ACID-PHASE ANAEROBIC DIGESTION: EFFECT OF VARYING INDUSTRIAL-TO-MUNICIPAL FEED RATIO AND SUITABILITY OF EFFLUENT FOR BATCH-CULTURE DENITRIFICATION

By

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A Thesis
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Department of Civil and Geological Engineering
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EXECUTIVE SUMMARY

This thesis presents the findings of research conducted at the Department of Civil and Geological Engineering's environmental engineering laboratories. The research investigated the effect of varying the starch-rich, industrial wastewater content in mixtures with municipal wastewater feeds for anaerobic contact digesters. The next portion of study investigated the suitability of a fermented 1:1 (by volume) feed mixture effluent for denitrification with batch cultures.

Two identical, laboratory-scale, completely-mixed, anaerobic contact digesters were employed. Each had a volume of 3L and was operated at an HRT of 30 hours, an SRT of 10 days, and at ambient temperature of 21.5 ± 1.5 ^{o}C. The industrial-to-municipal ratios tested were 1:3, 1:1, 3:1 and 100% industrial by volume. Steady-state, acidogenic conditions were achieved for all the runs except 100% industrial.

No pH control was provided for anaerobic digestion. It was observed that pH dropped substantially as the industrial component of the feed was increased. Highly acidic conditions with pH < 4.0 were achieved as the ratio increased to 3:1 and 100% industrial. This acidic response reflected an increase in VFA production.

Net VFA production was maximized at the 1:1 ratio, with an average reactor concentration of 800 mg/L (as acetic acid). The minimum average net VFA content observed during the 1:3 feed ratio and was 594 mg/L. Net specific VFA production rates were a maximum at the feed ratios of 1:1 and 3:1 and both

equaled 0.069 mg*VFA/mg*VSS*d. VFA production rates for the 1:3 feed ratio was only slightly lower at 0.065 mg*VFA/mg*VSS*d.

Nearly identical patterns were revealed for net SCOD production and net specific SCOD production rates. Net SCOD production was quite similar for the 1:3, 1:1, and 3:1 feed ratios ranging from 1,180 to 1,375 mg/L. The corresponding production rates were for the 1:3 and 3:1 feed ratios were nearly identical at 0.132 and 0.137 mg*SCOD/mg*VSS*d, respectively. The 1:1 ratio performance fell below those of 1:3 and 3:1 at 0.102 mg*SCOD/mg*VSS*d.

The performance of average net VFA production, average net VFA production rate, average net SCOD production, average net SCOD production rate, and VSS destruction were each improved significantly compared to 100% municipal feeds as reported by Banerjee (1997) and Maharaj (1999).

An increase in the industrial content also had an effect on the speciation of VFAs. Acetic acid content fell steadily from 69% with 100% municipal feeds to 51% at the 3:1 feed ratio. A much more dramatic drop was seen with respect to propionic acid as it fell from 27% with 100% municipal feed to 9, 15, and 9% at the next three feed ratios. The other VFA variety that was significantly affected was butyric acid. Butyric acid content rose from 2% with 100% municipal feeds to 18, 22, and 37% at the feed ratios of 1:3, 1:1, and 3:1, respectively. All feed ratios resulted in very little valeric, and especially isobutyric, or isovaleric acid production.

VSS destruction was seen to be a maximum of 79.2% at 1:1 feed ratio. The VSS destruction profile was quite bell-shaped with minimal destruction observed at the 100% municipal feed ratio and approximately equal destruction at

the 1:3 and 3:1 feed ratios near 66%. In addition, the bacterial population appeared most healthy at the 1:1 ratio as reflected in the maximum observed VSS/TS ratio.

Net ammonia production was strongly a function of feed ratio. At the 100% municipal and 1:3 feed ratios, ammonia was produced, while ammonia disappeared at the next three ratios. Ammonia production fell dramatically as the industrial content increased, indicating a strong dependence.

The anaerobic digestion research summarized in this thesis ought to be replicated with relative ease. It was shown that the hydrolysis-acidogenesis phase of anaerobic digestion can be prolonged indefinitely through the use of this equipment under the proper environmental and operational conditions.

The second phase of this study tested the suitability of 1:1 feed ratio fermented effluent for denitrification. It was hypothesized that the VFAs in the effluent would serve as an organic carbon source and enhance denitrification. This research was carried out in batch tests using 1,000 mL Erlenmeyer flasks seeded with prepared primary sludge. Nitrates were added in the form of 1M potassium nitrate solution. Tap water was added to standardize all flask volumes to 600 mL. The tests were conducted at ambient temperature (21 to 24 ^{o}C).

Initial tests were needed to streamline the seed preparation protocol and develop a meaningful sampling schedule. As a result, no directly applicable kinetic data was gained from these runs. However, in addition to developing a successful method, it was shown that denitrification was occurring. Nitrates were not consumed through other means as they persisted in the absence of seed. Also, nitrates were observed to completely disappear in conjunction with significant

recovery of pH and alkalinity. Such observations are highly indicative of biological denitrification.

In all test cases, denitrification followed zero-order kinetics. It was observed that the addition of the VFA effluent hampered denitrification rates. This was due to the low pH of the VFA effluent, with an average pH of 4.54.

With respect to VFA consumption, the denitrifier population had a preference for acetic acid. This VFA variety was available in the highest quantity and was exhausted most quickly. The next most abundant VFA was butyric acid and it too was consumed quickly. However, it was consumed only after acetate concentration began to decline. The third "choice" of the denitrifiers was propionic acid. Again, its concentration only saw significant decreases after the two aforementioned VFAs became limiting. The denitrifier population had no preference for valeric and especially isobutyric and isovaleric acids. The concentrations of these acids remained stable until acetate, butyrate, and propionate became limiting.

The apparatus and procedures employed in the course of the denitrification research are easily replicable; however, denitrification with batch cultures proved to have a strong dependence on seed preparation. Therefore, research of this sort will be dependent upon the seed. Differences in seed preparation, seed storage, conditions of transport, and conditions at the source will probably have a strong impact on denitrifier performance.

GLOSSARY OF ACRONYMS

ABR - anaerobic baffled reactor
ADP - adenosine diphosphate
AEBR - anaerobic expanded bed reactor
ATP - adenosine 5'-triphosphate
BNR - biological nutrient removal
BOD - biological oxygen demand
BPR - biological phosphorous removal
C:N - carbon-to-nitrogen ratio
CoA - coenzyme A
COD - chemical oxygen demand
DNRA - dissimilatory nitrate reduction to ammonia
DO - dissolved oxygen
DSS - digested sludge supernatant
F/M - food-to-microorganism ratio
Fp - flavoprotein
HAc - acetic acid
iHBu - isobutyric acid
nHBu - butyric acid
HPr - propionic acid
HRT - hydraulic retention time
iHVa - isovaleric acid
nHVa - valeric acid
Hy - hydrogenase
NAD - nicotinamide adenine dinucleotide
NADH - nicotinamide adenine dinucleotide (reduced)
PCP - pentachlorophenol
PFR - plug-flow reactor
P_i - inorganic phosphorous
QH_2 - ubiquinol
red - reductase
SCOD - soluble chemical oxygen demand
SOC - soluble organic carbon
SRT - solids retention time
SS - suspended solids
Std. dev. - standard deviation
TCOD - total chemical oxygen demand
TKN - total Kjeldahl nitrogen
TN - total nitrogen
TS - total solids
TSS - total suspended solids
UASB - upflow anaerobic sludge blanket
UCT - University of Cape Town
VFA(s) - volatile fatty acid(s)
VS - volatile solids
VSS - volatile suspended solids
WWTP - wastewater treatment plant
Y_{NET} - net yield

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In addition to Ms. Tingley, other people were also frequenters of the environmental engineering laboratories. The people of whom I write were the other research students. The support and camaraderie showed by these people is deeply appreciated. Indira Maharaj was very helpful showing me the ropes as I familiarized myself with the equipment and analysis techniques. From making tea to finding relevant articles, Greg Bujoczek was a smiling fixture in the laboratory. His wife Maja was also kind and only too willing to extend a helping hand. Laura Wytrykush and Chunhe Liu went through the program with me and we shared many good times. I wish them the best. Sarah Wakelin also entered the graduate program with us. She and I became instant friends, mostly thanks to her excellent sense of humour, her willingness to listen to my complaints, and her supportive words and deeds. I owe the environmental engineering program a great deal for introducing us.

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William Henry Smith

who valued hard work and sacrifice,
yet never cherished these above his family.

1 INTRODUCTION

The planet Earth is finite. With the exception of cosmic and solar radiation and the odd meteor, the planet Earth is a closed system. Matter neither leaves nor enters Earth in appreciable quantities. This must be considered by society as a whole as we usher in the new millennium and continue to "develop" the Earth. The carbon and nitrogen and other nutrients we consume and expel cannot leave the Earth. They must somehow be absorbed, transformed, and reconstituted into forms that not only suit our needs, but also not impair the natural cycles of the Earth. Of course, we too are destined to die and change form while remaining integral parts of our home: the Earth.

Unfortunately, human population and activity has grown to the point where it is beginning to stress the major nutrient cycles of the planet. The nutrient cycles are overloaded in many regions and locations where human population is so concentrated that our wastes cannot be absorbed, transformed, and reconstituted quickly enough. This is the point where the engineer enters the picture.

Engineered systems seek to enhance the ecosystem services provided by the interaction of many microorganisms to transform polluting, putrescent wastes into "clean," benign effluents. Such systems are often required to treat wastewater for the removal of excess carbon. One way to achieve this end is through anaerobic digestion. Anaerobic bacteria require no oxygen and produce less biomass than aerobic bacteria. They also produce methane gas, which can be used as a fuel. Intermediate products of anaerobic digestion are volatile fatty

acids or VFAs. These compounds are useful on their own apart from fueling subsequent methane production.

Not only are engineering systems involved in carbon recycling, but also nitrogen recycling. Nitrogen is removed from human wastes by a three-step process. The first step is ammonification, followed by the second called nitrification. The third step, called denitrification, requires readily digestible organic carbon compounds such as VFAs. A number of organic carbon substrates have been shown adequate for this purpose, but the advantage of using VFAs is that they can be produced cheaply and on-site. In this way, anaerobic digestion of primary sludge or wastewater achieves two goals simultaneously: first, excess carbon is removed from the waste during anaerobic treatment; second, the anaerobic effluent containing VFAs can be further degraded as VFAs furnish denitrification.

While this paper focuses on the use of VFAs for denitrification, VFAs are also used in phosphorus removal. Thus, VFA production during anaerobic treatment has become a major research topic. Such research has fostered the worldwide blossoming of installations that combine anaerobic VFA production and nutrient removal, which have been shown to be feasible and economically advantageous. Such trends are encouraging, as surface water quality is anticipated to improve through the judicious and determined application of this technology.

2 LITERATURE REVIEW

2.1 Anaerobic Digestion Processes and Fundamentals

2.1.1 Introduction

Anaerobic digestion occurs throughout the biosphere. It can be observed almost anywhere dead organic matter is present, especially in soils and sediments. The digestive tracts of animals, particularly ruminants, are another very important site of anaerobic digestion. In fact, much of the knowledge of anaerobic bacteria has been (and continues to be) gained from the study of the digestive systems of ruminants (Chynoweth and Isaacson, 1987). The absence of molecular oxygen characterizes anaerobic digestion as well as the production of methane gas. The production of other gases, such as hydrogen, carbon dioxide, and hydrogen sulfide, is also commonly associated with anaerobic digestion (Henry and Heinke, 1996).

2.1.2 Engineered Biological Treatment of Wastewater

Wastewater is treated biologically by bringing it into contact with microorganisms that consume its constituent organic matter, also referred to as its organic carbon. In addition to bacteria; fungi, algae, protozoa, nematodes, and rotifers are all found in the treatment vessel or film (Metcalf and Eddy, 1991). These organisms interact as a biological community to rid the wastewater of its biodegradable organic matter, both colloidal and soluble. However, bacterial

species are the chief consumers of this organic matter. Other microorganism species consume only minor quantities or prey on the bacteria themselves (Metcalf and Eddy, 1991).

Carbon-consuming bacteria may contact influent wastewater in a number of configurations. High volume wastewater treatment plants most often stabilize wastewater under aerobic conditions, although high volume anaerobic treatment of wastewater is not uncommon (Riggle, 1998; Barber and Stuckey, 1999). Aerobic treatment falls into either fixed-film or activated-sludge categories. The key difference between these categories is the state of mobility of the bacteria. Fixed-film processes capture and cultivate bacteria on the surfaces of immobile objects, be they grains of sand or large, flat structures. Influent wastewater passes over and nourishes these bacteria. Activated-sludge treatments feature free-floating bacteria which mix with the wastewater in a large treatment vessel.

The bacteria in question convert organic carbon into both carbon dioxide gas, which escapes into the atmosphere, and new cell tissue. This cell tissue constitutes the bulk of clarifier solids. Separation of settled bacterial solids generally occurs in a clarifier before and/or after the treatment process. In these ways, as escaping gas or as settled cell tissue, excess organic carbon is removed from the wastewater. The settled solids are collectively called sludge. Sludge tends to be high in strength (*i.e.* high oxygen demand). It is usually necessary to further treat the sludge in order to reduce its organic carbon content. This treatment often takes the form of anaerobic digestion (Metcalf and Eddy, 1991).

The widespread use of anaerobic digestion to treat wastes has been traditionally discouraged. It was thought that this form of treatment was

vulnerable to shock loads, high concentrations of residual solids, and the requirement of additional effluent treatment (Duran and Speece, 1997). As a result, anaerobic treatment was normally reserved for high-strength wastes such as sludges. However, many benefits of anaerobic treatment methods versus conventional aerobic treatment exist: decreased energy costs, decreased biomass synthesis, high pathogen kill rates, the production of energy-rich methane gas, and the production of a valuable soil conditioner (Duran and Speece, 1997; Ghosh *et al.*, 1975).

According to Riggle (1998), the number of anaerobic digestion systems, including those which treat wastewater, is increasing substantially worldwide. This trend will likely continue as anaerobic digestion is further studied and promising new technologies and applications are introduced. In fact, D'Addario *et al.* (1993) have even studied the feasibility of using municipal anaerobic digestion effluents as fuel additives!

2.1.3 Wastewater Composition and Anaerobic Treatability

Anaerobic bacteria are charged with the responsibility of converting putrescent organic carbon compounds into more benign, less polluting forms. As mentioned earlier, these forms may be gases or stabilized soluble molecules. What are the problematic organic compounds which must be treated? Generally speaking, municipal sludge contains three principle carbon compounds: carbohydrates, proteins, and lipids. These compounds are said to account for 80 to 100% of the volatile solids (VS) found in primary sludge (Elefsiniotis, 1993). Other

compounds are also found in small quantities in municipal sludge and fall under the heading of industrial waste in the following discussion. These compounds include surfactants (*e.g.* soap), nonmetals (*e.g.* arsenic), metals (*e.g.* cadmium), organic chemicals (*e.g.* toluene), and halogenated organic chemicals (*e.g.* pesticides) (Metcalf and Eddy, 1991). It should be noted that some organic compounds are not biodegradable within reasonable time frames due to inaccessibility or the presence of nonhydrolyzable linkages. As high as 70% or even 80% of volatile solids may be of this form (Parkin and Owen, 1986).

2.1.3.1 Carbohydrates

Carbohydrates are complex molecules which contain only carbon, hydrogen, and oxygen and have the general formula $(CH_2O)_n$ where n>2. Many carbohydrates are found in municipal wastewater such as sugars, starch, cellulose, and lignin. Only sugars are water soluble. Short carbohydrate molecules containing three to seven carbon atoms are called monosaccharides (Campbell, 1996). These molecules, joined by glycosidic linkages, form the building blocks for polysaccharides.

Starch is an example of a polysaccharide and of particular interest to this study. Starch is entirely comprised of glucose monomers joined by 1-4 glycosidic linkages. Two forms of starch exist: amylose and amylopectin. The simpler form, amylose, is a linear macromolecule with no branches. Amylose has molecular weights ranging from 150,000 to 600,000 and constitutes approximately 20% of starch. The other approximately 80% is represented by amylopectin. Amylopectin can be highly branched, usually off the sixth carbon

atom at twenty to twenty-five carbon intervals. Molecular weights of amylopectin run into the millions (Vollhardt, 1987).

2.1.3.2 Proteins

These macromolecules are the most complex known and account for over 50% of the dry weight of most cells. They exist without and within cells as support structures, storage molecules, transport molecules, motility facilitators, and enzymes (Campbell, 1996). Each type of protein molecule has a unique three-dimensional shape called its conformation. Despite this complexity, all proteins are formed of different combinations of the same twenty α-amino acids joined via peptide bonds. Amino acids invariably possess carbon, hydrogen, oxygen, and nitrogen atoms. In fact, proteins are the chief source of organic nitrogen in wastewater. Some amino acids also contain sulfur and phosphorous. As a result, most proteins will contain all six of these elements. Depending on its conformation, proteins may be either soluble or insoluble in water and may have molecular weights greater than 1,000,000 (Vollhardt, 1987).

2.1.3.3 Lipids

Lipids are molecules which are highly insoluble in water and therefore serve well as energy storage molecules and as membranes. Lipids are classed as fats, phospholipids, or steroids. What makes lipids so hydrophobic is their linear, non-polar, hydrocarbon "backbones," which are fatty acids of sixteen to eighteen carbon atoms in length (Prescott *et al.*, 1996). This hydrocarbon backbone is attached to a hydrophilic glycerol "head." This hydrophobic/hydrophilic structure

explains lipid suitability to membrane functions. In addition to this hydrocarbon structure, fats and steroids contain oxygen, while phospholipids contain oxygen, phosphorous, and nitrogen (Vollhardt, 1987).

2.1.3.4 Industrial Wastewater

This blanket term refers to all effluents from industrial sources. However, due to regulation, many highly toxic wastewaters are treated on site and therefore do not enter the municipal treatment plant in this toxic form. Those industrial wastewaters that do enter directly are normally liquid wastes boasting a high organic content. For example, Carrieri *et al.* (1993) studied the performance of anaerobic co-digestion of sewage sludge, olive mill effluents, cheese whey, and landfill leachate. They found that volumetric organic loading rates could be doubled without compromising the performance of the digester. Another study tracked the effect of anaerobic co-digestion of print pastes and primary sludge (Malpei *et al.*, 1998). In this case, municipal sludge digestion was not affected but the print pastes remained relatively unchanged. As these examples demonstrate, anaerobic co-digestion may or may not accommodate industrial wastewater. The situations are individual and must be evaluated separately. A summary of the anaerobic treatability of various industrial wastewaters is shown below in Table 2.1.

Low-strength wastewater and its anaerobic treatability are of particular interest to this study given the low strength of the wastewater studied here. Alderman *et al.* (1998) have analyzed the feasibility of employing anaerobic pretreatment of low-strength municipal wastewater in organically overloaded

plants. The authors employed an anaerobic expanded bed reactor (AEBR) and found it a viable option in some cases. Collins *et al.* (1998) conducted an investigation of anaerobic digestion applicability to treatment of low-strength wastewater sludge. The authors successfully destroyed 90% of sludge COD under a range of temperatures and organic loads using an AEBR. Even such severe conditions as $5^{O}C$ temperatures and low influent COD concentrations of 50 mg/L did not hamper reactor performance.

Table 2.1: Anaerobic Digestion Performance of Various Substrates

WASTE	COMPOUNDS EVALUATED	COMPOUND REDUCTION	REFERENCE
Olive mill effluent; pre-digested piggery effluent	Phenolic compounds, COD = 25 g/L	COD: 74%, phenols: >50%	Marques *et al.* (1998)
Olive oil mill effluent	Phenolic compounds, COD = 90 g/L	COD: 70-80%, lipids: <88%	Beccari *et al.* (1996)
Fish meal factory effluent	Protein = 10-30 g/L	80%	Guerrero *et al.* (1999)
Municipal landfill leachate	COD = 3.8-15.9 g/L	64-85%	Timur and Özturk (1999)
Toxic organic compounds	Chlorophenol and phthalate compounds	90%	Parker *et al.* (1994)
Toxic organic compounds	bis-2-ethylhexyl phthalate, *o*-cresol	55-65%	Parker *et al.* (1994)
Pentachlorophenol (PCP)	PCP = 35 mg/L, glucose	Glucose: 99%, PCP: 0%	Piringer and Bhattachrya (1999)
Agricultural pesticides	Dicamba, Metribuzin	Limited	Pavel *et al.* (1999)
Soap	Soap	80%	Prats *et al.* (1999)
High sulfate wastewater	COD = 2 kg/L, SO_4^- = 1 kg/L	COD: 90%, SO_4^-: 100%	Yamaguchi *et al.* (1999)

Starch and similar compounds were found to be responsive to anaerobic digestion. Chyi and Dague (1994) researched the conversion of cellulose, a carbohydrate very closely related to starch, into soluble COD. The authors

determined that a pH of 5.6 was optimal and a hydraulic retention time (HRT) of 48 hours was sufficient to convert 44% of colloidal COD to soluble COD products (*e.g.* VFAs and alcohols).

Starch is a major bi-product of potato processing industries, as is the case with the wastewater of this study. This wastewater is high in COD, nitrogen, phosphorous, and VSS. According to Abeling and Seyfried (1993), the most economical method of treating starch wastewater is with a two-stage anaerobic-aerobic process. The authors contend that this treatment reduces the said four measures to values under current discharge limits. COD reduction averaged 99%, TKN reduction was nearly total, phosphorous levels were reduced by 97% on average, and suspended solids were completely removed. The role of the anaerobic pretreatment phase is to convert colloidal starch molecules into soluble COD which is required for nutrient (N and P) removal. This aim was largely achieved and control of the anaerobic process was excellent.

Kwong and Fang (1996) conducted laboratory-scale tests to determine the applicability of anaerobic digestion to cornstarch wastewater. Two reactor configurations were used and variation between the reactors was slight. Both removed 95.3% COD at an HRT of 12 hours and with influent COD concentrations up to 45 g/L. Both reactors failed when organic loads reached 90 g*COD/L*d due to solids washout. The previous three studies cited are definitely encouraging to the notion of anaerobic treatment of starch wastewater.

However, the previous three studies treated wastewater with starch or similar compounds as the sole or predominant carbon source. The wastewater of this study contains complex mixtures of starch and municipal wastewater sludge.

Jeyaseelan and Matsuo (1995) compared the efficiencies of phase separated anaerobic digestion of two complex milk mixtures. One mixture, skim milk, contained 87% carbohydrates and proteins while the other, baby milk, contained 94% carbohydrates and lipids (the remaining 13% as lipids in the former and the remaining 6% as proteins in the latter). They found digestion efficiencies were "very much greater" for mixtures high in lipids than those high in proteins.

The apparent difficulty in digesting proteins was further validated in other studies. Breure *et al.* (1986a) studied the adaptation of complex anaerobic bacterial populations to glucose (carbohydrate) and gelatin (protein) substrates. In one investigation, bacteria reached steady-state with a glucose substrate, which was then abruptly switched to gelatin. After the switch, digestion stopped. When gelatin was added in conjunction with glucose, glucose digestion continued unabated while gelatin was less than 30% digested. These results indicate a preference for carbohydrate substrates by anaerobic bacteria.

In another variation of the above study, Breure *et al.* (1986b) showed when gelatin-adapted bacteria were fed glucose and gelatin, gelatin solubalization was not affected but subsequent rates of conversion of gelatin to acidic end products was reduced. In addition, protein degradation rates slowed as the substrate was diluted and as carbohydrate content increased. On the basis of these studies, the authors concluded that "anaerobic bacterial populations can loose their ability to degrade protein substrate" in the presence of carbohydrate (Breure *et al.*, 1986a) and in order to achieve maximum COD destruction, digestion of carbohydrates should be "spatially separated" from that of protein (Breure *et al.*, 1986b). This recommendation obviously cannot be implemented

when considering full-scale anaerobic digestion, but it does point to natural limitations of the process. Indeed, the authors note "complete degradation of protein in the presence of carbohydrates often cannot be achieved in anaerobic waste water treatment" (Breure *et al.*, 1986b).

2.1.4 Anaerobic Digestion Metabolism

The pathways by which organic molecules (especially proteins, lipids, and carbohydrates) in municipal and industrial sludges are converted to the signature anaerobic end-products have been studied in great detail. Novaes (1986) notes that Omelianski was one of the first researchers in this field to examine methane formation using a cellulose substrate in 1906. Since that time, much research and knowledge has accumulated.

2.1.4.1 Overview

Many models and theories linking organic molecules with the end-products exist, but most share certain common features. Some models, such as that proposed by Gujer and Zehnder (1983), divide the anaerobic digestion process into as many as six stages; while models like those presented by Wang (1994) and Metcalf and Eddy (1991) simplify anaerobic digestion into only three stages. Despite such an apparent variation in approach, these models agree upon the major stages of anaerobic digestion. A "compromise" model proposed by Novaes (1986) is pictured schematically in Figure 2.1. Figure 2.1 illustrates that anaerobic digestion occurs in four stages detailed below.

a. Hydrolysis

Large colloidal or soluble molecules are split into smaller molecules in order to be transferred into the bacterial cell and used as energy and carbon sources. Hydrolysis is facilitated through the bacterial excretion of extracellular enzymes. Complete hydrolysis would witness the production of monomers of the organic macromolecules. In anaerobic digestion, hydrolysis is often the rate-limiting step, especially with wastewaters high in volatile solids (Ghosh, 1987; Valentini *et al.*, 1997).

b. Acidogenesis

This stage of anaerobic digestion is responsible for the production of simpler, intermediate compounds from monomers produced during hydrolysis. Some important examples include volatile fatty acids (VFAs), alcohols, and diatomic hydrogen. Acidogenesis is a fermentation step, meaning organic molecules both accept and donate electrons.

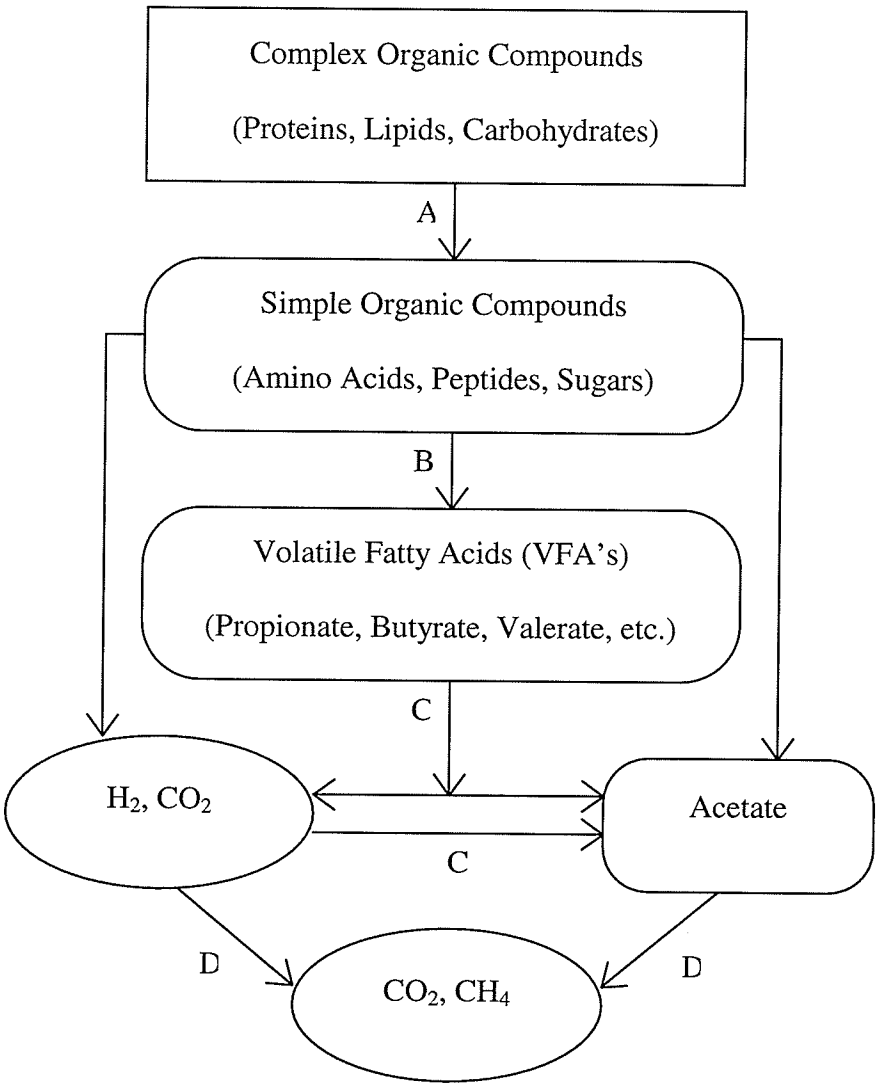
c. Acetogenesis

The name of this anaerobic digestion stage refers to the synthesis of acetic acid, the simplest VFA variety. Acetate (CH_3COOH) is formed by acetogenic bacteria from long chain VFA varieties and other intermediates formed during acidogenesis. A sub-group of acetogenic bacteria called homoacetogenic bacteria are able to synthesize acetate from CO_2 and H_2 gases (Novaes, 1986).

d. Methanogenesis

According to Novaes' model (1986), methanogenic bacteria perform the fourth and final phase of anaerobic digestion. This bacterial group is composed entirely of strict anaerobic archaebacteria which may survive on a variety of substrates (Prescott *et al.*, 1996). However, the principle substrates are CO_2, H_2, and especially acetate (Novaes, 1986). Methanogenic bacteria digest these substrates and produce methane gas as a waste product. Methanogenesis is often the rate-limiting step in anaerobic digestion since methanogenic bacteria are quite sensitive to low pH (Fox and Pohland, 1994) and normally exhibit slow growth rates.

Anaerobic digestion is often divided into two halves, with the twin aims of performance enhancement and aid of understanding. This convention places hydrolysis and acidogenesis in the first half and acetogenesis and methanogenesis in the second. It is commonly called phase separation (Fox and Pohland, 1994). Table 2.2 provides a list of advantages and disadvantages to phase separation. Among these advantages, phase separation allows the operator to tailor each half to its optimal nutritional, environmental, and operational conditions. However, phase separation sacrifices the syntrophic relationships between certain acidogenic and acetogenic bacteria. This latter effect is highly substrate specific. Fox and Pohland (1994) report that anaerobic digestion of fatty acids and aromatics responds poorly to phase separation, while that of carbohydrates and some proteins shows favorable performance. Therefore, the authors suggest that designers carefully examine the wastewater they wish to treat before employing phase separation.



A – Hydrolysis
B – Acidogenesis
C – Acetogenesis
D - Methanogenesis

Figure 2.1: Stages and Bacterial Groups Involved in Anaerobic Digestion
[insoluble compounds are denoted by rectangles, soluble compounds by rounded rectangles, and gases by ovals]
(Adapted from Novaes, 1986)

However, based on their research, Jeyaseelan and Matsuo (1995) discovered "[t]wo-phase operations compared with single-phase operations always produced better digestion efficiencies." Many studies support this conclusion including Fongastitkul *et al.* (1994) and Bhattachrya *et al.* (1996). Each found an increase in VS destruction when employing phase separation as compared to conventional complete-mix anaerobic digestion.

When anaerobic digestion occurs within a plug-flow reactor, it has been shown that phase separation develops naturally along the length of the reactor. Liu and Ghosh (1997) demonstrated how pH and VFA concentration varied with the distance from a laboratory-scale reactor inlet. These parameters indicated the dominance of the hydrolysis-acidogenesis phase within the first 50 cm of the reactor and acetogenesis-methanogenesis over the remainder of the reactor's length. VS destruction in the reactor was also satisfactory, peaking at 78% with an organic loading rate of 2.73 kg*VS/m^3*d. Citing this study, one concludes that phase separation is a natural phenomenon which engineers merely encourage through physical separation.

Table 2.2: Advantages and Disadvantages Associated with Phase Separation (Adapted from Fox and Pohland, 1994)

ADVANTAGES	DISADVANTAGES
Ability to isolate and optimize rate-limiting steps (hydrolysis and methanogenesis)	Disruption of syntrophic relationships
Separate fast-growing acidogenic bacteria from slow growing methanogens	Not applicable to all substrate types or applicability uncertain
Better resilience to shock loads	Difficulty with implementation and operation
Detoxification potential during initial phase	Lack of data with respect to full-scale experience and various substrates

2.1.4.2 Microbiology and Biochemistry of Hydrolysis-Acidogenesis

Of primary interest with respect to this study is the optimization of the hydrolysis-acidogenesis phase. Therefore, the microbiology, biochemistry, and conditions which enhance the hydrolysis and fermentation of carbohydrates, proteins, and lipids is explored. Special attention is paid to the production of the VFAs acetic, propionic, butyric, and valeric acids. Examples of bacterial species and genera capable of carbohydrate, protein, and lipid hydrolysis and fermentation are listed in Table 2.3.

a. Carbohydrate Metabolism

Cellulose, starch, and other carbohydrates are converted to their component monomers during hydrolysis. The chief monosaccharide observed during anaerobic digestion of municipal sludge is glucose. However, production of galactose, arabinose, fructose, and mannose is also common (Banerjee, 1997; Prescott *et al.*, 1996). Starch is a reserve polymer, meaning its readily-degradable structure promotes energy storage and is to be accessed in times of stress. Amylases, pullulanase, and glucoamylase are the enzymes secreted by hydrolytic bacteria to degrade starch (Gottschalk, 1986).

Glucose is the primary source of carbon and energy for anaerobic bacteria. Energy is derived from glucose through the metabolic pathway called glycolysis, shown in Figure 2.2. All monosaccharides and disaccharides of complex carbohydrates are eventually degraded to glucose or to one of the intermediate compounds shown in the six-carbon stage of glycolysis. As shown in Figure 2.2,

the final product of glycolysis is a compound called pyruvate. Compounds which are used to synthesize new cell matter are often derived from pyruvate.

Table 2.3: Examples of Bacteria Involved in the Hydrolysis and Fermentation of the Principle Organic Macromolecules

Characteristic	Bacteria	Reference
Carbohydrate Hydrolysis	*Acetovibrio celluliticus, Clostridium spp., Actinomycetes spp.*	Gujer & Zehnder, 1983; Prescott *et al.*, 1996.
Homoacetogenic Bacteria	*Acetobacterium wieringae, Acetobacterium woodii, Acetogenium kivui, Clostridium thermoaceticum, Clostridium aceticum*	Dolfing, 1988.
Glucose → HAc	*Clostridium spp., Acetobacterium spp.*	Kataoka *et al.*, 1997.
Pyruvate → HPr	*Propionibacterium spp.*	Prescott *et al.*, 1996.
Carbohydrate → HPr	*Megasphaera elsdenii, Clostridium propionicum, Propiogenium modestum*	Gottschalk, 1986.
Carbohydrate → Hbu	*Clostridium spp., Butyrivibrio spp., Eubacterium spp., Fuscobacterium spp., Butyrobacterium methylotrophicum*	Gottschalk, 1986; Schink, 1988.
Protein Hydrolysis	*Clostridium spp., Proteus vulgaris, Peptococcus anaerobicus, Bifidobacterium spp., Staphylococcus spp.*	McInerney, 1988; Gujer & Zehnder, 1983.
Thermophilic Protein Hydrolysis	*Thermobacteroides proteolyticus, Thermofilum pendens, Thermococcus celer, Desulfurococcus spp.*	McInerney, 1988.
Lipid Hydrolysis	*Ancurovibrio lipolytica, Clostridium perfringens, Treponema spp., Butyrivibrio fibrisolvens.*	McInerney, 1988.
Fatty Acid Hydrogenation	*Ruminococcus albus, Eubacterium spp., Butyrivibrio spp., Fescocillus spp.*, Protozoa.	McInerney, 1988.

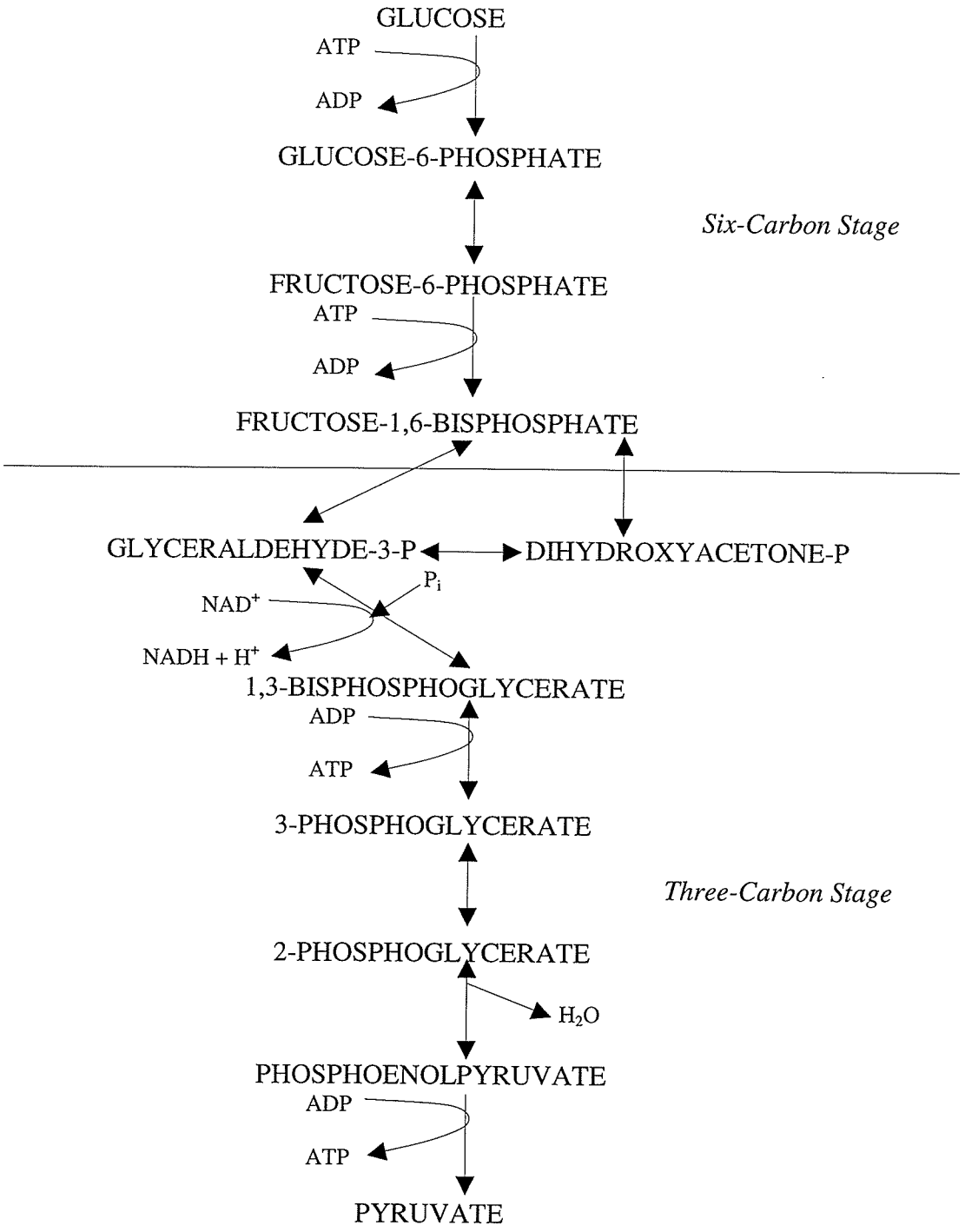


Figure 2.2: Glycolysis
(Adapted from Prescott *et al.*, 1996)

Acetic acid is formed during the degradation of pyruvate (Figure 2.3). The enzymes phosphotransacetylase and acetate kinase catalyze this conversion (Gottschalk, 1986). Bacterial species convert glucose to acetic acid according to the following reaction (Kataoka *et al*, 1997):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

Inorganic hydrogen and carbon dioxide gases produced during pyruvate degradation are combined by *Clostridium aceticum* to yield acetate according to the following reaction (Andrews and Pearson, 1965):

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$$

Homoacetogenic bacteria have been investigated since their discovery in 1942 for their high glucose-to-acetate conversion potential. Fontaine (cited in Dolfing, 1988), the discoverer of homoacetogens, reported the production of three moles of acetate for every mole of glucose. In one study, Ragsdale *et al.* (1983; cited in Dolfing, 1988) boasted 85% carbon conversion from glucose to acetate. However, Schink (1988) reports that homoacetogens have low tolerance to accumulating acetate concentrations.

Propionibacterium spp. facilitate the production of propionic acid from pyruvate (Prescott *et al.*, 1996). Vedamuthu (1988) designates propionate-forming bacteria as unique among heterotrophs for their CO_2-fixing system. The following reaction shows that acetic acid is formed at the same time:

$$3CH_3COCOOH + 3H_2 \rightarrow 2CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O$$

The preferred substrate for propionate-producing bacteria is lactate (Gottschalk, 1986). The following reaction describes this degradation (Gottschalk, 1986):

$$3CH_3CHOHCOOH \rightarrow 2CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O$$

Propiogenium modestum is reported to derive energy and produce propionate from succinate according to the following reaction:

$$CH_2CH_2(COOH)_2 \rightarrow CH_3CH_2COOH + CO_2$$

Butyric acid is another VFA by-product of pyruvate fermentation. This conversion results in the production of ATP (Figure 2.3). To explain butyric acid formation, Dolfing (1988) has proposed a cyclic pathway with acetyl-CoA and acetate as substrates. The enzymes phosphotransbutyrylase and butyrate kinase are active in butyric acid synthesis from glucose (Gottschalk, 1986).

Each of the VFA species may be further degraded to acetate and to other end products. This conversion to acetate is, of course, called acetogenesis and is impossible to avoid to some extent in the first phase of phase-separated anaerobic digestion. As a result, a complex mixture of carbohydrate substrates will usually yield a complex mixture of end products including VFAs, CO_2, H_2, water, etc. after the acidogenesis stage.

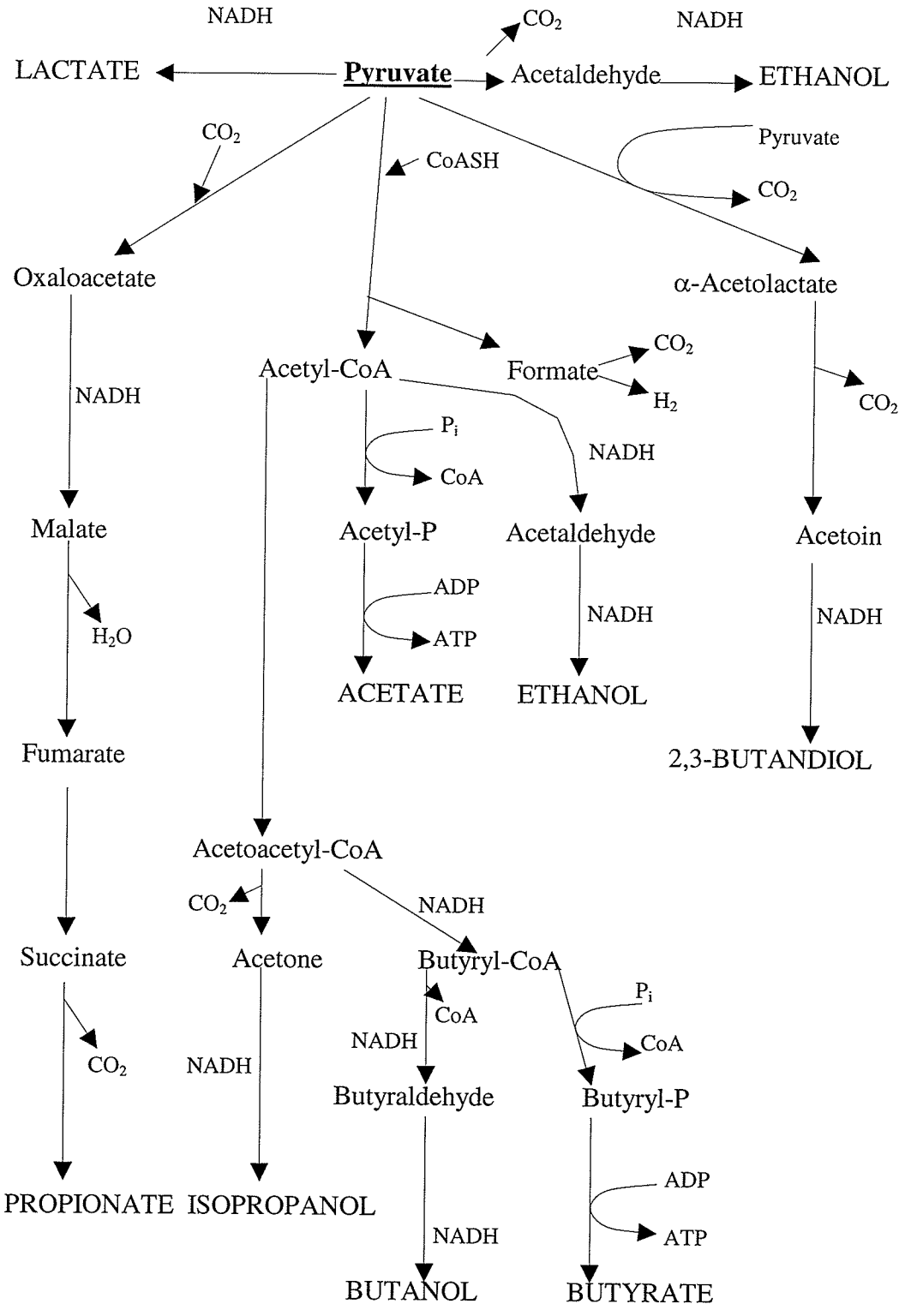


Figure 2.3: Fermentation Products of Pyruvate Degradation
[upper-case compounds indicate end products]
(Adapted from Prescott *et al.*, 1996)

b. Protein Metabolism

Generally speaking, a complex mixture of proteins will produce VFAs, CO_2, H_2, ammonia, and S^{2-} ions upon completion of hydrolysis-acidogenesis (McInerney, 1988). Hydrolytic bacteria are responsible for conversion of the quaternary, tertiary, and secondary structure of proteins to individual amino acids. These proteolytic bacteria are most often of the genera *Clostridium*, but others have been isolated from sewage sludge and thermophilic digesters. All these aforementioned bacteria secrete protein-specific enzymes called proteases to achieve hydrolysis (Prescott *et al.*, 1996).

