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Gabriel Thibault
AUTEUR DE LA THÈSE / AUTHOR OF THESIS

M.A.Sc. (Environmental Engineering)
GRADE / DEGREE

Department of Civil Engineering
FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

Effects of microwave irradiation on the characteristics and mesophilic anaerobic digestion of sequencing batch reactor sludge

TITRE DE LA THÈSE / TITLE OF THESIS

Kevin J. Kennedy
DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS

L. Fernandes

B. Ormeci

N. Ross

Gary W. Slater
LE DOYEN DE LA FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTDOCTORALES /
DEAN OF THE FACULTY OF GRADUATE AND POSTDOCORAL STUDIES

EFFECTS OF MICROWAVE IRRADIATION ON THE CHARACTERSITICS AND MESOPHILIC ANAEROBIC DIGESTION OF SEQUENCING BATCH REACTOR SLUDGE

by

Gabriel Thibault

A thesis submitted under the supervisor of

Dr. Kevin J. Kennedy

in partial fulfillment of the requirements

for the degree of Master of Applied Sciences in Environmental Engineering

Department of Civil Engineering

University of Ottawa

Ottawa, Canada

Your file Votre référence
ISBN: 978-0-494-14959-1
Our file Notre référence
ISBN: 978-0-494-14959-1

ABSTRACT

Wastewater treatment generates large quantities of sewage sludge whose disposal is expensive. Mainly because anaerobic digestion produces methane, which can be beneficially used as an energy source, this process has become the most popular means of stabilizing sewage sludge prior to spreading on agricultural land or disposal to landfills. Several pretreatment technologies have recently been developed to render sludge more degradable during anaerobic digestion. The purpose of this study is to investigate whether microwave irradiation can enhance the anaerobic degradability of aerobic sequencing batch reactor sludge.

Relationships relating microwave irradiation duration, microwave intensity, sludge concentration and the temperature reached by the sludge were developed. Sludge concentration in the 1.2-4.3% total solids range was found not to impact the temperature reached by the sludge. Three techniques were used to assess the impact of microwave irradiation on the size of the particles: visual analysis of sludge settling, microscopic analysis and particle size distribution analysis. A fraction of the particles larger than 100 μm were found to be broken down into smaller particles. The effects of the temperature of microwave treatment, microwave intensity and sludge concentration on the solubilization of the chemical oxygen demand (COD) were analyzed. Only the first variable was found to have a significant effect. The maximum soluble to total COD ratio (sCOD/tCOD) obtained using microwave irradiation to 85ºC was approximately 7%. The maximum sCOD/tCOD ratio of the sludge was found to be 57% using a strong dose of NaOH.

Two biochemical methane potential assays (BMP) were carried out to explore the effects of partial sludge treatment, temperature of microwave treatment, addition of a small dose of NaOH, multiple irradiation cycles and maintaining sludge temperature to 85ºC for 10 minutes after a single irradiation cycle. Partial pretreatment did not improve the anaerobic degradability. All other pretreatment conditions yielded similar improvements in biogas production. Specifically, the maximum biogas production observed represented a 16.2%

improvement over the control. These enhanced biogas production values were accompanied by a decrease in dewaterability as determined by the capillary suction test (CST).

Microwave irradiation of sludge to a temperature of 85ºC did not impact the dynamic viscosity and surface tension of the sludge.

RÉSUMÉ

Le traitement des eaux usées génère de grandes quantités de boue municipale. La gestion de ces boues coûte très cher aux municipalités. En vertu du fait que la digestion anaérobie émet du méthane, un gaz qui peut être utilisé comme source d'énergie, ce procédé est devenu une méthode populaire pour la stabilisation des boues municipales avant qu'elles soient épandues sur les terres agricoles ou enterrées dans des sites d'enfouissement sanitaire. Plusieurs technologies de prétraitement ont récemment été développées pour rendre la boue municipale plus dégradable lors de la digestion anaérobie. Le but de cette recherche est de déterminer si l'irradiation par micro-ondes de boue municipale provenant d'un réacteur biologique séquentiel peut améliorer la digestion anaérobie de celle-ci.

Des relations entre la durée de l'irradiation par micro-ondes, l'intensité du micro-onde, la concentration de la boue et la température atteinte par la boue ont été développées. La concentration de la boue dans l'étendue de 1.2-4.3% de matières sèches n'a pas eu d'impact sur la température atteinte par la boue. Trois techniques ont été utilisées afin d'évaluer l'impact de l'irradiation par micro-ondes sur la grosseur des particules : analyse visuelle de la sédimentation des particules, analyse microscopique et analyse granulométrique. Une fraction des particules plus grosses que 100 μm ont été brisées en de plus petites particules. Les effets de la température du traitement, de l'intensité du micro-onde et la concentration de la boue sur la solubilisation de la demande chimique en oxygène (DCO) ont été étudiés. Il a été déterminé que seulement la première variable a un effet significatif sur la DCO soluble. Le rapport maximale entre la DCO soluble et totale (sDCO/tDCO) avec traitement par micro-ondes de la boue jusqu'à une température de 85ºC fut de 7%. Le rapport sDCO/tDCO maximal de cette boue obtenu en utilisant une forte dose de NaOH fut de 57%.

Deux analyses biochimiques de potentiel en méthane ont été effectuées afin de déterminer les effets du traitement partiel de la boue, la température du traitement par micro-ondes, l'addition d'une petite quantité de NaOH, des cycles d'irradiation multiples et le maintient de la boue à une température de 85ºC pour dix minutes après un seul cycle d'irradiation. Le traitement partiel de la boue n'a pas amélioré la digestion anaérobie de cette boue. Toutes les

autres configurations de prétraitement ont amélioré la digestion anaérobie d'une façon semblable. Spécifiquement, la production maximale de biogaz observée constituait une amélioration de 16.2% par rapport aux échantillons contrôle. Ces bénéfices furent accompagnés par une diminution de la déshydration des boues tel que déterminé par le test de succion.

L'irradiation de boue par micro-ondes jusqu'à une température de 85ºC n'a pas eu d'impact sur la viscosité dynamique et sur la tension superficielle.

ACKNOWLEDGEMENTS

I wish to express my most sincere gratitude to Dr. Kevin Kennedy for providing me an opportunity to develop research, analytical, communication and interpersonal skills through this master's program. I appreciated his knowledge, kindness, easy-going nature and financial support.

I would like to acknowledge the high quality of teaching delivered by the environmental professors of the department. I thoroughly enjoyed the lectures and seminars offered and organized by Drs. Ronald Droste, Leta Fernandes, Kevin Kennedy, Roberto Narbaitz and Frédéric Tremblay.

A loud Thank You is also extended to Francisco Aposaga and Louis Tremblay for providing technical support and for their friendly nature. Francisco has been instrumental in the day-to-day assistance in the laboratory in providing equipment, reagents, filling empty gas tanks, etc. Louis was very kind in letting me use equipment from his department and his enthusiasm for science will be fondly remembered. I also want to thank Gabriela Fonseca for kindly showing me how to operate the viscometer and the tensiometer.

The cooperation provided by our competent and friendly administrative assistants was truly appreciated. Special thanks to Alain Boisvenue, Claire Focsaneanu and Yolande Hogan.

I would also like to thank Laura Seaman for delivering me regular shipments of sludge from Rockland. I am also grateful to the numerous friendly colleagues I have come across in my studies at the University of Ottawa. Your honest and friendly nature is cherished. In this regard, special thanks to Muna Albanna, Darren Ayyad, Cécile Bellec, Marcela Dereix, Berhe Entehabu, Jill Hass, Juan Marin-Hernandes, Barbara Minor, Ahn Nguyen, Laura Seaman, Martine Séguin, Ranya Sherif, Megan Storrar and Kate Zheng.

I believe that few students could succeed in graduate studies without the emotional support of family and friends. I am blessed in that regard and would like to extend my deepest appreciation to my family for their encouragement and for always having made my education a priority, my friends and squash partners for the good times and stress relief on the court. Most importantly, none of this would have been possible without the encouragement, unconditional love and patience of my beloved, Dahlia. I am extremely thankful for the sacrifices you have made and I adore you.

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NOMENCLATURE

Abs	Absorbance
β	Constant
C	Control
COD	Chemical Oxygen Demand, mg O_2/L
DIG	Anaerobically Digested Sludge
EP	Electrode Potential, mV
EXT	Activated Sludge from Extended Aeration
In	Inoculum
kGy	Kilo Gray
kHz	Kilo Hertz
Meq	Mili Equivalent
MeV	Mega Electron Volt
MW I	Microwave Intensity
MWt	Microwave Irradiation Time
OC	Specific Oxygen Consumption
R^2	Coefficient of Determination
SC	Sludge Concentration, %TS
sCOD	Soluble Chemical Oxygen Demand, mg O_2/L
SE	Standard Error
SH	Shear-gap Homogenizer
sTOC	Soluble Total Organic Carbon
T	Temperature Reached by the Sludge Upon Microwave Irradiation, ºC
tan (δ)	Dissipation Factor
tCOD	Total Chemical Oxygen Demand, mg O_2/L
Temp	Temperature, ºC
TS	Total Solids, weight %
tVFA	Total Volatile Fatty Acids
Var	Variance
VFA	Volatile Fatty Acids, mg/L

VS	Volatile Solids, weight %
μm	Micro Meter
W	Mass of Dry Evaporating Dish, g
X	Mass of Dry Evaporating Dish + Wet Sample, g
Y	Mass of Dry Evaporating Dish + Dry Sample, g
Z	Mass of Dry Evaporating Dish + Fixed Solids, g

LIST OF ABBREVIATIONS

AS	Activated Sludge
BMP	Biochemical Methane Potential Assay
BSS	Back-Scattering Spectroscopy
CFU	Colony Forming Unit
CHF	Capillary Hydrodynamic Fractionation
CRR	Cell Rupture Ratio
CST	Capillary Suction Time
DC	Disk Centrifuge
DNA	Deoxyribonucleic Acid
DR	Disintegration Rate
EC	Electrode Counter
GC	Gas Chromatograph
GS	Gravitational Sedimentation
HPH	High-pressure Homogenizer
HRT	Hydraulic Retention Time
HY	Hydrolysis Yield
IC	Inorganic Carbon
LALLS	Low Angle Laser Light Scattering
LC	Light Counter
MC	Microscope Counting
ORP	Oxidation-reduction Potential
PCS	Photon Correlation Spectroscopy
PETE	Polyethylene Terephthalate
PS	Primary Sludge
RNA	Ribonucleic Acid
ROPEC	Robert O. Pickard Environmental Centre
RPM	Revolutions Per Minute
SBM	Stirred Ball Mill
SBR	Sequencing Batch Reactor

SPC	Soluble Protein Concentration
SRT	Solids Retention Time
STP	Standard Temperature and Pressure
SVI	Sludge Volume Index
TFC	Time of Flight Counter
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
TSS	Total Suspended Solid
TWAS	Thickened Waste Activated Sludge
UH	Ultrasonic Homogenizer
VFA	Volatile Fatty Acid
VOC	Volatile Organic Compound
VSS	Volatile Suspended Solid
WAS	Waste Activated Sludge

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CHAPTER 1: INTRODUCTION

1.1 Background

Municipal wastewater treatment plants benefit society by reducing the amount of organic matter and the number of pathogens discharged to watercourses. Unfortunately, wastewater treatment also generates large quantities of sludge. The treatment and disposal of sludge is recognized as the most expensive part of municipal wastewater treatment and the most complex problem facing the sanitary engineer (Metcalf and Eddy, 2003). Sludge management includes pumping, grinding, screening, blending, thickening, digestion, conditioning, dewatering and disposal.

Typical large-scale wastewater treatment plants generate two main types of sludge: primary and waste activated sludge (WAS). Primary sludge consists of settleable organic and inorganic matter, is very offensive and readily degradable. WAS consists of the soluble-organics-consuming bacteria settled in the secondary clarifier and is difficult to degrade due to the cell wall surrounding bacteria. These two types of sludges are typically mixed together and stabilized. Options include alkaline stabilization, aerobic digestion, anaerobic digestion and composting.

Anaerobic digestion is the most popular sludge stabilization process. The advantages of this process include the production of methane which may be used as an energy source, the low production of waste sludge and potentially high organic loading rates. However, because a large portion of the organic material is bound in the cell wall in WAS, greater stabilization and methane production may be obtained by pretreating WAS before it is treated in anaerobic digesters. Such pretreatment processes aim to disrupt the bacterial cell wall and thus increase the amount of organic material available for digestion. Examples of pretreatment options that have been shown to enhance anaerobic digestion include alkaline, mechanical, thermal, ultrasound and steam explosion pretreatment. Another advantage of most pretreatment technologies is the significant reduction in the number of pathogens present in the sludge. Application of sludge to agricultural land thus becomes more attractive. Thermal pretreatment has been found to be particularly successful at both improving anaerobic digestion and reducing pathogen numbers but the vast amount of energy required to heat the sludge offset the advantages of increased methane and lower sludge disposal costs obtained. An innovative pretreatment option is the thermal pretreatment of sludge using microwave

irradiation. Microwave technology is capable of rapidly heating materials containing polar molecules (such as water) while using significantly less energy than conventional heating. In addition, microwave irradiation heats materials more uniformly.

In small communities, wastewater treatment in complex plants including primary and secondary treatment is not financially feasible. Instead, these municipalities treat wastewater using simpler processes such as the aerobic sequencing batch reactor (SBR). In such a plant, wastewater is first passed through screens or a macerator to remove coarse materials. It is then pumped to a basin until it is filled. The wastewater is then mixed vigorously during the react phase so that bacteria have sufficient oxygen to degrade the organic matter. Thereafter, mixing is stopped and the bacteria and other particles are allowed to settle to the bottom. Next, the supernatant is withdrawn, chlorinated and discharged to a watercourse. The sludge accumulating at the bottom of the basin during the settling phase is typically pumped to another basin, allowed to dry and then transported to a landfill for disposal. With the recent implementation of the Kyoto Protocols, an increasing number of small municipalities will be required to recover the energy content of the sludge they generate through anaerobic digestion. Pretreatment technologies will likely be called upon to boost the production of methane.

1.2 Research Objectives

The overall purpose of this research is to determine whether microwave irradiation pretreatment could enhance the anaerobic degradability of sequencing batch reactor sludge from Rockland, ON. This objective will be met through the following steps:

- Develop models relating the duration of microwave irradiation, microwave intensity, sludge concentration and the temperature reached by the sludge.
- Determine the effects of microwave irradiation on the size of sludge particles.
- Assess the maximum soluble to total chemical oxygen demand (sCOD/tCOD) of the sludge using a harsh pretreatment method.
- Establish whether temperature of microwave treatment, microwave intensity and sludge concentration have an effect on the sCOD/tCOD ratio of the sludge and, if so,

compare the maximum ratio obtained using microwave irradiation to the ratio yielded by the harsh treatment.

- Carry out two biochemical methane potential assays (BMP) to determine whether microwave irradiation can enhance the anaerobic degradability of SBR sludge. The variables investigated during the BMP assay will be developed by considering the results obtained thus far.
- Test whether the conditions that yielded the best results during the BMP assays have an effect on the viscosity and surface tension of the SBR sludge.

1.3 Layout of Thesis

This thesis is composed of five chapters and five appendices. Chapter 2 presents the literature review conducted during the course of this project. It lists related regulations, presents the basics of the anaerobic digestion of sludges, discusses four important relevant characteristics of sludge and offers a summary of previous research carried out in the field of sludge pretreatment technologies for enhancing anaerobic digestion. Chapter 3 describes the materials and methods used to conduct all experiments performed during this thesis work. Herein, the origins of the SBR sludge are discussed, the experimental protocols and analytical methods are listed and described and the sample preservation techniques are listed for each analytical test. Chapter 4 contains the results and discussion. The conclusions and recommendations for future work are enumerated in Chapter 5. The appendices include the raw data and other sections.

CHAPTER 2: LITERATURE REVIEW

2.1 Regulations

Guidelines and regulations pertaining to the spreading of biosolids on agricultural land follow three approaches: no net degradation, best achievable and the risk-based approach (Epstein, 2003). The former proposes the development of regulations that would insure the level of contamination of the soil is not negatively affected after spreading. This is very difficult to achieve considering, for example, that the background concentration in heavy metals of soils vary substantially in different areas. On the other hand, the best achievable method establishes required biosolids quality based on what the best available technology can accomplish. The risk-based approach sets regulations based on the assessed risks to humans and the environment.

The USEPA has used the risk-based approach and necessitated nine years to develop regulations for the spreading of biosolids on agricultural land with regards to pathogens, vector attraction, organics and heavy metals concentrations. The list of pollutants considered under the USEPA regulations is presented in Table 2.1.

Table 2.1: List of contaminants regulated by the "40 CFR Part 503" of the USEPA (Epstein, 2003).

ORGANICS	HEAVY METALS
Aldrin/dieldrin (total)	Arsenic
Benzene	Cadmium
Benzo(a)pyrene	Chromium
Bis(2-ethylhexyl)phthalate	Copper
Chlordane	Lead
DDT/DDE/DDD (total)	Mercury
Heptachlor	Molybdenum
Hexachlorobenzene	Nickel
Hexachlorobutadiene	Selenium
Lindane	Zinc
N-nitrosdimethylamine	
Polychlorinated biphenyls	
Toxaphene	
Trichloroethylene	

The section of 40 CFR Part 503 that deals with heavy metals classifies biosolids as high and low quality. The most probable number (MPN) of fecal coliforms and *Salmonella* sp. in the biosolids determines whether the product is classified as "class A" or "class B". Only class A biosolids may be applied to lawns and home gardens. Biosolids are referred to as Class A if the fecal coliform density is lower than 1,000 MPN/g of total solids (TS) or the *Salmonella* sp. density is less than 3 MPN per 4 g of TS. In addition to meeting one of these criteria, the biosolids must have been produced using one of six specific processes including a high pH-high temperature method. Class B biosolids are generally applied to agricultural land or disposed of in landfills. To meet class B requirements, biosolids must contain less than 2.0×10^6 MPN of fecal coliforms per g of TS or have been produced by a "process that significantly reduce pathogens" (Metcalf and Eddy, 2003). The other area of 40 CFR Part 503 deals with vector attraction reduction which is necessary to reduce the risks of infectious disease transmission by burrowing animals and birds. Vector attraction reduction must be carried out through one of ten possible options which essentially aim to reduce the volatile solids (VS) content of the biosolids or to create barriers between the biosolids and vectors (Epstein, 2003).

In Ontario, biosolids are regulated through Regulation 347 of the Environmental Protection Act. Producers of biosolids must apply for a certificate of approval from the Ministry of the Environment (MOE) prior to spreading biosolids on agricultural land. The ministry regulates potentially desirable constituents such as nitrogen, phosphorus, potassium, other nutrients and organic matter, as well as potentially undesirable constituents such as heavy metals, sodium, boron, industrial organic compounds and non-biodegradable constituents. In addition to the heavy metals listed in Table 2.1, the province of Ontario also monitors the chromium content of soils and biosolids. Moreover, issuing of the certificate of approval is contingent upon the type of crops grown by the farmer receiving the biosolids, the soil type, slopes, separation distances from watercourses, groundwater, bedrock, residences and the storage facilities employed (MOE and MAFR, 1996). The Ontario regulations are vague regarding the pathogenic requirements, simply stating that "before applying any waste to agricultural land, it must be treated in such a manner as to minimize the odor potential and reduce the number of pathogenic organisms ... to an acceptable level" (MOE and MAFR, 1996).

2.2 Anaerobic Digestion of Sludges

Anaerobic digestion is the most widely used sludge stabilization process. It is also used for wastewater treatment, industrial wastewater treatment and, mostly in Europe, for processing the organic fraction of municipal solid waste. The main advantages of anaerobic digestion are the production of methane which may be used as an energy source, the low production of waste sludge and potentially high organic loading rates.

There are a number of units in typical wastewater treatment plants that produce sludge which is commonly digested anaerobically. Examples include primary clarifiers (primary sludge), secondary clarifiers (WAS) and trickling filters (trickling-filter sludge). Relatively small rural communities often employ aerobic SBRs to treat the organic fraction of wastewater. These reactors are operated in four consecutive modes. First, the reactor is filled with wastewater. Next, the reactor is aerated and mixed vigorously so the aerobic bacteria can degrade the organic matter present in the wastewater. Subsequently, mixing is stopped and the suspended materials are allowed to settle to the bottom of the tank. The final phase is decantation when the supernatant is pumped out of the tank and chlorinated before discharge to a watercourse. A portion of the sludge accumulating at the bottom of the reactor is regularly removed. This is the sludge type that is used in this research. Figure 2.1 displays the operation of a SBR.

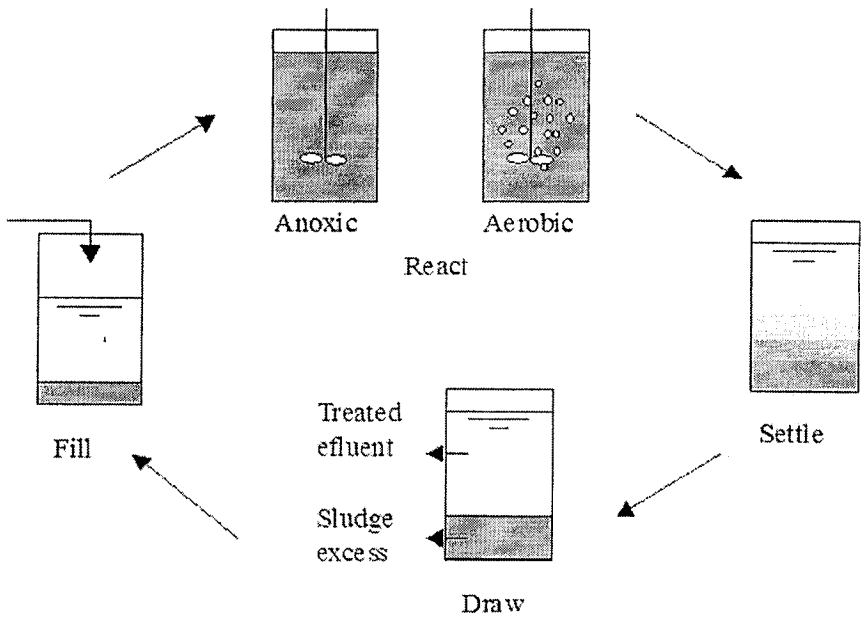


Figure 2.1: Operating modes in a sequencing batch reactor (EOLI, date unknown).

There are three steps to the anaerobic metabolism. The first, hydrolysis, involves the enzymatic disintegration of large and complex molecules into smaller ones which may be metabolized by the cells (Speece, 1996). Examples of products from this step include sugars, amino acids, and peptides. This step is widely regarded as the rate limiting step of the anaerobic digestion of sludge (Parkin and Owen, 1986). The second step is acidogenesis, the production of hydrogen and acetic, butyric and propionic acid. Finally, methanogenesis is the phase where methane and carbon dioxide are generated from acetate and hydrogen. This step is often the rate limiting step for easily degradable soluble wastewaters. A simplified diagram of the reactions involved is shown in Figure 2.2.

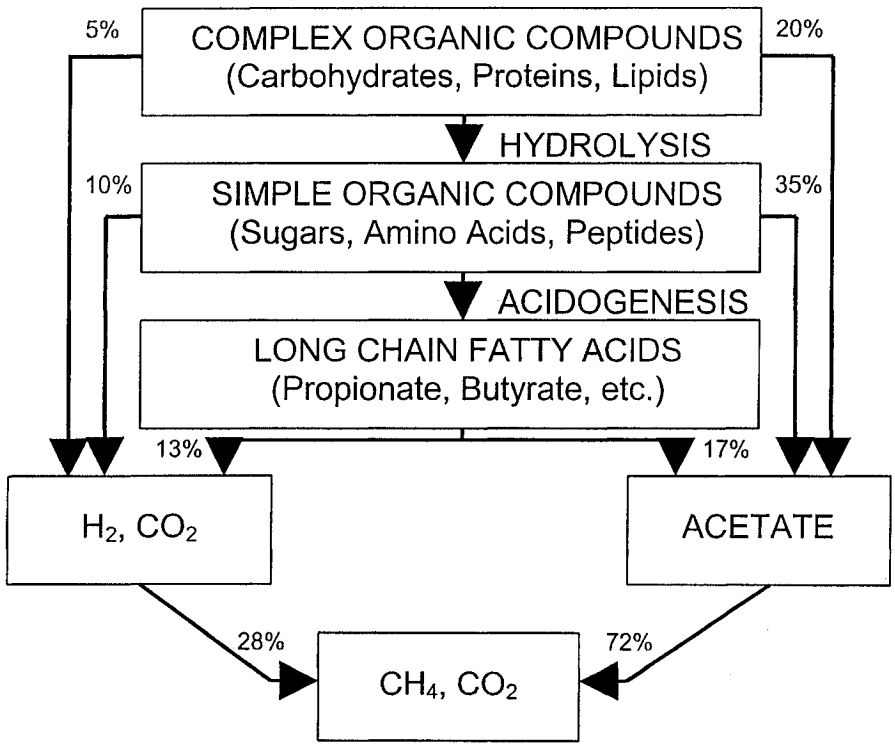


Figure 2.2: Simplified representation of the anaerobic metabolism (Speece, 1996).

There are thus two main groups of bacteria involved in anaerobic digestion: the acidophiles and the methanogens. The former group has an optimal pH between 5 and 6 but can tolerate a wide pH range and is generally considered robust. On the other hand, the methanogens have an optimal pH of around 7, a slower growth rate and are less tolerant to unfavorable environmental conditions. For those reasons, anaerobic reactors are operated at conditions most favorable to the methanogens.

A serious problem that must be avoided in anaerobic digestion is when the methanogens cannot degrade the acids produced by the acidophiles as fast as they are formed. As the acids accumulate, the pH decreases thus making conditions more favorable to the acidophiles and unfavorable for the methanogens which aggravates the situation. If this condition is allowed to persist, methane production may stop completely. Reactor recovery is typically a lengthy process.

Alkalinity is added to anaerobic reactors to allow for a temporary overproduction of volatile fatty acids (VFA). The desirable alkalinity is 1,000-5,000 mg/L as $CaCO_3$ and VFA concentration should be kept below 2,000 mg/L as acetic acid. However, the ratio of these two parameters (VFA/alkalinity) is said to be more important and should be kept below 0.3. Peak performance for mesophilic anaerobic digestion is attained at a temperature of 33-35 °C and a pH of 7.0-7.2.

Monitoring of the process is crucial to ensure proper treatment efficiency and that remedial action may be taken as soon as possible if needed. Typical tests include chemical oxygen demand (COD) reduction, VS removal, volatile suspended solids (VSS) concentration, pH, alkalinity, VFA concentration, ammonia concentration, biogas production and gas composition. The theoretical maximum yield of methane at 35°C is 0.39 L methane per g of COD removed. However, actual measured values in the range of 0.10-0.35 have been reported in the literature (Droste, 1997). Some of the reasons given for low values are gas leakage and conversion of some of the organics to compounds not oxidized during the COD test.

There is a long list of chemical compounds that may be toxic to the anaerobic consortium above specific concentrations (Speece, 1996). However, the literature is filled with examples of bacteria having successfully adapted to high concentrations of toxic compounds. This phenomenon is called acclimation. The procedure consists of simply feeding a reactor with the substrate containing the toxic compound(s) and monitoring the process until desired reactor performance is attained.

The test which is used to determine the potential for anaerobic degradation of a waste is the BMP assay. The waste sample is added to a serum bottle along with acclimated anaerobic biomass, a buffering solution and nutrients (if required). The bottle is incubated at 35°C and the gas production and composition are monitored. In addition, the COD is measured before and after the assay is conducted. The BMP assay allows the determination of the fraction of the COD that is anaerobically degradable, provides an estimate of degradation kinetics and may indicate toxicity but should not be used to design a large-scale anaerobic reactor. Such information must come from a pilot plant study that more closely simulates the operation of an actual anaerobic digester (Speece, 1996).

2.3 Important Relevant Characteristics of Sludge

2.3.1 Particle Size

As will be clearly demonstrated by numerous studies listed in section 2.4, particle size reduction is a key goal of pretreatment of sludge for anaerobic digestion. Depending on the pretreatment method employed, particle size reduction is typically accompanied by the release of cellular material due to cell rupture and modification of the material structure (Palmowski and Muller, 2003). These three mechanisms are responsible for the improvements in degradation rate and degree observed during anaerobic digestion of pretreated sludge. Palmowski and Muller (2003) summarized the roles of these mechanisms in Figure 2.3.

