferric chloride ($FeCl_3$) containing 2.1 mg/L ferric ion (Fe^{3+}) were added to the

influent of the other Cannibal system. The concentration of alum and ferric chloride in the influent of the control system were 6 and 3 mg/L respectively. These are similar to the Fe and Al found in municipal wastewater. For the second phase, the concentration of both alum and ferric chloride were the same as in the first phase for both Cannibal systems.

Analysis

Total solids, total suspended solids (TSS), total volatile solids, volatile suspended solids (VSS), and soluble COD were measured according to the Standard Methods (APHA et al., 1995). The protein concentration was determined by the Hartree (1972) modification of the Lowry et al. (1951) method, using bovine serum albumin as the standard. Polysaccharide was measured by the Dubois et al. (1956) method, using glucose as the standard. The pH was measured using an Accumet 910 pH meter (Fisher Scientific, Pittsburgh, Pennsylvania). Dissolved cations were measured using a Dionex ion chromatograph (Dionex, Sunnyvale, California). Methane sulfonic acid (30 mM) was used as the eluant at a flowrate of 1.0 mL/min.

Some samples were subjected to size separation, to determine the molecular weight distribution of the soluble protein. For this experiment, an aliquot of centrate was individually filtered through 1.5- and 0.45-µm membrane filters and the 30,000- and 1000-Dalton ultra filters. Ultrafiltration was performed at 414 kPa (60 psi) through Amicon YM30 (30 kDa) and YM 1 (1 kDa) partly hydrophilic membranes (Amicon, Beverly, Massachusetts).

Oxygen Uptake Rate. At the end of the second phase, the sludge in the anaerobic bioreactors was centrifuged using a Beckman J2-HS centrifuge (Beckman Coulter, Fullerton, California) operated at 9000 X g for 30 minutes at ambient temperature (25^{o}C). The centrate was decanted and added to activated sludge from the SBR, and the oxygen uptake rate was measured using a dissolved oxygen meter (YSI Model 57, Yellow Springs, Ohio). Oxygen uptake tests were conducted using 250 mL mixed liquor, 10 and 25 ml centrate from fast and slow feed systems respectively, and enough tap water to fill a 300-mL biochemical oxygen demand bottle.

Observed Yield. The observed yield was determined over a given range of operation as the TSS mass increase/COD mass utilization, using all the data over the range of operation for which the yield was determined.

Results and Discussion

The role of iron in sludge reduction. In the first phase, two Cannibal systems were operated-one with a higher iron concentration than the other. A sequencing batch reactor without a side-stream reactor was also run to represent a conventional activated sludge system and serve as a control. The measurement of the soluble chemical oxygen demand (COD) and total suspended solid (TSS) in the effluent and the sludge wasted was used to evaluate the system performance. The mixed liquor suspended solids concentration (MLSS) for all systems is shown in Figure 3-3. It can be seen that less variability was observed after

day 35. The loss of solids from the Cannibal systems was due to solids in the effluent and solids removed for sampling for measurement. No solids were intentionally wasted from the Cannibal system. However, daily wastage of settled sludge from the control system was performed to maintain the MLSS between 3,500 and 3,800 mg/L.

As shown in Figure 3-4, the total suspended solids in the effluent was similar for all systems and remained between 20-24 mg/L once steady-state was reached (after day 35) as evidenced by low variability in soluble COD and total suspended solid in the effluent. In Figure 3-5, the soluble COD in the effluent is shown. It can be seen that the effluent soluble COD was low and less variable as was the total suspended solid after day 35.

The accumulated solids over time are shown in Figure 3-6. It can be seen from Figure 3-6 that the Cannibal systems generated much less solids than the control system, as indicated by lower observed yield for both Cannibal systems. The solids accumulation in the Cannibal system with low and high iron in the influent was approximately 31% and 20% of the cumulative solid in the control, respectively. When the system performance of the two Cannibal systems is compared, the system with higher iron in the influent can be seen to generate less solids than the low iron system. The observed yield for the system receiving lower iron in the influent was 0.14, and that for the high iron system was 0.09. These data indicate that iron plays a role in solids reduction in the Cannibal system.

The cumulative solids was calculated by summation of total suspended solid in the effluent, the increase of mix liquor suspended solid in SBRs, and the solid lost due to sampling for the experiments.

Phase 2: The effect of substrate pressure on the Cannibal process performance

In this phase, two Cannibal systems were operated with different feeding patterns. One system was fed over a period of 5 minutes while the feeding period for the other system was 4 hours. The slow (4 hours) and fast feed (5 minutes) were used to provide low and high substrate pressure for those systems, respectively. The concentration of alum and ferric chloride was the same as phase 1 for both systems, one with low iron and one with high iron.

The data from figure 3-7 illustrate that a higher solid accumulation was found in the Cannibal system with low iron content in the influent compared to the system with the high iron concentration. On day 43, the feeding time for the system with high iron in the influent was increased from 5 minutes to 4 hours. As illustrated in figure 3-7, the reduced feed flow rate resulted in an increase in solids generation for the Cannibal system with high iron. However, when fast feed resumed, the solids generation returned to the lower level. Therefore, it can be seen that the fast feed mode gives better solids reduction than the slow feed mode. It is thought that fast feed operation provides an initial high food to microorganism ratio (F/M ratio) and this makes the system performance similar to a plug flow operation. In contrast, slow feeding provides a low F/M ratio and is similar to a complete mix operation. The data suggest that the characteristics of the sludge from two Cannibal systems are different. The sludge produced under slow feed does not respond as well to the Cannibal system as does sludge from the fast feed operation.

The generation of higher solids for the system with low substrate pressure operation is reflected in the observed yield data shown in Figure 3-8. The observed yields varied from

a high value of 0.31 mg TSS/mg COD for the Cannibal system with slow feed and low iron to 0.09 mg TSS/mg COD for the system with fast feed and a high iron concentration in the influent wastewater. These data show that the feed flow rate for the influent is an important factor and should be considered for the design of Cannibal systems. Nevertheless, it can be seen that the yield for the system with slow feed and low iron content is still less than the control system value of 0.45 (the conventional activated sludge) from phase 1. The Cannibal system provides some sludge reduction, regardless of the feeding pattern.

In general, the solids loss in the conventional activated sludge system is due to the wastage. For the Cannibal process, the solids production is decreased as indicated by the lower observed yield when compared to the control system, leading to the reduction of solid wastage. This is an advantage of the Cannibal process over the conventional activated sludge. The data in Figure 3-9 demonstrate that the sludge in the Cannibal system with fast feed and high iron concentration in the influent had a volatile fraction of approximately 0.70, while slow feed with high iron content and control systems had these values of 0.77 and 0.83, respectively. Therefore, it appears that a characteristic of solids destruction in the Cannibal system is high volatile solid reduction, resulting in the lower volatile component in the Cannibal system sludge than the control system. It can also be seen from Figure 3-8 that the observed yield of the Cannibal system operated with fast feed and high iron concentration in the influent was the lowest.

The oxidation-reduction potential (ORP) in anaerobic bioreactor of the Cannibal system operated under fast and slow feed was -264 mV and -108 mV, respectively. These data are in accord with the research from Saby et al. (2003). These researchers operated a

modified activated sludge system called the oxic-settling-anoxic process by recycling the settled activated sludge to an anoxic tank. They found that the amount of solid reduction was depended on the ORP. That is, a lower ORP in an anoxic reactor was associated with a higher solid reduction.

Mechanism of Floc Destruction

The structural composition of activated sludge flocs can be considered as microorganisms, organic matter in addition to microbial cells, and cations. (Frølund et al., 1996, Higgins and Novak., 1997). Extracellular polymeric substances (EPS) are a primary part of organic matter in which microorganisms, biopolymer, and other particles are embedded. Proteins were found to be the most abundant EPS component. (Frølund et al., 1996, Jourand et al., 1995, and Park et al., 2007). The structure of floc was proposed by Novak et al. (2003). They suggested that biopolymer that made up flocs in activated sludge consisted of protein and polysaccharide. Much of polysaccharide is associated with lectin-like protein and retained by divalent cations. In addition, proteins can be retained by ferric ion. Park et al., (2006) proposed that a major mechanism for solids reduction under an anaerobic environment was the destruction of Fe-associated EPS floc. They proposed that when ferric ion in the floc is reduced to ferrous ion, the material, primarily protein, bound to the iron would be released into solution, resulting in deflocculation and the released material can then be more easily degraded. Therefore, it is thought that the solids reduction taking place

in anaerobic bioreactor in the Cannibal process is similar to this concept.

The degradability of the returned material from the Cannibal reactor of the fast and slow feed systems was of interest. To determine this, the degradation rate and extent of soluble material from the anaerobic unit was determined. Sludge from those different anaerobic bioreactors was collected and centrifuged. The centrate was then fed to batch reactors containing mixed liquor suspended solid from the aerobic reactors and the oxygen uptake measured.

The results of the oxygen uptake measurement are presented in Figure 3-10. It can be seen that both the oxygen uptake rate and amount of oxygen used for the centrate from the fast feed system was higher than that in the slow feed system. These data show that the anaerobic bioreactor for the fast feed system contains more readily biodegradable material than that from the slow feed system.

As can be seen from Table 3-3, the protein concentration increased for the sludge discharged from both of anaerobic bioreactors. This is consistent with the study by Novak et al (2003) and Park et al (2006) who found that Fe-associated EPS flocs were disintegrated under anaerobic condition, resulting in deflocculation. The material that is bound to iron, primarily protein, is released and degraded. In addition, soluble protein in the anaerobic bioreactor of the fast feed system is higher than the system with slow feed. It appears that more Fe-associated EPS floc material was destroyed in the Cannibal system with fast feed operation. In addition, a higher portion of protein that passes through a 1 kDa ultrafiltration can be seen for the fast feed system compared to slow feed system. The

protein fraction less than 1 kDa can be considered as readily biodegradable material. Therefore, it is clear that operating the Cannibal system with fast feed to achieve high substrate pressure allows the system to generate more soluble biodegradable protein. The data also show that the protein released in the anaerobic bioreactor in fast feed system is more readily degraded than that for slow feed system in an aerobic environment (protein degradation in aerobic reactor is the difference between protein entering and leaving SBR). These data correspond with the data from oxygen uptake test. That is, more substrate uptake as indicated by protein degradation in fast feed system resulted in a greater rate of oxygen utilization.

The sludge settling characteristics in the aerobic reactors, as indicated by the sludge volume index (SVI) were excellent. The values of SVI for all systems were between 72-78 mL/g over the period of study. This implies that the EPS that was released from the floc and degraded was regenerated in the aerobic reactor because if the EPS was completely consumed, flocculation would deteriorate (Urbain et al., 1993).

For the fast feed condition, high substrate pressure may enhance gene expression, resulting in more substrate uptake and utilization, subsequent cell growth, and new cell generation. During cell synthesis, many proteins including enzymes are secreted from the cells for substrate degradation, or for binding to organic materials to transport them across cell membranes, or for other purposes. Those secreted proteins are included in the extracellular polymeric substances involved in floc formation (Park et al., 2007). The data in the Table 3-3 show that higher amounts of released protein (the difference between protein in the solution fed to aerobic reactor and to anaerobic reactor) were found in the

anaerobic bioreactor of the fast feed system. This suggests that, under high substrate pressure, more proteins were generated and involved in floc formation. It can be hypothesized that greater amounts of protein (including the protein from cell lyses) are released by microorganisms for bioflocculation purpose under high substrate pressure condition than under low substrate pressure.

Implications

The concept of increasing substrate pressure by feeding patterns, fast feed and slow feed, for solid reduction in the Cannibal system is new and additional research is needed to understand the solid loss mechanism under those two different conditions. However, it can be hypothesized that, under high substrate pressure condition, a higher frequency of gene expression occurs for microorganisms in anaerobic bioreactors, resulting in more extracellular protease secretion from the cells, more enzyme activity, and more solids reduction. In contrast, less production, less release, and less activity of the protease occurs under low substrate pressure, making it difficult for solids reduction.

Caccavo et al. (1996) reported on the influence of microbial Fe(III) reduction on the deflocculation of activated sludge under anaerobic condition. They found that cells of the dissimilatory metal-reducing bacterium Shewanella alga BrY oxidized hydrogen gas and reduced Fe (III) bound in the sludge floc, resulting in floc destruction. Interestingly, they also found that cells need to be bound to the Fe(III) associated EPS floc before the chemical

reduction could take place. Therefore, the amount of Fe (III) that can be reduced by Fe-reducing microorganisms, called microbially reducible iron, in the Fe (III)-associated EPS floc is of interest and needs further investigation.

Conclusions

The role of iron in sludge reduction in the Cannibal system was studied in the laboratory using a synthetic influent wastewater. The data indicate that the system with high iron in the influent produced less biological solids. It was observed that the solid generated in the Cannibal system receiving high iron in the influent is about a half of the solids generated in the same system with low iron content in the influent. This suggests that iron plays a crucial role for sludge reduction in the Cannibal process. In addition, a side-by-side operation of the Cannibal system was performed under high and low substrate pressure (fast and slow feed respectively). The system that was operated in the fast feed mode resulted in a significant reduction in the sludge generation. A reduction of approximately 64 % in solids was found for the fast feed system compared to slow feed system. It appears that the Cannibal process with high substrate pressure may produce floc structure that is more easily biodegradable than that from low substrate system. However, additional research need to be done to further clarify the specific mechanisms that account for this loss.

The study from phase 1 also support the research by Novak et al. (2007) who showed that the Cannibal system reduced a significant amount of biological solid generation compared with a control or non-Cannibal system.

There is no deterioration in system performance as indicated by high quality of the effluent, low amount of effluent total suspended solid and soluble COD, and low sludge volume index (SVI).

The reduction of iron is a primary mechanism for the sludge reduction under anaerobic condition (Park et al, 2006). After iron reduction, organic matter is released and solubilized. When the sludge and solubilized organic matter are returned to the SBR, it is rapidly degraded before it can be recoagulated by ferric ion in the mix liquor suspended solid.

The operation of the Cannibal system by fast and slow feed provides high and low substrate pressure to the systems. The sludge generated from slow feed system is more difficult to degrade than the system with fast feeding, resulting in more solids accumulation.

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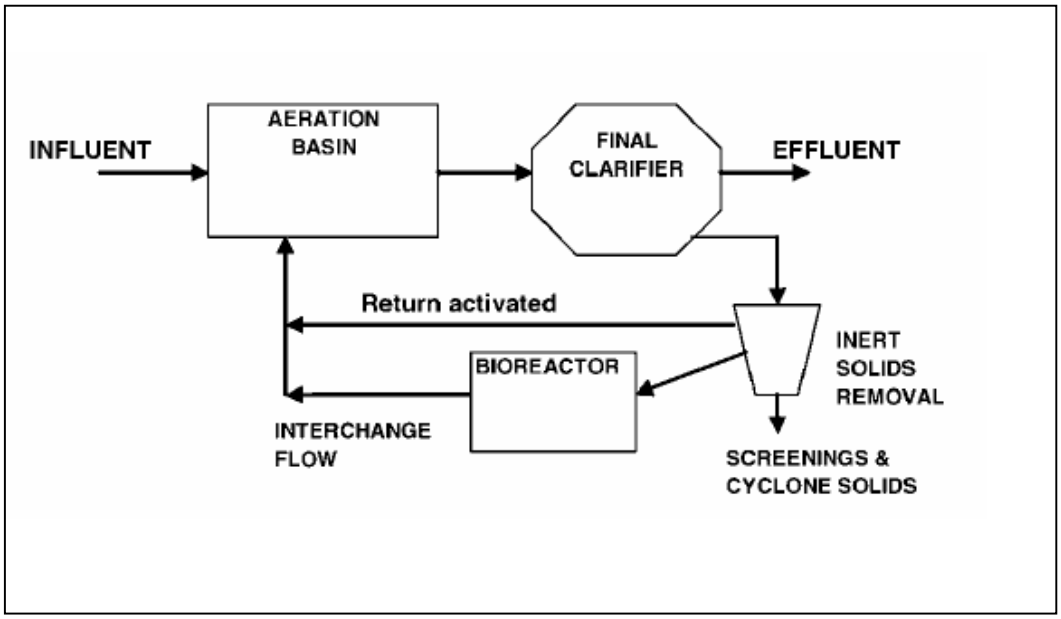

Figure 3-1. Flow scheme for the Cannibal process.

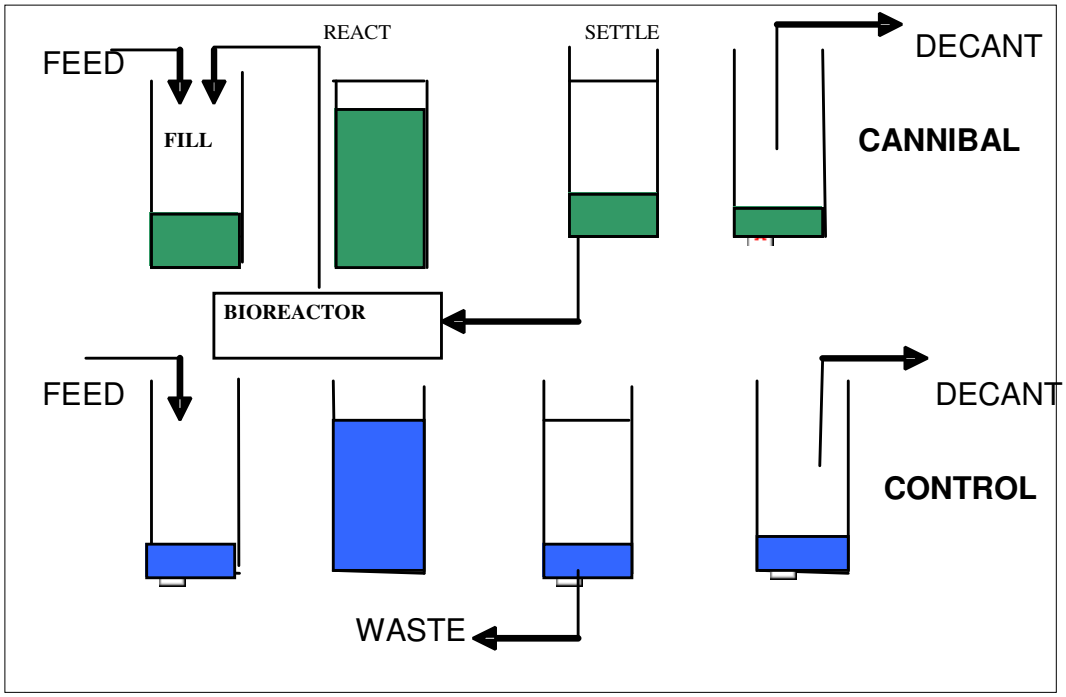

Figure 3-2. Laboratory operations for Cannibal and control systems.

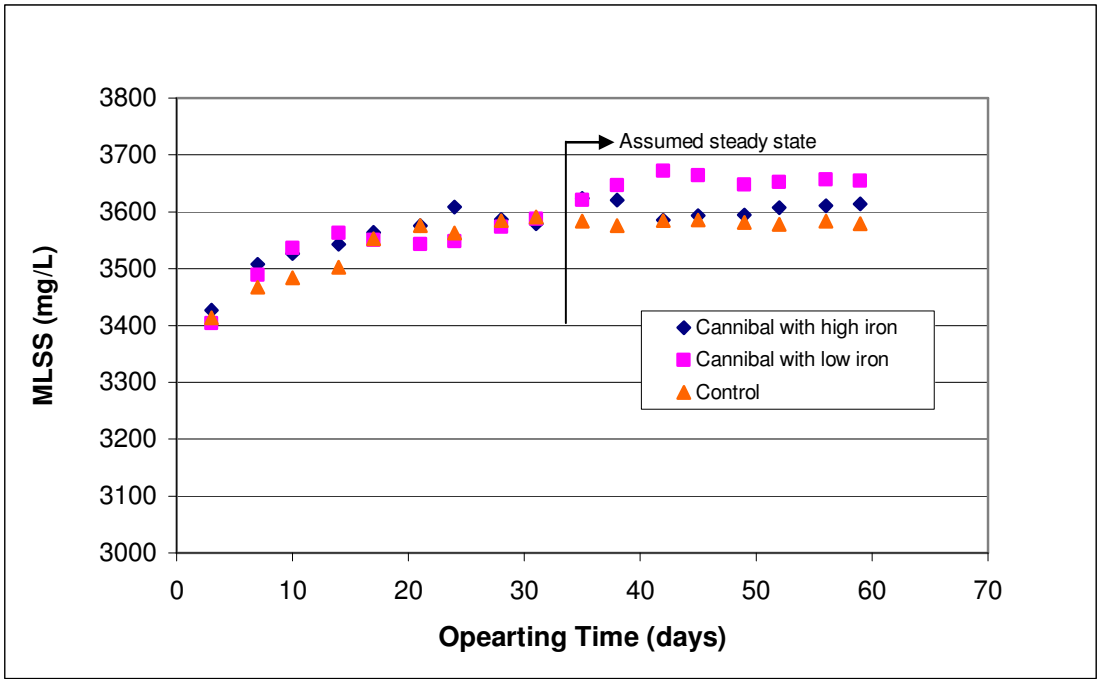

Figure 3-3. Mix liquor suspended solid (MLSS) for the Cannibal and control systems.

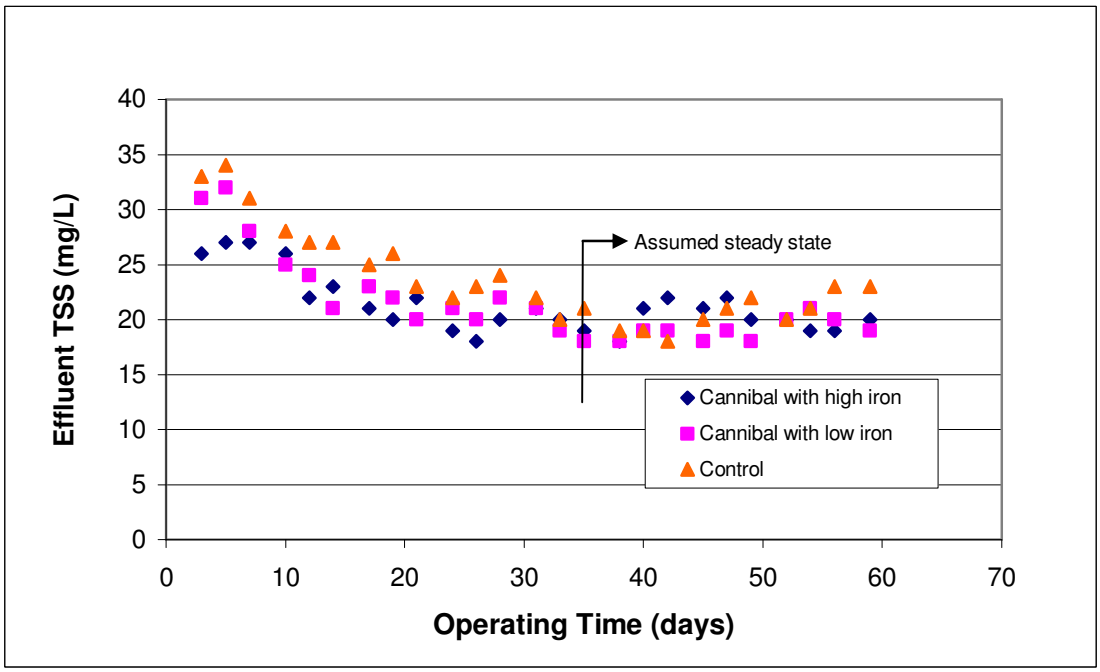

Figure 3-4. Effluent total suspended solid for the Cannibal and control systems.

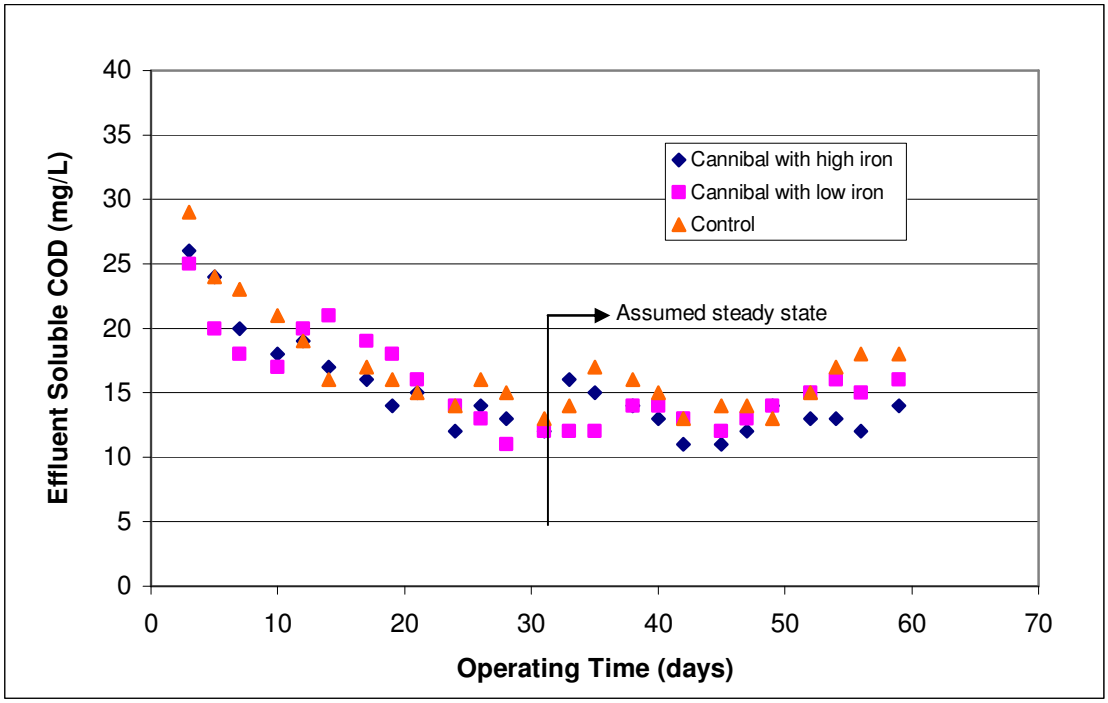

Figure 3-5. Effluent soluble COD for the Cannibal and control systems.

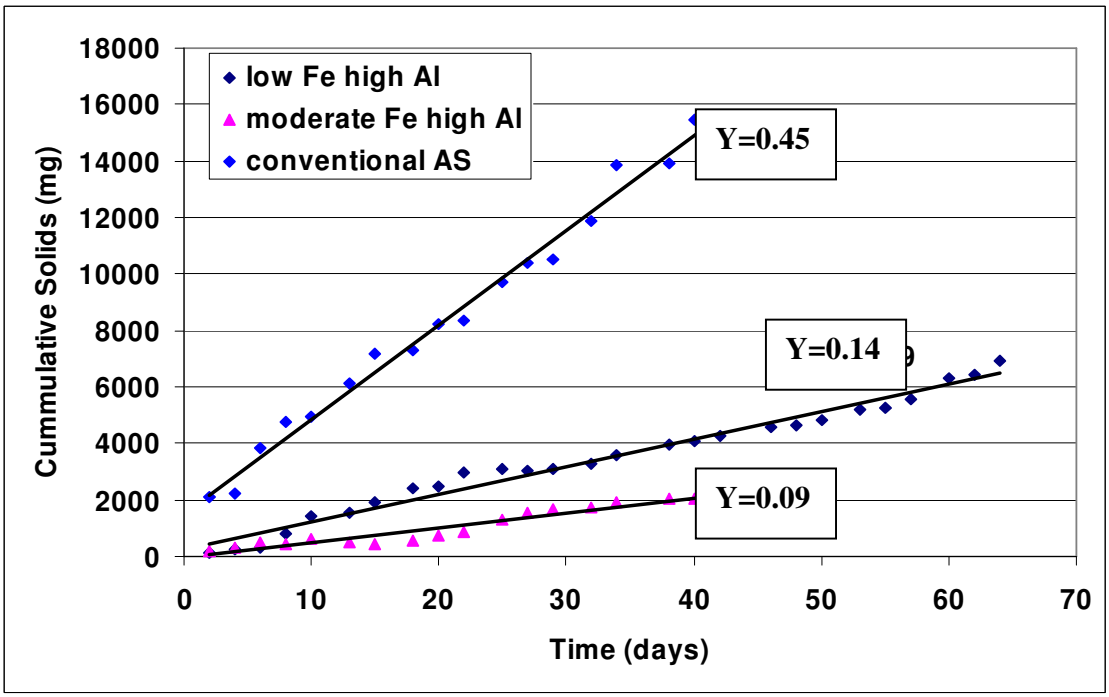

Figure 3-6. Effect of aluminum and iron on solid reduction.