It was once believed that protein hydrolysis rates were mainly dependent on the water solubility of the protein in its quaternary structure. However, research has shown some proteins in their tertiary arrangement to be more resistant to digestion than others in their quaternary structures. It is now believed that factors such as the number of disulfide bridges within the protein's quaternary structure, the characteristics of its tertiary structure, and the type of end group on the protein all play a significant part in the overall rate of digestion (McInerney, 1988).

Amino acids can be converted to pyruvate, acetyl-CoA, or an intermediate compound of the tricarboxylic acid (TCA) cycle through a process called transamination (Prescott *et al.*, 1996). Of course, once one of these compounds is produced, they can be digested as outlined in the previous section. Transamination is facilitated by the acid α-ketoglutarate which becomes the acceptor of the amino group or groups attached to the amino acid. The converted amino acid will not necessarily go on to produce energy; it may also serve as a

carbon source for cell synthesis. The excess nitrogen found in these amino groups is often expelled from the bacterium as ammonia.

c. Lipid Metabolism

McInerney (1988) reports that anaerobic lipid degradation is a major source of acetate. Therefore, this degradation is of particular importance to the syntrophic relationship between acidogenic, acetogenic, and methanogenic bacteria. Lipid hydrolysis frees long-chain fatty acids which are in turn reconfigured or incorporated directly into bacterial membranes. Maczulak *et al.* (1981; cited in McInerney, 1988) showed that high concentrations of free, long-chain fatty acids spare bacteria energy. It was reasoned that the building blocks for membrane components could be synthesized much more easily in this case.

Lipid hydrolysis first attacks glycerol ester linkages to separate glycerol, galactose, choline and other non-fatty acid components from the fatty acid lipid components (McInerney, 1988). The latter group includes compounds such as linoleic, linolenic, oleic, and stearic acids. Diglycerides are hydrolyzed more quickly than triglycerides. Phospholipids, sulpholipids, and galactolipids are all susceptible to certain hydrolytic bacteria depending on the specific enzymes they are able to secrete. These bacteria generally secrete one of two enzymes: lipases or phospholipases. Lipases sever ester bonds along fatty acid chains to produce shorter fatty acids, while phopholipids are hydrolyzed through the action of phopspholipases (Elefsiniotis, 1993). A wide variety of bacteria are able to perform lipid hydrolysis, some of which are presented in Table 2.3.

Once the fatty acids are separated from the non-fatty acid portion of lipids, the components can be digested or incorporated by bacteria. Non-fatty acid components are often converted to compounds of glycolysis, which are digested as outlined earlier. Other fermentative pathways are available as well. For example, choline is fermented by *Desulfovibrio desulfricans* to yield trimethylamine, acetate, and ethanol (McInerney, 1988).

The fatty acid portions of hydrolyzed lipids are fermented in two steps. The first step is hydrogenation of unsaturated fatty acids. For example, α-linolenic acid, γ-linolenic acid, and linolenic acid are saturated to stearic acid. The majority of hydrogenating bacteria fall into this group. Although hydrolyzation is normally thought to be required for fatty acid digestion, Schink (1988) reports that acetate can be produced by the action of anaerobic bacteria using unsaturated hydrocarbons as a substrate. However, this reaction is quite slow. Nonetheless, these reactions are important to the health of the bacterial population as it has been shown that a high concentration of polyunsaturated fatty acids inhibit bacterial growth (McInerney, 1988).

The next step in fatty acid digestion is called β-oxidation. This process sees long-chain fatty acids oxidized at the β-carbon bond as follows (Gujer and Zehnder, 1983):

$$R\text{-}(\text{-}CH_2CH_2\text{-})\text{-}COOH + 2H_2O \rightarrow R\text{-}COOH + CH_3COOH + 2H_2$$

Another oxidation reaction, α-oxidation, may play an important role in VFA speciation (McInerney, 1988). The α-carbon bond is severed to synthesize odd-numbered fatty acids from those with even numbers of carbon atoms. The converse of this process is also true. This form of oxidation has been shown in

Selenomonas ruminantium and is usually associated with membrane synthesis functions.

2.1.4.3 Conditions Influencing VFA Production

Hydrolysis-acidogenesis is an extremely complex process. Interconnections between various factors are difficult to characterize and must be approached carefully as wastewater characteristics, environmental conditions, reactor type, and operational parameters will all have a profound effect on VFA production. As a result, studies which assess such factors must be understood only to apply in their unique contexts. Daigger *et al.* (1993) also showed that laboratory-scale results do not necessarily translate well to full-scale. They found VFA production to be lower than predicted at the lab scale due to mechanical factors. Despite such difficulties, general patterns of VFA production are developed in this way and have been very instructive in the effort to better understand hydrolysis-acidogenesis.

The environmental conditions pH and temperature have been studied with regard to hydrolysis-acidogenesis. Elefsiniotis and Oldham (1994a) examined the influence of pH and found neither hydrolysis nor acidogenesis was significantly affected by varying the pH between 4.3 and 5.2. However, pH levels ranging from 5.9 to 6.2 resulted in a 25-30% decrease in COD solubilization and VFA production. Kugelman and Guida (1989) explored meso- and thermophilic anaerobic digestion with methanogenesis as the goal. Mesophilic digestion (T = 35^{O}C) resulted in better digestion of carbohydrates and lipids, but inferior organic

nitrogen breakdown compared to thermophilic digestion (T = 55^{O}C). With respect to VFAs, VFA production was highest at temperatures over 50^{O}C.

Operational factors have been studied as well. Zhang and Noike (1994) fed a complete-mix anaerobic reactor with a starch substrate and varied hydraulic retention time (HRT) from a minimum of 1.5 hours up to 250 hours. The reactor was not limited to hydrolysis-acidogenesis stages. They noted little effect on hydrolytic or H_2-producing bacterial populations over this range. However, the populations of homoacetogenic and sulfate-reducing bacteria were diminished as HRT increased from 6 hours to 250 hours. As these bacteria are important VFA-producers, this population dynamic will affect VFA production. Another investigation into the role of HRT was conducted by Elefsiniotis and Oldham (1994b). They reported a steady increase in hydrolysis and acidogenesis rates as HRT increased up to 12 hours. After this level was passed, hydrolysis and acidogenesis rates dropped moderately. The authors noted that the slight drop was likely due to superficial growth of gasifying bacteria in the reactor.

Elefsiniotis and Oldham (1994c) also probed the role played by solids retention time (SRT) in VFA production. At an HRT of 12 hours, SRT was adjusted from 10 to 20 days. A slight increase in VFA production was observed. While VFA levels remained fairly stable over this SRT range, the proportion of VFAs in soluble COD increased to 90% at an SRT of 20 days. This finding indicates that hydrolysis rates increased substantially with increasing SRT, while those of acidogenesis did not. When SRT was lowered to 5 days, VFA production dropped markedly.

An example of a study into the effect of combining factors was carried out by Banerjee (1997). This study related the effect of three parameters: HRT, temperature, and varying the industrial-to-municipal waste ratio of the feed. Banerjee found the optimal configuration for VFA production was an HRT of 30 hours, a temperature of 30^{O}C, and a 1:1 mixture of municipal and starch wastewater. Maharaj (1999) continued the work of Banerjee (1997) by exploring increased HRT and decreased temperatures on hydrolysis-acidogenesis of the same wastewater combinations. It was proved that a 1:1 mixture resulted in the highest VFA production at 25^{O}C and a 30 hour HRT. Maharaj found VFA speciation was also affected by the feed composition. Many of the findings of these studies are presented in this work in Chapter 5.

A number of studies have inquired into the role of the presence of VFAs and other acidogenesis end products on VFA production. Boone (1982) reported that H_2 greatly stimulates acetate synthesis, while Lin and Hu (1992) saw that the presence of acetic acid promoted iso-butyric acid formation. Mösche and Jördening (1999) explored propionate-to-acetate ratios by trying to establish the levels of inhibition at both ratio extremes. The authors found that there was little inhibition of substrate consumption due to high propionate-to-acetate ratios, although propionate bacteria are sensitive to low pH. Product inhibition (indicated by low propionate-to-acetate ratios) commenced at values of unity and lower. However, even very low propionate-to-acetate ratios failed to cause anaerobic digestion to cease altogether, despite being theoretically supported. Syutsubo *et al.* (1997) note in their study of thermophilic anaerobic digestion that propionate degradation is likely a rate-limiting step.

2.1.5 Anaerobic Reactor Configurations

Similar to aerobic wastewater treatment, anaerobic configurations may be either suspended- or attached-growth. Currently, there are several reactor choices, some of which are pictured in Figure 2.4. The results of this study were obtained with two suspended-growth, anaerobic contact reactors. As depicted in Figure 2.4, these reactors are completely mixed, provide solids recycle, and allow for gas evolution. Feeds enter the reactor chamber and are immediately dispersed throughout the reactor volume. Since biomass is uniformly distributed within the vessel, the effluent should have identical properties to the reacting liquor. Anaerobic contact processes are able to control HRT and SRT separately by varying the clarifier recycling rate. Such a feature allows for optimal HRT, which is normally much shorter than SRT. This feature also allows for HRT/SRT ratios much greater than unity which has been observed to encourage acidogenesis at the expense of methanogenesis (Banerjee, 1997; Maharaj, 1999). Conventional completely-mixed anaerobic digesters cannot accomplish this HRT/SRT uncoupling and therefore, require larger reaction vessels.

Choosing the appropriate anaerobic digestion reactor rests on many considerations. Paramount in this choice is characterizing the waste and identifying the desired end products of digestion. Recall that Fox and Pohland (1994) cautioned against blindly opting for reactors incorporating phase separation, for example, since "not all wastewaters are readily amenable to such treatment." Knowledge of the desired end products is essential for different

reactors may promote the synthesis of certain end products while discouraging others. Simple re-configuration of existing systems may also greatly improve performance (Speece *et al.*, 1997). Operational considerations should also enter into the decision. For example, Pagilla *et al.* (1997) showed that gas-mixed fermenters may promote foaming, but result in greater VS destruction than mechanically-mixed fermenters. Despite these factors, the overriding consideration is normally stabilization performance and cost. Some performance indicators of common anaerobic digesters are given in Table 2.4.

Table 2.4: Performance of Various Anaerobic Digesters

DIGESTER	$TCOD_{in}$ (mg/L)	%TCOD REMOVED	REFERENCE
Completely mixed anaerobic digester	62,800	60	Li *et at.* (1997).
Anaerobic contact process	1,500-5,000	75-90	Metcalf and Eddy (1991).
Temperature-staged anaerobic reactors	50,000-65,000	53% (VS removal)	Han and Dague (1997).
Temperature-staged anaerobic reactors	30,000	95	Duran and Speece (1997).
Anaerobic plug-flow reactor	61,310	74.2	Liu and Ghosh (1997).
Anaerobic baffled plug-flow reactor	550	80	Polprasert *et al.* (1992)*
Anaerobic baffled plug-flow reactor	115,800	70-88	Boopathy and Sievers (1991)*
Upflow anaerobic sludge blanket	5,000-15,000	75-85	Metcalf and Eddy (1991).
Anaerobic fluidized bed reactor	10,400	>60	Chen *et al.* (1997).
Anaerobic fixed bed reactor	10,000-20,000	75-85	Metcalf and Eddy (1991).
Anaerobic expanded bed reactor	5,000-10,000	80-85	Metcalf and Eddy (1991).

* - cited in Barber and Stuckey (1999).

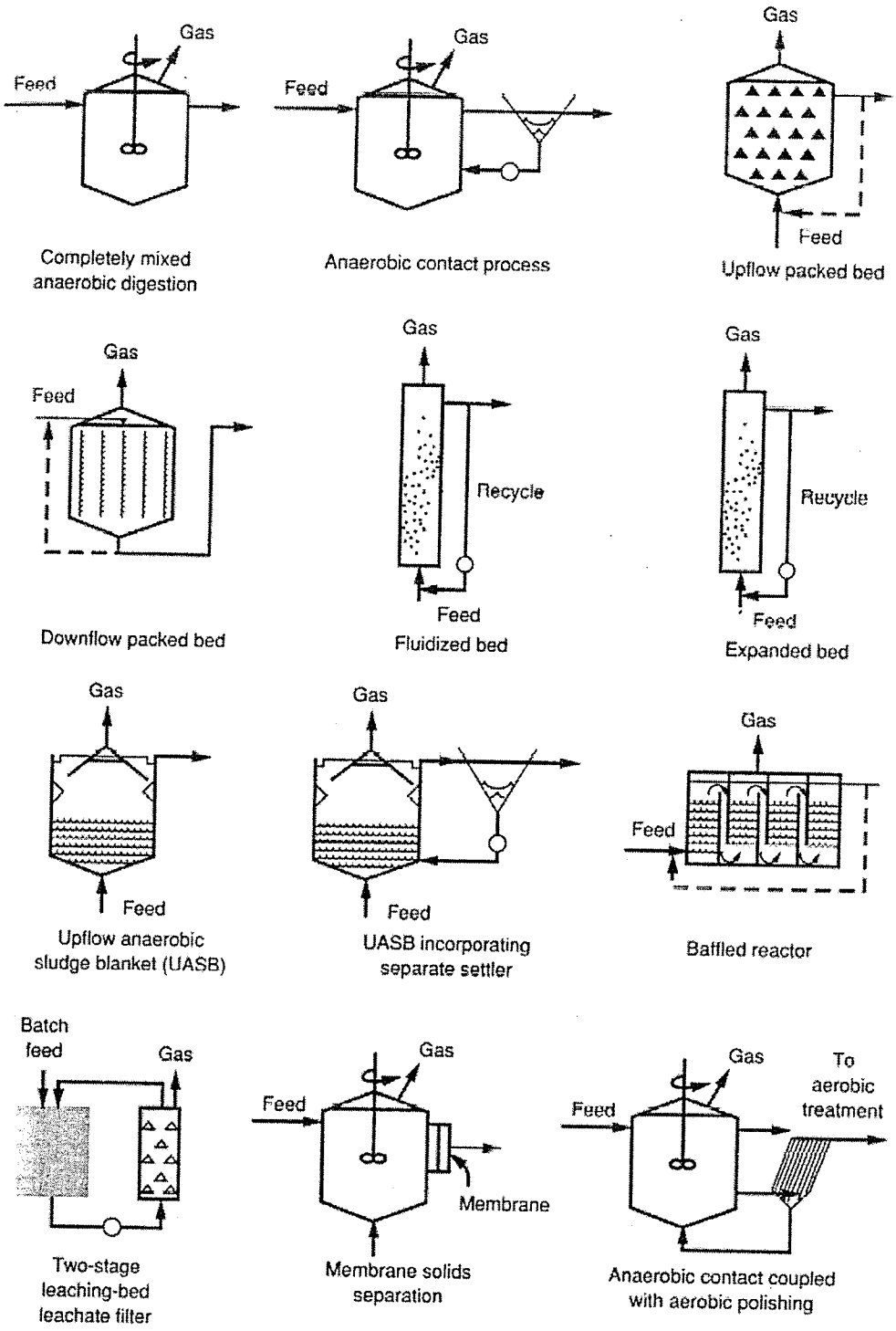


Figure 2.4: Common Reactor Configurations in Anaerobic Wastewater Digestion (Metcalf and Eddy, 1991)

2.2 Denitrification and Biological Nutrient Removal (BNR) Fundamentals

2.2.1 Introduction

Nutrient removal refers to the reduction of effluent phosphorous and nitrogen concentrations and is included in nearly all new wastewater treatment plant designs (Oleszkiewicz, 1994a). Older plants not incorporating such processes are being retrofitted or decommissioned worldwide (Brinch *et al.*, 1994; Wilson *et al.*, 1998). Nutrient removal can be achieved through purely chemical and physical means, but biological nutrient removal (BNR) is usually the most economical and sustainable method available (Randall *et al.*, 1992). Though both phosphorous and nitrogen removal are pressing concerns in the environmental engineering field, nitrogen removal is the focus of this study. Thus, the majority of this discussion will centre on nitrogen, although phosphorous is discussed in limited detail.

Excess nitrogen has become a problem in modern pre- and post-industrial societies. The sources of this nutrient, both natural and anthropogenic, are numerous and prolific. The negative effects of excess nitrogen have been observed in surface waters, groundwater, and in human health. Nitrogen is cycled naturally with microorganisms facilitating key nitrogen transformations that produce stable, non-toxic end products. These microbes thereby provide ecosystem services (Suzuki and Dressel, 1999); namely, ammonification, nitrification, and denitrification. However, modern society is overwhelming these

cycles and services. Therefore, engineered approaches to excess nitrogen removal are becoming more and more common in an effort to relieve this stress.

In the engineered setting, natural nitrogen transformations are enhanced through encouraging the growth of bacterial populations that perform the ecosystem services of nitrogen transformation. Conditions which provide optimal nutrient removal are sought and studied. One relatively new concept in denitrification and phosphorous removal is utilizing anaerobically-produced volatile fatty acids (VFAs) as the carbon source. This carbon source holds great cost-savings and long-term sustainability potential. Sludge produced during wastewater treatment must be stabilized regardless of the final disposal of the anaerobic effluent. By using anaerobically-digested effluent as a carbon source, external carbon need not be purchased and the anaerobic effluent is consumed, at least partially, during wastewater treatment.

Nitrogen and phosphorous removal can be achieved by a number of treatment options. Most of these are suspended-growth operations, especially on the large scale (Çeçen and Gönenç, 1995). Increasingly, particularly in Europe, the north-eastern United States, and parts of Asia, attached-growth methods are in use (M'Coy, 1997). Attached-growth reactors are reputed to require less space than conventional suspended-growth methods. However, the appropriateness of this technology to phosphorous removal is in doubt (Metcalf & Eddy, 1991). Hybrid reactors share characteristics of both suspended- and attached-growth systems and are now being designed and implemented. Hybrid reactors reportedly provide the space savings of attached-growth reactors with the

performance of suspended-growth nutrient removal systems. They are also promising with regard to the all-too-common case where retrofitting is desired.

2.2.2 Nitrogen Transformations in Natural Settings

Nitrogen, along with oxygen, carbon, hydrogen, phosphorous and sulfur, is one of the essential macro-nutrients required for the survival of all known livings things (Prescott *et al.*, 1996). Generally, these nutrients are recycled among the biosphere, the atmosphere, the hydrosphere, and the lithosphere. Within the biosphere, these cycles involve the assimilation of nutrients into bacterial tissues. This stage is decay, where formerly living tissues are digested by bacteria and other microorganisms.

Specific to nitrogen, this bacterial assimilation stage is mediated by several bacterial species and may be simplified to the following transitions (Oleszkiewicz, 1994b):

$$NH_2\text{-}CO\text{-}NH_2 + 2H_2O \rightarrow (NH_4)_2CO_3 \quad (A),$$

$$NH_3 \text{ or } NH_4^+ \rightarrow NO_3^- \text{ or } NO_2^- \quad (B), \text{ and}$$

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_{2(g)} \quad (C).$$

Reaction A is a hydrolysis reaction and called *Ammonification*. Reaction B is termed *Nitrification* while reactions C are collectively referred to as *Denitrification*.

The nitrogen cycle is complex and the human strain imposed on it represents one of the biggest pollution threats to the planet (Bodkin and Keller, 1995). A schematic representation of the nitrogen cycle is shown in Figure 2.5.

Cause for concern stems from the fact that nitrogen is often a limiting nutrient in both terrestrial and ocean ecosystems (Bodkin and Keller, 1995) and is harmful to human health in certain forms (Rajapakse and Scutt, 1999). It should be apparent that these processes are critical to maintaining the nitrogen balance of the biosphere. This critical importance is why engineered methods to reduce the burden on the nitrogen cycle have been developed and continue to be studied.

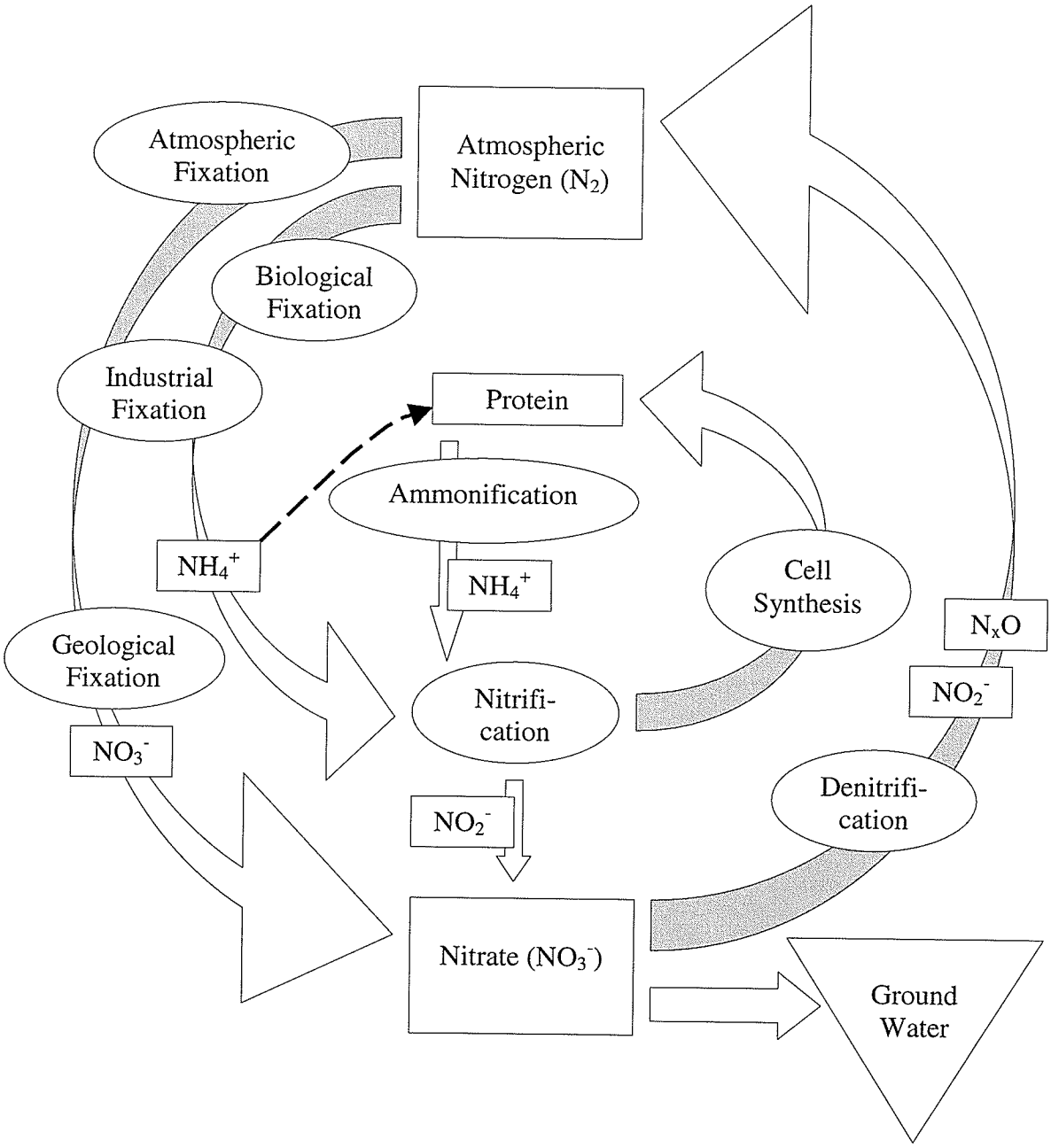


Figure 2.5: Simplified Schematic Diagram of the Nitrogen Cycle
(Rectangular components denote compounds, ovals denote processes)
(Adapted from USEPA, 1993)

2.2.3 Natural and Anthropogenic Sources of Nitrogen

In a balanced ecosystem, nitrogen influxes will be approximately equal to the nitrogen discharges when considered over long periods. The nitrogen present in the ecosystem at any given moment will be constantly cycling through it in many different forms. On a global scale, these principles apply as well. As Table 2.5 shows, the global nitrogen budget is believed approximately balanced.

Table 2.5: Approximate Global Nitrogen Budget
(Adapted from Tiedje, 1988)

Inputs	Input Rate (Tg*N/yr)	Outputs	Output Rate (Tg*N/yr)
Biological Fixation	140 – 310	Denitrification	130 – 435
Industrial Fixation	36 – 57	Sedimentary Burial	10
Combustion and Atmospheric Fixation	24 – 30	NET	-110 to +45

It should be apparent from the large variation in the figures provided that these numbers are estimates that are by no means uncontroversial. However, one point is certain; human activities are increasing the inputs of the nitrogen budget locally and globally, particularly through industrial fixation.

Sources of nitrogen are numerous, as are the pathways upon which nitrogen is transformed, despite the simplified impression given by Figure 2.5. They may be point or non-point sources; airborne or waterborne; gaseous, soluble, or solid. Due to this complexity and variety, nitrogen sources and pathways can be difficult to quantify.

Natural nitrogen fixation pathways are classed in three main categories. Atmospheric fixation is one of these categories. This term refers to reactions in the lower atmosphere that accompany lightning strikes to produce nitrogen oxide

gases. Some reports suggest that as much as 15% of all naturally-fixed nitrogen is due to lightning (USEPA, 1993). Another, less significant category of natural nitrogen sources is geological fixation. Volcanic eruptions are an example of such a nitrogen source.

By far the most significant single nitrogen fixation pathway, natural or anthropogenic, is biological fixation (Tiedje, 1988). Some details of the biochemistry of biological nitrogen fixation are discussed in Section 2.2.5.1. This process may exacerbate already troubled eutrophic lakes. For example, the USEPA (1993) studied the eutrophic Lake Mendota in Wisconsin. It is reported that 14% of the total nitrogen entering the lake was the result of the activities of biological nitrogen fixing bacteria. This example should bring to light the necessity of characterizing the source of wastewater effluent when setting effluent standards. Other natural nitrogen sources include decay of plant and animal tissues, dustfall, precipitation, and non-urban and non-agricultural runoff.

Anthropogenic, or human-induced nitrogen pathways and sources are numerous and increasing as time passes. Many industrial activities fix nitrogen, either as waste products or purposefully. Some major anthropogenic nitrogen sources include agricultural fertilizer runoff, landfill leachate, atmospheric deposition of particulate matter, industrial wastewaters, and domestic sewage (USEPA, 1993). Of particular interest to this study are the contributions of the latter two sources.

Industrial sources generally introduce nitrogen to surface waters through discharge of waste process water high in nitrogen or water used in flue gas scrubbers (USEPA, 1993). The primary industries at fault are chemical industries

involved in the manufacture of fertilizers and other nitrogenous compounds, pulp and paper industries of ammonia-based pulping, metal ore mining and smelting industries that employ nitric acid pickling, food processing industries through cooking waters or protein-rich process waters, and leather and textiles industries which discharge protein-rich waters. Power generating industries release nitrate-rich water from off-gas scrubbers. Nitrogen oxide gases, solubilized by scrubber waters, hydrolyze to nitrate in water. Many of these waters may be partially treated or discharged untreated to wastewater treatment plants.

Wastewater treatment plants are charged with accommodating these industrial inputs, in addition to domestic wastewater from sewer and septic systems. Raw domestic sewage will exhibit a wide range of total nitrogen (TN) concentrations, depending on the time of year, climate, and other factors. A typical range in the United States is 20 to 85 mg/L TN (USEPA, 1993). Of this total concentration, ammonia will normally comprise approximately 60% and organic nitrogen (such as amino acids) will constitute the remaining 40%. Small amounts of nitrate are also normally present. Conventional wastewater treatment helps reduce the organic nitrogen component significantly through bacterial cell synthesis and solids removal. However, most ammonia will pass through the effluent unless nitrification is provided. To achieve TN reductions greater than 30%, denitrification is required.

Some jurisdictions require very stringent nitrogen control of their wastewater treatment facilities. For example, River Oaks Advanced WWTP in Hillsborough, Florida cannot discharge effluent TN exceeding 1.2 mg/L (Yoder *et al.*, 1995). The Reno-Sparks WWTP of Reno, Nevada requires effluents to

contain no more than 2 to 4 mg/L ammonia (depending on the season) and 2 mg/L nitrate. Obviously, this level of treatment requires processes supplemental to conventional wastewater treatment. Therefore, biological nutrient removal (BNR) is a fundamental component of wastewater treatment in these jurisdictions, and is becoming so in many more.

2.2.4 Problems Associated with Excess Nitrogen Release

The need for nutrient removal is rarely disputed these days. Both phosphorous and nitrogen are applied to land and, indirectly, water (and in the case of nitrogen, air) in great quantities (Bodkin and Keller, 1995). Either may be a limiting nutrient in receiving waters. When the limiting nutrient enters a surface water ecosystem, growth of algae and other nutrient-starved organisms explodes. Sunlight is blocked from subsurface plants by mats of algae that cover the water's surface, leading to their demise. The death of the algae is assured since such populations cannot be sustained. Bacteria consume the dead algae, depleting surface waters of oxygen. This oxygen starvation eventually leads to the deaths of the majority of flora and fauna found in the ecosystem So many surface waters have experienced this boom and bust cycle of uncontrolled growth and death that the term "algal bloom" has been coined to describe it and entered common consciousness. Another ramification of this phenomenon is the tightening of nutrient regulations with respect to wastewater treatment (USEPA, 1993).

Algal bloom, or eutrophication, is more or less problematic, depending on the local conditions of the surface water. Van Luijn *et al.* (1999) examined

nitrogen fluxes in eutrophic lakes and found that fluxes were at a maximum at elevated temperatures, in muddy bottom lakes. The major thrust of this research is that effluent nitrogen (and by implication, phosphorous) standards ought to reflect the geology and climate of the catchment area. Indeed, effluent standards are evaluated and determined scientifically, on a region by region basis (USEPA, 1993).

Even with such considerations in mind, setting standards based solely on effluent characteristics is not a guarantee that excess nitrogen will no longer be a problem. Van Loosdrecht *et al.* (1997) have shown that wastewater treatment plants achieving very low effluent concentrations of phosphorous and nitrogen still become significant nutrient sources for surrounding ecosystems. Effluent nitrogen concentrations were improved in their study through the elimination of primary settling. However, excess nitrogen was simply transferred from the effluent to extra sludge production. Other environmental concerns were raised since energy demands and CO_2 emissions increased. Therefore, the authors caution that "the full environmental impact should also be considered for treatment processes."

The algal bloom phenomenon described above first caught the public's eye thanks to the drama and relative quickness of the destruction it caused. However, other effects related to nutrient loading from wastewater plants have shown themselves to be quite destructive. Surface water dissolved oxygen (DO) levels can be impaired due to nitrification, and waters can be polluted due to ammonia toxicity (USEPA, 1993). Partial nitrogen removal can produce harmful intermediate compounds such as nitrates. Rajapakse and Scutt (1999) catalogued

some of the suspected health dangers from consuming drinking water that contains nitrates. Included in their list are fifteen forms of cancer, birth defects, and thyroid difficulties. It is now known that infant methaemoglobinaemia, or "blue baby syndrome," results from consumption of nitrates in drinking water (MacPherson, 1992; USEPA, 1993). Nitrate concentration need only exceed 10 mg/L for the onset of this syndrome (USEPA, 1993), and groundwater drinking water supplies have reported nitrate levels greater than 100 mg/L in eastern and south-eastern England (MacPherson, 1992)!

2.2.5 Fundamentals of Nitrogen Transformations

Referring to Figure 2.5, one readily sees many nitrogen conversion processes at work in the nitrogen cycle. These processes are denoted by ovals and each of these is carried out by bacteria. In fact, some of these processes are almost exclusively bacterial in nature. Four fixation pathways are shown in Figure 2.5; however, only biological fixation is discussed in this section. Ammonification, nitrification, and denitrification all occur at different stages during BNR wastewater treatment. Ammonification occurs fairly readily, but nitrification and denitrification require special conditions to occur at an appreciable rate. Herein lies the challenge to the engineer: to determine conditions that promote BNR and to maintain their stability over long, perhaps indefinite periods.

2.2.5.1 Biological Nitrogen Fixation

Nitrogen is an highly abundant nutrient in the biosphere comprising 78% of atmospheric gases (Christopherson, 1994). However, it is found chiefly in the form of very unreactive nitrogen gas (N_2). This form is unavailable to most organisms; yet, certain key species are able to convert nitrogen gas to organic forms. This process is called biological fixation and before the advent of industrial fixation, was the principle means by which nitrogen entered the biosphere.

All nitrogen-fixing organisms are prokaryotic (Gottschalk, 1986). Nearly all prokaryotic orders and families contain bacteria able to fix nitrogen. Some of the species are shown in Table 2.6. These organisms are generally divided into free-living and symbiotic groups. For example, the nitrogen-fixing prokaryote *cyanobacteria* have evolved to develop symbiotic relationships with the aquatic fern Azolla. This symbiotic coupling is very important in aquatic ecosystems. Many plants such as legumes and the acacia tree house cysts of nitrogen-fixing *Rhizobium* on their roots (Gottschalk, 1986; Bodkin and Keller, 1995). Many nitrogen-fixing bacteria also dwell in the digestive tracts of ruminants, which accounts for the elevated ammonia content of their manure.

Table 2.6: Examples of Nitrogen-Fixing Bacteria
(Adapted from Gottschalk, 1986)

Group Characteristic or Common Name	Bacteria
Cyanobacteria	*Anabaena azollae,* *Gloeocapsa spp.*
Phototrophic bacteria	*Rhodospirillum rubrum,* *Rhodopsuedomonas capsulata,* *Chromatium vinosum*

Group Characteristic or Common Name	Bacteria
Obligate and Faculatative Aerobes	*Rhizobium japonicum,* *Frankia alni,* *Klebsiella pneumoniae*
Strict Anaerobes	*Clostridium pasteurianum,* *Desulfovibrio vulgaris*

2.2.5.2 Ammonification

Ammonification refers to the conversion of proteinaceous nitrogen and urea into new bacterial cell mass and ammonia (Oleszkiewicz, 1994b). Ammonification occurs quite readily and universally and is therefore often used as a measure of the age of wastewater. This process occurs via hydrolysis reactions and is facilitated by the enzyme urease according to the following reaction presented earlier:

$$NH_2\text{-}CO\text{-}NH_2 + 2H_2O \rightarrow (NH_4)_2CO_3$$

There seems to be very little study devoted to ammonification based on the lack of literature encountered. This finding likely reflects the ubiquity and relative ease with which ammonification occurs. If this process were a rate-limiting step or did not occur readily, it would certainly merit investigation and libraries of research would likely be available.

Ammonia is also formed during dissimilatory nitrate reduction to ammonium (DNRA) (Tiedje, 1988). However, this process, which could be described as a form of denitrification, is not well understood on a biochemical level. It has been observed in many ecosystems and settings, including wastewater treatment plants. Among the organisms known to perform DNRA in wastewaters are *Escherichia coli, Citrobacter spp., Klebsiella spp.,* and

Enterobacter aerogenes. These species may hold potential for treating nitrate-polluted groundwater or surface water in the future.

2.2.5.3 Nitrification

Nitrification is a two step process carried out by two different bacterial groups. In the first step, ammonia is converted to nitrite, while in the second step, nitrite is converted to nitrate. Both bacterial groups are autotrophic (though exceptions exist), deriving energy from the oxidation of these inorganic nitrogen compounds (USEPA, 1993). Molecular and atomic oxygen act as electron acceptors. Nitrifying bacteria are somewhat peculiar in that they synthesize inorganic carbon for new cell material (USEAP, 1993). The oxidation of ammonia to nitrite is carried out by the obligate chemolithotrophic bacteria *Nitrosomonas, Nitrosospira, Nitrosovibrio,* and *Nitrosococcus spp.* (Gottschalk, 1986) while *Nitrobacter, Nitrospina,* and *Nitrococcus spp.* are responsible for the oxidation of nitrite to nitrate.

The bacteria responsible for nitrification are among the most fragile in a typical WWTP (Oleszkiewicz, 1994a). Temperature, pH and alkalinity, dissolved oxygen (DO) concentration, inhibitory compounds, and carbon-to-nitrogen (C:N) ratio all affect nitrification rates (USEPA, 1993). Thus, operations must balance the needs of both nitrifying groups and account for changes in any or all of the factors mentioned above. This balance is often achieved by maximizing SRT (van Loosdrecht and Jetten, 1998). However, this response, if prolonged, may cause low F/M and C:N ratios. While these low ratios are not problems for nitrification, subsequent processes, particularly denitrification, may be hampered

(Oleszkiewicz, 1994a). Yuan *et al.* (2000) have proposed nitrification facilities may decrease SRT, and thus their size by 20% and more, through sludge storage and aeration.

Flora, *et al.* (1999a) have reported the additional influencing factors for attached-growth reactors: total ammonium loading, volumetric flow rate per unit biofilm surface area, biofilm thickness, and total biomass density. The factors already listed will have a bearing on the rates of nitrification in attached-growth reactors, as well.

2.2.5.4 Denitrification

Most often, nitrification is accompanied by denitrification. Nitrate and nitrite contamination result from incomplete nitrogen removal and nitrite is toxic to most life forms (Gottschalk, 1986). Also, nitrites have been shown to inhibit phosphorous uptake in BNR applications (Meinhold *et al.*). In addition to the removal of these compounds, denitrification is pursued because it is a much simpler process than nitrification (Oleszkiewicz, 1994a). In fact, denitrification occurs so readily that Koch *et al.* (1999) observed 37% of total denitrification took place in the secondary clarifier at a WWTP in Zurich! Denitrification is affected by variations in pH, temperature, and inhibitors, but much less so than nitrification.

Denitrification is carried out by heterotrophic bacteria and as such, requires organic carbon substrates. This carbon source has been traditionally methanol (USEPA, 1993). However, this compound must be purchased and therefore is subject to price fluctuations, shortages, or other difficulties. Much

investigation has centred on the suitability of employing VFAs produced on-site as a replacement for methanol, and many BNR facilities currently employ such a strategy.

a. Microbiology and Biochemistry

Denitrifiers are generally facultative aerobic bacteria able to use nitrogen oxides as electron acceptors when oxygen becomes limiting or with oxygen simultaneously (Tiedje, 1988). Although the process is normally thought of as anoxic in engineering practice, denitrifiers thrive when aerobic conditions gradually give way to anoxic or anaerobic conditions. This transition allows dentrifiers to generate the energy required to synthesize the enzymes necessary for denitrification.

As Tiedje (1988) reports, "among the biogeochemical cycles on earth, there are no inorganic biotransformations that are carried out by a wider distribution and diversity of organisms than is the case for denitrification." The many characteristics exhibited by denitrifiers is shown in Table 2.7. A particularly important bacterial species in wastewater treatment is *Hyphomicrobium.* This species has been found to be predominant in methanol-fed waste treatment systems. However, the predominant denitrifiers in nature are species of *Pseudomonas* and *Alcaligenes.* Although autotrophic dentrification is indicated in Table 2.7, van Loosdrecht and Jetten (1998) conclude that this conversion process plays only a minor role in wastewater treatment.

Both Patureau *et al.* (1998) and Zhang and Lampe (1999) indicate the feasibility of aerobic denitrification. The former study paid particular attention to

the bacteria *Microvirgula aerodenitrificans*. A single vessel containing aerobic nitrifiers and such denitrifiers was examined and recommended in conclusion. The cost-savings accrued by requiring only one reaction vessel are obvious. The latter study emphasized autotrophic denitrification to treat nitrate-contaminated waters by a sulfur:limestone process. Zhang and Lampe found this process to be well-adapted to pond systems since no external carbon is required and the responsible organisms are widely distributed in natural soils and sediments.

The kinetics of denitrification in terms of nitrate and carbon consumption are more or less uniform. Both Yatong (1996) and Bilanovic *et al.* (1999) found denitrification to follow zero-order kinetics. Bilanovic *et al.* (1999) noted that denitrification with a complex carbon source such as fermented effluent results in Monod-type kinetics as the preferred VFA species are sequentially consumed. However, when acetate, the preferred VFA type, is available in abundance, kinetics proceed according to zero-order.

Table 2.7: Known and Suspected Denitrifying Genera
(Adapted from Tiedje, 1988)

Distinctive Physiological Features		Genera
Organotrophic	Aerobic	*Pseudomonas*
		Alcaligenes
		Flavobacterium
		Paracoccus
		(Acinetobacter)
		Cytophaga
		(Gluconobacter)
		(Xanthomonas)
	Oligocarbophilic	*Hyphomicrobium*
		Aquaspirillium
	Fermentative	*Azospirillum*
		Janthinobacterium
		Bacillus
		Wolinella
	Halophilic	*Halobacterium*

Distinctive Physiological Features		Genera
		Paracoccus
	Thermophilic	*Bacillus*
		(Thermothrix)
	Sporeformer	*Bacillus*
	Magnetotactic	*Aquaspirillum*
	Nitrogen Fixing	*Rhizobium*
		Bradyrhizobium
		Azospirillum
		Pseudomonas
		Rhodopseudomonas
		Agrobacterium
	Animal or Pathogenic Association	*Neisseria*
		Kingella
		Moraxella
		Wolinella
Phototrophic		*Rhodospeudomonas*
Lithotrophic	H_2 Use	*Paracoccus*
		Alcaligenes
		Bradyrhizobium
		Pseudomonas
	S Use	*Thiobacillus*
		Thiomicrospira
		Thiospaera
		(Thermothrix)
	NH_4^+ Use	*Nitrosomonas*

Genera in parentheses indicate discontinued taxonomic status or unestablished, yet suspected, denitrification ability.

Æsøy and Ødegaard (1994) witnessed hyperbolic, Monod-type kinetics during their research of biofilm denitrification. This finding may be explained by the observations of Bilanovic *et al.* (1999). It is possible that certain VFA carbon sources were consumed before fully penetrating the biofilm, resulting in different rates across the film. The overall rate would appear Monod-like. Indeed, Çeçen and Gönenç (1995) approximated half-order dentrification with nitrate concentrations below 60 mg/L and zero-order denitrification otherwise. Again,

their research was conducted on biofilms and again the results can be understood in degrees of film penetration.

As presented earlier, denitrification is a multi-step process that converts nitrate (NO_3^-) to nitrite (NO_2^-) to nitric oxide (NO) to nitrous oxide (N_2O) to molecular nitrogen (N_2). Denitrifiers will preferentially use oxygen in place of nitrate or nitrite as an electron acceptor. When oxygen is present and methanol is the organic carbon source, denitrification proceeds according to the following reaction (USEPA, 1993):

$$O_2 + 0.93CH_3OH + 0.056NO_3^- \rightarrow 0.056C_5H_7O_2N + 1.04H_2O + 0.59H_2CO_3 + 0.056HCO_3^-$$

When no oxygen is present, nitrate and nitrite respectively are converted as follows:

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.056C_5H_7O_2N + 0.47N_2 + 1.68H_2O + HCO_3^-$$

$$NO_2^- + 0.67CH_3OH + 0.53H_2CO_3 \rightarrow 0.04C_5H_7O_2N + 0.48N_2 + 1.23H_2O + HCO_3^-$$

The nitrate reaction indicates a theoretical methanol demand of 2.47 g*CH_3OH/g*NO_3^--N. In practice, 2.5 to 3.0 grams of methanol carbon per gram of nitrate nitrogen are consumed. The consumption will increase in the presence of oxygen and nitrite. Such a situation is almost always encountered so methanol carbon consumption usually exceeds 3.0 g. Actual methanol consumption (or any carbon source, for that matter) is largely a function of the biomass yield. The amount of methanol consumed can be calculated with the following equation (USEAP, 1993):

$$COD/N = 2.86/(1-1.134*Y_{NET}).$$

Where Y_{NET} = net yield

These reactions also indicate that some alkalinity is restored during denitrification. About 50% of alkalinity destroyed during nitrification can be replaced during denitrification (Oleszkiewicz, 1994a).

The initial step of denitrification, in which nitrate is reduced to nitrite is facilitated by the membrane-bound enzyme nitrate reductase (Stouthamer, 1988). Nitrate reductase is apparently formed of three subunits in a ratio of 2:2:4. The enzyme has been observed to comprise 25% of total membrane protein. Nitrate reductase contains eight to twelve Fe-S groups (depending on the bacteria) and one molybdenum atom.

The step by which nitrite is reduced to NO or N_2O is not as well known as the nitrate reduction step. This uncertainty is likely the result of the characteristics of responsible enzyme, nitrite reductase. Two main types have been isolated from several bacteria. The first is called cytochrome *cd* (Stouthamer, 1988). The molecular weight is approximately 120,000. The second main nitrate reductase form contains two identical subunits, each with two copper atoms (Stouthamer, 1988). In this case, it has been observed that both nitrite and NO are reduced through the action of this nitrite reductase form. This observation helps elucidate why mixtures containing various amounts of NO and N_2O are found during incomplete nitrite reduction.