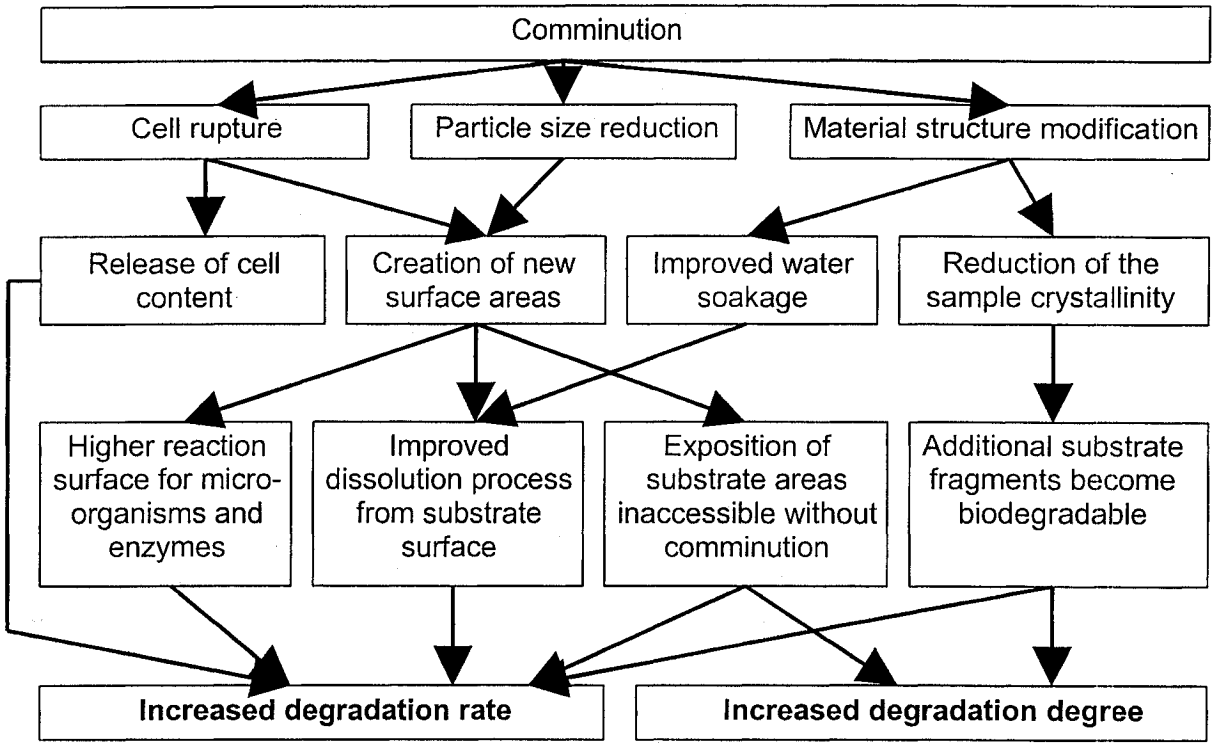


Figure 2.3: Connections between comminution and increased degradation rate and degree (Palmowski and Muller, 2003).

Numerous techniques exist to assess the particle size distribution of samples. These are classified in three groups: ensemble, counting and separation methods. The first group of techniques involves the analysis by computers of mixed data obtained simultaneously. This group includes low angle laser light scattering (LALLS), photon correlation spectroscopy (PCS) and back-scattering spectroscopy (BSS). The counting methods function by analyzing particles one at a time and sorting the information in different bins based on the size of the particles. The electrozone counter (EC), light counter (LC), time of flight counter (TFC) and microscope counting (MC) belong to this group. The separation methods work by exerting a force on the particles to separate them according to size. Examples include sieving, gravitational sedimentation (GS), disk centrifuge (DC) and capillary hydrodynamic fractionation (CHF). The advantages and disadvantages of these techniques are thoroughly listed by CPS Instruments (date unknown). This information is summarized in Appendix A.

Many factors must be considered when selecting a technique: expected range in particle size, shape of the particles, size of sample available, time available, accuracy required, whether

the mixture has particles with different optical properties, and whether the refractive index of the particles are known.

As will be seen in the next two sections, particle size and shape also influence the rheological characteristics and dewaterability of sludge samples.

2.3.2 Viscosity

The viscosity of sludge is a crucial parameter for the design of sludge mixing, pumping systems, and mass transfer. It is defined as the fluid's resistance to flow (shear) and is characterized by adhesive, cohesive or frictional properties. Newton believed the dynamic viscosity to be related to the shearing rate through equation 2.1.

$$\tau = \mu \frac{dv}{dy} \tag{2.1}$$

where τ is the shearing rate, μ is the dynamic viscosity and dv/dy is the velocity gradient. When the relationship between τ and μ is linear, as shown in equation 2.1, the fluid is said to be a Newtonian fluid. When the relationship is not linear, the fluid is called non-Newtonian. Sludge has been found to be a special type of non-Newtonian fluid called pseudo-plastic fluid. These can be modeled using equation 2.2.

$$\mu = k(\tau)^{n-1} \tag{2.2}$$

where k is the consistency index and n is the flow index. Because $n < 1$ for pseudo-plastic fluids, the form of the equation indicates that these fluids develop lower viscosity as the shearing rate is increased. The consistency index is the viscosity of the fluid when the shearing rate is 1 s^{-1} and the flow index is a measure of the degree to which the fluid differs from Newtonian behavior (Tanner, 2000).

The viscosity of colloidal dispersions is known to be affected by particle size, particle shape, the viscosity of the medium and the particle-particle and particle-medium interactions (Shaw, 1992). Sanin (2002) evaluated the effects of pH, ionic strength, solids concentration and flocculation properties on the viscosity of activated sludge, which is a colloidal dispersion. Solids concentration was shown to have the strongest effect on viscosity. In fact, 1.7% sludge yielded an apparent viscosity (μ/τ) seven times higher than 0.5% sludge. Also, the more

concentrated sludge exhibited more pronounced non-Newtonian behavior. No information could be found on the effects of the size of larger particles on the viscosity of sludge.

2.3.3 Dewaterability

Dewatering is the removal of a significant amount of moisture from sludge and biosolids prior to disposal. According to Metcalf and Eddy (2003), sludge dewatering is carried out for one or more of the following reasons:

- To reduce transportation costs
- To ease the manipulation of the sludge and biosolids
- To improve the calorific value prior to incineration
- To reduce the quantity of bulking agents or amendments prior to composting
- To render biosolids odorless and nonputrescible
- To reduce leachate generation during landfilling

Dewatering can be performed using several methods, such as by centrifugation, filter presses, drying beds, lagoons and heat drying. Typically, polymers are added to the sludge prior to dewatering so that greater moisture removals may be achieved.

The most common test employed to measure the dewaterability of sludge and biosolids is the capillary suction time (CST). This test involves placing a small sample of sludge in the middle of a filter paper and reporting the time needed for the water front to travel a specific distance. The faster the water is released from the sludge sample and travels on the filter paper, the better the dewaterability of the sludge is expected to be.

Upon carrying out this literature review, it seems that the factors affecting dewaterability are not well understood. Authors report dewaterability results but do not attempt to explain them. As will be seen in section 2.4, researchers have reported positive, negative and neutral effects of pretreatment of sludge on the dewaterability of digested sludge. Barber (date unknown) and Dereix *et al.* (2005) have reported positive effects; Kopp *et al.* (1997) and Haug and Stuckey (1978) have reported no change; Lin *et al.* (1997) and Weemaes *et al.* (2000) have reported a worsening of the dewaterability.

2.3.4 Foaming

Anaerobic digester foaming is a grave problem faced by many wastewater treatment plants. Barber (2005) presents an exhaustive review of the topic. Foaming may occur if surface active materials and hydrophobic materials are present. A lowering of the surface tension is caused by surface active agents, such as bio-surfactants. Examples of bio-surfactants include soluble microbial products, VFA, extracellular polymers and chelating agents. The lowering of the surface tension allows the hydrophobic materials to migrate to the liquid-gas interface which thus creates an inverted solids profile. This may result in the consequences listed in Table 2.2.

Table 2.2: Consequences of foaming during anaerobic digestion (Barber, 2005).

Effect	**Consequence**
Physical effects	a) Capacity loss b) Blockage of gas pipework c) Interference with monitoring/control d) Interference with floating roofs
Biological effects	a) Inversed solids profile b) Enrichment of cells around gas bubbles c) Microbial lysis d) Protein unfolding e) Metabolic breakdown due to reduced nutrient bioavailability f) Risk of environmental contamination due to bio-aerosols g) Loss of microorganisms, substrates and enzymes into froth h) High VFA/alkalinity ratio
Economic effects	a) Loss of electricity generated from biogas b) Increased oil consumption c) Increased polymer costs for post-digestion dewatering d) Increased personnel and maintenance costs e) Cost of anti-foaming agents f) Power consumption for mechanical breakers

Factors that may bring about or exacerbate the formation of foaming are the presence of fats, oils, grease, proteins, toxicants and refractory COD in primary treatment; nutrient removal in secondary treatment; excessive polymer addition during thickening; and low hydraulic retention time, intermittent operation, biogas mixing, inadequate mixing, bio-surfactants and nutrient limitations during anaerobic digestion. Filamentous microorganisms have been recognized as the main culprit in digester foaming because of their hydrophobic nature and

bio-surfactant producing capabilities. Since the growth of these organisms is favored in nitrifying activated sludge reactors, foam has been a common problem in digesters treating the effluent from these reactors.

Barber (2005) lists three approaches to foam treatment: prevention, indirect treatment and direct treatment. Prevention is the favored approach. Secondary sludge pretreatment has been found to be capable of reducing foaming. Pasteurization at 70°C for five minutes was shown to be capable of destroying *Microthrix parvicella*, a problematic filamentous microorganism (Barber, 2005). Pagilia *et al.* (2002) demonstrated that *Gordonia amarae*, another filamentous microorganism, could produce significant quantities of surfactant. Strains were isolated from activated sludge and grown in 250-mL flasks. Cultures were transferred to 8-L reactors that were fed acetate and hydrophobic hexadecane. The reactor fed acetate was characterized by a drop of surface tension from 70 to 55 dynes/cm within seven days. On the other hand, the surface tension in the reactor fed with hydrophobic substrate dropped to 40 dynes/cm within eight days. This clearly demonstrates that filamentous microorganisms may not only promote foaming due to their hydrophobic nature but also due to their capability to produce surfactants when hydrophobic substrates are available. Therefore, removal of these microorganisms is a key strategy in the control of foaming.

2.4 Pretreatment of Sludges for Anaerobic Digestion

2.4.1 Introduction

Over the past two decades, numerous experimental pretreatment methods have been developed to enhance the anaerobic digestion of municipal sludge. Most techniques can be classified as chemical, mechanical and thermochemical. One goal is to reduce the size of the particles, which results in a greater surface area per unit volume available for degradation (Muller *et al.*, 2004). Another goal is to disrupt the microorganisms in the sludge so that the cell-bound substrate may be released from the cell walls into the solution (Lin, Chang and Chang, 1997). Advantages of these pretreatment methods typically include enhanced VS reduction, increased methane production, smaller reactor volumes, lower disposal costs, improved disinfection, lower viscosity and less scum and foam production in anaerobic digesters. Disadvantages include increased polymer demand for dewatering, release of

nitrogen in the return wastewater and increase in ammonia concentration (Muller *et al.*, 2004). These problems are caused by the decrease in particle size and the improved digestion of proteins.

Muller *et al.* (2004) performed a cost analysis of the implementation of a pretreatment step prior to anaerobic digestion. The assumptions used are detailed in their study. It is clear that numerous factors affect the costs and revenues but Figure 2.4 gives a general idea of the significance of the different contributors to the costs of the operation. It is argued by the authors that pretreatment technologies are only financially beneficial if the costs of sludge disposal are high.

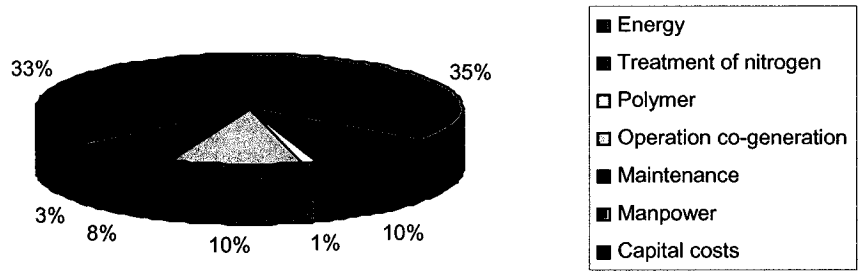


Figure 2.4: Cost factors of disintegration treatment for better digestion results (Muller *et al.*, 2004).

Below is a description of numerous pretreatment technologies along with the results obtained by researchers.

2.4.2 Chemical Pretreatment

2.4.2.1 Acids and Bases

Woodard and Wukasch (1994) developed a hydrolysis/thickening/filtration system to improve the solubilization of total suspended solids (TSS) and cake dryness. Although this process was not established as a pretreatment method for anaerobic digestion, the results of their solubilization study are worth mentioning. An acid dose of 4 g H_2SO_4 per g TSS were added to 1,000 mL of WAS at 25, 50, 70 and 90°C. The highest TSS solubilization was reached at a temperature of 90°C. After 30 minutes of contact, the TSS solubilization had already reached 67% and reached a maximum of 69% after one hour. Another experiment investigated the effect of acid dose when contacted with WAS at room temperature for 30

seconds. With only 1 g H_2SO_4 per g TSS, the TSS solubilization reached 55% and increased linearly at higher acid doses up to a value of 60% at a dose of 8 g H_2SO_4 per g TSS. During these two studies, the researchers observed that large quantities of carbon dioxide were being generated due to the "acidification of bicarbonate ions and the associated dissolution of calcium carbonate salts" (Woodward and Wukasch, 1994). This resulted in the flotation of the remaining suspended solids. This brought about the idea of recycling the subnatant (pellet) to the acidification stage with the goal of reducing the acid dose required. The addition of 0.5 g H_2SO_4 per g TSS to sludge and combining with five times the volume of subnatant yielded an improvement of the TSS solubilization from 38 to 59% after a contact time of only 30 seconds. Although the degree of solubilization reached by acidification in this study is impressive, it remains to be seen whether it would translate into similar improvements during anaerobic digestion. One serious drawback of this pretreatment option would be the large quantities of acids and bases required. Major gains in VS removal and methane production would be necessary to counterbalance this complication and to make this method competitive against the other pretreatment technologies.

Lin *et al.* (1997) conducted a study in which WAS at 1 and 2% TS was pretreated with sodium hydroxide at 20 and 40 meq/L for 24 hours at 25°C and under anoxic conditions. The untreated and pretreated sludge was digested in 1-L semi-continuous anaerobic reactors at solids retention times (SRT) of 20, 13, 10 and 7.5 days. The highest solubilization was achieved in the 1% sludge treated with 40 meq/L (pH = 12.3). For this sludge, the fraction of soluble to total COD was 38% as compared to 2% for the control. Digestion stabilized approximately 30% of the total COD in the control reactor, 43% in the reactor with 1% TS and 20 meq/L NaOH, 44% in the reactor with 1% TS and 40 meq/L and 44% in the reactor with 2% TS and 20 meq/L. For all reactors digesting pretreated sludge, the methane production was at least 19% greater than the control and at most 286% greater than the control. Pretreatment with NaOH worsened the dewaterability of the digested WAS as indicated by the increase in CST by a factor of 4 to 11 as compared to the untreated digested sludge.

Tanaka *et al.* (1997) compared the effects of chemical, thermal and thermochemical pretreatment on WAS. The sludge was contacted with different doses of NaOH in flasks and magnetically stirred for one hour. Increasing the dose of alkali from 0 to 0.6 g NaOH / g VSS improved the solubilization of the VSS linearly and reached a plateau at 15% for greater alkali doses. On the other hand, the methane production improved linearly with alkali dosage, reaching as high as 50% higher than the control at 1 g NaOH / g VSS.

The results of more studies on alkaline pretreatment are presented in section 2.4.12.

2.4.2.2 Ozone

One of the strongest oxidizing agents, ozone, has been found capable of solubilizing a significant portion of organics and, at the same time, to convert part of the COD to carbon dioxide. After generation from pure oxygen, ozone is applied by passing a gas stream through the sludge.

Weemaes *et al.* (2000) investigated the effects of ozonation at doses of 0.05-0.20 g O_3 / g COD on a mixture of primary and secondary sludge (ratio not specified). At a dose of 0.20 g O_3 / g COD, the changes listed in Table 2.3 were observed before and after ozonation.

Table 2.3: Effects of ozonation on sludge characteristics (Weemaes *et al.*, 2000).

	Before Ozonation	**After Ozonation**
tCOD (mg/L)	7,900 ± 500	4,900 ± 600
sCOD (mg/L)	60 ± 50	2,300 ± 100
TOC (mg/L)	2,900 ± 300	2,100 ± 200
sTOC (mg/L)	14 ± 2	1060 ± 60
IC (mg/L)	66 ± 10	2.45 ± 0.02
TSS (mg/L)	9,500 ± 1,200	3,800 ± 500
VSS (mg/L)	5,700 ± 600	1,800 ± 200
SVI (mL/g)	110	28
CST (s)	39	341
PH	7.8	4.9

It is important to note the large increase in soluble COD and significant decrease in total COD. The strength of ozonation is that it both increases the concentration of sCOD and

oxidizes a large portion of the organic matter to CO_2. Analysis of the off-gases confirmed that the decrease in tCOD was indeed due to oxidation and not to the release of volatile organic compounds (VOCs). The pH was lowered considerably upon ozonation and this would require the addition of alkalinity prior to digestion. Non-acclimated inoculum was mixed with sludges treated with 0.05, 0.1 and 0.2 g O_3 / g COD and digested for 30 days at 33°C in 1-L Erlenmeyer flasks. The reactor containing sludge treated with 0.1 g O_3 / g COD performed best by producing approximately 340 mL methane per g COD fed as compared to 120 mL methane per g COD for the control. The dewaterability of this same sludge after digestion improved to 115 seconds, almost as low as the value of 80 seconds recorded for the control sludge. Overall, this pretreatment contributed to a worsening of the dewaterability.

A wider range of ozone doses was investigated by Yeom *et al.* (2002). A 1.2%-TS sludge of undefined origin was ozonated at doses of 0.02-5 g O_3 / g TSS. The solubilization of the sludge particles improved from 0.8% for the untreated sludge to 9.1% at a dose of 0.02 g O_3 / g TSS, 19.6% at a dose of 0.05 g O_3 / g TSS, 23.9% at a dose of 0.1 g O_3 / g TSS, 32.7 g O_3 / g TSS and decreased at higher doses. At doses larger than 0.1 g O_3 / g TSS, the proportion of the particles that was mineralized became large, which resulted in a decrease in solubilized organics. Anaerobic digestion of sludges exposed to the same ozone dosages in 160-mL serum bottles for 30 days established an optimum dose of 0.2 g O_3 / g TSS where 165 mL CH_4 / g COD was produced as compared to 80 mL CH_4 / g COD for the control.

2.4.3 Mechanical Pretreatment

This method entails the subjection of microorganisms to strong and rapid pressure gradients. This action results in the rupture of the cell wall and the release of cell-bound substrate. Because the cytoplasm of microorganisms contains large quantities of proteins, the success of mechanical pretreatment is typically measured by comparing the soluble protein concentration (SPC) before and after pretreatment (Hwang *et al.*, 1997). Mechanical methods include cutting mills, jetting-and-colliding, high-pressure homogenizers, shear-gap homogenizers and stirred ball mills.

Hwang *et al.* (1997) subjected WAS samples to pressure gradients ranging from 5 to 50 bars using mechanical jetting-and-colliding under pressurized conditions. The sludge samples were concentrated for up to 48 hours using a gravity thickening system. Pretreatment at pressures varying from 0 to 30 bars on sludge thickened for less than 12 hours was compared to pretreatment of sludge thickened for more than 12 hours based on the cell rupture ratio (CRR), defined as the SPC after pretreatment divided by the maximum SPC. The CRR was highest for the WAS thickened for more than 12 hours at all pretreatment pressures. The highest CRR reached was approximately 40% at a pretreatment pressure of 30 bars. WAS pretreated at pressures varying between 0 and 50 bars were then digested anaerobically at 35°C for 26 days. Reactors digesting sludges treated at 30 and 50 bars behaved similarly and removed approximately 50% of the VS while the control reactor converted approximately 40% of the VS.

Similar work was carried out by Choi *et al.* (1997). In one phase of the study, WAS was pretreated five consecutive times under pressures of 5, 10, 20, 30, 40 and 50 bars. The pressure of 50 bars yielded a CRR of almost 90%. When thickened sludge was pretreated once at pressures of 10, 30 and 50, the resulting mean particle sizes were 37.0, 21.6 and 18.7 μm, respectively. Non treated thickened sludge had a mean particle size of 69.1 μm. The characteristics of WAS pretreated once at pressures of 30 and 50 bars are listed in Table 2.4. The soluble COD increased by factors of 6.5 and 8 at pressures of 30 and 50 bars, respectively. It is also interesting to note that both the alkalinity and the pH were increased as a result of pretreatment.

Table 2.4: Comparison of sCOD, total organic carbon (TOC), soluble proteins, alkalinity and pH before and after pretreatment at 30 and 50 bars (Choi *et al.*, 1997).

	Before Pretreatment	**Pretreated Once at 30 bars**	**Pretreated Once at 50 bars**
sCOD (mg/L)	152	990	1,250
TOC (mg/L)	90	780	1,010
Soluble Proteins (mg/L)	75	290	320
Alkalinity (mg/L)	229	280	330
pH	6.4	6.5	6.6

Nah *et al.* (2000) also evaluated the feasibility of mechanical jetting-and-colliding. WAS with TS concentration of approximately 16,000 mg/L was pretreated at pressures varying between 0 and 40 bars and the sCOD, soluble proteins and TSS were measured before and after pretreatment. The sCOD increased linearly from approximately 200 to 800 mg/L. Similarly, the SPC increased from 40 to 120 mg/L. The TSS decreased from 20,650 to 19,350 mg/L. The authors repeated the experiment with a pressure of 30 bars and measured more parameters. The results are compiled in Table 2.5. In this case, compared to untreated sludge, sCOD increased by a factor of 5.5, soluble proteins increased by a factor of 2, alkalinity increased by 23% and total soluble phosphorus increased by 18%. Also note that the ammonia concentration increased by approximately 20% following this mechanical treatment.

Table 2.5: Effects of jetting-and-colliding on sludge characteristics (Nah *et al.*, 2000).

Characteristics (mg/L)	**Before Pretreatment**	**After Pretreatment**
SCOD	155	854
TSS	20,057	18,912
STOC	105	740
Soluble Proteins	74	165
Alkalinity (as $CaCO_3$)	232	285
NH_3-N	51	62
Total Phosphorus	541	638

Four methods of mechanical pretreatment were compared by Kopp *et al.* (1997): high-pressure homogenizer (HPH), shear-gap homogenizer (SH), stirred ball mill (SBM) and ultrasonic homogenizer (UH). The duration of grinding (SBM, SH and UH) and the fluid pressure (HPH) were varied and the consumption of oxygen was monitored as follows:

$$DR_0 = [1 - (OC_m / OC_0)] * 100 \quad (2.3)$$

Where DR_0 is the disintegration rate, while OC_m and OC_0 are the specific oxygen consumption of the disintegrated and untreated sludge, respectively. Table 2.6 presents the specific energy needed for the disintegration ratio to reach 40 and 90%.

Table 2.6: Specific energy consumption required for sludge to reach a disintegration ratio of 40 and 90 % based on specific oxygen consumption (Kopp *et al.*, 1997).

	Specific Energy Required to Reach DR_0 (kJ/kg)	
Method	**40%**	**90%**
High pressure homogenizer	1,500	6,000
Shear-gap homogenizer	65,000	Not possible
Stirred ball mill	1,000	40,000
Ultrasonic homogenizer[1]	4,000	60,000

[1] Not designed for continuous operation

The shear-gap homogenizer could not produce a disintegration ratio of 90%. In fact, the maximum disintegration ratio reached by this technique is 45% at a specific energy of 100,000 kJ/kg. Based on these results, the high pressure homogenizer requires the least energy to disintegrate the sludge. WAS with TS of 0.8-1.5% with 70% VS was anaerobically digested at 35°C in continuous 20-L reactors. One experiment was performed at a HRT of four days. In this case, to avoid biomass washout, immobilization of the microorganisms was carried out using vertical bed material. The VSS removal efficiency reached by the high pressure homogenizer and stirred ball mill were 57.5 and 41%, respectively, as compared with 37% for the control. Another experiment was run to compare untreated sludge with WAS pretreated with a stirred ball mill at a HRT of 4-15 days with the biomass in suspension. At a HRT of 4 days the reactor holding the pretreated sludge could destroy 29% of the VSS while the control reactor could only destroy 22%. However, at the HRT of 15 days, both reactors could convert 52% of the VSS. This study indicates that pretreatment could be advantageous at low HRTs but that if digestion is continued for longer periods, the same extent of degradation would be reached by reactors digesting untreated sludge. Upon pretreatment, a decrease in dewaterability and an increase in polymer demand were observed. However, after 15 days of digestion, the dewaterability and polymer demand of treated and untreated sludges were very similar. According to Kopp *et al.* (1997), this is because the colloids produced by the pretreatment methods were adsorbed to the bed material during digestion.

A ball mill and cutting mill were used by Baier and Schmidheiny (1997) to pretreat numerous sludges: activated sludge with SRT = 5 days (AS1), activated sludge with SRT = 7

days (AS2), anaerobically digested sludge (DIG1), thickened anaerobically digested sludge (DIG2) and activated sludge from extended aeration (EXT). The operation of the ball mill was optimized by varying the revolution speed, ball material and ball size while the cutting speed of the cutting mill was varied. It was quickly discovered that the cutting mill could not solubilize the sludges as well as the ball mill and, as a result, only the ball mill was used for the remainder of the experiment. The results of the optimization of the ball mill are shown in Table 2.7. All samples were milled for nine minutes.

Table 2.7: Results of the optimization of the ball mill (Baier and Schmidheiny, 1997).

Test Conditions				Chemical Oxygen Demand (mg/L)		
Sludge	**Revolution Speed (rpm)**	**Ball Material**	**Ball Size**	**Total**	**Soluble Before Pretreatment (% of total)**	**Soluble after Pretreatment (% of total)**
AS1	2,000	Zircon	Coarse	20,200	5.5	12
AS1	3,200	Zircon	Coarse	20,200	5.5	16
AS1	4,200	Zircon	Coarse	20,200	5.5	18
AS1	2,000	Glass	Fine	20,200	5.5	18
AS1	**4,200**	**Glass**	**Fine**	**20,200**	**5.5**	**22**
AS2	3,200	Glass	Coarse	6,300	1	12
AS2	3,200	Glass	Fine	6,300	1	19
AS2	3,200	Zircon	Coarse	6,300	1	9
AS2	3,200	Zircon	Fine	6,300	1	12
DIG1	3,200	Glass	Coarse	31,200	0.5	3
DIG1	3,200	Glass	Fine	31,200	0.5	3
DIG2	3,200	Glass	Coarse	59,750	0.2	1
DIG2	3,200	Glass	Fine	59,750	0.2	1
EXT	**3,200**	**Glass**	**Coarse**	**31,130**	**0.1**	**4**
EXT	**3,200**	**Glass**	**Fine**	**31,130**	**0.1**	**4**

High rotational speeds and fine glass balls were found to be the best combination to solubilize the four sludges. Pretreatment increased the sCOD of the extended aeration sludge by a factor of 40. However, the sCOD of the resulting sludge is only 4% of the tCOD. Anaerobic digestion was performed on sludges pretreated with the ball mill in 1-L batch reactors at 35°C for 500 hours. Table 2.8 summarizes the results obtained. The biogas production is quantified as a percentage of the biogas produced in the control reactor after 500 hours. The authors report that the methane content of the biogas was 71-74% in all cases. The results obtained from the reactors digesting extended aeration sludge are encouraging. In

this case, pretreatment doubled the amount of VS degraded and boosted methane production by 24%. Baier and Schmidheiny (1997) report that the energy requirement for the operation of a ball mill is 1.0-1.25 kW per cubic meter of sludge per day.

Table 2.8: Biogas production and organics removal during anaerobic digestion test (Baier and Schmidheiny, 1997).