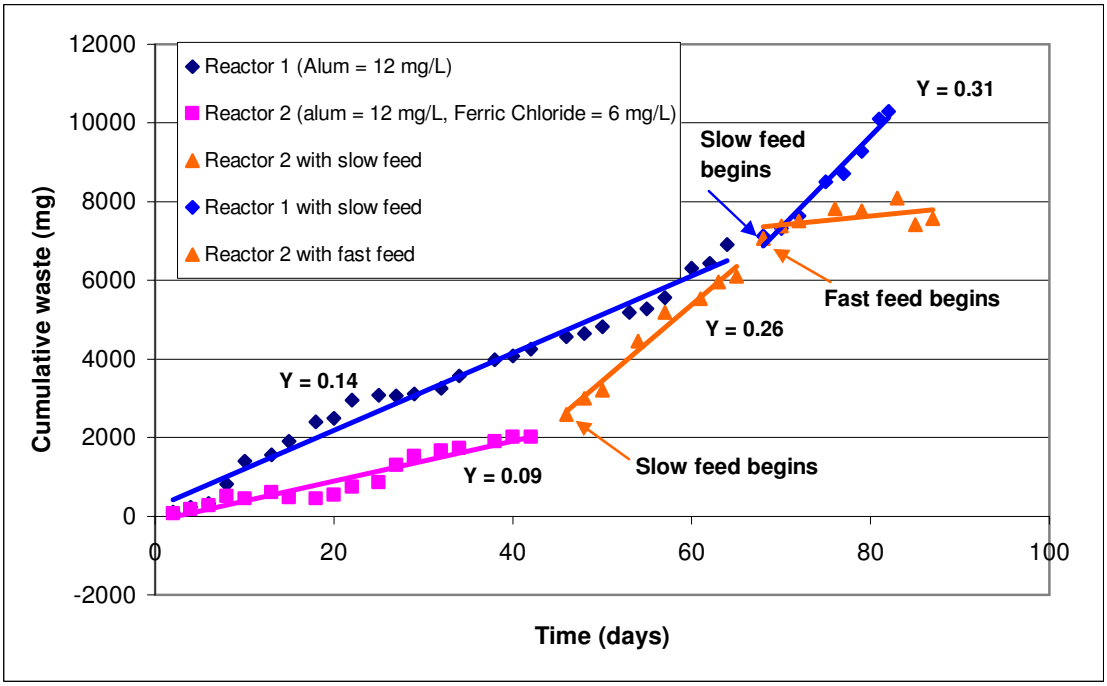

Figure 3-7. Effect of feeding pattern on solid reduction. (The unit of observed yield is mg TSS/mg COD)

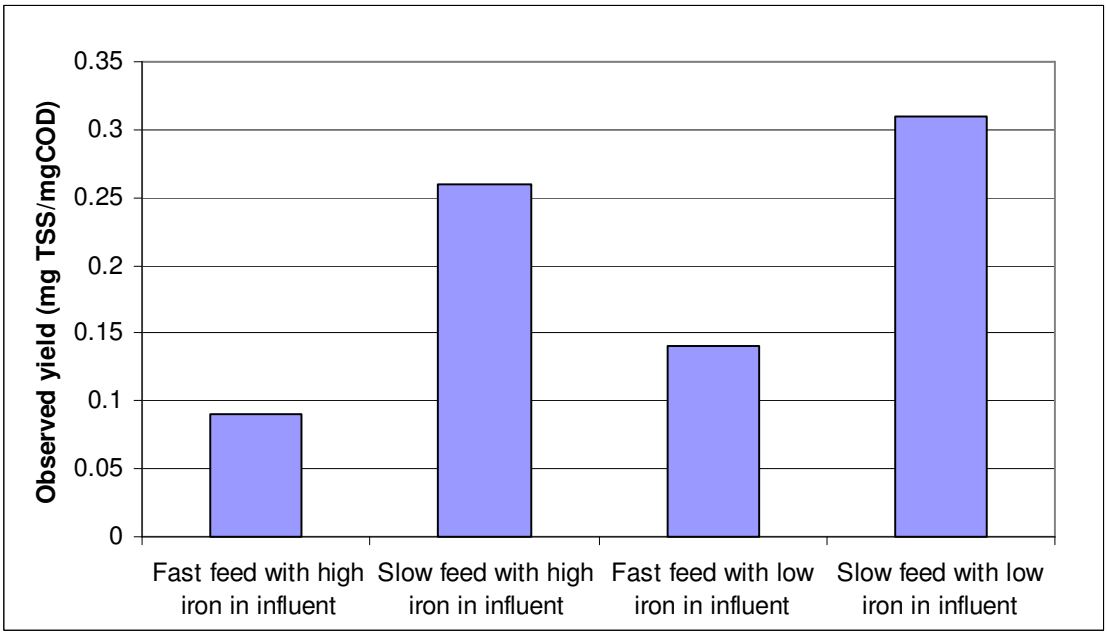

Figure 3-8. Observed yield for the Cannibal systems operated under different feeding patterns.

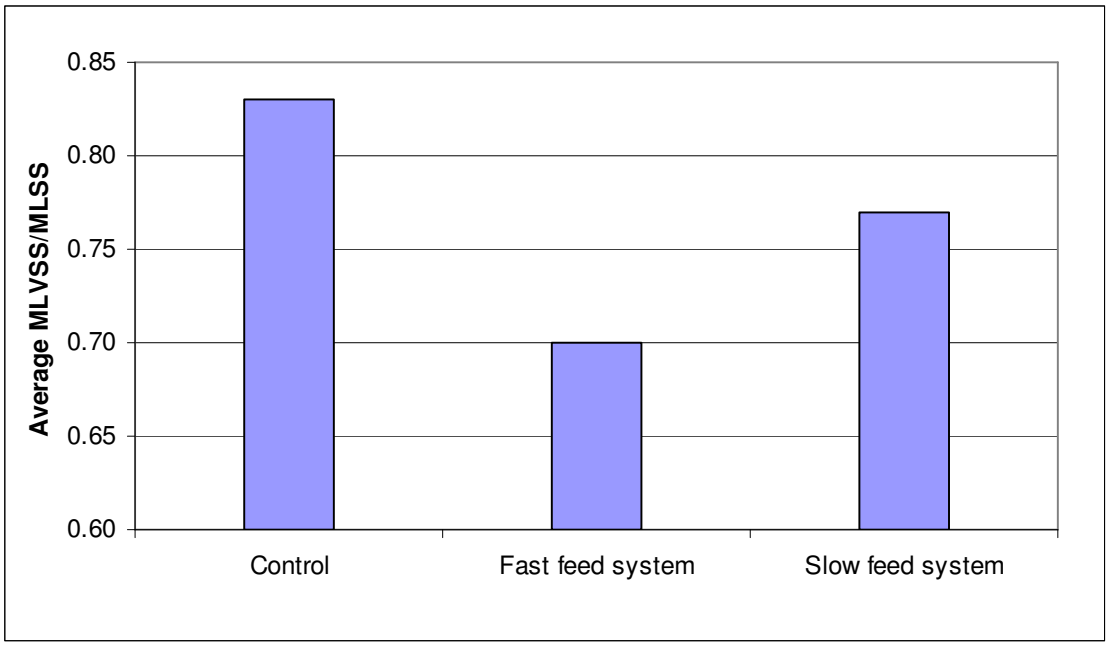

Figure 3-9. MLVSS/MLSS from three different reactors.

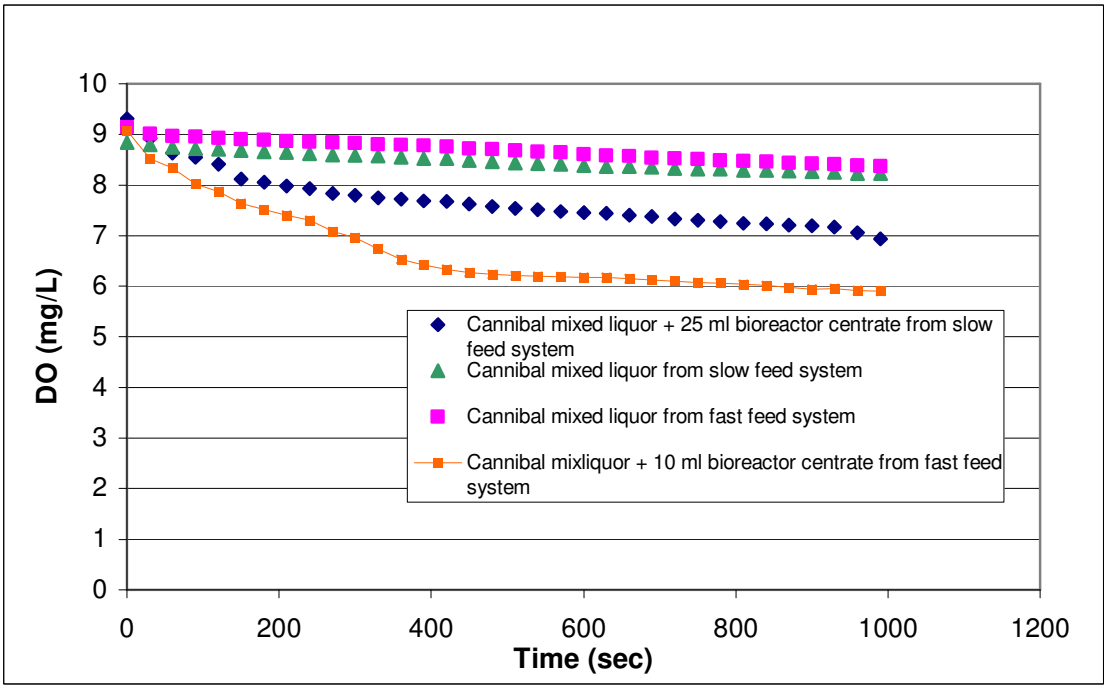

Figure 3-10. Oxygen uptake for centrate from anaerobic bioreactors in fast and slow feed systems

Table 3-1. Medium composition.

NAME	FORMULA	CONCENTRATION (mg/l)
Bacto Peptone	-	300mg COD/l
Sodium Acetate	CH_3COONa	100mg COD/l
Ammonium Chloride	NH_4Cl	57
Ammonium Bicarbonate	NH_4HCO_3	60
Potassium Phosphate	KH_2PO_4	44
Potassium Bisulphate	$KHSO_4$	34
Sodium Bicarbonate	$NaHCO_3$	394
Calcium Chloride	$CaCl_2.2H_2O$	220
Magnesium Sulfate	$MgSO_4.7H_2O$	150
Ferric Chloride	$FeCl_3$	-
Alum	$Al_2(SO_4)_3.18H_20$	-
Allylthiourea	-	6.0
Trace Element Solution	-	2ml/l

Table 3-2. Trace element solution composition.

NAME	FORMULA	CONCENTRATION (gm/l)
Citric acid	-	2.73
Hippuric acid	-	2
Ethylene Diamine Tetraacetic Acid, trisodium salt	$Na_3EDTA.4H_2O$	1.5
Ferric Chloride	$FeCl_3.6H_2O$	1.5
Boric Acid	H_3BO_3	.25
Zinc Sulphate	$ZnSO_4.7H_2O$	.15
Manganese Chloride	$MnCl_2.4H_2O$	.12
Copper (II) Sulphate	$CuSO_4.5H_2O$	.06
Potassium Iodide	KI	.03
Sodium Molybdate	$Na_2MoO_4.2H_2O$	.03
Cobalt chloride	$CoCl_2.6H_2O$	.03
Nickel (II) Chloride	$NiCl_2.6H_2O$	.03
Sodium Tungstate	$NaWO_4.2H_2O$	.03

Table 3. Protein data separated by molecular size at two different locations.

Location	Feed Pattern	Protein (mg/L)			
		1.5 µm	0.45 µm	30 kDa	1 kDa
Feed to aerobic reactor	Fast Feed	108.69	78.46	66.86	34.85
	Slow Feed	54.67	38.26	18.34	9.38
Feed to anaerobic reactor (settled activated sludge)	Fast Feed	17.29	12.48	9.04	4.57
	Slow Feed	12.89	8.34	5.97	2.88

Note: 30 kDa and 1 kDa refer to protein passing the 30 kDa and 1 kDa ultrafilter sizes respectively while other filter sizes refer to filtration without applied pressure.

Chapter 4. The Enhancement of Solid Reduction in the Cannibal Process

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The Enhancement of Solid Reduction in the Cannibal Process

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Abstract

An activated sludge system that incorporates a sidestream anaerobic bioreactor, called the Cannibal process, was studied by Novak et al. (2007) to evaluate its capability for activated sludge reduction. They found that this system generated about 60% less solids than conventional activated sludge without any negative effects on the effluent quality. Later, Khanthongthip et al (in preparation) studied this system operated with different feeding patterns. They found that for a sequencing batch reactor system, when the feed was provided to the Cannibal system over a period of 5 minutes (fast feed), the system generated less solids than the Cannibal system fed over a period of 4 hours (slow feed). In this study, the slow feed Cannibal systems were operated either with a selector unit or with intermittent aeration to increase substrate pressure and then determine if these modifications would increase solids destruction. It was found that the Cannibal system with intermittent aeration generated 35% less solids than the typical slow feed Cannibal system without any negative impact on the effluent quality. When the selector was used, a

maximum solids reduction of 48% could be attained, depending on the amount of sludge routed through the selector and the selector detention time. It is thought that either modification results the organisms being exposed to higher substrate concentrations (higher substrate pressure) and this results in the generation of a floc structure similar to those from the fast feed Cannibal system which is more susceptible to biodegradation through anaerobic/aerobic cycling.

Keywords: sludge, biosolids, activated sludge, solid reduction, intermittent aeration, selector, fast feed, slow feed.

Introduction

The activated sludge system is a process that uses microorganisms to oxidize organic matter in the wastewater to carbon dioxide and water by using molecular oxygen as a terminal electron acceptor. Although it has proven to be reliable, efficient, and capable of producing high effluent quality, it also produces significant quantities of excess sludge that require additional processing and disposal. Many sludge reduction technologies have been proposed, developed, and evaluated in terms of their sludge reduction potential, ease of implementation, impacts on plant operation and effluent quality, reliability and risk associated with the process, and relative capital and operating costs. However, most of these sludge reduction technologies either add more plant operating costs or are inappropriate in practical terms.

One of the most promising processes that have been developed to reduce sludge quantities is the "Cannibal system". This is a process capable of treating wastewater as well as reducing the sludge generated from biological wastewater treatment. It consists of an activated sludge system integrated with a sidestream anaerobic reactor (interchange reactor) and a recycle circuit (Figure 4-1). The operation involves the interchange of a portion of sludge between the aerobic and the anaerobic zone on daily basis. The biomass in the aerobic zone is allowed to settle before a portion of it is transferred to the anaerobic bioreactor. Then, the sludge is held in the anaerobic bioreactor for the specified detention time before it is recycled back to aerobic reactor. The hydraulic retention time (HRT) of the sidestream anaerobic bioreactor is in the range of 5-10 days and is fed approximately 10% per day of the mass of settled sludge from the aeration tank. A previous study by Novak et al. (2003), they demonstrated that the Cannibal process generated about 60% less solids than a conventional activated sludge system without any negative effects on the effluent quality or the settling characteristics of the activated sludge. That study was conducted under a specific operation in which the return sludge was retained in an anaerobic bioreactor for 10 days and the interchange rate (the exchange of sludge between anaerobic and aerobic bioreactor) was 10% per day by mass. It also used a sequencing batch reactor with substrate feeding taking place over a 5 minutes period.

The role of iron in sludge reduction in the Cannibal system was studied by Khanthongthip et al. (in preparation) using a synthetic influent wastewater. They found that the system with high iron in the influent produced less biological solids. They also found that the solids generated in the Cannibal system receiving high iron in the influent was

approximately a half of the solids generated in the same system with low iron content in the influent. This showed that iron plays a role in sludge reduction in the Cannibal system.

In addition, side-by-side operation of the Cannibal system was performed under high and low substrate pressure (fast and slow feed respectively). The fast feed was performed by providing the feed over a period of 5 minutes to the system to represent high substrate pressure while the synthetic wastewater was fed to the system in a period of 4 hours (slow feed) representing low substrate pressure. Fast feeding is designed to mimic a plug flow activated sludge system while the slow feed is designed to approximate a complete mix, continuous flow system. There was no deterioration found in system performance for either feeding pattern as indicated by high quality of the effluent, low amount of effluent total suspended solid and soluble COD, and low sludge volume index (SVI). However, the system that was operated in the fast feed mode resulted in a significant biological solid reduction compared to a non-Cannibal system. A reduction of approximately 64 % in solids was found for the fast feed system compared to slow feed system. This indicated that sludge generated from slow feed system was more difficult to degrade than that in the system with fast feed condition. It is thought that a high substrate pressure environment may produce a floc structure that is more biodegradable than that from low substrate pressure conditions. Therefore, in order to improve the capability of biological solid reduction for the Cannibal system operated under low substrate pressure (slow feed), an attempt to increase substrate pressure was made by two different strategies, one using intermittent aeration in the SBR and the other by introducing an aerobic selector in front of the SBR.

The objective of this study was to determine if solids reduction of the slow feed Cannibal system could be enhanced by increasing the transient substrate concentration by either shutting the air off for a time or by adding an aerobic selector. The system modifications were an attempt to increase substrate pressure so that the system could produce flocs that were structural similarly to those generated under the fast feed condition that were readily biodegradable in the Cannibal operation.

Materials and Methods

In this research, two operational phases were used. In the first phase, two Cannibal systems were run. Each of the Cannibal systems was operated as a sequencing batch reactor (SBR) with a sidestream anaerobic bioreactor. The first Cannibal system was operated using intermittent aeration while the other system was the typical Cannibal fast feed system. Sludge was not wasted from the Cannibal systems, but was returned from the anaerobic bioreactor to the main reactor. The volume in the react tanks was 3 L, and the feed was 2 liters/day for both systems. The sidestream anaerobic bioreactors had a 10-day hydraulic retention time (HRT) and were fed by 10%/day of mass of the settled sludge from the SBR. The volume of returned sludge from anaerobic bioreactors to the main reactor was the same as the feed volume. The HRT for all SBRs was 1.5 days. The SBRs were operated at 4 cycles/day. When intermittent aeration was used, the air supply was switched off for one hour at the first and the third hour in each feeding cycle. Mixing was not provided during the no-aeration period. The air supply for the typical Cannibal system was 5 hours during

the aeration period. A settling time of 40 minutes was used for both systems. All systems were fed with synthetic wastewater (see Table 4-1) with a feed period of 4 hours to provide the low substrate pressure (slow feed).

For the second phase, both Cannibal systems were operated as slow feed systems (4 hour feed period) but each contained a small bioreactor, called a selector, placed in front of the SBR, as shown in Figure 4-2. Both systems including the selectors were aerated for 5 hours during the react time in each cycle. The hydraulic retention time (HRT) in the selector was varied along with the quantity of biomass cycled through the selector. The selector hydraulic retention time (HRT) for the first Cannibal system was one hour while the initial selector HRT for the second Cannibal system was 30 minutes and later increased to 2 hours. Sludge in anaerobic bioreactors of both Cannibal systems was not returned to the SBRs but it was recycled to the selectors. The volume of returned sludge from anaerobic bioreactors to the selectors was the same as the feed volume. At the last stage of this phase, the settled sludge from the SBR in each Cannibal system was also cycled to the selector at various recycled ratios (the ratio between the volume of the settled sludge in the SBR pumped to the selector and the volume of the sludge from the anaerobic bioreactor recycled back to the selector). The ratio used for both systems in this study was 1:1, 2:1, and 3:1. The selector HRT used in this stage was 0.5, 1, 1.5, and 2 hours. The influent was fed directly to the selectors for both Cannibal systems.

A soluble synthetic feed with a chemical oxygen demand (COD) of 400 mg/L was used. The influent to the selectors in both systems was fed from a single tank. Feed was prepared every other day.

Analysis

Total solids, volatile solids, total suspended solids, and volatile suspended solids were measured using Standard Methods (APHA et al, 1995). The pH was measured using an Accumet 910 pH meter (Fisher Scientific, Pittsburgh, Pennsylvania).

Observed Yield. The observed yield was determined over a given range of operation as the TSS mass increase/COD mass utilization, using all the data over the range of operation for which the yield was determined. The increase in mass (cumulative solids) was calculated by summation of total suspended solid in the effluent, the increase of mix liquor suspended solid in SBRs, and the solid lost due to sampling for the experiments.

Results and Discussion

The role of intermittent aeration in sludge reduction. In the first phase, a side by side comparison of the slow feed Cannibal systems operated with and without the intermittent aeration was made. The Cannibal system with full air supply during the aeration period served as a control. The soluble chemical oxygen demand and total suspended solid in the effluent and the sludge generation represented by the observed yield were used to evaluate the system performance. The mixed liquor suspended solids concentration (MLSS) for both systems was between 3,600 and 3,900 mg/L, as shown in Figure 4-2. The solids loss from the Cannibal systems was the solids in the effluent and the solids collected for experiments

and testing. No solids were intentionally removed from either system. Both systems utilized in this study had been operated under slow feed condition for an earlier study so were at steady state when this study began

As can be seen from Figure 4-3, the effluent total suspended solids were similar for both systems and remained between 19 and 23 mg/L for the entire period of this study. In Figure 4-4, the soluble chemical oxygen demand (COD) in the effluent is shown and ranges from 14-19 mg/L. The low variability in effluent suspended solids and soluble COD indicate that the systems were performing well and were at steady state. The data in Figure 4-3 and 4-4 show that the slow feed Cannibal system with intermittent aeration produced a slightly better effluent quality than the typical slow Cannibal feed system as indicated by lower total suspended solids (TSS) and lower chemical oxygen demand (COD) in the effluent. The statistical analysis using two tail student t-test with 95 % confidential interval indicates that the sample means of effluent TSS between intermittent aeration and typical slow feed Cannibal systems are statistically different as they are for the effluent COD ($p<0.025$).

The data for accumulated solids over time are shown in Figure 4-5. As can be seen in Figure 4-5, the Cannibal system operated with the intermittent aeration generated less solids than the system with uninterrupted air supply during intermittent aeration. The cumulative solids for the Cannibal system with the intermittent aeration was approximately 65% as much solids as for the control. The observed yield (mg total suspended solid (TSS) / mg COD) for the slow feed with continuous aeration was 0.26 and that for the Cannibal system with the intermittent aeration was 0.21, as shown in Figure 4-6. For the fast feed

system with the same feed, the yield was 0.09 and for a non-Cannibal system, the yield was 0.45 (Khanthongthip, et al., in preparation). These data indicate that the interruption of air supply during aeration period plays a role in solids reduction in the Cannibal system under the slow feed condition. In addition, it can be seen from Figure 4-6 that operating the Cannibal system either by the fast feed (feeding is provided to the Cannibal system in a period of 5 minutes) or the slow feed with intermittent aeration results in the lower observed yield compared to the slow feed Cannibal system.

Determination of cumulative solids was made, by adding the solids increased in aerobic reactor to the solids lost in the effluent and the solids removed as samples for analysis and testing.

Phase 2: The effect of a selector on the Cannibal process performance

In this phase, two Cannibal systems were operated under slow feed conditions, but a selector was added. The selector, a small reactor placed in front of an aeration reactor (SBR), was used to expose the biomass to a period of high substrate prior to being added to the aeration basin of the SBR. Air was supplied to the selector during the aeration period for the SBR. The selector hydraulic retention time (HRT) for the first Cannibal system was one hour while the initial selector HRT for the second Cannibal system was 30 minutes and later increased to 2 hours. The purpose of using a selector was to increase the substrate pressure. It was thought that the selector could assist the slow feed Cannibal system to produce flocs that are structural similarly to those generated from the fast feed Cannibal system. In addition, the increase in the selector hydraulic retention time (HRT) were

anticipated the system to generate more amounts of flocs that are greater susceptible to the Cannibal system.

As can be seen from Figure 4-7, a higher cumulative solids was found in the Cannibal system with 30 minute selector HRT compared to the system with 1 hour selector HRT. On day 14, the HRT of the selector for the Cannibal system was changed from 30 minutes to 2 hours. The data from Figure 4-7 illustrate that the increase in selector HRT resulted in a reduction of solid generation in the Cannibal system. The data also reveal that less accumulated solids were found in the system with 2 hour selector HRT than the system with 1 hour selector HRT. This shows that a higher selector HRT, up to two hours, gives better solids reduction. It is thought that the slow feed operation provides a low food per microorganism ratio (F/M ratio) and this makes the system performance similar to a complete mix operation. However, when a selector with 1 hour HRT or higher was added to the slow feed Cannibal system, the substrate pressure was increased, resulting in higher F/M ratio. In addition, the increase in the selector HRT could help to decrease solids accumulation, making the system performance close to a plug flow operation. The data also indicate that the characteristics of the sludge from the Cannibal system at different selector HRTs produce sludge with different characteristics. The sludge produced under the lower selector HRT condition does not respond as well to the Cannibal system as does sludge from the higher selector HRT environment. The longest selector HRT was 2 hours. It is not known if a longer HRT would be beneficial.

The higher solids reduction for the Cannibal system with a selector placing in front of an aerobic reactor is reflected in the observed yield data shown in Figure 4-8. The

observed yield varied from a high value of 0.26 mg TSS/mg COD for the Cannibal system with slow feed to 0.18 mg TSS/mg COD for the Cannibal system with 2 hour selector HRT. These data show that adding a selector in front of an aerobic reactor is another alternative to increase the solids reduction for the slow feed Cannibal system.

In general, the formation of floc occurs in the aerobic reactor. By adding a selector in front of the aerobic reactor, a high F/M ratio is established in the selector. In this case, floc-forming microorganism can out-compete the filamentous bacteria. As a result, the filamentous bacteria that may cause poor settling can be eliminated. This can result in higher growth of floc-forming microorganisms, resulting in excellent flocculation as evidenced by the high quality of the effluent. Additionally, it can be hypothesized that the structure of flocs generated in the system with a selector HRT higher than one hour may be similar to that from the fast feed Cannibal system (feeding is provided to the Cannibal system over a period of 5 minutes) which is more susceptible to degradation than the flocs from the slow feed Cannibal system.

It can be seen that when a 30 minutes HRT selector was added to the slow feed Cannibal system, the solids reduction was not much different, as indicated by the observed yield data (0.25 and 0.26 for the slow feed Cannibal system with and without a selector, respectively). When intermittent aeration or a 1 hour HRT selector was used in the slow feed Cannibal systems, the solids reduction was similar, as indicated by the observed yield of 0.21 for both the slow feed Cannibal systems with intermittent aeration and a selector). This suggests that the performance of both systems is comparable. However, when the selector HRT was increased to 2 hours, the observed yield was reduced to 0.18, indicating

better system performance.

The effect of recycle ratio was studied at the last stage of this phase. The ratios used in this study were 1:1, 2:1, and 3:1. It can be seen from Figure 4-9.that when the recycle ratio is increased at the particular selector HRT, the observed yield decreased. The data also show that increasing the recycle ratio does not guarantee the reduction of the observed yield, as can be seen by the unchanged yield when the recycle ratio was increased from 2:1 to 3:1 and from 1:1 to 2:1for the slow feed Cannibal systems with 0.5 and 1 hour selector HRT, respectively. However, when 2 hour selector HRT was used in the system, the increase of any recycle ratio reduced the observed yield. This implies that the capability of the selector that has HRT of at least 2 hour in solids reduction assistance appears to be influenced by the recycle ratio. The data from both Figure 4-8 and 4-9 indicate that increasing either the selector HRT or the recycle ratio can enhance the performance of the slow feed Cannibal system. This suggests that both play important roles in biological solids reduction. Nevertheless, the data from Figure 4-9 indicate that increasing the selector HRT from 0.5 to 2 hours while the recycle ratio is maintained can result in more solids reduction than the increase in the recycle ratios from 1:1 to 3:1 while the selector HRT is unchanged, as indicated by the reduction of the observed yield of approximately 0.08 and 0.02 for the Cannibal system with the increase of the selector HRT and the recycle ratios, respectively. This implies that the selector HRT increment may play more vital role than the increase of the recycle ratios in solids reduction.