The exact role of NO in nitrite reductases is uncertain and may be as varied as the genera involved in denitrification (Stouthamer, 1988). Figure 2.6 shows four observed, yet quite different schemes positioning NO in nitrite reduction to N_2O. Pathway I has been observed in *Paracoccus denitrificans, Pseudomonas aeruginosa,* and *Alcaligenes spp.* Pathway II is valid for

Pseudomonas denitrificans (chiefly by X) and *Pseudomonas aureofaciens* (chiefly by Y and Z). Pathway III is a proposed variation of Pathway II whereby isotope exchange between NO_2^- and NO is enzyme-mediated. Stouthamer reported that some researchers have suggested that nitroxyl (HNO) should be a free intermediate in the reduction of nitrate and NO as shown in scheme IV. Such a process has been witnessed in *Pseudomonas stutzeri.*

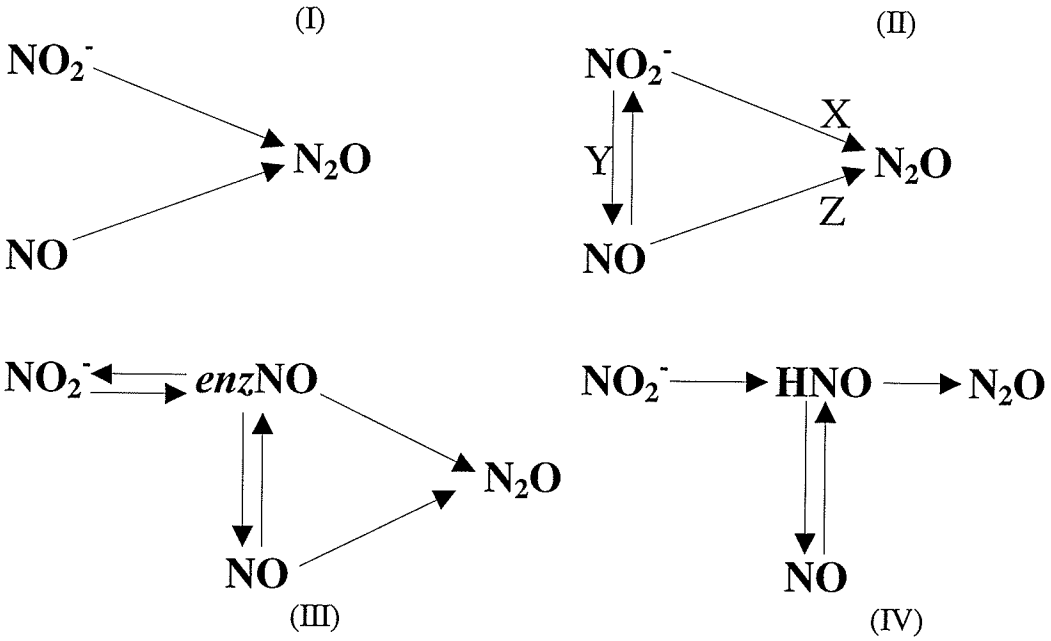


Figure 2.6: Four Observed Pathways Positioning NO in the Reduction of Nitrite to N_2O
(Adapted from Stouthamer, 1988)

The function of nitrous oxide reductase is understood even less than that of nitrite reductase. Estimates of the molecular weight of nitrous oxide reductase range from 25,000 to 120,000 (Stouthamer, 1988) and it is believed that the structure of the enzyme is dimeric and contains an unknown number of copper atoms.

Now that the structures and inner workings of nitrate, nitrite, and nitrous oxide reductase are somewhat clear, the overall picture of denitrification energetics can be presented. However, this picture varies among bacterial species. With this limitation in mind, the case of *Paracoccus denitrificans* is presented as a typical study. The processes of oxygen, nitrate, nitrite, and nitrous oxide respiration along with proton translocation and electron transport are shown in Figure 2.7.

Figure 2.7 shows that there are two cytochrome oxidases in *P. denitrificans*: cytochrome *o* and cytochrome aa_3. During NADH oxidation, Cytochrome aa_3 is activated in the presence of certain organic carbon substrates with methanol among them (Stouthamer, 1988). As Figure 2.7 shows, the use of this pathway results in three electron transfers. Other carbon sources (*e.g.* succinate) result in the activation of cytochrome *o* instead. In this case, only two electron transfers are initiated. This mechanism helps explain why certain, more energetic carbon sources are preferred over others by denitrifying bacteria.

Once anaerobic conditions prevail, *P. dentrificans* cannot synthesize cytochrome aa_3 (Stouthamer, 1988). The bacteria begin to respire nitrogen oxide compounds at this point. Due to the loss of this additional phosphorylation site under anaerobic conditions, anaerobic respiration is approximately 29 to 33% less efficient than aerobic. It has been shown that nitrite inhibits oxidase activity. Therefore, some nitrogen removal is expected even when aerobic conditions prevail. Research has also shown all nitrogen oxide reductions of denitrification are roughly equally energetic.

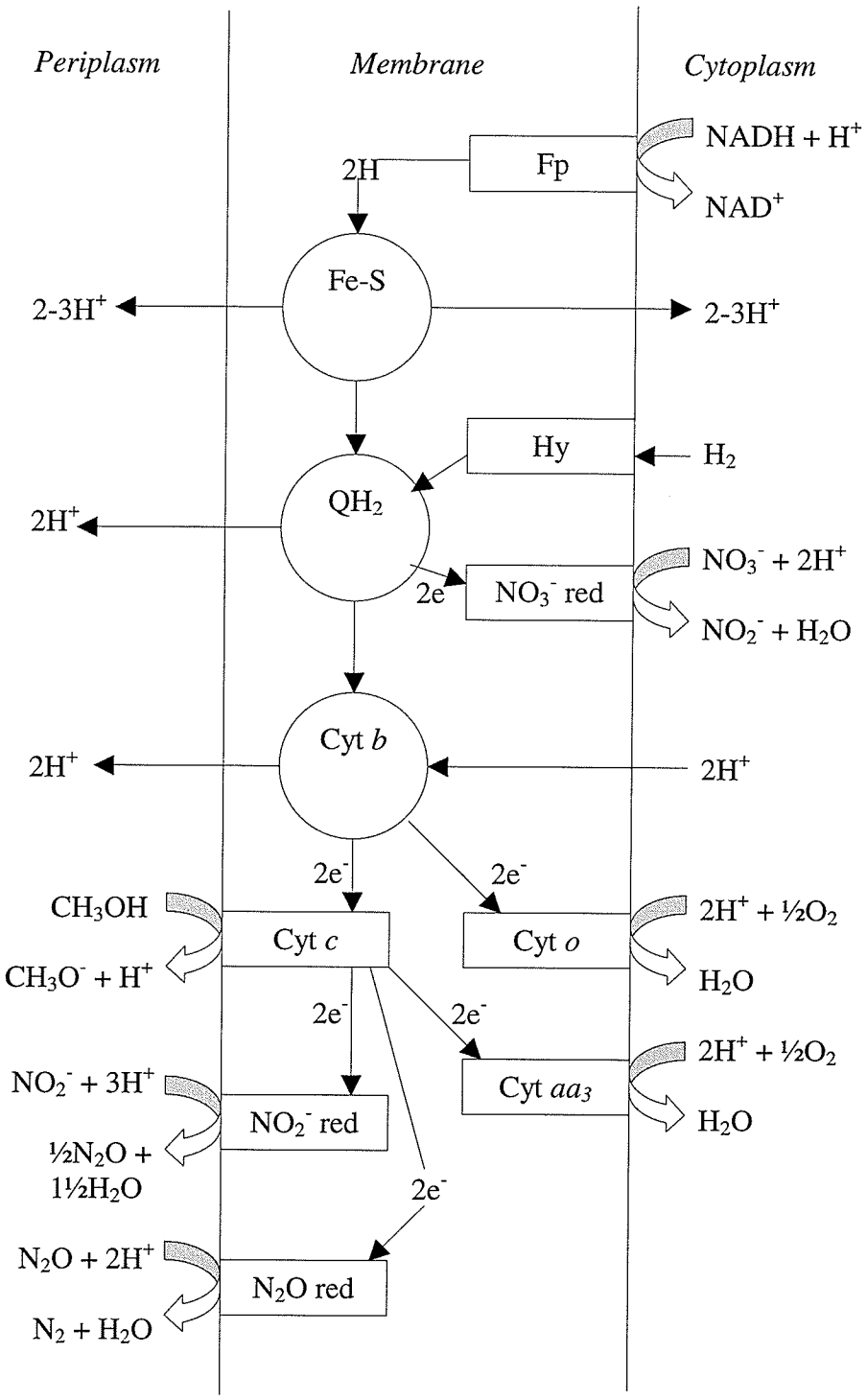


Figure 2.7: Respiration, Proton Translocation, and Electron Transport Across the Membrane of the Denitrifier *Paracoccus denitrificans* (Adapted from Stouthamer, 1988). [Note: Fp = flavoprotein, QH_2 = ubiquinol, Hy = hydrogenase, red = reductase]

Figure 2.7 also demonstrates how a proton gradient is created during denitrification. The periplasm is dosed with 7 or 8 protons per NADH and methanol molecule; whereas the cytoplasm runs a deficit of –2 to –3 protons under anaerobic conditions. This gradient drives the reduction of nitrite to N_2O and N_2O to nitrogen as shown on the periplasmic side of Figure 2.7. It was previously thought that the proton gradient was required to transport nitrate into and nitrite out of the cell. However, it is now known that this function occurs via nitrate/nitrite antiport action and consumes no energy. (Antiport action refers to the simultaneous transport of specific molecules into and out of the same cell and requires the presence of both molecules on the proper sides of the cell membrane.)

b. Factors of Influence

Conventional denitrification is a heterotrophic process and therefore tends to be less sensitive to environmental parameters than nitrification. Having said that, denitrification is affected by variation in temperature, pH, alkalinity, and the presence of inhibitory compounds. Denitrification rates are also strongly dependent on the organic carbon source, which is discussed in the next section.

Like all biological processes, denitrification is enhanced as temperature increases to approximately $50^{O}C$, after which denitrification is seriously impared (Caton, 2002). As Table 2.7 shows, many genera are able to perform denitrification. Some of these genera are thermophilic, while others are psychrophilic. Therefore, denitrification will be observed in all temperature ranges. However, temperature is reported to be a more significant factor below

$20^{O}C$ than above (USEPA, 1993). Optimal denitrification rates are achieved and maintained at pH levels between 6 and 8 (USEPA, 1993). Since alkalinity is produced during denitrification, pH control is less problematic.

Post-denitrification was recently added to the Blue Plains WWTP in Washington D. C. (Bailey *et al.*, 1998). It was found that denitrification was seriously inhibited due to phosphorous limitation. This lack of phosphorous also resulted in a poor settling quality of the sludge. When upstream chemical phosphorous removal processes were diminished, denitrification improved, though the problems did not completely disappear. Aspergren *et al.* (1998) experienced similar problems of phosphorous limitation with post-denitrification.

Nitrite is known to inhibit the metabolism of many organisms and therefore became the subject of studies involving denitrification. In one study, Oh and Silverstein (1999) found nitrite to accumulate in a denitrifying reactor when the carbon source, acetate, was limiting. However, nitrate did not accumulate. This finding could lead to the conclusion that high nitrite concentrations inhibit subsequent nitrogen conversions, or nitrate-reducing bacteria outcompeted nitrite-reducers. Bilanovic *et al.* (1999) settled this question by feeding a denitrifying culture large doses of nitrite. They found that nitrite accumulated at first, but soon returned to normal values as long as the carbon source was sufficient. Such findings are important to reactor design since significant nitrite concentrations might be found in effluents of reactors that are deficient in organic carbon.

c. Role of Organic Carbon

Traditionally, the organic carbon source for denitrification utilized in WWTPs has been methanol. However, within the last decade, research has turned to examining the use of VFAs produced in-house for this purpose. Other organic carbon sources have also been evaluated. This research is encouraged by the savings which would be achieved if VFAs are feasible. For example, MacDonald (1990) reported that methanol cost contributed 70% to the total operation and maintenance costs of the Rancho California Wastewater Reclamation Plant near San Diego. Most of the research has been encouraging and results from full-scale suspended- and attached-growth denitrification installments are being published. Denitrification rates found in the literature are summarized in Table 2.8. It should be noted that the results shown in Table 2.8 are not directly comparable since many different scales, reactor types, and actual and synthetic wastes and carbon sources are used in these studies. However, Table 2.8 does provide an idea of the denitrification rates and C:N ratios that are experienced at the bench, pilot, and full scales.

Table 2.8: Summary of Denitrification Rates Achieved with Various Organic Carbon Substrates

Org. C Source	Denitrification Rate	C:NO_3^--N Ratio	Reference
Molasses	0.36 kg*NO_x^--N/m^3*d	1.87	Çeçen & Gönenç, 1995
Natural Gas	0.26 kg*NO_3^--N/m^3*d	n/a	Rajapakse & Scutt, 1999
Methanol	1.9 kg*NO_3^--N/m^3*d	n/a	*ibid*
Methanol	n/a	1.4	Reising & Schroeder, 1996
Methanol	2.0 g*NO_3^--N/m^2*d	1.5 – 1.9	Aspergren *et al.*, 1998
Ethanol	2.5 g*NO_3^--N/m^2*d	1.5 – 1.9	*ibid*
Acetate	2.1 kg*NO_2^--N/m^3*d	0.84	Rahmani *et al.*, 1995
Acetate	n/a	1.35	Lee & Park, 1998
Acetate	n/a	2.0 – 3.0	Oh & Silverstein, 1999

Org. C Source	Denitrification Rate	C:NO_3^--N Ratio	Reference
VFA effluent	0.57 g*NO_3^--N/ g*VS*d	1.5 – 1.9	Æsøy & Ødegaard, 1994
VFA effluent	0.28 kg*NO_3^--N/ kg*VSS*d	n/a	Pavan *et al.*, 1998
VFA effluent	0.054 mg*NO_3^--N/ g*VSS*d	2.81	Llabres *et al.*, 1999
VFA effluent	0.144 g*NO_3^--N/ g*COD_{tot}*d	n/a	Moser-Engeler *et al.*, 1998
Acetate	0.091 g*NO_3^--N/ g*COD_{tot}*d	n/a	*ibid*
Propionate	0.041 g*NO_3^--N/ g*COD_{tot}*d	n/a	*ibid*
VFA effluent	0.19 g*NO_x-N/ g*VSS*d	n/a	Kristensen *et al.*, 1992#
VFA effluent	0.22 g*NO_x-N/ g*MLSS*d	n/a	Hatziconstantinou *et al.*, 1996
VFA effluent	0.36 g*NO_3^--N/ g*SS*d	n/a	Fass *et al.*, 1994

- Cited in Hatziconstantinou *et al.*, 1996.

Some studies have focused on employing regionally abundant carbon sources. Çeçen and Gönenç (1995) evaluated a submerged filter providing TN removal using molasses as the carbon source. They found a COD:NO_x-N ratio of approximately 5 and conversion rates equal to those found in literature. Molasses are generally cheap and abundant and therefore were a good substitute for methanol in the estimation of the authors. Also, the use of molasses did not exhibit a nitrite accumulation problem supposed common to all high-sugar wastes.

Yatong (1996) conducted a detailed laboratory assessment comparing a number of organic carbon sources for biological denitrifcation. Mixed synthetic VFAs, acetate, propionate, butyrate, valerate, methanol, ethanol, digested sludge supernatant (DSS), and endogenous carbon sources were all evaluated separately. Performance parameters of specific denitrification rate, specific carbon uptake

rate, and observed C:N ratio were all reported. A summary of Yatong's findings are given in Table 2.9.

Based on the results presented in Table 2.9, it appears that denitrification is optimized when pure, synthetic VFA mixtures are used. The next most effective carbon sources were acclimated digested sludge supernatant and pure acetate. This finding is somewhat surprising given the widespread use of methanol. The obvious implication is that methanol is not always the best-suited carbon source for denitrification. Yatong (1996) mentioned that both ethanol and methanol must be oxidized to the corresponding VFAs before they can be metabolized by denitrifiers and therefore, produce less energy than VFAs. As a result, these compounds displayed lower denitrification rates than other VFA compounds.

Table 2.9: Denitrification Performance of Various Organic Carbon Substrates (Adapted from Yatong, 1996)#

Org. C Source	Spec. DN Rate ($mg*NO_3^-$-N/mg*VSS*d)	Spec. C Uptake Rate (mg*C/mg*VSS*d)	C:N
Mixed VFA	0.754	1.792	2.37
Mixed VFA[@]	0.530	1.591	3.00
Acetate	0.603	1.236	2.05
Propionate	0.362	0.505	1.40
Butyrate	0.519	0.928	1.79
Valerate	0.487	0.929	1.91
Methanol	0.289	n/a	n/a
Ethanol	0.349	0.601	1.72
Ethanol+	0.415	0.635	1.53
DSS	0.575	1.212	2.12
DSS+	0.646	1.178	1.82
Endogenous	0.084	-	-

- All trials conducted at 25^{O}C with washed sludge acclimated at pH 7.5 and C:N = 1.5.

@ - Conducted at 20^{O}C.

+ - Sludge acclimated in tested compound.

The data also shows that propionate is the least effective VFA species for denitrification. Yatong (1996) explained this observation with a metabolic solution. Acetic acid can be directly inserted into the metabolic process without modification. Butyrate and valerate require some enzyme conversion before they can enter the metabolism of most denitrifiers. Propionate requires the most modification of the VFAs. This last statement is also validated by the work of Fass *et al.* (1994).

That VFAs are well-suited to BNR is frequently encountered in the literature. Moser-Engeler *et al.* (1998) report that methanol can cause a period of lag until the denitrifying population can adjust. They also remark that methanol is not suitable for phosphorous removal. Fass *et al.* (1994) showed that VFAs did not escape degradation in the denitrification chamber where VFA degradation was calculated to 99.9%. Llabres *et al.* (1999) saw BNR take place at modest VFA effluent-to-influent wastewater ratios of 0.0011 (by volume) which represented an insignificant additional burden on the heterotrophic COD-consuming bacteria.

VFA effluent from anaerobic digestion has also been shown to enhance biological phosphorous removal. Bacteria which facilitate this function absorb organic carbon and release phosphates under anaerobic conditions (Barnard, 1994). When aerobic conditions are again encountered, these bacteria re-absorb these phosphates plus excess phosphates in the wastewater. A number of studies have observed increased phosphorous removal rates when fermented VFAs are injected into reaction vessels. Pitman *et al.* (1991) and Randall *et al.* (1994) both found acetate to exhibit the greatest increases, while Randall *et al.* noted that

propionate did not aid in phosphorous removal. Randall *et al.* (1997) also showed that VFAs (except propionate) were superior to C_2 to C_5 alcohols, formate, and methanol.

On the horizon is the potential for the destruction of toxic anthropogenic compounds during denitrification. This development is especially exciting for three main reasons: (1) dentrifiers have the highest growth yield and are the most robust of all anoxic bacteria; (2) the most prevalent denitrier, *Pseudomonas*, is well-researched; and (3) the economy, solubility, and abundance of NO_3^- makes it an ideal additive to polluted sites to be treated (Tiedje, 1988). Denitrifiers have shown the ability to employ aromatic compounds, phthalate (use in plastics manufacture), phenol, nonionic detergents, and chlorinated solvents as organic carbon substrates.

2.2.6 Engineered Biological Nutrient Removal Systems

There are a number of suspended-growth BNR plants in use. They all operate on the principle of alternating anaerobic, anoxic, and aerobic conditions to maximize the activities of nitrifiers, denitrifiers, and phosphorous-absorbing bacteria. Oldham *et al.* (1994a; 1994b) presented five case studies of such reactor configurations. All these cases employed fermented VFAs as the carbon source.

A 5-stage Bardenpho process was implemented in Kelowna, British Columbia which achieved 0.4 mg/L NO_3^--N and 0.1 to 0.2 mg/L TP in the effluent. In Penticton, B. C., a modified University of Cape Town (UCT) process was installed which boasted effluent ammonia below 2 mg/L, effluent nitrate not

exceeding 4 mg/L, and effluent phosphorous less than 0.3 mg/L. This same design was employed in Westbank, B. C.; Kalispell, Montana; and Melby, Denmark with similar success.

Metcalf & Eddy (1991) reported that fixed-film technologies are limited to nitrogen removal only. However, Gibb *et al.* (1993) and Falkentoft *et al.* (1999) have had success with total BNR using fixed-suspended system at the pilot scale. Such a system incorporates free-floating, inert objects in a reaction vessel. Microbes attach themselves to these objects. Versus conventional BNR technologies, fixed-film systems offer the advantages of increasing the reactor biomass, lower sludge production, and lower capital and space requirements (Reardon, 1995). As Reardon reports, such fixed-film/suspended-growth hybrids are increasing in number in North America as their value is proved.

3 RESEARCH OBJECTIVES

This study seeks to explore the optimal starch wastewater-to-municipal wastewater ratio in anaerobic digestion in terms of volatile fatty acid (VFA) production. Two previous studies by Banerjee (1997) and Maharaj (1999) have partially explored this topic. These and other studies have also determined an optimal hydraulic retention time (HRT) of 30 hours and solids retention time (SRT) of 10 days. Therefore, these parameters were replicated by this study. A temperature of approximately 21^{O}C was employed. No pH control was exercised during this study.

Two solids-contact continuous anaerobic digesters identical to those used by Banerjee (1997) and Maharaj (1999) were employed in this portion of the study. VFA production and speciation, total Kjeldahl nitrogen (TKN), total and soluble chemical oxygen demand (TCOD and SCOD, respectively), pH, alkalinity, volatile and total solids (VS and TS, respectively), and total suspended solids and volatile suspended solids (TSS and VSS, respectively) were measured regularly over the course of this research.

Banerjee (1997) and Maharaj (1999) employed a starch-rich wastewater and municipal wastewater in a ratio of 1:1 and municipal wastewater only. The study reported herein replicated the 1:1 ratio and experimented with the new ratios of 1:3, 3:1, and starch wastewater only. The main aim of this experimentation was to determine the effect of altering this ratio on VFA production.

One of the anaerobic digesters was maintained in operation with a 1:1 starch-to-municipal feed ratio throughout the study. This digester produced a reliable VFA-rich effluent that became one of the feedstocks for the second portion of this study. This latter portion explored the topic of denitrification and the suitability of the VFAs produced during anaerobic digestion for this purpose.

The initial phases of exploration on this topic centred on determining a successful protocol to develop a reliable "seed" for future batch denitrification trials. The "seed" consisted of municipal sludge which had undergone a number of treatments.

A range of C:N (Carbon to Nitrogen) ratios were researched in batch denitrification cultures. These ratios ranged from approximately 0.1 to 5. In addition, influent C concentration (in the form of VFA-rich anaerobic digester effluent) was altered. C concentration ranged from roughly 15 to 500 mg/L. These results were analysed to determine the kinetics of denitrification with respect to the aforementioned parameters.

4 EXPERIMENTAL METHODS

4.1 Sources of Wastewater and Sludge

Two wastewater types were investigated in this study. The first was a prepared municipal wastewater. It was prepared by diluting concentrated primary sludge to a total solids (TS) level of 5,000 mg/L. Dilution was provided with tap water. The primary sludge was delivered every two weeks from the South End Pollution Control Centre in Winnipeg. The sludge was transported and stored in a plastic, translucent, 20 L jug. Storage occurred in a cold chamber at the University of Manitoba at 6^{O}C. The solids content of this sludge ranged from a low of 10,000 mg/L to a high of 63,000 mg/L.

The second prepared wastewater consisted of a diluted starch-rich, industrial sludge. This sludge was collected approximately every three weeks from the Old Dutch Food Company, located in Winnipeg. This plant processes potatoes into potato chips and therefore, the process water is very high in starch solids. In order to remove these solids, a series of settling tanks has been installed that catch the effluent and allow settling to occur. Then, the clear effluent is discharged to the sewer mains. The effluent collected for this study comes from a point in the line where no settling has yet occurred. The TS content of this water ranged from about 35,000 to 100,000 mg/L. Again, this sludge was diluted to 5,000 mg/L with tap water prior to use.

Skalsky and Daigger (1995) found that volatile fatty acid (VFA) production is enhanced during anaerobic digestion through the use of dilute

wastewater. Since VFA optimization was the primary aim of this study, such a dilute feed was employed. Hall (1992) has also shown that anaerobic contact processes perform better when solids levels are minimized. Since an anaerobic contact process was in place here, it seemed wise to dilute the sludges to the low levels employed (5,000 mg/L). In addition, Banerjee (1997) and Maharaj (1999) diluted wastewater to the same level during their investigations. Thus, in order to compare the results of this study with those gathered by the said authors, equal dilution was applied.

The sludge collected from the South End Water Pollution Control Centre was also used to provide the seed for denitrification experiments. Since denitrifying bacteria are ubiquitous (Tiedje, 1988), they could be expected to exist in this primary sludge. However, in order to concentrate the biomass and remove the soluble chemical oxygen demand (SCOD) of the sludge, sludge processing was required. The removal of SCOD is necessary since it interferes with the consumption of the external carbon substrate. This investigation employed the VFAs produced from the anaerobic contact digester effluent as the external carbon substrate.

SCOD removal was a multi-step centrifuge, decant, and wash process. Sludge was poured into 50 mL centrifuge tubes and then centrifuged for 10 minutes at 6,000 rpm. The centrifuge model no. HN-S was manufactured by IEC. The primary sludge settled very well after this treatment. Once this step was complete, the supernatant was decanted without the loss of solids. A small amount of tap water was then added to the tubes which were then vigorously agitated to break up the centrifuged solids. More tap water was added to restore

the 50 mL volume, and the process was repeated twice more. It was discovered that this treatment was sufficient to ensure the removal of most SCOD in the sludge.

4.2 Configuration of Apparatus

4.2.1 Anaerobic Digestion

Two identical laboratory-scale anaerobic contact reactors were employed over the course of this research. These reactors are pictured schematically in Figure 4.1 The anaerobic reactor was a cylindrical 4 L vessel with an internal diameter of 11.4 cm. The liquid level of this reactor was maintained at 3 L. The reactor vessel was constructed of plexiglass and stirred continuously at an unspecified rate. This rate was sufficient to ensure solids were suspended at all times. Stirring rods were inserted from the top of each reactor and powered by a modified Cole-Parmer pump. The stirring rods had two paddles mixing the bottom third of the reactor, and two paddles in the upper-middle third. Mixing was controlled with MasterFlex speed controllers. The reactor vessels had sampling ports 17 cm from their bases.

The wastewater feed mixtures were contained in 20 L buckets and stored at 6^{o}C. The buckets were mechanically stirred with modified Cole-Parmer pumps and controlled with MasterFlex speed controllers. The feed stirring rods had two paddles located at the ends of the rods near the bottoms of the feed buckets. The stirring rate was sufficient to suspend the solids of the feed

mixtures. Feeding occurred every 30 minutes for a duration of 1 minute again through the action of Cole-Parmer pumps and MasterFlex speed controllers.

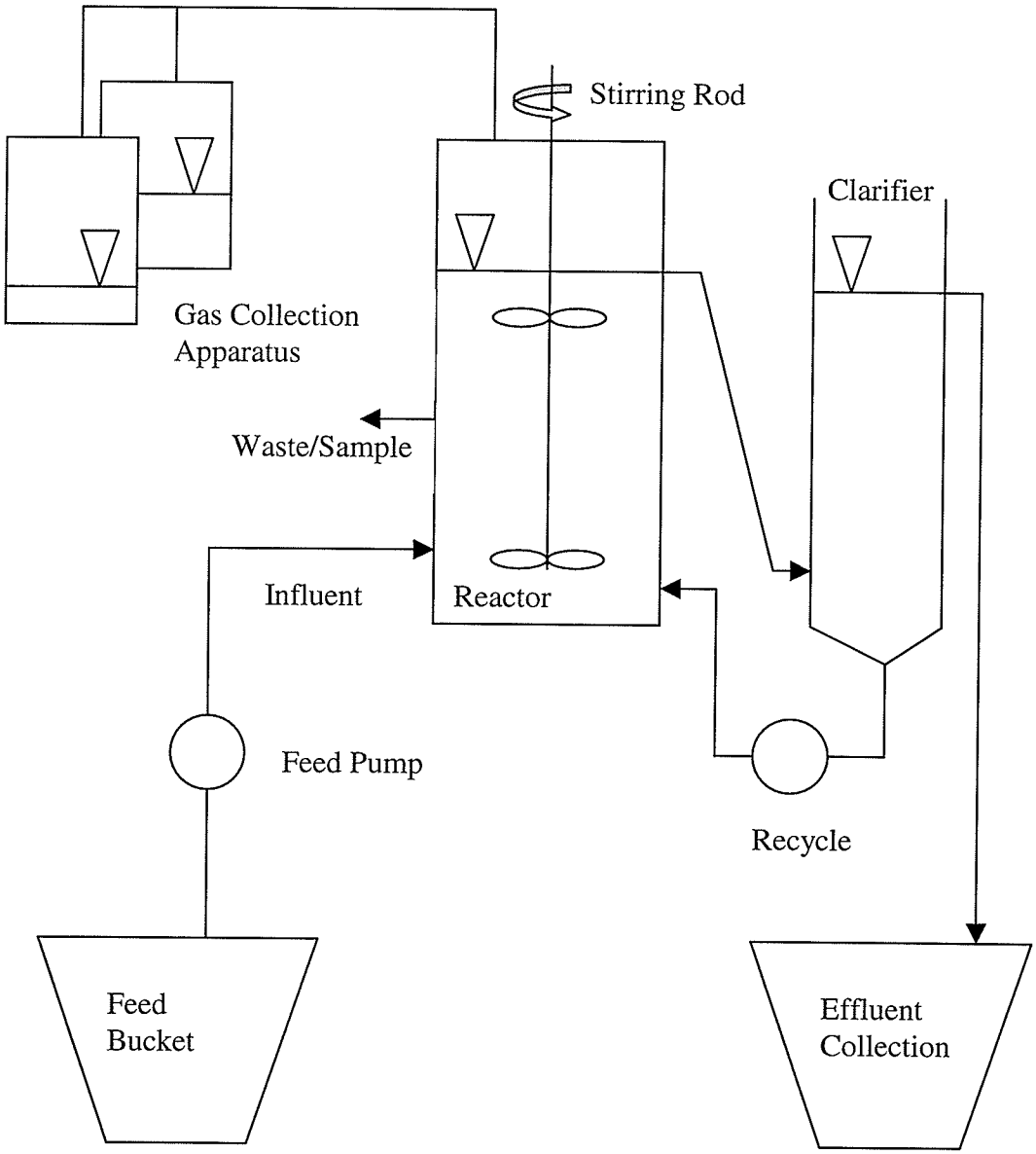


Figure 4.1: Schematic of the Anaerobic Contact Digesters

Recycle of the biomass from the clarifiers to the reactors was also facilited by way of Cole-Parmer pumps and MasterFlex speed controllers and coincided

with feeding. The plexiglass clarifiers had a modified Imhoff conical shape to aid in solids settling. The internal diameters of the two clarifiers were 10.8 cm and had a liquid volume of 2 L. The clarifier supernatent (*i.e.* the system effluent) was collected in 10 L plastic jugs and discarded after sampling. Some of this effluent was preserved and stored for no more than 1 week for use in denitrification batch tests.

Gas collection apparatus was fitted to the reactors. Each apparatus consisted of two 2.25 L flasks containing gas retaining fluid. One of these flasks was connected to the headspace of the reactor. When gas is produced in the reactor, the resultant pressure would push the level of the gas retaining fluid down in the flask connected to the reactor. The fluid level would rise in the second flask in this case. Through calibration of the fluid level in the first flask, the volume of reactor gas produced could be determined. In addition, a septum inserted in the connection between the first flask and the reactor provided a gas sampling port.

4.2.2 Denitrification

The apparatus employed in the denitrification investigation were much simpler. 1,000 mL Erlenmeyer flasks were used as the batch test vessels. The liquid level was set at 600 mL. T-Shaped magnetic stirrers were dropped to the bottom of these flasks and stirring was provided by Canlab model no. S58260-1 magnetic stirring bases. The rate of stirring was not prescribed, but was sufficient to allow the complete suspension of the reactor solids. These batch flasks were

filled with the prepared sludge seed and various quantities of nitrate-nitrogen (potassium nitrate as the source). The remaining volumes of the flasks were filled with tap water to the 600 mL level.

4.3 Operation and Sampling of the Research Systems

4.3.1 Anaerobic Digestion

The start-up of the anaerobic contact digester systems involved feeding the reactors continuously until the 3 L level had been reached. Only one of the reactors was used at the beginning of the study and was fed a 1:1 mixture of the industrial and municipal wastewaters. The other reactor systems, once put into use, were started up in the same manner with the same feed mixture. Once the reactor was full, feeding proceeded according to the once every 30 minutes for 1 minute regimen. An acclimation period of approximately two weeks was allowed to permit anaerobic conditions to prevail and the anaerobic population to establish itself. This acclimation period was provided at the onset of each new run.

A total of five runs were conducted over the course of this research. All five were conducted with an hydraulic retention time (HRT) of 30 hours at temperatures of 21^{O}C. The only varying factor was the feed industrial-to-municipal ratio. The feed ratio conditions are summarized in Table 4.1.

HRT was checked and maintained through the adjustment of the influent pump speed controller. Feed bucket volume was determined by measuring the height of the fluid level. (The volumes of the feed buckets were previously

calibrated according to fluid height.) The HRT was then calculated by dividing the reactor volume (3 L) by the difference between the current volume and the previous volume and multiplying by the time that had passed.

Table 4.1: Experimental Conditions

Run	Wastewater Feed		Ratio
	Industrial Content (%)	Municipal Content (%)	
1	50	50	1:1
A	25	75	1:3
B	50	50	1:1
C	75	25	3:1
D	100	0	100% Ind.

Solids retention time (SRT) was to be maintained at 10 days throughout this research. SRT was controlled by wasting quantities of reactor solids. SRT fluctuated mildly during the first four runs, but became quite large during run D due to the excellent settling qualities of the reactor solids of the mixture. Gas production and temperature were monitored on an approximate daily basis. Each run was conducted over a period of approximately 45 days.

Prior to detailed sampling during the runs, the system was allowed to reach a steady-state condition. This condition was assessed by monitoring pH and net VFA concentration. Fluctuations of more than ± 10% in pH, ± 20% in net VFA concentration, and/or obvious upward or downward trends in either parameter were interpreted as signs of instability.

Three samples from various points in the anaerobic system were taken about every two weeks. Sometimes this schedule was intensified or relaxed to accommodate other commitments. Samples were no larger than 100 mL and were drawn into plastic sups that were immediately sealed after sampling. The

first sample was taken from the influent buckets as stirring was taking place. The next was drawn from the reactor through the sampling port. The third and fourth samples were extracted from the clarifier before and after agitation. The final sample was taken from the effluent jug after it had been vigorously shaken. The total number of samples taken during each run was approximately 12 to 16 sample sets.

Alkalinity and pH, TS, total suspended solids (TSS), volatile solids (VS), volatile suspended solids (VSS), VFA content, and soluble COD content were evaluated for the influent, reactor, and effluent samples. The undisturbed clarifier sample was tested for VFA content only. The agitated clarifier sample was evaluated for VSS (to control SRT). Whenever possible, these tests were conducted on the day of sampling to avoid the need for sample preservation. Two out of three influent, reactor, and effluent samples were also analyzed for total Kjeldahl nitrogen (TKN) content, ammonia nitrogen content, and total COD. Each of these analyses was run with duplicate samples to help ensure the reliability of the results.

4.3.2 Denitrification

During the denitrification batch tests, 20 mL samples were extracted with two draws of a 10 mL pipette. Such samples were taken every hour from hours 0 through 7. Another sample was drawn around hour 14. The following day, hours 24 through 30 (in some cases hour 32) were sampled every 2 hours. Some denitrification tests saw samples drawn every two hours on the third day as well,

for hours 48 through 54. Usually, denitrification had ended by this time, but these samples established that this was indeed the case.

Denitrification runs were conducted in 1,000 mL Erlenmeyer flasks covered with parafilm. The total initial volume of the wastewater mixture was approximately 600 mL. Obviously, these were not perfectly anaerobic conditions but may be considered anoxic as oxygen could not enter the flasks.

There were four "ingredients" which went into most of the flasks. First, a "seed" consisting of washed and concentrated primary sludge solids were added to the flasks. The preparation of the seed presented special challenges which required the establishment of an effective protocol. This protocol is detailed in section 4.3.2.1. The seed provided a VSS concentration of approximately 5,000 mg/L once the wastewater mixture was fully prepared. The second ingredient was the synthetic 1 M potassium nitrate (KNO_3) solution added in appropriate amounts. The third ingredient was the fermented effluent from the anaerobic contact digester of Run B. It was sealed and allowed to settle for about three days before being added to the denitrification flasks to provide the desired initial VFA content. As a result, the solids content of this ingredient was very low. A summary of its characteristics is provided in Table 4.2. The fourth and final ingredient was tap water in order to standardize the volume at 600 mL. Not all flasks required tap water.

Table 4.2: Characteristics of the VFA Wastewater Source.

Parameter	Mean	Standard Deviation
pH	4.54	0.24
Alkalinity (mg/L as $CaCO_3$)	23	51
TS (mg/L)	3052	1697
VS (mg/L)	1698	1450
TSS (mg/L)	1916	1966
VSS (mg/L)	1655	1711
VFA (mg/L as acetate)	779	174
SCOD (mg/L)	1621	291
TCOD (mg/L)	4725	4051
NH_3-N (mg/L)	17.7	13.9
TKN (mg/L)	163.1	51.6

4.3.2.1 Final Seed Protocol Development

The knowledge gained during five initial runs allowed the development of a successful seed protocol applied during the final three runs. The wastewater mixture concocted for the first run contained all the ingredients outlined earlier. The seed used was both "fresh" and stored primary sludge. Unfortunately, the results were very erratic and provided little insight into secondary VFA production problems. The second run also did not provide firm indications of a problem with secondary VFA production.

The results of the third run demonstrated significant secondary VFA production for the first time. Such production masks the consumption of VFAs by denitrifying bacteria and renders C:N parameter meaningless. This secondary VFA production was observed despite efforts to treat the seed. In this case, loosely covered, fresh sludge was allowed to settle at room temperature for two days, after which the liquid was decanted. After decanting, tap water restored the

volume. After another two days of settling, the sludge was decanted again. However, this preparation was inadequate as explained above.

For the forth run, the fresh primary sludge was treated in the same manner outlined above. After decanting the second time, the sludge was centrifuged, decanted, filled with tap water, and agitated. This second treatment was repeated three times before being placed in the flasks. The magnitude of the secondary VFA production was diminished, but was still significant.

The seed protocol for the fifth run was exactly the same as that of the fourth with one exception. When the sludge was settling, 300 mg of KNO_3 crystals per L of seed were added to the storage cylinder. The rationale for this alteration was that it would provide some acclimation for the seed. The fifth run proved to be somewhat successful as VFA levels stabilized or fell over the run. However, initial VFA production was still observed. It was therefore necessary to adjust the seed protocol further for Run 6.

The seed for Run 6 was prepared in an identical fashion as it had been in Run 5. This time however, ten day old "stable" sludge was used. The rationale for this alteration was that the organic carbon in the sludge would be mostly hydrolyzed after ten days. Once the sludge was washed, most of the soluble organic carbon should be removed and little organic carbon would remain for hydrolysis. This protocol proved successful. Results showed VFAs were nearly completely consumed over the course of the run. Needless, to say, this protocol was adopted for Runs 7 and 8 and again proved to be successful.

4.3.2.2 Sampling Schedule

In addition to difficulties with seed protocol, the batch denitrification investigation was hampered by an initial ignorance of its corresponding time-scale. The first run spanned five days. As mentioned in the previous section, the first run provided little insight into the nature of these experiments since the data obtained was so erratic. The second run was basically another attempt at the first with aim of attaining better results. It was successful in that the results indicated difficulties that were heretofore unnoticed. Nitrates were seen to disappear in the space of one day. Therefore, the sampling schedule was amended to span hours, rather than days.

The third run took place over a time frame of 50 hours. Samples at hours 0, 5, 24, 28, and 51 were taken. However, it was observed that nitrates had disappeared by the hour 24 sample. It was therefore necessary to increase the sampling frequency between hours 0 and 24. This lesson was incorporated into Run 4. Samples were drawn at hours 0 through 8 and again at hours 24, 27, and 30. However, reactors having relatively low initial nitrate values (around 20 mg/L) achieved complete denitrification between hours 8 and 24. In addition, reactors with high initial nitrate concentrations had not achieved complete denitrification by hour 30.

This sampling schedule was slightly altered to produce a final schedule. Sample frequency was reduced during hours 0 to 8. Samples were taken at hours 0, 2, 4, 6, and 8 to provide enough time to conduct the required analyses. The following days, samples were taken at hours 24, 26, 28, 30, and sometimes 32.

Also, the time frame was extended to provide samples at hours 48, 50, 52 and sometimes 54 hours for reactors with high initial nitrate concentrations (between 100 and 200 mg/L). During Runs 6, 7, and 8, another sample was added for hour 14 or 15 to provide a nighttime value between hours 8 and 24. This finalized schedule proved to supply an adequate amount of data spaced over sufficient time to calculate kinetic parameters.

4.4 Analyses Methods

All samples were analyzed for nitrate nitrogen content and pH. Alkalinity, TS, TSS, VS, and VSS was assessed every third sample or so for the first day. VFA content was also determined for some of the samples. In order to determine that the sludge seed was not producing appreciable amounts of carbon substrate for denitrification, soluble organic carbon (SOC) was analyzed during one of the runs. It was observed that SOC did indeed drop over the course of the run, leading to the conclusion that only small quantities, if any carbon substrate available for denitrification was being produced. Most of these analyses were run in duplicate to ensure the reliability of the results. However, time constraints did not allow for duplication of pH and alkalinity analysis.

The different analyses that were undertaken over the course of this study are outlined in the following section. The apparatus and procedures by which these analyses were executed are also outlined here.

4.4.1 pH and Alkalinity

The values for pH and alkalinity were evaluated with a Fisher Accumet pH/ion meter (model no. 230) with a combination pH probe. Calibration was always provided before testing the actual samples. Standard buffer solutions of pH 4.0, 7.0, and, less frequently 10.0 were used for this purpose. 10 mL samples were drawn and placed on a magnetic stirrer and pH was read from this sample. Titration with 0.02N H_2SO_4 solution to a pH of 4.5 was then achieved. Titrant volume reveals the alkalinity in the sample, which is assumed exhausted at pH 4.5.

4.4.2 TS, VS, TSS, and VSS

Solids analysis for this study followed Methods 2540 B, D, and E of Standard Methods (A.P.H.A. *et al.*, 1992). Dishes for total and volatile solids samples were prepared by placing them in a furnace at 550^{O}C for 1 hour. Once cooled in a desiccator, the dishes were weighed on a Mettler AJ100 scale. A 5 or 10 mL volume of sample was placed in the dish using a 10 mL graduated pipette. Solids remaining in the pipette were washed into the dish with deionized water. These samples were then placed in a furnace at 105^{O}C for 5 hours. Once cooled in a desiccator, the samples were again weighed. The difference between this weight and that of the empty dish yields TS. To determine VS, the sample was then placed in the 550^{O}C furnace for 1 hour. The difference between this weight and the weight measured after 5 hours at 105^{O}C gives VS.

The preparation of crucibles for total and volatile suspended solids samples were also subjected to the same furnace conditions, but after Whatman 934-AH glass microfibre filters were fitted into them. This procedure involved placing the crucibles on a vacuum filter apparatus and washing the filter with deionized water. Using a 5 mL graduated pipette, 2 or 5 mL samples were injected into the crucibles mounted on the vacuum apparatus. Again, solids remaining in the pipette were washed into the crucibles with deionized water. The crucibles were then fired and weighed in the same way as the dishes outlined above to determine TSS and VSS.

4.4.3 Volatile Fatty Acids

Samples were analyzed for their acetate, propionate, iso-butyrate, n-butyrate, iso-valerate, and n-valerate composition. Two instruments working in concert provided this analysis. The first was the Antek 3000 gas chromatograph (GC), equipped with a flame ionization detector (FID), and an HP-FFAP cross-linked column of dimensions 10 m by 0.53 mm by 1.0 μm. The carrier gas for this instrument was helium. The second instrument was one of two integrators: a Waters 740 data module or a Shimadzu CR501 unit.

Sample preparation for VFA analysis involved a number of steps. First, the samples were injected into 1.5 mL micro-centrifuge tubes and centrifuged for 5 minutes in a IEC Centra-M centrifuge. After this step, samples were diluted to fall into the range of 0 to 100 mg/L for each VFA variety. Next, diluted samples were filtered through a 25 mm 0.45 micron nylon syringe filter into vials sealed

with air-tight caps. Should VFA analysis not be undertaken immediately after preparation, preservation was achieved with the injection of phosphoric acid and storage at $5^{O}C$. Samples were never stored beyond one week's time. Injection into the Antek unit was accomplished with a graduated 1.0 μL Hamilton syringe. Samples were injected in duplicate (sometimes triplicate) and further diluted if they were out of range (*i.e.* over 100 mg/L in any of the six VFAs).

4.4.4 Soluble Organic Carbon

The Persulfate-Ultraviolet Oxidation Method for soluble organic carbon analysis is described in section 5310 C of Standard Methods (A.P.H.A. *et al.*, 1995). Samples were initially prepared by centrifuging and filtering as described for VFA sample preparation. Once the solids had been removed from the samples, enough sulfuric acid was added to drop the pH between 2 and 3. This step removed alkalinity which would interfere with analysis. At this point, the samples were ready for analysis.

Analysis was conducted on a Dohrmann DC-80 Carbon Analyzer. Samples were manually injected into the analyzer where organic carbon was oxidized to CO_2 by persulfate in the presence of ultraviolet radiation. CO_2 was sparged continuously and carried to an infrared analyzer tuned to the signature CO_2 absorptive wavelength. A microprocessor read the peak areas and compared them to stored peak areas corresponding to calibration standards entered earlier. At this point, the Dohrmann display would indicate the soluble organic carbon (SOC) in mg/L.

4.4.5 Chemical Oxygen Demand

Section 5220 D of Standard Methods (A.P.H.A. *et al.*, 1992) was followed for this analysis. This passage describes the Closed Reflux Colorimetric Method. Once VFA samples were tested, they were saved and used in soluble COD analysis. 2.5 mL of the prepared sample was injected into a culture tube and a duplicate tube. To this sample were added 1.5 mL of digestion solution and 3.5 mL of the catalyst sulfuric acid. These two additives were injected into the tubes slowly down the sides of the tube in order to keep the temperature relatively low. The tubes were then capped and allowed to sit until analysis.