	Biogas Production (%)		Degradation (%)	
Sludge in reactor	**After 50 hours**	**After 500 hours**	**VS**	**COD**
Untreated AS2	35	100	38	51
Pretreated AS2	40	110	57	53
Untreated DIG1	52	100	28	21
Pretreated DIG1	56	97	28	22
Untreated DIG2	25	100	4.2	8.4
Pretreated DIG2	53	162	8.8	12.8
Untreated EXT	31	100	8.4	10.6
Pretreated EXT	37	124	16.5	13.9

2.4.4 Thermal Pretreatment

The pioneering work on thermal pretreatment performed by Haug and Stuckey (1978) was reviewed. WAS pretreated at 175°C for 30 minutes was digested anaerobically at 35°C in 2-L continuously stirred reactors. The reactors digesting pretreated WAS initially performed better than the control reactors but, after eight days of stable operation, gas production started to decrease and the pH and the concentration of VFA increased. It was subsequently demonstrated experimentally that ammonia toxicity was not to blame. A new experiment was carried out with two reactors digesting pretreated WAS diluted to 50% and two more reactors treating full strength pretreated WAS. The reactors digesting full strength pretreated WAS initially produced a large amount of biogas but again started to fail after eight days. The experimenters were more patient in this case and, after approximately 30 days, the gas production from these reactors increased and stable operation resumed. Thereafter, VS removal was 48.4% as opposed to 26.2% for the control. The 30 days during which acclimation of the bacteria occurred represents approximately two reactor volumes. On the other hand, the reactors treating half-strength WAS produced about twice the amount of biogas as the control and removed 41.4% of the VS compared to 31.4% for the control. Another means of eliminating the inhibition was to digest a 1:1 mixture of primary and waste

activated sludge. For this sludge mixture, pretreatment increased gas production by 13.5% and VS removal by 18%. Another experiment was carried out to determine the optimum pretreatment temperature in the 100-175°C range. The highest gas production and VS removal resulted from pretreatment at 175°C. The ammonia, alkalinity and sCOD concentrations in the effluent of the anaerobic digesters were also highest at these conditions. Again, WAS pretreated at 175°C experienced a period of inhibition while the samples pretreated at lower temperatures did not. More reactors were next fed WAS pretreated at 200 and 225°C and these digesters failed and acclimation had not occurred after 40 days of operation. Throughout these experiments, the dewaterability of sludge was monitored. For WAS, pretreatment to 100 and 135°C did not result in improved dewaterability whereas treatment at 175, 200 and 225°C did. The dewaterability after digestion was not measured for all pretreated samples but, from the scarce data presented, it seems that digestion did not significantly alter dewaterability characteristics.

A classic study by Stuckey and McCarty (1984) investigated the bioconvertibility and toxicity of some of the most important components of WAS: amino acids, DNA, RNA, bases in nucleic acid, proteins and carbohydrates. The methodology followed by the researchers is extensive and can be obtained in the original paper. The bioconvertibility of WAS after thermal pretreatment was evaluated at 150-275°C and the peak was found to occur at 175°C under both mesophilic (35°C) and thermophilic (55°C) conditions. Strangely, mesophilic digestion resulted in better bioconversion for the control and for all pretreated sludges. Next, the bioconvertibility of nitrogenous mixtures (amino acids, RNA, DNA and collagen) was evaluated. The bioconvertibility of all mixtures was high in the controls but was significantly lower for amino acids, RNA and DNA after heating to 200°C. The effects of the thermal pretreatment at 200°C was then evaluated for amino acids, DNA, RNA, collagen and albumin. Pretreatment severely increased the toxicity of DNA, RNA and albumin, slightly increased the toxicity of amino acids and did not significantly affect the toxicity of collagen. With and without pretreatment, toxicity was higher under mesophilic conditions. A similar experiment was carried out on pure nitrogen compounds: 19 amino acids, the five bases constituting nucleic acid and three carbohydrates. Of the 19 amino acids, eight were classified as highly degradable, another eight as moderately degradable and three (valine,

leucine and glutamic acid) as recalcitrant. Treatment at 200°C either decreased or did not affect the biodegradability of the amino acids. The bases were relatively degradable except for thymine which was found to be very refractory. Pretreatment of these bases did not significantly improve the biodegradability of thymine and had slightly positive or negative effects on the other four bases. The carbohydrates, ribose, deoxyribose and glucose, were all very biodegradable but pretreatment severely compromised their bioconvertibility. Another noteworthy finding of this study is that nitrogenous mixtures were more biodegradable than the individual nitrogenous compounds. For example, the bioconvertibility of the solution of amino acids was higher than the average bioconvertibility of the 20 amino acid solutions. Also, the fact that amino acids and bases in DNA and RNA often differ from each other by few functional groups allowed the authors to discover that structure greatly affects biodegradibility and toxicity. Specifically, it was noted that amino acids containing sulfur were most toxic. Moreover, untreated WAS was significantly less biodegradable than the nitrogenous mixtures and this seems to indicate that the organized order of the nitrogenous compounds in bacterial cells is responsible for the lower bioconvertibility.

The study by Tanaka *et al.* (1997) introduced in the previous section also studied the impact of thermal pretreatment of WAS in the range of 115-180°C. Both the solubilization of VSS and gas production improved at 115°C, reached a plateau until 150°C and improved again until 180°C. At 180°C, the solubilization of VSS reached approximately 30% and the methane production was 90% greater than for the control.

The CAMBI process is a thermal pretreatment option that involves thickening of sludge, heating to 165°C at a pressure of 12 bars in batch reactors. This pretreatment technology requires specialized equipment such as thickening/dewatering machinery, storage tanks, pumps able to carry high-solids streams, batch reactors and heat exchangers (Parker and Béland, 2003). A full-scale plant was built in Dublin, Ireland in 2001. Barnard *et al.* (2002) listed a number of reasons as to why the CAMBI process was selected:

- Sterilization of the sludge
- Destruction of foaming organisms
- Increase in methane production

- Generation of 34%-solids sludge which reduces transportation costs
- Surplus energy produced
- Automated process
- Operations are enclosed for adequate odor control
- Production of a marketable and safe end-product

The plant is equipped with screens, belt presses, storage silo, pulping tank, odor control system, batch reactors, flush tank, heat exchangers and centrifuge (Barnard *et al.*, 2002). The process diagram of this plant is shown in Figure 2.5.

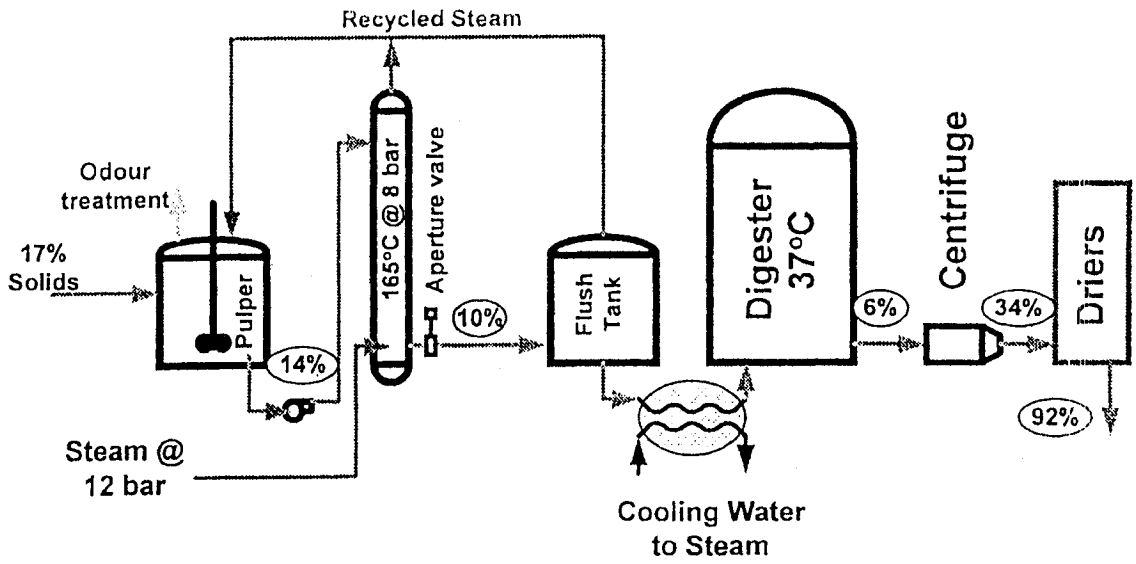


Figure 2.5: Diagram of the Dublin Bay sludge treatment train (Barnard *et al.*, 2002).

Start-up of the plant was characterized by two problems: lower than expected solids concentration in the hydrolysis reactors and blocking of the heat exchangers by fibers and fats. The second and most important problem was solved by mixing hydrolyzed and digested sludge prior to the heat exchangers. The plant is now capable of destroying approximately 70% of the VS (Abraham and Kepp, 2003).

A lower temperature pretreatment of WAS was investigated by Wang *et al.* (1997). Acclimated sludge was degraded in 1.5-L reactors at 36°C at HRTs of 10, 8, 6 and 4 days. At all HRTs, the WAS heated to 60, 80 and 100°C produced about 1.5 times more biogas than the control. The four pretreatment temperatures resulted in similar amounts of methane

generation. However, organic matter removal was highest at 100°C with 45% removed at a HRT of 10 days whereas the control reactor could destroy only 36%. Similar improvements were achieved at the other HRTs.

Research by Gavala *et al.* (2003) involved pretreating primary and secondary sludge (separately) at 70°C for 0, 1, 2, 4 and 7 days, followed by semi-continuous anaerobic digestion at 37°C in 1-L reactors at a SRT of 20 days. None of these pretreatment durations had significant positive effect on the methane potential and production rate during digestion of primary sludge. However, for secondary sludge, methane production rate improvements of 40, 60, 140 and 85% over the control were observed for pretreatment durations of 1, 2, 4 and 7 days. In this same experiment, improvements in methane potential of 20% were obtained for all pretreatment durations.

2.4.5 Thermochemical Pretreatment

As part of the study presented earlier, Haug *et al.* (1978) also studied the effects of thermochemical pretreatment on anaerobic digestion. Two reactors were fed WAS acidified to pH 1.2 and heated to 175°C for one hour and two more reactors fed with WAS dosed with sodium hydroxide to pH 12 and also heated to 175°C. These digesters initially performed better than the control but underwent inhibition after the fifth day and had not recovered by the 40th day of operation.

After determining the optimal conditions for chemical and thermal pretreatment for WAS, Tanaka *et al.* (1997) set up another experiment to study thermochemical pretreatment. With a pretreatment temperature of 130°C and alkali dosage varying between 0 and 0.5 g NaOH / g VSS, the VSS solubilization reached a plateau at 70% for dosages greater than 0.25 g NaOH / g VSS. Upon alkali addition, the pH of the sludge reached 11 with 0.1 g NaOH / g VSS. Addition of 0.25 g NaOH / g VSS or more resulted in a pH of 12. Batch tests were conducted for 20 days on a control and a sludge pretreated to 130°C and with 0.3 g NaOH / g VSS. After digestion, the control had removed approximately 35% of the COD whereas the reactor treating pretreated sludge removed approximately 47% of the COD.

The hydrolysis of pre-precipitated WAS by thermal and thermochemical pretreatment was studied by Smith and Goransson (1992). The sludge consisted of 4-5% TS and exhibited a COD content of 45,000-60,000 mg/L. The success of hydrolysis was defined by the hydrolysis yield (HY):

$$HY(\%) = \frac{sCOD_1 - sCOD_0}{tCOD - sCOD_0} \times 100 \qquad (2.4)$$

where the subscripts 0 and 1 indicate before and after treatment. Heating of the sludge in the range of 120-160°C for one hour yielded a HY of 15% at all temperatures. No improvement was observed when this treatment was carried out in conjunction with an addition of $Ca(OH)_2$ to pH 10-12. However, thermal treatment and addition of NaOH to the same pH range produced a yield of 40-60%. Thermochemical treatments using HCl and H_2SO_4 resulted in a hydrolysis yield of 30-50%. The dewaterability of the sludge treated by thermal alkaline and acidic treatment was compared by the CST test. The CST is said to have decreased dramatically upon thermo-alkaline treatment and is shown to decrease from 220 to 20 s after addition of a vague amount of sulfuric acid and heating to 160°C for one hour. Another interesting finding is that this same thermochemical treatment yielded a sludge with solids composed almost entirely of volatile matter (97-98%). It seems that this treatment releases inorganic matter from the sludge into solution. This theory was reinforced by metal analysis which showed that the concentration of all nine metals in the supernatant was slightly lower or equal to the concentration in the untreated sludge. This benefit of thermal-acidic treatment could make the land application of biosolids a much more viable option in a sludge disposal strategy.

2.4.6 Thermophilic Aerobic Pretreatment

This method involves the treatment of sludge in aerobic reactors operating at a temperature of 70-80°C. The key to this technique is the culture of thermophilic aerobic bacteria that are capable of solubilizing organic sludge. The procedure for isolating the desirable bacteria is described by Hasegawa *et al.* (2000).

Hasegawa *et al.* (2000) isolated a bacterium capable of such solubilization called *Bacillus Stearothermophilus*. It is a gram positive, rod-shaped microorganism that can grow in an

environment with pH in the range 5.0 to 8.5 and temperature of 55-70°C. The solubilization of VSS was tested at different pretreatment temperatures in a continuous-flow aerobic reactor. The optimal temperature was found to be 65°C where 45% of the VSS was solubilized. To confirm that the bulk of this solubilization was due to *Bacillus Stearothermophilus* and not to pretreatment temperature, sterilized WAS was shaken at 60 rpm at a temperature of 70°C for approximately three days. After one hour, the solubilization of the VSS had reached 20 to 25% and ultimately reached 25% after the three days. This clearly showed that *Bacillus Stearothermophilus* plays a significant role in the pretreatment of the sludge. The next step in the study was to operate continuous-flow aerobic reactors at 65°C at HRTs of 1.0, 1.5, 3.0 and 5.0 under aerobic (1-3 mg/L DO) and microaerobic (0-0.1 mg/L DO) conditions. Both aerobic and microaerobic reactors could solubilize approximately 40% of the VSS at all HRTs. On the other hand, the aerobic reactors produced no VFAs while the microaerobic reactors produced increasing amounts of VFAs as the HRT was increased (up to 2,000 mg/L at a HRT of 5 days). In the last phase, untreated and pretreated sludge were digested anaerobically for ten days at a temperature of 37°C and an undefined HRT. The control and the sludge pretreated aerobically behaved similarly and produced approximately 200 mL biogas per gram of volatile matter. The sludge pretreated microaerobically produced approximately 300 mL biogas per gram of volatile matter. Hasegawa *et al.* (2000) hypothesize that the bacterium was capable of producing extracellular enzymes and that this is the reason why the VSS could be solubilized. No attempt was made to isolate the enzymes.

2.4.7 Ultrasound Pretreatment

Low-frequency ultrasound (20 to 40 kHz) may be used as a mechanical pretreatment. In this case, the pressure gradient is achieved by cavitation. "Cavitation occurs when the local pressure in the aqueous phase falls below the evaporating pressure resulting in the explosive formation of small bubbles. These bubbles oscillate in the sound field over several oscillation periods, grow by a process termed rectified diffusion, and collapse in a non-linear manner" (Tiehm *et al.* 1997). The temperature and pressure in the bubbles can reach approximately 5,000 K and "several hundred atmospheres" (Tiehm *et al.*, 2001). This method is already well established in industry with numerous plants in Europe using ultrasound treatment prior

to anaerobic digestion (Barber, date unknown). The six sewage treatment plants analyzed by Barber (date unknown) enjoyed increases of biogas production of at least 20% using ultrasound pretreatment. In fact, two of these plants observed an increase in biogas production by more than 40%.

Tiehm *et al.* (1997) observed an increase in soluble COD concentration from 630 to 2,270 mg/L when a 53:47 mixture of primary and waste activated sludge was irradiated for 64 seconds at a frequency of 31 kHz. This treatment also resulted in an increase of sludge temperature from 15 to nearly 45°C. The particle size distribution of the sludge with and without pretreatment was also compared using the technique of laser light scanning. The median particle size of the untreated sludge was 165 μm and dropped to 135 and 85 μm for the sludge treated with ultrasound for 29.5 and 96 seconds, respectively. Five 150-L semi-continuous anaerobic digesters were next operated at HRTs of 22, 16, 12 and 8 days. In addition, a control reactor was run with a HRT of 22 days. On average, the control reactor achieved a 45.8% reduction in VS whereas the reactor digesting pretreated sludge at a HRT of 22 days removed 50.3% of the VS. The reactors operating at HRTs of 16 and 12 days removed more VS than the control reactor but the reactor operated with a HRT of 8 days could only destroy 44.3% of the VS. This showed that the pretreatment of sludge with ultrasound could be used to reduce the size of anaerobic digesters and/or to increase the removal of VS.

Experimental work performed by Wang *et al.* (1999) on the pretreatment of WAS using ultrasound showed an increase in soluble COD from 20 mg/L to 1,050 mg/L after 40 minutes of exposure to ultrasound at a frequency of 9 kHz. Under the same conditions, the SPC increased from 20 mg/L to 6,000 mg/L while the soluble carbohydrates increased from 20 mg/L to 1,730 mg/L. Digestion of a 3:1 ratio of seed sludge to pretreated WAS at 36°C yielded 350 mL methane per g VS added whereas digestion of the same ratio of seed sludge to untreated WAS only produced 205 mL methane per g VS added. The concentration of VFAs in the reactor containing pretreated WAS increased sharply at the beginning of digestion to a value of 1,700 mg/L and then decreased linearly until completion of the batch test. The VFA profile in the reactor containing the control was similar but only peaked to a

value of 1,100 mg/L. A linear relationship with R^2 of 0.994 was developed relating cumulative methane generation (mL methane per g VS added) and solubilization ratio, which once again shows that the key of pretreatment is the solubilization of the organics.

A wide range of ultrasound frequencies was explored by Tiehm *et al.* (2001). WAS was irradiated at frequencies of 41, 207, 360, 616, 1,068 and 3,217 kHz for four hours by the use of an ultrasound reactor equipped with disk transducers. Both the lowest median particle size (17 μm) and highest degree of COD solubilization (81%) were reached at the lowest frequency tested. The degree of solubilization was defined as the increase in soluble COD due to ultrasound pretreatment divided by the increase in soluble COD due to exposure to 0.5 mol/L NaOH for 22 hours. The next phase of the study involved the anaerobic digestion of WAS irradiated at a frequency of 41 kHz for 7.5, 30, 60 and 150 minutes. The digestion was carried out semi-continuously at a SRT of eight days at 37°C in 1-L reactors. The sludge sample irradiated for only 7.5 minutes did not show an increase in soluble COD and actually produced less biogas than the control. However, the reactor holding this sample was characterized by a higher oxygen utilization rate and greater VS reduction. This indicates that short irradiation exposure time can enhance the activity of the microorganisms in the sludge. The exposure duration that yielded the highest biogas production and VS degradation was 150 minutes. Despite this, after digestion, the supernatant of the sample irradiated for 150 minutes contained the highest soluble COD and ammonia concentrations. Thereafter, WAS samples were irradiated for 60 minutes at frequencies of 41, 207, 360 and 1,068 kHz. Once again, the best results were obtained at a frequency of 41 kHz as qualified by the degree of COD solubilization and VS removal during anaerobic digestion. A linear relationship could be fit to relate percent VS removal to the degree of COD solubilization with a R^2 of 0.94.

The COD solubilization and oxidation-reduction potential (ORP) in WAS were monitored upon ultrasonic and alkali addition by Chiu *et al.* (1997). Three pretreatment schemes were tested on WAS: 1) Exposure to sodium hydroxide for 24 hours in 1-L plastic bottles at room temperature at a dose of 40 meq/L, 2) Same exposure to NaOH followed by 20-kHz irradiation for 24 seconds per mL, and 3) Concurrent exposure to NaOH and irradiation (14.4 seconds per mL) for 24 hours. The third scenario led to the fastest initial hydrolysis rate:

211.9 mg/L/min. The second and third schemes both yielded a sCOD of approximately 10,500 mg/L compared to only 4,880 mg/L for the first scenario. The ORP curve during the three pretreatment scenarios behaved similarly during the first two hours. Indeed, the sharp decreases in ORP observed during this period accompanied by simultaneous increases in soluble COD indicate that hydrolysis took place during the first two hours. The concentration of VFA was also monitored during this experiment and the WAS pretreated simultaneously with NaOH and ultrasounds yielded a tVFA/tCOD ratio of 84% after 21 hours compared with 10% for untreated sludge.

Recent work by Dr. Hielscher from IWEtec has demonstrated that ultrasound treatment of only a portion of WAS prior to anaerobic digestion resulted in greater biogas production and improved dewaterability characteristics. Unfortunately, the original work does not seem to be published but Barber (date unknown) presents Figure 2.6 in a paper reviewing experiences with ultrasound treatment in industry. The figure indicates that, for pure primary sludge, pure secondary sludge and mixtures of the two sludges, the highest biogas yields were obtained by treating only 50-60% of the sludge with ultrasound. A hypothesis that stems from this observation is that the beneficial effect of solubilizing the sludge is counterbalanced by the release of toxic compounds. This could explain the success of partial pretreatment with ultrasound.

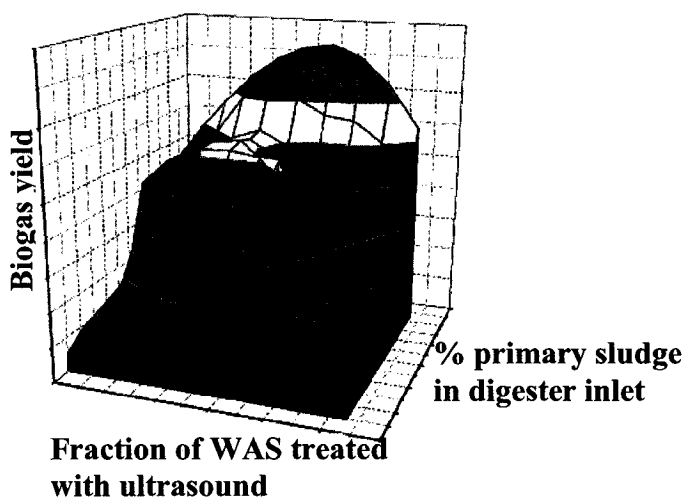


Figure 2.6: Effect of part-stream ultrasonic disintegration on biogas production from anaerobic digesters (Barber, date unknown).

Results from the operation of two full-scale 4,507 m^3 mesophilic anaerobic digesters operated at a 20-day SRT and fed a 40:60 mixture of primary sludge and WAS were reported by Brown *et al.* (2003). One reactor was fed a 40:60 mixture of primary and ultrasound-pretreated WAS while the other reactor acted as a control and was fed a 40:60 mixture of non-treated primary and waste activated sludge. WAS pretreatment was carried out for at least 1.5 seconds using five radial horn placed in series and operating at a frequency of approximately 20 kHz. The test reactor exhibited an increase in biogas production of approximately 50% compared to the control reactor. In addition, dewatering of digested biosolids from the test reactor yielded cake solids on average 1.2-2.6 percentage points drier than obtained using digested sludge from the control reactor. It was also reported by the authors that less foaming and lower effluent VFA concentrations were observed in the test reactor than in the control reactor.

2.4.8 Cell Lysate Pretreatment

The material released by microorganisms upon cell wall destruction is known as cell lysate. It contains enzymes and cofactors. These catalysts could be beneficial to boost anaerobic degradation of substrate. For this method to succeed it is essential that the lysate be released 'gently' or intensively over a short period of time so that the enzymes are not removed and toxic compounds are not leached out into solution (Dohanyos *et al.*,1997). According to these authors, most of the enzymes are inactivated in high-temperature thermal and high-pressure mechanical pretreatment methods.

Dohanyos *et al.* (1997) compared two methods of preparing cell lysate from anaerobic sludge: repeated freezing and thawing (lysateA) and heating to 100°C for 20 minutes (lysateB). The substrate was a 3.8%-TS mixture of primary and activated sludge. The anaerobic digestion was performed in 12-L batch reactors at 35°C. Table 2.9 is a summary of the results from this experiment. Both the methane production and the VS removal were highest in the reactor containing lysate prepared by freezing and thawing.

Table 2.9: Comparison of the effects on anaerobic digestion of lysate prepared by freezing and thawing and by heating (Dohanyos *et al.*, 1997).

	Control	LysateA	LysateB
Inoculum (g VS)	114.1	116.1	117.0
Substrate (g VS)	45.0	45.0	45.0
Lysate (g VS)	0	1.8	1.8
Methane production (L)	13.4	21.7	19.0
VS removed (%)	14.5	21.3	18.5

These same researchers carried out another study in which they added a lysate obtained by thermal heating to 100°C for 30 minutes to solutions of glucose, acetate, formate and propionate. The results of the anaerobic digestion experiment are not shown in the paper but the authors state that lysate did not boost methane production but increased VS removal in the reactors containing acetate, formate and propionate.

More work by Dohanyos *et al.* (1997) involved the use of a "lysis-thickening centrifuge" which is equipped with "a special impact gear which dissipates the kinetic energy generated by the centrifuge." A mixture of TWAS and primary sludge was digested anaerobically again at 35°C in 12-L batch reactors. Table 2.10 presents the outcome of the experiment.

Table 2.10: Results of batch anaerobic digestion of primary-TWAS mixture after pretreatment with "lysis-thickening centrifuge" (Dohanyos *et al.*, 1997).

Substrate	Substrate Dose (mg COD)	CH_4 Production (L)	Specific CH_4 Production (L/gCOD)	Increment of CH_4 Production (%)
WAS	169.1	14.3	0.09	-
TWAS	132.8	20.9	0.16	86.4
WAS + PS	290.3	48.9	0.17	-
TWAS + PS	187.6	39.1	0.21	24.0

The improvement by "lysis thickening" was more significant when the WAS was not mixed with primary sludge. Few studies on cell lysate pretreatment were found in the literature. It seems that the success of partial pretreatment by ultrasound as reported by Barber (date unknown) could be explained by a release of enzymes and cofactors in the treated portion and a dilution of the toxic compounds when mixing with untreated sludge. It would be

worthwhile to test partial microwave pretreatment and to investigate whether low intensity irradiation can limit the release of toxic compounds while at the same time break the cell walls and release the enzymes and cofactors.

2.4.9 Microsludge Pretreatment

Microsludge is a patented pretreatment technology that was developed by Paradigm Environmental Technologies Inc. It involves contacting sludge with sodium hydroxide for one hour, mechanical shearing for reducing particle sizes, 800-μm screens to remove non-cellular debris and high-pressure (12,000 psi) homogenization to break microbial cell walls. In the homogenizer, the sludge is accelerated to 305 meters per second in approximately 2 μs (Stephenson *et al.*, 2004). The homogenizer valve is shown in Figure 2.7.

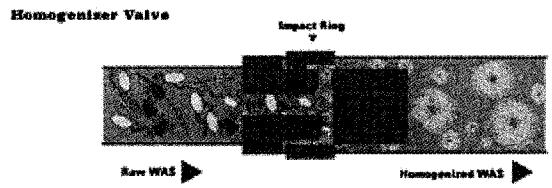


Figure 2.7: The Microsludge homogenizer valve (Paradigm Environmental Technologies Inc., 2004).

Stephenson *et al.* (2004) conducted a pilot-scale experiment at a SRT of 15 days with a 40:60 mixture of primary and secondary sludge. Anaerobic digestion of untreated feed yielded a 41% reduction in VS whereas microsludge-pretreated feed yielded a 71% reduction. The reactors treating pretreated feed were characterized by very low levels of VFA (less than 200 mg/L) and relatively high levels of ammonia (1,180 mg/L). A commercial plant is currently in operation in Chilliwack, B.C. to treat WAS. After four months of operation, reports indicated VS removals of 70, 67 and 64% at SRTs of 11, 9.5 and 6 days, respectively.

2.4.10 Steam Explosion Pretreatment

The steam explosion concept was developed by the Super Blue Box Recycling Corporation. The technology consists of subjecting sludge to a pressure of 150-600 psi and temperature of 180-260°C for five minutes in a reaction vessel and then releasing the sludge through a small orifice. This last action produces an explosion and this is what causes the cell wall of

microorganisms to be disrupted. Dereix *et al.* (2005) conducted BMP assays and semi-continuous experiments. Upon steam explosion treatment of a 30:70 mixture of thickened WAS and digested biosolids at pressures of 150, 300 and 600 psi and resulting temperatures of 180, 220 and 260°C, the soluble to total COD ratio increased from 7% to 13, 40 and 33%, respectively. The value obtained for the sample treated at a pressure of 300 psi is questioned by the author as the tCOD value obtained in this case was lower than for the other samples. Subsequent BMP assays of the same sludge yielded 28, 41 and 68% improvements in biogas production. The control showed a 28% removal in VS while pretreated samples yielded 38, 37 and 40% removals in VS. Semi-continuous anaerobic digestion at a SRT of 14 days of the same mixture pretreated at 300 psi yielded a 48% improvement in biogas production and improvement in VS removal from 32% to 34%. Results obtained at a SRT of only 8 days showed a 31% improvement in biogas production accompanied by an improvement in VS removal from 30 to 35%. The dewaterability of 15 effluent samples of the reactors treating control and pretreated samples was measured. Pretreatment had a positive effect on the dewaterability as the average CST of the control was 310 s compared to 205 s for the pretreated sample. It is not indicated whether these samples were obtained when the SRT was set at 8 or 14 days.