Mechanism of Floc Destruction

The structural composition of activated sludge flocs can be considered as microorganisms (predominantly floc-forming and filamentous bacteria), organic matter in addition to microbial cells, cations and anions (Higgins, M. J. et al., 1997). Extracellular polymeric substances (EPS) are a primary part of organic matter and can be composed of intracellular materials transported to the extracellular environment by active secretion or export, cellular components released by cell rupture, hydrolyzed or digested exocellular substances and materials adsorbed from the environment such as wastewater that has been fed to an activated sludge system (Nielsen, P. H. et al., 1998). Proteins are the most abundant in activated sludge EPS (Nielsen, P. H. et al, 1996). The viscous properties of EPS in activated sludge floc are responsible for microbial colonies (aggregation) and also bind cells to other particulate materials (cohesion), leading to the flocculent characteristic of activated sludge (Wingender, J., 1999).

The study of flocs structure was made by Novak et al (2003). They proposed that flocs comprise of two important biopolymer fractions. The first floc model is lectin-like proteins that are linked to polysaccharide and bridged by divalent cations (Ca^{2+}, Mg^{2+}) and the other one is biopolymer which is mainly proteins bounded to iron called Fe-associated EPS floc. They also suggested that lectin-like flocs were mainly degraded under aerobic condition while Fe-associated EPS flocs are readily degraded under anaerobic condition.

Park et al. (2006) found that the major mechanism for sludge degradation during anaerobic digestion was the reduction of iron with the release of iron associated organic matter, primarily protein, which is easily degraded. This mechanism was applied to the solid reduction in the Cannibal process by Novak et al (2003). That is, when settled sludge is cycled to an anaerobic bioreactor, iron is reduced, and organic matter is released and solubilized. The released materials and sludge are returned to the aerobic zone and is rapidly degraded before the released materials can be reflocculated. However, Khanthongthip et al. (in preparation) found that the sludge reduction was very different when the Cannibal systems were operated under different feed patterns. They found that sludges generated under the high substrate pressure (fast feed) were more susceptible to the Cannibal system than those generated from the low substrate pressure (slow feed).

The use of intermittent aeration in the slow feed Cannibal system showed the benefit in the solid reduction, as indicated by the decrease of the observed yield. It is thought that when the air is switched off, some of readily biodegradable organic matters are fermented to volatile fatty acids (VFAs) by facultative microorganisms. Aerobic microorganisms cannot transport and store or metabolize the organic matters due to the absent of a terminal electron acceptor, molecular oxygen. Consequently, some of organic substrates are accumulated in the solution, making higher substrate pressure. Because sulfate is presented in the solution, the facultative microorganism such as Fe(III)-reducing microorganisms can use the sulfate as a terminal electron acceptor and the VFAs as a substrate (Lovley et al, 1988). This can result in the production of free sulfide ions. These free sulfide ions are thought to be capable of reducing Fe(III) associated with EPS in flocs,

resulting in an increase in the deflocculation. However, it appears that Fe(III) reducers cannot conserve much energy for their growth, resulting in less activity (less new cell synthesis and less sulfide production) (Lovley et al, 2004). This suggests that although the sulfide production can help in floc disaggregation, the deflocculation does not deteriorate the effluent quality, as evident by the data in Figure 4-3. In addition, if the phosphorus accumulating organisms (PAOs) are present, they can transport VFAs into the cell and store them as polyhydroxyalkanoic acids (PHAs) and other carbon storage polymers, using energy from the cleavage of intracellular polyphosphate and releasing inorganic phosphorus to the solution. When the air is switched on, aerobic microorganisms can rapidly uptake organic matters due to the high substrate pressure. This makes microorganisms grow and later form flocs similar to those generated in the fast feed Cannibal system. Additionally, the PAOs can use the stored substrate (PHAs) for their growth and to provide energy for reforming polyphosphate by taking the phosphorus from wastewater. In this case, the PAOs do not help much in phosphorus removal during the period of intermittent aeration because the release and uptake of phosphorus were taking place in the same reactor. However, they can uptake the volatile fatty acids as a substrate during the periods without air supply, resulting in COD removal.

The use of a selector in the slow feed Cannibal system increased the solids reduction, as shown by the lower observed yield in Figure 4-8. In this case, the selector assists the system performance by increasing the substrate pressure. The data for the reduction of solids accumulation in Figure 4-7 imply that, under aerobic and slow feed condition with selector assistance, feed organics were converted to storage products that

were bound to iron to form flocs while much less material was stored and bound to iron under slow feed and aerobic environment. It can be hypothesized that the Cannibal system operated under slow feed condition with the selector assistance produces flocs that are structural similarly to those generated from the fast feed Cannibal system. Higher selector hydraulic retention time (HRT) can make the system generate more flocs that are susceptible to the Cannibal system. In addition, when the selector was used with the settled sludge transferred from the SBR, aerobic microorganisms can help to degrade materials recycled from the anaerobic bioreactor. It is thought that aerobic microorganisms were more active in the selector because high substrate was provided, resulting in greater amounts of solids reduction.

Implications

The utilizations of either the intermittent aeration or the selector addition in front of an aerobic reactor for the slow feed Cannibal system demonstrated the benefit in decreasing the solids production in the slow feed Cannibal system. This indicates that flocs generated under those two conditions are more responsive to the Cannibal system than the flocs from the Cannibal system operated under exclusively slow feed. This implies that the characteristics and structures of flocs produced in both conditions might be similar to those generated under fast feed environment. Consequently, additional research is needed to examine the characteristics and structures of those flocs generated under two different

environments. In addition, Khanthongthip et al. (in preparation) found that more amounts of microbially reducible iron flocs were disaggregated in the fast feed than the slow feed Cannibal system. They proposed that Fe(III)-reducing microorganisms might be more active in the fast feed than the slow feed environment. Therefore, the disintegration of microbially reducible iron flocs (the different between microbially reducible iron flocs in an aerobic reactor and those from an anaerobic bioreactor) in the slow feed Cannibal system with the intermittent aeration and that system with a selector addition is of interest and needs further investigation.

Conclusions

The increase of substrate pressure by operating the slow feed Cannibal system with the intermittent aeration showed the benefit to the system. It is thought that when the air is switched off, the substrate concentration is increased because aerobic microorganisms cannot take much substrate without a terminal electron acceptor. However, when the air is switched on, the microorganisms are able to take more substrate and electrons are rapidly transferred to the terminal electron acceptor which is molecular oxygen, resulting in the generation of higher amounts of biodegradable flocs compared with the flocs generated in the common slow feed Cannibal system.

The use of a selector assists the slow feed Cannibal system to decrease the solids production. It is thought that the selector increases substrate pressure and assists the system to generate flocs similar to those from the fast feed Cannibal system. Additionally, the increased substrate pressure can accelerate microbial activity to quickly degrade the

released materials and solids returned from the anaerobic bioreactor. The data suggest that the selector HRT of at least 2 hour appear to be necessary.

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Table 4-1. Medium composition.

NAME	FORMULA	CONCENTRATION (mg/l)
Bacto Peptone	-	300mg COD/l
Sodium Acetate	CH_3COONa	100mg COD/l
Ammonium Chloride	NH_4Cl	57
Ammonium Bicarbonate	NH_4HCO_3	60
Potassium Phosphate	KH_2PO_4	44
Potassium Bisulphate	$KHSO_4$	34
Sodium Bicarbonate	$NaHCO_3$	394
Calcium Chloride	$CaCl_2.2H_2O$	220
Magnesium Sulfate	$MgSO_4.7H_2O$	150
Ferric Chloride	$FeCl_3$	-
Alum	$Al_2(SO_4)_3.18H_20$	-
Allylthiourea	-	6.0
Trace Element Solution	-	2ml/l

Table 4-2. Trace element solution composition.

NAME	FORMULA	CONCENTRATION (gm/l)
Citric acid	-	2.73
Hippuric acid	-	2
Ethylene Diamine Tetraacetic Acid, trisodium salt	$Na_3EDTA.4H_2O$	1.5
Ferric Chloride	$FeCl_3.6H_2O$	1.5
Boric Acid	H_3BO_3	.25
Zinc Sulphate	$ZnSO_4.7H_2O$	.15
Manganese Chloride	$MnCl_2.4H_2O$	.12
Copper (II) Sulphate	$CuSO_4.5H_2O$	.06
Potassium Iodide	KI	.03
Sodium Molybdate	$Na_2MoO_4.2H_2O$	.03
Cobalt chloride	$CoCl_2.6H_2O$	.03
Nickel (II) Chloride	$NiCl_2.6H_2O$	.03
Sodium Tungstate	$NaWO_4.2H_2O$	.03

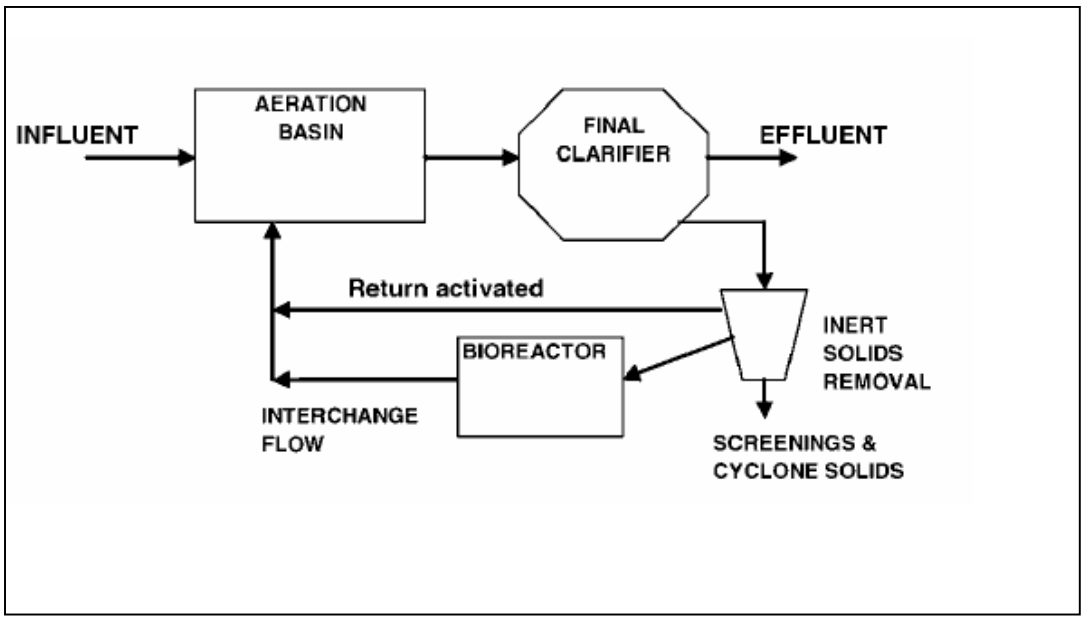

Figure 4-1(a). Flow scheme for the typical Cannibal process.

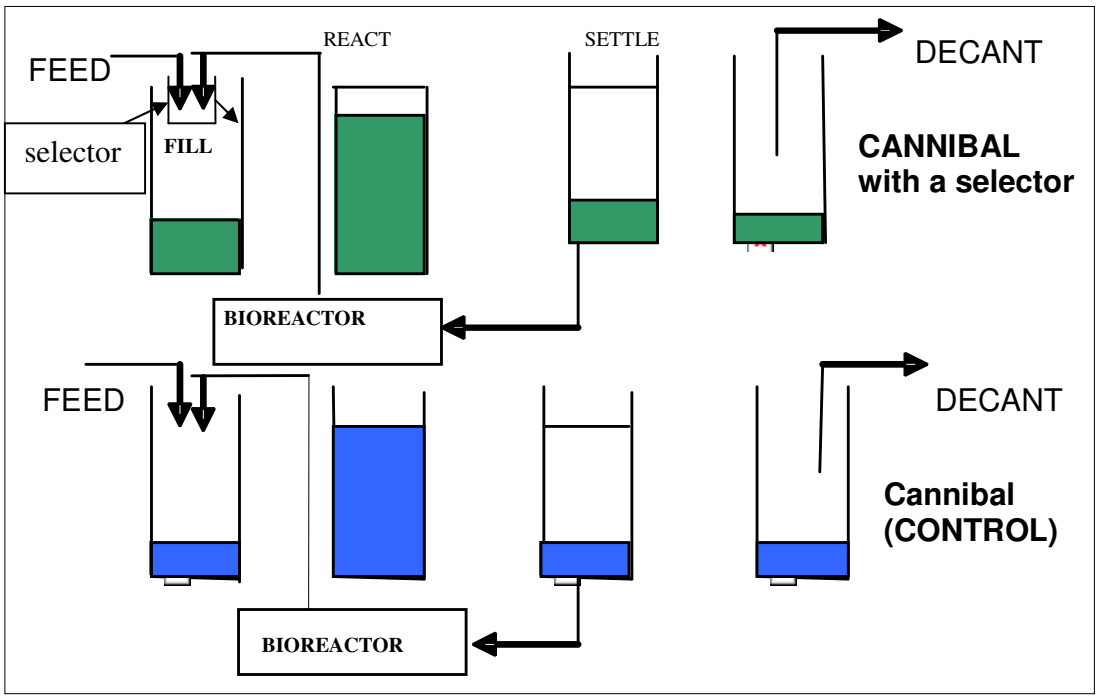

Figure 4-1(b). Laboratory operations for Cannibal and control systems.

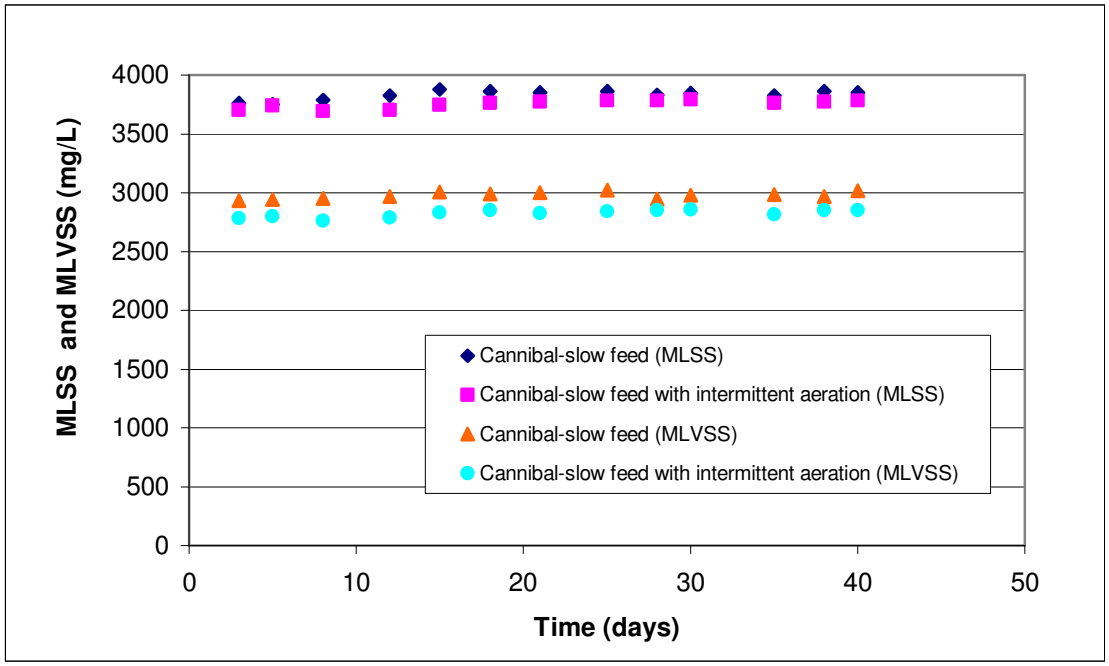

Figure 4-2. MLSS and MLVSS for the slow feed Cannibal and the slow feed Cannibal with intermittent aeration.

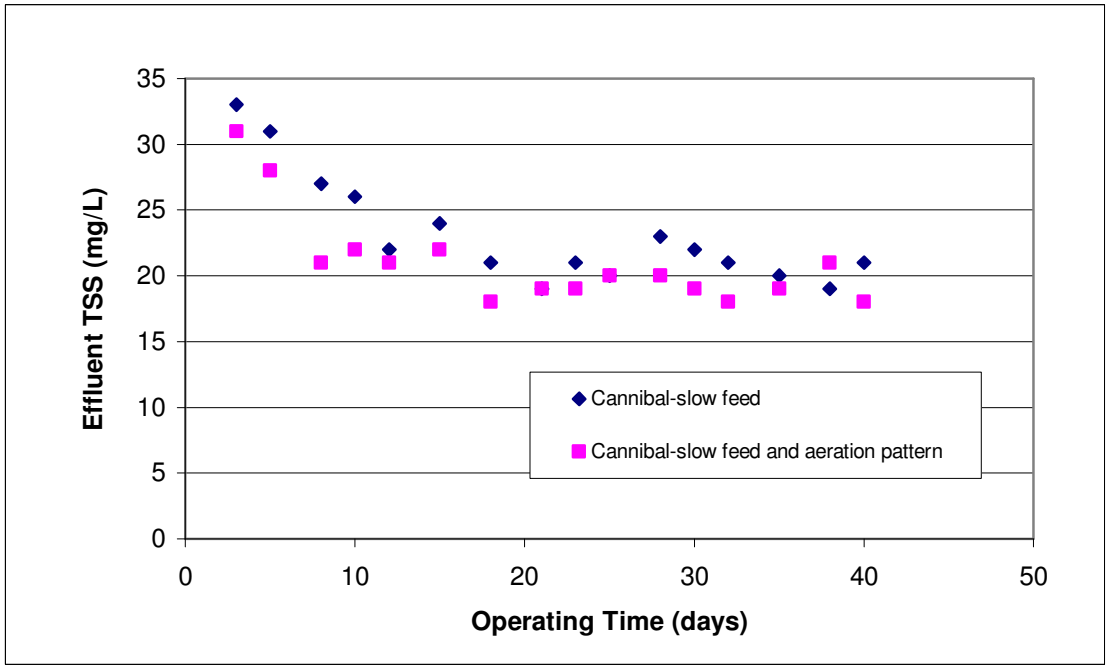

Figure 4-3. Effluent total suspended solid for the slow feed Cannibal system (control) and the slow feed Cannibal system with intermittent aeration.

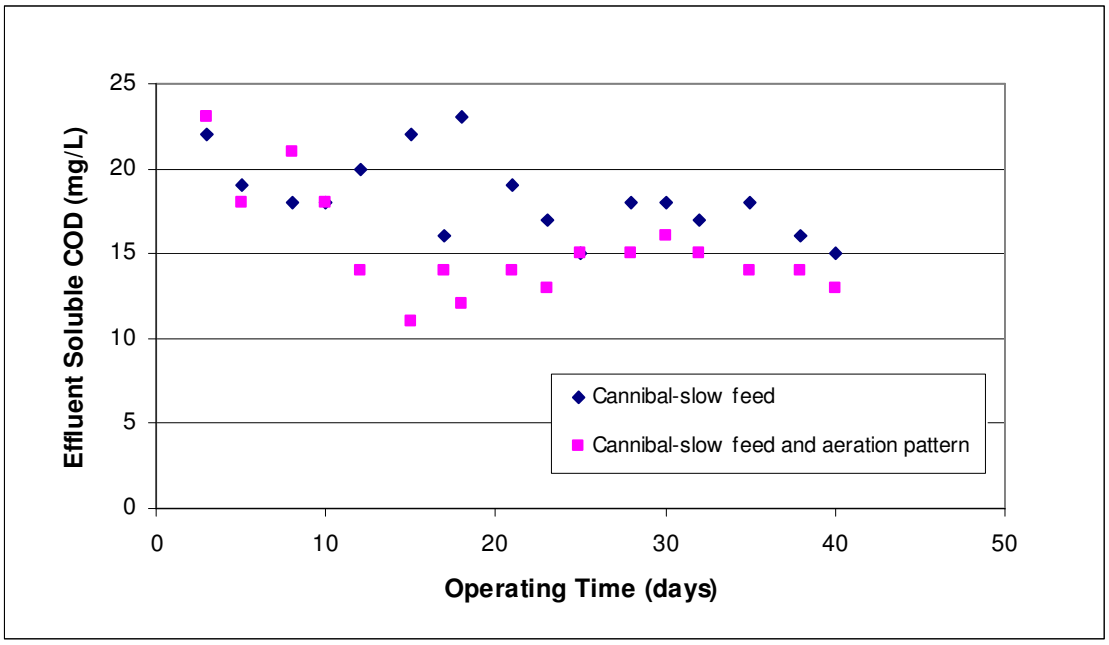

Figure 4-4. Effluent soluble COD for the slow feed Cannibal system (control) and the slow feed Cannibal system with intermittent aeration.

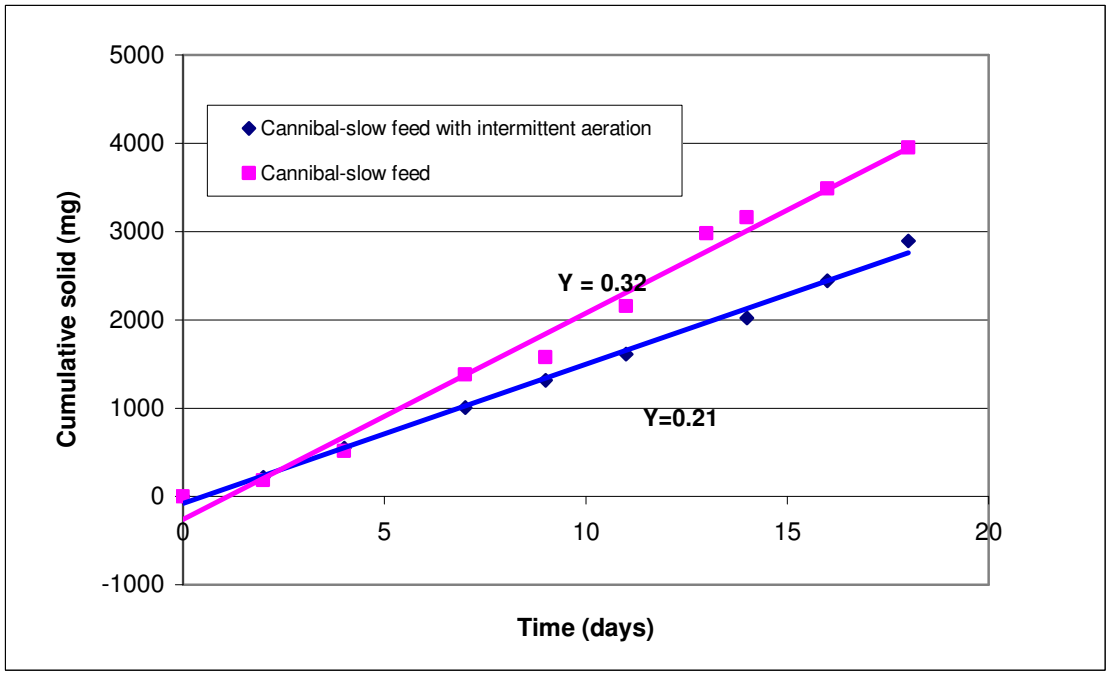

Figure 4-5. Effect of the intermittent aeration on solid reduction in the slow feed Cannibal system. (The unit of observed yield is mg TSS/mg COD)

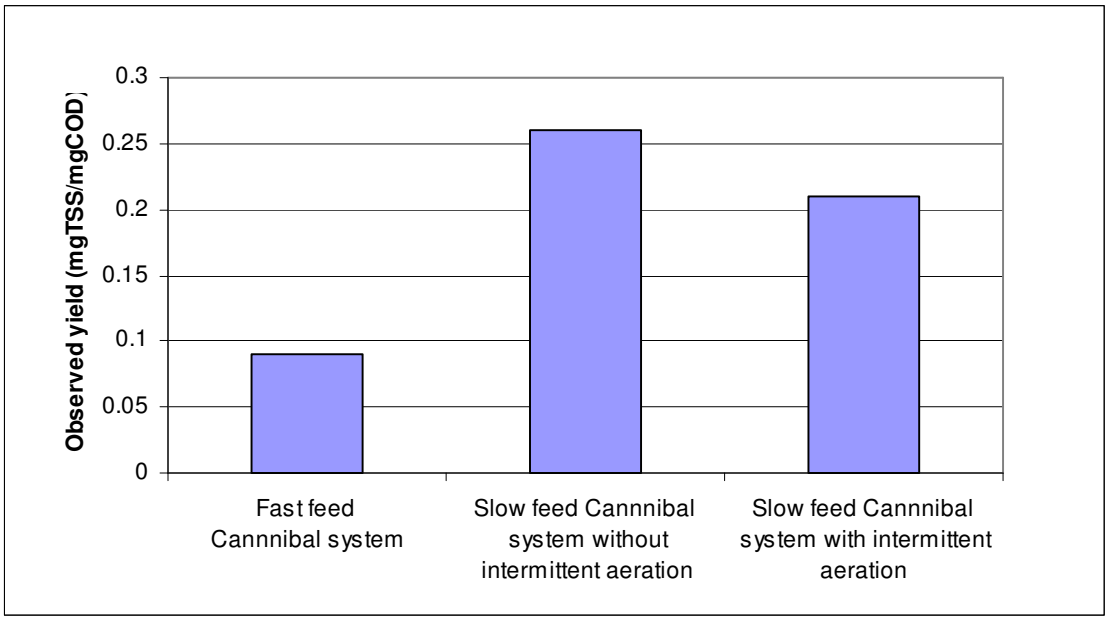

Figure 4-6. Observed yield for the fast and the slow feed Cannibal systems with and without intermittent aeration Fast feed data were taken from Khanthogthip et al. (in preparation)).

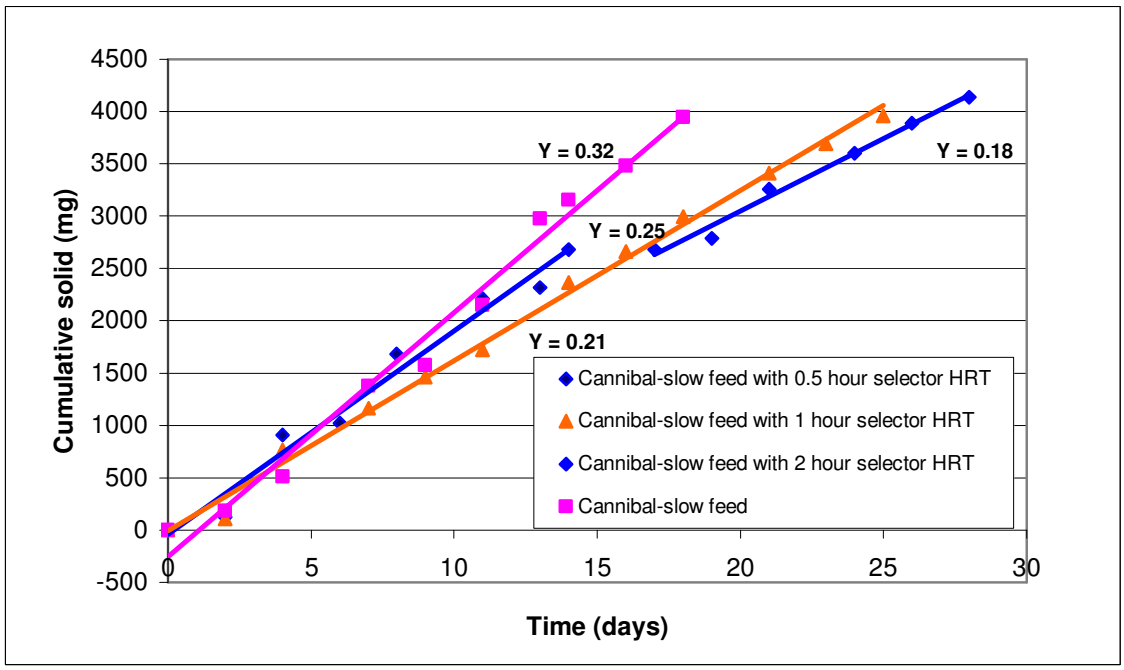

Figure 4-7. Effect of a selector on solids reduction in the slow feed Cannibal system.