Before analysis, the tubes were placed in racks and digested at 150^{o}C for 4 hours. After cooling, a Bausch & Lomb Spectronic 21 spectrophotometer was used to measure the COD level of the sample. The spectrophotometer was adjusted to 600 nm. Standard tubes were also read with the instrument ranging from COD concentrations of 0 to 1000 mg/L. These standard sample were used to prepare a standard curve onto which sample readings were superimposed.

Total COD analysis was exactly the same as soluble, except in this case, no filtration or centrifuging of the sample was required. It was necessary to dilute these samples substantially. Digestion of total COD samples also took 7 to 9 hours, much longer than that of soluble COD.

4.4.6 Total Kjeldahl Nitrogen

The preparation of these samples was quite involved. A 5 mL sample was placed in a digestion tube along with 50 mL of deionized water, 10 mL of concentrated sulfuric acid, and 2 kjeltabs. The tubes were then placed in a Tecator DS 20-1015 block digester that was controlled by a Tecator 1012 autostep controller. The procedure also required an Exhaust System 1013 Scrubber. After the tubes had cooled, 25 mL of deionized water was added to prevent potassium sulfate crystals from forming. Duplicate samples were digested for TKN analysis.

After this digestion process was complete and the tubes had cooled, their contents were emptied into 50 mL culture tubes. Deionized water was added to fill up to the 50 mL level. At this point, these samples would now be ready for analysis by the automated phenate method as described in Section 4500 NH_3 H of Standard Methods (A.P.H.A. *et al.*, 1992). However, a NaOH line was required during this analysis to neutralize the samples. This modification differs slightly from the description offered in the above volume. A Technicon automated system with an Auto-Analyzer II, a pump 111, a colorimeter with a 15 mm flow cell and 630 nm filters, and a dual pen recorder were all employed to this end. TKN standards were also analyzed to provide a standard curve.

4.4.7 Total Ammonia

Total ammonia analysis was also conducted according to the automated phenate method described in the previous section. In this instance however, no NaOH line is required since the samples are not nearly as acidic as the TKN samples.

Samples were prepared by filtering with #1 filter paper to ensure their clarity for colorimetry. Samples were then analyzed in the manner described above. Again, duplicate samples were provided and a standard curve was generated.

4.4.8 Nitrates

Section 4500-NO_3 F of Standard Methods (A.P.H.A. *et al.*, 1992) describes the nitrate analysis method used in this study. This method is called the Automated Cadmium Reduction Method since nitrates were reduced to nitrites in a packed cadmium column. Samples were first filtered with #1 filter paper, again to remove turbid interference. Once filtration was complete, nitrates were measured with a Technicon autoanalyzer with a NO_3/NO_2 manifold, which housed the cadmium column. The recorder was a GOW-MAC model 70-700. Again, a standard curve was required and duplicate samples were run.

4.5 Quality Assurance

For the anaerobic digestion portion of the research, each of the measurements was conducted in duplicate in an effort to provide greater confidence in the results. Analytical error observed between these duplicates was normally acceptable (*i.e.* within 20%). Certain parameters, such as pH, exhibited very small error approximately 1 to 2%. The largest errors of about 20% were observed for VFA, ammonia, TKN, and some solids measurements. The other parameters, namely alkalinity, COD, SOC, and nitrates, displayed errors near 10%.

The reader will note that this study borrows the results obtained by Maharaj (1999) and Banerjee (1997). This study does not attempt to duplicate the 100% municipal feed mixture and borrows exclusively from these previous studies. This strategy was adopted in an effort to concentrate on unknown feed mixtures and in light of the fact that Banerjee and Maharaj produced results with statistically insignificant differences when all other parameters were approximately equal. In addition, both Maharaj and Banerjee experienced significant problems with plugged tubing, pump failure, and clarifier overflow with this feed mixture. A discussion of the statistical compatibility of the results of this study compared to the other two studies in presented in section 5.1.3.4.

It was not possible to draw duplicate samples during the denitrification study. There were three reasons for this limitation. First, the volume required for each sampling period was approximately 20 mL. If duplicate samples were required, this volume would increase to 40 mL. Over the course of the run, this would result in a significant reduction in the total volume of the flasks. Second, an insufficient number of dishes were available to run duplicate VS, VSS, TS, and TSS samples. Third, and most importantly, there was insufficient time to perform duplicate analyses given the frequency of sampling and number of flasks per run. It is acknowledged that providing only single samples renders the results less reliable.

5 RESULTS AND DISCUSSION

5.1 Effect of Starch Feed Content on VFA Production Performance of Anaerobic Hydrolysis-Acidogenesis Investigation

The effect of varying the industrial-to-municipal wastewater ratio on the performance of the hydrolysis-acidogenesis stage of anaerobic digestion was investigated. This work represents the third such investigation conducted at the University of Manitoba's Environmental Engineering laboratories. This context allows for direct comparison amongst these studies. As a result, much of the previous work, conducted by Banerjee (1997) and Maharaj (1999), will be presented throughout this section.

5.1.1 General Characteristics

Some comment should be made regarding the nature of the raw influent, the mixed influent, the stability and acclimation of the reactor bacterial population, and the operational parameters of the reactor before delving into the implications of the results. Laying this groundwork ought to reveal that initial variations and variation inherent in the systems employed will have little or no impact on the results.

5.1.1.1 Nature of Raw Wastewater Components

Two raw waste components were used in this study. The primary, municipal sludge was dispatched from the South End Water Pollution Control Centre in

Winnipeg. Sources of wastewater to this plant are mainly residential, although there is some industrial activity in the plant catchment area. The second raw component used was a starch-rich industrial wastewater. This wastewater was produced at the Old Dutch Food Company potato chip plant in Winnipeg. At the plant, this wastewater passes through a number of settling tanks before draining into sewers. The starch wastewater used in this study was obtained before settling.

The raw sludge and industrial wastewater was stored at 6^{O}C in order to discourage biological activity. However, such processes could not be suppressed altogether, as the results shown in Table 5.1 indicate. Raw sludge and industrial wastewater were replaced approximately every 14 days. Therefore, the cold room study only spanned 21 days.

Table 5.1: VFA Content of Raw Components of Influent Wastewaters During Cold Storage Study

Municipal Sludge		Industrial Wastewater	
Day	VFA (mg/L as acetic)	Day	VFA (mg/L as acetic)
1	2067	1	661
4	2246	4	840
7	3189	7	1105
12	4156	12	1296
14	3790	14	1357
17	3550	17	1575
21	3934	21	1595
Mean:	3276	Mean:	1204
Std. Dev.:	824	Std. Dev.:	356

The cold room study reveals that some hydrolysis-acidogenesis took place in the raw component vessels. Maharaj (1999) noted limited acidogenic activity during cold storage as well. It should be noted that the cold room temperature climbed

to approximately 15^{O}C on day 11 due to a mechanical problem. This problem likely accounts for the highest VFA value on day 12.

Hydrolysis-acidogenesis cannot be expected to cease even at low temperatures encountered in cold storage. High volatile solids values in the raw wastewater components provide substrates for the bacteria in the raw components. This is particularly true for the easily-degradable starch wastewater. However, the observed increases in VFA content will not affect the final results as these are evaluated as net production, rather than gross.

5.1.1.2 Nature of Prepared Wastewater Feeds

The influent wastewater mixtures consisted of the four various industrial-to-municipal ratios: 1:3, 1:1, 3:1, and 100% industrial by volume. These different mixtures of raw primary sludge and industrial wastewaters were diluted in order to reduce total solids (TS) to 5,000 mg/L, or 0.5%, before being fed to the reactors. Experience has shown that tubing stress is reduced and pump and clarifier performance is enhanced by lowering TS levels. Further justification for this dilution is provided by Skalsky and Daigger (1995) who reported that diluted anaerobic reactors favoured increased VFA production over those high in solids. Influent wastewater characteristics for all four runs are summarized in Table 5.2.

The table shows that most parameters vary over the course of the runs, as reflected by the value of standard deviation. Even TS, which was the controlled variable, varies considerably, especially as the industrial content of the wastewater mixture increases. This variation is likely due to the nature of the starch solids. They were abundant, ranging from 7 to 12%. They also settled

rapidly and tended to congeal at the bottom of the storage vessel. The delivered starch water was agitated before analyzing for solid content and adding to the influent buckets, but it was impossible to suspend all the solids. As a result, TS values were limited in their accuracy and variation in TS resulted with each new agitation and drawing of the starch wastewater.

Table 5.2: Influent Wastewater Characteristics for All Four Runs (Industrial:Municipal by Volume)

Parameters	Run A (1:3)		Run B (1:1)		Run C (3:1)		Run D (Ind)	
	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.
pH	6.51	0.41	5.79	0.35	6.55	0.27	6.96	0.30
Alkalinity*	157	35	169	34	146	17	120	41
TS	5416	614	6259	1364	5489	1383	6643	1320
VS	4284	553	4680	1329	3876	1177	6182	1296
TSS	4944	853	5470	1842	4298	1334	5764	1507
VSS	3946	698	5082	1733	4072	1263	5676	1473
VFA**	90	130	127	96	12	20	9	15
S-COD	366	110	410	149	158	88	124	66
T-COD	6703	1881	5698	1671	5087	2166	6413	1427
Ammonia-N	75.8	54.9	53.1	20.9	22.2	15.8	52.4	43.5
TKN	305.1	26.5	221.2	51.9	195.2	37.4	139.5	34.4

Note: All parameters except pH are in mg/L.
* - as $CaCO_3$,
** - as acetic acid.

The VFA, COD, and nitrogen parameters displayed greater variation. Rain and melt water dilution, infiltration, plant clean-up and maintenance schedules, plant equipment failure, and plant process performance will each affect these parameters. In addition, these natural variations were compounded by the TS variation described earlier.

5.1.1.3 Acclimation and Stability of Reactor Populations

The microbial population within the anaerobic reactor is in a constant state of flux. Individual microbes continually die and new individuals are continually formed. This applies to entire species groups as well. Their relative numbers are never static. As operational and environmental conditions within the reactor change, the population ecology changes with them. Certain anaerobic bacteria will favour these altered conditions and will be able to 'out-compete' other bacteria.

Once the microbial population has fully adjusted to changes in the reactor, it is said to be in a state of dynamic equilibrium. That is, the population continues to vary somewhat as individuals perish and are born, but the overall population proportions remain essentially the same. This state of dynamic equilibrium is the purpose of allowing a short acclimation period before run data is gathered.

Maharaj (1999) allowed for an acclimation period of two to six times the HRT which proved to be sufficient. Banerjee (1997) acclimated the reactor population for eight to ten days. It was noted that a fifteen day acclimation period would be satisfactory. Drawing upon these findings, this study employed a fourteen day acclimation period. As the following discussion illustrates, the results of this study provide no hesitation in claiming this period was insufficient.

In order to assess stability after the acclimation period has expired, a number of factors are considered. One of these factors is pH. When pH fluctuates no more than 20%, stability has likely been achieved. In this study, pH fluctuations ranged from 2.4 to 5.8%; well within the range of stability. Another important factor in assessing reactor stability is VFA content. VFA variation in

the reactors of runs A, B, and C were 25, 22, and 21%, respectively, indicating borderline stability. That of run D was 42%, indicating some instability.

pH is an important indicator in anaerobic digestion. Successful methanogenesis requires pH values near neutrality (6.8-7.2); thus the low pH values encountered during this study indicate the successful suppression of methanogenesis (Kayhanian and Tchobanoglous, 1992). Indeed, no gas production was observed during any of the runs. In addition, influent pH was always at least one pH point higher than that found in the reactor. This suggests that hydrolysis-acidogenesis was successful for all four runs.

Further support for the success of hydrolysis-acidogenesis comes from the net VFA values observed in the reactors. Net VFA values increased with increasing industrial content starting from 596 mg/L (as acetate) in run A, peaking at 725 mg/L in run B and 762 mg/L in run D. Since VFAs are the principle end product of acidogenesis, it is safe to assume that hydrolysis-acidogenesis was successful in each run (Andrews and Pearson, 1965).

Analyses such as these revealed that run D never reached a steady-state level. This failure was attributed to the difficulty in controlling SRT. The starch solids settled so well in the clarifier that the return sludge was extremely high in solids. By the time clarifier solids were sampled, manipulated for analysis, and analyzed, two or three days had passed. At this point, the SRT value calculated would be an underestimation since, in the interim, many more solids had been pumped back into the reactor. In this fashion, SRT tended to spiral to higher and higher values. To compensate, sludge wasted from the clarifier was estimated

leading to further uncertainty in the control of SRT. It was hoped this action might provide stability in the run. However, stability was not achieved.

5.1.2 Influence of Industrial-to-Municipal Feed Ratio

This investigation sought to identify the effect of varying the influent industrial-to-municipal wastewater ratio on the performance of anaerobic hydrolysis-acidogenesis. The first three runs (runs A, B, and C) were conducted under identical environmental and operational conditions with the exception of this influent feed composition. (Run D did not stabilize to steady-state conditions owing to the difficulty in controlling SRT. However, results gathered from this run can still be consulted with this limitation in mind). HRT and SRT were controlled at 30 hours and 10 days, respectively. Temperature was limited to 21.5 $\pm$ 1.5^{O}C. It should be noted in comparing findings of this study to those of Banerjee and Maharaj, the three studies share a common HRT of 30 hours. However, temperature in Banerjee's study averaged 22^{O}C, while that of Maharaj's study was 25^{O}C.

As indicated earlier, anaerobic bacteria are sensitive to these operational parameters. The reactor population will respond to these variations by shifting the proportions of its bacterial composition. For example, some bacteria can tolerate limited food availability and will therefore thrive under low HRT conditions, since those that cannot adapt to the limited food provided under low HRT will survive only in small numbers. The success or failure of these bacterial

populations will express themselves through measures such as pH, VFA production, VFA speciation, and VSS conversion to soluble COD (SCOD).

5.1.2.1 pH

Generally speaking, carbohydrates are more easily digested than lipids and especially proteins, as the work of Breure *et al.* (1986a, 1986b) has shown. Based on this logic, one would expect to see a greater degree of hydrolysis and acidogenesis in reactors fed with such influent mixtures. VFAs are the major end product of hydrolysis-acidogenesis, along with some alcohols and gases (Novaes, 1986). Therefore, a higher degree of hydrolysis-acidogenesis should be accompanied by a lower pH resulting from high acid concentrations.

This line of reasoning is supported by the pH patterns displayed during this study. Figure 5.1 shows the variation in pH over the course of the four runs. It seems that as the high-starch, industrial portion of the wastewater increased from 25% to 100% by volume, the pH dropped. Again, it should be noted that run D never attained steady-state operation.

5.1.2.2 Net VFA Production

All four runs successfully produced VFAs throughout their duration. This seems to indicate that VFA production is feasible for all industrial-to-municipal mixtures tested. However, run D showed a high degree of instability in this regard with an average variation of 42%. Instability of this magnitude may render starch-only wastewater impractical to anaerobic hydrolysis-acidogenesis under the conditions of this study. Table 5.3 displays the average net VFA

production for all four runs, as well as their average specific VFA production rates.

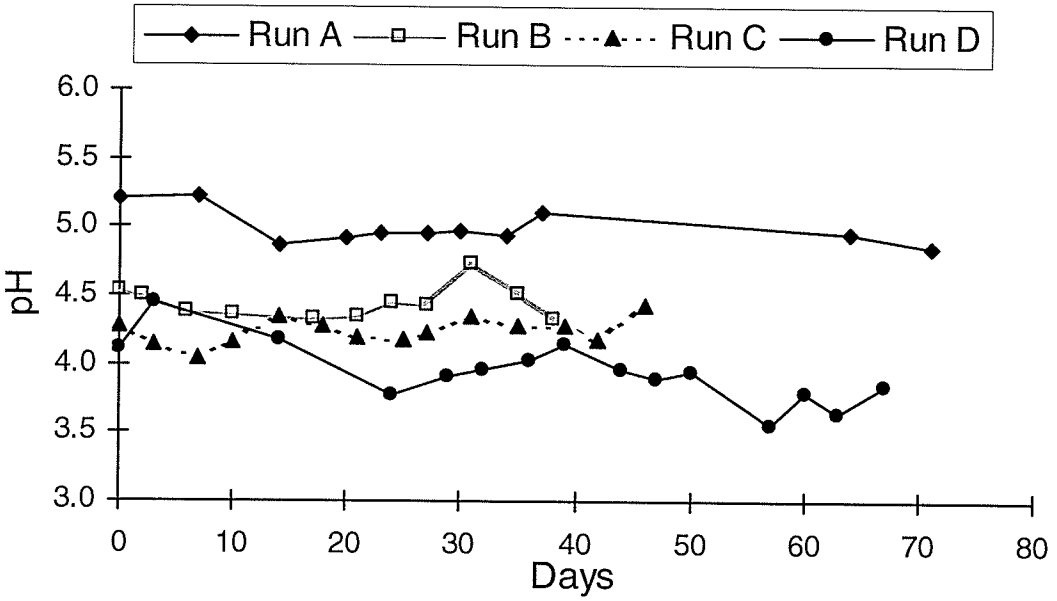


Figure 5.1: Reactor pH Variations for Runs A, B, C, and D

Table 5.3: Average Net VFA Production and Average Specific VFA Production Rates with Varying Industrial-to-Municipal Feed Ratios

Feed Ratio	Reactor VSS (mg/L)	Net VFA (mg/L as acetic acid)	Specific VFA Production Rate (mgVFA/mgVSS*d)
Mun. only*	13,631	408	0.030
1:3	6,467	594	0.065
1:1	8,686	800	0.069
3:1	7,379	690	0.069
Ind. only	39,707	784	0.015

* - Average of results from Banerjee (1997) and Maharaj (1999).

Figure 5.2 illustrates the net VFA production patterns of the four runs. The aforementioned instability of Run D is apparent in this figure. Net VFA production is only slightly greater than 200 mg/L as acetate at the beginning of the run. This production rises throughout run D to values greater than 1,200

mg/L by its completion. The fact that this rise is fairly gradual gives credit to the hypothesis presented earlier in which instability was caused by spiraling SRT.

The figure also shows that net VFA production seems to be stimulated by increasing the proportion of high-starch wastewater in the feed. Run A, which contains only 25% starch wastewater by volume, shows the lowest net VFA gains. Runs B and C improve on this production. In fact, the highest net VFA value of 1,543 mg/L (as acetate) was observed during run B and its value is labeled. (However, this value is well outside the remainder of the data for run B and is therefore treated as an outlier.) The net VFA production numbers for runs B and C are quite similar. Given the magnitude of their standard deviations, net VFA production of runs B and C are essentially equal.

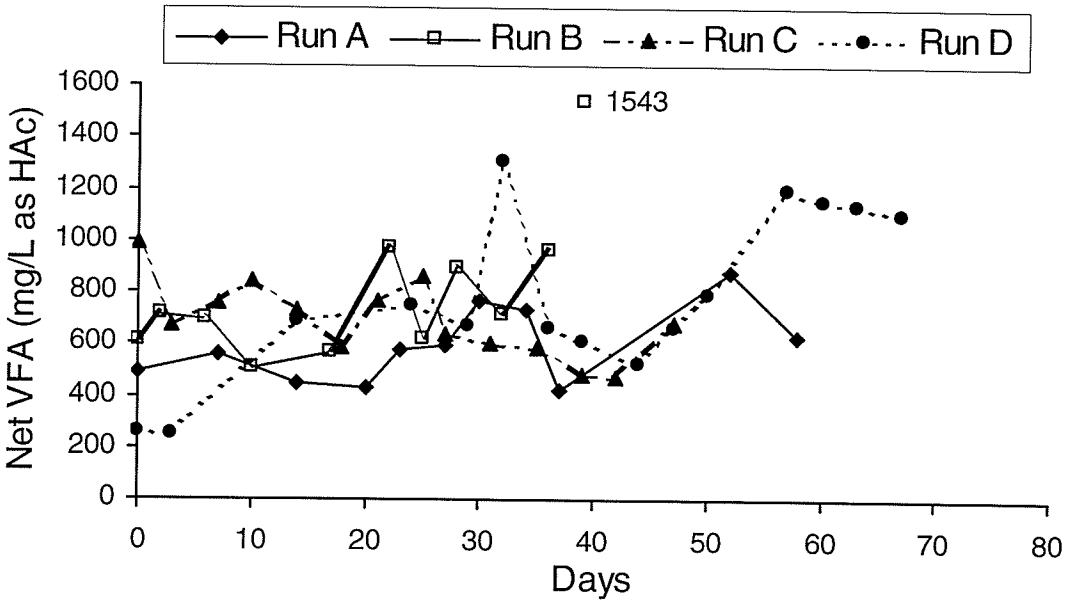


Figure 5.2: Variation in Net VFA Production for Runs A, B, C, and D

It seems that increasing the starch content of the feed stimulates increased VFA production. Figure 5.3 presents the average net VFA production from all four runs and the municipal-only data from Banerjee (1997) and Maharaj (1999).

The figure clearly shows that the municipal-only feed did not perform nearly as well as the other ratios in terms of VFA production. The municipal-only feed also resulted in a modest specific VFA production rate of 0.030 mgVFA/mgVSS*d. It seems clear that including at least some starch wastewater in the feed stimulates VFA production and VSS destruction.

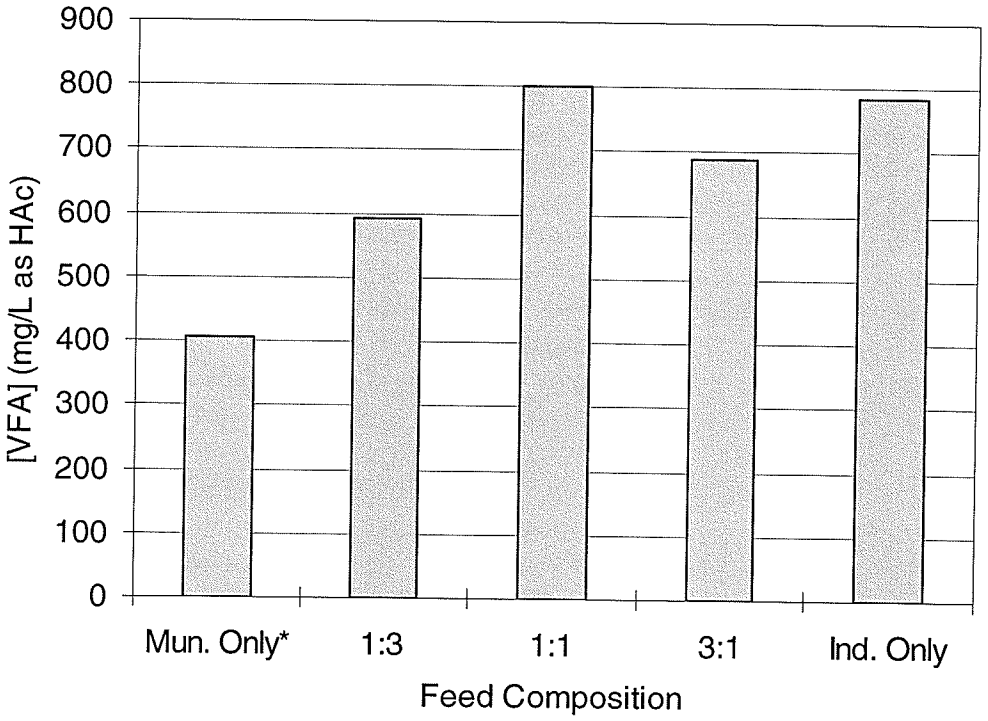


Figure 5.3: Average Net VFA Production as a Function of Varying Industrial-to-Municipal Feed Ratio
(* - Average of data from Banerjee [1997] and Maharaj [1999])

Figure 5.3 also illustrates a trend that leads one to believe that increasing the starch content of the feed yields greater VFA production. An optimum feed ratio appears to be the 1:1 feed ratio. (As noted earlier, the results of run D are unreliable. Therefore, this data should be treated with skepticism.)

Although VFA production during run D was very high at times, it also was low at others. As noted earlier, this run likely never achieved stable

operation. Even if steady-state conditions prevailed, Table 5.3 shows that the specific VFA production rate suffered compared to those of the other ratios. While ratios of 1:3, 1:1, and 3:1 boast rates approaching 0.07 mgVFA/mgVSS*day, run D fed with only starch wastewater only managed a rate of 0.015 mgVFA/mgVSS*d. That rate is well under that of even the municipal-only feed, which achieved 0.030 mgVFA/mgVSS*d. Hence, it appears that a starch-only feed is disadvantageous in terms of VSS destruction compared to the other feed mixtures under the given conditions.

The explanation for the higher net VFA production despite the low specific VFA production rate observed during run D lies in the VSS values. Reactor VSS content during run D was three to five times higher than those observed during all other runs. Thus, reactor microbes during run D had much more substrate to feed upon than their counterparts during the other runs. Also, this substrate-rich environment probably supported an elevated microbial population. Thanks to elevated VSS values, run D saw the maximum net VFA production observed during this study.

Unfortunately, stability and production rates were compromised to achieve such performance. The feed of run D may have supported a large population due to abundant substrate, but it also selected bacteria best able to digest starch. This latter feature created a less diverse population than those observed in runs A, B, and C, or by Banerjee (1997) and Maharaj (1999). The municipal component of the wastewater feed is much more complex and therefore promotes the growth of many microbial populations in the same reactor. This likely accounts for the more stable VFA production observed during the

other runs as compared to run D. Referring to Figure 5.2, there were no significant jumps or crashes in VFA production of the first three runs, unlike run D. These patterns likely reflect the population dynamics within the reactors.

5.1.2.3 VFA Speciation

Samples were analyzed not only for their overall VFA content, but also for their content of individual VFA compounds, since the presence or absence of these compounds reveals much about the prevailing metabolic pathways of digestion. The various VFAs that were identified were acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid, and n-valeric acid.

Figure 5.4 illustrates the composition of reactor VFAs for all four runs plus those found by Banerjee (1997) and Maharaj (1999) for municipal-only feeds. Some patterns are apparent from an examination of this figure. First, it is obvious that acetic acid is the dominant VFA product for all industrial-to-municipal feed mixtures. However, its dominance diminishes with increasing industrial content in the feed. The compound which seems to make up for this loss in acetate is n-butyric acid. Examining Figure 5.4 reveals a near jigsaw fit between the acetate and n-butyrate profiles over the spectrum of influent ratios. Municipal-only feed resulted in almost no n-butyrate production. N-Butyrate production jumped to 18 and 22% when 25 and 50% starch wastewater were fed to the reactor, respectively. Production of n-butyrate nearly doubled as starch content increased to 75 and 100% of the feed. N-Butyrate levels rose to 37 and 34% respectively at these ratios.

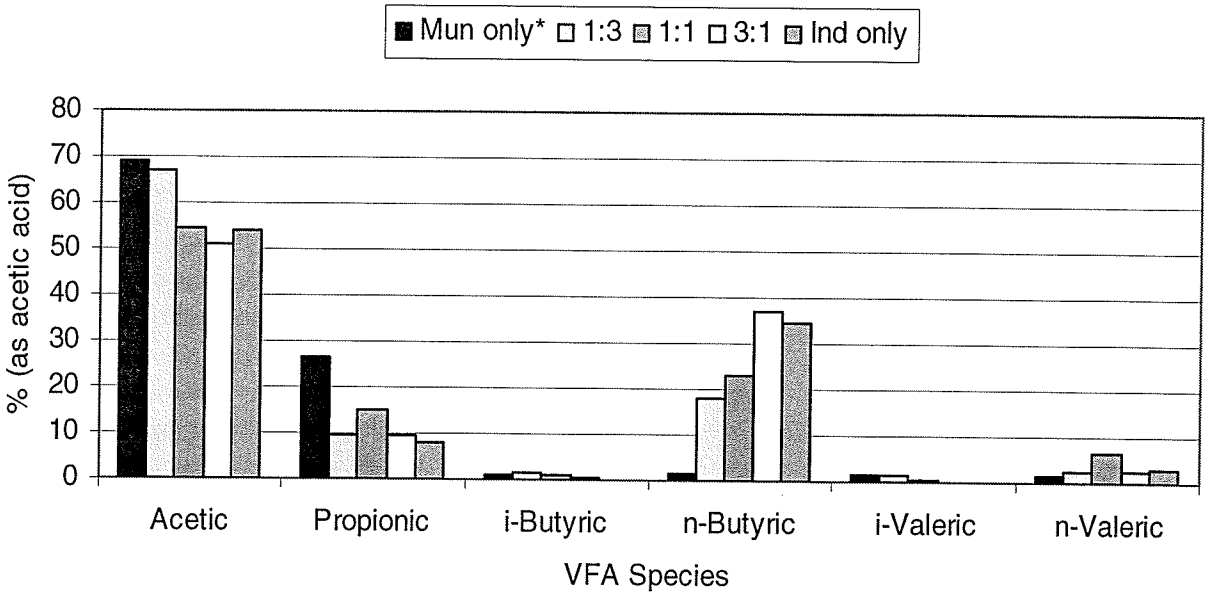


Figure 5.4: Change in VFA Distribution as a Function of Varying Industrial-to-Municipal Feed Ratio
(* - Average of data from Banerjee [1997] and Maharaj [1999])

Patterns observed by Banerjee (1997) and Maharaj (1999) with respect to propionic acid production were observed again during this study. They saw propionic levels decrease as the starch content of the feed increased. During this study, propionate content rose from 9% during run A to 15% during run B. After this gain, propionic acid content fell back to 9 and 8% during runs C and D, respectively. Despite this modest increase at the 1:1 ratio, it seems safe to suggest that propionate levels tend to be suppressed with the addition of starch carbohydrates as a substrate.

The only other VFA compound that appeared in appreciable concentrations was n-valeric acid. Municipal-only feed to the reactors yielded very little n-valerate production (1%). Runs A, C, and D saw n-valerate numbers of 3 to 4%. N-Valerate comprised 7% of the total during run B. Judging from

these observations, n-valeric acid production seems to increase as the starch content of the feed increases to 25 and 50%. However, after this level is reached, n-valerate production plateaus near 5%. Given the small production and relatively large variation between n-valerate numbers, it is difficult to draw general conclusions with confidence, however.

5.1.2.4 Metabolism of VFA Production

It appears that altering the industrial-to-municipal feed ratio does have an impact on the degradation pathways employed by the anaerobic bacteria performing hydrolysis-acidogenesis. When no starch feed is included, acetic and propionic acids are the favoured end products. As starch wastewater is fed, the predominance of acetic acid drops somewhat, while propionic acid levels fall sharply. N-Butyric acid levels rise quickly with rising starch content, indicating metabolic pathways that lead to the production of this acid are in use.

An overview of the anaerobic bacterial metabolism of carbohydrates, proteins, and lipids was presented in section 2.1.4. Recall that the digestion of each of these compounds yields acetic acid. It is therefore not surprising that acetic acid is the predominant VFA compound produced from all feed mixtures.

Why was an increase in n-butyric acid observed as starch content increased in the feed? Gottschalk (1986) states that butyrate fermentation is one of most energy efficient uses of glucose, yielding 3 ATP per glucose molecule. Such metabolic activity might explain the increase in n-butyrate with starch feed content observed during this study and during those of Banerjee (1997) and Maharaj (1999). In addition, butyrate and acetate production are often

coincidental in mixed bacterial cultures (Gottschalk, 1986). Gottschalk presents fermentation mixtures of *Clostridium butyricum, C. perfringens,* and *C. acetobutylicum* showing that acetate and butyrate are produced in equal amounts and are the sole VFAs produced. Perhaps the increase in starch content encourages the growth of such bacterial cultures that do not produce propionic acid, which might account for the diminished propionate content.

Recall from section 2.1.4 that lactic acid is the preferred substrate of propionate-producing bacteria according to the following reaction (Gottschalk, 1986):

$$3 \text{ lactate} \rightarrow 2 \text{ propionate} + \text{acetate} + CO_2$$

Lactic acid bacteria have complex nutritional requirements which must be provided for by their environments. An example of such an environment suggested by Gottschalk is the animal intestinal tract. Municipal wastewaters likely contain such bacteria and the substrates these bacteria require. As the starch component of the feed increases, the nutritional composition becomes more simplified, possibly leaving the nutritional requirements of lactic acid bacteria unsatisfied. Thus, lactate production would drop, thereby dropping propionic acid production drastically and acetic acid production to a lesser degree.

Although the situation is likely a result of a number of interactions, this explanation does match the observations of this study and the studies of Banerjee (1997) and Maharaj (1999). In all three cases, increasing starch content was met with a significant decrease in propionate and a modest drop in acetate. Further

study dedicated to monitoring lactate content as well as VFA content is required to confirm or disprove this hypothesis.

Data presented in the previous sections revealed that very little iso-butyric, iso-valeric, or n-valeric acids were produced, regardless of the influent composition. Elsden and Hilton (1978) report that these compounds are normally associated with protein digestion. The obvious implication is that these feed mixtures contained little protein, or protein digestion was inhibited somehow. Recall that Breure *et al.* demonstrated carbohydrate inhibition of protein digestion (1986a, 1986b). However, these compounds were observed at their lowest levels even when no starch wastewater was added to the feed. It is still possible that carbohydrates in the municipal wastewater could be inhibitory to protein decay.

5.1.2.5 Hydrolysis Performance

The ability of the bacterial population to convert large, insoluble macromolecules into monomeric, soluble molecules is alternately called hydrolysis, solubilization, and liquefaction. The relative success of this faculty can be measured through SCOD (soluble COD) values. Average net SCOD values are presented in Figure 5.5.

The results shown in Figure 5.5 demonstrate that increasing the starch content of the feed improves the degree of solubilization in the reactor. Municipal-only feed yielded 654 mg/L SCOD, which was approximately half of the 1,203 mg/L SCOD produced when 25% starch was added to the wastewater. Increasing starch content beyond 25% seemed to have little effect. Substrate solubilization actually decreased when starch content reached 50%, only to rise

again as starch content equaled 75%. However, the variation between the SCOD levels observed at the 1:3, 1:1, and 3:1 ratios was only approximately ± 100 mg/L. In other words, these production levels only vary slightly from a statistical vantage. A continuation of this plateau was observed during run D with the industrial-only feed.

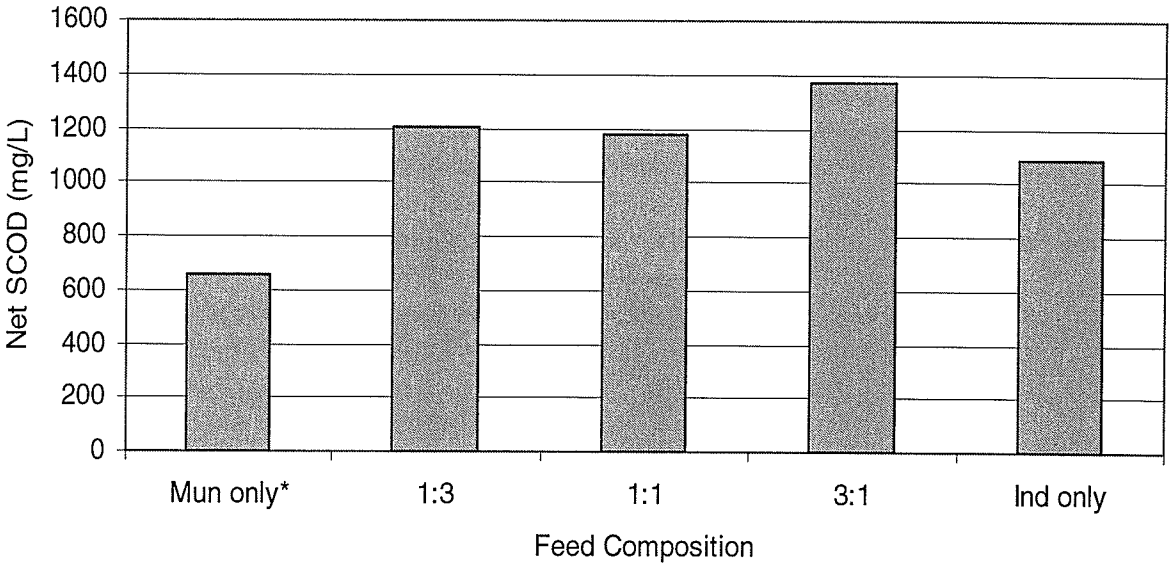


Figure 5.5: Average Net SCOD Production as a Function of Varying Industrial-to-Municipal Feed Ratio
(* - Average of data from Banerjee [1997] and Maharaj [1999])

Similar findings to those mentioned above in terms of specific SCOD production rates were observed. Table 5.4 presents these findings. A low production rate was observed when municipal-only feed was in use as compared to feeds containing a municipal/industrial mixture. There was a small drop in value when starch content reached 50%. A similar drop was observed with respect to net SCOD production; however, its value was too small to be significant. In the case of production rates however, this drop is larger. To be

certain this drop is a result of a change in starch content, further investigation is required.

Table 5.4: Average Specific SCOD Production Rates over the Spectrum of Industrial-to-Municipal Feed Ratios

Feed Ratio	Avg. Net SCOD (mg/L)	Specific SCOD Production Rate (mgSCOD/mgVSS*d)
Mun. only*	654	0.048
1:3	1,203	0.132
1:1	1,180	0.102
3:1	1,375	0.137
Ind. Only	1,085	0.021

* - Average of data from Banerjee (1997) and Maharaj (1999).

The trend revealed by the VSS destruction data of Table 5.5 is somewhat peculiar. In this case, the 1:1 ratio showed the best hydrolysis performance, destroying an average of 79.2% of VSS despite the lower SCOD production rate. This significant difference may be due, in part, to the higher reactor VSS values observed at this ratio. Run B (1:1) boasted the highest reactor VSS values, apart from run D (industrial only feed). It is likely that this abundance of substrate at the 1:1 ratio accounts for the higher VSS destruction despite the low SCOD production rates. Therefore, the reliability of this finding; that VSS destruction is a maximum at the 1:1 ratio, is dubious and deserves further research.

Table 5.5: Average VSS Destruction at the Feed Ratios

Feed Ratio	VSS Destruction (%)
Mun. only*	55.9
1:3	65.9
1:1	79.2
3:1	67.3
Ind. only	61.9

* - Average of data from Banerjee (1997) and Maharaj (1999).

A finding consistent between net SCOD production, specific SCOD production rates, and VSS destruction is the drastic decline of all these parameters when starch makes up the entire feed. Very low production, production rates, and VSS destruction are observed in this case. Recall Borzacconi *et al.* (1997) who warned that anaerobic digestion of "easily putrescible waste" could have an inhibitory effect on the acidogenic phase. Perhaps the situation described by the authors is similar to that observed with the easily-degraded, starch-only feed. The extremely low pH, VFA production rates, SCOD production rates, and VSS destruction provide compelling evidence in support of this idea.

5.1.2.6 Acidogenesis Performance

After having examined hydrolysis performance in terms of SCOD production, it is useful to analyze acidogenesis performance. This stage can be assessed by determining the VFA-to-SCOD ratio. This measure indicates the proportion of SCOD represented by VFAs and thereby reveals the performance of acidogenic bacteria that facilitate this conversion. The results of this analysis are provided in Table 5.6.

Table 5.6: VFA-to-SCOD Ratio over the Spectrum of Feed Ratios

Feed Ratio	Net VFA (mg/L as COD)	Net SCOD (mg/L)	VFA:SCOD (%)
Mun. only*	435	654	66.5
1:3	634	1,230	51.5
1:1	854	1,180	72.4
3:1	736	1,375	53.5
Ind. only	837	1,085	77.1

* - Average of data from Banerjee (1997) and Maharaj (1999).

Table 5.6 seems to indicate that acidogenic performance is optimized at municipal-only, 1:1, and starch-only feed ratios. It is interesting to note that acidogenic performance appears to be at a maximum with the starch-only feed. As the previous section showed, rates of hydrolysis were at a minimum under this influent condition. The predominance of acidogenic bacterial populations when starch is the sole substrate likely resulted in the high proportion of VFAs in the SCOD.

5.1.2.7 Net Ammonia Production

Ammonia is a major end product of protein fermentation. Hence, its presence is an indicator of the relative protein content of the environment from which a sample is drawn. Ammonia is released into the reactor liquor during deamination as individual amino acids are stripped of amino (NH_2) groups. Figure 5.6 displays net ammonia production as a function of increasing the starch content of the feed.

As this figure shows, ammonia tended to disappear for influent mixtures of 50% starch wastewater and greater. The mechanism of ammonia disappearance is not clear. It could be due to adsorption on starch molecules. However, it is likely anaerobic bacteria assimilated some free ammonia as a nitrogen source. Of course, bacteria still consume ammonia during the other runs as well. However, there seems to be an abundance of ammonia when these influent mixtures are fed.

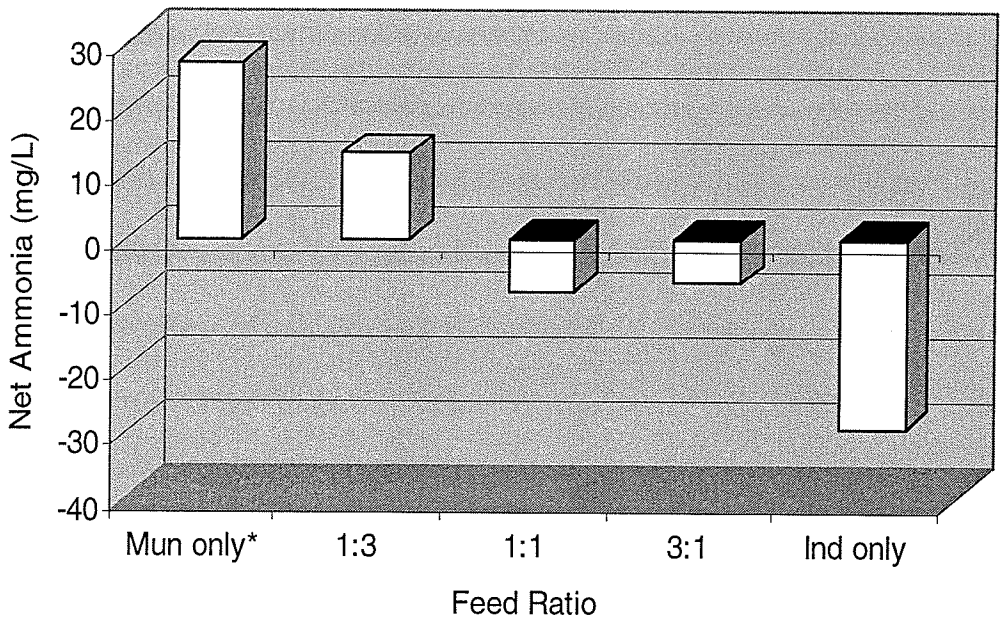


Figure 5.6: Average Net Ammonia Production Versus Feed Ratio
(* - Average of data from Banerjee [1997] and Maharaj [1999])

It would seem that influent mixtures high in municipal wastewater provide more ammonia-nitrogen than the bacteria require. This finding is not surprising since the protein content of the feed decreased as the starch content increased. In other words, more protein was fed to the reactors when more municipal wastewater was in the feed. Net ammonia content and production rates are summarized in Table 5.7.

An additional factor explaining the lack of ammonia in 50% and greater starch ratios is provided by Breure *et al.* (1986a, 1986b). These studies demonstrated that protein degradation is inhibited as carbohydrates are fed to anaerobic bacterial cultures. In the extreme case where 100% starch wastewater

was fed to the bacterial culture in the reactor, VFA production was observed to peak and fall possibly reflecting the nitrogen-starved condition of the reactor. Bacterial populations would fall, at which time remaining individuals received nitrogen from the dead bacteria. This nitrogen release would spur further starch digestion reflected in rising VFA production until nitrogen once again became a limiting nutrient.

Table 5.7: Net Ammonia Production and Specific Ammonia Production Rates at the Five Feed Ratios

Feed Ratio	Net Ammonia Production (mg/L)	Specific Ammonia Production Rate ($mgNH_3$-N/mgVSS*d)
Mun. only*	27.2	0.00200
1:3	13.2	0.00145
1:1	-8.0	-0.00069
3:1	-6.7	-0.00067
Ind. only	-29.2	-0.00057

* - Average of data from Banerjee (1997) and Maharaj (1999).

It is likely this effect would produce a high degree of variation in reactor VSS values. This study did observe this effect; however, difficulties in controlling SRT likely contributed to this observation. As a result, it is uncertain whether the high VSS variation was affected by the phenomenon of soaring and crashing bacterial populations.

5.1.3 General Commentary

In the previous section, the collected data was analyzed to highlight major trends and draw general conclusions. In this section, data will be examined to allow comment on the performance of the anaerobic systems. Commentary regarding TCOD and TKN conservation and ease of replication will be more quantitative in

nature; while that describing clarifier performance, gas production, and potential applications will be more qualitative.

5.1.3.1 TKN and TCOD Conservation

TKN and total COD (TCOD) are grouped together since both of these parameters should be conserved in acidogenic systems if steady-state exists and gases are not produced. Performing TKN and TCOD mass balances will reveal any inconsistencies in this respect.

The anaerobic systems employed in this study are fed a certain amount of nitrogen in the influent. Some of this nitrogen is assimilated into reactor bacteria, while some will escape in the effluent. This nitrogen will exist in many forms, all of which should be organic and therefore, detected by TKN analysis. One major exception is nitrogen gas which will be liberated if denitrification takes place in the systems. However, the low pH values observed in these systems all but precludes this possibility. By performing TKN analysis of influent, reactor, and effluent samples, the change in TKN in the systems was monitored. These findings are presented in Table 5.8.

Table 5.8: TKN Flux During Runs A, B, C, and D

Run	TKN Reduction (%)
A	33.8
B	19.3
C	-10.3
D	33.3

TKN flux for runs B and C was under 20%, which is considered acceptable. Flux for run D was, not surprisingly, well over 20% since this run never reached steady-state operation as mentioned before. However, the finding

that run A saw a 33.8% TKN reduction is surprising. There was a significant loss of clarifier solids early in run A which would have allowed some TKN loss. Perhaps this event is responsible for the significant TKN reduction. However, it is more likely that the mathematical assumption of absolute steady-state deserves most of the blame.