2.4.11 Electron Beam Pretreatment

Shin and Kang (2003) explored the effects of electron beam pretreatment on the solubilization of organics and anaerobic degradation of WAS. The >1.5% TS sludge samples were irradiated by a 1-MeV electron accelerator at doses of 0.5, 1, 3, 6 and 10 kilo Grays (kGy). The soluble COD of the WAS was initially 50 mg/L and reached a plateau of 1,255 mg/L when irradiated at a dose of 10 kGy. The maximum possible sCOD of the sludge was determined to be 11,600 mg/L (by exposing the sludge to NaOH for 26 hours) and the total COD of the sludge was 15,300 mg/L. Therefore, approximately 11% of the maximum sCOD was solubilized whereas the control only contained 0.5%. Comparison of soluble proteins and carbohydrates before and after a 10-kGy electron beam irradiation indicates that the proteins increased from 14.4 to 397.3 mg/L and the carbohydrates improved from 5.9 to 116.8 mg/L. Interestingly, the authors state that the pretreated sludge (10 kGy) left at room temperature for 24 hours after irradiation developed significantly higher levels of sCOD than

right after irradiation. The authors speculate that this is most likely due to "uncontrolled enzymatic activity as a result of enzyme release on cell disintegration." Four 18-L semi-continuous reactors were also run at HRTs of 20, 15 and 10 days to treat non-irradiated sludge and WAS irradiated with 1, 3 and 6 kGy. The reactor digesting the sludge irradiated with 6 kGy performed best at all HRTs. At a HRT of 20 days it could destroy 60.3% of VS whereas the control reactor could only destroy 36.7%. Under the same conditions, the biogas production was 236 and 82 L/m^3/day for the reactor digesting treated and untreated WAS, respectively.

2.4.12 Direct Technology Comparisons

The effects of chemical, thermal, thermochemical and ultrasonic pretreatment on the solubilization and anaerobic digestion of WAS were directly compared by Kim *et al.* (2003). The first phase of their study involved the optimization of each method for the sludge at their disposal (except for thermal pretreatment for which a temperature of 121°C, pressure of 1.5 atmospheres and 30 minutes were used). The following results were obtained:

- Chemical pretreatment: at pH 12, NaOH solubilized the sludge better than KOH, $Mg(OH)_2$ and $Ca(OH)_2$. The optimal NaOH dose was found to be 7 g/L.
- Ultrasonic pretreatment: A frequency of 42 kHz was employed and the maximum solubilization occurred after a 120-minute exposure.
- Thermochemical pretreatment: Again, 7 g/L NaOH were contacted with the sludge after it had been heated to 121°C for 30 minutes.

The particle size distributions for the sludge treated under these conditions were evaluated using a laser particle size analyzer. Table 2.11 summarizes the results obtained. The thermochemical method was the most effective at reducing the size of the particles.

Table 2.11: Particle size distribution of WAS before and after pretreatment (Kim *et al.*, 2003).

	Particle Size of 10, 50 and 90th Percentiles (μm)		
Treatment Method	**10%**	**50%**	**90%**
Control	24	219	450
Chemical	11	58	186
Thermal	10	47	153
Thermochemical	2	29	144
Ultrasounds	10	46	240

The same sludges were anaerobically digested in 1-L batch reactors at a temperature of 37°C. Prior to digestion, the pH of all samples was lowered to 6.7 using HCl. Table 2.12 lists the results of the digestion experiment. The sludge pretreated with the thermochemical method had the highest sCOD before digestion, VS removal and methane production. It would be interesting to investigate whether NaOH addition after microwave irradiation could produce similar or even better results.

Table 2.12: Comparison of effectiveness of four pretreatment methods for the solubilization and anaerobic digestion of WAS (Kim *et al.*, 2003).

Pretreatment Method	**sCOD Before Digestion (mg/L)**	**sCOD After Digestion**	**VS Removal (%)**	**Methane Production (L / m^3 WAS)**
Control	2,250	1,100	20.5	2,500
Chemical	12,200	7,200	29.8	1,400
Thermal	5,000	3,200	32.1	3,400
Thermochemical	22,200	8,750	46.1	3,400
Ultrasonic	5,000	3,400	38.9	3,050

The relationship between the dose of sodium hydroxide added to an industrial sludge and the solubilization of the COD was studied by Penaud *et al.* (1999). The dose was varied between 0 and 28.1 g NaOH/L. The percent solubilization curve was found to closely follow the pH response of the sludge with solubilization reaching a plateau of 60-65% at 5 g NaOH/L where the pH also reached a maximum value of 12. The same alkali doses were again applied in a second experiment but this was followed by heating of the sludge to 140°C for 30 minutes. Again, the rate of solubilization and pH of the sludge were closely related but a maximum solubilization of 75-85% was observed for doses greater than 4.6 g/L.

The performance of four alkalis was compared by raising the pH to 12 using NaOH, KOH, $Mg(OH)_2$ and $Ca(OH)_2$ and again heating the sludge to 140°C for 30 minutes. The percent solubilization reached by the four alkalis are listed in Table 2.13. At room temperature, NaOH yielded the highest solubilization but when followed by thermal treatment, KOH was most successful at solubilizing the sludge. The authors explain the poor performance of the dibasic salts by arguing they were only partially dissolved.

Table 2.13: COD solubilization percentage achieved by four alkalis with and without thermal treatment (Penaud *et al.*, 1999).

	Percent COD Solubilization (%)	
Alkali	**At Ambient Temperature**	**Followed by Heating at 140°C**
NaOH	60.4	71.6
KOH	58.2	83.7
$Mg(OH)_2$	29.1	55.6
$Ca(OH)_2$	30.7	51.1

Biodegradibility and biotoxicity tests were also performed on sludge pretreated by different doses of NaOH with and without thermal pretreatment. At ambient temperature, the optimal NaOH concentration was 5 g/L where biodegradability was 52% and very little toxicity. When followed by thermal treatment, optimum NaOH dosage was also 5 g/L where the biodegradability peaked at 58% and toxicity was again low. Higher NaOH additions severely lower biodegradability and slightly increase toxicity. Toxicity tests were also carried out for sludge pretreated with the other salts listed in Table 2.13. The values reported for those runs are in the same range as when NaOH is the alkali agent, which would indicate the low biodegradability values recorded are not caused by sodium cations. Penaud *et al.* (1999) conclude their work by hypothesizing that the low biodegradability of the pretreated sludge was caused by the newly solubilized molecules. Overall, the results reported in this study are in close agreement with the ones published by Kim *et al.* (2003) although a lower NaOH optimum dose is proposed (5 g/L compared to 7 g/L).

Muller *et al.* (1998) compared the disintegration of WAS by a high-pressure homogenizer, and a shear-gap homogenizer, a stirred ball mill and an ultrasonic homogenizer. It was demonstrated that the high-pressure homogenizer and stirred ball mill required the least

energy to reach a high degree of disintegration DR_0 (based on oxygen consumption as defined previously). It was also observed that the amount of energy that must be expended to reach a certain degree of disintegration is inversely proportional to the suspended solids concentration of the sludge being treated. WAS of sludge ages of 3 and 13 days treated with the high-pressure homogenizer using a pressure differential of 400 bars was digested for five days in continuous 20-L reactors and compared with the performance of control reactors digesting untreated WAS of the same origin. According to the researchers, the degree of VSS degradation attained in pretreated sludge was 10 to 20% higher than in the control. As expected, the sludge with lower sludge age was most easily degraded although pretreatment improved VSS removals by similar extents for both sludges. The digested sludge was next treated a second time by the high-pressure homogenizer at pressure differentials of 200 and 400 bars and by ozonation. A further increase in the degree of disintegration (based on COD) was reached. In fact, a linear correlation could be developed between the degree of disintegration (based on COD) obtained by pretreatment and the degree of disintegration (based on VSS) reached in a second anaerobic treatment. This suggests that the success of pretreatment on anaerobic digestion enhancement is not influenced by whether mechanical or chemical treatment was used but simply on the degree of disintegration attained. Once again, anaerobic sludges having undergone pretreatment required higher polymer doses. As compared to the control, the return flow after anaerobic digestion of the pretreated samples exhibited higher ammonia and TKN concentrations while the carbon to nitrogen ratio was not impacted.

In a more recent study, Muller *et al.* (2004) evaluated the effects of four pretreatment methods on the specific energy use, degree of disintegration, degree of degradation, polymer demand and concentration of sCOD and ammonia. The methods investigated are stirred ball-mill, ozonation, lysate centrifugation and ultrasounds. The source and type of sludge used is not stated. Table 2.14 summarizes the results of the experiment. No information is given in the report regarding the methodology used when treating the sludge with the four techniques.

Table 2.14: Effects of four pretreatment methods on the characteristics of sludge (Muller *et al.*, 2004).

Characteristic	Treatment Method			
	Stirred Ball-mill	**Ozonation**	**Lysate Centrifugation**	**Ultrasounds**
Specific energy (kWh/m^3)	21.0	49.5	11.0	28.0
Degree of disintegration (%)	23.0	35.0	5.0	17.5
Increase in degree of degradation (%)	14.0	20.0	8.0	10.0
Increase in sCOD concentration (%)	19.0	59.0	8.5	2.0
Increase in ammonia concentration (%)	11.0	16.5	11.0	5.0
Increase in polymer demand (%)	7.5	29.0	6.0	10

2.5 Microwave Irradiation

2.5.1 Basic Principles

Microwave irradiation is a form of electromagnetic radiation characterized by frequencies ranging between 300 MHz and 300 GHz. Figure 2.8 presents the energy, frequency and wavelength associated with the different forms of electromagnetic radiation. Heating in microwave ovens occurs due to the friction generated by the rapid oscillation of water molecules. Since water has a resonant frequency of 2.45 GHz, microwave ovens have been designed to produce radiation at this exact energy so that the maximum possible energy may be consumed (NMABCETS, 1994). Chemical and physical properties dictate the degree to which a sample is suitable to microwave heating. The applicability of a sample to microwave heating can be characterized by the dissipation factor (tan δ), which is the ratio of the input power absorbed by the sample (loss factor) to the ability of the sample to obstruct the passage of microwave energy (dielectric constant). In microwave engineering, materials are classified into three groups: the absorbers (such as water and food), transparents (such as glass) and reflectors (such as metals).

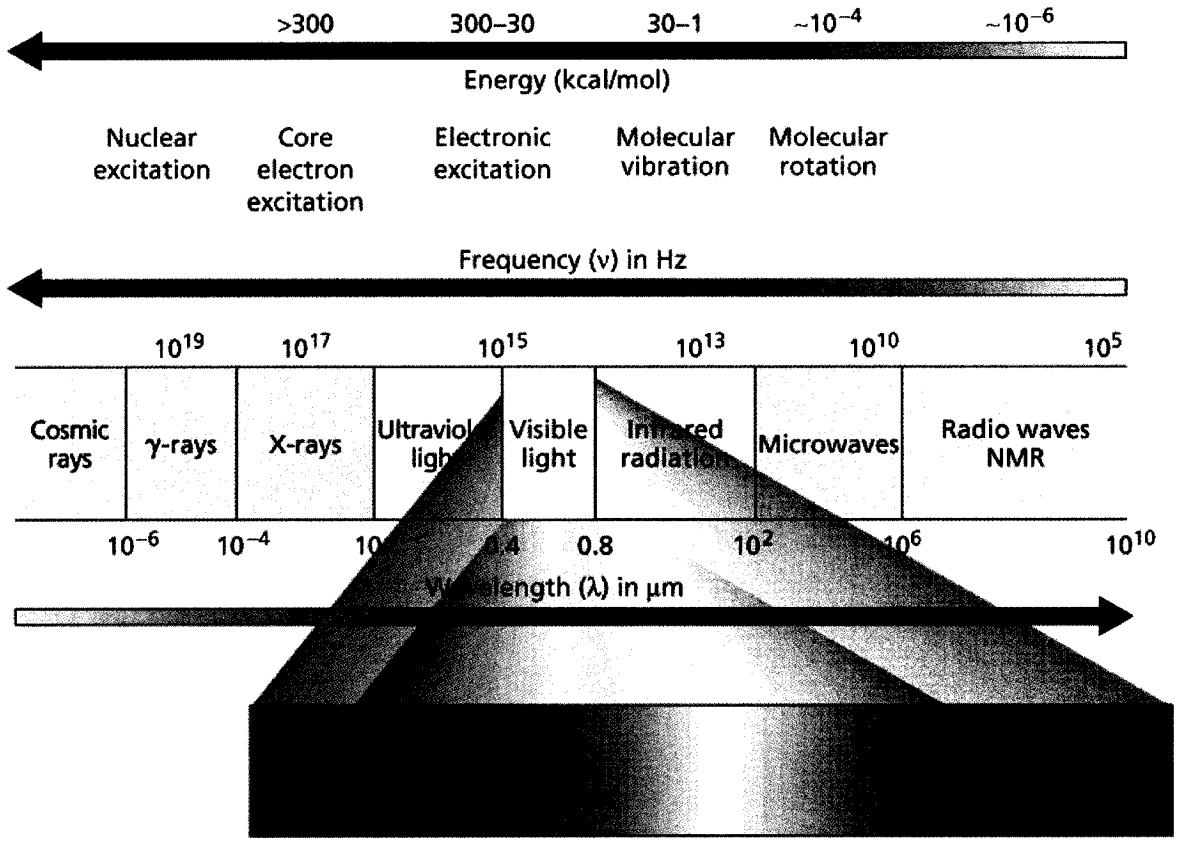


Figure 2.8: Electromagnetic spectrum (Huskey, date unknown).

Microwave ovens are constituted of six important components: the microwave cavity, turntable, wave generator (magnetron), wave guide, mode stirrer and circulator. Figure 2.9 displays the role of these components in microwave ovens. The wave guide propagates into the cavity the microwave energy delivered by the wave generator. This energy is dispersed in the oven cavity by the mode stirrer. The energy that is not absorbed by the sample is dissipated to a dummy load by the circulator to protect the wave generator.

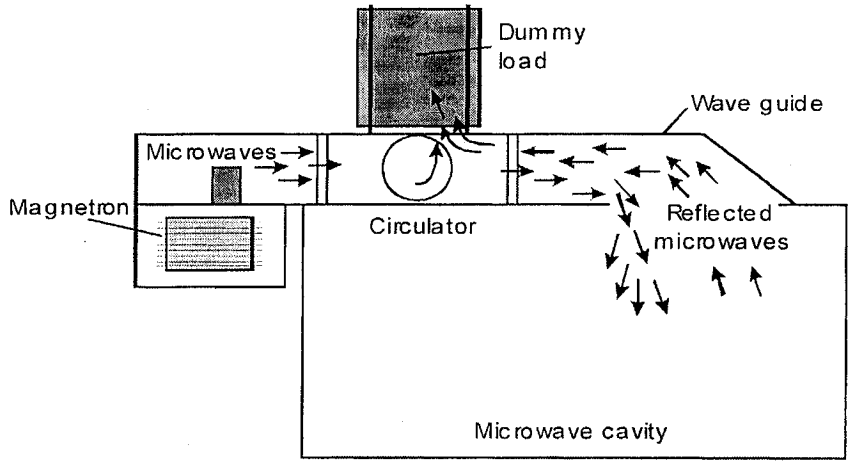


Figure 2.9: Schematic of a circulator deflecting the reflected microwaves to a dummy load (Kingston and Jassie, 1988).

Compared to conventional heating, microwave heating offers high heating rates, uniform heating, clean energy transfer, energy savings, and reduced equipment size. Disadvantages include difficult measurement of temperature, high initial costs and complexity of the system.

Because of these advantages, microwave technology is applied for numerous applications: cooking, sludge dewatering, microwave plasma processing of materials, minerals processing, waste processing and recycling and the sterilization of medical wastes. Interestingly, microwave irradiation is also used in the oil and gas industry to separate oil from water and soil in emulsions and sludge generated during the lifting, transportation and processing of oil (IPRC, date unknown). Figure 2.10 shows the microwave separation technology process.

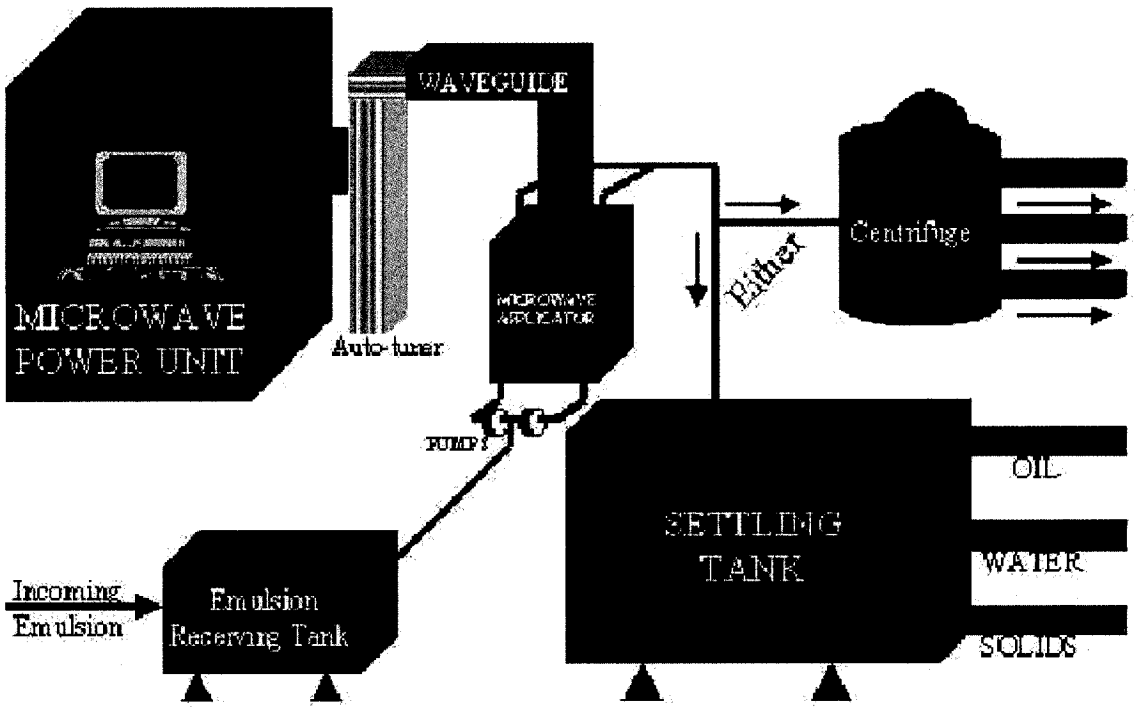


Figure 2.10: Microwave separation technology process (IPRC, date unknown).

In these emulsions and sludges the solids are encapsulated by the water which is in turn surrounded by oil. The surfactants in the mixture are composed of two ends. One is hydrophilic and is attracted to the water while the other is oleophilic and is attracted to the oil. This acts to stabilize the mixture. The concept behind the microwave separation technology application is that microwave energy activates the oil, water and solids selectively so that the three phases absorb different amounts of energy. The rapid oscillation of the polar end of surfactants causes a disruption of the surfactant molecules. Upon microwave irradiation, the recovery of the oil may be accomplished rapidly in a centrifuge or over a longer period in a settling tank.

2.5.2 Microwave Irradiation as a Pretreatment Method

Hong (2002) investigated the use of microwave irradiation to enhance the anaerobic digestion of anaerobically digested, primary and waste activated sludge and to destroy pathogens. Upon microwave heating to 70°C, the sCOD/tCOD ratio of the primary sludge increased from 12 to 13% whereas the sCOD/tCOD ratio of the WAS increased from 8.5 to 18%. BMP assays were conducted in 160-mL serum bottles at 35°C. Primary sludge

irradiated to 85 and 100°C yielded 11.9 and 22.7% improvements in biogas production compared to the control. WAS irradiated to 85 and 100°C yielded 11.45 and 15% improvements in biogas production compared to the control. The poorer performance of the pretreatment to enhance biogas production on WAS than primary sludge is argued by the author to be due to a lesser penetration depth of microwaves in WAS. The effectiveness of conventional and microwave heating to destroy pathogens were compared for primary and secondary sludge. For primary sludge containing 10^6 colony forming units (CFU) of fecal coliforms, sludge necessitated heating to 65°C using microwave irradiation and 85°C using conventional heating for all fecal coliforms to be destroyed. For WAS containing approximately 4 x 10^5 colony forming units (CFU) of fecal coliforms, sludge necessitated heating to 85°C using both microwave and conventional heating for all fecal coliforms to be destroyed. Anaerobic digestion was also performed in 6-L semi-continuous reactors. The anaerobic seed was acclimated for a three-month period. The feed consisted of a 50:50 mixture of primary and waste activated sludge. One reactor was fed untreated sludge whereas the other two reactors were fed sludge heated to 60°C conventionally and using microwave irradiation. The SRT was set to 20, 15, 10, 7.5 and 5 days. Unfortunately, the author either did not measure gas production and COD/VS removal or decided not to report the data. The effluent from the reactor fed with microwave-heated sludge contained significantly less fecal coliforms than the reactor fed conventionally-heated sludge and both reactors consistently produced Class A sludge.

Park *et al.* (2004) demonstrated that microwave irradiation of a WAS sample to boiling temperature increased the sCOD/tCOD ratio from 2% to 22%. However, irradiation to a reduced temperature of 91.2°C yielded a sCOD/tCOD ratio of 19%. Semi-continuous anaerobic digestion was performed in 5-L reactors at a temperature of 35°C. The performance of reactors fed control and pretreated sludge was compared at SRTs of 15 and 10 days. The pretreated sludge was irradiated to a temperature of 91.2°C. At a SRT of 15 days, the reactor fed pretreated sludge showed a 24.5% improvement in biogas production accompanied by an improvement in VS removal from 23.0 to 25.9%. At a SRT of 10 days, an improvement of 36.6% in biogas production was observed, along with an increase in VS removal from 23.2 to 25.5%. Additional work at a SRT of 8 days (only for reactor fed

pretreated sludge) showed that such high loading rates were possible as the process was still stable, as demonstrated with low effluent VFA concentration (65 ± 14 mg/L), high biogas production rate (240 ± 8 mL/L/day) and high VS removal (23.3 ± 0.4%).

Eskicioglu *et al.* (2004) investigated the effects of temperature, microwave intensity and sludge concentration on the solubilization and batch anaerobic digestion of WAS. Microwave intensity was found not to have an effect on the COD solubilization and batch anaerobic digestion. After irradiation to 75°C, the sCOD of 1.4% TS sludge increased from 700 to 3,500 mg/L while the sCOD of 5.4% TS sludge increased from 2,500 to 12,000 mg/L. A BMP assay conducted on 3% TS WAS irradiated to 96°C produced 17% more biogas than the control. No inhibitation and lag phase were observed.

CHAPTER 3: MATERIALS AND METHODS

3.1 Origins of the Sludge Tested

The sludge tested in this study was obtained from the municipal wastewater treatment plant in Rockland, ON. The facility is operated by the Ontario Clean Water Agency. Wastewater treatment at the plant involves screening, degritting, aerobic digestion and settling in a SBR and chlorination of the effluent prior to discharge to the Ottawa River. The operation of SBRs was described in section 2.2. There are three rectangular SBRs available, each with an effective volume of 2,270 m^3 and water depth of 5.5 m (ECO Equipment Systems Inc., 1997). The four stages and associated duration are the following: fill (2.67 hours), mix (3.33 hours), settle (1 hour) and decant (1 hour). The design data includes a wastewater flow rate of 6,800 m^3/day, BOD_5 of 150 mg/L, TSS of 350 mg/L, effluent BOD_5 of 15 mg/L and effluent TSS of 15 mg/L. Mass balance results indicate a HRT of one day, SRT of 12 days, mixed liquor suspended solids concentration of 3,334 mg/L, sludge volume index of 120 and food to microorganism ratio of 0.235. The sludge is currently air-dried and disposed of in a landfill.

3.2 Sludge Sampling

The dilute nature of the sludge collected at the bottom of the SBRs by pumps necessitated that the sludge be concentrated on site prior to transportation to the laboratory. On a pick-up day, the technician in charge of the plant would fill two 200-L barrels with sludge. The sludge was allowed to settle for a few hours before the supernatant was pumped to a drain and the concentrated sludge was pumped to 20-L buckets and transported to the laboratory. This technique yielded sludge varying between 1.5 and 3% TS, depending on the timing of the operator in pumping sludge to the barrels after the pumps are turned on upon completion of the decanting stage.

In the laboratory, the sludge from different buckets was mixed a few times in empty buckets to ensure homogeneity (although this was not done sufficiently during BMP assay #1). Each sample was obtained upon thorough mixing of a bucket with a stirring rod.

3.3 Experimental Protocols

3.3.1 Microwave Calibration

A calibration experiment was necessary to obtain a model relating sample final temperature to microwave irradiation time, microwave intensity and sludge concentration. Refer to section 3.3.3 for a description of the microwave oven specifications.

The first such experiment was conducted in 2-L polypropylene containers. Sludge obtained from Rockland was centrifuged to a concentration of 4.3% TS. Sixteen 850-mL samples were prepared by dilutions at concentrations between 1.2 and 4.3% TS. The samples were irradiated at an intensity of 100% for durations ranging between 45 and 270 seconds. Upon microwave irradiation, the sludge was stirred vigorously and its temperature was measured using a thermocouple probe inserted in the middle of the sample. This calibration experiment demonstrated that sludge concentration in the range of 1.5-4.3% TS does not influence the irradiation time needed to reach specific temperatures. The resulting model was used to pretreat sludge for the BMP assays.

The second experiment was carried out on 400-mL samples in 1-L polypropylene containers. It was required so that the study that investigated the factors affecting COD solubilization could be performed. In this case, the sludge concentration was kept constant at 3% TS but the microwave intensity was varied between 60 and 100%. Otherwise, the same procedure as that described in the previous paragraph was employed.

3.3.2 Acclimation of the Anaerobic Seed

Acclimation of the anaerobic seed was carried out in a 20-L tank placed on a shaker rotating at 50 rpm and located in a hot room maintained at a temperature of 35°C. The tank was initially filled with 10 L of digested WAS obtained from the Robert O. Pickard Environmental Centre (ROPEC). The SRT was set at 25 days by the daily withdrawing of 400 mL of digested sludge and addition of a 400-mL 50:50 mixture of irradiated (to 85°C) and non-treated sludge from Rockland. To assess the degree of acclimation, gas production was measured daily using a wet-tip meter. In addition, total COD, TS, VS, pH and VFAs were measured bi-weekly while gas composition was measured weekly. The acclimation of

the seed was continued until these parameters stabilized and this required two months. Figure 3.1 is a picture of the acclimation tank and setup.

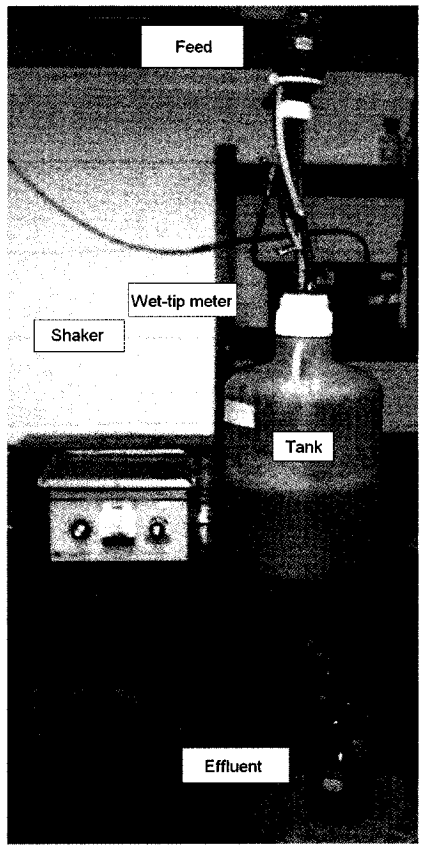


Figure 3.1: Acclimation tank and setup.

3.3.3 Microwave Pretreatment of Sludge

As described in section 3.3.1, pretreatment of sludge was carried out in 1-L and 2-L polypropylene containers. A Panasonic microwave oven with a magnetron power consumption of 1,460 W and operating at a frequency of 2.45 GHz was used. The oven was equipped with a rotating cooking tray. The model number is NN-S963 and the oven cavity dimensions are 278 mm x 469 mm x 470 mm.