(The unit of observed yield is mg TSS/mg COD)

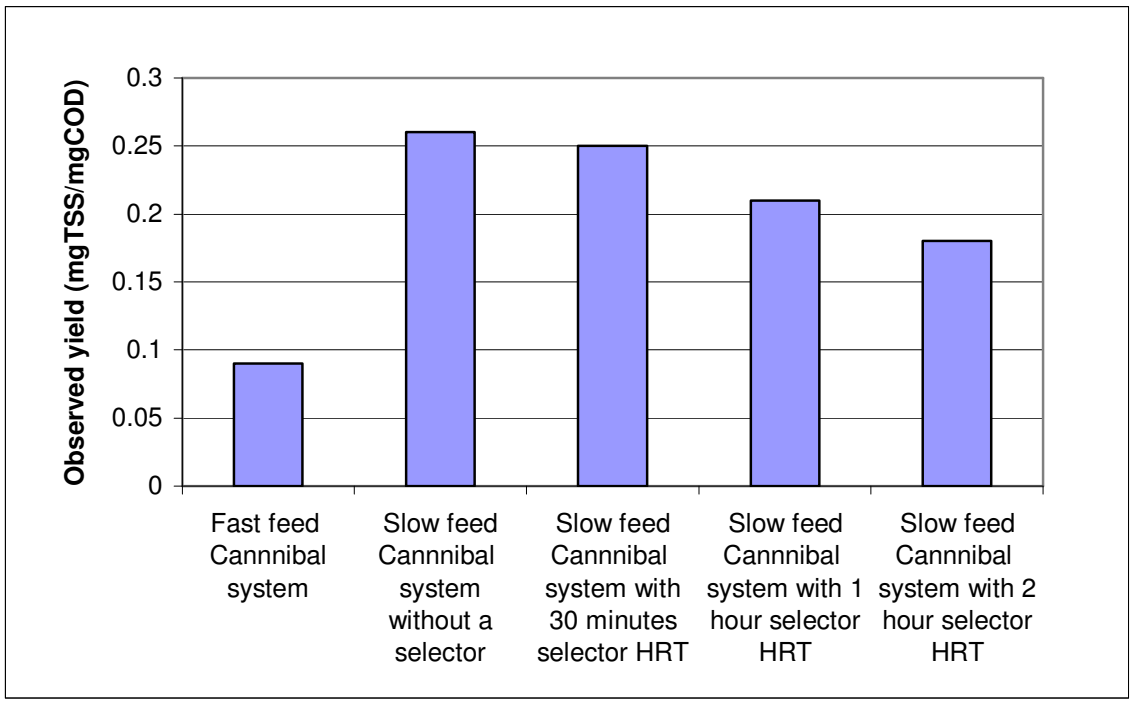

Figure 4-8. Observed yield for the fast and the slow feed Cannibal systems with different selector hydraulic retention time (HRT). (Fast feed data were taken from Khanthogthip et al. (in preparation))

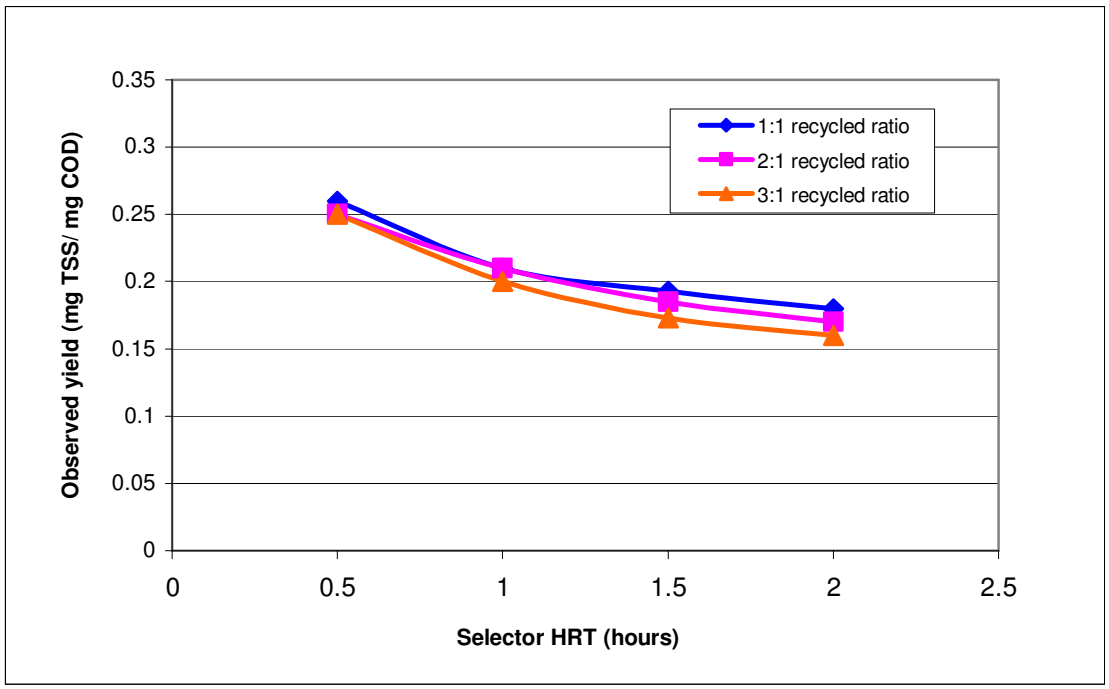

Figure 4-9. Effect of the various selector HRT and recycle ratios on the observed yield for the slow feed Cannibal systems.

Chapter 5. The Mechanisms for Sludge Reduction in the Cannibal Process under High and Low Substrate Pressure

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The Mechanisms for Sludge Reduction in the Cannibal Process under High and Low Substrate Pressure

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Abstract

The mechanisms for sludge reduction in the Cannibal process operated under high and low substrate pressure were studied to gain an insight in the performance of this innovative sludge reduction process. Side-by-side Cannibal sequencing batch reactor (SBR) activated sludge systems were operated, one receiving the substrate over a period of 5 minutes (fast feed) during the feed cycle and the other receiving the substrate over 4 hours (slow feed). The different feeding times resulted in a spike in the substrate concentration or high substrate pressure for the fast feed system while the substrate concentration remained low and constant in the slow feed system. It was found that larger amounts of proteins and polysaccharides accumulated in the anaerobic bioreactor of the fast feed system which had enhanced biological solids reduction, as reflected by lower observed yield. Greater amounts of 1 kDa protein as well as ammonium ions were found in the fast feed system and this showed that the released proteins were readily biodegradable. Additionally, it was found

that greater amounts of sulfate were used in the fast feed Cannibal system, indicating more free sulfide ion generation. The negative charges of the free sulfide ions can help to reduce Fe(III) in Fe-associated EPS flocs, resulting in higher floc destruction. Hydrogen was also produced in anaerobic reactors of both systems but it was not high enough to interfere the system performance. The greater activity of both sulfate reducers and methanogens in the fast feed system led to more volatile fatty acid and hydrogen utilization, more fermentation, and more floc destruction.

Keywords: sludge, biosolids, activated sludge, solids reduction, sulfate-reducing microorganisms, methanogens, hydrogen partial pressure, digestion.

Introduction

The activated sludge process is the primary method used for treating municipal wastewater before its discharge into receiving waters. Despite its usefulness in treating a wastewater, from various sources, the process also produces excess biomass (waste activated sludge), which needs to be properly treated prior to its ultimate disposal. A significant cost in the activated sludge process also lies in solids handling processes downstream of the activated sludge system (Crites and Tchobanoglous, 1998), rendering proper and efficient treatment of sludge of great importance.

A wide range of processes that can reduce the mass of biological sludge generated from wastewater treatment processes have been proposed. Many of these processes have

been evaluated in terms of their sludge reduction potential, ease of implement into existing facilities, impacts on plant operation and effluent quality, reliability and risk associated with the process. These processes included anaerobic-aerobic configurations in the activated sludge process, anaerobic treatment; chemical, mechanical, and thermal strategies to cause cell disruption, the addition of chemicals that uncouple cellular energy utilization, the use of protozoa for bacteria consumption, and extreme solid retention time (SRT) processes. However, most of these either add more plant operating costs or are inappropriate from a practical point of view.

One of the most promising sludge reduction processes is anaerobic-aerobic cycling known as the Cannibal process. This is an activated sludge process modified by incorporating an anaerobic sidestream reactor for return activated sludge prior to returning it to the aerobic reactor. A previous study by Novak et al. (2007) demonstrated that this process generates about 60% less solids than conventional activated sludge system without any negative effects on the effluent quality or settling characteristics. That study was conducted under a specific operation in which the return sludge was retained in an anaerobic bioreactor for 10 days and the interchange rate (the exchange of sludge between anaerobic and aerobic bioreactor) was 10% per day by mass. Later, Khanthongthip, P. et al. (in preparation) studied the influence of substrate pressure on the performance of this system and found that the system produced less solid reduction when it was operated under low substrate pressure which was represented by feeding influent wastewater to a sequencing batch reactor over 4 hours (slow feed) in each cycle. However, when high substrate pressure was provided by feeding the influent to the system over 5 minutes, the

solid reduction was higher. Therefore, it is thought that the sludge generated from a high substrate pressure system might be more susceptible to degradation than that from a low substrate pressure system.

In order to understand the mechanism of solids loss in the Cannibal system operated under different substrate pressures, it was essential to characterize the floc structure and behavior under aerobic and anaerobic conditions. In general, the structural composition of activated sludge flocs can be considered as microorganisms, organic matter in addition to microbial cells, cations, and anions (Higgins, M. J. et al., 1997). From a biological point of view, bioaggregates such as activated sludge flocs or biofilms consist of cells and organic matter exterior to cells, which has been characterized as extracellular polymeric substances (EPS) or exocellular biopolymers. EPS can be composed of intracellular materials transported to the extracellular environment by active secretion or export, cellular components released by cell rupture, hydrolyzed or digested exocellular substances, and materials adsorbed from the raw wastewater that has been fed to an activated sludge system (Nielsen, P. H. et al., 1998). In general, activated sludge EPS consist of lipids, nucleic acids, humic substances, proteins, and polysaccharides (Frølund, B. et al., 1996). A number of recent studies have shown that proteins are the most abundant EPS component in activated sludge (Nielsen, P. H. et al, 1996). The viscous properties of EPS in activated sludge floc are responsible for microbial colonies (aggregation) and also bind cells to other particulate materials (cohesion), leading to the flocculent characteristic of activated sludge (Wingender, J., 1999). In addition, the negative charges of EPS bind to cations for floc formation. EPS in activated sludges and biofilms also promote cell-cell recognition and

communication (quorum sensing) and protect cells from severe environmental conditions such as turbulence, dehydration, antibiotics and biocides (Wingender, J., 1999).

Novak et al. (2003) proposed that flocs consist of two important biopolymer fractions, which are divalent cation-bound biopolymer and Fe-associated biopolymers. They also found that aerobic digestion of activated sludge released calcium (Ca^{2+}) and magnesium (Mg^{2+}) into solution in conjunction with volatile solid destruction and accumulation of polysaccharide. In contrast, when the sludge underwent anaerobic digestion, a large amount of protein was released but no increase in divalent cations was found.

Therefore, those forms of flocs are expected to be presented in sidestream anaerobic bioreactor. Park et al. (2006) proposed that the major mechanism for sludge degradation during anaerobic digestion was the reduction of iron with the release of iron associated organic matter, primarily protein, which is easily degraded. Novak et al. (2007) used this mechanism to describe the solid reduction in the Cannibal process. They suggested that when settled sludge was cycled to an anaerobic bioreactor, iron was reduced, and organic matter was released and solubilized. The released materials and sludge were returned to the aeration basin and were rapidly degraded. However, recent research in our lab has shown that up to 70 % of the solids loss that occurs in the Cannibal process can occur in the anaerobic bioreactor. That is, while the mechanism proposed by Novak et al. (2007) accounts for some of the solid loss, solids loss across the anaerobic bioreactor is also important.

The objective of this study was to thoroughly investigate the mechanisms of the solids loss across the anaerobic bioreactor in the Cannibal system. These studies included characterization of anaerobic bioreactor such as the release of protein and polysaccharide, volatile fatty acids (VFA) and gas production, and cations and anions in the solution. This study was also expected to provide a better understanding of solid reduction when this system was operated under high and low substrate pressure.

Materials and Methods

In this study, two Cannibal systems were operated under fast and slow feed conditions in which the feeding was provided to the Cannibal systems in a period of 5 minutes and 4 hours, respectively. The system configuration and operation can be found in Khanthongthip et al., (in preparation). The hydraulic retention time (HRT) for both systems was 10 days and later was reduced to 5 days. The compositions of synthetic wastewater are shown in Table1. Soluble COD, volatile fatty acids, gases, proteins, polysaccharides, cations, and anions in the solution phase in anaerobic bioreactor of fast and slow feed Cannibal system were measured to compare solid reduction capability. Additionally, solution phase materials were fractionated using regular filtration and ultrafiltration to determine the molecular weight distribution of biopolymers, protein and polysaccharide in the anaerobic bioreactors of the fast and slow feed systems. For this experiment, all sludge samples were centrifuged at 9000 g for 15 minutes. An aliquot of centrate was individually filtered

through 1.5- and 0.45-µm membrane filters and the 30,000- and 1000-Dalton ultra filters. Ultrafiltration was performed at 414 kPa (60 psi) through Amicon YM30 (30 kDa) and YM 1 (1 kDa) partly hydrophilic membranes (Amicon, Beverly, Massachusetts). The terms "soluble or solution" refers to the material that can be filtered through 0.45-µm.

Activated sludge from the aerobic reactors of the fast and slow feed systems, that the anaerobic bioreactors were operated with 10 day HRT, was anaerobically digested at room temperature (21^{o}C) and the release of soluble COD, proteins, and polysaccharides was monitored over a certain period of time to see the release rate of these materials. For this experiment, 200 ml of settled sludge from the SBR of each system was placed in a 300 ml batch reactor without mixing. Distilled water was added to the reactor to make up for evaporative loses. A rubber stopper with a gas bag to serve as a gas collector was placed on the top of the reactor and tightly sealed to prevent gas leakage and to insure anaerobic conditions in order to provide an environment similar to the anaerobic bioreactor.

Analysis

The protein was determined by the Hartree et al. (1972) modification of the Lowry et al. (1951) method to account for the interference of phenolic compounds and humic substances in protein quantification. The standard was bovine serum albumin. Polysaccharide was measured by phenol-sulfuric acid method of Dubois et al. (1956) using glucose as the standard. The soluble cations, calcium (Ca^{2+}), potassium (K^{+}), ammonium (NH_4^{+}), magnesium (Mg^{2+}), and sodium (Na^{+}), and anions, were determined by a Dionex

(Sunnyvale, California) ion chromatography. Chemical oxygen demand (COD) was measured using Standard Methods (APHA et al, 1995). The pH was measured using an Accumet 910 pH meter (Fisher Scientific, Pittsburgh, Pennsylvania).

The gases were measured using Shimadzu Gas Chromatograph-14A with a Thermal Conductivity Detector (TCD). The gases of primary interest were methane and hydrogen. Volatile fatty acids (VFA) were determined using a Shimadzu Gas Chromatograph equipped with a Flame Ionization Detector (FID).

Results and Discussion

In this study, a side by side comparison of reactor characteristics associated with solids reduction in the anaerobic bioreactors of the Cannibal systems with high substrate pressure (fast feed) and low substrate pressure (slow feed) was made. The hydraulic retention time (HRT) in the anaerobic bioreactors of both systems was 10 days and later was reduced to 5 days. Soluble chemical oxygen demand (COD), volatile fatty acids (VFA), gases, proteins, polysaccharides, and cations and anions in the solution phase were measured to evaluate solids reduction processes and associated changes in constituents. In addition, the concentration of soluble COD, proteins, and polysaccharides was monitored in the anaerobic bioreactor to quantify the amount of these materials under fast and slow feed conditions. As can be seen from Table 5-3, more proteins were found in the 5 and 10 day HRT anaerobic bioreactors of the fast feed system than in the slow feed Cannibal system. It

is thought that under anaerobic conditions, proteins, and to a lessor extent, polysaccharide, are released to solution when iron is reduced (Novak et al., 2007). The lower concentrations of proteins and polysaccharides in the 5 day detention time reactor is associated with lower solids loss in the Cannibal system as measured by Easwaran et al. (2006). Greater amounts of 1- kDa molecular weight protein were found in the anaerobic bioreactor of the fast feed system. These small proteins are thought to be readily biodegradable.

The data from Table 5-4 show that higher amounts of ammonium ions accumulated in the fast feed systems compared to the slow feed systems. This indicates that some of the protein in the anaerobic bioreactor of this system was degraded. These data support the data in Table 5-3 which shows that proteins in the anaerobic bioreactor of the fast feed Cannibal system were more readily biodegradable than in the slow feed system. The data for protein accumulation imply that, under aerobic and fast feed conditions, feed organics were converted to storage products that were bound to iron to form flocs while much less material was stored and bound to iron under low substrate pressure (slow feed). Therefore, when flocs formed under fast feed condition were recycled to the anaerobic bioreactor, much more biopolymer, primarily protein, was released, solubilized, and degraded when compared to the system operating under slow feed conditions.

It is known that the release of ammonium ions results from degradation of proteins. In general, a mixture of proteins contains approximately 16 % nitrogen (Wrolstad et al., 2005). As shown in Table 5-4, the concentration of ammonium ions found in the anaerobic bioreactors of fast and slow feed Cannibal systems is 174 and 93 mg/L. This indicates that approximately 1090 and 580 mg/L of proteins were degraded in the 10 day HRT anaerobic

bioreactors of the fast and slow feed systems. Therefore, it can be seen that greater amounts of protein were released and degraded in the anaerobic bioreactor of the fast feed system than the slow feed system. Also, it can be seen that the degraded protein in the anaerobic bioreactor of the slow feed system was about 53% of that in the fast feed system.

As shown in Table 5-4 and 5-5, some amounts of solution polysaccharide coupled with divalent cations, calcium and magnesium, were found in anaerobic bioreactors of both Cannibal systems. This suggests that other types of flocs organics, perhaps lectin proteins which usually degraded under aerobic environments (Novak et al., 2003), were deflocculated in both Cannibal systems.

It is possible that the accumulation of higher levels of protein, polysaccharide, and cations in the fast feed system is a result of cell lysis. Cell lysis could occur when aerobic sludge is added to the anaerobic reactor during daily feeding. When the cells lyse, the products from cell lysis such as intracellar and extracellular protein, polysaccharide, and the materials from cytoplasm (potassium, sodium, calcium, and magnesium) are released into solution.

The release of materials from floc destruction was studied to evaluate the susceptibility of the flocs generated under the fast and the slow feed conditions during deflocculation in anaerobic condition. This was done by measuring the accumulation of the materials (e.g., protein, polysaccharide, soluble COD, ammonium ions) that were released from the floc during anaerobically floc destruction into the solution over a period of time. The release of soluble COD, proteins, and polysaccharides from aerobic sludge taken from SBRs of fast and slow feed systems with 10 day HRT in the anaerobic bioreactors are

shown in Figures 5-1, 5-2, and 5-3, respectively. It can be seen that greater amounts of proteins and polysaccharides were released from the fast feed Cannibal sludge. This is consistent with the higher levels of protein and polysaccharide found in the anaerobic bioreactor of the fast feed system. In addition, more ammonium ions were generated in the fast feed system as shown in Figure 5-4. This indicates that the released proteins are being biodegraded.

The accumulation of biopolymers, proteins and polysaccharides in the anaerobic bioreactors of both the fast and slow feed Cannibal systems is reflected in the observed yield data shown in Figure 5-5. It can be seen that the yields varied from a high of 0.36 mg TSS/mg COD for the Cannibal system with slow feed and 5 day HRT in anaerobic bioreactor to 0.12 mg TSS/mg COD for the system with fast feed and 10 day HRT in the anaerobic bioreactor. The higher soluble protein and polysaccharide found in the anaerobic bioreactor of the fast feed system results in the lower yield while lower amount of biopolymers found in the slow feed system resulted in higher yield. This indicates that more floc material was disaggregated and degraded in the fast feed system.

It is likely that both the feed patterns and the HRT of the anaerobic bioreactors play a role in solids reduction. Both fast feed Cannibal system with 10 and 5 day anaerobic HRT resulted in a lower observed yield than the systems with slow feed. This indicates that feed patterns play a role in solids reduction regardless of the HRT in the anaerobic bioreactors.

After floc disaggregation, the released biopolymers, proteins, polysaccharides, and lipids, can be hydrolyzed to amino acids, sugar, and long-chain fatty acids, respectively. Amino acids and sugars can be further degraded by fermentative reactions in which organic

compounds serve as both electron donors and acceptors. As shown in Figure 5-6(a), the principal products of fermentation are intermediate degradable products such as propionic and butyric acids and the methane precursors, acetic acid and hydrogen gas. The production of hydrogen gas is very important to the proper function of anaerobic processes. Hydrogen is one of the primary substrates for metanogenesis and can be produced by fermentation. If hydrogen formation does not occur and reduced organic products are formed, they will accumulate in solution because they cannot be used as substrates for methane production. Acetate and hydrogen gas are the usual electron donors for methanogens (Muyzer et al., 2008). Reactions leading from long chain fatty acids, sugar, amino acids, and reduced compounds to acetic acid and hydrogen gas are thermodynamically unfavorable under standard conditions, having positive free energies. Thus, when the hydrogen partial pressure is high, these reactions will not proceed, but fermentations can still take place. When the partial pressure of hydrogen gas is 10^{-4} atmospheres (atm.) or less, the reactions are thermodynamically favorable and can proceed (McCarty et al., 1986), leading to the end products, acetic acids and hydrogen gas. The end products can be further converted to methane by methanogens. This means that the microorganisms that produce hydrogen gas are linked to methanogens that use hydrogen gas as a substrate. When the hydrogen is continuously removed by hydrogen-utilizing microorganisms, the partial pressure of hydrogen gas can be kept low enough to allow the production of hydrogen and acetic acids. The partial pressures of hydrogen gas in anaerobic bioreactors of both Cannibal systems are shown in Table 5-7. It can be seen that hydrogen partial pressure in both Cannibal systems were less than 10^{-4} atm. This suggests that the

activity of hydrogen-utilizing microorganisms in both Cannibal systems was high enough to maintain hydrogen gas at a level for active fermentation. Consequently, the Cannibal systems could help to reduce biological solids regardless of the feed patterns.

In anaerobic environments, sulfate-reducing microorganism can compete with other anaerobes including fermentative microorganisms, proton-reducing microorganism, homoacetogens, and methanogens for available common substrates (Grady et al., 1999). The degradation of organic matter under sulfate-reducing conditions is different from the degradation under methanogenic conditions. Methanogens can use a limited number of substrates, while sulfate reducer can use a wider variety of substrates. Generally, hydrogen, carbon dioxide, and acetate are the most important and well known substrates for methanogens while organic acids such as lactate, propionate, and butyrate are common substrates for sulfate-reducing microorganisms (Gerard et al., 2008).

The amount of sulfate in the influent was approximately 82 mg/L for both the fast and slow feed Cannibal systems. As can be seen from Figure 5-7, higher amounts of sulfate were released from the aerobic sludge of the fast feed system. This implies that more sulfate might be stored in the flocs of the fast feed system. When those flocs were transferred to an anaerobic bioreactor, they are deflocculated; resulting in more sulfate release into solution. Higher amounts of sulfate as a terminal electron acceptor for sulfate-reducing microorganisms in the fast feed system may provide excess sulfate for this type of microorganism and make them more active when compared to the slow feed system. In this situation, sulfate reducers can compete with methanogens for the common substrates, hydrogen and acetate (Stams et al., 2003). Having higher affinity and lower threshold

values for hydrogen, hydrogen-utilizing methanogens and homoacetogen (i.e., a bacterium that produces acetate as the sole product from sugar fermentation or from hydrogen and carbon dioxide) are easily out-competed by hydrogen-utilizing sulfate-reducing microorganisms (Weijma, J. et al., 2002). Nevertheless, many sulfate reducers need acetate as a carbon source (Brysch, K. et al., 1987). In general, acetate-utilizing sulfate reducers can compete with acetate-utilizing methanogens, but this competition is not clear-cut as for hydrogen (Schönheit et al., 1982). Therefore, it is most likely that methanogens use acetate as a substrate to produce methane gas under the fast feed condition.

The slow feed operation for the Cannibal system seems to provide limited amounts of sulfate when compared to the system operated as fast feed. Under insufficient sulfate condition, sulfate-reducing microorganisms compete with each other for the available sulfate. Laanbroek et al. (1984) found that sulfate reducers use hydrogen, lactate, and ethanol as substrates, but not propionate and acetate. Therefore, hydrogen-utilizing methanogens are replaced by hydrogen-utilizing sulfate reducers.

The data from Table 5-6 demonstrate that there was less sulfate left in the anaerobic bioreactor of fast feed system than slow feed system. This suggests that greater amounts of sulfate were utilized by sulfate reducers in the fast feed system with hydrogen serving as a substrate. Consequently, more sulfide ions were generated in this system. It is thought that some of free sulfide ions can reduce Fe (III) in Fe-associated EPS flocs to Fe (II) and bind to Fe (II) to form FeS, resulting in floc destruction and the materials bound to iron are released into solution. The fast feed system contains higher amounts of substrate (hydrogen) and electron acceptors (sulfate) than the slow feed system. It is therefore likely

that sulfate-reducing microorganisms in the fast feed system are more active than that in the slow feed system. The higher activity of these organisms can be a factor in the greater solid reduction in the fast feed system.

It seems that methanogens in both the fast and slow feed systems use acetate as a substrate rather than hydrogen because they are unable to compete with hydrogen –utilizing sulfate reducers. However, the greater amounts of acetate available as a substrate for methanogens in the fast feed system lead to more methane generation as shown in Table 5-7. This suggests that methanogens under fast feed conditions were more active than that in slow feed condition.

The data for volatile fatty acid accumulation are demonstrated in Table 5-8. It can be seen that more volatile fatty acids were found in the fast feed systems than that in slow feed systems. This indicates that fermentative microorganisms in this system are more active.

Conclusions

Two Cannibal systems with high and low substrate pressure (fast and slow feed) were operated in the laboratory to study the solid reduction mechanisms. The fast feed system showed higher release of protein as well as ammonium ions accumulation in the anaerobic bioreactor than the slow feed system. In addition, greater amount of polysaccharide and divalent cations, calcium and magnesium, were found in the anaerobic bioreactor of the fast feed system. The protein and polysaccharide found in anaerobic bioreactor of the fast feed

system were more biodegradable. This was demonstrated by comparing the amount of 1 kDa protein. The 1 kDa protein in the anaerobic bioreactor of the fast feed system was much higher than that in the slow feed system, indicating higher amounts of readily biodegradable protein. The greater release and degradation of biopolymers in the fast feed system were the results of higher floc disintegration, as indicated by a much lower observed yield. The partial pressure of hydrogen gas was not exceeded 10^{-4} atm. in both Cannibal systems, indicating that fermentation in the anaerobic bioreactors could be maintained and the systems work without hydrogen interference. The sulfate-reducing microorganisms and methanogens in the fast feed system were more active than those in the slow feed system. The higher sulfate utilization led to the production of more free sulfide ions in the fast feed system than the slow feed system. These free sulfide ions are thought to be capable of reducing Fe(III) associated with EPS in flocs, resulting in an increase in deflocculation in the fast feed system. Additionally, the higher activity of methanogens in the fast feed environment resulted in larger amounts of methane production. The greater activity of both sulfate reducers and methanogens in the fast feed system led to more volatile fatty acid and hydrogen utilization, more fermentation, and more floc destruction.

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Table 5-1. Medium composition.

NAME	FORMULA	CONCENTRATION (mg/l)
Bacto Peptone	-	300mg COD/l
Sodium Acetate	CH_3COONa	100mg COD/l
Ammonium Chloride	NH_4Cl	57
Ammonium Bicarbonate	NH_4HCO_3	60
Potassium Phosphate	KH_2PO_4	44
Potassium Bisulphate	$KHSO_4$	34
Sodium Bicarbonate	$NaHCO_3$	394
Calcium Chloride	$CaCl_2.2H_2O$	220
Magnesium Sulfate	$MgSO_4.7H_2O$	150
Ferric Chloride	$FeCl_3$	-
Alum	$Al_2(SO_4)_3.18H_20$	-
Allylthiourea	-	6.0
Trace Element Solution	-	2ml/l

Table 5-2. Trace element solution composition.

NAME	FORMULA	CONCENTRATION (gm/l)
Citric acid	-	2.73
Hippuric acid	-	2
Ethylene Diamine Tetraacetic Acid, trisodium salt	$Na_3EDTA.4H_2O$	1.5
Ferric Chloride	$FeCl_3.6H_2O$	1.5
Boric Acid	H_3BO_3	.25
Zinc Sulphate	$ZnSO_4.7H_2O$	.15
Manganese Chloride	$MnCl_2.4H_2O$	.12
Copper (II) Sulphate	$CuSO_4.5H_2O$	.06
Potassium Iodide	KI	.03
Sodium Molybdate	$Na_2MoO_4.2H_2O$	.03
Cobalt chloride	$CoCl_2.6H_2O$	.03
Nickel (II) Chloride	$NiCl_2.6H_2O$	.03
Sodium Tungstate	$NaWO_4.2H_2O$	.03

Table 5-3. Molecular distribution of protein in anaerobic bioreactor of the Cannibal system operarted under different feed patterns.