Like TKN, TCOD should be conserved throughout the anaerobic systems. If there is significant CO_2 or CH_4 gas production, this assumption no longer holds; however, these systems witnessed only very minor gas production. The results of the TCOD mass balance are presented in Table 5.9.

Table 5.9: TCOD Flux During Runs A, B, C, and D

Run	TCOD Reduction (%)
A	50.4
B	12.9
C	-1.5
D	31.8

The results of Table 5.9 are quite similar to those of Table 5.8. Runs B and C showed good TCOD and TKN conservation, while those of runs A and D are poor.

5.1.3.2 Gas Production

Many of the metabolic pathways of anaerobic digestion see the production of gases, principally carbon dioxide. However, hydrogen gas should also be produced in significant quantities according to the pathways. Hydrogen gas is also a major substrate of homoacidogenic bacteria and its presence enhances acetate production (Boone, 1982). Production of small amounts of nitrogen gas

can also be expected due to denitrification. Methane gas has also been observed during the hydrolysis-acidogenesis phase of anaerobic digestion (Ghosh, 1987).

This study saw very small amounts of biogas produced during any of the runs. Both nitrogen and methane gases were not anticipated as these gases are the end products of processes that were suppressed. Hydrogen gas, if it was produced to any degree, was expected to be consumed. The negligible gas production of this study and others justify this expectation (Banerjee, 1997; Maharaj, 1999). It is somewhat surprising that very little carbon dioxide gas was produced. It is possible that small amounts of soluble CO_2 escaped with the liquid effluent; however, it is most likely that the gas collection apparatus leaked and most CO_2 produced was lost to the atmosphere.

5.1.3.3 Clarifier Performance

Clarifier performance will have an important impact on the overall performance of biological digestion systems. Efficient recycling of acclimated biomass and prevention of solids escape through the effluent are the primary goals of clarification. These aims can be better achieved if good settling is achieved. For the most part, clarifier performance of this study was excellent. However, the clarifier of run A experienced bulking and solids escape. This clarifier also experienced a major overflow event caused by solids bulking and subsequent plugging of the effluent line. Never during any of the other runs did such an event take place. The excellent clarifier performances of runs C and D, as well as the poorer performance of run A are reflected in the effluent solids concentrations displayed in Table 5.10. Although clarifier B never had bulking or overflow

problems, its performance was roughly equal to that of clarifier A when the effluent numbers are compared.

Table 5.10: VSS-to-TS and TSS-to-TS Ratios for Run A, B, C, and D Effluents

Run	VSS (mg/L)	TSS (mg/L)	TS (mg/L)	VSS/TS	TSS/TS
A	1587	2398	3653	0.434	0.656
B	1654	1916	3052	0.542	0.628
C	1125	1770	3158	0.356	0.560
D	925	1004	2948	0.314	0.341

Comparing the VSS/TS ratios between runs A and B seem to indicate that clarifier A outperformed clarifier B. However, it should be noted that this anomaly is due to the nature of the solids. Run A had more inert solids than run B, which suppressed its effluent VSS values. Thus, the VSS/TS ratio appears lower than that of run B. However, more of these inert solids will pass through the effluent in run A than in run B. An examination of the TSS/TS ratio for these runs quantifies this phenomenon. The effluent TSS/TS ratio for run A is 0.656 and is much higher than its corresponding VSS/TS ratio of 0.434. The effluent TSS/TS ratio for run B is 0.628 and is only slightly higher than its VSS/TS ratio of 0.542. This comparison shows that the clarifier performances of runs A and B are roughly identical in terms of solids escape. The TSS/TS ratios also show that clarifiers C and D performed better than those of A and B.

The scum layer of clarifier A was thick and dense. It also had a foamy portion on its underside. This foam consisted largely of gas bubbles indicating that there may have been methanogenesis taking place in clarifier A. Maharaj (1999) noted the same phenomenon in clarifiers during municipal-only feed runs. Since these descriptions are qualitative, it is difficult to say if the 25% starch

content of run A resulted in clarifier performance improvement over municipal-only feeds.

It was observed that clarifier performance improved as the starch content of the feed increased. In fact, this improvement was so drastic that when the feed consisted of only starch, the solids content of the reactor spiraled out of control due to the extremely high return sludge solids concentration. The bulking and overflow troubles of run A disappeared once starch content reached 50% in run B. Runs C and D showed excellent settling characteristics in the clarifiers as well. However, run C, with 75% starch feed seems to be the optimal ratio when clarifier performance is the concern.

5.1.3.4 Feasibility of Replication

In order to ensure the universality of the results of this study, a comparison between the two anaerobic systems employed was undertaken. Run B was conducted on a different system than runs A, C, and D. However, these runs cannot be compared because different influent treatments become a second variable. A preliminary run called run 1 was conducted at the 1:1 ratio on the other system from run B. Therefore, these runs could be compared to determine if there is a significant difference between the results gathered from the two systems.

According to t-test analysis (Moore, 1995), there was no significant difference between the results of the two systems in terms of VFA production rate, TSS destruction rate, or VSS destruction rate at a 95% confidence interval. Thus, it is safe to extrapolate from this finding and make the assumption that the

results gathered over the course of this study are reproducible. A summary of the findings of the t-test are given in Table 5.11.

Unfortunately, it was not possible to statistically compare the results within the same system since no runs were repeated on the same system. However, Maharaj (1999) did perform this comparison on the same equipment used in this study and determined no difference in VFA and SCOD production rates. However, she did note a difference in terms of TSS and VSS destruction. Maharaj attributed these apparent differences to the fact that one of the runs had not reached steady state until quite some time had passed.

Table 5.11: Results of the t-Tests Comparing Runs 1 and B

System, Run	VFA Prod. Rate (mgVFA/mgVSS*d)		VSS Destruction (%)		TSS Destruction (%)	
	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
1, 1	0.054	0.179	74.2	18.9	74.2	20.1
2, B	0.070	0.159	79.2	11.0	77.7	11.3
Significant Difference?	No		No		No	

Throughout this study, reference has been made to studies conducted by Banerjee (1997) and Maharaj (1999). In order to ensure these studies are compatible with this study, statistical comparisons (t-tests) of the VFA production, VFA production rate, and percent VSS destruction results from the studies were performed. The three studies employed nearly identical control parameters, as summarised in Table 5.12 below.

Table 5.12: Control Parameters of Runs from Banerjee's, Maharaj's, and this Study Chosen for Statistical Comparison

Study	System	Run	HRT (hrs)	Temp. (^{O}C)	Feed Ratio
Banerjee (1997)	1	3	30	22	1:1
Maharaj (1999)	A	1	30	25	1:1
This Study	2	B	30	21	1:1

As Table 5.12 indicates, the control parameters for these three runs are nearly identical, thereby providing an excellent comparison between the three studies.

The results of these runs were compared and are summarized in Table 5.13 below. Table 5.13 also indicates that the statistical analyses revealed no difference between the results. As a result, comparisons between the three studies can be made with confidence and the feasibility of repeating these types of studies is enhanced.

Table 5.13: Results of the t-Tests comparing the results of Banerjee's and Maharaj's studies with this study

Study, System, Run*	Net VFA Production (mg/L)		VFA Production Rate (mg VFA/mg VSS*d)		% VSS Destruction	
	Mean	St. dev	Mean	St. dev.	Mean	St. dev.
B, 1, 3	783	145	0.041	0.008	77.0	9.3
M, A, 1	769	131	0.045	0.014	74.6	7.6
S, 2, B	725	163	0.070	0.159	79.2	11.0
Significant Difference?	No		No		No	

* - B = Banerjee (1997), M = Maharaj (1999), S = This Study.

Maharaj (1999) also demonstrated that there was no statistical difference between the results of her study and those obtained by Banerjee two years earlier. This finding further supports the repeatability and compatibility of the three studies.

5.1.3.5 Limitations

When considering the results presented in this section, it is important to bear certain limitations in mind. Possibly most limiting is the fact that this study did

not conduct a run fed with 100% municipal feed. This proved to be a difficult run in previous studies. Pumps and clarifiers often clogged resulting in overflows and added variability in the data. For this reason, and the fact that two previous studies had gathered data for this feed mixture, a 100% municipal run was not conducted.

It should also be noted that this study relies heavily on the results of studies conducted by Banerjee (1997) and Maharaj (1999). This was intentional. This study was intended to explore a range of feed mixtures that were not addressed by the previous two studies. However, the apparatus and the optimal HRT and SRT revealed in the previous two studies were adopted, thereby allowing direct comparison between the three studies for runs of nearly equal temperature. Nonetheless, the reader should remember that some of the data were generated elsewhere.

5.1.3.6 Engineering Significance

VFA production and optimization has become an important research topic because the economic and environmental benefits of applying this research have enormous potential. On-site production of VFAs through anaerobic digestion of sludge waste is economical since it performs two services at once. On one hand, anaerobic digestion will stabilize the putrescent sludge. Harnessing methane gas or marketing stabilized sludge as a soil conditioner may realize further economic benefit. On the other hand, VFAs may be produced in such a quantity to provide a compatible carbon source for nutrient removal. One such study by Brinch *et al.* (1994) explored the demanding situation where existing plants are to be upgraded

to perform nutrient removal. This is an especially challenging situation since the plant cannot physically expand without an interruption of service and significant capital expense. The study concludes that such on-site production of VFAs for phosphorous and nitrogen removal is "a most viable process." Many other studies confirm the viability of VFAs for nutrient removal. Therefore, the findings of this study should be of interest to communities that wish to commence, enhance, or resume nutrient removal programs. Cheap production of renewable and plentiful VFAs will reduce the operating costs of plants that rely upon methanol or other carbon outside sources.

This study has shown that VFA production can be greatly enhanced through the addition of starch wastewater into an anaerobic municipal wastewater digester. Net VFA production is nearly doubled with industrial-to-municipal ratios as modest as 1:3 compared to municipal-only feeds, with a 1:1 ratio as the optimum. Carbon destruction is also increased as starch-rich wastewater increases from 0 to 50% of the feed mixture. These findings could be especially important to communities such as Portage la Prairie, Manitoba in which a significant portion of the wastewater comes from potato-processing industries. They may also be reassuring to communities in which potato-processing industries are expanding.

5.2 Investigation of Denitrification with Fermented VFA Effluent as Carbon Source

A total of eight denitrification batch test runs were carried out over the course of this study. Each run consisted of either five or six batch reactors under various initial conditions. Of the eight runs, the first five were unsuccessful due to the interference of volatile fatty acid (VFA) production of the seed. VFAs produced in the reaction vessel masked VFA consumption by denitrifying bacteria during these runs. Also, the first four runs were not successful because the rate of denitrification outpaced the initial sampling schedule. By the fifth run, an effective seed protocol and sampling schedule were developed, which allowed for more rewarding exploration over the final three runs.

It was determined that tracking changes in VFA concentration over the course of the runs would provide unreliable kinetic results. It appeared that additional simultaneous carbon consumption occurred during denitrification. However, it was also determined that no appreciable soluble organic carbon was produced during the last two runs. As a result, only nitrate disappearance was used to calculate denitrification kinetics. The aforementioned carbon limitations should be considered when interpreting C:N data as well.

Other quantitative observations supported the conclusion that denitrification was taking place. Both pH and alkalinity increased over the course of the runs; a finding consistent with the chemistry presented in Chapter 2. Most runs also showed nitrate values eventually reached zero again indicating a healthy

denitrifying population. This population also exhibited growth reflected in an increase in VSS values over the course of the runs.

5.2.1 Initial Conditions

As shown in Table 4.2, significant amounts of nitrogen in the forms of ammonia and TKN are present in the anaerobic digester effluent, which serves as the VFA source. However, these compounds should have little effect on denitrification. There are no nitrates in this source and no conversion of ammonia to nitrate or nitrite by nitrification can take place due to the anoxic conditions and short SRT. Second, the table shows that there is much more organic carbon available as SCOD in addition to VFA. Therefore, the actual C:N ratio will be somewhat higher than that predicted based solely on VFA content. This effect will only be important when considering reactors to which a significant amount of fermented effluent has been added.

5.2.2 Preliminary Runs

The first five runs were largely unsuccessful in terms of gathering useful kinetic data. However, they provided invaluable insight into the limitations of the seed and the sampling period. These insights were incorporated into the final seed protocol and sampling schedule which are described in full detail in sections 4.3.2.1 and 4.3.2.2, respectively.

In order to ensure nitrates were being biologically removed through denitrification, it was necessary to provide proof that nitrates were not

disappearing through other means. Nitrates could be removed from the reaction flasks by adsorption of the glassware or through volatilization. Reactor 5 of Run 3 provided proof to discount these mechanisms. Only tap water and 90 mg/L KNO_3 were included in the flask. As shown in Figure 5.7, nitrate levels remained at approximately 90 mg/L, indicating that no other nitrate consumption mechanism was present.

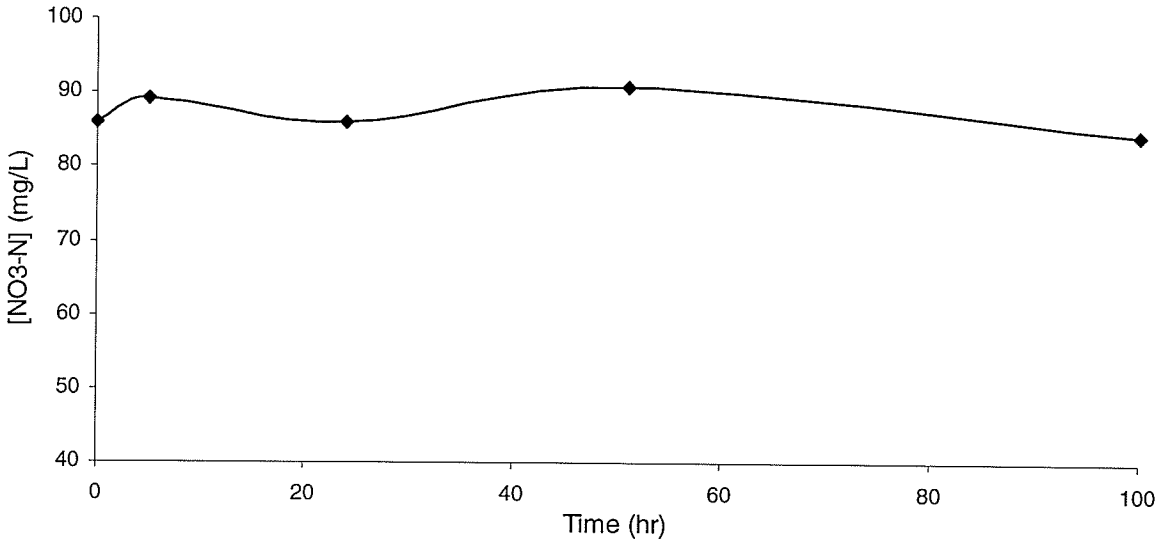


Figure 5.7: Persistence of Nitrates in the Presence of No Seed

5.2.3 Kinetics of Denitrification

Data applicable for the calculation of kinetic rate constants were obtained during Runs 6, 7, and 8. A variety of initial nitrate concentrations, VFA concentrations, and C:N ratios were tested. It was found that all runs proceeded under zero-order conditions. The organic carbon source was normally VFAs added to the flasks; however, in certain runs, little or no organic carbon was added. In these cases,

denitrification still took place. Table 5.14 provides a summary of the kinetic rate constants on a reactor-by-reactor basis.

Table 5.14: Initial Reactant Concentrations and Kinetic Rate Constants for the Reactors of Runs 6, 7, and 8

Run	Reactor	$[NO_3\text{-}N]_{initial}$ (mg/L)	$[VFA\text{-}C]_{initial}$ (mg/L)	C:N	k_0 (mg/[L*hr])
6	1	50.8	183.5	3.61	1.89
	2	60.6	245.7	4.05	2.20
	3	111.7	345.5	3.09	2.32
	4	107.2	522.8	4.88	1.76
	5	202.8	441.0	2.17	3.13
	6	204.3	539.4	2.64	n/a
7	1	0.0	143.6	n/a	n/a
	2	53.1	18.1	0.34	2.43
	3	53.5	234.9	4.39	1.54
	4	117.8	180.2	1.53	3.92
	5	116.8	391.6	3.35	2.21
	6	215.5	156.5	0.73	6.30
8	1	0.0	174.0	n/a	n/a
	2	13.1	30.3	2.31	0.53
	3	53.2	34.7	0.65	2.33
	4	106.4	41.3	0.39	4.60
	5	225.2	30.0	0.13	5.93
	6	113.8	369.0	3.24	2.02

5.2.3.1 Effect of Initial Nitrate Concentration

A quick survey of Table 5.14 shows that there is a high degree of variability between the zero-order kinetics rate constants. Reactors operated with seemingly similar initial conditions produced quite different kinetic rate constants. The source of this variability likely lies with the seed. The other flask ingredients: the nitrate solution, the fermented VFA effluent, and tap water, should not produce a great deal of variability between the different reactors. The remaining ingredient, the seed, has been seen to vary quite significantly depending on its preparation. It is very likely that other differences at the source will have a significant impact on

the characteristics of the seed. These differences could include plant SRT, pH, and temperature, among many others. Variation of this kind is beyond the control of this researcher and is therefore inherent in research of this nature. It is therefore more appropriate to compare results within a run, rather than between runs.

However, it can also be seen that this variability seems to follow certain general trends. One of these trends can be matched to initial nitrate concentration. During Run 6, the maximum rate constant was 3.13 mg/L*hr. This value corresponded to an initial nitrate concentration of 202.8 mg/L. Again in Runs 7 and 8, the maximum rate constants were observed when initial nitrate concentration was also a maximum. The values of these maximum rate constants were not very close, but the fact that all the maxima were observed in conjunction with maximum nitrate concentrations is significant. The converse of this finding can also be seen for reactor 2 of Run 8. This reactor was operated with an initial nitrate concentration of only 13.1 mg/L. This minimum concentration yielded the minimum observed kinetic rate constant of 0.53 mg/L*hr. This profile corresponding to initial nitrate concentration is shown clearly in Figure 5.8.

Judging by Figure 5.8, it would appear that the initial nitrate concentration is the controlling factor for denitrification kinetics. However, further scrutiny dispels this conclusion. The other initial nitrate concentrations tested were approximately 50 and 100 mg/L. Fortunately, denitrification performance with these concentrations can be compared within runs. Within Run 6, reactors 3 and 4 were operated with approximately identical initial nitrate concentrations of 111.7 and 107.2 mg/L, respectively. Despite near equality of nitrate conditions,

the kinetic rate constants of these reactors were not. The k_0 value for reactor 3 was 2.32 mg/L*hr, while that of reactor 4 was 1.76 mg/L*hr. Indeed, this inequality of rate constants was observed for all reactors operated with near-equal initial nitrate concentrations. Obviously, other factors affect denitrification kinetics besides initial nitrate concentration. In fact, USEPA (1993) indicate that nitrate concentration will have no effect on suspended-growth denitrification rates.

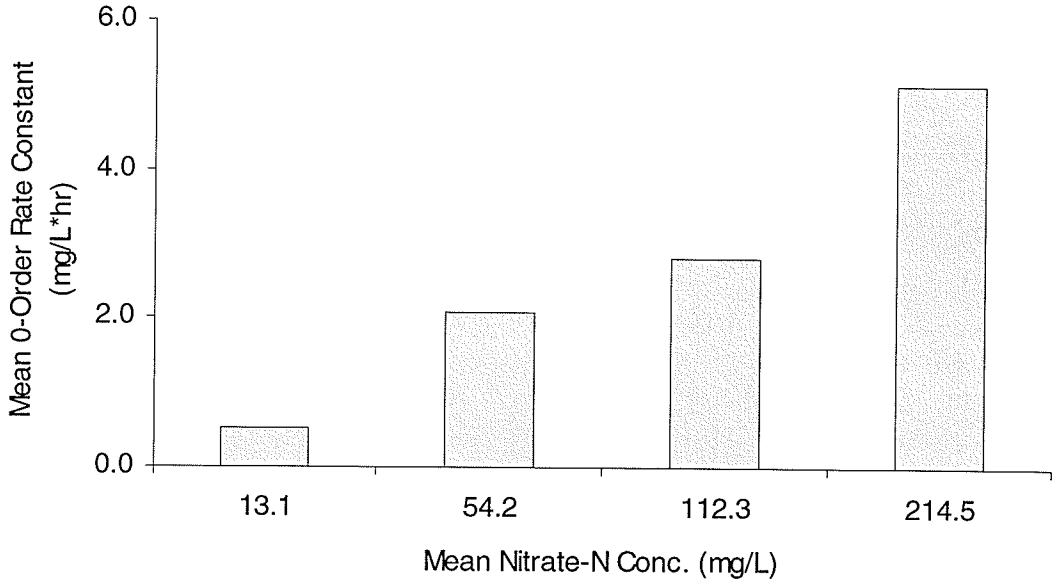


Figure 5.8: Effect of Initial Nitrate Concentration on the Value of Denitrification Kinetic Rate Constant

5.2.3.2 Effect of Initial C:N Ratio

One of the other factors which may bear on the rate of denitrification is the initial carbon-to-nitrogen (C:N) ratio. For the purposes of this research, this ratio is specific to nitrate-nitrogen and VFA-carbon except in Run 7, in which soluble organic carbon is also measured. It would be anticipated that abundant carbon, expressed as a high C:N ratio, would result in high denitrification kinetic rates, as

discovered by Yatong (1996). However, the results here show the opposite. In fact, denitrification kinetic rate constants appear to fall as the C:N ratio increases within the same run! This finding is presented in Figure 5.9 and holds regardless of initial nitrate concentration.

Sludge storage during seed preparation was designed to exhaust available organic carbon. No doubt, in the process, endogenous carbon was exploited as a carbon source by the bacterial population. This switch to endogenous carbon explains the efficiency with which denitrification occurred in the presence of little or no VFA effluent. This switch can also explain why denitrification with endogenous carbon would outperform denitrification with the addition of VFA effluent. This effect is further explored during the discussion of endogenous carbon.

Although pains were taken to minimize the VSS content of the VFA effluent, some biomass would have passed through and into the reaction flasks. These heterogenous biosolids convert organic carbon to anaerobic end-products, and would therefore compete with the acclimated denitrifiers found in the seed. According to this explanation, denitrification kinetic rates would then reduce correspondingly. Thus, the greater the input of VFA effluent, the greater the reduction in reaction kinetics.

Based on these data, the effect of the VFA effluent seems to be clear. However, it is likely that such findings are not universally applicable. The majority of the literature boasts of the success of denitrification using fermented effluent as a carbon source. The findings here seem to contradict those boasts. However, the complexity of the sludge seed and VFA effluent characteristics

render these findings highly specific. Despite this, it is important to note that this effluent may not be applicable in every situation. Therefore, the refrain of environmental engineering should be heeded: each case is unique and requires independent verification and investigation before full-scale application. Recall Fox and Pohland (1994) who echoed this warning as applied to phase-separated anaerobic digestion.

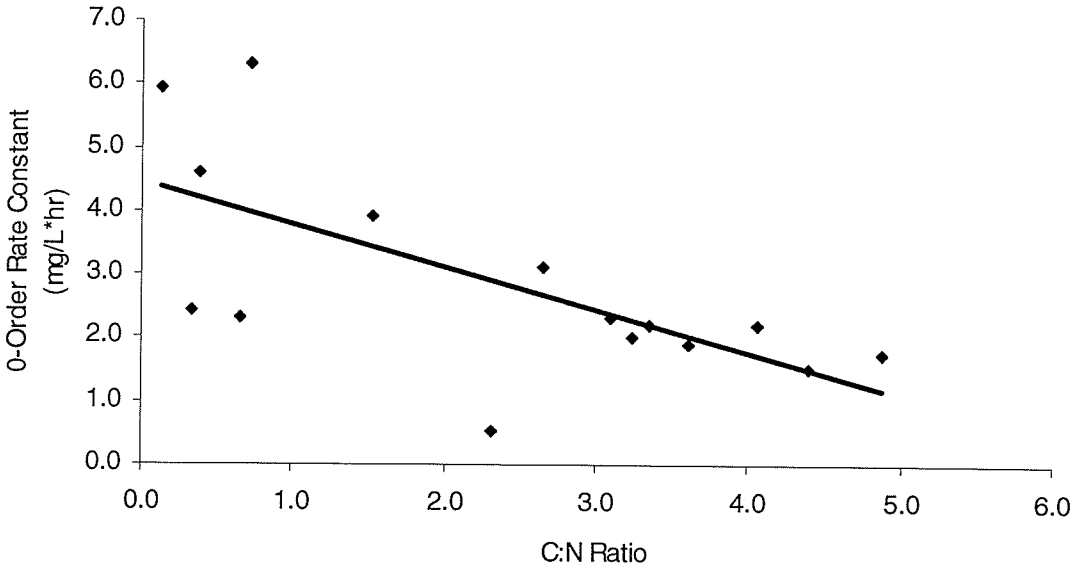


Figure 5.9: Effect of Initial C:N Ratio on Denitrification Kinetic Rate Constant

5.2.3.3 Effect of Organic Carbon Type

As mentioned earlier, two carbon types were tested during this research, VFAs and endogenous carbon. Endogenous carbon outperformed VFAs, reflected in greater denitrification kinetic rate constants. This surprising finding is likely due to a combination of a number of factors.

a. Preferential VFA Consumption

What of the runs which did contain significant quantities of VFAs? Which were consumed first and which were consumed later? Were other organic carbon compounds consumed at the expense of VFAs? These questions can be answered by dissecting the composition of VFA-carbon over the time scale of those denitrification reactions.

In Run 7, soluble organic carbon (SOC) was measured along with VFA content. This parameter was included in order to ensure that other organic carbon forms were not being consumed preferentially to VFAs. These trends are shown in Figure 5.10 where reactors 5 and 6 are only presented as typical cases for the sake of clarity. The figure shows that the disappearance of VFAs mirrored that of SOC in general. In other words, other organic carbon forms were not being consumed prior to VFA consumption. The figure also shows that no other SOC production was occurring. Figure 5.10 also reveals another trend with respect to carbon consumption. As VFAs became limiting, SOC consumption generally did not compensate. In other words, the denitrifiers turned to endogenous carbon as VFAs disappeared.

Of course, these findings may only apply to Run 7 since parallel data for Runs 6 and 8 are lacking. However, given the consistency of the other trends across the three runs, it seems safe to project this finding onto Runs 6 and 8.

Now that it has been shown that VFAs are consumed in preference to other carbon forms (besides endogenous carbon), the question of preference within the VFAs themselves arises. Again, all three runs showed the same

approximate behaviour in this regard. Reactor 5 of Run 6 is chosen as an illustrative example because it had the typical profile and was operated with an abundance of VFA effluent. This example is illustrated in Figure 5.11.

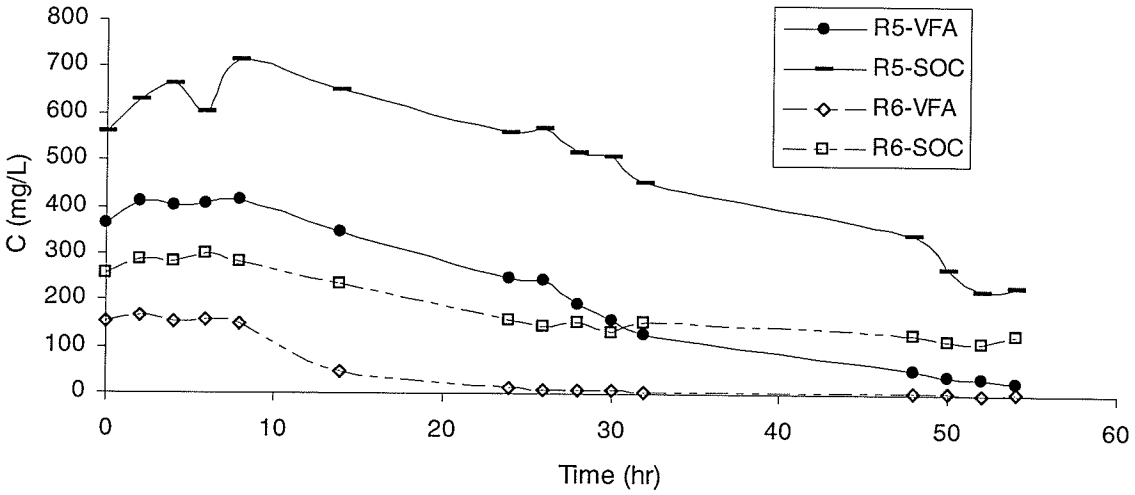


Figure 5.10: VFA and SOC Disappearance During Denitrification

Figure 5.11 demonstrates that acetic acid (HAc-C) is the preferred VFA form for denitrification. This finding is consistent with other research found in the literature such as Yatong (1996). Acetate is almost entirely consumed and only when it is limiting do the other VFA concentrations begin to decline. The next most readily consumed VFA species is butyric acid (denoted by nHBu-C).

The third "choice" of the denitrifiers is propionic acid (HPr-C). This finding is in contrast to other research, such as Fass *et al.* (1994) which claim that propionic acid is not metabolized and may actually inhibit denitrification. It is possible that there is some inhibition here that may account for the decrease in denitrification kinetic constants when VFA effluent is added to the reaction flasks. However, this explanation is not likely, given the relatively quick

consumption of acetate and butyrate. To fully explore this question would require further study.

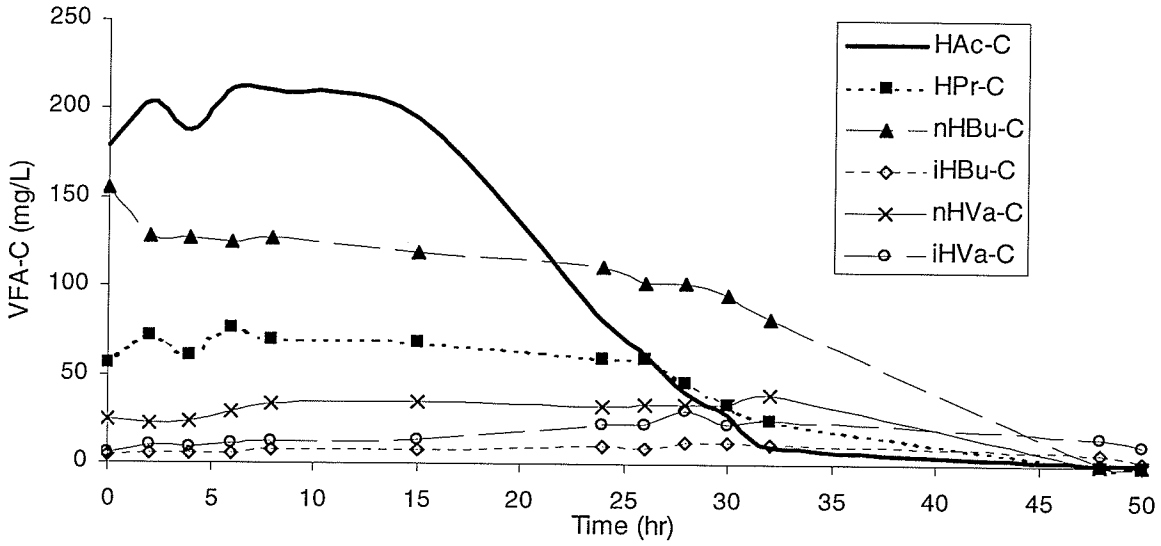


Figure 5.11: Preferential VFA Consumption

It can also be seen in Figure 5.11 that isobutyric (iHBu-C), valeric (nHVa-C), and isovaleric (iHVa-C) acids are used lastly as an organic carbon source. In fact, their concentrations actually increase (though very modestly) until the concentrations of the other three VFA species become depleted. Other reactors also witnessed an increase in concentration of both isomers (*i.e.* iHBu-C and iHVa-C) as the run proceeded. It seems obvious that denitrifying bacteria will preferentially use acetate and butyrate as organic carbon. Only when these are limiting are isobutyric, isovaleric, and especially valeric acids consumed. The role of propionate is not clear; however, based on the findings of this research, propionate is consumed after acetate and butyrate become limiting.

b. Endogenous Carbon

Certain reactors of Runs 7 and 8 contained nearly no VFAs to serve as an organic carbon source. Although some organic carbon was present in the seed, this quantity was quite low. As a result, the denitrifying population turned to endogenous carbon for their carbon source. The word "turned" is probably misleading since the seed was acclimated to exploit endogenous carbon during storage for seed preparation. As a result, it was simpler for these bacteria to continue to use endogenous carbon. In contrast, flasks filled with VFA effluent encouraged the seed population to "switch" to this more energetic carbon source. This switch took time as the bacteria worked to produce the necessary enzymes.

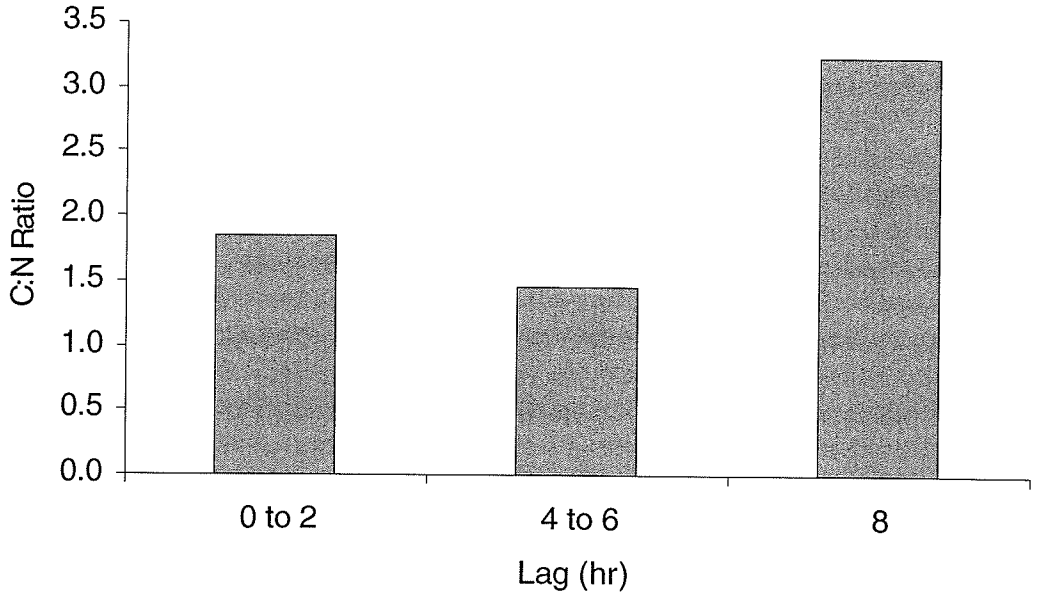


Figure 5.12: Effect of VFA Content on Lag Time

This effect is displayed in Figure 5.12. Low C:N ratios reflect at least the partial use of endogenous carbon for denitrification while high C:N ratios indicate the presence of abundant VFA effluent. While delays of the onset of

denitrification between zero to six hours shows an unclear relationship with C:N ratio, the same cannot be said for lags of eight hours. The figure clearly shows that a greater lag was exhibited in those reactors which were fed large amounts of VFA effluent with respect to the nitrates present. This finding appears to validate the earlier explanation whereby VFAs encourage a switch in organic carbon source for the denitrifying bacteria.

This seeming preference for endogenous carbon probably reflects the time required for the denitrifiers to make the necessary adjustments to other carbon types. In other words, it is not likely that denitrifiers prefer endogenous carbon to external sources, but rather they require time to acclimate to the new or different organic carbon type. The literature is in agreement on this topic not only for denitrification, but for all biological systems.

5.2.4 General Commentary

Successful denitrification was accomplished in all the flasks during all of the runs. However, it could only be quantified during the final three. However, all eight saw complete or near complete disappearance of nitrates as well as recovery of alkalinity and pH. These last two observations are hallmarks of denitrification and are predicted by the denitrification stoichiometry. Seed age was a determining factor in the performance of the denitrification flasks and played a crucial role in the success of the final seed protocol. Indicators of the seed age were biomass growth (measured as VSS) and secondary VFA production.

5.2.4.1 Alkalinity and pH Recovery

All runs indicated recovery of pH and alkalinity. These observations are good evidence for denitrification. According to USEPA (1993), alkalinity recovery is 3.57 g as $CaCO_3$ per g of nitrate nitrogen reduced to nitrogen gas. USEPA also states that deviation in practice from this theoretical recovery is normally small. Table 5.15 summarizes the recovery observed for the flasks of this study. It should also be noted that the addition of VFAs and any oxidation of carbon within the flasks will consume alkalinity.

Table 5.15: Recovery of pH and Alkalinity During Denitrification

Run	Reactor	$pH_{initial}$	pH_{final}	Alkalinity Recovery (mg*$CaCO_3$ per mg NO_3-N)
6	1	5.48	6.50	6.7
	2	4.89	6.57	5.9
	3	4.92	6.80	6.4
	4	4.63	6.73	7.0
	5	4.65	7.03	7.0
7	2	6.48	6.74	4.3
	3	4.96	6.50	6.3
	4	5.40	7.04	4.3
	5	4.76	6.62	5.6
	6	5.29	7.69	3.7
8	2	6.47	6.57	11.0
	3	6.58	6.74	5.1
	4	6.45	6.90	3.8
	5	6.46	7.27	3.4
	6	4.83	6.75	5.9

The table clearly shows that pH and alkalinity were recovered for all the flasks. It also shows alkalinity recovery either higher or approximately equal to that suggested by USEPA. This finding is likely due to the fact that these reactors were closed systems in which acids, once neutralized, were not replaced with acids from the influent.

Table 5.13 also illustrates that flasks with little or no VFA effluent addition produce less alkalinity. These flasks are identified by relatively neutral initial pH values. This finding is curious since much of alkalinity produced should be consumed in acidic flasks as pH is raised. Indeed, all the flasks demonstrate final pH values near or even above 7.0.

5.2.4.2 Seed Age

The effect of the age of the seed has already been shown. Older, acclimated seed produced a bacterial population which provided denitrification while preventing secondary VFA production. Some of the limitations of this seed are discussed here. Typically, this population would be adapted to endogenous carbon, thereby suppressing denitrification kinetic rate constants when VFA effluent served as the carbon source. This endogenous condition of the seed is reflected in VSS values and in comparison with "fresh" seed, such as that used in Run 5.

a. Volatile Suspended Solids

VSS was not measured for all of the runs due to a lack of time. However, the VSS values determined for Run 7 are considered representative for all the successful denitrification runs due to their relative consistency. VSS values for Run 5 are to be accepted as typical for fresh seed.

Table 5.16 shows that VSS of Run 5 increased rapidly from hour zero onward. Such an increase is an obvious sign of exponential-phase growth of the bacterial population. The table also shows that this growth stabilized fairly quickly indicating that the stationary phase was also reached. Such growth is

typical of batch cultures and indicates the presence of no toxic or inhibitory effect from the other flask components. More importantly, it indicates a fresh seed.

Table 5.16: VSS (in mg/L) Variation over the course of Run 5

Reactor	Time (hr)			
	0	6	24	30
1	4800	10600	10250	9000
2	3800	9750	8850	8000
3	2700	10950	10100	9100
4	4200	11450	9350	9600
5	2750	11050	11400	8550

Table 5.17: VSS (in mg/L) Variation over the course of Run 7

Reactor	Time (hr)				
	0	4	6	32	54
2	6960	7000	7720	6740	n/a
3	7060	7100	5800	7820	n/a
4	9540	8180	8420	n/a	7780
5	9880	8700	9200	n/a	8100
6	7960	8200	8760	n/a	7260

In contrast to Table 5.16, Table 5.17 shows that VSS values remained constant over Run 7. It is obvious that the seed of this run was in the stationary phase, perhaps even bordering on the endogenous phase. As a result, the population remained more-or-less unchanged and the addition of VFAs had little impact on the population.

b. Secondary VFA Production

Faster denitrification was achieved when endogenous carbon served as the carbon source, which was largely attributed to the seed. It is also most likely that the fresh seed population was better able to consume the VFA effluent as an organic carbon source than the acclimated seed. These beg the question: why was non-acclimated seed not used? The reason is that the fresh seed also produced

substantial amounts of internal VFAs which would render any C:N analysis useless and mask VFA consumption. Figure 5.15 shows the secondary VFA production observed during Run 5.

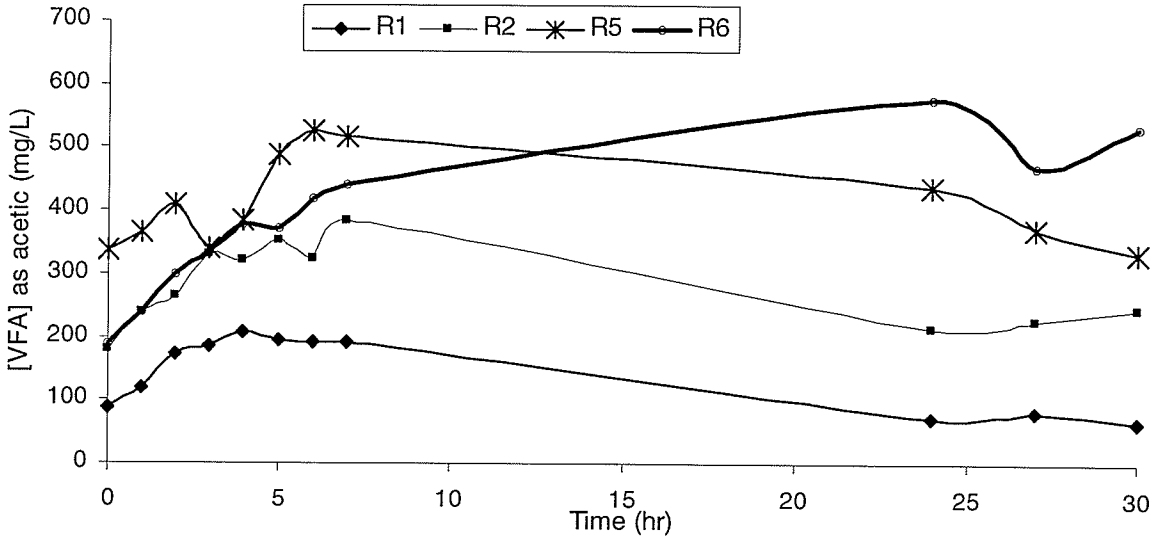


Figure 5.13: Secondary VFA Production Concurrent with Denitrification During Run 5

Reactor 6 of Run 5 contained no nitrates and therefore shows secondary VFA consumption with no denitrification. However, Reactors 1, 2, and 5 contained nitrates. Examining these latter reactors in seclusion might lead one to think that successful denitrification runs had been achieved. However, there was obviously secondary VFA production occurring.

This observation is in vivid contrast to Run 7. Reactor 1 of Run 7 also contained no nitrates. The VFA profile of this run is shown in Figure 5.14. It reveals that VFAs were actually consumed, leading to the conclusion that there is modest carbon consuming bacterial activity occurring thanks to this acclimated seed. However, no secondary VFA production will interfere with these data.

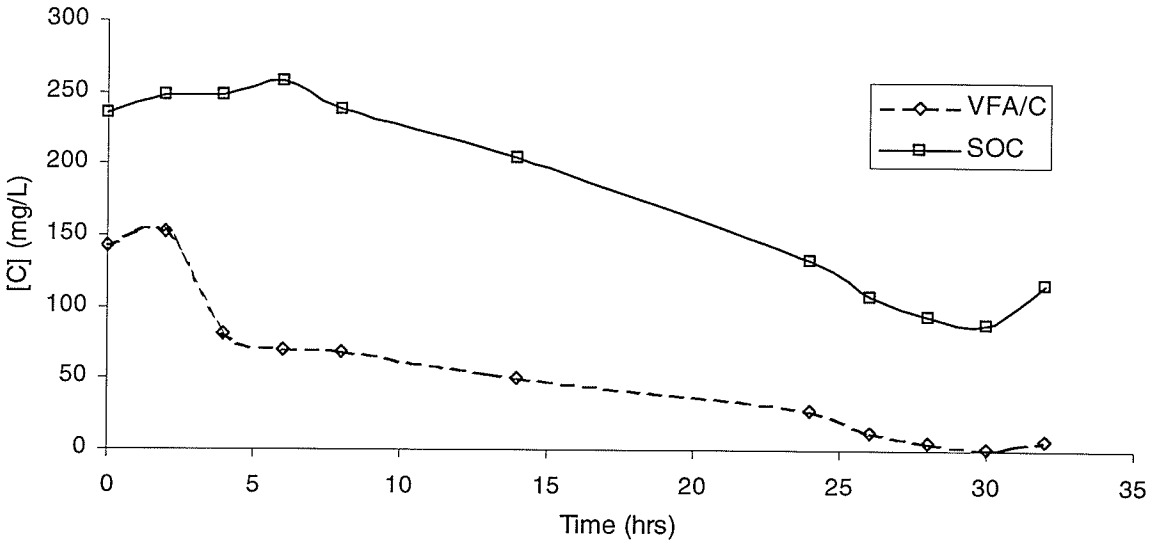


Figure 5.14: Carbon Profile for Reactor 1 of Run 7

5.2.4.3 Feasibility of Replication

The nature of this type of research makes it highly case specific. The seed characteristics may change substantially depending on the source, conditions at the source, preparation, and storage. Therefore, it is likely impossible to duplicate the results found here. Indeed, results were highly variable when the same seed was used within the same experiment!

However, the apparatus used in this experiment was very simple and little or no variation will arise from it. This is the chief attraction of this type of batch experiment; the ease of layout, design, and replication. As a result, this type of study often forms the initial survey into a matter of research and replication of this study will not be difficult while bearing the limitations of the following section in mind. It is hoped that this study can provide such an initial investigation.