Upon microwave irradiation of a sludge sample, the container and sample were left on the counter for at least one hour so the sludge reached room temperature again. The container was then placed on the balance and evaporated water was replaced with distilled water. Figure 3.2 shows the microwave pretreatment setup.

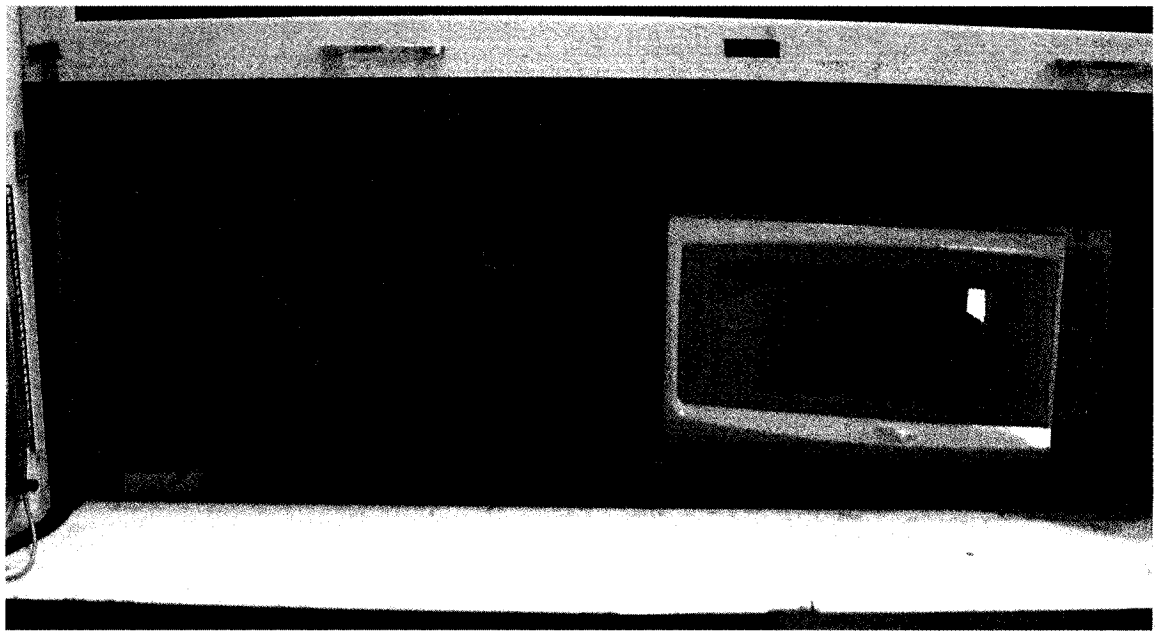


Figure 3.2: Microwave pretreatment setup.

3.3.4 Visual Analysis of Sludge Settling

Fresh 2.5% TS Rockland sludge was separated into five 850-mL portions. One portion was not treated while the other four were irradiated to 40, 55, 70 and 85°C in 2-L polypropylene containers. A 25-mL graduated cylinder was employed to pour 20 mL of distilled water into five 100-mL graduated cylinders. Upon cooling of the sludge samples and addition of distilled water to replace evaporated water, 80 mL of each sample was transferred to the 100-mL graduated cylinders. The contents of the five graduated cylinders were stirred and the solids were allowed to settle overnight. The next day, a picture was taken.

3.3.5 Microscopic Analysis of Sludge

Rockland sludge was obtained and separated into two portions. One portion was directly transferred to a 2-L glass bottle. Two 850-mL samples were irradiated until the temperature reached 85°C. Upon cooling and addition of distilled water to replace evaporated water, samples were transferred to a 2-L glass bottle. The bottles were stored in the refrigerator at 4°C.

An Olympus BX40 microscope equipped with 2x, 20x and 100x UMPlan Fl objectives was used to observe microscopic features of sludge samples. Pictures were obtained using a Polaroid digital camera model DMC1 connected to a computer. The software Polaroid DMC Direct version 1.0 was installed on the computer. Drops of sludge samples were placed on 25 mm x 75 mm microscope slides using disposable 14.6-cm long borosilicate glass Pasteur pipets. Initially, no modifications were made on the sludge samples. The result was poorly discernible structures. Improvements were made to the technique. First, staining of the microscope slides with methyl blue was carried out to render the microscopic structures distinguishable. This was accomplished by placing drops of samples on the slides and letting the drops dry. In the meantime, a few grams of methyl blue powder was added to distilled water in a beaker. The mixture was thoroughly mixed until a uniform blue solution developed. The slides were soaked with methyl blue solution which was allowed to react for 1 minute. The slides were next rinsed gently with distilled water until all excess methyl blue was drained. Finally, the slides were dried by applying a gentle pressure to the slides using Kimwipes® task wipers. The second improvement to the technique was the separation of particles by size. Three sieves were used for this purpose: #40 (420μm), #80 (177μm) and #200 (74μm). For example, to observe particles larger than 74μm but smaller than 177μm, untreated sludge was passed through sieves #80 and #200, as shown in the top frame of Figure 3.3. The larger sieve (#80) was removed and the smaller sieve (#200) was flushed with a large quantity of water to ensure that all particles smaller than 74μm were removed (see middle frame of Figure 3.3). Next, sieve #200 was turned upside down and flushed with water which was collected in the pan (see lower frame of Figure 3.3). The water in the pan was mixed thoroughly and separated into two portions: one which was irradiated to 85°C and the other one was the control. A similar procedure was used to observe particles larger than 177μm and smaller than 420μm.

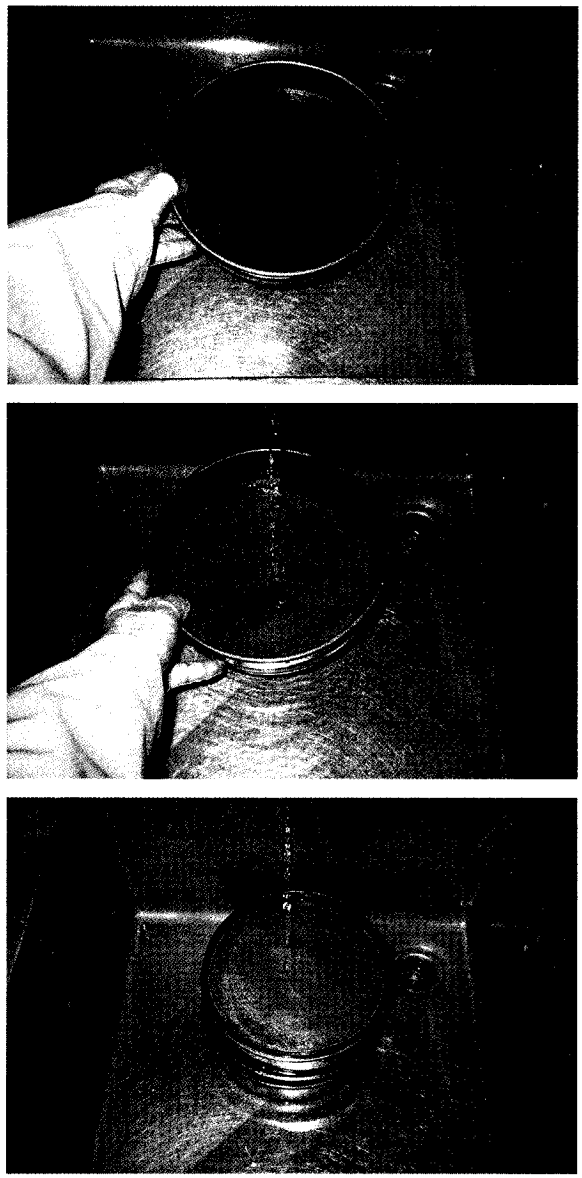


Figure 3.3: Procedure used to sort particles by size.

3.3.6 Particle Size Distribution

Rockland sludge was obtained and separated into two portions. One portion was directly transferred to a 2-L glass bottle. Two 850-mL samples were irradiated until the temperature reached 85°C. Upon cooling and addition of distilled water to replace evaporated water, samples were transferred to a 2-L glass bottle. The bottles were transported to Accutest Laboratories Ltd, Ottawa, ON. The total suspended solids concentrations were determined according to Standard Method 2540D. The particle size analysis was performed by using five

47-mm-diameter sieves with the following pore sizes: 100, 60, 30, 11 and 1 μm. No replicates were analyzed by Accutest Laboratories, Ltd.

3.3.7 Determination of Maximum Soluble COD of Sludge

Rockland sludge was obtained and a total solids test was performed in duplicates. Sixteen sets of 2-g amounts of NaOH were weighed on the balance and transferred to 150-mL glass bottles. A 100-mL graduated cylinder was employed to transfer 100 mL of well-mixed sludge to the first 150-mL bottle. Removal of oxygen from the bottle was accomplished by circulating nitrogen gas through the stopper for one minute. The bottle was capped with a rubber stopper and placed on a shaker rotating at 50 rpm at room temperature. The time was recorded. This procedure was repeated for all 16 bottles. The bottles were prepared in a random order.

Control samples were immediately prepared for sCOD and tCOD analysis as described in Table 3.1 in section 3.5. The samples in the 16 bottles were allowed to react with the NaOH for durations of 1, 2, 3, 6, 9, 12, 15 and 24 hours. At the end of these reaction periods, bottles were removed from the shaker, shaken vigorously, uncapped and prepared for sCOD and tCOD analysis as described in Table 3.1. The sCOD and tCOD tests were performed over the next two days.

3.3.8 Determination of Factors Affecting the Solubilization of COD

This study investigated the effects of temperature (microwave irradiation time), microwave intensity and sludge concentration on the solubilization of COD in the sludge. As will be fully described in section 4.4.1, a 2^3 factorial experiment was employed. Rockland sludge was centrifuged to 4.4% TS and diluted to 1.5, 2.75 and 4.0% by mixing the necessary mass of 4.4% TS sludge and distilled water in the 1-L polypropylene containers. The samples were prepared and irradiated in a random order. The code for the random number generator used is presented in Appendix E. Upon cooling and addition of distilled water to replace evaporated water, samples were transferred to 450-mL polyethylene terephthalate (PETE) containers. After all samples were treated, they were centrifuged and preserved as described in sections

3.4.9 and 3.5, respectively. Total and soluble COD determinations were performed over the next two days.

3.3.9 Biochemical Methane Potential Assays

Rockland sludge was obtained in 20-L buckets and brought to the laboratory. It was attempted to homogenize the sludge as described in section 3.2. The samples were prepared and irradiated in a random order. The code for the random number generator used is presented in Appendix E. Upon cooling, distilled water was added to the samples to replace evaporated water. In the first BMP assay the duplicate samples were not mixed prior to transferring them to batch bottles and 450-mL plastic bottles. However, since partial pretreatment was investigated in this study, treated and untreated sludge was mixed in a 1-L bottle. In the second BMP assay the duplicate samples were mixed in a 3-L PETE container. In both cases, 500 mL of a well-mixed sample was transferred to the appropriate 1-L Wheaton® borosilicate glass batch bottle while the remaining sludge (350 mL) was placed in 450-mL PETE bottles. The pH of the samples was measured in the 450-mL bottles. The 450-mL bottles were placed in the refrigerator at 4°C after pH measurement. Once all samples had been transferred to the appropriate bottles, alkalinity was added to the 1-L batch bottles (1 g/L of $NaHCO_3$ and 1 g/L of $KHCO_3$). Next, 160 mL of anaerobic seed was added to 1-L batch bottles using a 200-mL graduated cylinder. Two 500-mL batch bottles were filled with 350 mL of anaerobic seed only. Immediately after the addition of seed to a bottle, removal of oxygen from the bottle was accomplished by circulating nitrogen gas through the stopper for two minutes. Black halobutyl stoppers with internal diameter of 18 mm and outside diameter of 43 mm were used. The manometer apparatus was then used to ensure the pressure in the bottles was atmospheric at the beginning of the BMP assay. The bottles were placed in a shaker rotating at a speed of 30 rpm and maintained at 35°C. Gas production was measured daily and sometimes twice daily while gas composition was measured irregularly. The shaker and batch bottles are shown in Figure 3.4.

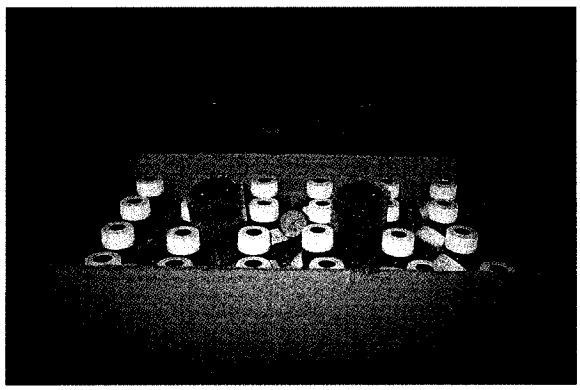


Figure 3.4: Biochemical methane potential assay setup.

The samples stored in the 450-mL bottles were separated into different bottles depending on the preservation technique needed for each sample (refer to section 3.5). Alkalinity, dissolved ammonia, pH, soluble and total COD, total and volatile solids and volatile fatty acids were measured at the beginning and end of the BMP assays. The dewaterability of the sludge samples was evaluated after anaerobic digestion. All tests were carried out within a week of being placed in the refrigerator. The appropriate sample bottles were removed from the fridge at least three hours before carrying out all tests (except for solids) so that samples could reach room temperature.

At the end of the two BMP assays, the biogas production was measured one last time, the bottles were shaken vigorously and the bottles were uncapped. Samples were again transferred to a variety of bottles depending on the preservation technique needed for each sample.

3.3.10 Effects of Microwave Irradiation on Viscosity and Surface Tension

The studies investigating the effects of microwave irradiation on the viscosity and surface tension of sludge were carried out in the same week. Rockland sludge was obtained and separated into two portions. One portion was directly transferred to two 450-mL PETE bottles. An 850-mL sample was irradiated until the temperature reached 85°C. Upon cooling and addition of distilled water to replace evaporated water, samples were transferred to two 450-mL PETE containers. The 450-mL bottles were stored in the refrigerator. The appropriate bottles were removed from the fridge at least three hours before measuring

viscosity and surface tension. The procedures used to measure these parameters are given in sections 3.4.6 and 3.4.8.

3.4 Analytical Methods

3.4.1 Alkalinity

Standard Method 2320B was employed to measure alkalinity. Sludge samples of 50 mL were placed in 125-mL Erlenmeyer flasks and titrated using 0.02 and 0.1N sulfuric acid dispensed through a 50-mL Kimax® burette. A stirring rod was inserted in the Erlenmeyer flask which was placed on a Thermix® stirrer model 120MR which was set at speed #4. The quantity of acid needed to reach pH of 8.3 and 4.6 was recorded. This was accomplished by using a Fisher Accumet® Model 925 pH meter. The electrode was thoroughly rinsed with distilled water and dried with Kimwipes® task wipers between each sample.

3.4.2 Ammonia

Dissolved ammonia ($NH_{3(aq)}$ and NH_4^+) concentration was measured according to Standard Method 4500D. Sludge samples were centrifuged with a relative centrifugal force of 8,484 for 20 minutes in a Sorval model RC-5C centrifuge equipped with a GSA rotor. Dupont Instruments stainless steel tubes were used for the centrifugation. An Orion ammonia electrode model 95-12 hooked to a Fisher Accumet® model 750 pH/ion meter was used for the measurement of ammonia. Calibration curves were prepared each time the probe was used using 10, 100 and 1,000 mg NH_3-N/L solutions. One mL of 10N NaOH solution was added to the standard solutions and supernatant samples prior to each measurement to ensure a pH greater than 11. Analyses were carried out on 50-mL supernatant samples poured in 125-mL Erlenmeyer flasks which were set on a Thermix® stirrer model 120MR which was set at a speed #4. The electrode was thoroughly rinsed with distilled water and dried with Kimwipes® task wipers between each sample. The electrode was immersed in a solution containing 10 mg NH_3-N/L between measurements. The electrode was immersed in a solution containing 1,000 mg NH_3-N/L when not in use. The membrane on the electrode was replaced the day before measurements were made.

3.4.3 Biogas Composition

Biogas samples were collected by inserting the tip of a 1-mL syringe into the stopper of the batch bottles. Representative samples were ensured by collecting and withdrawing gas twice before finally collecting a 1-mL sample. Half the sample was wasted in the air and the rest was inserted into the injection port of the gas chromatograph (GC). The equipment was a Hewlett Packard model 5710A gas chromatograph. The GC was equipped with a thermal conductivity detector that used helium as the carrier gas and a model 3380A integrator. National Instruments™ LabVIEW version 6.0 was installed on the accompanying computer. The composition of the biogas in nitrogen, methane and carbon dioxide was recorded directly from the computer monitor in percentages.

3.4.4 Biogas Production

The water displacement method was employed to measure the production of biogas from the batch bottles. A 20G1½ needle connected to a U-tube manometer was used to puncture the stopper of the bottles. A valve was opened and this allowed the biogas to displace water in the manometer until the gas in the bottle reached atmospheric pressure. The change in water level in the manometer was recorded and multiplied by the area of the water column to obtain the volume of biogas present in the bottle.

3.4.5 Dewaterability

Standard Method 2710G was employed to measure the capillary suction time of the digested sludge. A Fann® capillary suction timer was utilized. The stainless steel reservoir was placed on the CST paper such that the 1-cm diameter funnel opening was at the top. A 5-mL syringe was used to inject 2 mL of sludge sample into the reservoir. The timer counted the time required for the water released by the sludge to travel between two reference marks. The distance between the two reference marks was 6.3 mm. Duplicate measurements were made on each sample.

3.4.6 Dynamic Viscosity

The dynamic viscosity of sludge samples was measured with a Brookfield Viscometer, model LVF. Proper calibration of the viscometer was verified using a standard of known

viscosity: glycerin. Measurements were carried out by filling stainless steel centrifuge tubes and immerging the #2 cylindrical spindle into the liquid. The rotor speed was varied between 6 and 60 rpm and the resulting readings on the 0-100 scale were recorded. These readings were multiplied by conversion factors to obtain viscosity values. The conversion factors were obtained from the user manual and depend on the spindle and rotor speed used.

3.4.7 pH

The pH was measured using a Fisher Accumet® Model 925 pH meter equipped with a glass electrode. Sample temperature was obtained using a mercury thermometer before each measurement and this value was input to the pH meter. The electrode was rinsed with distilled water, dried with Kimwipes® task wipers and immersed in Thermo Orion® pH 7 buffer solution between each measurement.

3.4.8 Surface Tension

A Kruss tensiometer model K12 was employed to measure the surface tension of samples. The plate method was used. The plate is made of roughened platinum, has a wetted length of 40.2 mm, length of 19.9 mm and thickness of 0.2 mm. The sample vessel was thoroughly cleaned by rinsing with water, drying with acetone and flaming with a Bunsen burner. The plate was cleaned by rinsing with warm tap water, rinsing with distilled water and annealing to red-hot with a Bunsen burner. The sample vessel was filled with sludge and placed in the vessel. A total of three measurements were taken for each sample. The method basically involves raising the liquid level until it touches the plate. The force acting on the balance is measured and the surface tension is calculated using the equation shown in Figure 3.5.

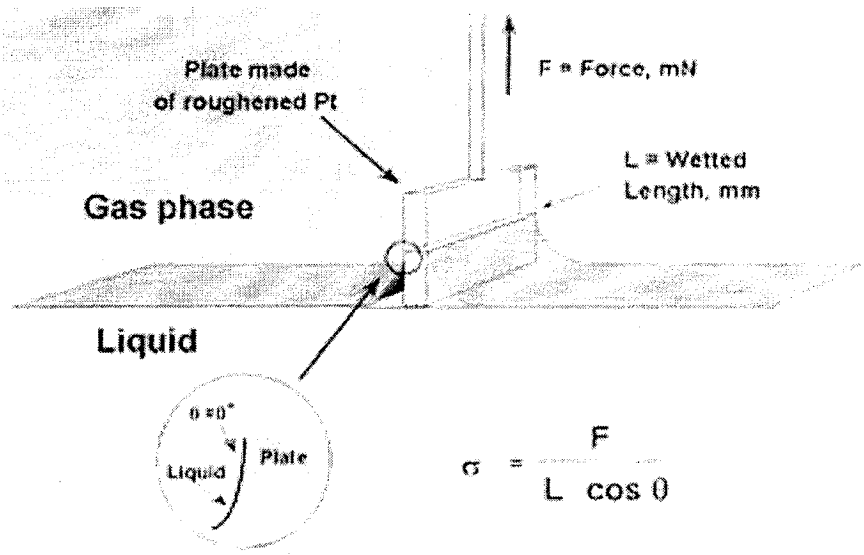


Figure 3.5: The plate method for measuring surface tension (Kruss, date unknown).

3.4.9 Total and Soluble Chemical Oxygen Demand

Chemical oxygen demand was measured using the closed reflux, colorimetric Standard Method 5220D. Soluble COD determination necessitated centrifugation of samples and filtration of the resulting supernatant. The first operation was conducted with a relative centrifugal force of 8,484 for 20 minutes in a Sorval model RC-5C centrifuge equipped with a GSA rotor. The second operation was carried out by pouring supernatant samples on Metricel 47-mm diameter sterile 0.45μm filters and by applying a vacuum using a Little Giant vacuum pump model 13154.

Volume measurements of sludge samples were carried out using a modified 5-mL serological pipet. The narrow tip of the pipet was cut off to minimize plugging. The pipet was re-calibrated by filling the pipet with different quantities of distilled water and weighing the mass of water delivered. New marks were etched on the pipet. Sample dilution was carried out in a 100-mL volumetric flask for the total COD determination and directly in the Kimax tubes for the soluble COD determination. In both cases, 10 mL of solution was added to the tubes, along with 6 mL of digestion solution and 14 mL of sulfuric acid reagent. The tubes were mixed with a Fisher vortex Genie 2^{TM} prior to digestion in a Precision mechanical convection oven model 20 for three hours. The tubes were allowed to cool overnight while protected from light by covering with a box. The absorbance of the solutions in the tubes was

measured using a Coleman Spectrophotometer model 295. The outside surface of the tubes were soaked in a bucket containing soap and dried with a clean rag and Kimwipes® task wipers prior to taking readings. Calibration curves were prepared each time one of the reagents was prepared and consisted of 12 tubes containing the following standards: three blanks, one 200 mg/L, three 300 mg/L, one 400 mg/L, three 500 mg/L and one 700 mg/L. The standards were prepared by diluting a COD stock solution containing 850 mg/L of potassium hydrogen phthalate (theoretical COD of 1,000 mg/L). This stock solution was kept for a maximum of three months. Procedures for preparing the digestion solution and sulfuric acid reagent can be found in Standard Methods.

3.4.10 Total and Volatile Solids

Total and volatile solids were analyzed according to Standard Method 2540G. Before use, porcelain evaporating dishes were scrubbed in a bucket containing soap, contacted with 8.5% sulfuric acid overnight and ignited for 40 minutes in a Thermolyne muffle furnace model F62730 at 550°C. The evaporating dishes were allowed to cool for 40 minutes in a Precision mechanical convection oven model 23 maintained at 105°C and for 40 minutes in dessicators. A dish was weighed on a Sartorius model 2001MP@ analytical balance (W). The dish was placed on a paper towel and a vigorously-mixed sludge sample was poured in the dish. The dish with sludge sample was weighed on the analytical balance (X) and placed in the oven. This procedure was repeated for all samples. The evaporating dishes were left in the oven until all water had evaporated (this typically took at least 12 hours). The dishes were subsequently transferred to dessicators for 40 minutes and weighed on the analytical balance (Y). Four dishes were placed in the muffle furnace for forty minutes while the other dishes were returned to the oven. All dishes were eventually ignited for 40 minutes. Dishes were then transferred to dessicators and weighed on the analytical balance (Z). Percent total solids and volatile solids were calculated using equations 3.1 and 3.2.

$$\%TS = 100 \times \frac{Y - W}{X - W} \qquad \text{Equation 3.1}$$

$$\%VS = 100 \times \frac{Y - Z}{X - W} \times \frac{\%TS}{100} = \frac{Y - Z}{X - W} \times \frac{Y - W}{X - W} \qquad \text{Equation 3.2}$$

3.4.11 Volatile Fatty Acids

Determination of volatile fatty acids concentrations was accomplished using a Hewlett-Packard gas chromatograph model 5840A equipped with a flame ionization detector, an automatic sampler, an injection port (temperature of 250°C), a Chromosorb 101 packed column (temperature of 180°C) and an integrator model 5840. The carrier gas was helium saturated with formic acid and flowed at a rate of 15 mL/min.

The GC was calibrated by injecting a vial containing 0.5 mL of an internal standard containing 2,000 mg/L isobutyric acid and 0.5 mL of VFA standard mixture containing 2,000 mg/L of acetic, butyric and propionic acid. Calibration was repeated until 2,000 ± 50 mg/L of each acid was measured.

Sludge samples prepared as indicated in section 3.5 were centrifuged further in an Eppendorf centrifuge model 5415 at 12,000 rpm for five minutes. Equal amounts of supernatant and internal standard were added to vials and mixed for a few seconds on a Fisher vortex Genie 2™. The vials were covered with a layer of parafilm and placed in the refrigerator until ready to use.

3.5 Sample Preservation

Upon sludge pretreatment and at the conclusion of the BMP assays, sludge samples were separated into five portions according to the preservation requirements of the analytical methods. Table 3.1 presents the preservation methods employed. After each use, all bottles and caps were thoroughly rinsed with tap water, scrubbed with a metal brush and rinsed twice with distilled water.

Table 3.1: Preservation methods employed.

Analyses Required	Bottle Size (mL)	Bottle Material	Sample Type	Preservation	Max. Storage Time Allowed
sCOD	70	HDPE	Filtered Supernatant	Added 2 mL H_2SO_4 / L sample, refrigerated	7 days
Alkalinity	130	HDPE	Sludge	Refrigerated	24 hours
Ammonia	240	HDPE	Supernatant	Added 2 mL H_2SO_4 / L sample, refrigerated	7 days
VFA					
tCOD	22	Unknown	Sludge	Added 2 mL H_2SO_4 / L sample, refrigerated	7 days
TS	240	HDPE	Sludge	Refrigerated	7 days
VS					
CST					

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Microwave Calibration

Initial experiments were needed to develop relationships between the dependent variable (sludge temperature) and the three independent variables: irradiation time, microwave intensity and sludge concentration. To allow flexibility in the choice of sample size in later experiments, microwave calibration was performed using 400-mL and 850-mL SBR sludge samples. The procedure used during the microwave calibration experiments is described in section 3.3.1.

4.1.1 850-mL Samples

The first microwave calibration experiment was carried out to establish the relationship between microwave irradiation time and sludge concentration on the temperature reached by 850-mL sludge samples. The sludge concentration was varied between 1.2 and 4.3% TS while the irradiation time was manipulated to obtain sludge temperatures below 90°C. It was decided to operate at this temperature to avoid the unpredictable and dangerous behavior of partially boiling liquids in microwave ovens.

A total of 15 SBR sludge samples were irradiated at a microwave intensity of 100%. Figure 4.1 shows the relationship between microwave irradiation time and the temperature reached by the sludge samples. The R^2 obtained was 0.9829. Analysis of the data presented in Appendix C established that sludge concentration in the range of 1.2 to 4.3% TS did not influence the sludge temperature reached. The final model is presented in Equation 4.1.

$$T = (0.2256 * MWt) + 23.862 \qquad (4.1)$$

where T is the temperature reached by the sludge (°C) and MW_t is the microwave irradiation time (s).

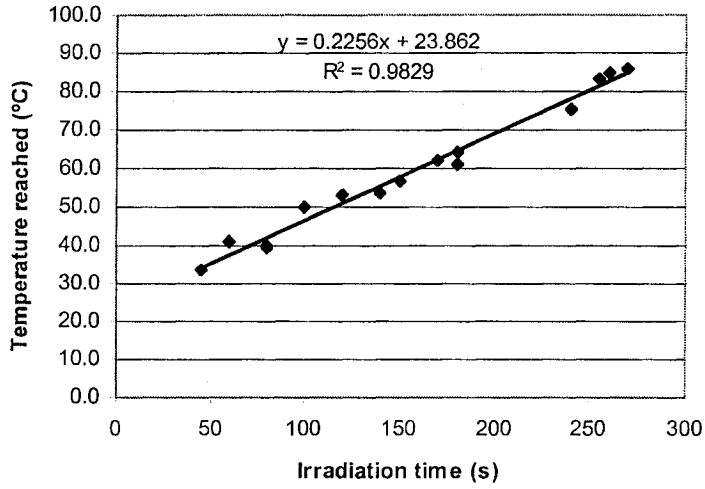


Figure 4.1: Temperature reached versus irradiation time for 850-mL SBR sludge samples between 1.2 and 4.3% TS at 100% microwave intensity.