Hydraulic retention time (HRT) in anaerobic bioreactor (days)	Feed patterns	Protein (mg/L)			
		1.5 µm	0.45 µm	30 kDa	1 kDa
10	Fast feed	118.69	78.46	66.86	34.85
	Slow feed	54.67	38.26	18.34	9.38
5	Fast feed	69.82	47.84	39.80	19.58
	Slow feed	15.04	7.04	4.14	2.48

Table 5-4. Cations in anaerobic bireacotor of the Cannibal system operated under different feed patterns.

HRT (days)	Feed Pattern	Na^+ (mg/l)	NH_4^+ as N (mg/l)	K^+ (mg/l)	Mg^{2+} (mg/l)	Ca^{2+} (mg/l)
10	Fast feed	154.74	174.54	66.88	32.94	94.48
	Slow feed	122.16	93.84	44.64	24.58	56.43
5	Fast feed	126.97	102.51	50.69	21.78	74.46
	Slow Feed	115.93	68.38	37.21	10.64	44.85

Table 5-5. Molecular distribution of polysaccharide in anaerobic bioreactor of the Cannibal system operarted under different feed patterns.

Hydraulic retention time (HRT) in anaerobic bioreactor (days)	Feed patterns	Polysaccharide (mg/L)			
		1.5 µm	0.45 µm	30 kDa	1 kDa
10	Fast feed	53.23	27.59	23.69	20.61
	Slow feed	34.48	16.09	8.75	6.54
5	Fast feed	28.71	16.91	13.37	11.18
	Slow feed	25.54	10.32	5.86	4.37

Table 5-6. Anions in anaerobic bireacotor of the Cannibal system operated under different feed patterns.

HRT (days)	Feed Pattern	Cl^- (mg/l)	NO_2^- as N (mg/l)	NO_3^- as N (mg/l)	PO_4^{3-} as P (mg/l)	SO_4^{2-} (mg/l)
10	Fast feed	175.14	NA	1.66	73.48	4.34
	Slow feed	165.58	NA	2.83	46.16	14.54
5	Fast Feed	182.44	NA	2.67	54.06	8.31
	Slow Feed	173.24	NA	3.42	34.11	17.11

Table 5-7. Gases accumulation in anaerobic bireacotor of the Cannibal system operated under different feed patterns.

HRT (days)	Feed Pattern	Gases		
		Methane (percent)	Hydrogen (ppm)	Hydrogen partial pressure (atm)
10	Fast feed	9.64	29.68	0.029×10^{-6}
	Slow feed	5.08	10.86	0.010×10^{-6}
5	Fast feed	5.84	18.56	0.018×10^{-6}
	Slow feed	2.54	6.87	0.0069×10^{-6}

Table 5-8. Volatile fatty acid in anaerobic bireacotor of the Cannibal system operated under different feed patterns.

HRT (days)	Feed Pattern	Acetic acid	Propionic Acid	Isobutyric Acid
10	Fast feed	108.48	39.46	18.78
	Slow feed	56.28	18.74	7.98
5	Fast feed	68.46	21.68	10.28
	Slow feed	29.54	12.36	4.67

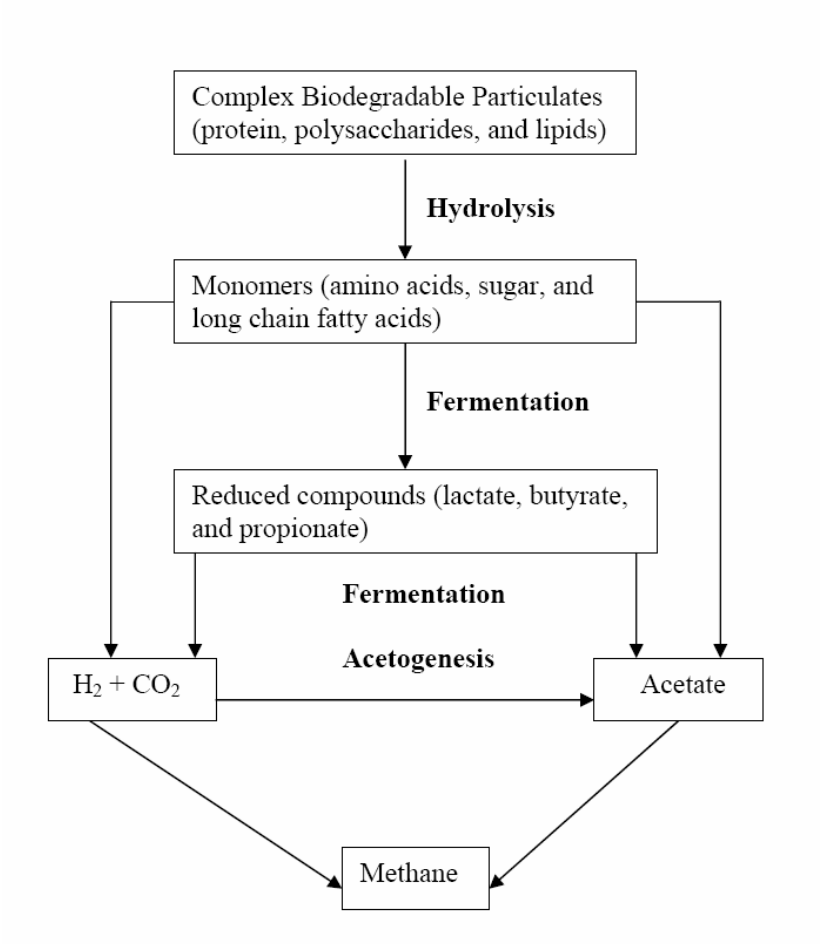

Figure 5-1(a). The pattern of microbial degradation of complex organic matter in anaerobic conditions in the absence of sulfate.

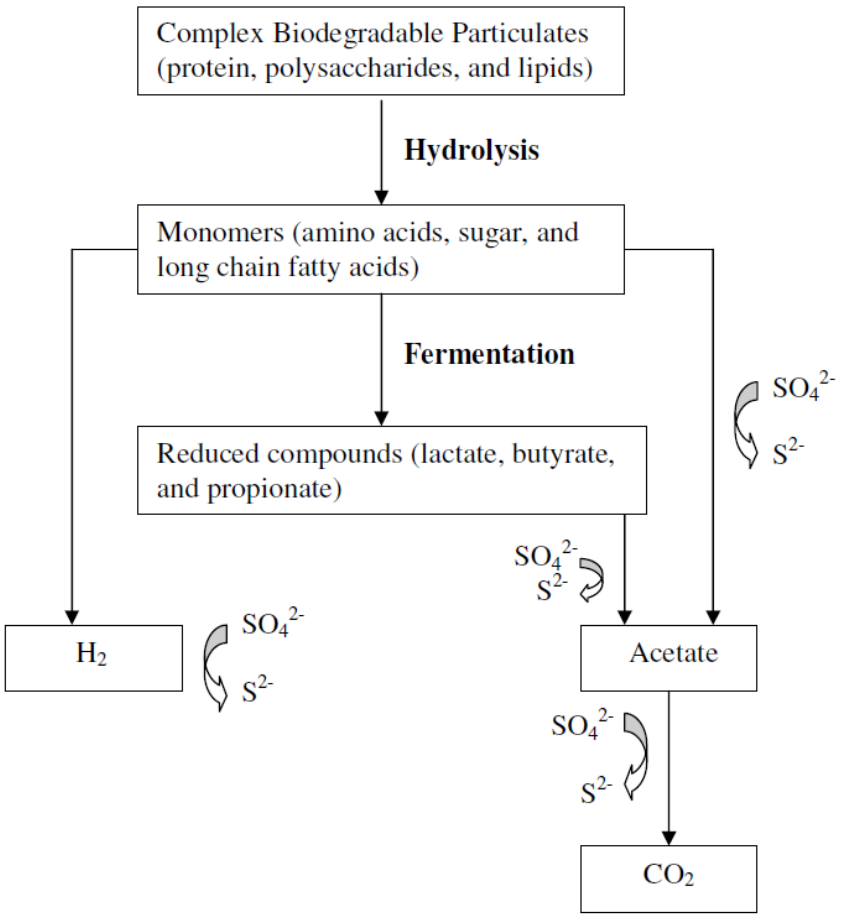

Figure 5-1(b). The pattern of microbial degradation of complex organic matter in anaerobic conditions in the presence of sulfate.

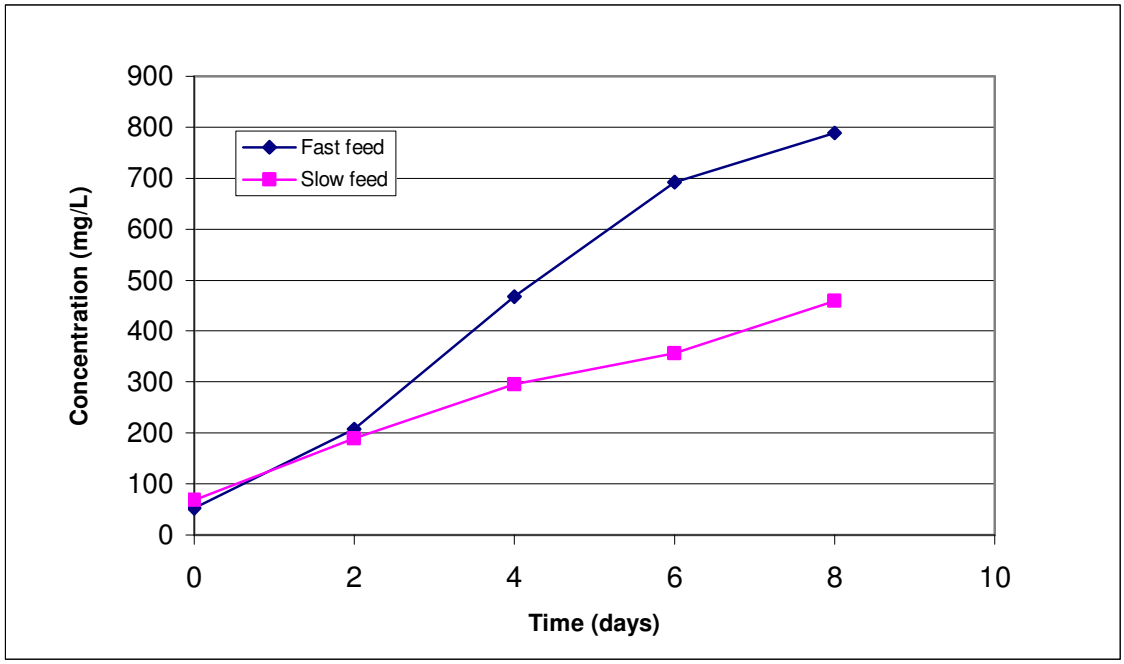

Figure 5-2. The release of soluble COD from aerobic sludge of fast and slow feed systems with 10 day HRT anaerobic bioreactors (batch test).

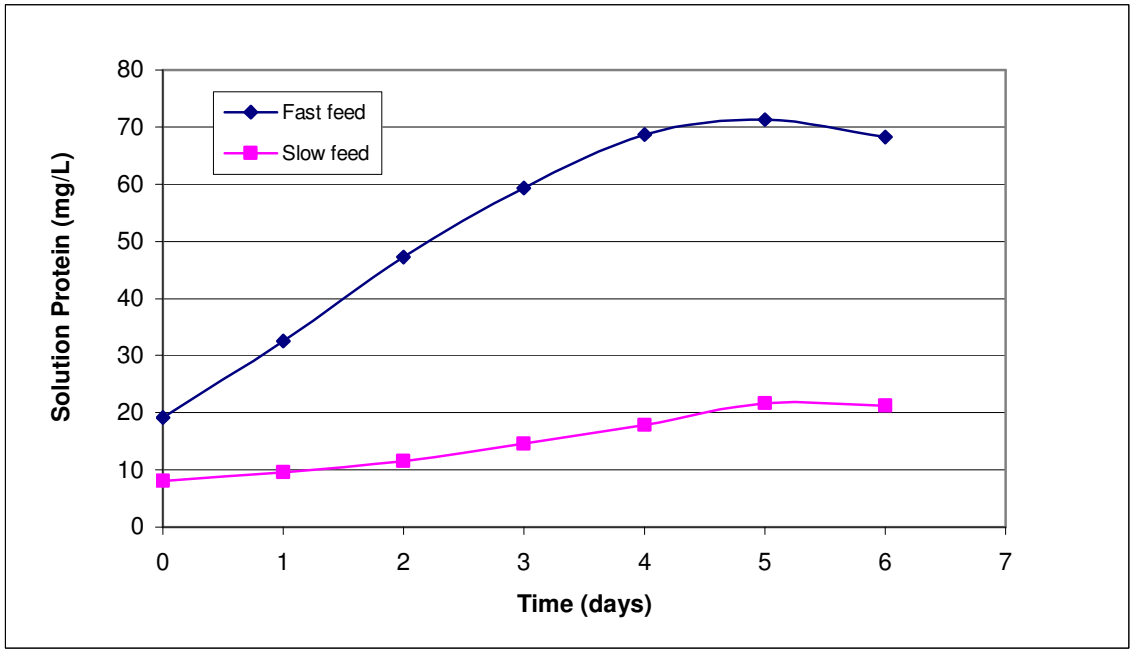

Figure 5-3. The release of protein from aerobic sludge of fast and slow feed systems with 10 day HRT anaerobic bioreactors (batch test).

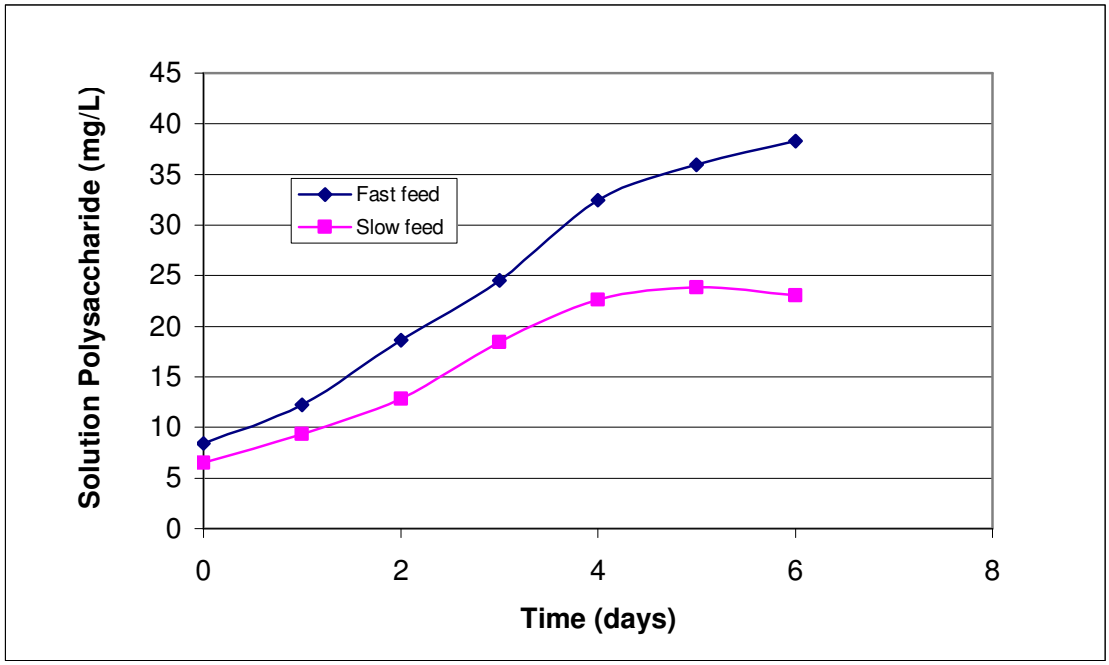

Figure 5-4. The release of polysaccharide from aerobic sludge of fast and slow feed systems with 10 day HRT anaerobic bioreactors (batch test).

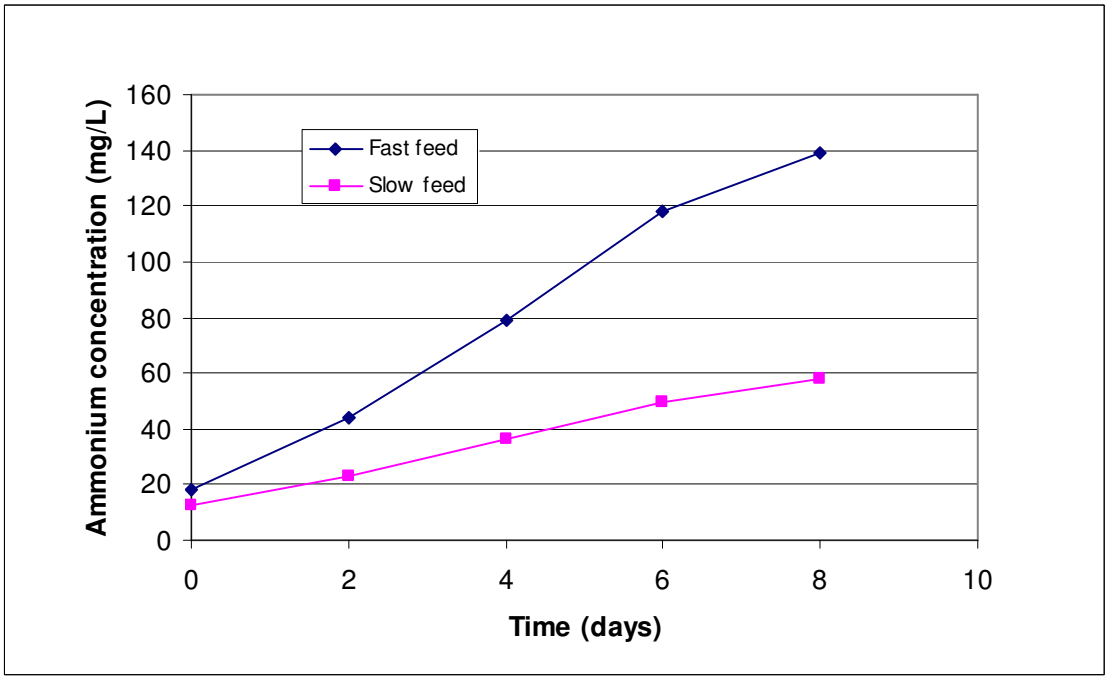

Figure 5-5. The release of ammonium ions from aerobic sludge of fast and slow feed systems with 10 day HRT anaerobic bioreactors (batch test).

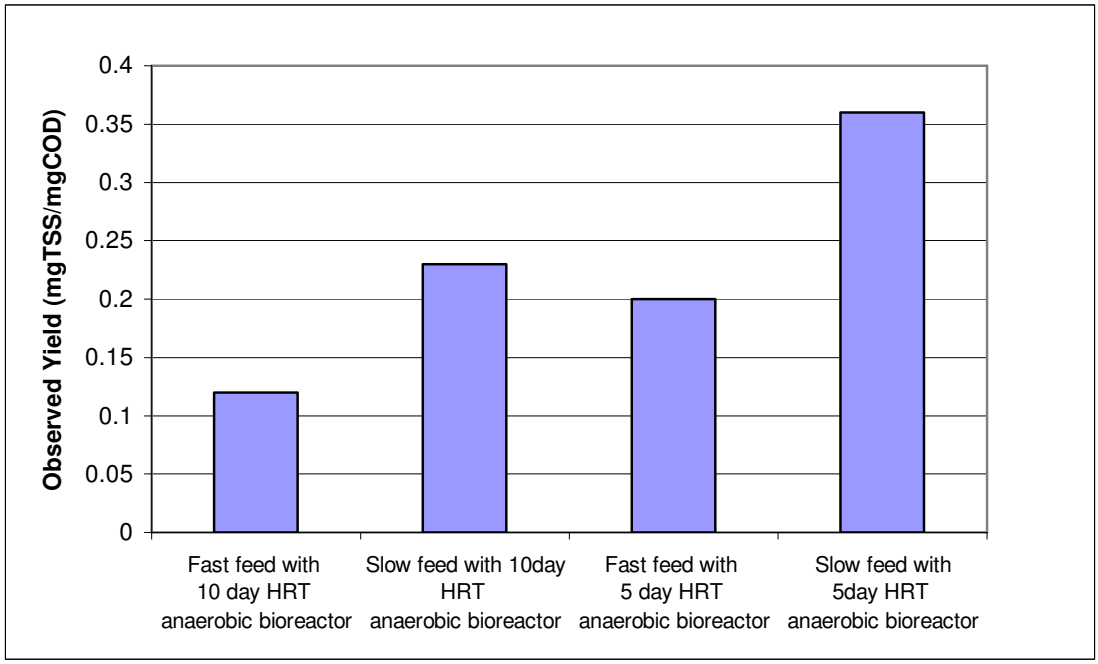

Figure 5-6. Observed yield for the Cannibal systems under different operation.

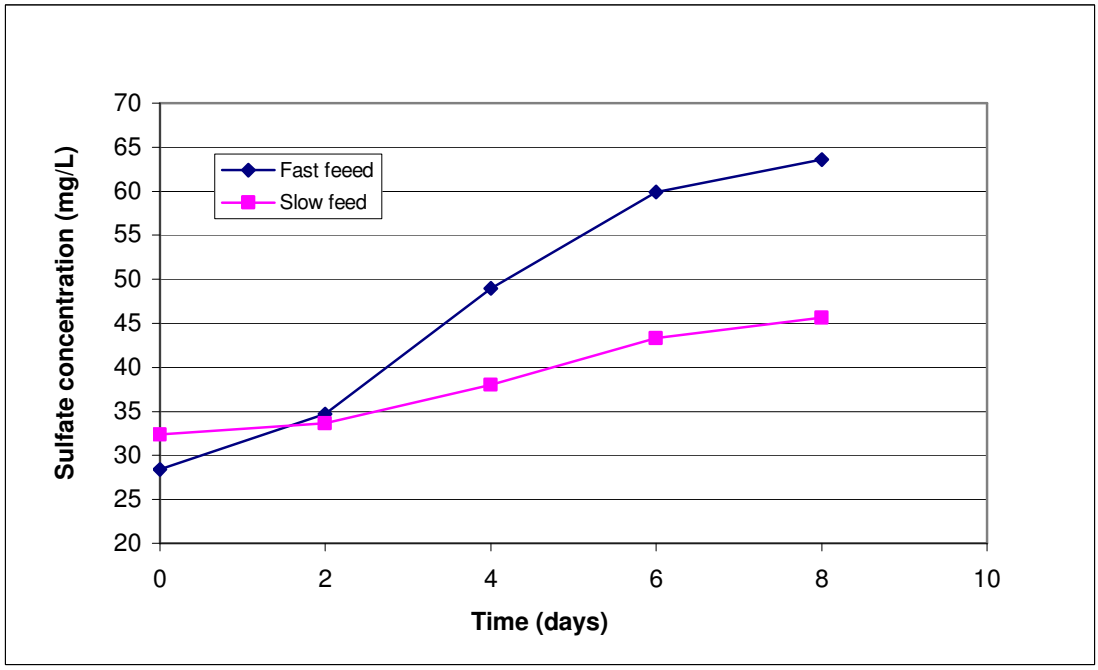

Figure 5-7. The release of sulfate from aerobic sludge of fast and slow feed systems with 10 day HRT anaerobic bioreactors.

Chapter 6. The Role of Microbially Reducible Iron flocs and Extracellular proteases on the Sludge Reduction in the Cannibal process

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The Role of Microbially Reducible Iron flocs and Extracellular proteases on the Sludge Reduction in the Cannibal process

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Abstract

An activated sludge system that incorporates a sidestream anaerobic bioreactor, called the Cannibal process, was studied by Novak et al. (2007) to evaluate its capability for sludge reduction. They found that this system generated about 60% less solids than conventional activated sludge without any negative effects on the effluent quality. Later, Khanthongthip et al (in preparation) studied this system operated with different feeding patterns. They found that for a sequencing batch reactor system, when the feed was provided to the Cannibal system over a period of 5 minutes (fast feed), the system generated less solid than the Cannibal system fed over a period of 4 hours (slow feed). In this study, the use of microbially reducible iron and extracellular proteases as indicators of the amount of sludge reduction in the Cannibal systems operated under high and low substrate pressure (fast and slow feed, respectively) was investigated. It was found that the generation of microbially reducible iron in aerobic reactors operated as sequencing batch reactors (SBRs) was

somewhat higher in the fast feed system compared to the slow feed system. However, the destruction of biological floc in the anaerobic bioreactor of the fast feed Cannibal system was much greater than in the slow feed system. This indicates that Fe(III) reducers were more active in the fast feed than the slow feed environment. In addition, the proteolytic enzyme activity of chymotrypsin and elastase was investigated to see their effect on protein hydrolysis. It was found that the activity of both proteases in the fast feed system was greater than that in the slow feed system. The higher activity of the proteases in the fast feed environment indicated that a greater amount of proteins were hydrolyzed in a shorter period of time compared to the slow feed system.

Keywords: solids reduction, sulfate-reducing microorganisms, methanogens, Fe(III)-reducing microorganisms, microbially reducible iron, extracellular proteases, protein hydrolysis, protease activity.

Introduction

The activated sludge process is the primary method used for treating municipal wastewater before its discharge into receiving waters. Despite its usefulness in treating a variety of wastewater sources, the process also produces a byproduct, excess sludge, that needs to be properly treated prior to its ultimate disposal. A significant cost in the activated sludge process also lies in solids handling processes downstream of the activated sludge system (Crites et al., 1998.), rendering proper and efficient treatment of sludge of great importance.

A wide range of processes that can reduce sludge generated from biological wastewater treatment processes have been proposed. Many of these processes have been evaluated in terms of their sludge reduction potential, ease of implement into existing facilities, impacts on plant operation and effluent quality, reliability and risk associated with the process. However, most of these are either add more plant operating costs or are inappropriate from a practical point of view.

One of the novel and promising sludge reduction processes is anaerobic-aerobic cycling or the Cannibal process. This is an activated sludge process modified by incorporating an anaerobic bioreactor as a sidestream reactor for return activated sludge prior to returning it to the aerobic reactor. A previous study by Novak et al. (2007) demonstrated that this process generated about 60% less solids than conventional activated sludge system without any negative effects on the effluent quality or settling characteristics. That study was conducted under a specific operation in which the return sludge was retained in an anaerobic bioreactor for 10 days and the interchange rate (the exchange of sludge between anaerobic and aerobic bioreactor) was 10% per day by mass. Later, Khanthongthip et al. (in preparation) studied the influence of substrate pressure on the performance of this system and found that the system produced less solid reduction when it was operated under low substrate pressure which was represented by feeding influent wastewater to a sequencing batch reactor over 4 hours (slow feed) in each cycle. However, when high substrate pressure was provided by feeding the influent to the system over 5 minutes, the solid reduction was higher. They hypothesized that the sludge generated from

high substrate pressure system might be more susceptible in degradation than that from low substrate pressure system.

Novak et al. (2007) investigated the mechanism for the solids reduction in the Cannibal process. They showed that when settled sludge was cycled to an anaerobic bioreactor, iron was reduced, and organic matter was released and solubilized. The released materials and sludge were returned to the aeration basin and were rapidly degraded. However, recent research in our lab has shown that up to 70 % of the solids loss that occurs in the Cannibal process can occur in the anaerobic bioreactor. That is, while the mechanism proposed by Novak et al. (2007) accounts for some of the solid loss, solids loss across the anaerobic bioreactor is also important. Later, Khanthongthip et al. (in preparation) investigated the mechanisms for the loss of biological solids across the anaerobic bioreactor in the Cannibal system operated under fast and slow feed conditions. They found that greater amounts of biopolymers (protein and polysaccharide) were accumulated in the fast feed than the slow feed Cannibal system, indicating more flocs disaggregation in the fast feed system.

Because Fe(III)-reducing microorganisms are often found in anaerobic environments, it is thought that this type of microorganism may be present in the anaerobic bioreactor of the Cannibal system. Caccavo et al. (1996) reported on the influence of microbial Fe(III) reduction on the deflocculation of activated sludge under anaerobic condition. They found that cells of the dissimilatory metal-reducing bacterium Shewanella alga BrY oxidized hydrogen gas and reduced Fe (III) bound in the sludge floc, resulting in floc destruction. Interestingly, they also found that cells need to be bound to the Fe(III)

associated EPS floc before the chemical reduction could take place. Therefore, the amount of Fe (III) that can be reduced by Fe-reducing microorganisms, called microbially reducible iron, in the Fe (III)-associated EPS floc is of interest and needs further investigation.