5.2.4.4 Limitations

As in the previous portion of this study, the reader should be aware of certain limitations inherent in this research. Firstly, VFA, pH and alkalinity, and solids analyses were conducted on single samples. A lack of time, sample volume, and/or resources led to this limitation.

The source of another, more significant limitation on these data was the nature of the seed. Although denitrifying bacteria are nearly ubiquitous, the contents of competing bacteria, putrescent compounds, and other interfering factors will vary in the seed depending upon many factors. These may include geographic location, time of year, plant conditions, and so on. As a result, replication of this study under identical conditions could yield different results, as the nature of the seed will almost certainly be different.

The above rationale may also apply to the fermented VFA source, although this effect is likely less pronounced. Depending upon specific conditions in the anaerobic contact reactor, the VFA source may contain more or fewer VFAs, nitrogen compounds, competing bacteria, etc. Also, the pH of the VFA source will vary in this way. Therefore, it would likely be difficult to exactly duplicate the characteristics of the VFA source and thus, the individual results gathered here were specific to this study.

5.2.4.5 Engineering Significance

The sludge used as seed in this study should provide excellent denitrification in a large-scale installation. In this study, it was necessary to rid the seed of its secondary VFA production characteristics; however, in full-scale, such a property

would benefit denitrification. In fact, simultaneous VFA production and denitrification is probably a syntrophic relationship and should be encouraged. Secondary VFA production would also enhance phosphorous removal in such a BNR plant. The sludge has also shown its ability to provide denitrification under endogenous circumstances. As a result of these characteristics, this sludge would likely encounter little difficulty adapting to denitrification requirements. The sludge, despite extended storage of up to two weeks, still provided denitrification under carbon-depleted conditions. Therefore, it is likely that this sludge could serve as seed for remote biological denitrification if required.

The success encountered in denitrification with this sludge reflects the ubiquity of denitrifiers in nature. As a result, it is probable that any secondary or even primary sludge will provide some level of denitrification. This is likely independent of location and the variables that come with it. However, it should be remembered that sludge characteristics are highly case specific. Therefore, this should always be verified in each individual case.

The wide applicability of treatment plant sludges to denitrification might indicate their ability to serve as seeds for treatment of nitrite-rich wastewaters or to remove harmful carbon compounds. In this latter case, these compounds may well serve as carbon sources for denitrifiers. USEPA (1993) identifies a number of carbon compounds which may perform this role. There may be a day when acclimated denitrifier populations will digest industrial and agricultural chemicals in this way.

6 RECOMMENDATIONS

6.1 Anaerobic Digestion

A number of recommendations can be made which might draw upon the findings of this and related research.

1) Considering the increase in VFA and SCOD production achieved through the use of the starch-rich industrial wastewater mixed with municipal wastewater, it would likely be beneficial to conduct similar research with different organic industrial feeds. The effects of high protein or lipid wastewaters could be elucidated.
2) Other anaerobic digesters, in addition to the solids contact configuration used in this research, should be simulated. The effect of the digester type itself could then be observed. It could also be observed if industrial-to-municipal ratios would be equally beneficial.
3) Employing the VFAs produced from anaerobic digestion in nitrogen and phosphorous removal studies should be pursued.
4) The 100% industrial feed content trial should be run again with an emphasis on controlling SRT. The true effect of this ratio could then be discovered.

6.2 Denitrification

In the future, denitrification studies employing complex seed cultures such as the sludge used in this study should heed the recommendations presented here. Apart from such recommendations, much more research opportunity is available.

1) Biosolids in the VFA effluent should be removed through filtration or other means. Competition from these biosolids could be partially responsible for the poor denitrification performance when VFA effluent is added to the batch cultures.

2) The low pH of the VFA effluent appears to be responsible for the poor performance of flasks containing it. Therefore, some form of pH control in the flasks should be employed to reduce this effect or at least determine this effect.

3) Denitrification with a synthetic VFA effluent would provide a useful comparison to that with anaerobically-digested VFA effluent.

4) In addition to denitrification with VFA effluent, other carbon sources should be investigated. In particular, methanol ought to be researched. There is a good deal of literature that provides comparison for methanol. Individual VFA types might also be studied.

5) As well as these traditional carbon sources, more exotic carbon sources should be studied. Regionally abundant compounds available for local industries should be investigated. Perhaps

investigations into the effect of harmful carbon compounds would also be useful, such as hydrocarbons.

6) Since the denitrifier population favoured the consumption of acetic and butyric acids, starch wastewater should be included in anaerobic digestion feeds when the effluent is expected to supply organic carbon to denitrification processes. Recall raising the starch content of anaerobic digesters increases the content of these acids.

7 CONCLUSIONS

Since this study was divided into anaerobic digestion and denitrification sections, the accompanying conclusions and recommendations will also be presented in this manner.

7.1 Anaerobic Digestion

This research into the role of the industrial-to-municipal ratio on anaerobic digestion has generated a number of conclusions. The conclusions presented here were obvious and statistically sound.

1) An increase in the industrial content always resulted in improved settling performance in the clarifiers.
2) An increase in the starch-rich industrial content of the feed yielded a drop in pH in the anaerobic reactor and effluent. This drop continued as the industrial content was varied from 0 to 100%. At 100% industrial feed, the pH was very acidic, often below 4.0.
3) Net VFA and SCOD production rates, net VFA and SCOD production, and VSS destruction were optimized at the industrial-to-municipal ratio of 1:1.
4) An increase in the industrial content of the feed was accompanied by a modest drop in the production of acetic acid, and a substantial drop in propionic acid production. At the same time, a significant increase in butyric acid production was observed.

5) Replication of this research is feasible. This has been proved in comparisons between the two systems employed in this study and systems used in studies conducted by Banerjee (1997) and Maharaj (1999).

7.2 Denitrification

With regard to the batch culture denitrification study, a number of conclusions were garnered.

1) All denitrification runs proceeded according to zero-order kinetics.
2) Flasks containing anaerobically-digested VFA effluent generally yielded lower kinetic rate constants than those flasks containing little or no VFA effluent.
3) Acetic acid was consumed in preference to the other VFA species. The next most favoured VFA type was butyric acid followed by propionic acid. Valeric and especially isobutyric and isovaleric acids were consumed only after these VFA species became limiting.
4) Replication of the research conducted here is feasible but is dependent upon the seed condition.

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APPENDIX A: ANAEROBIC DIGESTION STUDY DATA

Table A.1: Influent VFA, SCOD, and TCOD

Run 1										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
May-13	54.19	2.86	4.28	2.53	3.67	0.00	63.33		n/a	n/a
May-20	0.00	0.00	0.00	0.00	0.00	0.00	0.00		n/a	n/a
May-27	3.26	0.00	0.00	0.00	0.00	0.00	3.26		n/a	n/a
Jun-01	6.69	0.00	0.00	0.00	0.00	0.00	6.69		n/a	n/a
Jun-05	9.58	1.47	4.89	0.85	0.00	0.00	14.69		n/a	n/a
Jun-10	0.00	0.00	0.00	0.00	0.00	0.00	0.00		n/a	n/a
Jun-15	0.00	0.00	0.00	0.00	0.00	0.00	0.00		n/a	n/a
Jun-19	117.97	66.82	10.19	37.25	12.95	7.81	217.13		n/a	n/a
Jun-24	0.00	0.00	0.00	0.00	0.00	0.00	0.00		n/a	n/a
Jun-29	0.00	0.00	0.00	0.00	0.00	0.00	0.00		n/a	n/a
Jul-03	0.00	0.00	0.00	0.00	0.00	0.00	0.00		n/a	n/a
Jul-08	24.93	0.00	0.00	0.00	0.00	0.00	24.93		n/a	n/a
Jul-13	58.25	0.00	0.00	0.00	0.00	0.00	58.25		n/a	n/a
Jul-17	98.26	54.37	6.70	28.40	7.58	2.68	172.66		n/a	n/a
Jul-22	0.00	0.00	0.00	0.00	0.00	0.00	0.00		n/a	n/a
Mean	24.88	8.37	1.74	4.60	1.61	0.70	37.40		n/a	n/a
St. dev.	39.09	21.35	3.23	11.60	3.78	2.09	67.76		n/a	n/a
Run A										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
29-Jul	1.94	0.00	0.00	0.00	0.00	0.00	1.94		198	7838
05-Aug	270.09	199.92	0.00	0.00	0.00	0.00	433.43		434	5115
12-Aug	0.00	0.00	0.00	0.00	0.00	0.00	0.00		243	7021
18-Aug	0.00	0.00	0.00	0.00	0.00	0.00	0.00		279	5660
21-Aug	4.39	0.00	4.04	0.00	6.10	0.00	10.74		n/a	-
25-Aug	53.35	73.43	1.45	3.62	3.46	1.24	119.56		461	-
28-Aug	18.82	7.60	0.00	0.00	0.00	0.00	25.03		379	4843
01-Sep	28.55	15.69	0.00	0.48	0.73	0.00	42.13		n/a	-
04-Sep	28.55	15.69	0.00	0.48	0.73	0.00	42.13		n/a	-
19-Sep	44.35	95.34	3.42	6.29	3.29	0.86	131.31		479	-
25-Sep	63.66	136.77	0.00	5.34	3.33	1.20	181.71		452	9743
Mean	46.70	49.50	0.81	1.47	1.60	0.30	89.82		366	6703
St. dev.	77.35	68.38	1.51	2.40	2.09	0.52	129.60		110	1881

Table A.1 cont.

Run B										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
10-Nov	48.31	16.62	0.00	0.00	0.00	0.00	61.89		394	3700
12-Nov	72.31	28.12	0.00	0.85	2.34	0.58	97.58		382	4888
16-Nov	114.76	93.32	5.78	4.68	4.89	1.80	202.07		n/a	9299
20-Nov	117.16	130.02	9.00	11.90	9.34	3.35	245.11		541	4548
27-Nov	69.38	107.39	9.80	3.78	11.97	0.51	173.72		507	4548
1-Dec	103.01	116.94	9.51	7.01	9.37	3.33	217.29		496	-
4-Dec	133.51	125.72	11.82	2.25	7.16	1.95	251.17		643	6245
7-Dec	3.86	12.94	4.04	2.52	3.60	1.13	21.70		134	6924
11-Dec	2.52	2.20	0.64	1.17	0.81	1.15	6.71		279	5906
15-Dec	86.38	15.15	6.73	10.54	6.01	3.11	115.90		443	5227
18-Dec	0.87	0.00	0.00	0.00	0.00	0.00	0.87		279	-
Mean	68.37	58.95	5.21	4.06	5.05	1.54	126.73		410	5698
St. dev.	48.76	54.66	4.52	4.12	4.11	1.27	95.93		149	1671
Run C										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
16-Oct	2.02	0.00	0.00	0.00	0.00	0.00	2.02		116	6749
19-Oct	3.17	0.00	0.00	1.19	0.00	0.00	3.98		89	8654
23-Oct	1.76	0.00	0.00	0.00	0.00	0.99	2.34		171	-
26-Oct	37.19	11.98	1.24	0.42	1.85	0.00	49.20		207	2665
30-Oct	2.63	0.00	0.00	0.55	0.00	0.51	3.30		225	5660
3-Nov	1.59	0.00	0.00	0.00	0.00	0.00	1.59		152	-
6-Nov	18.82	7.60	0.00	0.00	0.00	0.00	25.03		192	6768
10-Nov	2.19	0.00	0.00	0.00	0.00	0.00	2.19		67	2196
12-Nov	2.34	0.60	0.00	4.39	0.00	0.00	5.82		124	3045
16-Nov	0.67	0.00	0.00	0.00	0.00	0.00	0.67		90	-
20-Nov	3.29	0.98	0.00	1.80	0.00	0.00	5.32		327	3214
24-Nov	36.27	21.26	2.45	6.45	3.32	1.60	62.60		316	-
27-Nov	1.23	0.00	0.00	0.00	0.00	0.00	1.23		90	6233
1-Dec	0.86	0.00	0.00	0.00	0.00	0.00	0.86		45	5688
Mean	8.15	3.03	0.26	1.06	0.37	0.22	11.87		158	5087
St. dev.	12.93	6.36	0.71	1.97	0.98	0.49	19.81		88	2166

Table A.1 cont.

Run D										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
12-Jan	7.96	16.26	0.00	0.00	0.00	0.00	21.25		129	4718
15-Jan	3.94	0.74	0.00	0.68	0.67	0.69	5.81		100	8790
26-Jan	0.00	0.00	0.00	1.11	0.86	0.59	1.61		n/a	-
05-Feb	6.44	1.64	0.00	0.00	0.00	0.51	8.08		224	4211
09-Feb	4.66	0.00	0.00	0.75	0.00	0.00	5.16		44	5776
12-Feb	2.80	0.00	0.00	0.00	0.00	0.00	2.80		214	-
16-Feb	5.11	0.00	0.00	0.00	0.00	0.00	5.11		100	6061
19-Feb	3.46	0.68	0.51	0.61	0.68	0.62	5.55		25	7341
24-Feb	5.05	0.00	1.28	0.00	0.00	0.00	5.92		n/a	-
27-Feb	3.15	0.00	0.00	0.00	0.00	0.00	3.15		119	7911
02-Mar	2.01	0.00	0.00	0.00	0.00	0.00	2.01		186	6203
09-Mar	2.22	0.78	0.41	0.69	0.83	0.61	4.45		138	-
12-Mar	1.92	0.00	0.00	0.00	0.00	0.53	2.23		195	5776
15-Mar	36.43	27.01	0.00	5.38	0.00	0.00	62.16		91	7341
19-Mar	4.06	0.00	0.63	0.00	0.00	0.00	4.49		44	-
Mean	5.95	3.14	0.19	0.61	0.20	0.24	9.32		124	6413
St. dev.	8.66	7.79	0.37	1.37	0.35	0.30	15.35		66	1427

Table A.2: Reactor VFA, SCOD, and TCOD

Run 1										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
May-13	250.94	86.98	0.00	134.78	9.52	10.78	425.85		n/a	n/a
May-20	363.61	121.86	11.79	125.33	14.98	34.58	585.82		n/a	n/a
May-27	671.24	126.01	0.00	148.64	16.52	52.23	915.99		n/a	n/a
Jun-01	588.62	55.79	0.00	103.00	0.00	26.27	719.89		n/a	n/a
Jun-05	445.59	24.81	50.56	72.30	19.61	19.52	572.66		n/a	n/a
Jun-10	299.90	59.33	0.00	65.87	0.00	11.80	400.24		n/a	n/a
Jun-15	523.53	80.59	0.00	237.13	24.92	38.04	788.12		n/a	n/a
Jun-19	500.76	99.58	0.00	315.56	0.00	30.32	815.15		n/a	n/a
Jun-24	365.45	85.92	0.00	139.33	0.00	21.54	543.34		n/a	n/a
Jun-29	534.90	88.07	0.00	201.53	0.00	35.42	765.12		n/a	n/a
Jul-03	483.58	64.22	0.00	297.81	0.00	25.87	754.37		n/a	n/a
Jul-08	787.99	127.21	0.00	671.82	0.00	70.50	1391.56		n/a	n/a
Jul-13	498.19	90.24	0.00	401.48	0.00	55.65	878.45		n/a	n/a
Jul-17	347.94	138.14	0.00	357.65	0.00	39.92	728.19		n/a	n/a
Jul-22	414.28	186.40	0.00	337.94	8.52	60.51	837.63		n/a	n/a
Mean	471.77	95.68	4.16	240.68	6.27	35.53	741.49			
St. dev.	142.03	39.69	13.19	161.78	8.77	17.71	238.76			
Run A										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
29-Jul	281.39	96.87	0.00	160.07	13.01	18.84	488.43		1705	14586
05-Aug	638.45	152.25	19.08	272.90	29.07	48.55	994.60		1070	-
12-Aug	317.42	58.59	0.00	106.77	0.00	21.46	450.72		888	8053
18-Aug	308.42	72.38	0.00	73.94	0.00	20.10	429.80		1433	7508
21-Aug	386.70	96.01	14.27	117.92	21.77	34.35	588.30		n/a	-
25-Aug	465.97	121.98	11.88	171.05	12.22	27.12	713.51		2068	10230
28-Aug	538.80	107.75	13.24	191.51	16.74	26.96	792.17		1614	18942
01-Sep	551.84	99.76	11.34	165.08	13.45	26.67	777.25		n/a	-
04-Sep	340.62	60.93	0.00	89.49	7.15	13.48	463.56		n/a	-
19-Sep	618.82	221.68	16.51	214.26	24.24	61.11	1007.50		1977	-
25-Sep	466.28	182.63	12.56	226.49	12.96	44.58	812.35		1796	8597
Mean	446.79	115.53	8.99	162.68	13.69	31.20	683.47		1569	11319
St. dev.	128.34	51.27	7.44	61.77	9.18	14.58	213.05		417	4529

Table A.2 cont.

Run B										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
10-Nov	282.12	131.73	14.04	344.60	5.79	64.10	675.43		1557	15205
12-Nov	265.21	158.80	5.50	299.33	3.61	354.70	813.02		1421	11811
16-Nov	452.05	222.47	14.27	332.47	5.01	43.02	898.52		n/a	24029
20-Nov	364.05	218.05	17.81	239.87	10.79	52.61	755.21		1801	11472
27-Nov	383.00	220.60	20.11	180.91	15.80	53.07	740.81		781	11811
1-Dec	660.47	314.04	27.57	277.46	17.41	85.38	1185.51		2027	-
4-Dec	521.69	215.46	22.90	173.32	9.11	54.26	868.80		1575	12151
7-Dec	578.03	199.63	20.10	176.10	12.12	54.14	913.89		1349	19956
11-Dec	463.71	126.24	13.65	167.75	10.41	36.34	718.06		1735	13169
15-Dec	716.84	152.33	17.48	274.20	13.28	50.91	1077.97		1589	19277
18-Dec	1027.43	192.01	10.40	423.16	16.25	91.90	1543.59		2061	-
Mean	519.51	195.58	16.71	262.65	10.87	85.49	926.44		1590	15431
St. dev.	221.75	53.31	6.07	84.10	4.69	90.82	256.57		368	4569
Run C										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
16-Oct	637.66	129.47	13.14	309.54	16.70	35.01	993.90		1614	8597
19-Oct	369.97	69.36	0.00	344.61	0.00	15.32	670.66		1433	10775
23-Oct	350.87	73.60	0.00	467.83	0.00	45.44	756.78		1705	-
26-Oct	462.19	93.39	0.00	469.15	0.00	59.16	893.23		1796	14042
30-Oct	327.41	73.17	0.00	469.95	0.00	47.70	735.74		1796	11319
3-Nov	259.03	67.98	0.00	355.54	0.00	43.51	582.63		1161	-
6-Nov	538.80	107.75	13.24	191.51	16.74	26.96	792.17		1696	8107
10-Nov	346.77	123.23	11.25	546.84	13.24	49.73	865.10		1628	8126
12-Nov	235.79	76.74	0.00	472.00	0.00	37.61	642.51		1718	7786
16-Nov	226.97	63.79	0.00	451.11	0.00	31.62	605.34		1447	-
20-Nov	312.22	55.50	0.00	333.97	0.00	15.56	594.48		1581	7447
24-Nov	302.68	47.08	0.00	285.66	0.00	18.46	546.82		1310	-
27-Nov	257.51	47.79	0.00	245.90	0.00	11.02	470.74		1174	14916
1-Dec	397.20	69.54	0.00	315.88	0.00	22.50	682.67		1400	12193
Mean	358.93	78.46	2.69	375.68	3.33	32.83	702.34		1533	10331
St. dev.	118.22	25.84	5.36	103.89	6.67	15.01	146.30		215	2729

Table A.2 cont.

Run D										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
12-Jan	190.89	29.21	0.00	86.30	0.00	11.80	280.55		0	14526
15-Jan	162.11	41.21	3.30	71.94	0.00	12.38	254.06		174	20635
26-Jan	447.00	51.68	6.24	273.64	3.41	17.86	692.61		n/a	-
05-Feb	293.10	48.06	0.00	576.55	0.00	50.38	755.20		1844	28630
09-Feb	291.70	51.13	0.00	466.04	0.00	41.48	675.71		1730	29199
12-Feb	685.94	112.95	6.67	706.27	0.00	82.30	1312.84		1920	-
16-Feb	470.27	81.21	3.48	161.11	0.00	29.70	666.33		1199	29199
19-Feb	464.40	76.72	3.54	106.67	0.00	21.28	614.76		1085	28914
24-Feb	376.69	86.74	0.00	106.29	0.00	15.35	529.08		n/a	-
27-Feb	452.05	104.51	1.88	170.28	0.00	19.66	666.41		781	27207
02-Mar	453.87	119.67	3.43	334.09	0.00	26.60	797.46		971	23506
09-Mar	598.17	114.43	0.00	675.56	0.00	76.90	1197.61		1464	-
12-Mar	559.23	84.54	0.00	702.74	0.00	76.46	1152.52		1806	22937
15-Mar	534.27	89.36	3.43	769.91	0.00	106.37	1197.24		1540	28630
19-Mar	509.19	104.94	4.87	642.90	0.00	123.24	1109.17		1199	-
Mean	432.59	79.76	2.46	390.02	0.23	47.45	793.44		1209	25338
St. dev.	146.71	29.24	2.38	267.23	0.88	36.64	331.49		612	4898

Table A.3: Effluent VFA, SCOD, and TCOD

Run 1										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
May-13	250.74	79.15	0.00	138.60	11.68	12.42	424.10		n/a	n/a
May-20	195.91	70.69	0.00	55.89	10.78	21.00	310.47		n/a	n/a
May-27	616.44	134.91	0.00	184.52	18.00	58.48	897.47		n/a	n/a
Jun-01	594.77	69.86	0.00	101.19	13.44	27.74	745.08		n/a	n/a
Jun-05	505.82	30.89	49.25	81.49	18.43	22.14	644.07		n/a	n/a
Jun-10	254.10	58.31	0.00	64.78	0.00	21.05	358.30		n/a	n/a
Jun-15	309.38	56.91	0.00	82.64	0.00	21.72	425.01		n/a	n/a
Jun-19	386.08	74.03	0.00	279.06	0.00	28.55	653.67		n/a	n/a
Jun-24	273.78	83.13	0.00	149.03	0.00	23.34	457.06		n/a	n/a
Jun-29	296.02	89.48	0.00	130.91	0.00	31.59	476.97		n/a	n/a
Jul-03	515.42	65.86	0.00	182.20	0.00	24.02	707.61		n/a	n/a
Jul-08	630.43	114.05	0.00	475.39	0.00	44.65	1074.08		n/a	n/a
Jul-13	628.85	138.43	0.00	563.11	0.00	74.40	1169.74		n/a	n/a
Jul-17	456.64	141.13	0.00	454.16	0.00	47.26	909.47		n/a	n/a
Jul-22	409.84	159.24	0.00	347.17	0.00	67.25	816.26		n/a	n/a
Mean	421.61	91.07	3.28	219.34	4.82	35.04	671.29			
St. dev.	153.86	37.52	12.72	165.38	7.31	18.88	264.71			
Run A										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
29-Jul	286.72	113.73	0.00	204.49	24.67	29.38	550.88		1251	2675
05-Aug	452.68	135.97	0.00	161.14	32.70	45.65	673.46		1614	-
12-Aug	461.13	119.07	0.00	145.97	30.18	38.35	698.26		1251	3582
18-Aug	411.66	106.14	0.00	111.67	0.00	27.94	590.97		1524	3582
21-Aug	332.51	75.51	0.00	93.84	0.00	21.09	470.60		n/a	-
25-Aug	237.81	58.86	3.92	69.25	8.09	13.31	348.39		979	3128
28-Aug	348.57	79.75	10.66	115.02	12.00	18.90	517.61		1705	1540
01-Sep	676.51	126.91	15.81	216.75	17.34	31.76	967.67		n/a	-
04-Sep	444.49	83.20	9.48	129.44	12.58	22.03	627.56		n/a	-
19-Sep	315.93	117.72	13.98	45.31	20.37	20.90	476.81		253	-
25-Sep	343.49	122.34	10.79	101.88	12.55	33.71	547.48		1433	1767
Mean	391.95	103.56	5.88	126.80	15.50	27.55	588.15		1251	2712
St. dev.	118.73	25.03	6.33	52.64	10.90	9.47	160.23		465	889

Table A.3 cont.

Run B										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
10-Nov	525.30	154.30	18.71	451.48	18.43	114.28	1050.06		1987	9028
12-Nov	293.24	149.13	13.44	341.27	8.26	61.02	630.23		1692	9299
16-Nov	221.91	143.93	9.80	253.05	0.00	38.60	541.46		n/a	11336
20-Nov	415.20	214.99	24.52	240.04	13.85	43.12	804.77		1846	3869
27-Nov	373.18	249.97	19.62	236.02	14.68	37.71	782.56		1912	3191
1-Dec	469.37	353.61	29.50	258.36	20.60	66.70	1005.93		1575	-
4-Dec	389.58	120.09	21.03	108.32	7.08	38.35	602.62		1258	2173
7-Dec	559.42	232.86	26.64	193.88	15.46	53.30	940.49		1349	2173
11-Dec	375.35	124.04	16.11	137.15	12.84	38.40	611.34		1735	985
15-Dec	445.38	114.70	19.37	194.32	19.34	43.94	722.03		1118	476
18-Dec	561.83	147.74	6.46	244.44	9.03	38.65	881.68		1735	-
Mean	420.89	182.30	18.65	241.67	12.69	52.19	779.38		1621	4725
St. dev.	106.84	73.44	6.95	93.61	6.15	22.92	174.15		291	4051
Run C										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
16-Oct	345.15	50.71	8.38	121.00	12.67	21.84	495.10		1251	1087
19-Oct	483.21	95.14	3.88	356.03	13.10	37.80	780.70		2159	6304
23-Oct	411.61	95.24	0.00	457.24	11.42	37.21	829.86		1614	-
26-Oct	430.63	100.42	0.00	482.78	0.00	63.57	879.31		2068	1767
30-Oct	387.16	100.50	0.00	526.91	0.00	76.72	873.73		1977	2902
3-Nov	371.96	99.27	0.00	568.50	0.00	78.75	887.09		2068	-
6-Nov	348.57	79.75	10.66	115.02	12.00	18.90	517.61		1899	7054
10-Nov	345.83	98.81	6.35	504.70	6.31	68.24	818.94		1718	3840
12-Nov	311.13	88.56	0.00	490.96	0.00	41.46	742.69		1673	3384
16-Nov	325.55	80.00	4.58	510.98	0.00	43.07	767.84		1650	-
20-Nov	395.54	80.55	15.70	509.49	0.00	40.98	843.63		1717	5081
24-Nov	351.37	63.68	4.96	348.68	0.00	26.62	660.23		2124	-
27-Nov	373.64	58.93	5.87	282.51	0.00	15.96	627.83		1672	5688
1-Dec	356.27	88.27	31.18	307.34	19.59	33.09	690.22		1581	5008
Mean	376.08	84.62	6.22	420.55	4.85	45.02	761.32		1790	4212
St. dev.	46.45	16.97	8.76	128.28	6.95	20.49	116.15		268	1953

Table A.3 cont.

Run D										
Date	VFA Concentration						Total			
	Acetic	Propionic	i-butyric	n-butyric	i-valeric	n-valeric	(mg/L		SCOD	TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	as acetic)		(mg/L)	(mg/L)
12-Jan	228.48	25.38	1.76	126.71	0.00	13.17	344.57		98	815
15-Jan	180.80	42.70	3.43	91.55	0.00	10.37	267.84		591	1833
26-Jan	255.10	38.39	4.19	122.55	2.39	10.08	380.23		n/a	-
05-Feb	400.12	77.86	9.96	822.16	0.00	69.87	1072.33		1882	2333
09-Feb	309.92	60.79	3.21	498.92	0.00	54.84	734.27		2072	3851
12-Feb	586.55	106.16	8.24	871.89	0.00	84.92	1323.46		2072	-
16-Feb	414.88	60.16	4.56	261.64	0.00	41.49	669.98		1275	2997
19-Feb	519.12	82.93	4.02	149.14	0.00	24.75	705.89		1123	2333
24-Feb	415.64	82.09	0.00	113.57	0.00	17.48	570.43		n/a	-
27-Feb	425.05	87.05	3.85	121.33	0.00	17.12	591.61		743	4230
02-Mar	410.45	125.87	0.00	212.33	0.00	16.61	667.86		895	3471
09-Mar	559.33	125.59	2.93	678.89	0.00	62.80	1163.86		1464	-
12-Mar	537.76	98.15	3.44	655.68	0.00	84.57	1117.20		2072	2807
15-Mar	497.54	72.86	0.00	591.26	0.00	84.18	1009.81		1692	3661
19-Mar	458.91	96.83	4.34	601.78	0.00	86.64	1002.33		1464	-
Mean	413.31	78.85	3.60	394.63	0.16	45.26	774.78		1342	2833
St. dev.	122.90	29.82	2.76	287.17	0.62	31.23	323.72		630	1037

Table A.4: Influent Ammonia and TKN

Run 1
No Data

Run A		
Date	NH3-N (mg/L)	TKN (mg/L)
29-Jul	n/a	-
05-Aug	n/a	-
12-Aug	87.6	328.8
18-Aug	103.8	303.1
21-Aug	147.3	333.9
25-Aug	115.0	287.7
28-Aug	0.0	344.2
01-Sep	7.0	-
04-Sep	69.9	-
19-Sep	n/a	290.3
25-Sep	n/a	308.3
01-Oct	n/a	287.7
08-Oct	n/a	262.0
Mean	75.8	305.1
St. dev.	54.9	26.5

Run B		
Date	NH3-N (mg/L)	TKN (mg/L)
10-Nov	41.7	308.3
12-Nov	33.2	-
16-Nov	n/a	-
20-Nov	66.4	-
27-Nov	68.1	178.9
1-Dec	67.2	197.4
4-Dec	50.2	271.3
7-Dec	74.0	215.9
11-Dec	52.7	160.5
15-Dec	69.8	215.9
18-Dec	7.7	-
Mean	53.1	221.2
St. dev.	20.9	51.9

Run C		
Date	NH3-N (mg/L)	TKN (mg/L)
08-Oct	24.1	-
16-Oct	18.0	153.7
19-Oct	35.9	157.1
26-Oct	54.7	242.8
30-Oct	16.3	191.4
03-Nov	47.3	174.3
10-Nov	21.0	198.2
12-Nov	11.3	160.6
20-Nov	7.4	-
24-Nov	13.6	-
27-Nov	10.5	229.1
01-Dec	6.0	249.6
Mean	22.2	195.2
St. dev	15.8	37.4

Run D		
Date	NH3-N (mg/L)	TKN (mg/L)
12-Jan	110.8	138.6
15-Jan	116.2	110.8
05-Feb	16.1	120.1
09-Feb	17.9	147.8
16-Feb	23.2	212.5
19-Feb	n/a	138.6
24-Feb	n/a	-
27-Feb	80.4	92.4
02-Mar	76.9	110.8
09-Mar	23.2	157.0
15-Mar	6.9	166.3
Mean	52.4	139.5
St. dev	43.5	34.4

Table A.5: Reactor Ammonia and TKN

Run 1
No Data

Run A		
Date	NH3-N (mg/L)	TKN (mg/L)
29-Jul	n/a	-
05-Aug	n/a	-
12-Aug	48.9	1109.7
18-Aug	26.3	755.2
21-Aug	13.1	1310.1
25-Aug	160.2	1125.1
28-Aug	224.7	1317.8
01-Sep	142.5	-
04-Sep	7.0	-
19-Sep	n/a	1148.2
25-Sep	n/a	701.3
01-Oct	n/a	1086.6
08-Oct	n/a	539.4
Mean	89.0	1010.4
St. dev.	86.0	277.2

Run B		
Date	NH3-N (mg/L)	TKN (mg/L)
10-Nov	5.1	289.8
12-Nov	6.8	-
16-Nov	n/a	-
20-Nov	20.4	-
27-Nov	30.6	640.8
1-Dec	22.1	807.1
4-Dec	17.0	825.5
7-Dec	40.0	788.6
11-Dec	38.3	696.2
15-Dec	57.0	881.0
18-Dec	8.5	-
Mean	24.6	704.1
St. dev.	16.8	199.8

Run C		
Date	NH3-N (mg/L)	TKN (mg/L)
08-Oct	35.7	-
16-Oct	19.6	974.0
19-Oct	11.4	1179.5
26-Oct	14.7	1111.0
30-Oct	16.3	1093.9
03-Nov	17.1	802.8
10-Nov	8.5	1162.4
12-Nov	17.0	939.8
20-Nov	11.3	-
24-Nov	14.2	-
27-Nov	11.3	1316.5
01-Dec	8.5	1316.5
Mean	15.5	1099.6
St. dev	7.3	171.0

Run D		
Date	NH3-N (mg/L)	TKN (mg/L)
12-Jan	3.6	554.2
15-Jan	5.4	360.2
05-Feb	5.4	517.3
09-Feb	7.1	563.5
16-Feb	3.6	572.7
19-Feb	10.7	600.4
24-Feb	5.4	-
27-Feb	7.1	628.1
02-Mar	5.4	572.7
09-Mar	4.6	637.4
15-Mar	4.6	581.9
Mean	5.7	558.8
St. dev	2.0	78.0

Table A.6: Effluent Ammonia and TKN

Run 1
No Data

Run A		
Date	NH3-N (mg/L)	TKN (mg/L)
29-Jul	n/a	-
05-Aug	n/a	-
12-Aug	119.9	298.0
18-Aug	6.8	259.4
21-Aug	26.2	208.1
25-Aug	81.2	95.0
28-Aug	48.9	118.2
01-Sep	66.7	-
04-Sep	29.6	-
19-Sep	n/a	246.6
25-Sep	n/a	118.2
01-Oct	n/a	146.4
08-Oct	n/a	123.3
Mean	54.2	179.2
St. dev.	38.4	74.7

Run B		
Date	NH3-N (mg/L)	TKN (mg/L)
10-Nov	5.1	234.4
12-Nov	6.0	-
16-Nov	n/a	-
20-Nov	2.6	-
27-Nov	8.5	86.6
1-Dec	33.2	142.0
4-Dec	20.4	160.5
7-Dec	25.5	215.9
11-Dec	45.9	178.9
15-Dec	15.3	123.5
18-Dec	14.5	-
Mean	17.7	163.1
St. dev.	13.9	51.6

Run C		
Date	NH3-N (mg/L)	TKN (mg/L)
08-Oct	31.3	-
16-Oct	16.3	126.3
19-Oct	14.7	208.5
26-Oct	14.7	119.5
30-Oct	14.7	177.7
03-Nov	11.4	184.5
10-Nov	2.8	273.6
12-Nov	2.3	167.4
20-Nov	3.4	-
24-Nov	2.8	-
27-Nov	4.5	256.5
01-Dec	4.5	249.6
Mean	10.3	195.9
St. dev	8.7	55.6

Run D		
Date	NH3-N (mg/L)	TKN (mg/L)
12-Jan	8.9	157.0
15-Jan	3.6	92.4
05-Feb	3.6	64.7
09-Feb	5.4	27.7
16-Feb	7.1	73.9
19-Feb	7.1	36.9
24-Feb	5.4	-
27-Feb	7.1	64.7
02-Mar	5.4	46.2
09-Mar	4.6	46.2
15-Mar	4.6	73.9
Mean	5.7	68.4
St. dev	1.7	36.7

Table A.7: Influent pH, Alkalinity (as $CaCO_3$), TS, VS, TSS, and VSS

Run 1						
Date	pH	Alkalinity (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
13-May	7.02	170	6320	5340	4150	3225
20-May	7.33	245	8170	6900	7850	6850
27-May	7.00	175	5960	5070	5550	4775
01-Jun	7.03	165	4570	3730	4025	3425
05-Jun	6.64	115	3780	2990	3475	2825
10-Jun	6.74	215	4750	4120	5225	4650
15-Jun	6.30	160	5020	4430	5000	4525
19-Jun	6.94	235	5720	4940	5475	5075
24-Jun	6.89	215	6320	5780	5375	5100
29-Jun	7.14	210	7520	6720	7275	6575
03-Jul	6.71	80	7180	6350	7250	6550
08-Jul	6.67	85	6110	5240	5850	5200
13-Jul	6.77	100	5510	4840	5125	4550
17-Jul	6.22	125	5470	4700	5050	4500
22-Jul	6.92	105	4810	4100	4675	4125
Mean	6.82	160	5814	5017	5423	4797
St. dev.	0.29	56	1184	1097	1232	1187

Run A						
Date	pH	Alkalinity (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
29-Jul	7.05	215	5940	4780	5825	4725
05-Aug	6.11	100	5320	4210	4975	3975
12-Aug	6.77	150	4430	3550	4325	3500
18-Aug	6.88	135	4950	4000	4350	3600
21-Aug	7.16	185	5910	4680	5900	4725
25-Aug	6.06	175	6310	5180	5775	4725
28-Aug	6.14	135	6000	4800	5800	4750
01-Sep	6.28	115	5430	3990	5100	3925
04-Sep	6.67	150	4750	3550	4425	3275
19-Sep	n/a	n/a	5120	4100	5325	4200
25-Sep	n/a	n/a	n/a	n/a	5450	4225
01-Oct	6.12	195	n/a	n/a	3725	2875
08-Oct	6.37	170	n/a	n/a	3300	2800
Mean	6.51	156.82	5416	4284	4944	3946
St. dev.	0.41	35	614	553	853	698

Table A.7 cont.

Run B						
Date	pH	Alkalinity (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
10-Nov	5.60	120	5610	3710	3650	3325
12-Nov	5.61	130	5340	3630	3975	3550
16-Nov	5.83	165	6100	4560	4500	4200
20-Nov	5.83	180	6040	4600	4700	4400
27-Nov	5.64	145	4950	3210	4525	4200
01-Dec	5.46	150	4520	2970	3725	3375
04-Dec	5.24	150	5920	4940	5275	5050
07-Dec	6.03	205	5820	4460	6100	5800
11-Dec	6.06	215	8130	6230	9450	8675
15-Dec	5.85	175	7470	5980	6400	5875
18-Dec	6.53	220	8950	7190	7875	7450
Mean	5.79	169	6259	4680	5470	5082
St. dev.	0.35	34	1364	1329	1842	1733

Run C						
Date	pH	Alkalinity (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
16-Oct	6.63	125	n/a	n/a	n/a	n/a
19-Oct	6.74	135	n/a	n/a	n/a	n/a
23-Oct	6.70	135	7150	6440	6825	6400
26-Oct	6.54	155	6930	5170	6525	6250
30-Oct	6.57	144	7010	4290	4525	4300
03-Nov	6.91	125	7230	3540	3650	3325
06-Nov	6.60	185	4570	2810	3225	2950
10-Nov	6.68	145	5260	3950	4050	3825
12-Nov	6.58	140	4040	2610	3050	2800
16-Nov	6.75	160	3870	2930	4225	4100
20-Nov	5.79	150	4190	2910	3475	3325
24-Nov	6.62	160	5080	4240	3650	3625
27-Nov	6.47	165	3990	2810	2800	2750
01-Dec	6.22	125	6550	4810	5575	5225
Mean	6.55	146	5489	3876	4298	4073
St. dev.	0.27	17	1383	1177	1334	1263

Table A.7 cont.

Run D						
Date	pH	Alkalinity (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
12-Jan	6.94	167.5	7690	7250	7825	7775
15-Jan	7.05	135	8860	8410	8775	8650
26-Jan	6.99	32.5	8290	8070	8300	8250
05-Feb	6.74	90	6800	6350	5900	5775
09-Feb	6.67	80	6900	5900	5600	5400
12-Feb	6.93	80	6570	6010	5550	5300
16-Feb	6.66	100	6620	5690	5325	5200
19-Feb	6.53	75	8520	7960	4500	4900
24-Feb	7.02	125	n/a	n/a	6200	6025
27-Feb	7.24	140	5320	4830	4675	4875
02-Mar	7.65	150	6110	5760	4700	4650
09-Mar	7.38	150	4480	4190	3400	3350
12-Mar	6.98	162.5	5940	5750	6025	5600
15-Mar	6.64	147.5	5810	5690	5250	5075
19-Mar	7.10	160	5090	4690	4440	4310
Mean	6.96	120	6643	6182	5764	5676
St. dev.	0.30	41	1320	1296	1507	1473

Table A.8: Reactor pH, Alkalinity (as $CaCO_3$), TS, VS, TSS, and VSS

Run 1						
Date	pH	Alkalinity (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
13-May	5.26	190	11720	5140	5575	4400
20-May	4.86	150	8400	6650	7900	6325
27-May	4.44	35	7230	5800	6525	5475
01-Jun	4.50	0	6190	4990	5450	4625
05-Jun	4.79	80	4910	3920	4175	3500
10-Jun	4.87	125	4610	3510	4450	3575
15-Jun	4.30	0	3870	3040	3250	2750
19-Jun	4.45	0	7070	5790	6650	5725
24-Jun	4.72	80	9700	8220	8950	7600
29-Jun	4.45	0	9200	7620	8800	7450
03-Jul	4.44	0	10870	9410	10350	9175
08-Jul	4.20	0	9160	7860	8725	7675
13-Jul	4.30	0	4450	3800	9025	8050
17-Jul	4.44	0	7520	6370	6625	5800
22-Jul	4.46	0	7460	6540	6175	5525
Mean	4.56	44	7491	5911	6842	5843
St. dev.	0.28	65	2390	1877	2069	1876

Run A						
Date	pH	Alkalinity (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
29-Jul	5.20	195	7830	5780	9675	7675
05-Aug	5.23	195	7230	5730	6650	5375
12-Aug	4.88	120	5870	4680	5300	4325
18-Aug	4.92	130	6370	5200	5750	4875
21-Aug	4.96	150	6340	5040	5650	4550
25-Aug	4.96	165	9750	7460	9350	7325
28-Aug	4.97	160	11710	8930	11200	8850
01-Sep	4.94	140	9800	7360	8675	6725
04-Sep	5.11	135	7140	5460	6350	4975
19-Sep	n/a	n/a	14260	11040	13375	10500
25-Sep	n/a	n/a	n/a	n/a	8350	6500
01-Oct	4.96	205	n/a	n/a	8150	6400
08-Oct	4.86	145	n/a	n/a	7550	6000
Mean	5.00	158	8630	6668	8156	6467
St. dev.	0.13	29	2720	2034	2357	1795

Table A.8 cont.

Run B						
Date	pH	Alkalinity	TS	VS	TSS	VSS
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
10-Nov	4.53	0	9410	7320	8650	7425
12-Nov	4.50	0	7600	5860	6825	5850
16-Nov	4.38	0	7430	5760	6600	5825
20-Nov	4.37	0	8540	6830	7775	6875
27-Nov	4.33	0	9250	6990	8200	7350
01-Dec	4.36	0	7030	5340	6500	6075
04-Dec	4.45	0	13680	11380	12950	11775
07-Dec	4.44	0	12000	10270	10900	9950
11-Dec	4.74	70	11910	9500	10150	9475
15-Dec	4.52	0	14560	12300	12975	11675
18-Dec	4.33	0	15450	13280	14525	13275
Mean	4.45	6	10624	8621	9641	8686
St. dev.	0.12	21	3027	2837	2852	2673

Run C						
Date	pH	Alkalinity	TS	VS	TSS	VSS
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
16-Oct	4.29	0	20380	16640	9625	8425
19-Oct	4.15	0	15300	12270	6800	6075
23-Oct	4.05	0	11420	10190	11200	10250
26-Oct	4.17	0	15570	12850	15075	14225
30-Oct	4.34	0	11440	8190	10350	9750
03-Nov	4.28	0	9740	5620	6625	5975
06-Nov	4.19	0	10980	7340	9000	8150
10-Nov	4.18	0	7810	6110	7025	6625
12-Nov	4.23	0	7450	5940	6750	6250
16-Nov	4.35	0	7680	6460	7475	7100
20-Nov	4.28	0	6320	4770	5025	4825
24-Nov	4.28	0	4010	2980	2800	2900
27-Nov	4.19	0	4690	3490	3650	3500
01-Dec	4.44	0	11930	9550	10125	9250
Mean	4.24	0	9087	8029	7966	7379
St. dev.	0.10	0	3360	3882	3221	2950

Table A.8 cont.

Run D						
Date	pH	Alkalinity (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
12-Jan	4.11	0	15280	14590	15200	14975
15-Jan	4.46	0	17120	16570	18200	17900
26-Jan	4.18	0	32630	32180	33325	33050
05-Feb	3.77	0	40380	39270	52450	52025
09-Feb	3.92	0	37140	35720	38325	37925
12-Feb	3.97	0	33860	33040	36425	36075
16-Feb	4.04	0	33300	32570	36600	36450
19-Feb	4.14	0	30520	29330	43575	43125
24-Feb	3.96	0	32530	31350	38725	38650
27-Feb	3.89	0	27640	27320	29900	29700
02-Mar	3.95	0	27060	26510	26850	26750
09-Mar	3.55	0	58430	57460	61300	60650
12-Mar	3.80	0	61260	60250	57875	56850
15-Mar	3.64	0	44040	43600	65900	65200
19-Mar	3.84	0	28450	27880	46920	46280
Mean	3.95	0	34643	33843	40105	39707
St. dev.	0.23	0	12696	12581	14876	14679

Table A.9: Effluent pH, Alkalinity (as $CaCO_3$), TS, VS, TSS, and VSS

Run 1						
Date	pH	Alkalinity (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
13-May	6.13	165	1410	990	725	625
20-May	5.75	205	1250	870	350	325
27-May	4.54	0	7700	6130	2875	2325
01-Jun	4.62	70	3190	2460	2400	1950
05-Jun	4.72	65	3100	2380	2325	1850
10-Jun	5.30	160	910	630	225	300
15-Jun	5.00	130	1260	1000	450	425
19-Jun	4.50	0	1870	1480	925	1100
24-Jun	4.91	110	1670	1410	600	700
29-Jun	4.88	110	1480	1070	700	600
03-Jul	4.51	0	1430	1050	675	575
08-Jul	4.29	0	2580	1950	1700	1325
13-Jul	4.18	0	2770	2190	1800	1600
17-Jul	4.45	0	2000	1490	1025	875
22-Jul	4.47	0	2210	1740	1200	1150
Mean	4.81	68	2322	1789	1198	1048
St. dev.	0.54	74	1645	1328	827	638

Run A						
Date	pH	Alkalinity (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
29-Jul	5.26	175	2230	1670	1350	1075
05-Aug	5.01	185	8700	6370	7075	5775
12-Aug	4.77	170	3690	2810	2850	2300
18-Aug	4.89	125	3290	2610	2525	2050
21-Aug	4.91	130	2700	2150	1825	1550
25-Aug	5.14	140	1380	1130	575	525
28-Aug	5.31	185	1850	1430	1450	1225
01-Sep	4.83	130	4550	3240	3600	1000
04-Sep	4.84	100	5220	3850	4375	950
19-Sep	n/a	n/a	2920	1990	2200	1575
25-Sep	n/a	n/a	2940	1880	600	375
01-Oct	4.99	170	4250	2240	1050	800
08-Oct	4.98	160	5380	3200	1700	1425
Mean	4.99	152	3777	2659	2398	1587
St. dev.	0.18	28	1924	1359	1795	1374

Table A.9 cont.