4.1.2 400-mL Samples

The second microwave calibration experiment was carried out to establish the relationship between microwave irradiation time and microwave intensity on the temperature reached by 400-mL sludge samples. The microwave intensity was varied between 60 and 100% while the irradiation time was manipulated to obtain sludge temperatures below 90°C. The sludge concentration was kept constant at approximately 3% TS. A total of 14 samples were irradiated to develop the three models. Figure 4.2 presents the results of this calibration experiment and Equations 4.2, 4.3 and 4.4 present the linear models obtained for microwave intensities of 60, 80 and 100%, respectively. Note the reduced irradiation times required to reach specific temperatures when 400-mL samples are used. According to Equations 4.1 and 4.4, irradiation durations of 271 and 133 seconds are required to reach 85°C by the 850 and 400-mL samples, respectively at 100% microwave intensity.

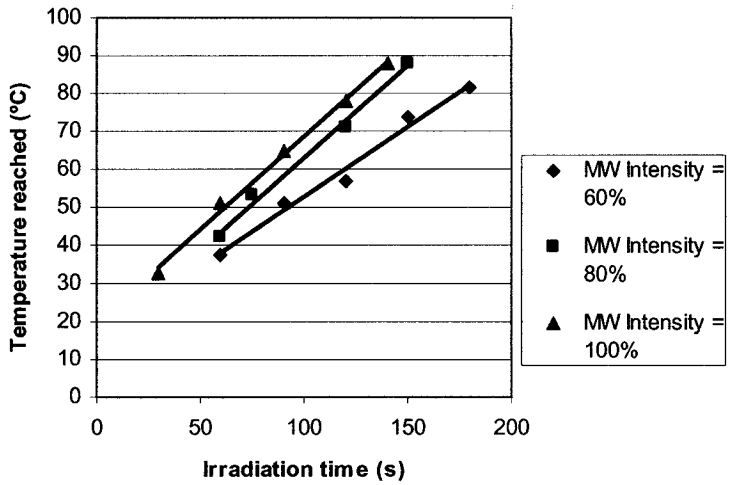


Figure 4.2: Temperature reached versus irradiation time for 400-mL samples.

$$T = (0.3683 * MWt_{60\%}) + 15.9 \tag{4.2}$$

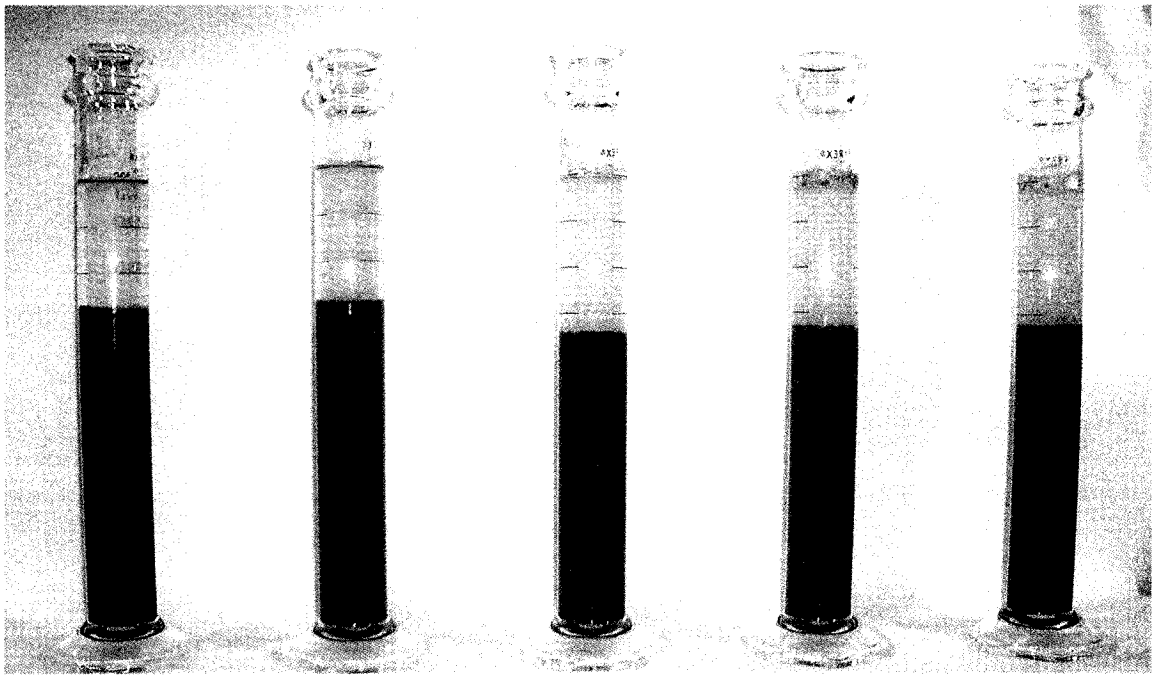
$$T = (0.4879 * MWt_{80\%}) + 14.099 \tag{4.3}$$

$$T = (0.4935 * MWt_{100\%}) + 19.49 \tag{4.4}$$

4.2 Effects of Microwave Irradiation on Particle Size of the Sludge

4.2.1 Visual Analysis of Sludge Settling

A preliminary test was also conducted to assess whether microwave irradiation can decrease particle size. This was accomplished by visually comparing supernatant turbidity of settled sludge samples. One sample was not treated while the other four were irradiated in the microwave oven to temperatures of 40, 55, 70 and 85°C. The sludge samples, along with 20 mL volumes of distilled water were mixed in five 100-mL graduated cylinders. This appropriate amount of distilled water added to increase the size of the supernatant was attained by trial and error. The picture shown in Figure 4.3 was taken approximately 15 hours after the beginning of the experiment.



Control 40°C 55°C 70°C 85°C

Figure 4.3: Qualitative comparison of suspended and colloidal particle distribution.

The supernatant of the control and 45°C samples are transparent and the supernatant occupies approximately 28 mL. On the other hand, the supernatant of the samples irradiated to 55, 70 and 85°C occupies approximately 33 mL although the supernatant of the 85°C sample seems to be the most turbid. It appears that microwave irradiation of sludge samples to temperatures greater than 40°C is capable of converting a portion of the suspended particles into colloidal particles. This positive first experiment led to the search for a more quantitative particle size analysis method to confirm that microwave irradiation is capable of reducing the size of particles in SBR sludge. The results of this literature review are presented in section 2.3.1 and Appendix A.

4.2.2 Microscopic Analysis

Attempts were made to use a light microscope to compare the particle size of control and irradiated samples. Two scenarios were seen as desirable. The first was that the difference in particle size between the two samples would be so pronounced that only two pictures would be necessary to show that microwave irradiation is successful in reducing particle size. The

second was that the particles would be spherical and easily sorted into particle size bins to allow the visual determination of particle size distribution of each sample.

A first attempt was made by simply placing a drop of well-mixed sludge sample on microscope slides, as described in section 3.3.5. The slides were placed under an Olympus BX40 microscope, observed under magnifications of 20 and 200 times and the pictures shown in Figures D.1-D.4 were taken using a digital camera. As seen on the photos, the features are not easily discernible and it is not clear whether one of the two desirable scenarios was met. It does seem, however, that the sample irradiated to 85°C contains less filamentous microorganisms than the control sample.

A second attempt was made to improve the clarity of the structures. This involved removing the small particles from the samples, separating the particles into size groups and staining the microscope slides with methyl blue. The detailed procedures can be found in section 3.3.5. The first such trial entailed filtering a sludge sample through 177 and 74 μm sieves and re-suspending the particles caught by the 74 μm sieve into water. This filtered sludge was separated into two portions, one of which was irradiated to 85°C. Drops were placed on microscope slides which were subsequently stained with a methyl blue dye. The pictures that were obtained are shown in Figures D.5-D.10. With the background material removed it is much easier to observe the size and shape of the structures. Most particles are long and thin with few spherical particles and so particle size distribution based on these pictures would be very difficult. In addition, there is no flagrant difference between the size of the particles as observed through these pictures. A more analytical method will be required to confirm that microwave irradiation is capable of reducing particle size. For completeness, the above procedure was repeated by filtering sludge samples through 420 and 177 μm sieves in order to check whether microwave irradiation is effective in breaking up larger particles. The pictures thus obtained are presented in Figures D.11-D.16. Microscopic pictures at a magnification of 200 times were also taken and these are shown in Figures D.17-D.28. Again, these pictures do not display conclusive evidence that the irradiated samples contain smaller particles.

4.2.3 Particle Size Distribution Analysis

Due to lack of the required equipment, Accutest Laboratories Ltd. was contracted to carry out particle size analysis of two sludge samples using five small-diameter sieves with pore size of 100, 60, 30, 11 and 1 μm. One-Liter samples of control and microwave irradiated (to 85°C at 100% microwave intensity) sludge were sent to their laboratory for analysis. No replicates were analyzed by Accutest Laboratories. The results of the particle size distribution analysis shown in Figure 4.4 show that the sludge sample irradiated to 85°C contains a smaller concentration of particles larger than 100 μm than the control sample. As a consequence, a re-distribution of particle sizes is observed in the smaller size bins. In particular, note the increase in the mass of particles in the 11-30 and 60-100 μm size ranges in the irradiated sample. This indicates that microwave irradiation of SBR sludge is capable of breaking down large SS particles into smaller ones thus increasing the surface area available for degradation.

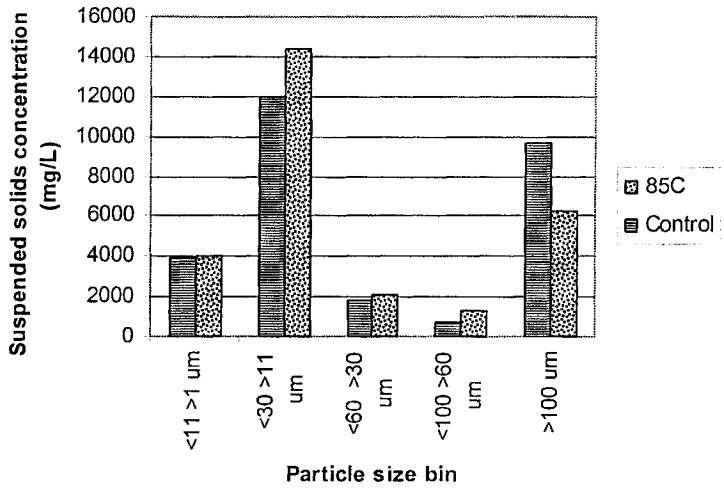


Figure 4.4: Particle size comparison of control and microwave irradiated samples in the 1-100 μm size range.

4.3 Determination of the Maximum Soluble COD of the Sludge

Prior to investigating the effects of microwave irradiation on the solubilization of SBR sludge, it was desired to quantify the maximum achievable COD solubilization of SBR sludge. This was accomplished by modifying a technique utilized by Chiu *et al.* (1997) and Tiehm *et al.* (2001). Sludge samples were contacted with 20 g/L NaOH for durations of 1, 2, 3, 6, 9, 12, 15 and 24 hours. The tCOD of the sludge was measured on six samples and was determined to be 11,645 ± 450 mg/L. The sCOD of each sludge sample was measured at the

end of the experiment. The sCOD/tCOD ratios were calculated and are shown in Figure 4.5. Error bars represent the standard deviation of the sCOD/tCOD ratios of duplicate samples. The sCOD/tCOD ratio of untreated sludge is low (1.7%), indicating that the COD of this sludge is mainly exerted by particles larger than 0.45 μm. The solubilization of the sludge with the strong dose of NaOH occurred very rapidly. Within an hour, the sCOD/tCOD ratio increased from 1.7 to 42.4%. After 24 hours, the ratio reached approximately 57%. This value will be useful in assessing the success of microwave irradiation in solubilizing COD.

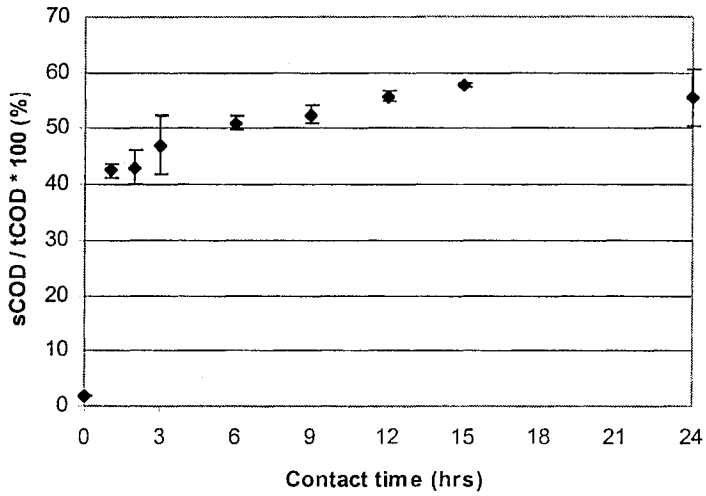


Figure 4.5: Determination of the maximum sCOD/tCOD ratio of the SBR sludge.

4.4 Determination of Factors Affecting the Solubilization of COD

A 2^3 factorial experiment with central point was employed to investigate which of three factors have an impact on the solubilization of COD and to compare the extent of the solubilization with the maximum sCOD/tCOD ratio of this sludge obtained in the experiment described in section 4.3. A model resulting from this experiment could take the form below:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_1 x_2 + \beta_5 x_1 x_3 + \beta_6 x_2 x_3 + \beta_7 x_1 x_2 x_3 \qquad (4.5)$$

where the β symbols are constants and the x symbols are the values of the three factors.

4.4.1 Design of the Factorial Experiment

The three factors were the temperature reached by the sludge (T), microwave irradiation intensity (MW I) and sludge concentration (SC). Figure 4.6 is a schematic representation of the factorial experiment and Table 4.1 presents the experimental data points. Note that three

control samples were added to supplement the factorial experiment. The sludge temperature was varied between 45 and 85°C, microwave intensity between 60 and 100% and the sludge concentration between 1.5 and 4.0% TS. It was deemed worthwhile to explore the microwave intensity variable as, for a specific temperature, sludge irradiated at an intensity of 60% would spend more time in the oven than sludge irradiated at an intensity of 100%. Sludge concentration was also deemed of interest as it influences the quantity of polar solvent acting to heat the matrix. Sludge samples of specific concentrations were prepared by centrifuging two buckets of sludge to 4.42% TS and diluting with distilled water to the desired concentrations.

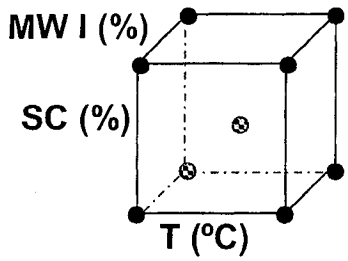


Figure 4.6: Schematic representation of the 2^3 factorial experiment.

Table 4.1: Experimental data points of the 2^3 factorial experiment.

Sample	Temperature (°C)	Sludge Concentration (%)	Microwave Intensity (%)
1	45	1.5	60
2	45	1.5	100
3	45	4	60
4	45	4	100
5	65	2.8	80
6	85	1.5	60
7	85	1.5	100
8	85	4	60
9	85	4	100
C	-	4.4	-

4.4.2 Results

Table 4.2 shows the results of this experiment. The sCOD/tCOD ratio of the control samples was approximately 1.4%, which is similar to the value obtained in the experiment described in section 4.3. Increasing the temperature reached by the sludge in the microwave oven in the 45-85°C range has a positive effect on the solubilization of the COD. The sCOD/tCOD ratio approximately doubled when sludge was heated to 45°C in the microwave oven. A large increase in the ratio was observed when the temperature of microwave irradiation was 65°C and increased slightly further for samples treated to 85°C, reaching an ultimate value of approximately 7%.

Table 4.2: sCOD/tCOD ratio reached by the sludge samples in the factorial experiment.

Sample	Temperature (°C)	Sludge Concentration (%)	Microwave Intensity (%)	sCOD/tCOD * 100 (%)
1A	45	1.5	60	3.0
1B	45	1.5	60	3.3
2A	45	1.5	100	3.2
2B	45	1.5	100	2.9
3A	45	4.0	60	3.0
3B	45	4.0	60	3.1
4A	45	4.0	100	2.5
4B	45	4.0	100	3.4
5A	65	2.8	80	5.9
5B	65	2.8	80	6.0
6A	85	1.5	60	6.8
6B	85	1.5	60	6.0
7A	85	1.5	100	5.7
7B	85	1.5	100	5.8
8A	85	4.0	60	6.5
8B	85	4.0	60	7.1
9A	85	4.0	100	5.8
9B	85	4.0	100	6.2
Control A	-	4.4	-	1.4
Control B	-	4.4	-	1.3
Control C	-	4.4	-	1.4

A plot of the sCOD/tCOD ratio versus the temperature of microwave treatment and sludge concentration was created and is shown in Figure 4.7. A plot of the sCOD/tCOD ratio versus

the temperature of microwave treatment and microwave intensity was also created and is shown in Figure 4.8.

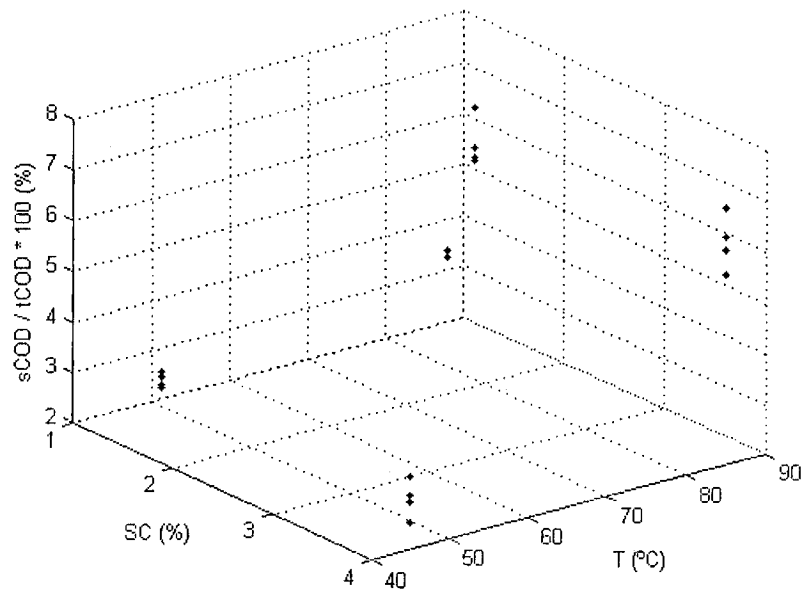


Figure 4.7: Effect of the temperature of microwave treatment and sludge concentration on COD solubilization.

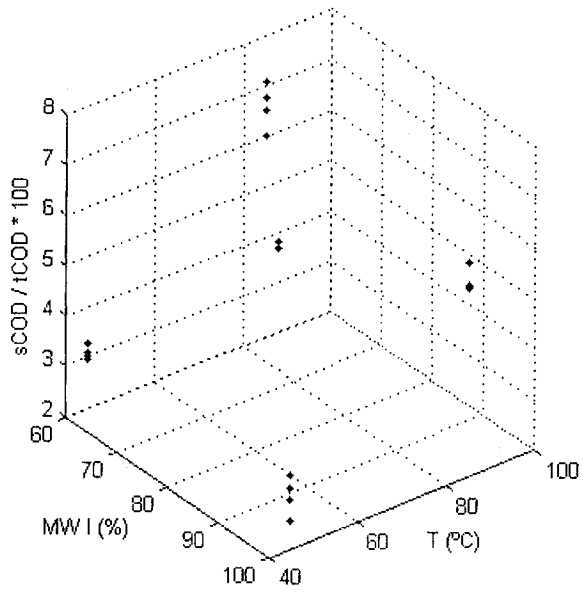


Figure 4.8: Effect of the temperature of microwave treatment and microwave intensity on COD solubilization.

Visually, sludge concentration and microwave intensity do not seem to affect the sCOD/tCOD ratio. One goal of this experiment is to develop a relationship of the form shown in Equation 4.5 between any statistically significant variables and the sCOD/tCOD ratio reached by the sludge. For this reason, it is sought to confirm that sludge concentration and microwave intensity do not have significant effects on the sCOD/tCOD ratio.

4.4.3 Statistical Analysis

The procedure described by Berthouex and Brown (2002) in chapter 27 of "Statistics for Environmental Engineers" was employed to analyze the results of the factorial experiment. The coded factorial matrix is based on Table 4.1 and is shown in Table 4.3. X_1, X_2 and X_3 represent the factors temperature reached, sludge concentration and microwave intensity, respectively. X_{12}, X_{13}, X_{23} and X_{123} represent two and three-factor interactions. The last two columns of Table 4.3 contain the average sCOD/tCOD ratio of each run, along with the standard deviation associated with its duplicate measurements. Note that the duplicate measurements were not carried out on the same sample but rather on two different samples that were irradiated under the same conditions. Again, the random number generator presented in Appendix E was utilized to determine the order that samples were pretreated and that COD measurements were made.

Table 4.3: 2^3 factorial design matrix.

Run	X_0	X_1	X_2	X_3	X_{12}	X_{13}	X_{23}	X_{123}	y	s^2
1	1	-1	-1	-1	1	1	1	-1	3.15	0.03
2	1	-1	-1	1	1	-1	-1	1	3.06	0.03
3	1	-1	1	-1	-1	1	-1	1	3.02	0.01
4	1	-1	1	1	-1	-1	1	-1	2.98	0.41
5	1	1	-1	-1	-1	-1	1	1	6.39	0.30
6	1	1	-1	1	-1	1	-1	-1	5.77	0.00
7	1	1	1	-1	1	-1	-1	-1	6.79	0.16
8	1	1	1	1	1	1	1	1	6.00	0.12

The results of the statistical analysis are presented in Table 4.4. The effect of the main three factors and combinations thereof was calculated by multiplying the appropriate X matrix by the y matrix and dividing by 4. The variance of each main effect and interaction was

calculated according to Equation 4.6. The 95% confidence intervals were calculated using Equation 4.7. SE represents the standard error.

$$Var(effect) = (\frac{1}{4})^2 [Var(y_1) + Var(y_2) + ... + Var(y_8)] \quad (4.6)$$

$$Effect \pm t_{v=8,\alpha/2=0.025} SE(effect) \quad (4.7)$$

Table 4.4: Results of the statistical analysis.

	Effect	Variance of Effect	95% Confidence Interval	Lower Boundary	Upper Boundary	Signifi-cant?
Main effect of factor 1	3.1850	0.0664	0.4123	2.7727	3.5973	Yes
Main effect of factor 2	0.1050	0.0664	0.4123	-0.3073	0.5173	No
Main effect of factor 3	-0.3850	0.0664	0.4123	-0.7973	0.0273	No
Inter. of factors 1 and 2	0.2100	0.0664	0.4123	-0.2023	0.6223	No
Inter. of factors 1 and 3	-0.3200	0.0664	0.4123	-0.7323	0.0923	No
Inter. of factors 2 and 3	-0.0300	0.0664	0.4123	-0.4423	0.3823	No
Inter. of factors 1, 2 and 3	-0.0550	0.0664	0.4123	-0.4673	0.3573	No

Effects were seen as significant if the 95% confidence interval of an effect did not contain zero. According to this analysis, only the temperature of microwave irradiation has a significant effect on the sCOD/tCOD ratio. Therefore, Equation 4.5 can be simplified to model the data obtained in this experiment.

4.4.4 Development of a Mathematical Relationship

Because sludge concentration and microwave intensity do not significantly affect the sCOD/tCOD ratio, the simplest possible model is a linear equation relating the temperature of microwave treatment and the sCOD/tCOD ratio. Figure 4.9 displays this relationship and the resulting model while Figure 4.10 presents the residuals of this model.

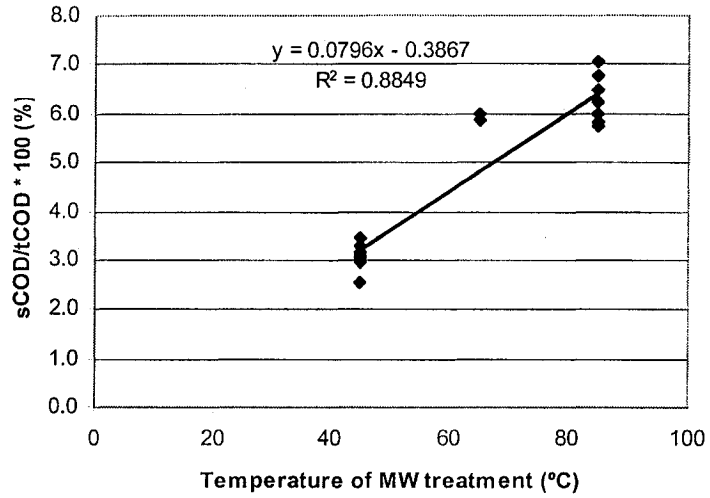


Figure 4.9: Linear relationship between the temperature of microwave treatment and the sCOD/tCOD ratio.

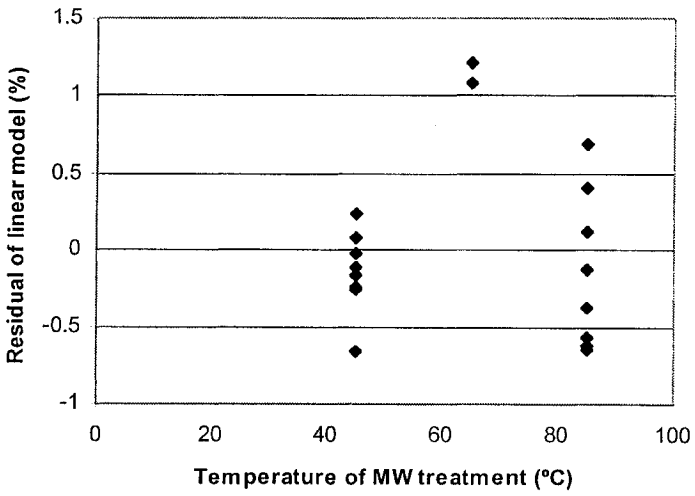


Figure 4.10: Residuals of the linear model relating the temperature of microwave treatment and the sCOD/tCOD ratio.

The two figures convey that a linear equation is not appropriate to model the data. Indeed, the sCOD/tCOD ratio reaches a plateau when the temperature of microwave treatment is 65°C. For this reason, it was attempted to model the data using a second-order equation. Figure 4.11 displays this relationship while Figure 4.12 presents the residuals of this model.

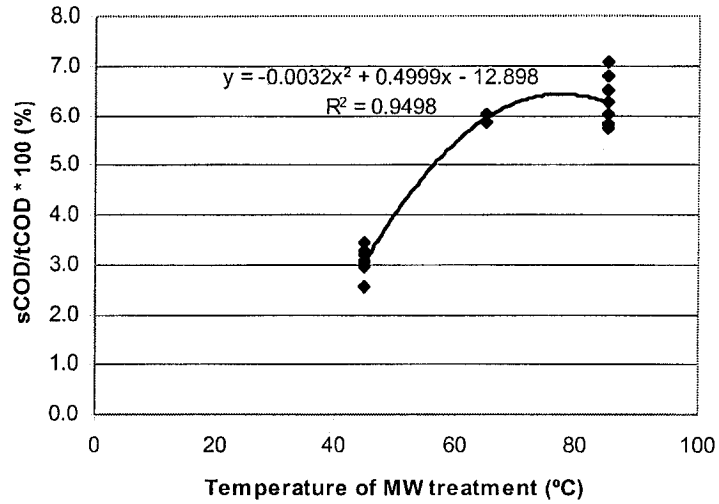


Figure 4.11: Second-order relationship between the temperature of microwave treatment and the sCOD/tCOD ratio.

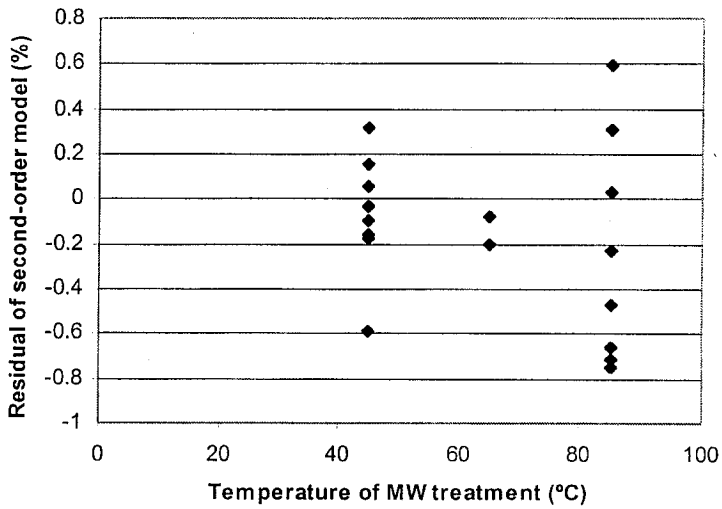


Figure 4.12: Residuals of the second-order model relating the temperature of microwave treatment and the sCOD/tCOD ratio.