Because proteins are the most abundant EPS component in activated sludge flocs (Nielsen et al., 1996), hydrolysis of protein can increase the solids reduction. In general, chemical protein hydrolysis at room temperature, atmospheric pressure, and neutral pH is slow. If this reaction needs to be increased, proteolytic enzymes must be presented and active. Therefore, it is essential to examine the activity of extracellular proteases to see their effects on the solids reduction in the fast and slow feed Cannibal system.

The objective of this study was to thoroughly investigate the role of microbially reducible iron, and the extracellular protease activity on solids reduction in the Cannibal system. This study was expected to provide a better understanding of the impact of microbially reducible iron and the protease activity on solid reduction when the Cannibal system was operated under different circumstances, especially under high and low substrate pressure.

Materials and Methods

Microbially reducible iron.

In this experiment, samples from settled sludge entering and leaving the 10 day hydraulic retention time (HRT) anaerobic bioreactors of fast and slow feed Cannibal systems were

analyzed for microbially reducible iron in the flocs by using the hydroxylamine-extractable iron chemical assay (Lovley et al., 1987). The difference between the amount of microbially reducible iron in the settled sludge leaving the SBR and that in the sludge leaving the anaerobic bioreactor was used to quantify the relative amount of solids reduction by iron reducing microorganisms from those two systems.

Approximately 0.1 g. of dry sludge was transferred to 5 ml of 0.25 M HCI in a glass scintillation vial of known weight. The sludge and acid were mixed with gentle swirling for 30 seconds. The weight of the added sludge was determined. After 1 hour at room temperature, a 0.1-ml sample of the extract was added to 5 ml of ferrozine (1 g/liter) in 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer at pH 7. After being mixed for 15 seconds, the mixture was filtered through a 0.45-µm membrane filter. The amount of Fe (II) was determined by measuring the absorbance at 562 nm wavelength of the filtrate. Fe (II) would not be oxidized and Fe (III) would not be reduced during the extraction. Another sample of the sludge was extracted by the same procedure as that described above with the exception that the extractant was 5 ml of 0.25 M hydroxylamine hydrochloride in 0.25 M HCl. Under acidic conditions, hydroxylamine reduced Fe (III) to Fe (II).

The amount of hydroxylamine-reducible Fe (III) can be calculated as the difference between the Fe(II) measured in the hydroxylamine and HCI extractions.

Extracellular protease activity

Approximately a 100-ml. sample taken from each of anaerobic bioreactors in fast and slow feed Cannibal systems was added to a volumetric flask to allow the settling of the sludge over a night. An aliquot of supernatant was individually filtered through 0.45 µm and the filtrate was transferred to another volumetric flask with a sealed cap. The filtrate was stirred by a magnetic stirrer overnight to allow the hydrolysis of protein in the solution by proteases. This avoids the interference of the protein already presented in the solution with the synthetic substrate. A mixture was prepared by adding 3 ml of each stirred sample from the fast and slow feed system into a tube of 8 mg synthetic substrate, *p*-nitrophenyl acetate, and 5 ml of distilled water. The absorbance was read at 420 nm wavelength from a spectrophotometer every 10 minutes for one hour. Another mixture was prepared in the same manner but 10-µL of 5.7-M. diisopropylfluorophosphate (DFP) was also added to irreversibly inhibit the serine proteases (DFP bound to serine in catalytic triad and permanently destroyed active site of the serine proteases (e.g., trypsin, chymotrypsin, elastases). The absorbance of phenol was also read using the same approach as the first mixture. The elastase activity in anaerobic bioreactors of both Cannibal systems was measured using commercially available synthetic substrate, N-succinyl-L-Ala- L-Ala- L-Ala-p-nitroanilide. For this experiment, approximately 8 ml of each sample from fast and slow feed system prepared in the same manner as for the analysis of chymotrypsin activity was transferred to 5 and 3.5 mg of the synthetic substrate in a glass tube respectively. The

solution was then mixed together. The rate of substrate hydrolysis (the formation of p-nitroanilide) was measured by spectrophotometer at 410 nm wavelength.

Results and Discussion

The role of microbially reducible iron in biological solid reduction

Because Fe(III)-reducing microorganisms are often found in anaerobic environments, it is thought that this type of microorganism would be present in the anaerobic bioreactor of the Cannibal system. For Fe-associated EPS floc, Fe(III)-reducing microorganisms can transfer electrons from substrate (organic acid such as acetate or hydrogen) to Fe(III) in the floc and material bound to iron will be released, resulting in floc destruction. In this study, it was believed that under fast feed conditions (high substrate pressure) feed organics are converted to storage products that are bound to iron within the flocs. Under low substrate pressure, much less material binds to iron. It is also believed that flocs from high substrate condition are more available for degradation by Fe(III)-reducing microorganisms than those from low substrate condition. Therefore, the amount of microbially reducible iron was examined to see the activity Fe(III) reducers under fast and slow feed conditions.

Hydroxylamine extraction is a method used to measure microbially reducible iron in the flocs because it is selective for this type of floc. If microbially reducible iron is present, then there is the potential for iron reduction by microbes. This method is faster than directly

finding the activity of iron-reducing organisms so it can be a useful indicator of the availability of microbially reducible iron in the flocs.

The measurement of microbially reducible iron from settled sludge leaving the SBR indicated the generation of these flocs while this analysis in sludge leaving anaerobic bioreactors demonstrates those flocs remaining after 10 days under anaerobic conditions. The difference between these two is a measure of the solids reduction by iron reducing microorganisms in both Cannibal systems.

The data for microbially reducible iron are illustrated in Figure 6-1 and 6-2. It can be seen from Figure 6-1 that more microbially reducible iron flocs were generated in the fast feed system than that in the slow feed system. The data also show that a lesser amount of microbially reducible iron was found in the anaerobic bioreactor of the system with the fast feed condition. The difference between microbially reducible iron flocs generated in an aerobic reactor (SBR) and that found in the anaerobic bioreactor of the fast feed Cannibal system was much higher than that in the system with the slow feed operation. This indicates that more microbially reducible iron was disaggregated in the anaerobic bioreactor of the fast feed than the slow feed Cannibal system. As shown in Figure 6-2, the degradation of microbially reducible iron flocs was approximately 70 and 40 percent for fast and slow feed systems, respectively. These data show that Fe(III)-reducing microorganisms are more active in the fast feed than the slow feed environment.

The Fe(III)-reducing microorganisms can metabolize acetate, long chain fatty acids, and other metabolic intermediates produced by fermentative organisms such as hydrogen and alcohol. The most common substrates for iron reducers to conserve energy and support

their growth are organic acids, particularly acetate (Lovley et al., 2004). Acetate is the key intermediate and likely to be the most important electron donor for Fe(III) reduction in many environments. A unique aspect of the *Geobacter* TCA cycle is the presence of citrate synthase genes. These genes can allow *Geobacter* to oxidize acetate via the TCA cycle (Lovley et al., 2004). Hydrogen is another electron donor found to support the growth of Fe(III)-reducing microorganisms. Many of these microorganisms including some of *Geobacter* and *Shewanella* species can oxidize hydrogen with the reduction of Fe(III) (Lovley et al., 2004).

Khanthongthip et al. (in preparation) found that the accumulation of acetate in the anaerobic bioreactors of the fast and the slow feed Cannibal systems were approximately 108 and 56 mg/L, respectively. This indicates that a greater amount of acetate is available to serve as an electron donor for iron reducers in the fast feed than the slow feed system. In addition, they also found that methane gas production in the anaerobic bioreactors of the fast and the slow feed system were approximately 29 and 10 ppm, respectively. This suggests that methanogens in the fast feed environment were more active than the slow feed condition. Because Fe(III)-reducing microorganisms, methanogens, and other microorganisms can utilize acetate and hydrogen as sources of electron donors, it can be thought that those microorganisms can compete each other for limited amounts of electron donors available in the anaerobic bioreactor of the slow feed system. This can be a factor for less methane gas production in the slow feed system.

The electron acceptor, Fe(III), is insoluble and cannot diffuse into microbial cells for the reduction reaction. Therefore, it is necessary for iron reducers to have Fe(III)

reduction outside the cell. Various mechanisms have been proposed as possible strategies that these microorganisms might use to transfer electrons to the extracellular Fe(III) complex. Direct contact between microorganisms and the solid-phase Fe(III) complex was shown to be a requirement for this reduction reaction (Lovley et al., 2004). The other active mechanism that can be used to alleviate the need for iron reducers to be directly attached to the insoluble Fe(III) complex is electron shuttling. Soluble external electron shuttles such as humic substances can be used as mediators to complete the transfer of electrons to the solid-phase terminal electron acceptor. The quinone moieties in these compounds can be reduced to the hydroquinone state. When Fe(III) is available in the environment, the hydroquinone can reduce Fe(III) to Fe(II), regenerating the oxidized form (Lovley et al., 1999).

It is likely that more available substrate (acetate), coupled with more active of extracellular electron carriers (e.g., molecule of extracellular quinone might go through multiple cycles of oxidation and reduction reaction.) in the fast feed system could make Fe (III)-reducing microorganisms more active than that in the slow feed system.

The Fe (III)-reducing microorganism activity can result in the deflocculation and the release of material bound to Fe (III), primarily protein. The released proteins can be used as a substrate for fermentative microorganisms. If iron reducers are absent, the flocs may not be deflocculated biologically. In addition, if the fermentative microorganisms are less active, macromolecules such as protein can be accumulated in the reactor. This can result in the decrease of the iron reducer activity because cumulative products, primarily proteins, can reduce the activity of Fe (III) reductase (an enzyme at the outer membrane).

Therefore, both microorganisms need to work together for proper biological solid reduction. Nevertheless, the high concentration of proteins found in the anaerobic bioreactor of the fast feed system by Khanthongthip et al. (in preparation) were not at the level to interfere the activity of Fe(III) reducers because this microorganisms were still active, as indicated by the data from Figure 6-1 and 6-2.

It should be noted that the amount of microbially reducible iron measured in this study is not an intrinsic value but it is a relative value. It should be used to get some idea on the possibility of the Fe(III) that can be reduced by Fe(III)-reducing microorganisms. It should also be kept in mind that nitrate can interfere with the activity of iron reducers because nitrate can be diffused into microbial cell and reduced at the periplasms, resulting in fewer electrons transferred out of the cell for Fe(III) reduction. However, this is not a case in this study because there was a little amount of nitrate found in the anaerobic bioreactors of both systems.

Some field data were collected to determine if the Microbially reducible iron (MRI) seen in the laboratory reactor could also be seen in the field data. Nine wastewater treatment plants, which operated with the Cannibal systems, were investigated. The MRI in the aerobic and the anaerobic reactors for each plant and the MRI removal data are shown in Figure 6-3 and 6-4, respectively.

The data in Figure 6-3 illustrate that plant A to D contains larger amount of MRI in the aerobic reactors than that in plants M to P. This may imply that the aerobic environments in plant A to D are more favorable to generate MRI floc than plant M to P. In addition, the MRI in the anaerobic bioreactors of plant A to D is less than plant M to P. The

difference between MRI in the aerobic and the anaerobic reactors is higher in plant A to D than plant M to P. This indicates that more MRI removal was taken place in plant A to D. It can be thought that Fe(III)-reducing microorganisms in plant A to D might be more active, resulting in a larger MRI floc destruction compared to those of plant M to P.

It can be seen, from Figure 6-4, that there are three different groups of the Cannibal wastewater treatment plants, according to their performances. Plant A to D show at least 63% of MRI removal with lower observed yield compared to plant M to P and are considered as good performance while plant M to P demonstrate poorer performance with the maximum MRI removal of 35%. Plant E shows an intermediate performance as can be seen by the removal of MRI at 45%. These data support the observation from the laboratory data, as shown in the Figure 6-1 and 6-2, that more MRI removal plays a role in higher solids reduction (lower observed yield). (The observed yield data were provided by Siemens Water Technologies, Corp.)

Extracellular protease activity

Activated sludge flocs generally consist of cells and organic matter exterior to cells, which has been characterized as extracellular polymeric substances (EPS) or exocellular biopolymers. In general, activated sludge EPS consist of lipids, nucleic acids, humic substances, proteins, and polysaccharides (Frølund et al., 1996, Dignac et al., 1998, and Higgin et al., 1997). Many researchers have shown that proteins are most abundant in activated sludge EPS (Frølund et al., 1996, Dignac et al., 1998, and Nielsen et al., 1996)

and thereby the destruction of flocs depends on the rate of protein hydrolysis. The hydrolysis of proteins requires proteolytic enzymes. This led to an investigation of the activity of the proteases to quantify their impacts on biological solids reduction.

In order to hydrolyze protein under atmospheric pressure, room temperature, and neutral pH in a short period of time, it is essential to have an enzyme to accelerate this reaction (hydrolysis). Enzymes are catalysts that are vital for living cells to maintain their cellular activity. The rates of reaction are increased tremendously by enzyme catalysis. The power of enzyme catalysis is usually manifested through the nature of the active site where particular residues or cofactors react with substrates, leading to series of chemical reactions which require much less activation energy when compared to the uncatalyzed reaction and resulting in the increase in reaction rate. The enzymes commonly used to hydrolyze (or digest) proteins are called proteolytic enzymes or proteases. The proteases are generally divided into four major groups: (1) acid proteases, (2) diisopropylfluorophosphate (DFP)-sensitive alkaline proteases, (3) metal chelator-sensitive neutral proteases, and (4) thiol proteases. (Matsubara et al., 1971). The most widely distributed proteolytic enzymes among microorganisms are the serine proteases which are generally identified by their sensitivity to organophosphorus reagent such as DFP. These enzymes are almost entirely extracellular (Matsubara et al., 1971). Therefore, the activity of these proteases was examined to evaluate their effects on the protein hydrolysis.

In this research, chymotrypsin and elastase are the serine proteases selected to study their activities because chymotrypsin can hydrolyze the peptide bonds at amino acids with large side chain while elastase can cleave these bonds on amino acids with small side chain.

It should be noted that both of these proteolytic enzymes were already presented in the sludge or secreted from microorganisms (extracellular proteases) including the proteases that mimic chymotrypsin or elastase. The activity, behaviors, or characteristics such as active site (catalytic triad) and specificity of substrate cleavage of the mimiced proteases might be the same or similar to chymotrypsin or elastase but the whole structure (the sequence of amino acids, the amount of amino acids, or folding patterns) might be different. Therefore, these enzymes should be called "large hydrophobic side chain degrading serine protease (LCDS)" and "small side chain degrading serine protease (SCDS)" in stead of chymotrypsin and elastase, respectively. LCDS can cleave the carboxyl side of the aromatic side chain, tyrosine, tryptophan, and phenylalanine and the large hydrophobic residue such as methionine. In addition, LCDS can hydrolyze the ester bond. Although this is not important physiologically, ester bond hydrolysis is of interest because of its close reaction rate to peptide bond hydrolysis. This is a reason to select p-nitrophenyl acetate as a synthetic substrate for LCDS. The analysis of LCDS activity for each sample consists of two steps. The first step was the measurement of LCDS activity without an inhibitor while the inhibitor was included in the other step. The LCDS activity was calculated by the difference of the enzyme activity from those two steps. The analysis of LCDS activity had been performed for three times during three consecutive weeks. Figure 6-5 and 6-7 illustrate the activity of LCDS in the anaerobic bioreactors of the fast and the slow feed Cannibal system. The slope of each graph indicates the rate (activity) of enzymatic reaction for the designated system. The data suggest that the activity of this protease in the fast feed system was higher than that in the slow feed system. However, the

hydrolysis of ester bond in the synthetic substrate was not only associated with chymotrypsin but also other enzymes such as esterase. This could overestimate the protease activity. Consequently, an inhibitor called diisopropylfluorophosphate (DFP) was introduced to the enzyme assay to inhibit LCDS activity. DFP binds irreversibly to the serine located in catalytic triad in the active site of this enzyme and inactivates this protease. It can be seen from Figure 6-6 and 6-8 that the enzyme activity decreased after adding the inhibitor. The difference in the enzymatic activity with and without the inhibitor indicates the intrinsic rate of the enzymatic reaction, as shown in Figure 6-9. It is clear that the LCDS activity in the anaerobic reactor of the fast feed system is higher than the slow feed system. In addition, Khanthongthip et al. (in preparation) found that more protein was released in the fast feed system than the slow feed system. This highly released protein, which is a substrate for the proteases in the anaerobic bioreactor of the fast feed system, can promote protease activity. This is accordance with the Michaelis-Menton equation in that when the substrate concentration is high, the equation will yield high reaction rate (enzyme activity).

SCDS is a digestive enzyme similar to LCDS. The catalytic triad (serine, histidine, aspartate) are present in their active sites for both proteases, making them to have nearly identical catalytic mechanisms. SCDS can cleave the carboxyl side of uncharged nonaromatic residues such as alanine, glycine, valine, and serine. Therefore, SCDS was chosen as a representative of serine protease family in the hydrolysis of proteins containing small side chain amino acids while LCDS was another protease in this family selected for hydrolysis of proteins having large side chain amino acid residues. In addition, Park et al

(2008) used cation exchange resin extraction (CER) to extract the EPS from the sludge taken from wastewater treatment plants and found SCDS. However, they did not perform the enzyme analysis to see if this protease was active. Therefore, the analysis of this protease activity was conducted in this research to examine its activity under the dynamic circumstances. A commercially available synthetic small peptide fragment, N-Succinyl-L-alanyl-L-alanyl-L-alanyl-p-nitroanilide, was selected as a synthetic substrate for SCDS. The succinyl group was employed in peptide substrate to increase the water solubility. In addition, this peptide terminated with N-succinyl instead of N-acetyl can interact more favorably with SCDS. The cleavage of the peptide bond (or scissile bond) at the carboxyl site of L-alanine located in front of p-nitroanilide by this protease resulted in the release of the product, p-nitroanilide, that can be detected by light absorbance using the spectrophotometer. The first two L-alanyl in the substrate help to ensure the precise alignment of the substrate and the catalytic triad and thereby the selectivity of catalysis. The activity of SCDS in anaerobic bioreactors of the fast and the slow feed systems was 0.0009 and 0.0004 per minute, as shown by the slope of data from Figure 6-10 and 6-11, respectively. The highly accumulated protein in the anaerobic bioreactor of the fast feed system found by Khanthongthip et al. (in preparation) is thought as a factor to enhance the activity of this protease by increasing substrate binding to the active site of this enzyme.

The DFP-sensitive alkaline proteases such as the serine proteases including LCDS and SCDS require divalent cation like Ca^{2+}, Mg^{2+}, Mn^{2+}, and Co^{2+} or a combination of these cations to maximize their activity. These cations were found to enhance the stability of these alkaline proteases (Paliwal., N. et al., 1994). It was believed that these cations

protect the enzyme against denaturation and play a significant role to maintain the active conformation of the enzyme. (Kumar., C.G. et al., 1999). As can be seen from Table 6-1, higher divalent cations (calcium and magnesium) were found in the anaerobic bioreactor of the fast feed system and this could assist to prevent the conformation of change of protease structure and promote the protease activity (both LCDS and SCDS), resulting in greater protein degradation as indicated by more ammonium ion accumulation in the anaerobic bioreactor of this system. These data are accordant with the study from Allison et al. (1991) who also found that this protease activity was stimulated by divalent cations.

It can be seen that the activity of LCDS and SCDS was higher in the fast feed than the slow feed system. This might result from: (1) higher amounts of each protease in the fast feed system. (2) higher amounts of divalent cations found in the anaerobic bioreactor of the fast feed system. (The greater amount of divalent cations can help to stabilize the enzyme structure and protect them from denaturation.) (3) more protein can increase enzyme activity (most of the proteins come from cell lysis products such as cell membrane and intrcellular and extracellular proteins). (4) less inhibitor or interference. (Further investigation is needed to verify this)

The light absorbance rate for LCDS and SCDS in fast feed system is approximately 0.0025 and 0.0009 per minute respectively and that is 0.0015 and 0.0004 per minute for slow feed system. This suggests that SCDS was less active than LCDS. It also implies that LCDS play a more important role than SCDS for biological solids reduction in both systems.

It is interesting to note that various environments and mix cultures in anaerobic bioreactors can make the activity of LCDS and SCDS in this study different from those typical LCDS and SCDS in the pure culture. For instance, if the inhibitors or interferences were present in anaerobic bioreactors, the activity of the enzymes could be lower when compared to those in the pure culture. In addition, the neutral pH could reduce the activity of these proteases because these proteolytic enzymes worked at maximum capability under alkaline condition. As a result, the analysis of protease activity under mix culture in this study might be different from that in the pure culture.

It should be also noted that the activities (the reaction rate) of these extracellular proteases are not intrinsic values but they are relative values. It should be used to compare among the same proteases that use the same substrate to measure their activity. However, the enzyme may be in the different environment. For instance, LCDS activity measured from samples taken from the fast and the slow feed Cannibal system in this study can be compared because the measurement of this protease activity used the same substrate which was p-nitrophenylacetate. However, the activity was different because the enzyme was in the various environments.

Some field protease data were collected from nine Cannibal wastewater treatment systems as was done for microbially reducible iron (MRI). The SCDS was selected to be investigated because this was found in the sludge by Park et al. (2008). The activity of SCDS in anaerobic bioractors for each plant is shown in Figure 6-12.

It can be seen from the data in Figure 6-12 that SCDS activity is higher in plants A to D than in plants M to P. This suggests that the environment in the anaerobic bioreators of

plants A to D is more favorable for enhancing the activity of SCDS in plants A to D than in plants M to P. This results in larger protein hydrolysis and higher solids reduction in plants A to D.

Because plants A to D show lower observed yield than plants M to D, it is clear that the SCDS activity data support the laboratory data, as shown in Figure 6-10 and 6-11, in which more SCDS activity is associated with higher solids reduction.

Conclusions

The roles of microbially reducible iron and extracellular protease activity for biological solid reduction in the Cannibal process operated under high and low substrate pressure were studied. The fast feed Cannibal system resulted in higher microbially reducible iron flocs destruction compared to the slow feed Cannibal system. A reduction in the microbially reducible iron flocs of approximately 70% was seen for the fast feed system while 40% reduction of this type of flocs was showed in the slow feed system. This demonstrates the higher activity of Fe(III)-reducing microorganisms in the fast feed than the slow feed system. The greater activity of iron reducers made the fast feed system more effective in biological solid reduction compared to the slow feed system.

The activity of extracellular proteolytic enzymes, LCDS and SCDS, was also investigated to see their capability of protein hydrolysis in the anaerobic bioreactors of the fast and the slow feed Cannibal system. The fast feed system demonstrated greater protease activity than the slow feed system. The higher activity of the proteases in the fast feed

operation indicated that more amounts of protein were hydrolyzed in a shorter period of time. In addition, more divalent cations in the fast feed solution could assist to stabilize the structure of the proteases, preventing them to be denatured and sustaining the active site in the original position.

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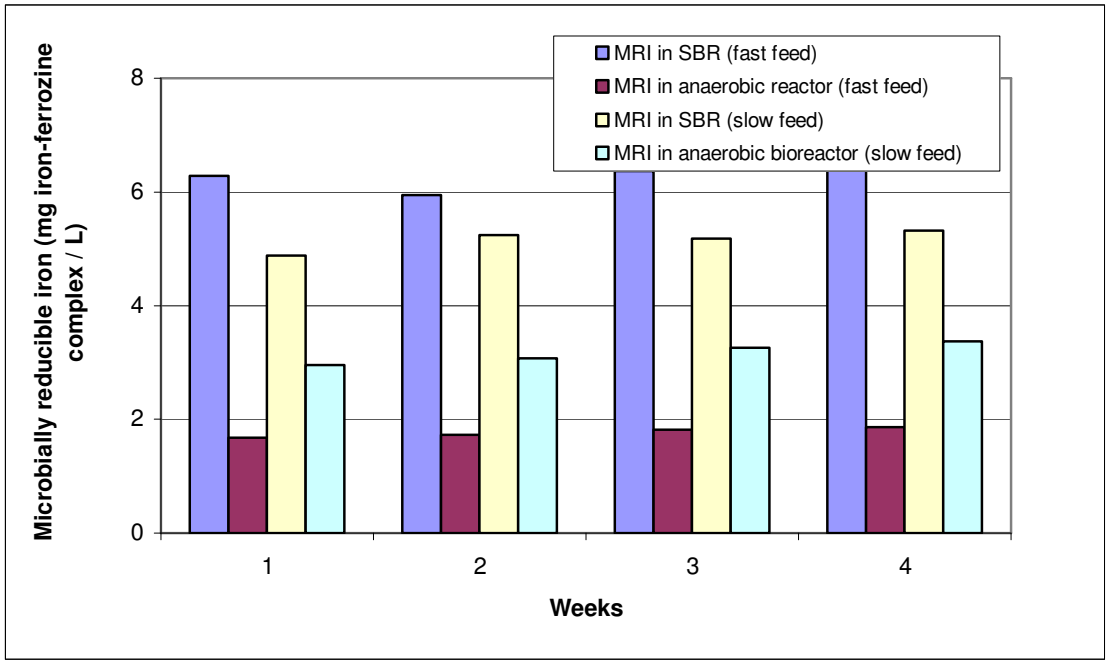

Figure 6-1. Microbially reducible iron (MRI) in both SBRs and anaerobic bioreactors of fast and slow feed Cannibal systems.

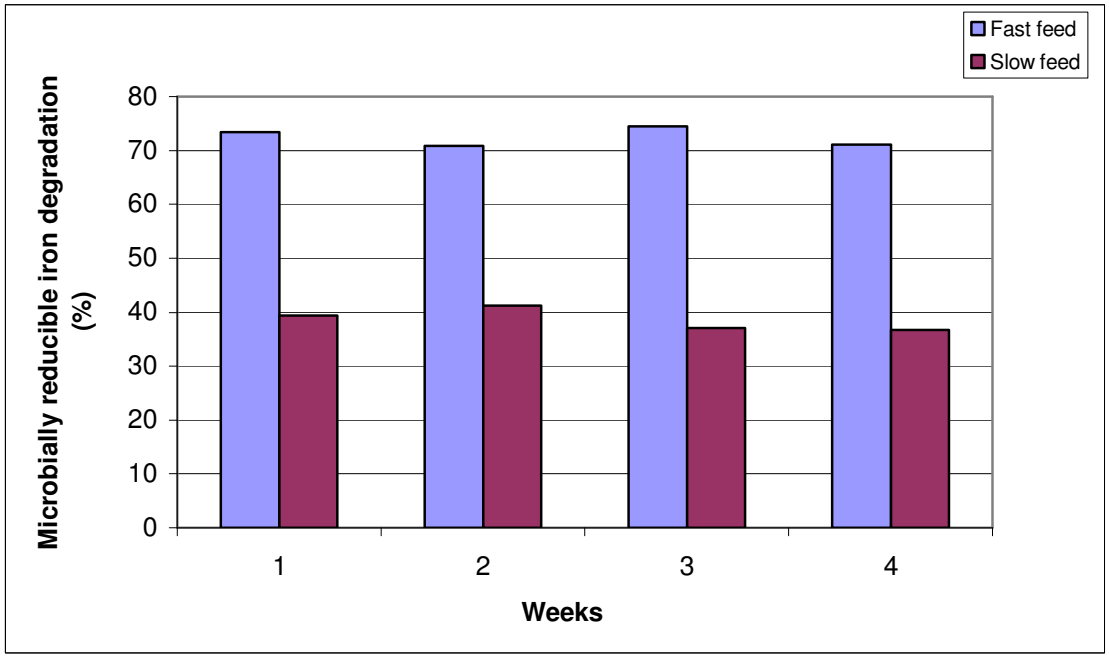

Figure 6-2. Microbially reducible iron (MRI) degradation in fast and slow feed Cannibal systems.