Run B						
Date	pH	Alkalinity	TS	VS	TSS	VSS
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
10-Nov	4.29	0	6500	4620	5250	4575
12-Nov	4.44	0	6110	4390	6300	5425
16-Nov	4.42	0	3330	2110	2100	1850
20-Nov	4.44	0	2210	1140	975	975
27-Nov	4.29	0	1840	590	525	400
01-Dec	4.45	0	1490	740	950	775
04-Dec	4.58	0	2220	1230	1150	950
07-Dec	4.48	0	3180	1260	1200	1175
11-Dec	4.90	115	1940	590	600	350
15-Dec	5.04	135	2390	970	1175	900
18-Dec	4.59	0	2360	1040	850	825
Mean	4.54	23	3052	1698	1916	1655
St. dev.	0.24	51	1697	1450	1966	1711

Run C						
Date	pH	Alkalinity	TS	VS	TSS	VSS
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
16-Oct	4.57	35	2630	1590	350	175
19-Oct	4.17	0	4580	2760	1325	1125
23-Oct	4.10	0	1390	980	800	575
26-Oct	4.15	0	2300	840	725	600
30-Oct	4.17	0	3670	n/a	1075	950
03-Nov	4.18	0	5100	2120	2100	875
06-Nov	4.16	0	6650	4710	5375	975
10-Nov	4.18	0	3520	2310	2750	1575
12-Nov	4.21	0	2190	1160	1225	1025
16-Nov	4.15	0	2470	1690	3000	2025
20-Nov	4.20	0	2840	1770	1900	1775
24-Nov	4.19	0	2340	1480	2075	2050
27-Nov	4.18	0	1900	930	875	925
01-Dec	4.45	0	2100	1120	1200	1100
Mean	4.22	3	3039	1805	1770	1125
St. dev.	0.13	9	1506	1046	1296	550

Table A.9 cont.

Run D						
Date	pH	Alkalinity (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
12-Jan	4.04	0	3930	3630	3200	3200
15-Jan	4.40	0	2070	1780	1200	1150
26-Jan	4.27	0	1530	1380	1175	1075
05-Feb	3.77	0	3250	2800	1225	875
09-Feb	3.84	0	3400	2900	1375	1275
12-Feb	3.84	0	3360	2730	1050	975
16-Feb	4.01	0	3550	3020	1025	1025
19-Feb	3.98	0	4540	3980	1000	1000
24-Feb	3.97	0	3960	3400	725	725
27-Feb	4.01	0	2390	2290	475	475
02-Mar	4.04	0	3010	2730	675	600
09-Mar	3.71	0	2490	2260	900	825
12-Mar	3.71	0	2260	2110	500	325
15-Mar	3.69	0	1900	1920	300	200
19-Mar	3.83	0	2580	2050	230	150
Mean	3.94	0	2948	2599	1004	925
St. dev.	0.20	0	860	723	702	719

Table A.10: System HRT

Run 1				
Date	Vol. diff. (mL)	Time diff. (hrs)	Flow (mL/hr)	HRT (hrs)
09-May	1560	26.0	60.0	50.0
11-May	10042	43.5	230.9	13.0
12-May	3670	24.0	152.9	19.6
13-May	3583	22.0	162.9	18.4
14-May	1750	25.5	68.6	43.7
15-May	3667	25.0	146.7	20.5
17-May	4333	49.0	88.4	33.9
19-May	4250	46.5	91.4	32.8
20-May	2667	21.5	124.0	24.2
21-May	2250	23.5	95.7	31.3
22-May	1920	23.5	81.7	36.7
24-May	6083	51.0	119.3	25.2
25-May	1750	19.0	92.1	32.6
26-May	1833	25.5	71.9	41.7
27-May	2767	24.0	115.3	26.0
28-May	1733	23.5	73.7	40.7
29-May	1500	25.5	58.8	51.0
31-May	4833	50.5	95.7	31.3
01-Jun	1833	21.0	87.3	34.4
02-Jun	2500	20.0	125.0	24.0
04-Jun	4667	55.5	84.1	35.7
05-Jun	2250	25.5	88.2	34.0
08-Jun	6250	66.5	94.0	31.9
09-Jun	2083	19.5	106.8	28.1
10-Jun	2500	23.5	106.4	28.2
12-Jun	4500	48.5	92.8	32.3
15-Jun	6200	70.5	87.9	34.1
18-Jun	2000	26.0	76.9	39.0
19-Jun	1850	20.5	90.2	33.2
20-Jun	2400	28.0	85.7	35.0
22-Jun	3667	45.0	81.5	36.8
23-Jun	2250	22.5	100.0	30.0
24-Jun	2083	24.5	85.0	35.3
25-Jun	1833	23.5	78.0	38.5
27-Jun	4000	49.0	81.6	36.8
29-Jun	3583	46.5	77.1	38.9
01-Jul	4250	49.5	85.9	34.9
02-Jul	1083	21.0	51.6	58.2
03-Jul	1833	23.5	78.0	38.5
04-Jul	2333	26.0	89.7	33.4
08-Jul	7750	93.5	82.9	36.2
17-Jul	1500	20.0	75.0	40.0
19-Jul	4667	50.5	92.4	32.5
22-Jul	5833	71.0	82.2	36.5
			Mean	33.8
			St. dev.	8.4

Table A.10 cont.

Run A				
Date	Vol. diff. (mL)	Time diff. (hrs)	Flow (mL/hr)	HRT (hrs)
23-Jul	2583	21.5	120.2	25.0
24-Jul	2000	23.5	85.1	35.3
26-Jul	3667	46.5	78.9	38.0
29-Jul	6000	70.0	85.7	35.0
03-Aug	10000	116.0	86.2	34.8
04-Aug	2667	27.5	97.0	30.9
05-Aug	2000	23.0	87.0	34.5
07-Aug	5333	53.0	100.6	29.8
08-Aug	1833	22.0	83.3	36.0
10-Aug	3667	46.0	79.7	37.6
12-Aug	4667	48.0	97.2	30.9
14-Aug	5250	45.0	116.7	25.7
17-Aug	6950	72.5	95.9	31.3
18-Aug	1133	19.5	58.1	51.6
19-Aug	2500	23.5	106.4	28.2
20-Aug	2167	27.0	80.2	37.4
21-Aug	2250	22.0	102.3	29.3
22-Aug	2250	25.5	88.2	34.0
24-Aug	4333	47.5	91.2	32.9
25-Aug	3250	25.5	127.5	23.5
28-Aug	6750	70.0	96.4	31.1
31-Aug	3000	24.0	125.0	24.0
01-Sep	1000	17.5	57.1	52.5
04-Sep	8167	73.0	111.9	26.8
06-Sep	4167	44.0	94.7	31.7
17-Sep	1167	23.0	50.7	59.1
18-Sep	2750	24.5	112.2	26.7
20-Sep	4667	48.5	96.2	31.2
22-Sep	4500	53.5	84.1	35.7
25-Sep	6667	66.0	101.0	29.7
			Mean	33.7
			St. dev.	8.2

Table A.10 cont.

Run B				
Date	Vol. diff. (mL)	Time diff. (hrs)	Flow (mL/hr)	HRT (hrs)
11-Nov	2333	23.5	99.3	30.2
12-Nov	2000	24.5	81.6	36.8
13-Nov	2333	28.0	83.3	36.0
14-Nov	2167	19.5	111.1	27.0
16-Nov	4500	45.5	98.9	30.3
17-Nov	2417	23.0	105.1	28.6
20-Nov	4083	43.0	95.0	31.6
23-Nov	6667	73.0	91.3	32.9
25-Nov	2500	21.5	116.3	25.8
27-Nov	5500	56.5	97.3	30.8
29-Nov	3500	40.5	86.4	34.7
01-Dec	4500	48.0	93.8	32.0
03-Dec	4500	47.0	95.7	31.3
05-Dec	2500	23.5	106.4	28.2
07-Dec	4000	47.5	84.2	35.6
10-Dec	5833	72.0	81.0	37.0
11-Dec	2333	27.5	84.8	35.4
12-Dec	2000	22.0	90.9	33.0
14-Dec	4400	44.5	98.9	30.3
15-Dec	2600	28.0	92.9	32.3
18-Dec	6000	69.5	86.3	34.8
			Mean	32.1
			St. dev.	3.2

Run C				
Date	Vol. diff. (mL)	Time diff. (hrs)	Flow (mL/hr)	HRT (hrs)
09-Oct	2167	28.0	77.4	38.8
11-Oct	5167	44.0	117.4	25.5
20-Oct	2250	22.5	100.0	30.0
21-Oct	2000	24.5	81.6	36.8
23-Oct	4250	43.5	97.7	30.7
24-Oct	2750	30.5	90.2	33.3
25-Oct	1833	22.5	81.5	36.8
26-Oct	2167	22.5	96.3	31.2
29-Oct	6000	70.0	85.7	35.0
30-Oct	2750	24.0	114.6	26.2
31-Oct	1833	24.5	74.8	40.1
01-Nov	2150	23.0	93.5	32.1
02-Nov	2250	24.5	91.8	32.7
03-Nov	2333	25.0	93.3	32.1
05-Nov	4167	46.5	89.6	33.5
06-Nov	1833	23.5	78.0	38.5
07-Nov	2000	24.0	83.3	36.0
09-Nov	5000	52.5	95.2	31.5

Table A.10 cont.

Run C cont.				
Date	Vol. diff.	Time diff.	Flow	HRT
	(mL)	(hrs)	(mL/hr)	(hrs)
11-Nov	2333	23.5	99.3	30.2
12-Nov	2000	24.5	81.6	36.8
13-Nov	2333	28.0	83.3	36.0
14-Nov	2167	19.5	111.1	27.0
16-Nov	4500	45.5	98.9	30.3
17-Nov	2417	23.0	105.1	28.6
20-Nov	4083	43.0	95.0	31.6
23-Nov	6667	73.0	91.3	32.9
25-Nov	2500	21.5	116.3	25.8
27-Nov	5500	56.5	97.3	30.8
29-Nov	3500	40.5	86.4	34.7
01-Dec	4500	48.0	93.8	32.0
03-Dec	4500	47.0	95.7	31.3
			Mean	32.6
			St. dev.	3.7

Run D				
Date	Vol. diff.	Time diff.	Flow	HRT
	(mL)	(hrs)	(mL/hr)	(hrs)
20-Dec	3250	36.0	90.3	33.2
03-Jan	4583	49.0	93.5	32.1
12-Jan	3000	26.0	115.4	26.0
28-Jan	2233	24.0	93.1	32.2
05-Feb	6417	68.0	94.4	31.8
07-Feb	4833	51.5	93.9	32.0
09-Feb	4750	46.5	102.2	29.4
11-Feb	4500	49.0	91.8	32.7
12-Feb	1833	21.0	87.3	34.4
15-Feb	6833	72.5	94.3	31.8
16-Feb	1833	19.0	96.5	31.1
19-Feb	2083	20.0	104.2	28.8
21-Feb	4500	49.5	90.9	33.0
24-Feb	5667	70.0	81.0	37.1
26-Feb	2833	31.0	91.4	32.8
27-Feb	2000	22.0	90.9	33.0
03-Mar	8333	97.0	85.9	34.9
04-Mar	2083	18.0	115.7	25.9
11-Mar	3000	24.0	125.0	24.0
12-Mar	2833	24.0	118.1	25.4
15-Mar	7167	72.0	99.5	30.1
16-Mar	3000	24.0	125.0	24.0
17-Mar	3000	29.5	101.7	29.5
19-Mar	4000	42.0	95.2	31.5
			Mean	30.7
			St. dev.	3.5

APPENDIX B: DENITRIFICATION STUDY DATA

Table B.1: Experimental Results for Run 1

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
0	1	51.84	24.14	17.9	6.29	100	10000	7000
	2	73.56	34.80	20.7	6.51	180	17250	9250
	3	61.31	29.12	17.0	6.35	160	14750	8000
	4	125.33	62.20	20.0	6.16	150	12750	10500
	5	144.99	71.27	0.0	6.25	170	13250	10250
	6	70.33	33.74	0.8	6.55	200	17000	10750
24	1	0.00	0.00	16.2	n/a	n/a	n/a	n/a
	2	0.00	0.00	20.8	n/a	n/a	n/a	n/a
	3	0.00	0.00	17.8	n/a	n/a	n/a	n/a
	4	0.00	0.00	0.0	n/a	n/a	n/a	n/a
	5	54.98	20.52	17.8	n/a	n/a	n/a	n/a
	6	0.00	0.00	0.8	n/a	n/a	n/a	n/a
96	1	0.00	0.00	0.0	n/a	n/a	n/a	n/a
	2	0.00	0.00	23.1	n/a	n/a	n/a	n/a
	3	0.00	0.00	19.6	n/a	n/a	n/a	n/a
	4	105.71	43.59	0.0	n/a	n/a	n/a	n/a
	5	119.45	46.05	0.0	n/a	n/a	n/a	n/a
	6	0.00	0.00	3.6	n/a	n/a	n/a	n/a
120	1	0.00	0.00	0.0	n/a	n/a	n/a	n/a
	2	0.00	0.00	1.4	n/a	n/a	n/a	n/a
	3	0.00	0.00	20.2	n/a	n/a	n/a	n/a
	4	148.88	59.50	23.2	n/a	n/a	n/a	n/a
	5	7.51	0.62	4.5	n/a	n/a	n/a	n/a
	6	0.00	0.00	0.0	n/a	n/a	n/a	n/a

Table B.2: Experimental Results for Run 2

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
0	1	390.28	208.30	16.1	6.39	480	11375	9025
	2	426.56	224.98	16.4	6.39	460	12475	10125
	3	535.46	283.27	15.5	6.11	480	11800	9725
	4	533.77	289.69	17.0	6.12	420	11525	9300
	5	396.64	214.23	0.0	5.98	380	11900	9550
	6	485.04	260.47	0.0	6.08	400	12100	9850
24	1	536.28	248.65	n/a	6.67	760	n/a	n/a
	2	630.94	303.76	n/a	6.80	640	n/a	n/a
	3	742.33	358.34	n/a	6.62	560	n/a	n/a
	4	536.07	275.37	n/a	6.91	820	n/a	n/a
	5	742.46	374.16	n/a	6.63	580	n/a	n/a
	6	586.99	293.34	n/a	6.45	520	n/a	n/a
48	1	606.03	272.69	n/a	7.03	600	n/a	n/a
	2	914.18	460.77	n/a	6.89	660	n/a	n/a
	3	868.41	440.96	n/a	7.05	600	n/a	n/a
	4	870.98	478.08	n/a	7.08	760	n/a	n/a
	5	918.34	443.32	n/a	6.60	540	n/a	n/a
	6	881.23	477.30	n/a	6.88	580	n/a	n/a
72	1	588.99	262.07	n/a	6.95	600	n/a	n/a
	2	777.21	401.20	n/a	6.63	580	n/a	n/a
	3	710.03	378.21	n/a	6.81	640	n/a	n/a
	4	779.03	393.74	n/a	6.89	720	n/a	n/a
	5	828.97	400.84	n/a	6.69	640	n/a	n/a
	6	709.77	351.86	n/a	6.78	600	n/a	n/a
120	1	954.81	427.72	n/a	n/a	n/a	n/a	n/a
	2	1229.28	611.93	n/a	n/a	n/a	n/a	n/a
	3	823.19	441.19	n/a	n/a	n/a	n/a	n/a
	4	53.74	34.16	n/a	n/a	n/a	n/a	n/a
	5	1064.90	497.35	n/a	n/a	n/a	n/a	n/a
	6	689.60	334.23	n/a	n/a	n/a	n/a	n/a
192	1	883.97	363.78	n/a	5.92	440	n/a	n/a
	2	1383.66	670.18	n/a	5.33	500	n/a	n/a
	3	1049.12	517.10	n/a	5.48	580	n/a	n/a
	4	650.65	261.95	n/a	7.20	480	n/a	n/a
	5	1387.88	663.86	n/a	5.63	520	n/a	n/a
	6	1376.60	664.88	n/a	5.55	540	n/a	n/a

Table B.2 cont.

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
216	1	870.52	361.64	n/a	6.19	380	n/a	n/a
	2	1511.16	747.91	n/a	5.28	480	n/a	n/a
	3	1299.90	635.56	n/a	5.31	420	n/a	n/a
	4	662.05	277.62	n/a	6.56	480	n/a	n/a
	5	989.58	474.74	n/a	5.48	500	n/a	n/a
	6	1338.72	594.59	n/a	5.54	500	n/a	n/a
312	1	1204.27	558.43	n/a	5.59	480	6250	4625
	2	1549.17	703.92	n/a	5.77	500	6700	5200
	3	1478.47	730.82	n/a	5.48	560	5450	3975
	4	949.31	441.67	n/a	6.55	560	6875	5000
	5	1491.77	727.13	n/a	5.51	560	6875	5300
	6	1322.80	613.88	n/a	5.56	580	7350	5700

Table B.3: Experimental Results for Run 3

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
0	1	94.20	50.72	23.3	6.37	170	5650	4525
	2	181.83	97.81	58.1	6.28	220	9700	7875
	3	150.21	82.04	24.5	6.34	170	6125	4925
	4	136.13	74.56	60.5	6.01	180	4550	3675
	6	152.57	84.79	86.0	6.42	200	5625	4600
5	1	161.43	73.85	7.3	6.33	330	n/a	n/a
	2	149.09	66.56	47.8	6.57	340	n/a	n/a
	3	202.40	95.38	13.7	6.28	360	n/a	n/a
	4	415.36	214.57	59.7	5.85	310	n/a	n/a
	6	213.56	107.40	89.2	5.97	320	n/a	n/a
24	1	277.61	126.69	0.0	6.09	400	n/a	n/a
	2	191.16	88.19	0.0	6.61	760	n/a	n/a
	3	305.03	137.75	0.0	6.03	470	n/a	n/a
	4	365.27	163.84	0.0	6.46	740	n/a	n/a
	6	227.00	111.11	86.0	5.84	310	n/a	n/a
51	1	283.79	124.44	0.0	6.40	400	n/a	n/a
	2	314.50	145.06	0.0	6.73	710	n/a	n/a
	3	545.16	259.48	0.0	5.97	460	n/a	n/a
	4	774.51	361.87	0.0	6.47	710	n/a	n/a
	6	460.63	219.19	90.8	5.67	380	n/a	n/a
100	1	959.99	421.56	0.0	5.28	390	8200	7075
	2	158.83	65.10	0.0	6.37	590	8175	7000
	3	1254.94	543.16	0.0	5.23	340	8925	7425
	4	1275.27	593.78	0.0	5.25	490	8675	7250
	6	902.51	454.64	84.4	5.16	620	9175	7850

Table B.4: Experimental Results for Run 4

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
0	1	215.64	126.85	32.4	5.11	n/a	6550	5650
	2	655.79	390.20	88.6	4.74	n/a	10050	8400
	3	408.83	246.01	25.3	4.85	n/a	10550	9050
	4	989.87	594.74	78.5	4.70	n/a	9550	8250
	5	1063.38	648.69	103.8	4.69	n/a	8200	6800
3	1	514.21	290.54	38.5	4.96	n/a	15800	12900
	2	1221.93	704.92	92.4	4.81	n/a	18700	15050
	3	860.53	484.69	32.3	4.79	n/a	18500	14650
	4	1373.98	794.07	86.1	4.65	n/a	16600	13450
	5	872.67	515.00	110.1	4.62	n/a	16900	13550
6	1	592.42	334.81	32.9	5.04	n/a	18050	14050
	2	1209.71	682.35	87.3	4.92	n/a	18800	15200
	3	1108.59	618.69	33.4	4.90	n/a	18450	14400
	4	1282.66	748.54	83.5	4.83	n/a	17000	13450
	5	1628.05	936.88	103.8	4.76	n/a	18200	14600
24	1	430.26	206.82	0.0	5.87	n/a	n/a	n/a
	2	1478.86	729.57	86.1	5.25	n/a	n/a	n/a
	3	1250.48	600.22	7.1	5.35	n/a	n/a	n/a
	4	660.33	361.11	79.7	5.02	n/a	n/a	n/a
	5	1294.62	632.53	97.4	4.84	n/a	n/a	n/a
30	1	378.82	188.85	0.0	5.84	n/a	15650	12350
	2	626.08	308.69	16.5	5.90	n/a	17250	13550
	3	918.45	445.83	0.0	5.74	n/a	13800	11150
	4	952.45	467.91	54.4	5.30	n/a	12400	9800
	5	1834.48	864.29	40.5	5.18	n/a	13350	10800

Table B.5: Complete Nitrate Values for Run 4

Hour	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Reactor 5
0	32.4	88.6	25.3	78.5	103.8
1	35.4	68.3	33.4	64.5	97.4
2	23.8	55.7	26.8	65.8	122.8
3	38.5	92.4	32.3	86.1	110.1
4	40.5	92.4	30.4	62.8	60.7
5	32.9	54.7	24.3	88.6	107.6
6	32.9	87.3	33.4	83.5	103.8
7	45.6	97.4	35.9	89.9	115.2
24	0.0	86.1	7.1	79.7	97.4
27	0.0	64.5	0.0	77.2	87.3
30	0.0	16.5	0.0	54.4	40.5

Table B.6: Experimental Results for Run 5

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
0	1	86.92	48.10	19.0	5.81	30	5350	4800
	2	180.08	93.08	44.6	5.40	100	4300	3800
	3	120.01	63.16	16.6	5.58	90	3050	2700
	4	249.49	129.92	50.2	5.06	90	4850	4200
	5	338.94	176.42	96.9	4.94	80	3300	2750
	6	189.03	97.27	0.0	5.19	80	4200	3600
1	1	120.41	66.35	19.9	5.44	n/a	n/a	n/a
	2	239.28	127.63	45.9	5.24	n/a	n/a	n/a
	3	197.86	106.23	18.3	5.34	n/a	n/a	n/a
	4	302.13	160.85	57.1	5.13	n/a	n/a	n/a
	5	364.33	192.06	96.9	5.07	n/a	n/a	n/a
	6	240.78	127.14	0.0	5.14	n/a	n/a	n/a
2	1	174.81	96.81	18.7	5.39	n/a	n/a	n/a
	2	265.78	139.81	46.7	5.31	n/a	n/a	n/a
	3	204.26	108.95	19.9	5.31	n/a	n/a	n/a
	4	337.96	180.38	46.3	5.17	n/a	n/a	n/a
	5	408.94	214.81	84.8	5.09	n/a	n/a	n/a
	6	298.75	158.70	0.0	5.12	n/a	n/a	n/a
3	1	184.81	97.13	22.8	5.39	230	n/a	n/a
	2	332.49	174.93	58.4	5.41	180	n/a	n/a
	3	258.46	136.20	23.4	5.39	190	n/a	n/a
	4	347.64	183.93	62.3	5.18	160	n/a	n/a
	5	341.32	182.31	148.0	5.12	220	n/a	n/a
	6	332.87	171.53	0.0	5.25	120	n/a	n/a
4	1	207.38	112.89	17.0	5.36	n/a	n/a	n/a
	2	322.32	169.23	57.1	5.38	n/a	n/a	n/a
	3	269.78	143.12	16.6	5.41	n/a	n/a	n/a
	4	382.11	202.12	49.3	5.18	n/a	n/a	n/a
	5	384.73	207.94	131.5	5.19	n/a	n/a	n/a
	6	377.81	201.93	0.0	5.19	n/a	n/a	n/a
5	1	195.28	106.12	17.3	5.31	n/a	n/a	n/a
	2	352.42	184.14	44.1	5.32	n/a	n/a	n/a
	3	268.50	139.66	16.3	5.35	n/a	n/a	n/a
	4	390.12	205.61	44.1	5.18	n/a	n/a	n/a
	5	490.31	255.11	85.7	5.13	n/a	n/a	n/a
	6	372.57	198.03	0.0	5.12	n/a	n/a	n/a

Table B.6 cont.

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
6	1	193.28	104.44	16.3	5.37	200	12850	10600
	2	325.48	166.96	43.3	5.42	230	11250	9750
	3	314.95	162.15	14.9	5.49	240	13300	10950
	4	407.67	214.20	42.4	5.25	240	13400	11450
	5	525.26	271.52	116.0	5.17	240	13600	11050
	6	420.15	218.23	0.0	5.24	180	13150	10750
7	1	191.81	103.27	13.8	5.5	n/a	n/a	n/a
	2	383.94	195.34	39.8	5.38	n/a	n/a	n/a
	3	329.66	168.37	12.8	5.42	n/a	n/a	n/a
	4	454.32	239.61	38.9	5.18	n/a	n/a	n/a
	5	518.35	271.05	76.2	5.21	n/a	n/a	n/a
	6	442.38	226.33	0.0	5.19	n/a	n/a	n/a
24	1	73.77	41.17	0.0	5.87	330	12600	10250
	2	214.22	107.64	0.0	6.27	380	9950	8850
	3	310.63	148.64	0.0	5.74	300	11550	10100
	4	443.15	217.30	0.0	5.8	410	10500	9350
	5	439.62	218.14	29.4	5.93	470	13000	11400
	6	576.64	258.05	0.0	5.22	200	12600	10400
27	1	82.49	46.85	0.0	5.97	n/a	n/a	n/a
	2	225.47	112.12	0.0	6.08	n/a	n/a	n/a
	3	295.85	142.79	0.0	5.75	n/a	n/a	n/a
	4	467.45	227.80	0.0	5.87	n/a	n/a	n/a
	5	373.00	183.07	8.2	6.25	n/a	n/a	n/a
	6	471.25	210.12	0.0	5.25	n/a	n/a	n/a
30	1	64.74	35.55	0.0	6.06	360	10600	9000
	2	245.00	119.57	0.0	6.21	410	9100	8000
	3	342.94	160.99	0.0	5.69	300	10050	9100
	4	472.21	228.01	0.0	5.9	440	10700	9600
	5	335.32	166.57	0.0	6.33	680	9650	8550
	6	533.83	237.72	0.0	5.2	210	11600	9950

Table B.7: Experimental Results for Run 6

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
0	1	315.43	183.48	50.8	5.48	150	n/a	n/a
	2	442.75	245.73	60.6	4.89	100	n/a	n/a
	3	621.31	340.39	116.6	4.92	80	n/a	n/a
	4	987.62	537.26	102.2	4.63	70	n/a	n/a
	5	771.29	425.16	227.9	4.65	50	n/a	n/a
	6	988.75	539.38	204.3	4.69	80	n/a	n/a
2	1	291.48	168.84	46.7	5.64	290	n/a	n/a
	2	486.23	270.59	53.1	5.24	120	n/a	n/a
	3	603.12	335.52	106.8	5.13	230	n/a	n/a
	4	1031.91	548.12	98.7	4.97	250	n/a	n/a
	5	832.79	443.45	179.4	5.14	310	n/a	n/a
	6	999.76	540.89	196.2	5.03	250	n/a	n/a
4	1	275.31	154.32	45.6	5.58	240	n/a	n/a
	2	476.16	263.08	52.5	5.32	190	n/a	n/a
	3	633.44	349.58	113.7	5.23	230	n/a	n/a
	4	946.85	510.97	104.5	4.97	270	n/a	n/a
	5	769.79	413.63	189.1	5.22	360	n/a	n/a
	6	927.71	508.40	237.8	5.06	300	n/a	n/a
6	1	315.18	171.20	39.8	5.75	250	n/a	n/a
	2	471.69	258.47	49.6	5.38	190	n/a	n/a
	3	644.96	353.40	113.7	5.25	240	n/a	n/a
	4	948.84	509.39	109.1	4.99	270	n/a	n/a
	5	864.28	460.94	193.9	5.21	360	n/a	n/a
	6	1024.91	572.22	175.5	5.11	300	n/a	n/a
8	1	293.91	166.97	36.9	5.93	290	n/a	n/a
	2	425.45	235.15	44.4	5.47	200	n/a	n/a
	3	640.29	348.27	107.9	5.26	280	n/a	n/a
	4	931.95	508.11	121.8	5.08	290	n/a	n/a
	5	858.53	461.60	223.9	5.29	390	n/a	n/a
	6	989.25	540.40	168.5	5.12	320	n/a	n/a
15	1	203.50	127.19	21.4	6.26	310	n/a	n/a
	2	332.79	198.85	25.4	5.85	270	n/a	n/a
	3	543.27	305.07	76.8	5.61	310	n/a	n/a
	4	831.60	457.67	89.5	5.23	310	n/a	n/a
	5	808.53	438.34	177.8	5.52	400	n/a	n/a
	6	985.25	540.97	157.0	5.23	350	n/a	n/a

Table B.7 cont.

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
24	1	166.41	100.07	7.8	6.37	420	n/a	n/a
	2	183.35	126.56	6.0	6.37	360	n/a	n/a
	3	422.40	250.12	54.8	6.06	440	n/a	n/a
	4	644.48	381.72	55.7	5.73	430	n/a	n/a
	5	510.21	317.22	152.4	6.20	580	n/a	n/a
	6	780.47	455.41	98.1	5.86	500	n/a	n/a
26	1	148.40	90.35	0.9	6.66	450	n/a	n/a
	2	137.38	100.95	3.2	6.60	410	n/a	n/a
	3	390.50	232.23	49.6	6.33	430	n/a	n/a
	4	612.17	367.62	71.9	5.95	450	n/a	n/a
	5	450.82	289.34	148.9	6.36	620	n/a	n/a
	6	724.67	422.63	142.0	5.94	470	n/a	n/a
28	1	114.36	73.42	0.0	6.50	490	n/a	n/a
	2	86.60	69.33	0.7	6.72	460	n/a	n/a
	3	365.70	218.45	52.5	6.39	470	n/a	n/a
	4	559.87	345.76	68.4	5.89	450	n/a	n/a
	5	387.48	266.44	163.9	6.50	640	n/a	n/a
	6	746.21	443.31	107.3	5.97	480	n/a	n/a
30	1	89.51	59.51	0.0	6.62	n/a	n/a	n/a
	2	55.29	43.12	0.0	6.57	n/a	n/a	n/a
	3	345.65	205.60	39.2	6.26	n/a	n/a	n/a
	4	474.41	295.79	62.0	5.84	n/a	n/a	n/a
	5	319.31	226.97	139.7	6.22	n/a	n/a	n/a
	6	655.55	391.06	94.7	5.69	n/a	n/a	n/a
32	1	48.17	36.17	0.0	6.53	n/a	n/a	n/a
	2	30.39	23.53	0.0	6.46	n/a	n/a	n/a
	3	298.28	182.72	38.7	6.25	n/a	n/a	n/a
	4	446.89	290.16	53.7	5.83	n/a	n/a	n/a
	5	249.44	193.44	114.3	6.25	n/a	n/a	n/a
	6	622.62	370.95	92.3	5.73	n/a	n/a	n/a
48	1	completed	completed	completed	completed	completed	n/a	n/a
	2	completed	completed	completed	completed	completed	n/a	n/a
	3	35.96	29.08	0.0	6.80	790	n/a	n/a
	4	319.21	222.19	29.7	6.58	670	n/a	n/a
	5	28.72	24.96	63.7	6.88	1000	n/a	n/a
	6	492.39	331.07	92.9	6.29	560	n/a	n/a

Table B.7 cont.

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
50	1	completed	completed	completed	completed	completed	n/a	n/a
	2	completed	completed	completed	completed	completed	n/a	n/a
	3	35.58	26.68	0.0	6.80	800	n/a	n/a
	4	278.67	191.93	19.7	6.73	680	n/a	n/a
	5	17.26	14.60	52.5	7.03	1100	n/a	n/a
	6	402.21	275.36	99.8	6.49	570	n/a	n/a

Table B.8: Experimental Results for Run 7

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	SOC (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
0	1	262.32	143.62	267.4	0.6	5.27	140	7740	6840
	2	24.76	14.52	71	53.9	6.48	140	8060	6960
	3	409.46	223.90	373	55.9	4.96	110	8540	7060
	4	321.77	180.15	281.4	117.8	5.40	150	10940	9540
	5	684.05	365.29	561.6	113.8	4.76	100	11400	9880
	6	276.27	154.34	255.4	227.5	5.29	140	9360	7960
2	1	279.00	152.80	281.2	0.0	5.34	180	n/a	n/a
	2	50.36	29.26	123.4	53.4	6.42	180	n/a	n/a
	3	487.12	267.95	377	53.9	5.30	150	n/a	n/a
	4	338.48	187.20	320.7	108.8	5.61	200	n/a	n/a
	5	728.26	408.80	627.2	119.7	5.08	210	n/a	n/a
	6	303.12	168.34	287.8	193.6	5.73	190	n/a	n/a
4	1	293.39	162.26	282	0.0	5.87	180	8980	7780
	2	27.12	15.46	132.2	53.4	6.45	200	8000	7000
	3	391.69	212.66	450.4	52.9	5.41	180	8020	7100
	4	296.41	159.77	297.2	109.8	5.74	220	9580	8180
	5	737.47	400.65	663.6	99.8	5.17	250	10140	8700
	6	276.61	152.51	283.3	207.6	5.83	220	9380	8200
6	1	254.31	139.99	291.6	0.0	5.96	210	7660	5980
	2	23.36	13.17	130.3	51.9	6.49	200	9320	7720
	3	466.64	257.99	447.6	54.9	5.47	170	7180	5800
	4	323.41	173.26	303.9	105.8	5.91	220	10520	8420
	5	740.28	404.44	602.2	99.8	5.25	230	11240	9200
	6	290.22	159.07	297.4	225.5	5.90	210	10560	8760
8	1	246.55	138.25	270.1	0.0	6.01	200	n/a	n/a
	2	12.36	6.99	107.2	45.4	6.49	200	n/a	n/a
	3	399.92	212.10	427.4	28.9	5.62	190	n/a	n/a
	4	312.82	172.51	298.5	103.8	5.85	210	n/a	n/a
	5	747.90	416.77	715	97.8	5.36	230	n/a	n/a
	6	271.06	148.51	282.2	223.5	5.96	220	n/a	n/a
14	1	179.42	101.28	231.9	0.0	6.06	220	n/a	n/a
	2	3.94	1.85	105.7	10.5	6.64	300	n/a	n/a
	3	302.27	175.88	400.8	49.9	5.83	230	n/a	n/a
	4	157.34	102.00	246.1	75.8	6.20	290	n/a	n/a
	5	612.46	345.53	652.2	81.8	5.50	260	n/a	n/a
	6	67.24	45.07	233.6	167.6	6.28	290	n/a	n/a

Table B.8 cont.

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	SOC (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
24	1	42.59	28.67	151.5	0.0	6.20	290	n/a	n/a
	2	5.15	2.23	161.2	0.0	6.74	370	n/a	n/a
	3	124.03	93.26	265.3	15.2	6.15	310	n/a	n/a
	4	54.91	44.54	202.1	35.1	6.50	430	n/a	n/a
	5	373.93	247.59	559.2	52.4	5.97	370	n/a	n/a
	6	14.30	11.27	157.1	100.8	6.54	480	n/a	n/a
26	1	18.39	12.82	124.1	0.0	6.29	260	n/a	n/a
	2	4.85	1.94	67.9	0.0	6.63	370	n/a	n/a
	3	84.44	65.90	252.7	15.6	6.26	350	n/a	n/a
	4	41.78	34.47	193.7	19.2	6.66	500	n/a	n/a
	5	355.79	245.84	567.8	43.9	6.03	390	n/a	n/a
	6	12.39	9.29	146.6	73.8	6.65	530	n/a	n/a
28	1	9.77	5.79	108	0.0	6.26	250	n/a	n/a
	2	3.67	1.47	80.4	0.0	6.63	330	n/a	n/a
	3	49.45	38.97	n/a	12.0	6.30	370	n/a	n/a
	4	24.25	20.04	158.9	8.4	6.91	580	n/a	n/a
	5	266.25	191.29	516.8	45.1	6.15	420	n/a	n/a
	6	13.53	9.89	155.6	46.9	7.08	650	n/a	n/a
30	1	3.75	1.50	101.2	0.0	6.29	250	n/a	n/a
	2	4.84	1.94	81.3	0.0	6.64	320	n/a	n/a
	3	25.51	19.48	174.1	6.0	6.41	410	n/a	n/a
	4	12.85	9.91	156.7	0.0	7.04	660	n/a	n/a
	5	207.92	157.81	508.2	43.1	6.25	420	n/a	n/a
	6	8.74	6.80	133.4	20.0	7.37	790	n/a	n/a
32	1	11.35	7.22	132.7	0.0	6.30	240	7240	6560
	2	3.30	1.32	81.2	0.0	6.68	320	7360	6740
	3	12.11	8.62	132.3	1.3	6.50	440	8920	7820
	4	16.64	10.90	151	0.0	6.99	680	n/a	n/a
	5	163.83	128.69	454.6	44.7	6.27	440	n/a	n/a
	6	9.05	5.93	152.4	0.0	7.69	930	n/a	n/a
48	1	completed	completed	completed	completed	comp.	completed	completed	completed
	2	completed	completed	completed	completed	comp.	completed	completed	completed
	3	completed	completed	completed	completed	comp.	completed	completed	completed
	4	4.53	1.81	129.7	0.0	6.84	600	n/a	n/a
	5	70.91	50.48	343.8	10.4	6.51	680	n/a	n/a
	6	9.81	5.48	129.4	0.0	7.10	950	n/a	n/a

Table B.8 cont.

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	SOC (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
50	1	completed	completed	completed	completed	comp.	completed	completed	completed
	2	completed	completed	completed	completed	comp.	completed	completed	completed
	3	completed	completed	completed	completed	comp.	completed	completed	completed
	4	4.89	1.95	120	0.0	6.74	630	n/a	n/a
	5	52.71	37.78	269.3	0.6	6.62	750	n/a	n/a
	6	6.71	3.33	114.4	0.0	7.07	930	n/a	n/a
52	1	completed	completed	completed	completed	comp.	completed	completed	completed
	2	completed	completed	completed	completed	comp.	completed	completed	completed
	3	completed	completed	completed	completed	comp.	completed	completed	completed
	4	6.72	2.99	107.5	0.0	6.80	610	n/a	n/a
	5	41.29	32.55	224	0.0	6.71	780	n/a	n/a
	6	3.90	1.92	111.9	0.0	7.09	940	n/a	n/a
54	1	completed	completed	completed	completed	comp.	completed	completed	completed
	2	completed	completed	completed	completed	comp.	completed	completed	completed
	3	completed	completed	completed	completed	comp.	completed	completed	completed
	4	4.41	1.76	124.5	0.0	6.81	620	9320	7780
	5	33.51	26.77	231.8	0.0	6.78	790	9360	8100
	6	6.80	3.07	126.3	0.0	7.11	940	8800	7260

Table B.9: Experimental Results for Run 8

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
0	1	316.35	173.69	0.0	5.32	70	n/a	n/a
	2	39.02	24.14	12.4	6.47	140	n/a	n/a
	3	38.33	23.83	50.7	6.58	130	n/a	n/a
	4	61.05	37.42	106.4	6.45	200	n/a	n/a
	5	47.63	30.05	225.2	6.46	150	n/a	n/a
	6	689.15	368.96	113.8	4.83	120	n/a	n/a
2	1	332.29	182.40	0.0	5.73	200	n/a	n/a
	2	50.63	30.45	10.6	6.44	210	n/a	n/a
	3	64.88	39.52	51.3	6.39	220	n/a	n/a
	4	75.26	45.19	106.4	6.36	220	n/a	n/a
	5	62.14	37.96	210.3	6.28	200	n/a	n/a
	6	741.97	402.62	100.2	5.03	180	n/a	n/a
4	1	364.17	199.53	0.0	5.78	210	n/a	n/a
	2	56.19	34.31	15.8	6.38	220	n/a	n/a
	3	66.33	40.67	53.2	6.33	200	n/a	n/a
	4	88.61	53.25	103.9	6.27	220	n/a	n/a
	5	64.09	37.88	212.8	6.23	210	n/a	n/a
	6	722.60	387.01	91.6	5.08	200	n/a	n/a
6	1	253.87	149.71	0.0	5.74	210	n/a	n/a
	2	54.66	32.07	13.5	6.36	210	n/a	n/a
	3	67.43	41.57	43.5	6.38	220	n/a	n/a
	4	93.22	55.05	88.1	6.27	230	n/a	n/a
	5	64.87	37.25	182.3	6.24	210	n/a	n/a
	6	654.61	350.67	89.1	5.14	240	n/a	n/a
8	1	310.41	169.53	0.0	5.79	220	n/a	n/a
	2	51.59	30.37	10.9	6.32	210	n/a	n/a
	3	63.50	37.74	40.4	6.33	240	n/a	n/a
	4	91.94	55.41	97.3	6.29	240	n/a	n/a
	5	67.43	39.64	170.0	6.24	200	n/a	n/a
	6	669.29	363.36	84.0	5.17	220	n/a	n/a
14	1	267.25	141.87	0.0	5.84	230	n/a	n/a
	2	17.89	11.37	0.4	6.57	280	n/a	n/a
	3	16.50	13.32	19.5	6.42	270	n/a	n/a
	4	26.05	19.78	60.4	6.44	310	n/a	n/a
	5	17.12	13.68	153.6	6.37	290	n/a	n/a
	6	511.23	304.00	60.4	5.59	290	n/a	n/a

Table B.9 cont.

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
24	1	210.43	111.55	0.0	6.05	240	n/a	n/a
	2	8.54	4.78	0.0	6.36	260	n/a	n/a
	3	5.26	2.94	0.0	6.74	400	n/a	n/a
	4	1.26	0.50	1.2	6.86	560	n/a	n/a
	5	0.69	0.28	116.3	6.67	480	n/a	n/a
	6	330.71	221.82	58.2	5.93	380	n/a	n/a
26	1	125.84	67.84	0.0	6.11	250	n/a	n/a
	2	6.00	3.27	0.0	6.31	280	n/a	n/a
	3	3.83	1.92	0.0	6.62	420	n/a	n/a
	4	1.36	0.54	0.0	6.90	600	n/a	n/a
	5	0.83	0.33	89.1	6.97	520	n/a	n/a
	6	357.34	242.39	53.2	6.00	400	n/a	n/a
28	1	93.96	52.88	0.0	6.29	270	n/a	n/a
	2	2.87	1.30	0.0	6.56	280	n/a	n/a
	3	6.90	2.90	0.0	6.57	400	n/a	n/a
	4	2.28	0.91	0.0	6.88	610	n/a	n/a
	5	0.90	0.36	49.8	6.84	600	n/a	n/a
	6	319.93	217.33	40.0	6.16	410	n/a	n/a
30	1	89.97	50.08	0.0	6.26	270	n/a	n/a
	2	2.67	1.21	0.0	6.41	270	n/a	n/a
	3	13.70	6.27	0.0	6.63	410	n/a	n/a
	4	2.81	1.12	0.0	6.99	600	n/a	n/a
	5	1.08	0.43	19.5	7.27	750	n/a	n/a
	6	294.23	203.58	46.9	6.10	420	n/a	n/a
48	1	completed	completed	completed	completed	completed	completed	completed
	2	completed	completed	completed	completed	completed	completed	completed
	3	completed	completed	completed	completed	completed	completed	completed
	4	4.01	2.13	0.0	6.60	580	n/a	n/a
	5	141.58	60.94	0.0	6.90	850	n/a	n/a
	6	240.27	187.50	9.2	6.68	700	n/a	n/a
50	1	completed	completed	completed	completed	completed	completed	completed
	2	completed	completed	completed	completed	completed	completed	completed
	3	completed	completed	completed	completed	completed	completed	completed
	4	1.87	0.75	0.0	6.68	560	n/a	n/a
	5	166.66	71.55	0.0	6.94	870	n/a	n/a
	6	159.24	120.42	0.0	6.75	790	n/a	n/a

Table B.9 cont.