The final model relating the temperature of microwave treatment and the sCOD/tCOD ratio of the sludge is shown in Equation 4.8.

$$\frac{sCOD}{tCOD} \times 100 = -0.0032T^2 + 0.4999T - 12.898 \qquad (4.8)$$

This model is applicable to Rockland SBR sludge characterized by a TS concentration ranging between 1.5 and 4.0% and heated to a temperature between 45 and 85°C in a microwave oven operating at a frequency of 2.45 GHz and at an intensity varying between 60

and 100%. The maximum sCOD/tCOD ratio obtained by microwave irradiation is approximately 7%. This is significantly lower than the maximum ratio of the sludge (57%) obtained with a strong dose of NaOH. Despite this, advantages of microwave irradiation such as its low power consumption of microwave irradiation and addition of no chemicals to the sludge encourage us to pursue this research. The next step is to carry out BMP assays to observe the extent of the improvement in biogas production and solids destruction that can be achieved by pretreating sludge by microwave irradiation.

4.5 Biochemical Methane Potential Assays

4.5.1 First Biochemical Methane Potential Assay

4.5.1.1 Design of the Experiment

The variables investigated in the first BMP assay were the temperature reached by sludge samples and the percent of sludge samples that were pretreated. As mentioned in section 2.4.7, Barber (date unknown) observed the highest biogas yield when pretreating 50-60% of the waste activated sludge in primary/WAS mixtures with ultrasound. In this experiment, the percent of the sludge sample pretreated was varied between 0 and 100% and the temperature reached by the treated fraction was varied between 45 and 85°C. Table 4.5 presents the pretreatment conditions of the 24 samples (each experimental condition was investigated in duplicate). The samples were prepared in a random order. Pretreated and untreated portions were mixed together and 500 mL of the mixture was added to batch bottles while the other portion was placed in the fridge for analysis. Volumes of 160 mL of acclimated inoculum were added to the batch bottles. Two 500-mL batch bottles were filled with 350 mL of inoculum to assess the extent of the stabilization of the anaerobic seed.

Table 4.5: Experimental conditions of the samples in BMP assay #1.

Sample	Percent of Sludge Sample Pretreated (%)	Temperature Reached by Pretreated Fraction (°C)
1	20	45
2	20	65
3	20	85
4	60	45
5	60	65
6	60	85
7	100	45
8	100	55
9	100	65
10	100	75
11	100	85
C	0	-

4.5.1.2 Results

At the beginning of the assay, the pH of all samples was approximately 6.1 while the inoculum samples had a pH of 6.9. Because the pH was lower than 8.3 in all cases, samples were devoid of phenolphthalein alkalinity. Therefore, all alkalinity was in the bicarbonate form. The alkalinity of all samples ranged between 330 and 900 mg/L while the inoculum samples contained 2,770 mg/L (as $CaCO_3$). To buffer against sudden rises in VFA during anaerobic digestion, approximately 1 g/L each of $NaHCO_3$ and $KHCO_3$ were added to all bottles. This acted to raise the alkalinity of the samples by 2,190 mg/L as $CaCO_3$ (calculated). The NH_3-N concentration of all samples ranged between 20-70 mg/L while the inoculum samples contained approximately 400 mg/L. The volatile fatty acids content of the samples was less than 130 mg/L acetic acid and 100 mg/L propionic acid. The inoculum was devoid of volatile fatty acids.

Analysis of the tCOD, VS and gas production data showed that the sludge in the three buckets used during the preparation of samples had not been properly homogenized. Plots showing the effect of the order in which samples were prepared on the initial tCOD and VS of the samples were made and are shown in Figures 4.13 and 4.14. The first 11 samples were prepared with sludge taken from one bucket, the next 12 samples were prepared with sludge from a second bucket and the last sample was prepared with sludge from a third bucket. The

average tCOD and VS in each bucket was calculated and later used to calculate tCOD and VS destruction. The second bucket was the most concentrated. Its tCOD was 22,921 ± 932 mg/L and its VS was 1.40 ± 0.11%. The first bucket had tCOD of 18,592 ± 770 mg/L and VS of 1.185 ± 0.077%. Note that non-digested sludge is more difficult to accurately measure than digested sludge as it is more viscous and more concentrated (greater dilution factor required to measure tCOD).

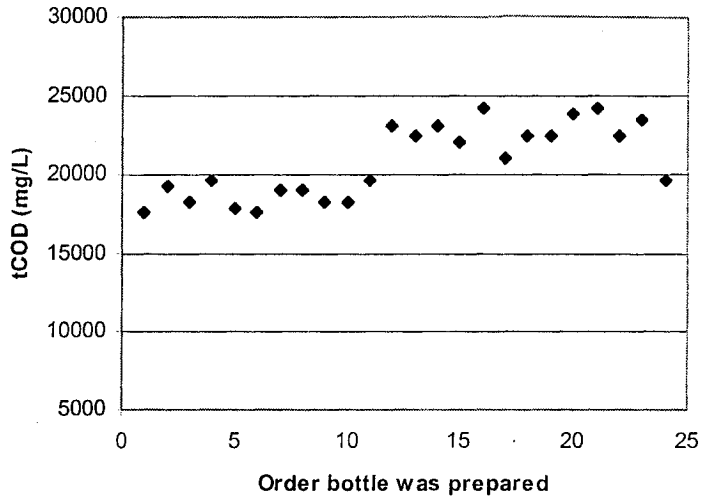


Figure 4.13: Initial tCOD of samples in BMP assay #1.

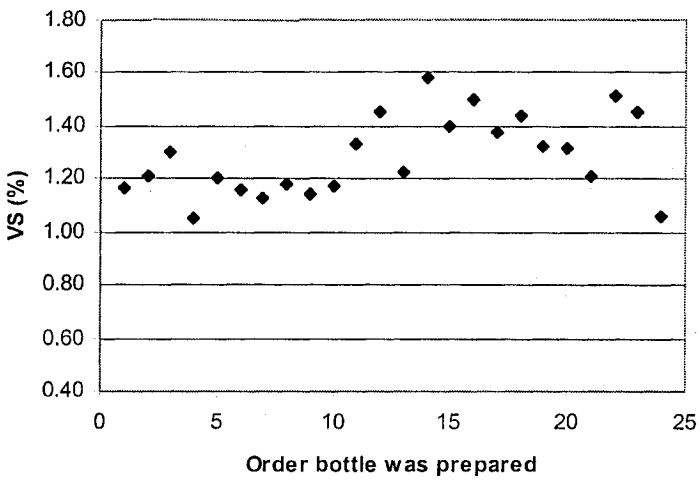


Figure 4.14: Initial VS of samples in BMP assay #1.

The sCOD of the sludge before anaerobic digestion was monitored to check/confirm the results obtained in section 4.4. The sCOD/tCOD ratios of sludge samples that were 100%

pretreated to temperatures between 45 and 85°C are shown in Figure 4.15. The curve illustrates the model developed in section 4.4. The fit is respectable considering that the two experiments were conducted using two different batches of sludge. The control data points were arbitrarily located on the x-axis at a temperature of 20°C. The results are very similar to the ones shown in Table 4.2 for the control and samples irradiated to 45 and 85°C. For samples irradiated to 65°C the sCOD/tCOD ratio was 7.2 ± 0.5% compared to 5.9 ± 0.1% obtained in section 4.4. These results indicate that microwave irradiation to 85°C does not yield appreciable improvements in the solubilization of COD compared to samples irradiated to 65°C and 75°C. This is consistent with Figure 4.3 which showed similar turbidities for samples irradiated to 70 and 85°C. It remains to be seen whether samples irradiated to 65, 75 and 85°C will be degraded to similar extents during anaerobic digestion.

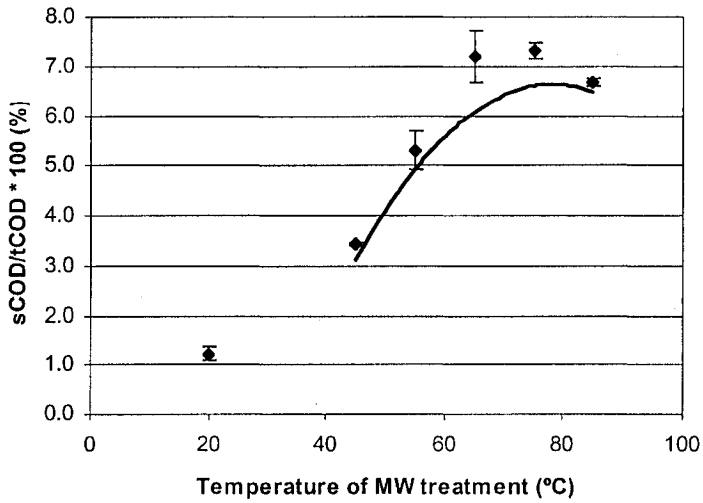


Figure 4.15: sCOD/tCOD ratio of sludge samples that were 100% irradiated.

The frequency of gas production measurements was initially bi-daily and was later lowered to once daily. Figure 4.16 shows the cumulative biogas production recorded from bottles containing sludge samples that were 20% pretreated to temperatures varying between 45 and 85°C. The cumulative biogas production curves from the control samples are illustrated using smoothed lines to enhance clarity. Figure 4.16 again indicates that the sludge in the three buckets was not properly homogenized. To remedy the situation, biogas readings from the bottles containing sludge from buckets 1 and 3 were normalized according to Equation 4.9.

The readings shown in Figure 4.16 were thus normalized and the result is shown in Figure 4.17.

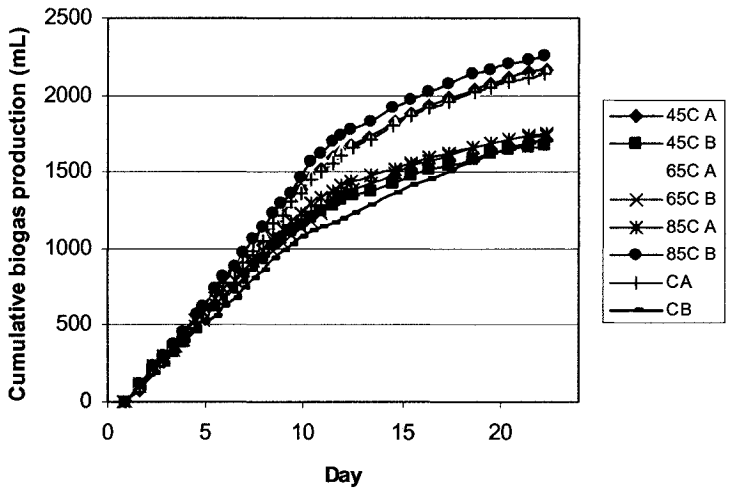


Figure 4.16: Biogas production from bottles containing samples that were 20% pretreated.

$$BiogasAdjustmentFactor = \frac{tCOD_{bucket2}}{tCOD_{bucketi}} \tag{4.9}$$

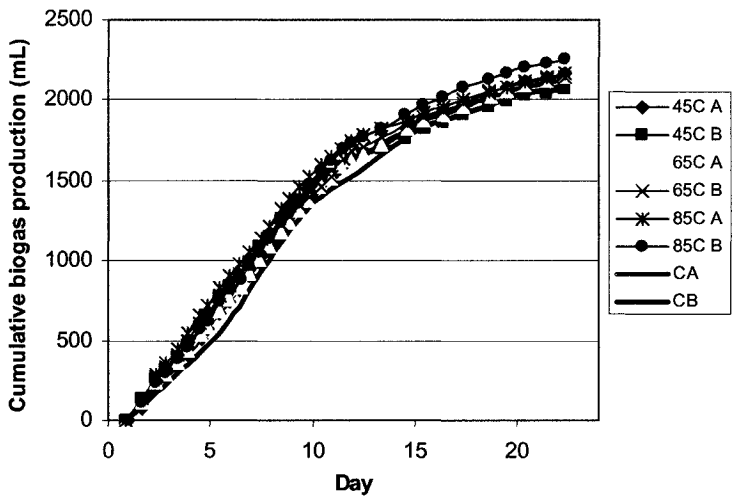


Figure 4.17: Normalized biogas production from bottles containing samples that were 20% pretreated.

No lag phase was exhibited by the samples that were 20% pretreated as demonstrated by the rapid biogas production recorded at the beginning of the assay. No signs of inhibition were observed as all samples generated a steady supply of biogas during the assay. The ultimate

amount of biogas produced by all pretreated samples are approximately the same as that produced by the controls. Pretreatment of only 20% of sludge samples to temperatures between 45 and 85°C does not improve biogas production.

The normalized gas production recorded from bottles containing sludge samples that were 60% pretreated to temperatures varying between 45 and 85°C is shown in Figure 4.18. Again, no lag phase or evidence of inhibition were observed. In this case, there is a definite improvement (> 7% based on bottle 85°C A) in biogas production from the samples whose pretreated fraction reached 85°C.

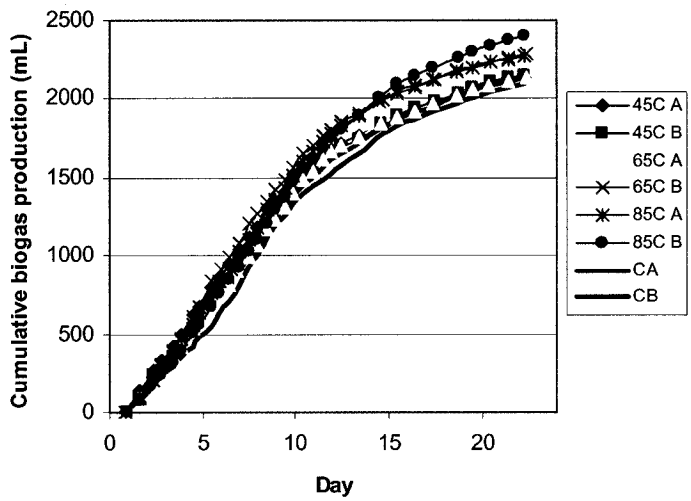


Figure 4.18: Normalized biogas production from bottles containing samples that were 60% pretreated.

The gas production recorded from bottles containing sludge samples that were 100% pretreated to temperatures varying between 45 and 85°C is shown in Figure 4.19. Again, no lag phase or evidence of inhibition were observed. Pretreatment of sludge samples to temperatures lesser than 65°C did not result in conclusive improvements in biogas production. However, pretreatment to 65°C yielded a 10.8% improvement, pretreatment to 75°C yielded a 10.9% improvement and pretreatment to 85°C yielded a 16.2% improvement. Hence, despite similar solubilization of COD at microwave irradiation temperatures of 65, 75 and 85°C, the samples heated to 85°C produced more biogas than the ones heated to 65 and 75°C. This may be hypothesized to be due to a more successful disruption of particles larger than 100 μm into particles larger than 0.45μm at a temperature of 85°C than at 65 and 75°C.

This would render more organic matter available for anaerobic degradation. It can be observed in Figure 4.19 that all samples were characterized by an initial biogas production rate of approximately 150 mL per day. The difference between the best pretreatment conditions and the control was the duration of this maximum production rate. Hence, pretreatment of this SBR sludge by microwave irradiation does not affect the rate of degradation but enhances the degradability of the substrate.

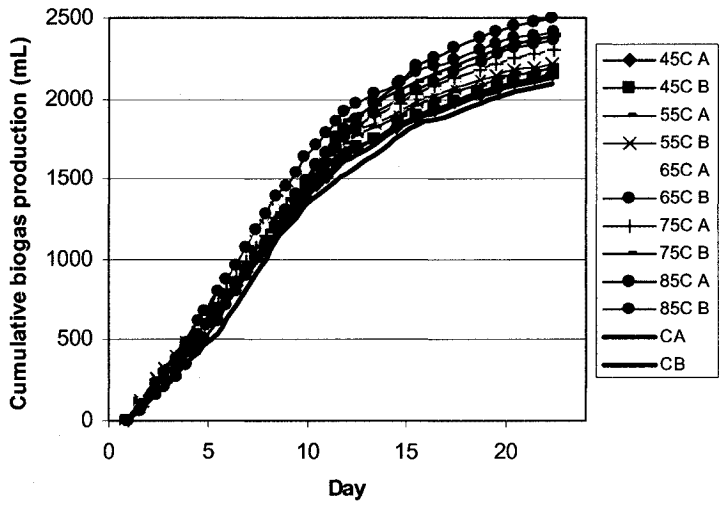


Figure 4.19: Normalized biogas production from bottles containing samples that were 100% pretreated.

The cumulative biogas production curves from the degradation of samples pretreated to 45°C are shown in Figure 4.20. The graph clearly shows that microwave irradiation of sludge to 45°C is not sufficient to improve the anaerobic degradability of this sludge.

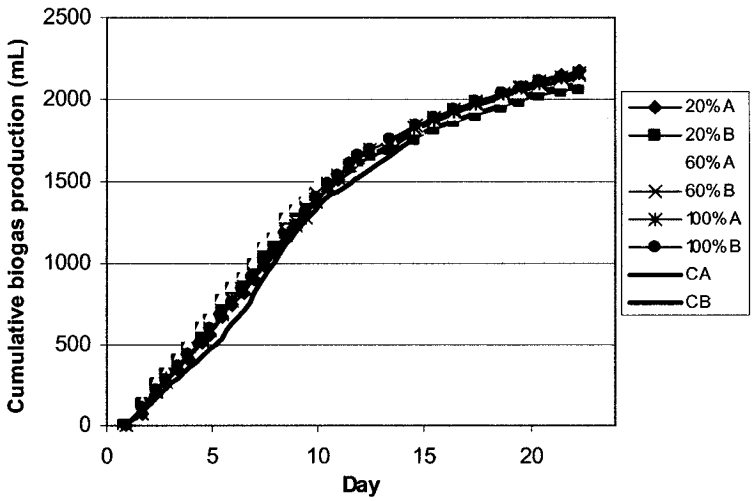


Figure 4.20: Normalized biogas production from bottles containing samples irradiated to 45°C.

The cumulative biogas production curves from the degradation of samples pretreated to 65°C are shown in Figure 4.21. As mentioned earlier, pretreatment to 65°C is capable of producing significant improvements in biogas production. The two samples that were 60% pretreated showed variability.

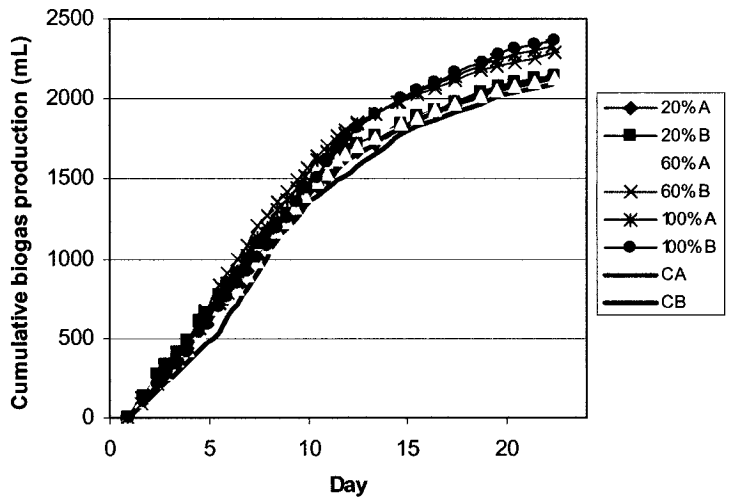


Figure 4.21: Normalized biogas production from bottles containing samples irradiated to 65°C.

The cumulative biogas production curves from the degradation of samples pretreated to 85°C are shown in Figure 4.22. The two samples that were 20% pretreated showed variability and

did not yield appreciable improvements in biogas production. Treatment of 60% of sludge samples to 85°C yielded improvements of 7.3 and 13.1% in biogas production for the two samples. Sludge that was completely treated to 85°C yielded an average improvement of 16.2% in biogas production for the two samples.

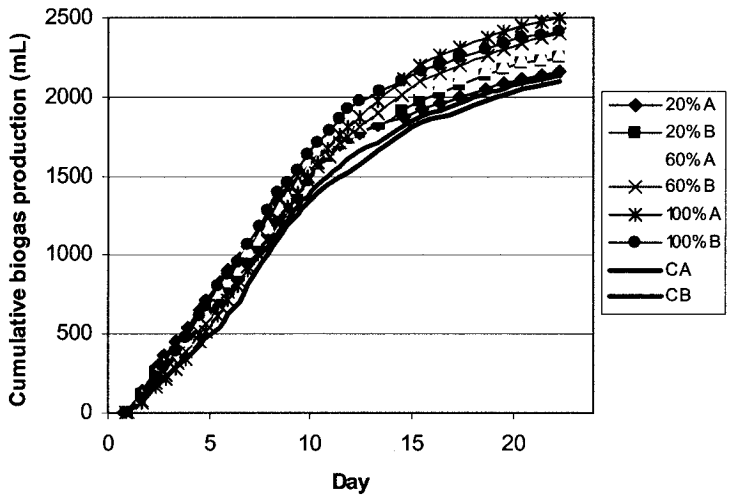


Figure 4.22: Normalized biogas production from bottles containing samples irradiated to 85°C.

Another way to assess whether partial pretreatment was effective in improving anaerobic digestion is to compare the total biogas production values obtained and the expected values. Figure 4.23 displays the total biogas production from samples whose pretreated fraction was heated to 85°C (and the controls). Because 342 mL extra biogas was produced by treating 100% of sludge samples (2,458 – 2,116), 2,184 mL of biogas was expected from the samples that were 20% pretreated (2,116 mL + 342 mL * 20%). Similarly, 2,321 mL of biogas was expected from the samples that were 60% pretreated. Had partial pretreatment been successful at improving biogas production, the experimental total biogas productions obtained would have been significantly greater than the theoretical values calculated above. Because this is not the case here, partial pretreatment was found not to have a positive influence on the anaerobic digestion of microwave irradiated SBR sludge at temperatures lesser than 85°C.

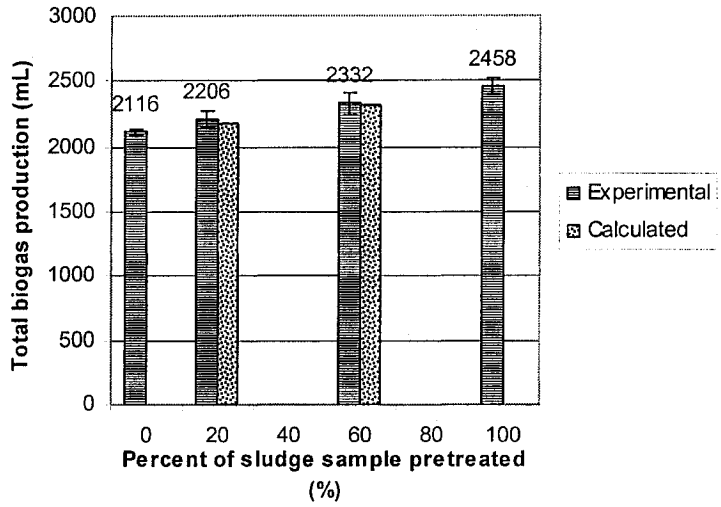


Figure 4.23: Total biogas production of samples whose pretreated fraction was heated to 85°C.

The biogas production curves from the bottles containing 350 mL of inoculum are shown in Figure 4.24. Because the bottles containing the pretreated and control samples contained 160 mL of inoculum, the anaerobic seed contributed only approximately 119 mL of biogas in the bottles containing pretreated and control samples. This low gas production in the bottles containing the inoculum illustrates that the SRT of 25 days used in feeding the acclimation tank was appropriate in producing a stable and active anaerobic seed.

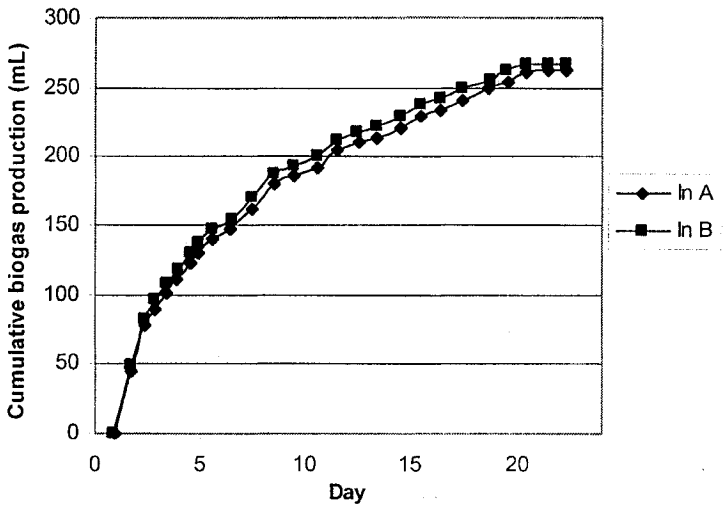


Figure 4.24: Biogas production from the bottles containing inoculum in BMP assay #1.

The composition of the biogas was measured four times during the assay. The first reading taken from each bottle was the lowest of the four readings, except for the bottle containing one replicate of the inoculum. During the last three measurements, the biogas was composed of 70-75% methane and 20-25% carbon dioxide.

The bottles were all uncapped on the same day, once daily biogas production from all bottles represented less than 1% of the total biogas production. The pH of samples was recorded immediately after the uncapping of a bottle. It was found to be approximately 7.2 for all pretreated and control samples and 7.5 for the inoculum samples. These values are appropriate for anaerobic digestion. The alkalinity of all samples ranged between 3,200 and 4,100 mg/L as $CaCO_3$. The alkalinity was all in the bicarbonate form. This alkalinity content was suitable for proper buffering against sudden rises in VFA. Gas chromatography of all centrifuged sludge samples at the end of the BMP assay detected no VFA.

The sCOD of all samples after anaerobic digestion ranged between 255 and 455 mg/L. This illustrates that a portion of the soluble organic matter was recalcitrant to anaerobic degradation. In addition, the sCOD concentration of the control and inoculum samples was higher than it was at the beginning of the BMP assay. This simply demonstrates that some of the COD that was solubilized by the anaerobic microorganisms was recalcitrant to degradation.

The dissolved ammonia (NH_4^+ and NH_3) concentration in all samples at the end of the BMP assay was in the 325-520 mg/L range. According to Speece (1996), ammonia concentrations in the 200-1,000 mg/L range pose no adverse effects on anaerobic biological activity. Because ammonia is produced during the anaerobic digestion of nitrogen-rich organic matter (such as proteins), it was hypothesized that high ammonia contents would be measured in samples that received the most intense pretreatment and showed the greatest VS destruction and biogas production. Figure 4.25 shows the relationship between biogas production and ammonia concentration in the sludge samples and confirms the aforementioned hypothesis. The ammonia content of the inoculum samples did not change significantly during the BMP

assay, implying that most nitrogen-rich organic matter had been digested in the acclimation tank.

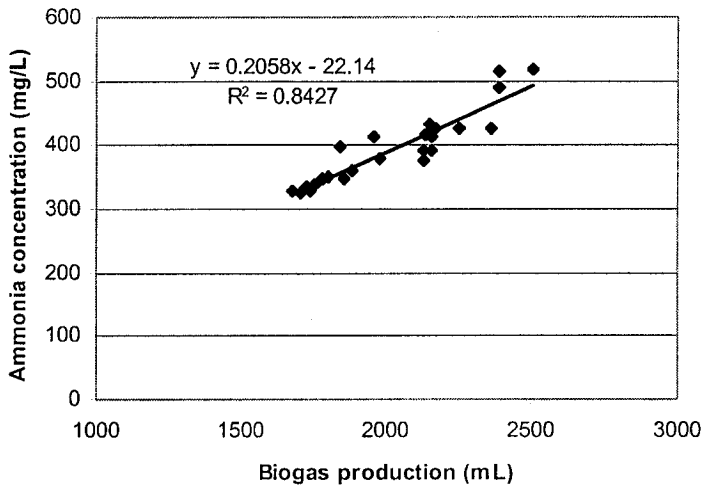


Figure 4.25: Relationship between ammonia concentration and biogas production in BMP assay #1.