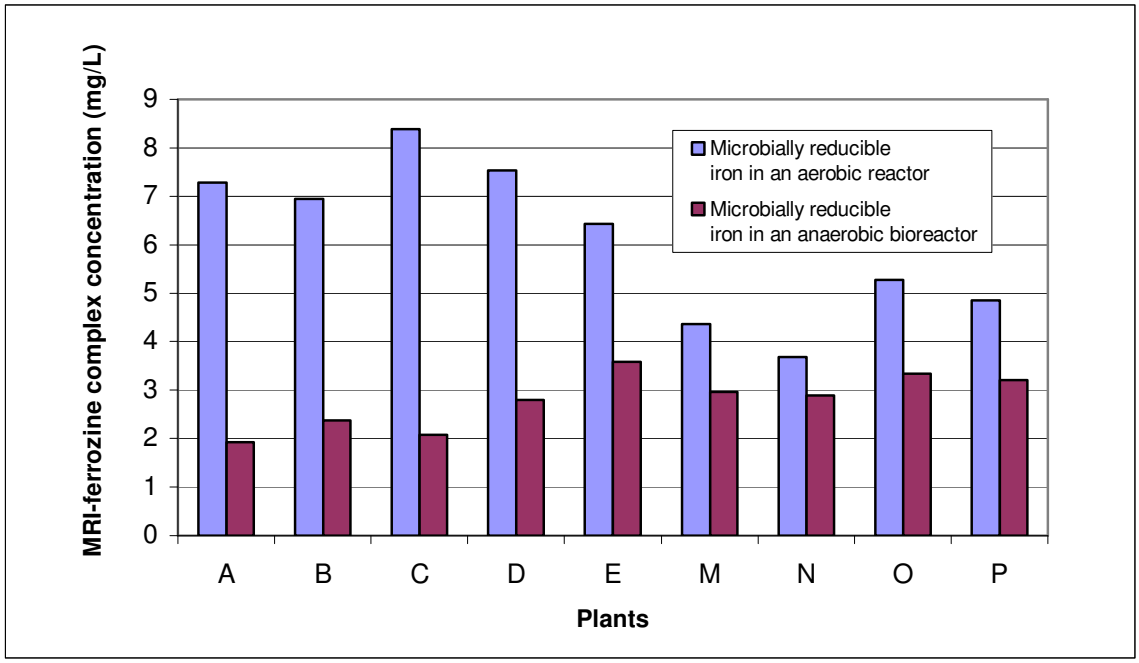

Figure 6-3. Microbially reducible iron (MRI) in the aerobic and the anaerobic bioreactors of nine Cannibal wastewater treatment plants.

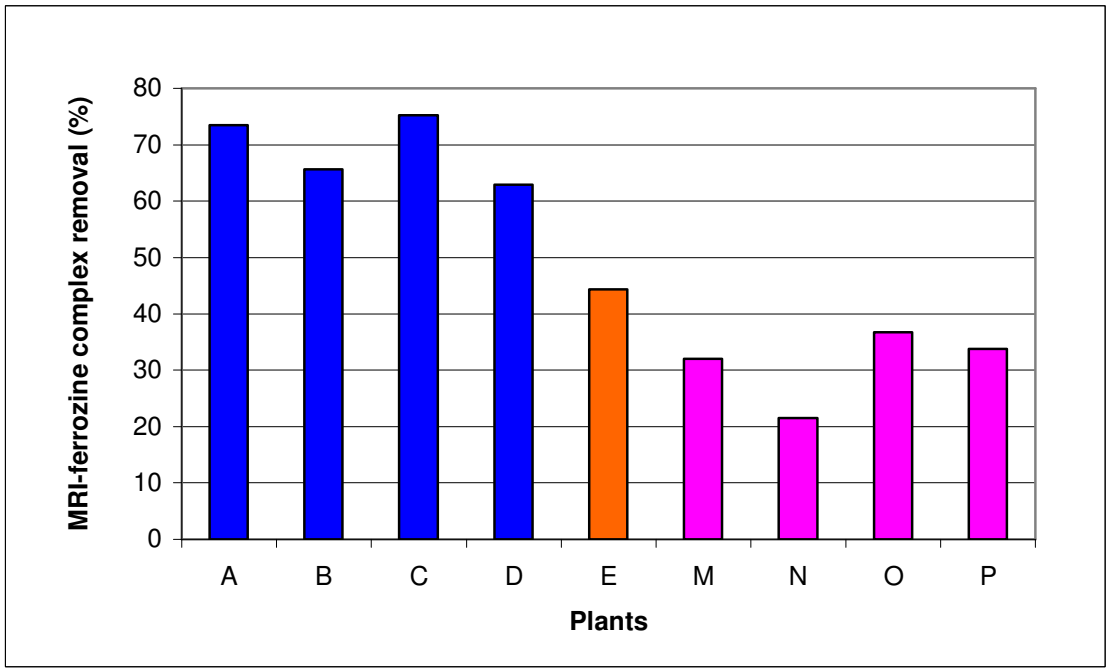

Figure 6-4. Microbially reducible iron (MRI) degradation in nine Cannibal wastewater treatment plants.

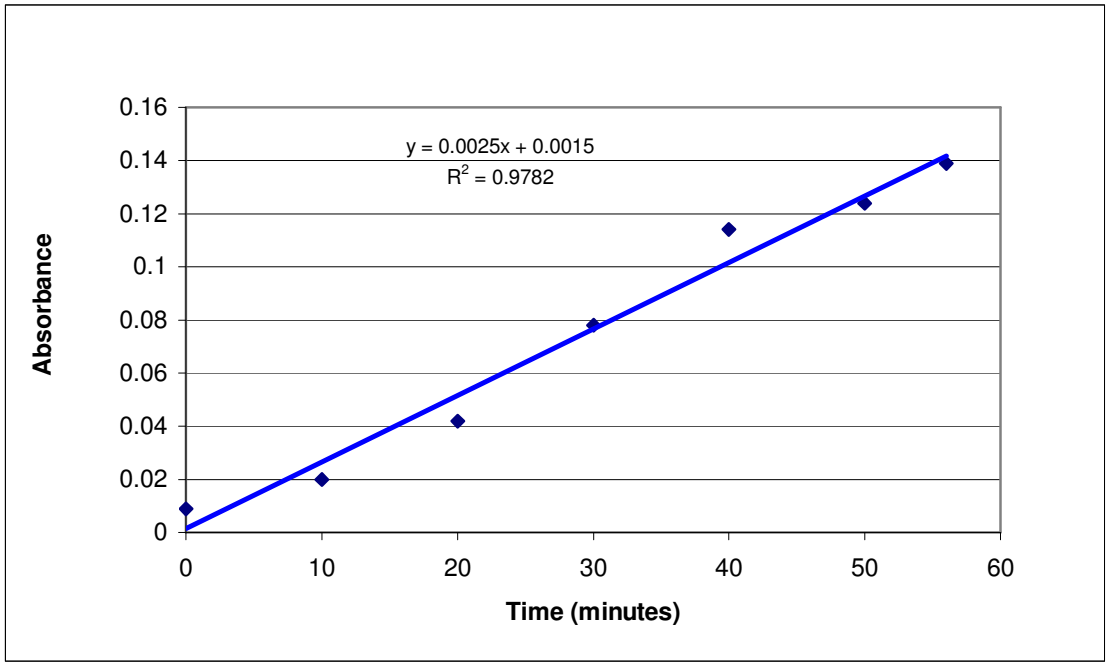

Figure 6-5. Uninhibited LCDS activity in anaerobic bioreactor of the slow feed Cannibal process during the first week of testing.

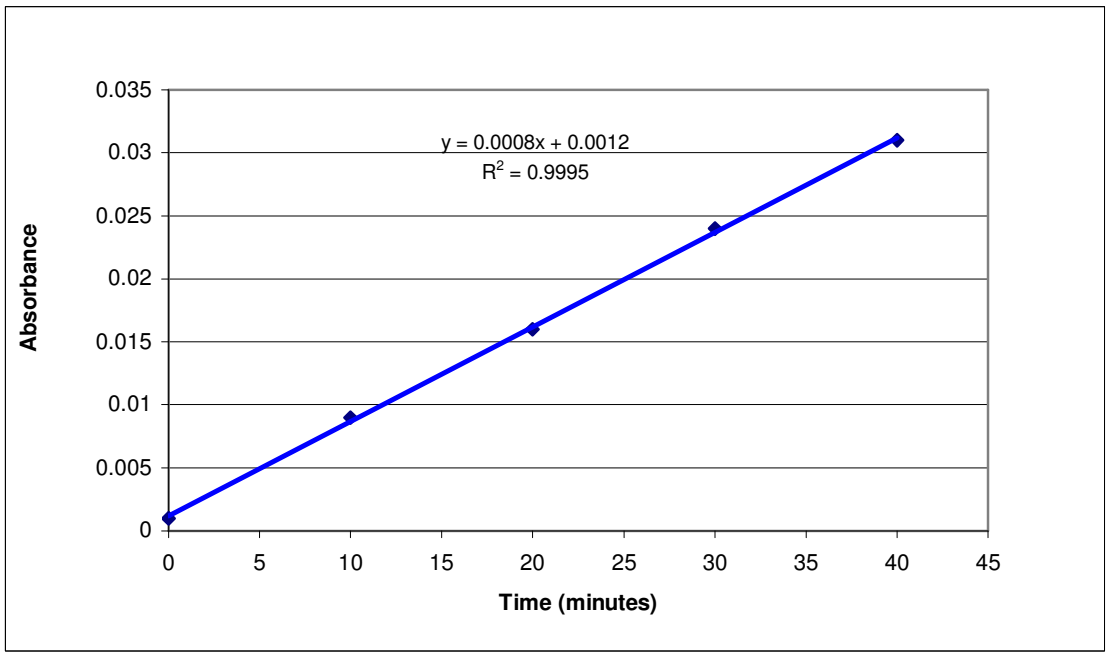

Figure 6-6. Inhibited LCDS activity in anaerobic bioreactor of the slow feed Cannibal system during the first week of testing.

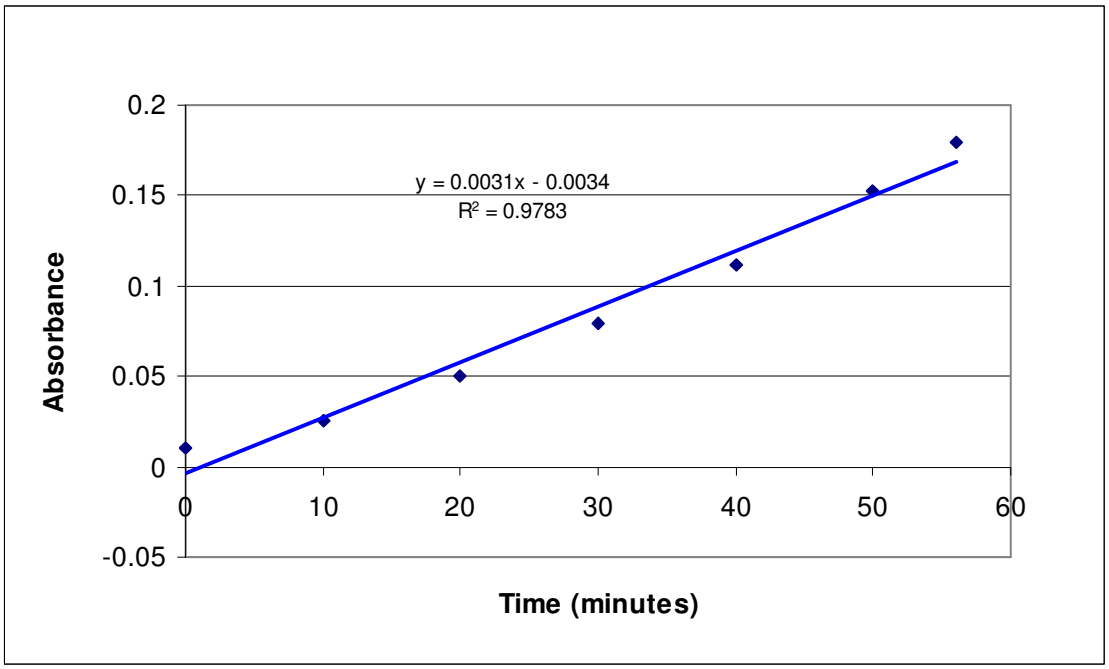

Figure 6-7. Uninhibited LCDS activity in anaerobic bioreactor of the fast feed Cannibal system during the first week of testing.

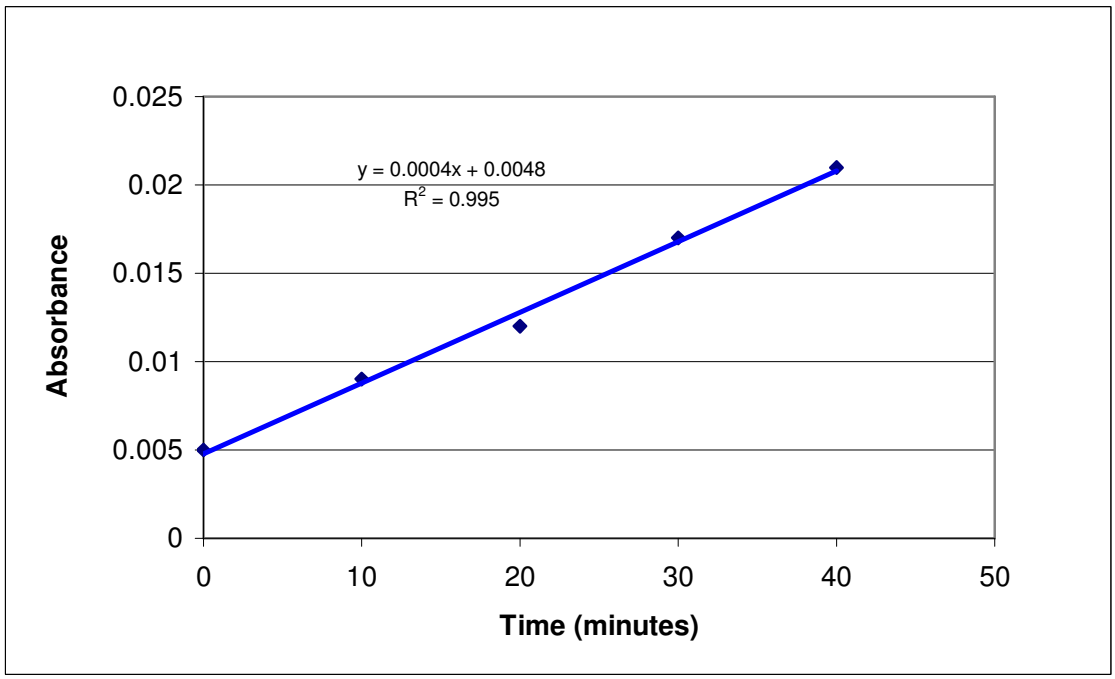

Figure 6-8. Inhibited LCDS activity in anaerobic bioreactor of the fast feed Cannibal system during the first week of testing.

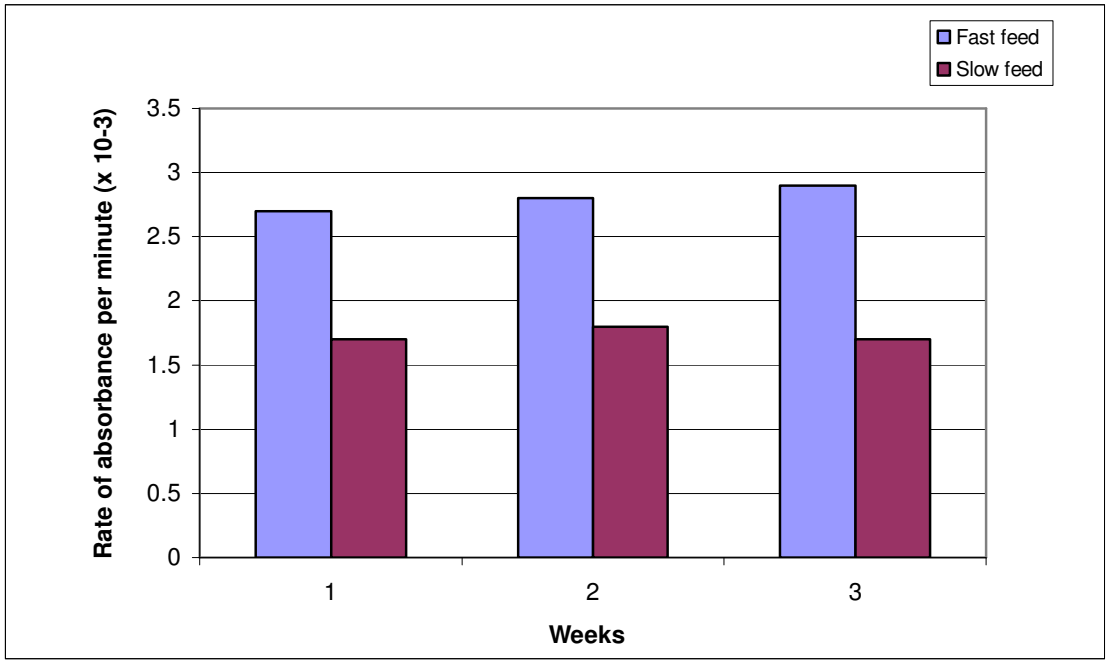

Figure 6-9. LCDS activity in anaerobic bioreactor of the slow and fast feed Cannibal system during three consecutive weeks.

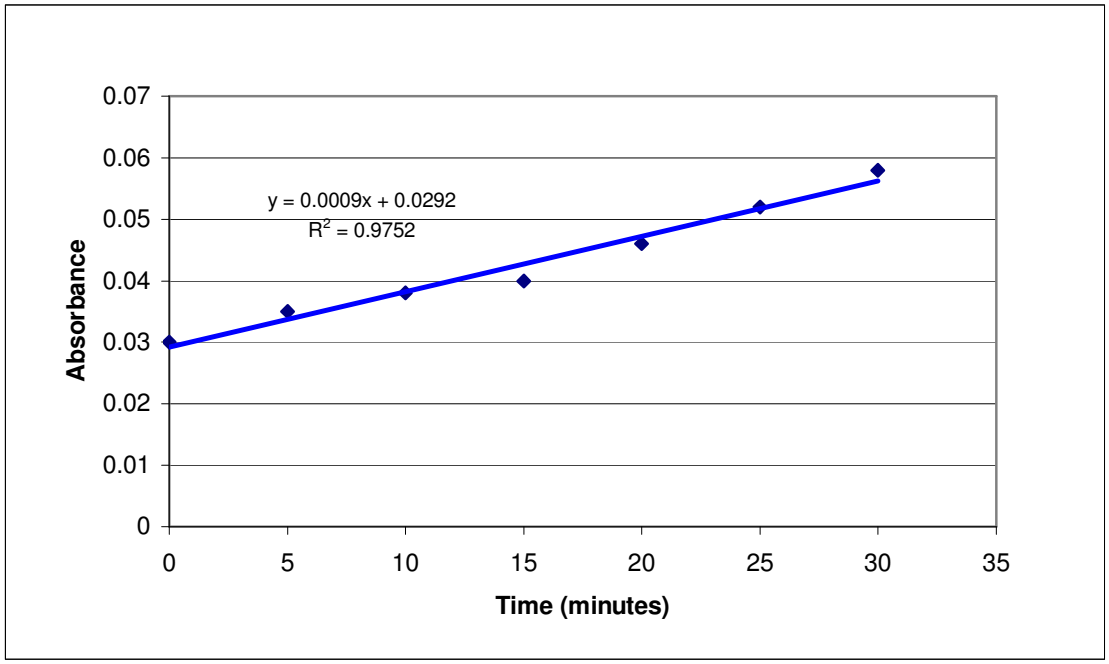

Figure 6-10. SCDS activity in anaerobic bioreactor of the fast feed Cannibal system.

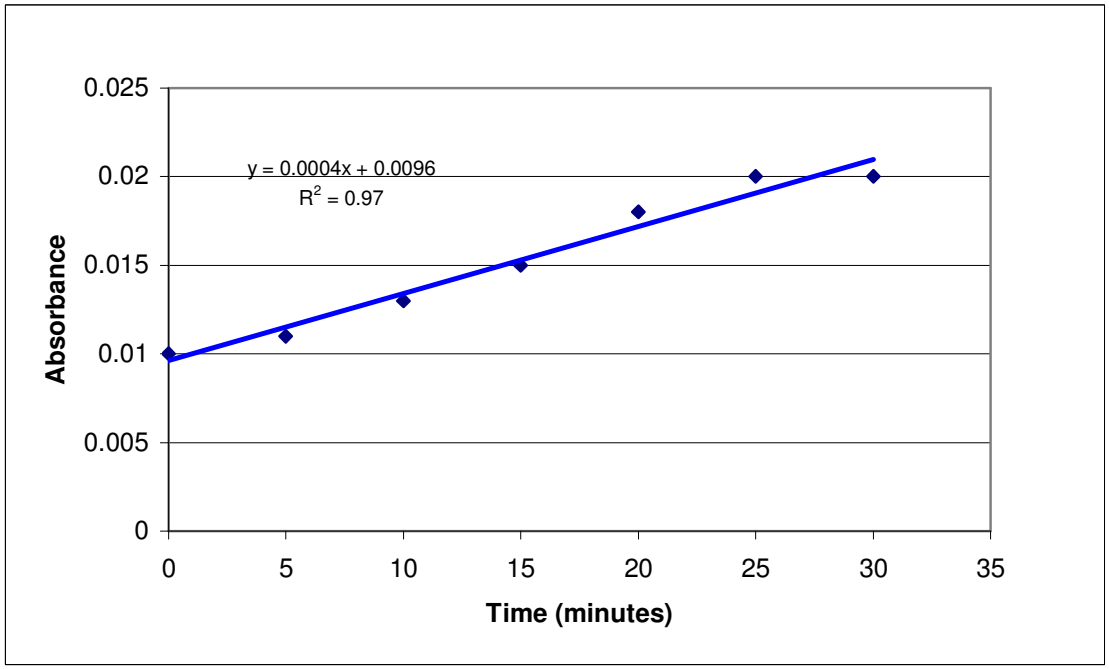

Figure 6-11. SCDS activity in anaerobic bioreactor of the slow feed Cannibal system.

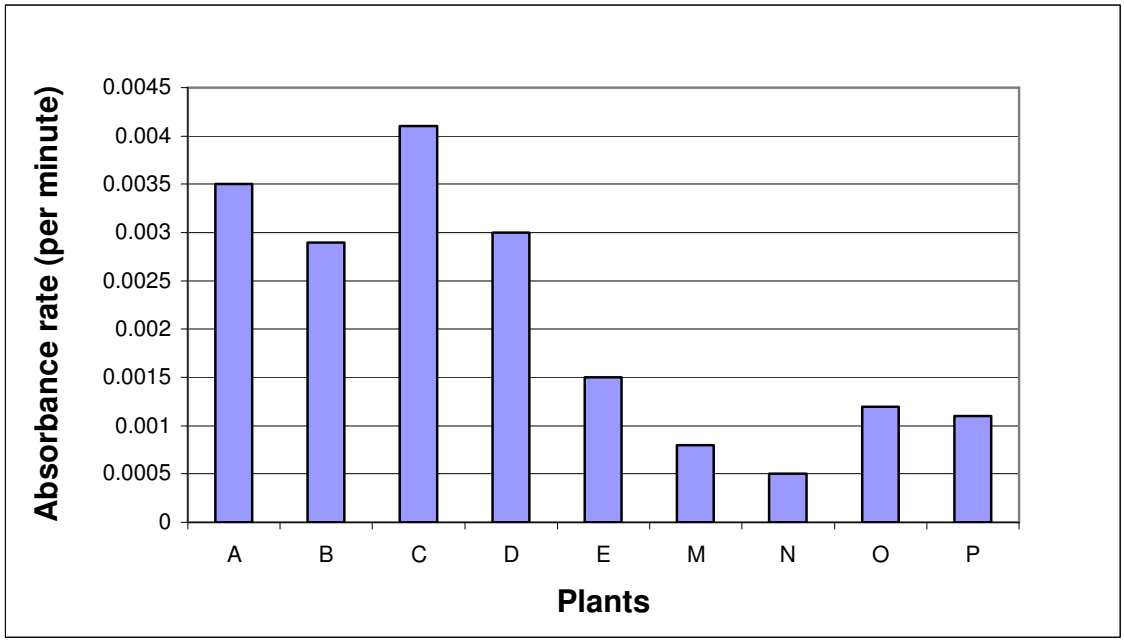

Figure 6-12. SCDS activity in anaerobic bioreactors of nine Cannibal wastewater treatment plants.

Table 6-1. Cations in anaerobic bireacotor of the Cannibal system operated under different feed patterns.

HRT (days)	Feed Pattern	Na^+	NH_4^+ as N	K^+	Mg^{2+}	Ca^{2+}
		mg/l	mg/l	mg/l	mg/l	mg/l
10	Fast feed	154.74	174.54	66.88	32.94	94.48
	Slow feed	122.16	93.84	44.64	24.58	56.43

Note: Data were taken from Khanthongthip, P. and Novak, J.T., (in preparation) The Mechanisms for Sludge Reduction in the Cannibal process under High and Low Substrate Pressure.

Chapter 7. The Structure of the Cannibal Sludges flocs generated under the fast and the slow feed conditions and their Digestibility

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The Structure of the Cannibal Sludges flocs generated under the fast and the slow feed conditions and their Digestibility

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Abstract

An activated sludge system that incorporates a sidestream anaerobic bioreactor, called the Cannibal process, was studied by Novak et al. (2007) to evaluate its capability for sludge reduction. They found that this system generated about 60% less solids than conventional activated sludge without any negative effects on the effluent quality. Later, Khanthongthip et al. (in preparation) studied this system operated with different feeding patterns. They found that for a sequencing batch reactor system, when the feed was provided to the Cannibal system over a period of 5 minutes (fast feed), the system generated less solid than the Cannibal system fed over a period of 4 hours (slow feed). In this study, the cation exchange resin (CER) and sulfide addition procedures were utilized to extract the Cannibal sludge flocs generated under the fast and the slow feed conditions to gain a fundamental insight on the structure of those flocs. In addition, the fast and the slow feed Cannibal sludge was anaerobically and aerobically digested to evaluate the effect of feeding patterns on their digestibility. It was found that the readily biodegradable (1 kDa.) CER and sulfide

extracted protein is larger in the flocs from the fast feed than the slow feed Cannibal system. When these two different types of flocs were anaerobically digested, the major fractions to be degraded were the sulfide extractable proteins. It was found that the proteins in the fast feed Cannibal sludge flocs were degraded more than the slow feed Cannibal sludge flocs under anaerobic conditions. Consequently, it can be thought that the influence of the high substrate pressure (fast feed) is to generate larger readily biodegradable proteins in the fast feed than the slow feed Cannibal sludge flocs. Because the 1 kDa. sulfide extracted proteins released from the fast feed Cannibal sludge floc are more biodegradable than that from the slow feed Cannibal sludge under an anaerobic environment, it can also be thought that the 1 kDa. proteins generated under the fast feed condition may consist of either many simple amino acids that are easily degradable, or amino acids that microorganisms cannot produce but need to consume for their survival.

Keywords: sludge, protein, polysaccharide, cation exchange resin extraction, sludge digestion, sulfide extraction, fast feed, slow feed.

Introduction

The activated sludge process is widely used for treating wastewater prior to its discharge to receiving water. Despite its usefulness in high organic matter removal efficiency, it remains a costly operation due to the large expense associated with excess sludge disposal. Over the past decades, operational and technological approaches to reduce biological solids

generation have been developed but many of these have encountered problems with implementation, effluent quality, reliability of the processes, and capital and operating cost (Macarie et al., 2000., Frankin et al., 2001., Lettinga et al., 2001.). In general, most of these sludge reduction technologies either add significantly more costs or are inappropriate in practical terms.

A promising process that has been recently developed to reduce activated sludge production and minimize the above problems is called the Cannibal process. This is an activated sludge process modified by incorporating an anaerobic sidestream reactor for return activated sludge prior to returning it to the aerobic reactor. A previous study by Novak et al. (2007) demonstrated that this process generates about 60% less solids than conventional activated sludge system without any negative effects on the effluent quality or settling characteristics. Later, Khanthongthip et al. (in preparation) studied the influence of substrate pressure on the performance of this system and found that the system produced less solid reduction when it was operated under low substrate pressure which was represented by feeding influent wastewater to a sequencing batch reactor over a period of 4 hours (slow feed) in each cycle. However, when high substrate pressure was provided by feeding the influent to the system over a period of 5 minutes, the solids reduction was higher.

In general, the structural composition of activated sludge flocs can be considered as a mixture of microorganisms, organic matter in addition to microbial cells, cations, and anions (Higgins et al., 1997). A number of studies have reported that extracellular polymeric substances (EPS) account for the major organic fraction (Frølund et al., 1996).

They originate from cell lysis, cell metabolism, and organic matter absorbed from the raw wastewater (Urbain et al., 1993, Nielsen et al., 1998, Park et al., 2007). A number of recent studies have shown that proteins are the most abundant EPS component in activated sludge (Nielsen et al, 1996). The viscous properties of EPS in activated sludge floc are responsible for microbial colonies (aggregation) and also bind cells to other particulate materials (cohesion), leading to the flocculent characteristic of activated sludge (Wingender et al., 1999). The polyanionic property of EPS and cations are significantly structural components as binding agents within biopolymeric matrix (Urbain et al., 1993). The multivalent cations are important as ionic bridges with EPS and maintain the flocs structure, leading to the stability of bioflocculation.