Hour	Reactor	VFA (mg/L as acetate)	VFA-C (mg/L)	NO3-N (mg/L)	pH	Alkalinity (mg/L as CaCO3)	TSS (mg/L)	VSS (mg/L)
52	1	completed	completed	completed	completed	completed	completed	completed
	2	completed	completed	completed	completed	completed	completed	completed
	3	completed	completed	completed	completed	completed	completed	completed
	4	2.00	0.80	0.0	6.69	550	n/a	n/a
	5	152.53	65.77	0.0	6.97	870	n/a	n/a
	6	171.96	129.65	0.0	6.75	790	n/a	n/a
54	1	completed	completed	completed	completed	completed	completed	completed
	2	completed	completed	completed	completed	completed	completed	completed
	3	completed	completed	completed	completed	completed	completed	completed
	4	1.59	0.64	0.0	6.80	570	n/a	n/a
	5	179.75	76.71	0.0	7.02	850	n/a	n/a
	6	165.95	116.92	0.0	6.90	780	n/a	n/a

Table B.10: Complete VFA Data for Run 1

Reactor	Day	HAc (mg/L)	avg. (mg/L)	HPr (mg/L)	avg. (mg/L)	iHBu (mg/L)	avg. (mg/L)	nHBu (mg/L)	avg. (mg/L)	iHVa (mg/L)	avg. (mg/L)	nHVa (mg/L)
r1	0	42.50		2.07		5.67		1.75		3.15		0.00
		44.11	43.31	2.02	2.04	5.76	5.72	1.94	1.84	2.65	2.90	0.00
r2		60.34		2.95		5.40		7.54		1.88		2.35
		59.86	60.10	2.76	2.86	5.18	5.29	7.44	7.49	1.89	1.88	2.07
r3		51.58		2.60		5.09		5.88		1.59		1.85
		47.96	49.77	2.50	2.55	4.70	4.90	6.06	5.97	1.61	1.60	1.92
r4		90.59		13.93		8.43		16.33		4.34		3.89
		93.25	91.92	13.22	13.58	9.06	8.75	17.13	16.73	4.70	4.52	3.88
r5		105.25		15.89		8.49		17.89		4.76		4.28
		110.31	107.78	16.42	16.15	9.39	8.94	18.24	18.06	5.64	5.20	4.34
r6		55.84		2.80		5.20		7.28		2.05		2.33
		56.81	56.33	3.24	3.02	5.10	5.15	8.59	7.94	2.11	2.08	2.39
r1	1	0.00		0.00		0.00		0.00		0.00		0.00
			0.00		0.00		0.00		0.00		0.00	
r2		0.00		0.00		0.00		0.00		0.00		0.00
			0.00		0.00		0.00		0.00		0.00	
r3		0.00		0.00		0.00		0.00		0.00		0.00
			0.00		0.00		0.00		0.00		0.00	
r4		0.00		0.00		0.00		0.00		0.00		0.00
			0.00		0.00		0.00		0.00		0.00	
r5		51.02		0.00		0.00		0.00		3.56		0.00
		51.58	51.30	0.00	0.00	0.00	0.00	0.00	0.00	3.81	3.68	0.00
r6		0.00		0.00		0.00		0.00		0.00		0.00
			0.00		0.00		0.00		0.00		0.00	

Table B.10 cont.

Reactor	Day	HAc (mg/L)	avg. (mg/L)	HPr (mg/L)	avg. (mg/L)	iHBu (mg/L)	avg. (mg/L)	nHBu (mg/L)	avg. (mg/L)	iHVa (mg/L)	avg. (mg/L)	nHVa (mg/L)
r1	4	0.00	0.00	0.00		0.00		0.00		0.00		0.00
					0.00		0.00		0.00		0.00	
r2		0.00	0.00	0.00		0.00		0.00		0.00		0.00
					0.00		0.00		0.00		0.00	
r3		0.00	0.00	0.00		0.00		0.00		0.00		0.00
					0.00		0.00		0.00		0.00	
r4		92.74		15.09		0.00		0.00		0.00		0.00
		88.25	90.50	15.34	15.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00
r5		100.32		8.22		3.72		0.00		6.94		0.00
		101.02	100.67	7.42	7.82	3.58	3.65	0.00	0.00	7.69	7.31	0.00
r6		0.00		0.00		0.00		0.00		0.00		0.00
			0.00		0.00		0.00		0.00		0.00	
r1	5	0.00		0.00		0.00		0.00		0.00		0.00
			0.00		0.00		0.00		0.00		0.00	
r2		0.00		0.00		0.00		0.00		0.00		0.00
			0.00		0.00		0.00		0.00		0.00	
r3		0.00		0.00		0.00		0.00		0.00		0.00
			0.00		0.00		0.00		0.00		0.00	
r4		130.74		14.62		2.88		0.00		3.99		0.00
		125.07	127.91	13.50	14.06	2.66	2.77	0.00	0.00	4.31	4.15	0.00
r5		0.00		0.00		0.00		0.00		5.63		0.00
		3.08	1.54	0.00	0.00	0.00	0.00	0.00	0.00	6.31	5.97	0.00
r6		0.00		0.00		0.00		0.00		0.00		0.00
			0.00		0.00		0.00		0.00		0.00	

Table B.11: Complete VFA Data for Run 2

Reactor	Day	HAc (mg/L)	avg. (mg/L)	HPr (mg/L)	avg. (mg/L)	iHBu (mg/L)	avg. (mg/L)	nHBu (mg/L)	avg. (mg/L)	iHVa (mg/L)	avg. (mg/L)	nHVa (mg/L)	avg. (mg/L)
r1	0	222.81		80.55		12.43		73.58		18.82		13.53	
		243.84	233.32	92.66	86.60	12.69	12.56	95.69	84.63	19.21	19.02	16.18	14.85
r2		273.22		102.73		15.73		93.76		16.22		11.78	
		245.17	259.19	91.80	97.27	16.50	16.11	76.55	85.15	20.08	18.15	15.99	13.89
r3		339.89		118.17		18.45		131.51		23.71		14.27	
		310.73	325.31	110.61	114.39	16.70	17.57	112.66	122.09	19.32	21.51	15.62	14.95
r4		310.40		122.38		21.15		158.37		25.66		19.05	
		306.19	308.30	100.53	111.46	14.56	17.85	130.08	144.22	20.91	23.29	15.58	17.31
r5		237.30		89.31		14.49		84.63		18.03		14.37	
		220.99	229.15	91.81	90.56	13.14	13.82	105.95	95.29	20.62	19.32	11.94	13.15
r6		278.15		89.59		13.64		130.12		18.07		17.89	
		300.23	289.19	100.06	94.82	13.67	13.66	129.75	129.93	17.67	17.87	15.95	16.92
r1	1	448.54		52.10		26.48		23.17		31.72		9.51	
		431.48	440.01	53.89	52.99	19.39	22.93	19.89	21.53	28.61	30.16	7.23	8.37
r2		459.02		107.12		22.57		47.10		33.70		14.57	
		485.02	472.02	101.24	104.18	27.31	24.94	41.49	44.29	31.33	32.51	10.85	12.71
r3		571.94		103.67		23.83		64.01		37.90		17.42	
		547.07	559.51	111.25	107.46	23.97	23.90	73.94	68.97	34.66	36.28	17.81	17.61
r4		334.03		129.15		22.68		73.04		27.40		14.49	
		354.38	344.20	119.26	124.21	24.29	23.49	70.75	71.89	30.81	29.11	13.51	14.00
r5		501.65		114.06		28.42		91.46		45.29		16.86	
		539.81	520.73	120.56	117.31	32.13	30.27	96.79	94.13	50.90	48.09	26.60	21.73
r6		408.93		124.77		17.87		74.24		23.11		14.92	
		389.60	399.27	127.53	126.15	19.37	18.62	68.60	71.42	27.64	25.37	13.42	14.17
r1	2	520.02		48.65		28.69		3.27		35.81		1.66	
		520.02	520.02	52.83	50.74	30.27	29.48	3.94	3.60	35.68	35.74		1.66
r2		628.02		174.82		41.85		69.71		68.69		20.42	
		628.02	628.02	194.19	184.50	54.04	47.95	61.04	65.38	85.40	77.04	23.22	21.82
r3		579.55		191.31		53.27		41.04		72.62		23.81	
		579.55	579.55	213.86	202.58	47.93	50.60	42.57	41.80	87.71	80.17	21.07	22.44
r4		464.27		282.85		60.67		88.90		88.20		44.89	
		464.27	464.27	282.85	282.85	59.90	60.28	92.93	90.92	77.10	82.65	36.42	40.65
r5		696.12		142.79		45.12		25.58		74.51		13.65	
		696.12	696.12	143.40	143.09	45.05	45.08	29.93	27.76	83.18	78.84	17.89	15.77
r6		521.86		191.75		48.08		88.62		109.30		38.95	
		521.86	521.86	229.25	210.50	53.24	50.66	118.79	103.70	84.76	97.03	46.31	42.63

Table B.11 cont.

Reactor	Day	HAc (mg/L)	avg. (mg/L)	HPr (mg/L)	avg. (mg/L)	iHBu (mg/L)	avg. (mg/L)	nHBu (mg/L)	avg. (mg/L)	iHVa (mg/L)	avg. (mg/L)	nHVa (mg/L)	avg. (mg/L)
r1	3	509.69		31.35		33.21		0.00		40.28		0.00	
		532.79	521.24	27.64	29.49	31.14	32.17	0.00	0.00	33.57	36.92	0.00	0.00
r2		489.59		159.63		48.34		63.37		72.87		28.87	
		542.53	516.06	148.91	154.27	43.59	45.97	68.24	65.81	76.74	74.81	21.81	25.34
r3		469.58		176.52		51.12		48.53		80.44		27.43	
		405.00	437.29	158.62	167.57	59.21	55.16	45.21	46.87	88.42	84.43	29.03	28.23
r4		488.09		159.65		56.11		9.81		76.01		18.47	
		571.21	529.65	192.58	176.11	57.48	56.80	11.02	10.41	89.87	82.94	18.57	18.52
r5		628.61		121.08		47.65		17.22		84.36		10.85	
		632.32	630.46	127.78	124.43	46.40	47.02	15.29	16.26	75.08	79.72	12.34	11.60
r6		493.97		116.81		58.71		4.18		84.86		6.90	
		535.92	514.94	126.88	121.85	58.93	58.82	3.93	4.06	78.56	81.71	7.89	7.39
r1	4	831.35		113.60		32.03		21.21		34.60		9.92	
		774.32	802.84	122.15	117.87	25.23	28.63	14.59	17.90	29.04	31.82	7.86	8.89
r2		865.23	865.23	237.09	237.09	47.76	47.76	96.26	96.26	56.96	56.96	65.69	
r3		493.52	493.52	215.48	215.48	54.94	54.94	51.38	51.38	79.40	79.40	58.56	
r4		32.63	32.63	0.00	0.00	0.00	0.00	0.00	0.00	35.90	35.90	0.00	
r5		846.10	846.10	146.77	146.77	47.65	47.65	34.96	34.96	59.28	59.28	13.10	
r6		511.05	511.05	120.71	120.71	47.29	47.29	16.84	16.84	56.31	56.31	5.23	
r1	7	885.52		0.00		11.69		0.00		21.06		0.00	
		843.34	864.43	0.00	0.00	10.78	11.23	0.00	0.00	19.36	20.21	0.00	0.00
r2		1023.48		241.97		40.98		102.62		53.36		55.00	
		1042.31	1032.90	197.24	219.61	43.76	42.37	119.91	111.27	59.90	56.63	58.14	56.57
r3		719.68		223.87		39.81		65.99		52.38		52.61	
		770.52	745.10	205.50	214.69	35.96	37.89	61.14	63.56	46.80	49.59	50.37	51.49
r4		653.74		7.27		0.00		0.00		0.00		0.00	
		632.24	642.99	9.06	8.17	2.89	1.44	0.00	0.00	0.00	0.00	0.00	0.00
r5		1040.46		200.03		39.22		67.66		77.38		44.57	
		1093.13	1066.80	213.84	206.94	51.26	45.24	67.30	67.48	76.36	76.87	57.27	50.92
r6		1014.76		240.94		46.04		59.94		71.89		59.42	
		1032.15	1023.46	260.37	250.65	49.38	47.71	59.02	59.48	60.84	66.36	63.84	61.63
r1	8	790.91		8.76		22.07		0.00		20.52		0.00	
		887.17	839.04	8.62	8.69	15.63	18.85	0.00	0.00	18.67	19.59	0.00	0.00
r2		1033.55		236.69		39.44		142.87		72.84		92.30	
		1155.77	1094.66	256.73	246.71	38.64	39.04	140.96	141.91	58.40	65.62	87.78	90.04
r3		959.51		260.19		46.84		67.76		46.89		68.54	
		907.34	933.43	274.61	267.40	41.19	44.01	74.52	71.14	51.39	49.14	69.50	69.02
r4													
		621.07	621.07	25.11	25.11	6.18	6.18	19.16	19.16	5.40	5.40	0.00	0.00
r5		758.36		188.39		30.59		41.72		43.52		35.62	
		731.80	745.08	157.39	172.89	33.26	31.93	48.58	45.15	51.24	47.38	42.01	38.81
r6		1195.21		168.16		44.06		0.00		33.64		14.84	
		1090.06	1142.64	146.66	157.41	44.31	44.19	5.82	2.91	55.80	44.72	16.01	15.42

Table B.11 cont.

Reactor	Day	HAc (mg/L)	avg. (mg/L)	HPr (mg/L)	avg. (mg/L)	iHBu (mg/L)	avg. (mg/L)	nHBu (mg/L)	avg. (mg/L)	iHVa (mg/L)	avg. (mg/L)	nHVa (mg/L)	avg. (mg/L)
r1	12	1023.18		162.21		44.64		52.79		55.79		21.73	
		904.34	963.76	173.30	167.75	32.73	38.69	45.91	49.35	48.42	52.10		21.73
r2		1326.43		135.20		65.46		60.14		75.21		8.88	
		1276.61	1301.52	151.45	143.33	60.90	63.18		60.14	64.80	70.01	9.09	8.98
r3		1070.75		279.42		56.65		104.10		84.89		67.30	
		1044.43	1057.59	268.59	274.01	62.15	59.40	104.10	104.10	77.19	81.04	61.49	64.39
r4		748.32		94.77		24.55		74.54		37.93		18.66	
		795.97	772.14	89.16	91.97	21.81	23.18	82.24	78.39	35.90	36.91	18.94	18.80
r5		1067.68		272.71		55.36		84.24		80.38		61.47	
		1130.18	1098.93	244.00	258.36	68.28	61.82	80.59	82.42	72.69	76.54	69.09	65.28
r6		1074.20		176.81		56.62		43.44		59.67		21.85	
		1038.88	1056.54	194.36	185.58	55.35	55.98	42.56	43.00	57.99	58.83	20.78	21.32

Table B.12: Complete VFA Data for Run 3

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	0	54.71	21.41	3.02	21.72	4.09	4.64
r2		106.90	41.48	4.47	38.47	6.16	13.84
r3		84.02	36.42	4.02	35.84	4.98	10.75
r4		76.31	30.17	2.46	38.80	2.45	9.50
r6		83.31	34.17	3.64	42.14	3.44	13.77
r1	5	131.91	20.09	7.08	2.85	9.54	1.23
r2		131.55	0.00	16.94	2.11	6.36	1.39
r3		167.03	0.00	24.97	13.89	9.12	5.95
r4		272.48	73.26	13.47	75.98	16.44	21.00
r6		152.43	29.67	6.60	26.18	12.37	12.34
r1	24	234.97	21.44	11.11	4.80	23.41	0.84
r2		153.10	27.78	5.67	5.80	12.82	0.00
r3		269.85	0.00	21.79	7.97	25.31	0.00
r4		325.21	0.00	27.37	6.91	28.38	0.00
r6		180.59	0.00	24.09	20.46	19.80	7.44
r1	51	250.17	23.61	4.90	3.49	14.65	0.00
r2		250.08	51.43	9.55	3.61	20.94	1.91
r3		417.15	84.21	19.12	27.55	35.16	11.41
r4		621.98	86.53	35.37	28.30	58.01	7.33
r6		360.34	63.50	3.74	25.43	29.86	18.64
r1	100	817.63	129.37	10.74	21.32	16.13	9.04
r2		154.86	2.47	0.00	0.00	3.32	0.00
r3		1092.07	156.49	19.13	7.22	19.73	9.26
r4		976.11	242.08	21.32	84.03	22.98	27.23
r6		588.91	229.15	40.24	78.76	46.76	30.16

Table B.13: Complete VFA Data for Run 4

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	0	93.31	69.14	3.90	68.55	3.35	24.60
r2		288.75	185.22	9.59	216.94	9.17	94.95
r3		166.79	135.93	6.04	123.53	6.45	66.02
r4		449.12	233.38	14.53	338.77	12.62	172.95
r6		473.55	229.03	12.92	386.72	13.53	207.84
r1	3	227.12	218.46	0.00	110.11	8.44	48.56
r2		519.10	479.33	14.16	328.10	16.37	115.92
r3		383.34	363.54	11.45	174.70	9.71	80.81
r4		655.77	387.09	17.79	407.92	17.96	171.87
r6		377.87	272.10	11.58	286.84	11.93	105.37
r1	6	263.76	250.02	7.78	113.37	10.45	60.61
r2		577.30	423.85	15.66	281.30	18.90	123.27
r3		511.39	461.11	0.00	222.13	12.49	104.82
r4		587.95	392.79	13.74	361.00	17.59	183.47
r6		762.48	521.69	20.74	424.52	24.30	206.46
r1	24	305.27	111.87	23.74	0.00	24.76	4.85
r2		967.89	473.82	41.96	43.61	64.52	46.88
r3		834.41	433.45	32.53	12.23	35.61	17.79
r4		337.44	243.33	22.39	95.93	28.43	45.37
r6		942.58	243.81	27.98	87.59	34.57	91.33
r1	30	271.33	66.94	27.52	9.03	36.44	10.96
r2		418.92	187.68	28.56	0.00	46.52	11.89
r3		627.29	273.81	40.98	0.00	59.32	7.86
r4		649.67	257.44	30.72	38.86	40.96	35.57
r6		1397.95	372.51	38.42	20.81	47.81	108.30

Table B.14: Complete VFA Data for Run 5

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	0	46.01	25.38	2.89	17.68	2.55	7.90
r2		113.36	44.46	1.99	29.20	1.85	13.68
r3		71.69	31.27	1.25	24.20	1.09	8.12
r4		156.44	55.66	3.78	49.39	3.43	15.82
r5		216.51	71.49	3.28	62.05	2.33	30.78
r6		123.82	36.03	1.74	38.67	1.47	12.49
r1	1	63.40	37.16	2.46	24.72	2.32	11.48
r2		132.11	79.44	3.09	41.35	2.19	18.14
r3		108.33	65.76	2.09	33.38	2.56	17.19
r4		176.70	78.04	4.57	64.21	4.27	20.83
r5		218.32	92.78	3.27	73.86	2.83	27.13
r6		142.66	64.56	1.88	46.85	2.36	18.30
r1	2	86.70	66.42	3.33	25.91	3.14	20.52
r2		154.26	83.71	3.30	38.32	3.10	21.96
r3		112.31	70.36	2.45	31.38	2.57	16.82
r4		188.09	107.75	4.31	61.82	5.12	23.35
r5		237.23	127.26	3.84	66.79	3.27	30.03
r6		170.79	91.44	2.81	50.53	3.04	25.65
r1	3	95.48	78.44	3.65	28.67	4.46	0.97
r2		191.66	107.30	3.78	47.72	3.47	27.23
r3		144.77	91.94	4.01	32.27	3.26	20.27
r4		203.54	98.04	5.09	61.58	6.11	25.41
r5		195.97	97.08	3.36	64.50	3.59	30.00
r6		201.13	102.56	3.15	45.84	2.97	21.75
r1	4	109.15	71.92	4.05	33.82	5.31	17.90
r2		191.61	94.10	3.88	46.99	4.42	28.11
r3		148.84	96.83	3.48	35.24	5.59	20.63
r4		223.14	110.04	6.66	64.08	7.40	28.02
r5		209.17	125.23	5.40	66.40	5.59	35.70
rr6		212.94	116.32	4.35	65.76	5.06	32.37
r1	5	102.68	71.11	4.57	24.69	5.76	18.97
r2		207.97	111.89	4.32	42.83	6.23	29.28
r3		157.25	90.40	3.59	29.73	4.47	20.47
r4		227.16	117.36	6.14	62.29	8.17	26.54
r5		293.27	146.80	5.19	72.45	6.03	35.03
r6		216.14	110.03	4.35	57.18	5.61	36.17

Table B.14 cont.

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	6	104.78	65.67	5.04	25.84	6.58	16.85
r2		201.90	95.91	3.92	37.17	5.50	23.75
r3		188.95	106.14	4.45	26.95	5.72	24.67
r4		243.55	112.95	6.66	65.95	8.91	29.05
r5		321.69	149.35	5.65	76.83	6.43	36.61
r6		250.90	131.09	3.95	54.35	5.03	33.04
r1	7	105.44	66.42	5.61	18.40	6.85	19.91
r2		239.42	117.31	4.92	39.75	6.92	24.05
r3		202.38	108.18	5.44	24.17	8.41	23.41
r4		261.60	146.47	7.86	59.80	10.68	35.08
r5		305.67	161.47	6.22	70.07	6.94	41.91
r6		272.17	139.20	5.15	43.61	5.66	33.85
r1	24	34.24	32.15	9.39	0.95	10.57	0.00
r2		131.02	80.28	8.32	1.87	13.24	4.89
r3		213.59	97.08	9.60	2.84	13.46	2.26
r4		295.93	139.07	14.35	2.15	22.89	15.12
r5		296.36	124.29	13.40	5.02	22.63	26.95
r6		459.01	125.77	8.01	3.33	9.15	3.00
r1	27	36.86	35.96	10.45	1.40	13.03	0.88
r2		143.72	76.47	10.93	2.30	14.34	3.09
r3		197.33	99.49	11.04	1.62	12.81	1.81
r4		319.50	137.46	15.12	2.72	28.47	11.46
r5		252.02	112.45	10.89	0.00	22.31	14.56
r6		375.21	105.85	7.09	0.00	8.05	0.00
r1	30	34.01	23.00	8.59	0.00	10.36	0.00
r2		165.61	73.15	11.78	1.79	15.47	2.18
r3		253.23	85.14	10.97	2.40	16.99	1.76
r4		322.84	144.52	16.94	2.49	23.33	7.37
r5		227.31	88.00	14.88	8.68	21.54	12.54
r6		428.27	114.06	7.63	2.56	9.22	0.00

Table B.15: Complete VFA Data for Run 6

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	0	163.51	52.64	13.28	106.55	12.85	33.40
r2		253.27	78.10	7.54	132.71	8.37	42.71
r3		365.59	101.96	8.35	194.68	11.97	45.76
r4		582.86	157.16	10.53	344.43	14.99	43.31
r5		446.57	114.75	8.84	284.63	10.49	41.93
r6		581.49	167.42	0.00	328.87	18.59	59.95
r1	2	145.95	60.05	7.79	103.07	8.56	26.92
r2		274.60	80.59	9.45	167.23	10.49	32.52
r3		335.13	119.18	10.07	187.58	15.03	45.90
r4		643.37	170.11	14.42	288.21	24.68	48.74
r5		510.57	148.81	9.83	236.51	16.48	39.04
r6		599.44	171.80	16.00	293.03	20.03	63.65
r1	4	152.57	50.75	6.16	86.20	8.54	22.56
r2		275.94	79.63	7.73	146.72	10.64	40.10
r3		357.66	122.03	10.12	200.61	14.18	40.87
r4		578.94	146.25	11.52	281.29	20.43	62.45
r5		469.65	124.06	10.08	233.02	15.75	40.35
r6		542.24	160.27	15.04	283.60	22.42	64.07
r1	6	184.51	63.19	6.75	83.47	9.17	20.63
r2		276.44	84.35	9.69	132.21	10.62	39.65
r3		370.85	119.41	9.86	205.69	13.79	36.46
r4		577.68	153.80	11.03	295.06	14.96	47.54
r5		530.04	158.07	10.67	230.17	19.29	50.18
r6		569.30	179.11	14.22	364.01	20.98	66.30
r1	8	161.14	50.71	7.92	90.46	9.43	31.82
r2		242.56	77.46	8.48	131.10	11.05	30.46
r3		379.20	110.79	8.72	194.41	15.77	38.72
r4		559.55	132.27	13.62	309.94	21.08	53.18
r5		526.02	143.00	13.65	234.39	20.79	58.30
rr6		580.20	174.18	13.37	301.05	22.06	66.91
r1	15	84.58	39.62	9.09	81.80	14.95	26.83
r2		158.35	60.96	11.71	125.18	15.46	37.73
r3		300.89	100.34	10.66	167.82	21.43	44.35
r4		484.73	136.26	11.88	265.43	18.84	60.12
r5		486.55	140.28	13.67	218.69	23.13	60.05
r6		567.70	186.68	15.57	290.04	31.14	65.12

Table B.15 cont.

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	24	83.42	29.17	13.73	34.71	19.60	24.85
r2		56.55	24.32	14.77	93.34	20.37	36.08
r3		202.95	86.76	15.64	143.45	24.96	43.18
r4		313.65	110.80	12.92	263.70	24.94	62.92
r5		204.04	121.66	17.58	203.55	38.69	56.48
r6		375.92	179.10	15.10	278.48	33.76	64.88
r1	26	72.77	27.31	12.98	26.03	23.90	21.52
r2		31.39	16.39	14.75	74.08	24.99	29.46
r3		186.71	84.97	15.59	116.60	33.03	42.15
r4		280.97	110.73	13.81	273.20	23.36	53.17
r5		154.91	121.80	17.37	188.65	38.01	57.05
r6		354.33	156.01	19.34	256.57	34.96	58.08
r1	28	52.14	16.36	12.89	18.01	25.33	21.90
r2		12.94	5.35	14.96	41.46	24.40	27.98
r3		175.37	77.49	16.05	103.51	28.76	48.58
r4		233.42	104.17	15.85	273.02	20.52	54.88
r5		100.65	95.38	23.42	188.82	52.05	57.07
r6		350.94	145.82	20.49	301.18	30.84	65.70
r1	30	34.01	17.17	15.46	13.07	20.32	17.11
r2		11.09	5.22	14.68	12.93	23.70	12.19
r3		166.74	76.34	16.28	91.32	32.98	40.42
r4		190.28	92.89	11.23	234.76	20.45	48.39
r5		69.47	70.64	22.80	176.36	39.20	56.57
r6		303.04	127.68	17.95	276.93	25.06	55.00
r1	32	11.26	8.59	14.27	3.24	22.73	7.81
r2		6.61	2.93	10.72	3.34	15.90	4.17
r3		136.51	56.48	17.41	91.81	33.91	36.06
r4		153.07	83.39	10.90	261.25	20.75	47.41
r5		25.89	50.55	21.38	151.87	41.99	67.02
r6		290.31	131.25	16.80	231.64	25.94	68.69
r1	48	n/a	n/a	n/a	n/a	n/a	n/a
r2		n/a	n/a	n/a	n/a	n/a	n/a
r3		5.11	5.20	13.63	1.59	26.57	1.01
r4		85.09	50.33	14.33	199.61	23.23	56.86
r5		2.70	0.99	11.57	1.65	27.54	0.00
r6		139.95	113.49	20.92	269.51	38.17	66.68

Table B.15 cont.

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	50	n/a	n/a	n/a	n/a	n/a	n/a
r2		n/a	n/a	n/a	n/a	n/a	n/a
r3		9.75	4.46	9.37	2.16	23.60	0.76
r4		83.09	43.00	14.19	151.13	28.37	52.75
r5		3.56	0.00	3.76	0.00	18.60	0.34
r6		103.57	83.65	17.06	253.15	29.01	49.25

Table B.16: Complete VFA Data for Run 7

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	0	155.20	42.12	3.93	81.08	5.66	19.41
r2		11.79	7.20	0.85	4.86	1.79	3.65
r3		239.85	69.37	5.91	130.53	8.03	25.79
r4		182.12	46.26	5.13	119.18	6.22	22.81
r5		430.74	95.80	8.29	200.50	10.84	44.70
r6		156.51	43.15	5.01	95.29	6.25	21.13
r1	2	166.01	39.70	4.50	93.76	6.01	17.03
r2		20.48	23.37	0.00	7.84	6.48	2.76
r3		281.64	88.62	9.88	142.29	8.70	41.13
r4		194.04	55.29	5.66	115.22	6.55	22.08
r5		404.55	119.66	10.90	260.07	16.01	53.96
r6		171.94	55.97	4.40	91.19	8.69	25.77
r1	4	167.66	50.01	3.99	98.05	7.10	18.88
r2		13.91	7.86	1.33	3.80	2.74	2.85
r3		233.36	70.47	6.46	108.07	9.65	28.87
r4		181.51	47.91	5.55	81.19	7.85	20.39
r5		447.50	103.34	9.81	239.23	12.70	48.01
r6		159.29	48.36	4.71	86.65	7.09	19.28
r1	6	150.14	41.16	5.79	71.66	7.98	22.15
r2		13.13	5.29	1.65	2.46	3.07	2.21
r3		269.11	72.94	8.87	159.32	10.75	28.77
r4		201.68	50.26	5.70	85.60	9.06	22.25
r5		435.51	122.67	10.74	236.87	15.35	45.31
r6		169.60	49.84	5.71	88.35	7.33	19.45
r1	8	139.78	35.56	4.23	88.21	6.14	18.82
r2		6.93	2.54	1.35	1.36	1.82	0.73
r3		253.31	63.62	5.83	102.00	8.17	27.69
r4		179.34	52.76	5.07	106.15	6.34	18.37
r5		424.13	111.51	11.57	278.55	12.42	46.77
r6		157.12	51.27	4.99	77.53	6.56	20.27
r1	14	104.00	21.09	4.63	59.28	7.56	17.28
r2		3.26	0.00	0.00	1.00	0.00	0.00
r3		156.58	44.73	7.73	120.99	9.55	26.77
r4		58.21	23.10	6.27	86.94	7.60	20.79
r5		338.65	92.41	10.01	232.00	12.45	44.13
r6		20.39	12.23	2.58	40.35	3.74	9.15

Table B.16 cont.

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	24	16.15	5.16	6.30	12.16	9.43	6.97
r2		4.29	1.05	0.00	0.00	0.00	0.00
r3		17.72	18.29	6.88	95.96	11.55	24.58
r4		5.25	2.21	6.87	40.47	12.06	14.41
r5		116.45	68.39	10.81	236.13	14.06	42.39
r6		2.63	1.53	5.40	1.49	9.04	0.70
r1	26	6.36	2.33	3.25	3.94	7.31	1.58
r2		4.85	0.00	0.00	0.00	0.00	0.00
r3		9.27	10.13	8.27	61.97	11.60	20.69
r4		3.85	1.49	7.25	25.67	12.20	12.07
r5		91.52	59.34	11.21	247.21	17.21	50.05
r6		3.72	0.00	5.35	0.97	7.42	0.00
r1	28	5.22	1.48	1.30	1.31	2.65	0.00
r2		3.67	0.00	0.00	0.00	0.00	0.00
r3		6.68	5.39	8.38	25.82	12.26	13.32
r4		4.01	0.00	8.15	5.03	12.88	6.26
r5		50.99	50.05	9.53	193.40	15.61	45.58
r6		4.55	0.00	5.33	1.31	7.57	0.00
r1	30	3.75	0.00	0.00	0.00	0.00	0.00
r2		4.84	0.00	0.00	0.00	0.00	0.00
r3		6.15	2.21	7.03	4.74	12.52	3.67
r4		3.49	0.00	5.25	0.93	8.75	0.00
r5		22.02	32.90	9.14	180.70	12.62	37.65
r6		2.08	0.00	3.62	1.44	5.45	0.00
r1	32	4.48	2.56	1.67	2.65	1.74	1.37
r2		3.30	0.00	0.00	0.00	0.00	0.00
r3		3.92	1.60	4.44	0.00	6.56	0.00
r4		6.78	2.52	3.97	2.14	6.19	0.00
r5		12.57	22.28	13.15	134.42	15.63	39.49
r6		4.16	0.00	0.00	4.58	3.00	0.00
r1	48	n/a	n/a	n/a	n/a	n/a	n/a
r2		n/a	n/a	n/a	n/a	n/a	n/a
r3		n/a	n/a	n/a	n/a	n/a	n/a
r4		4.53	0.00	0.00	0.00	0.00	0.00
r5		20.29	15.69	19.91	2.43	26.54	11.82
r6		5.63	2.22	0.00	1.58	2.20	0.00

Table B.16 cont.

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	50	n/a	n/a	n/a	n/a	n/a	n/a
r2		n/a	n/a	n/a	n/a	n/a	n/a
r3		n/a	n/a	n/a	n/a	n/a	n/a
r4		4.89	0.00	0.00	0.00	0.00	0.00
r5		12.74	13.58	20.35	0.00	20.44	5.06
r6		4.68	1.73	0.00	0.00	1.05	0.00
r1	52	n/a	n/a	n/a	n/a	n/a	n/a
r2		n/a	n/a	n/a	n/a	n/a	n/a
r3		n/a	n/a	n/a	n/a	n/a	n/a
r4		5.19	1.88	0.00	0.00	0.00	0.00
r5		4.48	9.22	20.43	1.47	21.97	2.42
r6		3.31	0.00	0.00	0.00	1.01	0.00
r1	54	n/a	n/a	n/a	n/a	n/a	n/a
r2		n/a	n/a	n/a	n/a	n/a	n/a
r3		n/a	n/a	n/a	n/a	n/a	n/a
r4		4.41	0.00	0.00	0.00	0.00	0.00
r5		4.45	4.16	19.63	0.00	19.96	0.90
r6		6.20	0.00	0.00	0.00	1.01	0.00

Table B.17: Complete VFA Data for Run 8

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	0	186.85	47.69	5.70	102.08	8.45	20.51
r2		15.67	11.74	1.31	10.29	3.57	6.37
r3		14.32	12.85	1.41	10.64	3.88	5.12
r4		23.26	21.91	2.02	15.68	4.96	8.35
r5		17.22	15.41	1.50	14.11	4.10	8.08
r6		424.36	110.80	6.89	204.08	11.33	40.36
r1	2	192.16	59.83	3.46	104.48	8.75	21.25
r2		21.79	16.42	1.45	10.68	4.48	7.66
r3		26.55	20.44	2.20	17.28	5.53	8.68
r4		31.07	26.32	2.17	17.63	6.61	9.01
r5		24.51	21.00	1.93	16.46	4.95	8.53
r6		446.99	115.31	7.81	232.96	15.95	46.24
r1	4	211.27	68.69	4.08	106.51	9.36	26.97
r2		23.37	17.18	1.44	14.38	5.61	7.98
r3		26.37	22.15	2.08	16.22	6.79	9.18
r4		37.34	28.97	2.55	22.06	7.04	11.36
r5		28.97	20.42	2.02	13.08	4.93	8.90
r6		447.15	118.26	7.05	198.47	15.88	49.90
r1	6	119.45	54.24	3.09	102.96	7.78	22.46
r2		26.11	16.04	1.45	10.33	4.42	8.20
r3		28.76	18.95	2.03	15.01	6.88	12.79
r4		41.75	30.14	2.69	20.25	7.12	11.93
r5		32.96	17.23	1.79	13.83	4.33	7.87
r6		403.64	107.07	6.29	185.77	14.77	40.51
r1	8	184.21	52.59	4.82	89.65	8.24	23.74
r2		25.58	12.76	1.80	10.26	5.50	7.02
r3		30.31	16.35	2.40	13.42	6.82	8.58
r4		40.67	27.99	2.82	18.06	10.03	14.05
r5		33.11	17.72	2.10	12.90	7.71	8.64
r6		399.93	112.78	7.77	201.31	16.02	42.87
r1	15	166.24	47.97	3.68	67.53	7.64	14.90
r2		8.33	2.83	1.87	2.39	4.87	2.51
r3		2.25	2.75	2.76	3.90	5.89	6.79
r4		4.77	6.37	2.47	7.01	7.35	9.00
r5		2.47	3.79	2.52	2.60	7.61	6.08
r6		248.13	76.25	7.90	233.83	16.41	44.73

Table B.17 cont.

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	24	134.48	40.92	4.71	29.21	13.24	19.73
r2		4.59	2.52	0.00	1.28	1.74	0.00
r3		3.15	1.30	0.00	0.00	1.78	0.00
r4		1.26	0.00	0.00	0.00	0.00	0.00
r5		0.69	0.00	0.00	0.00	0.00	0.00
r6		98.32	68.23	7.82	191.61	17.03	52.08
r1	26	73.50	33.51	4.53	15.99	10.15	8.51
r2		3.40	1.75	0.00	0.78	1.09	0.00
r3		3.19	0.00	0.00	0.00	1.09	0.00
r4		1.36	0.00	0.00	0.00	0.00	0.00
r5		0.83	0.00	0.00	0.00	0.00	0.00
r6		90.64	83.57	8.63	231.60	14.38	44.45
r1	28	47.41	32.52	5.01	8.64	10.89	7.27
r2		2.08	0.97	0.00	0.00	0.00	0.00
r3		6.15	0.91	0.00	0.00	0.00	0.00
r4		2.28	0.00	0.00	0.00	0.00	0.00
r5		0.90	0.00	0.00	0.00	0.00	0.00
r6		76.36	87.09	7.62	196.41	15.56	41.01
r1	30	47.02	30.73	4.77	6.26	10.75	6.81
r2		1.94	0.90	0.00	0.00	0.00	0.00
r3		11.86	0.96	0.00	0.00	1.80	0.00
r4		2.81	0.00	0.00	0.00	0.00	0.00
r5		1.08	0.00	0.00	0.00	0.00	0.00
r6		65.90	71.34	7.11	193.55	15.18	41.28
r1	32	25.60	25.18	7.78	2.64	21.26	6.13
r2		1.85	0.88	0.00	0.00	0.00	0.00
r3		14.24	0.98	0.00	0.00	1.51	0.00
r4		2.64	0.00	0.00	0.00	0.00	0.00
r5		2.39	0.00	0.00	1.11	0.00	0.00
r6		70.94	75.86	10.85	195.04	16.39	42.86
r1	48	n/a	n/a	n/a	n/a	n/a	n/a
r2		n/a	n/a	n/a	n/a	n/a	n/a
r3		n/a	n/a	n/a	n/a	n/a	n/a
r4		2.26	1.05	0.00	1.31	0.00	0.00
r5		128.37	8.90	1.88	3.03	4.40	0.00
r6		18.06	34.11	8.63	214.98	18.03	53.11

Table B.17 cont.

Reactor	Hour	HAc (mg/L)	HPr (mg/L)	iHBu (mg/L)	nHBu (mg/L)	iHVa (mg/L)	nHVa (mg/L)
r1	50	n/a	n/a	n/a	n/a	n/a	n/a
r2		n/a	n/a	n/a	n/a	n/a	n/a
r3		n/a	n/a	n/a	n/a	n/a	n/a
r4		1.87	0.00	0.00	0.00	0.00	0.00
r5		151.79	9.83	2.71	2.96	5.04	0.00
r6		23.73	19.10	7.64	125.63	16.92	32.42
r1	52	n/a	n/a	n/a	n/a	n/a	n/a
r2		n/a	n/a	n/a	n/a	n/a	n/a
r3		n/a	n/a	n/a	n/a	n/a	n/a
r4		2.00	0.00	0.00	0.00	0.00	0.00
r5		137.36	10.98	2.71	2.42	4.57	0.00
r6		30.17	19.12	8.11	122.37	18.57	44.67
r1	54	n/a	n/a	n/a	n/a	n/a	n/a
r2		n/a	n/a	n/a	n/a	n/a	n/a
r3		n/a	n/a	n/a	n/a	n/a	n/a
r4		1.59	0.00	0.00	0.00	0.00	0.00
r5		165.01	10.25	3.00	1.69	5.39	0.00
r6		46.02	16.12	9.33	108.05	15.21	30.21

APPENDIX C: KINETIC RATE CONSTANT CALCULATION

(The data for this example are taken from Run 6, Reactor 1 of this study.)

The first step in calculating the kinetic rate constant is creating a data table in the following manner:

Table C1: Kinetic Rate Constant Data

Time (hrs)	$[NO_3^-$-N] (mg/L)	LN$[NO_3^-$-N]	1/$[NO_3^-$-N]
0	50.8	3.93	0.020
2	46.7	3.84	0.021
4	45.6	3.82	0.022
6	39.8	3.68	0.025
8	36.9	3.61	0.027
15	21.4	3.06	0.047
24	7.8	2.06	0.127
26	0.9	-0.08	1.083
28	0.0	Error	Error

The data must be graphed in three ways to determine the order of the reaction in the following manner:

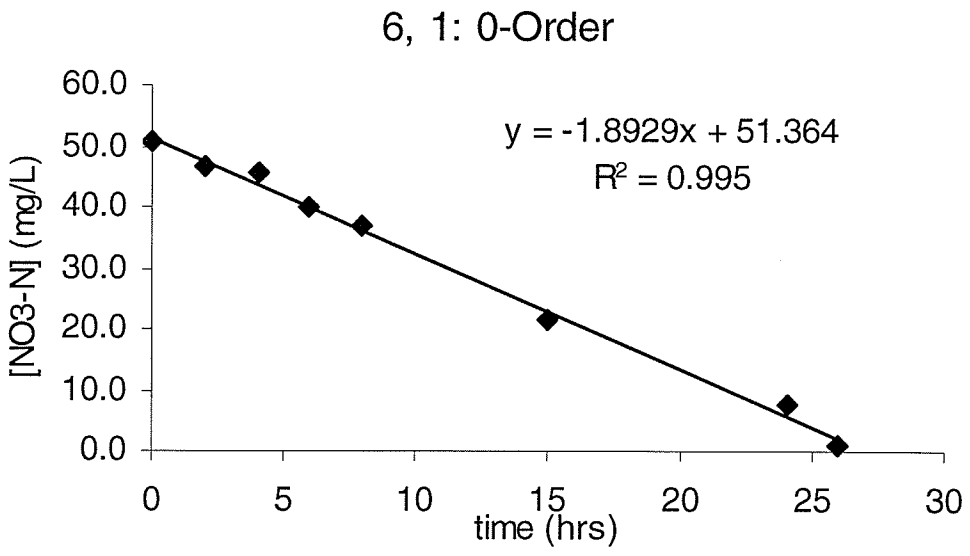

Figure C1: Zero-Order Data

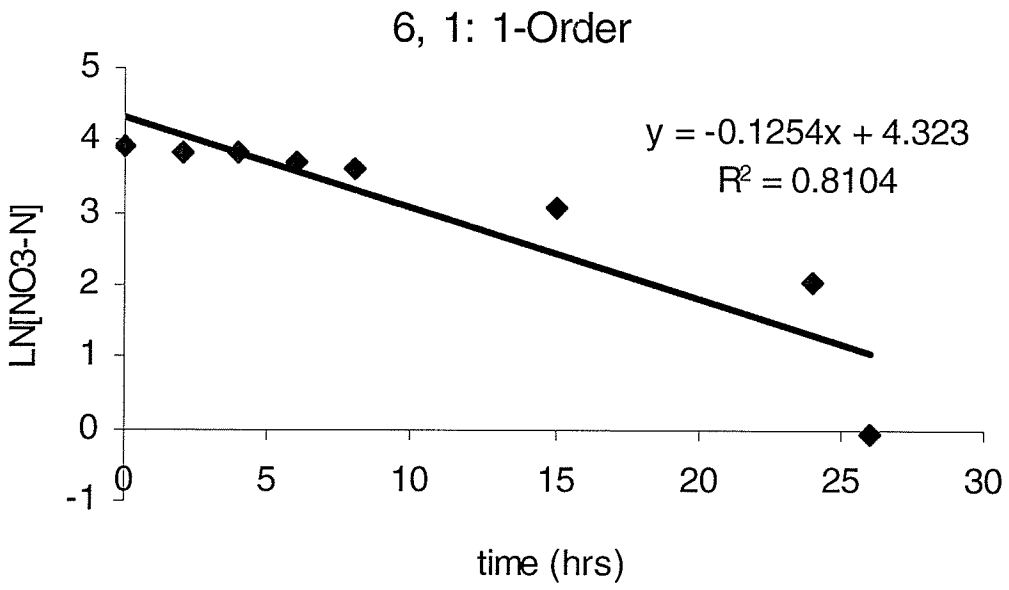

Figure C2: First-Order Data

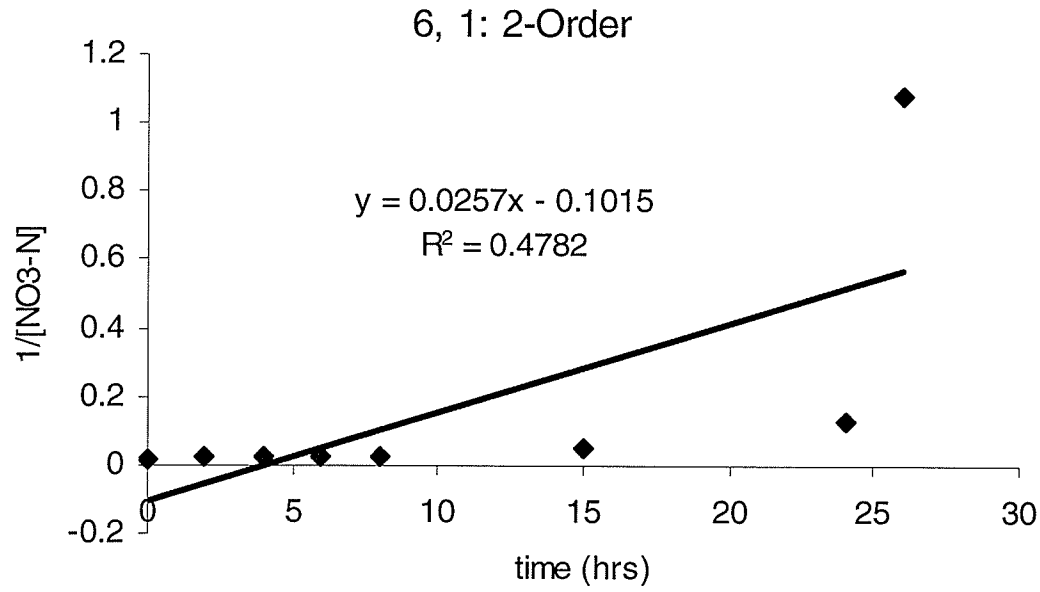

Figure C3: Second-Order Data

A cursory review of Figures C1, C2, and C3 reveal that the zero-order data provide the best approximation of the data as reflected in the correlation

coefficient (R^2) near unity. The manipulation of the example data has shown that the reaction is zero-order.

The equation of the linear trendline possesses a slope of 1.89 mg/L*hr. This value corresponds to the zero-order kinetic rate constant. In this fashion, the order and kinetic rate constants of each of the reactions was determined.