Novak et al. (2003) proposed that flocs consist of two important biopolymer fractions, which are divalent cation-bound biopolymer and Fe-associated biopolymers. They also found that aerobic digestion of activated sludge released calcium (Ca^{2+}) and magnesium (Mg^{2+}) into solution in conjunction with volatile solid destruction and accumulation of polysaccharide. In contrast, when the sludge underwent anaerobic digestion, a large amount of protein was released but no increase in divalent cations was found.

Therefore, those forms of flocs are expected to be presented in sidestream anaerobic bioreactors. Park et al. (2006) proposed that the major mechanism for sludge degradation during anaerobic digestion was the reduction of iron with the release of iron associated organic matter, primarily protein, which is easily degraded. Novak et al. (2007) used this mechanism to describe the solid reduction in the Cannibal process. They suggested that

when settled sludge was cycled to an anaerobic bioreactor, iron was reduced, and organic matter was released and solubilized. The released materials and sludge were returned to the aeration basin and were rapidly degraded. However, the structure and component of the sludge flocs generated in SBRs of the fast and the slow feed Cannibal systems are still not understood. Consequently, the investigation of flocs structure and their composition is needed to gain more insight on the system performance.

The objective of this study was to attempt to elucidate the nature of the sludge flocs generated under two different circumstances, high and low substrate pressure (fast and slow feeding). The fast feed condition is generated by providing the synthetic influent wastewater to the Cannibal system in a period of 5 minutes while the influent fed to the system in a period of 4 hours is called the slow feed condition. Two different extraction methods, cation exchange resin (CER) and sulfide extraction, were utilized to examine the floc structure. In addition, the digestibility of two different sludges was also studied under anaerobic and aerobic environment. The results of this research are expected to assist understanding the composition of flocs generated from two different environments and their digestibility under anaerobic and aerobic conditions.

Materials and Methods

The flocs structure and their composition were studied using extraction techniques developed by Park et al., (2007). The extraction methods utilized in this research were cation exchange resin (CER) and sulfide extraction. The sludge samples were taken from

lab-scale SBRs operating in the Cannibal mode using side-by-side fast and slow feed Cannibal systems.

The CER extraction was performed by adding a cationic resin to the sludge sample. The negatively charged resin will pull divalent cations (calcium and magnesium) out of the lectin-like floc, resulting in the release of materials bound to the cations that are lectin-like protein and polysaccharide. Wilen et al. (2003) found that this exchange mainly occurred between divalent cations and resin-Na^+ but not with either Al or Fe. This led to select the CER procedure to extract calcium and magnesium bound EPS.

The sulfide extraction was performed by adding sodium sulfide (Na_2S. $9H_2O$) to the sludge sample. Sulfide can reduce Fe(III) to Fe(II) and pull Fe(II) out of the floc matrix and form FeS, resulting in significant Fe-associated EPS floc disintegration and the materials bound to iron, primarily protein, are released to the solution.

Extracellular Polymeric Substance Extraction

All sludge samples were centrifuged at 12,000 g for 15 minutes at 3^oC to remove soluble EPS that is initially in the samples. This created a sludge pellet that was used for EPS extraction. For CER extraction, the pellet was resuspended with a low strength of phosphate buffer saline (PBS) (2 mM KH_2PO_4, 6 mM Na_2HPO_4, and 10 mM NaCl). The dose of CER (Dowex 50 x 8, Na^+ form, 20-50 mesh) was 60 g resin/g VS. The use of a buffer solution for the CER procedure is critical since the functional group must be ionized for the resin to work. Carboxyl group of carboxymethyl (CM) resin must be unprotonated,

carrying a fixed negative charge that interact with molecule carrying a positive charge, so the pH must be high enough to achieve this (pH > pKa of carboxyl group ($-COO^-$).Without a buffer, the resin and a portion of EPS can be protonated, resulting in less negative charge in the resin and reprecipitation of proteins and the extraction may not be efficiently performed. The extraction was performed for 1 hr with 600 rpm in a four-baffled extraction beaker for extraction enhancement. When the extraction was completed, the resin beads were removed by filtering through a nylon wire mesh (250 μm). After that, samples were centrifuged at 12,000 g for 15 minutes at 3°C and the supernatant was used to quantify biopolymers.

The procedure for sulfide extraction was similar to the method of Nielsen and Keiding (1998). This method consisted of two important steps, an iron-reducing step by addition of a sulfide molecule and physical iron extraction step by another sulfide molecule. The sludge pellet from the prior centrifugation was resuspended with 10 mM NaCl solution and sulfide addition in 200 mL flask, sealed with a rubber cap to maintain air-free condition, and purged with N_2 gas. The dose of sulfide was determined by the concentration of Fe in the sludge samples, basically using a molar ratio of S^{2-}/Fe at 2.0 or greater, using the stock solution of $Na_2S•9H_2O$ (600 mM, pH ~7.5). The sludge mixture gradually turned black with gentle mixing on a shaker overnight. Then, samples were centrifuged at 12,000 g for 15 minutes at 3°C and the supernatant was used to quantify sulfide extractable biopolymers.

EPS size separation was performed to examine the molecular distribution of biopolymers, protein and polysaccharide. Aliquots of supernatant were filtered through 1.5

µm, 0.45 µm, and 1 kDa. Ultrafiltration was performed by filtering an aliquot through 1 kDa membrane under 60 psi pressure. The filtration through 1.5 µm and 0.45 µm was carried out using a vacuum pump.

Digestibility Study

Sludge samples were collected from the aerobic zones of the SBRs operated as Cannibal systems under fast and slow feed conditions. In order to study the digestibility of the two different sludges under anaerobic and aerobic conditions, sludge samples were batch digested for 30 days under anaerobic (at 35^{o}C) and aerobic (at room temperature) environments. Approximately 300 ml of settled sludge from the SBRs of each Cannibal system was placed in 500 ml batch reactors. The mixing was continuously provided by magnetic stirrers. Distilled water was added to the reactor to make up for evaporative loses.

In anaerobic digestion, a rubber stopper with a gas bag to serve as a gas collector was placed on the top of the reactor and tightly sealed to prevent gas leakage. For aerobic digestion, air stones were placed at the bottom of the reactor to provide oxygen to the system. After digestion was completed, the CER and sulfide extraction were performed. The CER extraction for anaerobic digested sludge was performed under N_2 to minimize the chemical changes that might result from contact with air during the extraction. The quantity and molecular distribution of biopolymers (proteins and polysaccharides) were determined.

For the soluble phase of undigested and digested sludge, analyses of cations, protein, and polysaccharide were conducted. In this experiment, sludge samples were

centrifuged at 12000 g for 15 minutes at 3^{o}C and aliquots of centrate were filtered through 0.45-µm filters. Then, aliquots of filtrate were used to determine cations, protein, and polysaccharide.

Analysis

The extracted protein was determined by Hartree et al. (1972) modification of the Lowry et al. (1951) method to account for the interference of phenolic compounds and humic substances in protein quantification. The standard was bovine serum albumin. Polysaccharide was measured by phenol-sulfuric acid method of Dubois et al. (1956) using glucose as the standard. The soluble cations, calcium (Ca^{2+}), potassium (K^{+}), ammonium (NH_4^{+}), magnesium (Mg^{2+}), and sodium (Na^{+}) were determined by a Dionex (Sunnyvale, California) ion chromatography. Total iron (Fe) was analyzed according to U.S. Environmental Protection Agency Method 3050B (U.S.EPA, 1996). Total solids, volatile solids, total suspended solids, and volatile suspended solids were measured using Standard Methods (APHA et al, 1995). The pH was measured using an Accumet 910 pH meter (Fisher Scientific, Pittsburgh, Pennsylvania).

Results and Discussion

Novak. et.al. (2003) studied the mechanism of floc destruction during anaerobic and aerobic digestion and found that there were two significant forms of flocs presented in the

activated sludge system. They proposed that lectin-like proteins bind to polysaccharides that are cross-linked to adjacent lectin-like proteins with divalent cations bridging negatively charged sites on the exocellular biopolymers. Therefore, cross-linking of polysaccharides and cation bridges act as to stabilize the biopolymer network for this type of floc. Additionally, the role of Fe(III) in EPS flocculation was also addressed by these authors. They suggested that Fe(III) selectively binds to specific proteins to generate Fe(III)-associated EPS floc. They also found that the Fe-associated EPS flocs were primarily degraded under anaerobic conditions while divalent cation linked EPS flocs (lectin floc) were deflocculated under an aerobic environment. Park et al. (2007) demonstrated that Ca^{2+} and Mg^{2+} in lectin floc were removed by CER (cation exchange resin) extraction, resulting in the release of proteins and polysaccharides bound to the divalent cations, while Fe in Fe(III) associated EPS was unaffected by this procedure. However, they showed that iron in Fe(III) associated EPS flocs could be removed from the flocs by sulfide extraction.

The first phase of this research was to study the structure of the Cannibal sludge flocs generated under the fast and the slow feed condition. The data in Table 7-1 show that some amounts of polysaccharide were released by sulfide extraction of undigested fast and slow feed Cannibal sludge. This indicates that it is not only protein but also carbohydrate binding to Fe(III) for floc formation in the fast and the slow feed Cannibal systems. The data also reveal that polysaccharide was released into solution under anaerobic digestion. This is supported with a study by Khanthongthip et al. (in preparation) who found that solution phase polysaccharide accumulated in anaerobic bioreactors of the fast and the slow

feed Cannibal systems. It is thought that Fe(III) in an aerobic reactor of the Cannibal system binds to protein, polysaccharide, and glycoproteins, resulting in Fe(III) centered flocs. Therefore, when the settled sludge from the aerobic reactors are cycled to anaerobic condition in anaerobic bioreactors of the Cannibal systems, this floc type including lectin flocs can be degraded as indicated by the accumulation of protein, polysaccharide, and divalent cations (Khanthongthip et al., in preparation).

As demonstrated in Table 7-1, it can be seen that the polysaccharide fraction that passes a 0.45 μm filter of the slow feed Cannibal sludge flocs increased after anaerobic digestion. This implies that the biopolymers in slow feed Cannibal sludge might be more extractable after anaerobic digestion. Additionally, it can be thought that the polysaccharide fraction that passed the 1.5 filter was degraded to a smaller size so that it could pass through the 0.45 μm filter, resulting in the increase in the polysaccharide that could be filtered through the 0.45 μm filter after anaerobic digestion.

It can be seen, from Table 7-2 and 7-3, that the fraction of protein in the undigested fast feed Cannibal sludge, extracted by both sulfide and CER procedures that pass a 1k Dalton molecular weight ultrafilter is higher than that in the undigested slow feed Cannibal sludge. The data also indicate that the decrease of the 1 k Da. CER and sulfide extracted protein is higher in the fast feed than the slow feed Cannibal sludge after anaerobic digestion. This suggests that, during anaerobic digestion, more 1 kDa. CER and sulfide extracted proteins from the fast feed Cannibal sludge were released and degraded. Additionally, the data reveal that the portion of the protein that did not pass the 1 kDa ultrafilter is lower in the fast feed than the slow feed Cannibal sludge. The protein larger

than 1 k Dalton can be considered as slowly biodegradable while the material that passes the 1 k filter is readily biodegradable. Therefore, it can be thought that an effect of the fast feed (high substrate pressure) is to generate more readily biodegradable protein in the floc than the slow feed. The data in Table 7-4 demonstrate that the fraction of readily biodegradable polysaccharides (1 kDa.) is also higher in the fast feed Cannibal sludge but it is less than the protein.

The ratio of protein to polysaccharide in extracted biopolymers of undigested Cannibal sludge is considerably different between extraction methods and feeding patterns, as indicated by the data in Table 7-5. The data show that the ratio of protein to polysaccharide from the CER extraction of the undigested slow feed Cannibal sludge is less than that for the sulfide extraction. This is consistent with Park et al. (2007) who found that sulfide extraction for activated sludge always resulted in the higher ratio of protein to polysaccharide than extraction using cation exchange resin (CER). They concluded that sulfide-extracted EPS was rich in protein while polysaccharide comprised an important fraction in CER-extracted EPS. However, this is not a case with the undigested fast feed Cannibal sludge. The data in Table 7-5 also demonstrate that the ratio of protein to polysaccharide from extraction using CER is higher than the extraction using sulfide addition for this sludge type. This suggests that there were more polysaccharides binding to Fe(III) in Fe(III)-associated EPS floc in the fast feed than the slow feed Cannibal system. The larger fraction of polysaccharide in the Fe(III)-associated EPS floc in the fast feed Cannibal system also support the higher release of polysaccharide from the fast feed than the slow feed Cannibal sludge during anaerobic digestion, as shown in Table 7-6.

Anaerobic and aerobic digestion of the fast and the slow feed Cannibal sludge

Protein is the most abundant organic compound in activated sludge EPS (Frølund et al., 1996). Therefore, if solids are to be reduced in the Cannibal system, it is likely that degradation of this type of EPS will be significant. Novak et al (2003) postulated that the large release of protein from floc during anaerobic digestion was due to iron reduction and subsequent loss of binding between protein and ferric iron. In addition, Park et al (2006) proposed that the reduction of iron was a primary mechanism for sludge under an anaerobic environment, resulting in the release of iron-associated protein that is easily degradable. Therefore, it is thought that the release mechanisms in the Cannibal anaerobic bioreactor are similar to those in an anaerobic digester. If it is a case, substrate pressure (feeding patterns) should influence digester performance.

In the second phase of this research, the fast and the slow feed Cannibal sludge was batch digested under both anaerobic and aerobic conditions for 30 days. It was observed from the solution-phase following anaerobic and aerobic digestion that cation bound biopolymers react differently. The data in Table 7-6 illustrate that a large protein and ammonium concentration was detected in the solution of anaerobically digested fast and slow feed Cannibal sludge. In contrast, more polysaccharide along with a substantial increase in divalent cations was found in the fast and the slow feed sludge solution following aerobic digestion. The increase of ammonium concentration in the sludge

solution after anaerobic digestion of the fast and the slow feed Cannibal sludge indicates that proteins were primary degraded under the anaerobic environment. Additionally, larger amount of protein accumulation was seen in the anaerobically digested fast feed Cannibal sludge solution. This implies that the structure of the fast feed Cannibal sludge flocs may compose of higher amounts of proteins that are more biodegradable in the anaerobic condition than the Cannibal flocs generated under the slow feed condition.

In Figure 7-1 and 7-2, 1 kDa. proteins extracted from undigested and digested fast and slow feed Cannibal sludge using the CER and sulfide extraction procedures are compared to determine if a particular cation bound proteins specifically respond to anaerobic or aerobic conditions. The 1 kDa proteins are used because this protein size is considered as readily biodegradable materials. The extraction data in Figure 7-1 clearly show that CER extracted protein were primarily released under aerobic condition, resulting in smaller amount of protein remaining in the Cannibal sludge floc compared to anaerobic condition. The release of Ca^{2+} and Mg^{2+} along with protein and polysaccharide into the solution, as shown in Table 7-6, suggests that lectin flocs were degraded under aerobic condition. This implies that the degradation of lectin flocs under aerobic environment results in the release of the CER extracted protein. In addition, the data in Figure 7-1 and Table 7-6 show that the CER extracted proteins and divalent cations were released during anaerobic digestion. The data also demonstrate that the fast feed Cannibal sludge floc released more CER-target protein and divalent cations than the slow feed Cannibal sludge floc into the solution. This is in accordance with the study by Khanthongthip et al. (in preparation) who found more accumulation of solution protein, polysaccharide, and

divalent cations in the anaerobic bioreactor of the fast feed than the slow feed Cannibal systems. This implies that some lectin floc generated in the Cannibal system can be degraded under anaerobic conditions. In contrast to the CER-targeted proteins, sulfide extracted proteins were mainly released under anaerobic condition, as demonstrated in Figure 7-2. The large protein accumulation found in the solution from anaerobic digestion, as shown in Table 7-6, along with the dramatically reduction of protein in the flocs, as illustrated in Figure 7-2, indicates that the released proteins from Fe(III) associated EPS floc destruction are the sulfide extracted proteins.

Because the sulfide extracted proteins are selectively degraded under an anaerobic environment, a comparison of the fast feed with the slow feed Cannibal sludge can be made to evaluate their degradability under anaerobic conditions. It can be seen, from Figure 7-2, that the fast feed Cannibal sludge flocs contain more 1 kDa. sulfide extracted protein than the slow feed Cannibal sludge flocs. The data also show that approximately 78 % of 1 kDa. proteins were released from the fast feed Cannibal flocs while only about 45 % of the same size proteins were released from the slow feed Cannibal flocs. This resulted in less proteins remaining in the fast feed Cannibal sludge floc and higher protein accumulation in solution, as shown in Table 7-6. In addition, a larger ammonium concentration was found in the solution of anaerobic fast feed Cannibal sludge. This indicates that the 1 kDa. sulfide extracted proteins generated under the fast feed environment are more anaerobically degradable than that produced in the slow feed condition. It can be hypothesized that the 1 kDa. proteins generated under the fast feed condition may consist of either many simple amino acids such as alanine, serine, or glycine that microorganisms can easily degrade or

amino acids that microorganisms cannot produce but need to consume for their survival. However, additional research is needed to clarify this.

As shown in Table 7-6, the release of polysaccharides into solution after anaerobic digestion of the fast and the slow feed Cannibal sludge indicates that polysaccharide also binds to Fe(III) to form Fe(III) associated EPS flocs. It is likely that some glycoprotsins are involved (Park et al., 2007).

The results obtained in this study suggest that the Cannibal sludge flocs consist of different biopolymers associated with different cations. The feeding patterns (fast and slow feed) made an impact on the composition and structure of floc formation. This resulted in different behavior of each cation associated biopolymer when subjected to anaerobic and aerobic digestion.

Conclusions

Based on the data collected in this study, it can be seen that there are two different types of flocs generated in the fast and the slow feed Cannibal systems. One is divalent cations associated with protein and polysaccharide, called the lectin floc, while the other is iron primarily associated with protein and is generally called Fe (III) associated EPS floc. The lectin floc degrades primarily under aerobic digestion and the other during anaerobic digestion. This is consistent with the study by Novak at el., (2003) who proposed two new flocs models. Specific conclusions from this research are:

- It appears that polysaccharide also binds to Fe(III) in addition to protein to generate a new floc in the fast and the slow feed Cannibal systems.
- The amount of polysaccharide bound to Fe(III) is higher in the fast than the slow feed Cannibal flocs.
- The fast feed Cannibal sludge flocs contain more readily, but less non-readily biodegradable protein, than the slow feed Cannibal sludge flocs.
- The protein concentration in solution was substantially higher than the polysaccharide concentration under anaerobic environments while polysaccharide was primarily remained in the solution under aerobic conditions.
- The anaerobic and aerobic digestion of the fast feed Cannibal sludge resulted in larger soluble protein and polysaccharide accumulation than the slow feed Cannibal sludge.
- The release of the protein during aerobic digestion was CER extracted protein while it was sulfide extracted protein released under anaerobic digestion.
- The sulfide extracted proteins in the fast feed Cannibal flocs are more released and degraded than that in the slow feed Cannibal flocs under anaerobic conditions

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Table 7-1. Molecular distribution of polysaccharide in the undigested and digested sludge, extracted by using sulfide addition, from the Cannibal system operated under different feeding patterns.

Feed patterns	size	Polysaccharide extracted from undigested sludge (mg/g VS)	Polysaccharide extracted from anaerobically digested sludge (mg/g VS)	Polysaccharide extracted from aerobically digested sludge (mg/g VS)	Solution polysaccharide after anaerobic digestion
Fast feed	1.5µm	4.94	2.66	3.22	-
	0.45µm	3.51	1.73	2.12	43.56
	1 kDa	2.14	0.75	1.14	-
Slow feed	1.5µm	5.29	3.71	3.69	-
	0.45µm	3.21	3.55	2.36	28.35
	1 kDa	1.57	0.76	0.96	-

Table 7-2. Molecular distribution of protein in the undigested and digested sludge, extracted by using sulfide addition, from the Cannibal system operated under different feeding patterns.

Feed patterns	size	Protein extracted from undigested sludge (mg/g VS)	Protein extracted from anaerobically digested sludge (mg/g VS)	Protein extracted from aerobically digested sludge (mg/g VS)	Solution protein after anaerobic digestion
Fast feed	1.5µm	6.88	4.43	5.43	-
	0.45µm	5.64	2.81	4.10	183.56
	1 kDa	4.18	0.94	3.48	-
Slow feed	1.5µm	10.27	7.87	8.59	-
	0.45µm	7.11	4.42	5.54	98.35
	1 kDa	3.10	1.72	2.83	-

Table 7-3. Molecular distribution of protein in the undigested and digested sludge, extracted by using cation exchanged resin (CER), from the Cannibal system operated under different feeding patterns.

Feed patterns	size	Protein extracted from undigested sludge (mg/g VS)	Protein extracted from anaerobically digested sludge (mg/g VS)	Protein extracted from aerobically digested sludge (mg/g VS)
Fast feed	1.5µm	17.45	14.10	8.37
	0.45µm	11.42	7.38	4.79
	1 kDa	7.86	6.38	2.28
Slow feed	1.5µm	29.70	26.07	21.78
	0.45µm	10.88	8.86	6.85
	1 kDa	6.61	5.94	3.51

Table 7-4. Molecular distribution of polysaccharide in the undigested and digested sludge, extracted by using cation exchanged resin (CER), from the Cannibal system operated under different feeding patterns.

Feed patterns	size	Polysaccharide extracted from undigested sludge (mg/g VS)	Polysaccharide extracted from anaerobically digested sludge (mg/g VS)	Polysaccharide extracted from aerobically digested sludge (mg/g VS)
Fast feed	1.5µm	10.85	7.64	5.23
	0.45µm	7.54	5.24	3.21
	1 kDa	4.26	2.62	1.15
Slow feed	1.5µm	19.53	14.05	11.24
	0.45µm	11.37	8.36	6.22
	1 kDa	5.22	3.56	2.16

Table 7-5. The ratio of protein and polysaccharide in extracted biopolymers of the undigested fast and slow feed Cannibal sludge.

Feed Patterns	Size	Protein/polysaccharide from CER extraction	Protein/polysaccharide from sulfide extraction
Fast feed	1.5µm	1.61	1.39
	0.45µm	1.52	1.61
	1 kDa	1.85	1.78
Slow feed	1.5µm	1.52	1.94
	0.45µm	0.96	2.22
	1 kDa	1.27	2.61

Table 7-6. Changes in solution cations and solution biopolymers by 30-day batch anaerobic and aerobic digestion of the fast and slow feed Cannibal sludge.

Feeding patterns	Sludge	NH_4^+ (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	Solution protein (mg/L)	Solution polysaccharide (mg/L)
fast	Undigested (before digestion)	-	53.18	21.36	8.35	5.21
	Anaerobic digested	284.23	125.54	41.58	183.56	43.56
	Aerobic digested	35.43	188.64	63.18	48.21	83.17
slow	Undigested (before digestion)	-	33.65	14.35	7.24	6.31
	Anaerobic digested	176.29	78.53	32.61	98.35	28.35
	Aerobic digested	21.38	136.84	50.32	25.34	56.38

Note: Solution biopolymers are defined as materials that can pass a 0.45 µm filter

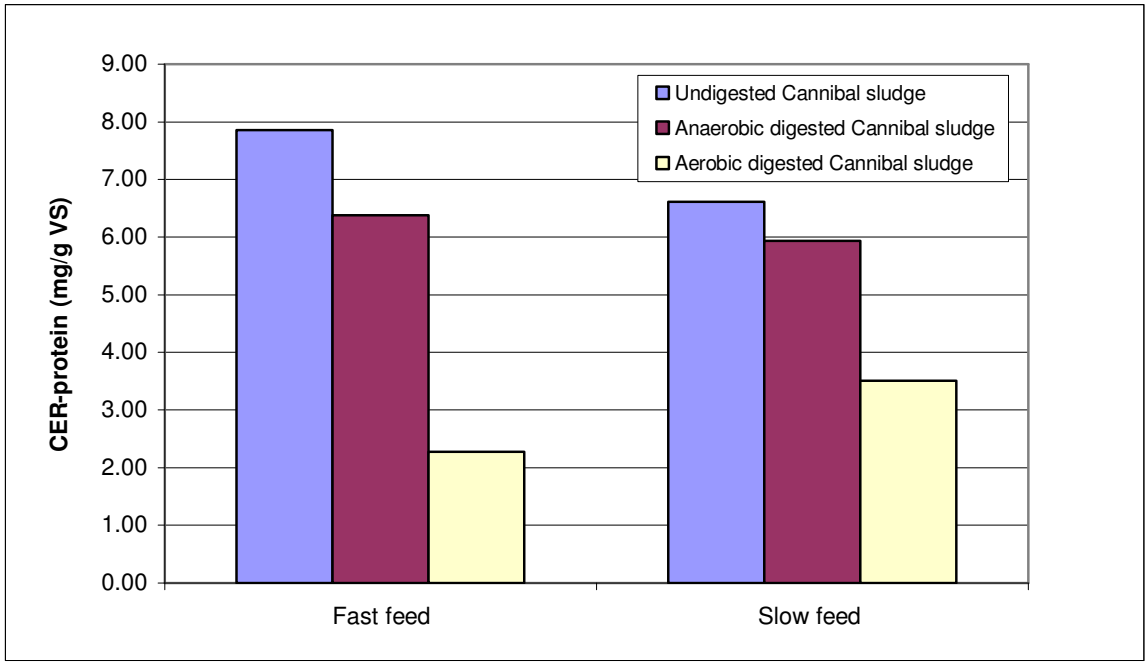

Figure 7-1. The content of 1 kDa. CER extracted protein in the fast and the slow feed undigested, anaerobically digested, and aerobically digested Cannibal sludge flocs.

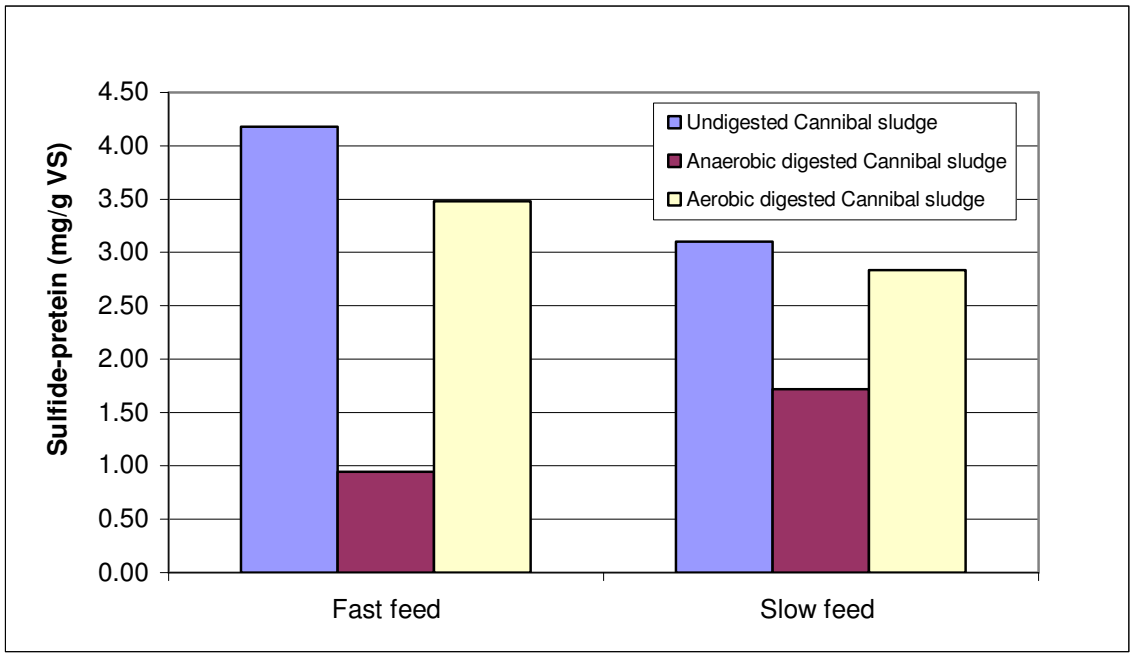

Figure 7-2. The content of 1 kDa sulfide extracted protein in the fast and the slow feed undigested, anaerobically digested, and aerobically digested Cannibal sludge flocs.