The tCOD and VS removal during the BMP assay were calculated by taking into account the remaining tCOD contributed by the inoculum in the bottles containing control and pretreated samples. This was accomplished according to Equation 4.10.

$$\frac{(tCOD_1 * 0.5L) - [(tCOD_2 * 0.66L) - (tCODinoc_2 * 0.16L)]}{tCOD_1 * 0.5L} * 100 \qquad (4.10)$$

where $tCOD_1$ is the tCOD of sludge samples before the BMP assay, $tCOD_2$ is the tCOD of sludge samples after the BMP assay and $tCODinoc_2$ is the average tCOD of the inoculum samples at the end of the BMP assay. The yield of methane from each sludge sample was calculated according to Equation 4.11.

$$\frac{totalCH_4 production}{[\frac{(tCOD_1 * 0.5L) + (tCODinoc_1 * 0.16L)}{0.66L} - tCOD_2] * 0.66L} = \frac{mLCH_4}{gCODremoved} \qquad (4.11)$$

Table 4.6 shows the tCOD removal and yield of methane calculated for each control and pretreated sample. The second column of the table describes the pretreatment conditions associated with the sample labels. The first number is the percent of the sample that was pretreated and the second number is the temperature reached by the pretreated fraction. The tCOD removals obtained are consistent with the total biogas production measured, as

evidenced by the rather consistent methane yield shown in Table 4.6. The average methane yield was 0.28 L CH_4 per g COD removed at standard temperature and pressure (STP). This is less than the theoretical value of 0.39 L CH_4 per g COD removed. However, actual measured values in the range of 0.10-0.35 have been reported in the literature (Droste, 1997). Some of the reasons given for low values are gas leakage and conversion of some of the organics to compounds not oxidized during the COD test. Although minute biogas leakage cannot be ruled out, that the difference between the theoretical and measured values could be accounted for by biogas leakage is unlikely as such biogas leakage would have been more pronounced for the bottles having produced the most biogas (because of the higher pressure in those bottles) and this would have contributed to lower methane yields for pretreated samples than for the controls. As this is not the case, the low biogas yields are most likely due to the conversion of some of the organics to compounds not oxidized during the COD test. The average tCOD removal in the control samples was 45%. The highest tCOD removal which was observed in the samples pretreated to 85°C, was 51%. This is a 6-percentage point improvement over the control. There is some variability in the tCOD removal rates of duplicate samples due to the difficulty of obtaining homogeneous samples and the high dilution factors required when performing the COD analysis. In general, however, the tCOD removal rates confirm the biogas results discussed earlier that microwave irradiation of sludge can significantly improve anaerobic degradability.

Table 4.6: tCOD removal and methane yield obtained in BMP assay #1.

Sample	Pretreatment Conditions (%/°C)	tCOD Removal (%)	tCOD Removal (%)	L CH_4 Produced Per g COD Removed (L/g)
1A	20/45	47	46	0.27
1B		45		0.26
2A	20/65	44	43	0.28
2B		42		0.30
3A	20/85	46	45	0.27
3B		44		0.30
4A	60/45	43	44	0.28
4B		46		0.28
5A	60/65	44	46	0.28
5B		48		0.27
6A	60/85	46	48	0.29
6B		50		0.28
7A	100/45	44	46	0.28
7B		47		0.26
8A	100/55	50	50	0.27
8B		50		0.27
9A	100/65	48	48	0.28
9B		47		0.29
10A	100/75	48	48	0.28
10B		48		0.29
11A	100/85	48	49	0.30
11B		51		0.28
CA	0/-	43	45	0.29
CB		46		0.27

The VS removal in the samples was calculated according to Equation 4.10 (replace tCOD symbols by VS). The values thus obtained are shown in Table 4.7. The average VS removal in the control samples was 49%. The average VS removal of the samples that were fully treated to 85°C was 56% which represents a 7-percentage point improvement. This concurs with the tCOD removals and biogas production data thus providing further confidence in the results indicating improved anaerobic degradability of SBR sludge pretreated with microwave irradiation.

Table 4.7: VS removal in BMP assay #1.

Sample	Pretreatment Conditions (%/°C)	VS Removal (%)	VS Removal (%)
1A	20/45	48	50
1B		52	
2A	20/65	45	49
2B		53	
3A	20/85	53	51
3B		49	
4A	60/45	50	50
4B		50	
5A	60/65	54	52
5B		50	
6A	60/85	51	51
6B		51	
7A	100/45	53	51
7B		50	
8A	100/55	53	52
8B		52	
9A	100/65	52	52
9B		52	
10A	100/75	53	54
10B		55	
11A	100/85	56	56
11B		57	
CA	0/-	49	49
CB		50	

The capillary suction time (dewaterability) of the sludge samples was measured in duplicate at the end of the assay. Because the dewaterability of sludge samples depends partly on the sludge concentration, the fact that the sludge from the three buckets contained different concentrations prevents the use of a plot of CST versus temperature of microwave irradiation. Instead, Figure 4.26 presents the relationship between CST and biogas production for each of the three buckets. The control samples are represented by red markers. Because the best pretreatment conditions yielded the most biogas, this plot permits the indirect analysis of the effect of microwave irradiation on the CST. Overall, the plot displays the worse dewaterability conditions at the pretreatment settings that produced the most biogas.

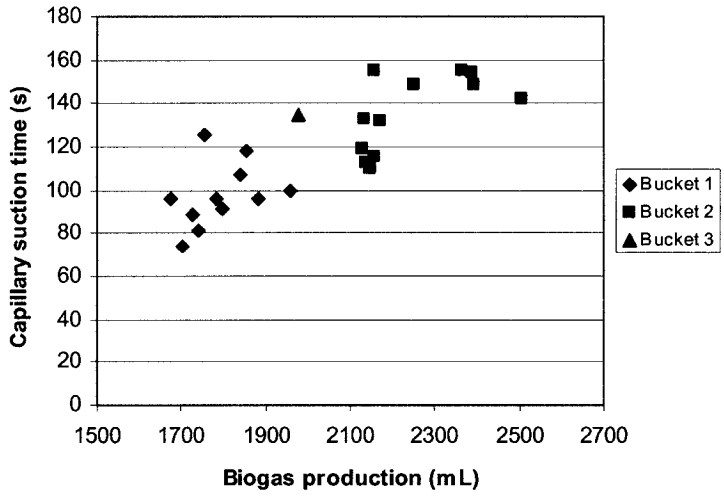


Figure 4.26: Capillary suction time versus biogas production in BMP assay #1.

4.5.2 Second Biochemical Methane Potential Assay

4.5.2.1 Design of the Experiment

The positive results obtained in BMP assay #1 led to the search for variables that could further enhance the anaerobic degradability of SBR sludge. One idea was to contact the sludge with a small dose of NaOH. Table 4.8 presents the dose of NaOH used in four studies reviewed in chapter 2. Because this chemical would be expensive to use in industry and its use requires the subsequent neutralization of sludge, it was desired to use a small dose that would weaken cell membranes but its impact on anaerobic biodegradability would not completely mask the effect obtained from microwave irradiation. The NaOH dose used in this study was 2 g/L which is in the low range of the values presented in Table 4.8. Note that a dose of 20 g/L was employed to measure the maximum sCOD/tCOD ratio of the sludge in section 4.3.

Table 4.8: NaOH doses used in sludge pretreatment in the literature.

Authors	Dose of NaOH Used
Lin *et al.* (1997)	0.8 and 1.6 g/L
Tanaka *et al.* (1997)	~3 g/L
Penaud *et al.* (1999)	4-5 g/L
Kim *et al.* (2003)	7 g/L

One other variable that was investigated in BMP assay #2 is the multiple microwave irradiation of sludge. The third and final variable was to use the "Keep Warm" feature of the microwave oven at the end of a single irradiation cycle so that sludge may be kept at 85°C an extra ten minutes. Table 4.9 presents the pretreatment conditions of the 20 samples. The number of microwave irradiation cycles was varied between 0 and 3 both for untreated and NaOH-treated sludge. The "Keep Warm" feature was investigated after a single irradiation cycle on untreated and NaOH-treated sludge. All microwave irradiated samples were heated to 85°C.

One bucket of sludge was contacted with 2 g/L NaOH overnight and neutralized with 6N. HCl before sample preparation. Sludge irradiated multiple times was cooled to room temperature by storing in the freezer for 40 minutes between irradiation cycles. Distilled water was used to replace evaporated water after sludge had reached room temperature after each microwave irradiation cycle. The samples were prepared in a random order. In this case, however, duplicates were prepared at the same time and mixed together before being transferred to batch and test bottles. Volumes of 160 mL of inoculum (acclimated to 50% pretreated sludge irradiated to 85°C) were added to the batch bottles.

Table 4.9: Experimental conditions of the samples in BMP assay #2.

Sample	Number of MW Treatment Cycles	Exposed to NaOH?	Keep Warm?
1	1	no	no
2	2	no	no
3	3	no	no
4	0	yes	no
5	1	yes	no
6	2	yes	no
7	3	yes	no
8	1	no	yes
9	1	yes	yes
C	0	no	no

4.5.2.2 Results

At the beginning of the assay, the pH of all samples was between 6.0 and 7.0 while the inoculum had a slightly higher pH at 7.2. Because the pH was lower than 8.3 in all cases, samples were devoid of phenolphthalein alkalinity. Therefore, all alkalinity was in the bicarbonate form. The alkalinity of all samples ranged between 400 and 800 mg/L while the inoculum samples contained 2,750 mg/L (as $CaCO_3$). To buffer against sudden rises in VFA during anaerobic digestion, approximately 1 g/L each of $NaHCO_3$ and $KHCO_3$ were added to all bottles. This acted to raise the alkalinity of the samples by 2,190 mg/L as $CaCO_3$ (calculated). The NH_3-N concentration of all samples ranged between 11-72 mg/L while the inoculum samples contained approximately 595 mg/L. The volatile fatty acids content of the samples was less than 110 mg/L acetic acid and 115 mg/L propionic acid. The inoculum samples were devoid of volatile fatty acids.

Analysis of the tCOD and VS showed that the sludge in the two buckets was properly homogenized during the preparation of samples in BMP assay #2. Plots showing the effect of the order in which samples were prepared on the initial tCOD and VS of the samples were made and are shown in Figures 4.27 and 4.28. The sludge from the NaOH-contact bucket contained 16,617 ± 564 mg/L of tCOD and 1.038 ± 0.056% VS while the sludge from the other bucket contained 16,304 ± 624 mg/L tCOD and 1.032 ± 0.079% VS. In subsequent calculations, tCOD of 16,461 mg/L and VS of 1.035% were employed.

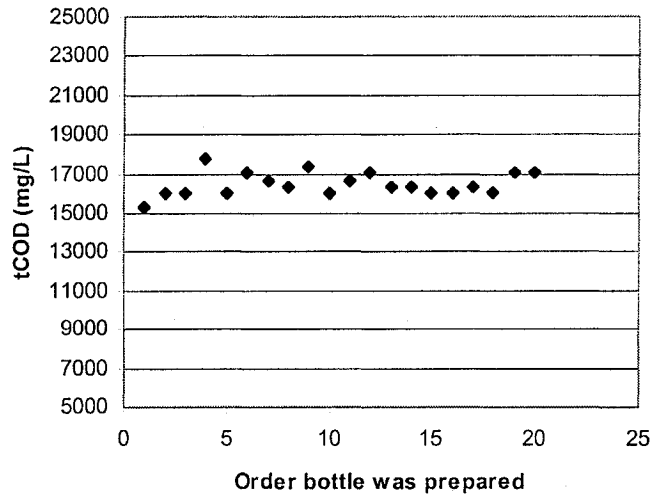


Figure 4.27: Initial tCOD of samples in BMP assay #2.

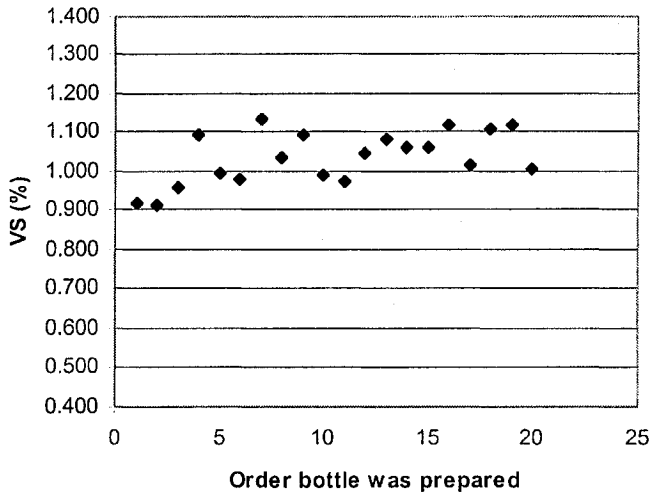


Figure 4.28: Initial VS of samples in BMP assay #2.

Analysis of the solids composition data for the untreated and NaOH-treated sludge showed that these two groups of samples had the same amount of volatile solids but different quantities of fixed solids. Figure 4.29 illustrates this observation. The error bars represent the standard deviation of the 10 measurements made on untreated and NaOH-treated sludge. The difference in fixed solids between the untreated and NaOH-contacted samples is 0.33 ± 0.06% which is equivalent to 3.3 g/L. A total of 2 g/L NaOH and 1.8 g/L HCl were added to the NaOH-treated sludge during pretreatment. Therefore, 0.5 g/L is not accounted for and must have been consumed in the solubilization of the COD.

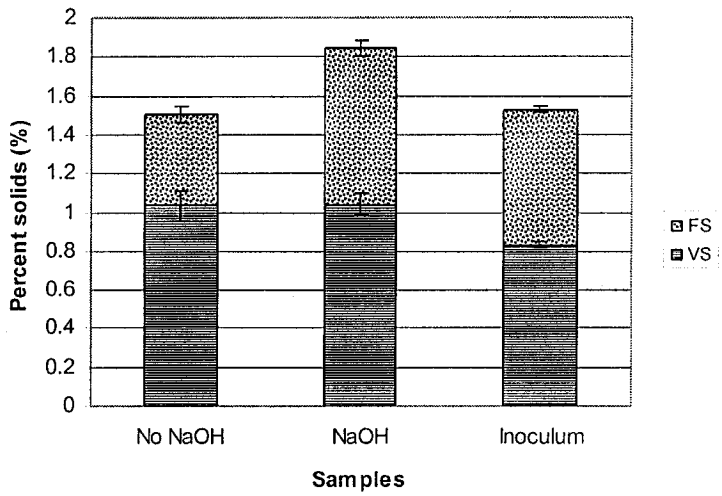


Figure 4.29: Comparison of the solids composition of the sludge and inoculum samples.

The effects of microwave irradiation cycles and addition of NaOH on the solubilization of COD were monitored. The sCOD/tCOD ratio of untreated and NaOH-treated sludge irradiated 0, 1, 2 and 3 times is shown in Figure 4.30. The sCOD/tCOD ratio of the control sample was 2.06 ± 0.03% which is higher than the 1.72 ± 0.04% obtained in section 4.3, 1.35 ± 0.09% obtained in section 4.4 and 1.23 ± 0.13 mg/L obtained in BMP assay #1. These values illustrate the variability of the Rockland SBR sludge. Meager improvements in COD solubilization were observed when irradiating untreated sludge twice and thrice compared to sludge that had been irradiated a single time. Addition of 2 g/L of NaOH to sludge brought the sCOD/tCOD ratio to 16.3 ± 0.3%. The effects of NaOH and microwave irradiation were not additive but microwave irradiation did yield higher sCOD/tCOD ratios than those obtained by NaOH treatment alone. Multiple irradiation cycles had a positive effect on the solubilization of NaOH-treated sludge, as evidenced by the increase of the sCOD/tCOD ratio from 18.8 ± 0.3% when sludge was irradiated once to 21.7 ± 0% when sludge was irradiated thrice. Keeping the sludge at 85°C for an additional 10 minutes after a single irradiation cycle did not yield appreciable improvements in the solubilization of COD.

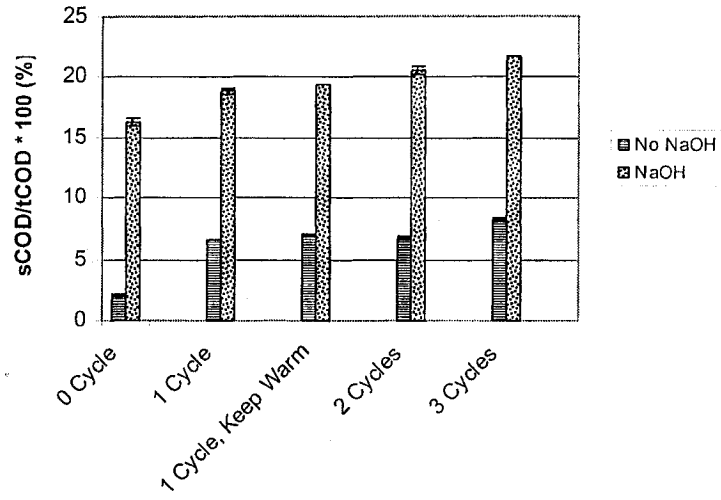


Figure 4.30: sCOD/tCOD ratio of sludge samples in BMP assay #2.

Biogas production was measured once daily. On the morning of the third day bottle 2B was found shattered in the shaker. This was most likely due to a weakness in the bottle. Figure 4.31 shows the cumulative biogas production recorded for untreated samples irradiated 0, 1, 2 and 3 times to a temperature of 85°C. The cumulative biogas production curves from the control samples are illustrated using smoothed lines to enhance clarity. No lag phase and no signs of biomass inhibition were observed. The untreated sludge samples irradiated 1, 2 and 3 times produced similar quantities of biogas. The improvement in biogas production ranged between 8.7 and 13.9%. It can be observed in Figure 4.31 that all samples were characterized by an initial biogas production rate of approximately 190 mL per day. The difference between the best pretreatment conditions and the control was the duration of this maximum production rate. Hence, the pretreatment conditions investigated in this BMP assay do not affect the rate of degradation but enhance the degradability of the substrate.

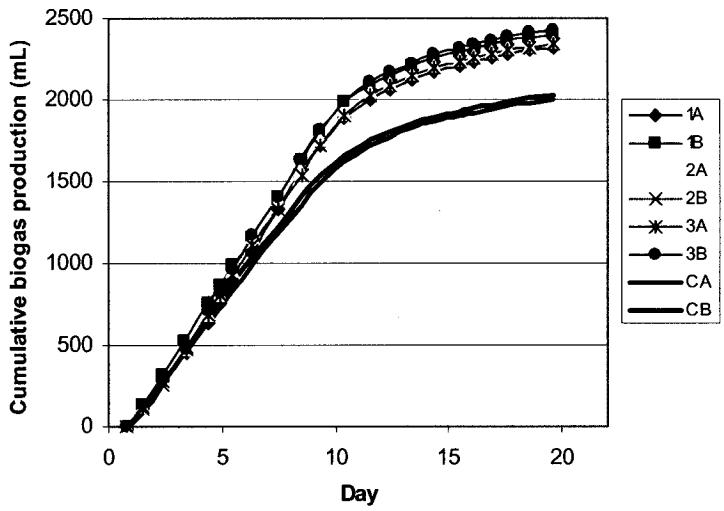


Figure 4.31: Biogas production of untreated samples irradiated 0, 1, 2 and 3 times.

Figure 4.32 shows the cumulative biogas production recorded for NaOH-treated samples irradiated 0, 1, 2 and 3 times to a temperature of 85°C. No lag phase and no signs of biomass inhibition were observed even though the biomass had not been acclimated to NaOH-treated sludge. The NaOH-treated sludge samples irradiated 1, 2 and 3 times produced similar quantities of biogas. The improvement in biogas production ranged between 10.3 and 16.8%.

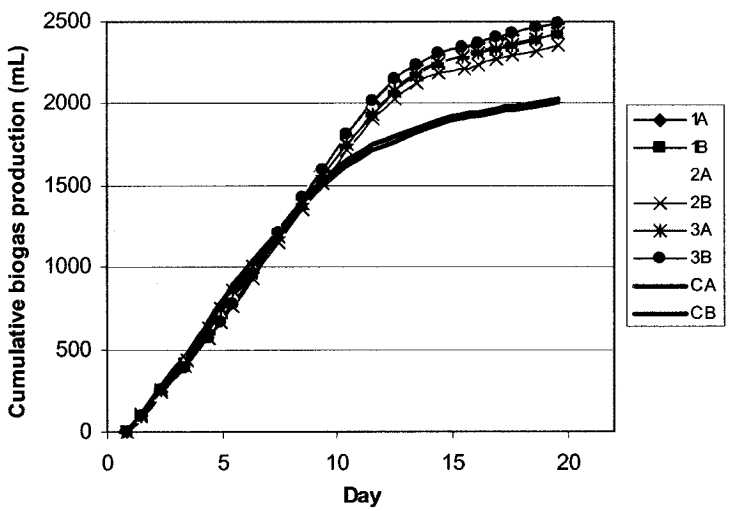


Figure 4.32: Biogas production of NaOH-treated samples irradiated 0, 1, 2 and 3 times.

Figure 4.33 shows the cumulative biogas production recorded for untreated and NaOH-treated samples irradiated once to a temperature of 85°C and maintained at that temperature for an extra 10 minutes. The untreated and NaOH-treated sludge samples presented in Figure

4.33 produced similar quantities of biogas. It is interesting to note that the NaOH-treated samples that were not heated in the microwave oven produced comparable amounts of biogas than the samples that were irradiated. As observed in Figures 4.31, 4.32 and 4.33, all pretreated samples generated similar amounts of biogas despite the different extent of COD solubilization obtained by the different pretreatment settings. This would indicate that all the pretreatment settings render the sludge more anaerobically digestible but up to a maximum extent. In light of these findings, the simplest pretreatment condition should be employed to enhance the anaerobic digestibility of Rockland SBR sludge and this would entail a single microwave irradiation cycle to 85°C on sludge not previously contacted with NaOH.

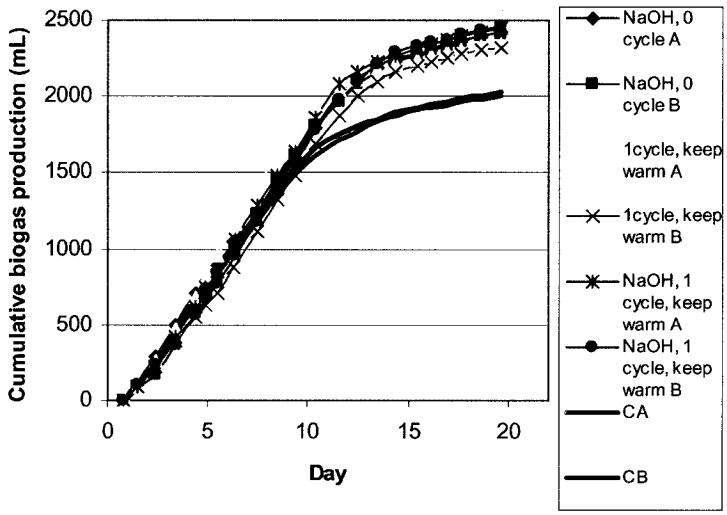


Figure 4.33: Biogas production of untreated and NaOH-treated samples not irradiated and irradiated once and kept at 85°C for 10 extra minutes.

The biogas production curves from the bottles containing 350 mL of inoculum are shown in Figure 4.34. Because the bottles containing pretreated and control sludge contained 160 mL of inoculum, the anaerobic seed contributed only approximately 111 mL of biogas in the bottles containing pretreated and control samples.

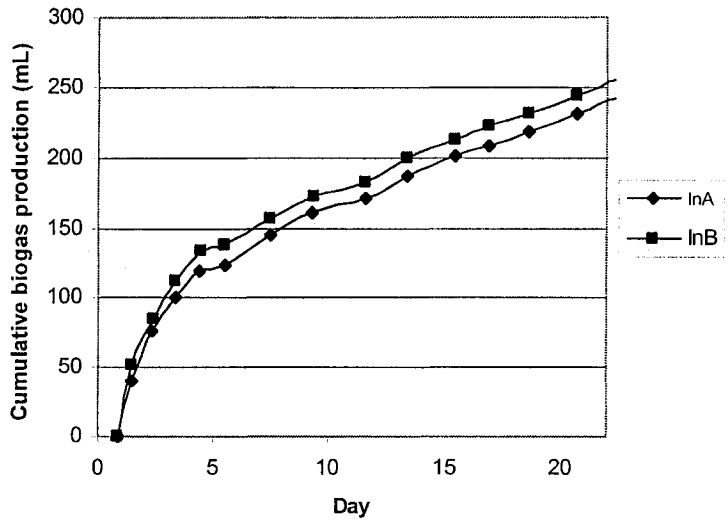


Figure 4.34: Biogas production from the bottles containing inoculum in BMP assay #2.

The composition of the biogas was measured twice during the assay. For each bottle, the two readings were very similar. In all cases, biogas was composed of 60-67% methane and 33-40% carbon dioxide.

The bottles were all uncapped on the same day, once daily biogas production from all bottles represented less than 1% of the total biogas production. The pH of samples was recorded immediately after the uncapping of a bottle. It was found to be approximately 7.5 for all pretreated and control samples and 7.7 for the inoculum samples. These values are appropriate for anaerobic digestion. The alkalinity of all samples ranged between 3,400 and 3,800 mg/L as $CaCO_3$. The alkalinity was all in the bicarbonate form. This alkalinity content was suitable for proper buffering against sudden rises in VFA. Gas chromatography of all centrifuged sludge samples at the end of the BMP assay detected no VFA.

The sCOD of all samples after anaerobic digestion ranged between 290 and 470 mg/L. This illustrates that a portion of the soluble organic matter was recalcitrant to anaerobic degradation. In addition, the sCOD concentration of the inoculum samples was higher than it was at the beginning of the BMP assay. This simply demonstrates that some of the COD that was solubilized by the anaerobic microorganisms was recalcitrant to degradation.

The dissolved ammonia (NH_4^+ and NH_3) concentration in all samples at the end of the BMP assay was in the 400-510 mg/L range. These ammonia concentrations pose no adverse effects on anaerobic biological activity. Figure 4.35 shows the relationship between biogas production and ammonia concentration in the sludge samples. The ammonia content of the inoculum samples did not change significantly during the BMP assay, implying that most nitrogen-rich organic matter had been digested in the acclimation tank.

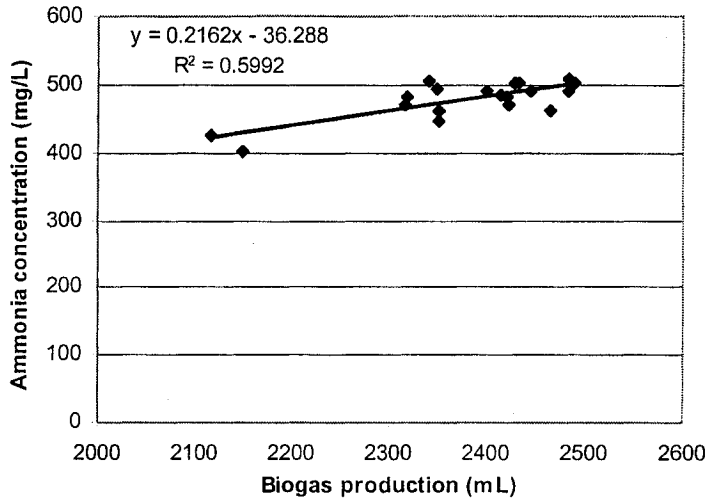


Figure 4.35: Relationship between ammonia concentration and biogas production in BMP assay #2.

The tCOD and VS removal during the BMP assay were calculated by taking into account the remaining tCOD and VS contributed by the inoculum in the bottles containing control and pretreated samples. This was accomplished according to Equation 4.10. The yield of methane from each sludge sample was calculated according to Equation 4.11. Table 4.10 shows the tCOD removal and yield of methane calculated for each control and pretreated sample. The tCOD removals obtained are consistent with the total biogas production measured, as evidenced by the rather consistent methane yield shown in Table 4.10. The average methane yield was 0.33 L CH_4 per g COD removed at STP. This is higher than the average yield value obtained in BMP assay #1 (0.28 L CH_4 / g COD removed). This again exemplifies that the Rockland SBR sludge has widely varying characteristics. The average tCOD removal in the control samples was 45%. The tCOD removal in all pretreated samples varied between 50 and 54 %, which represents a 5 to 9 percentage point improvement in tCOD removal.

Table 4.10: tCOD removal and methane yield obtained in BMP assay #2.

Sample	Pretreatment Conditions			tCOD Removal (%)	tCOD Removal (%)	L CH_4 Produced Per g COD Removed (L/g)
	NaOH?	# of Cycles	Keep Warm?			
1A	no	1	no	49	50	0.33
1B				52		0.33
2A	no	2	no	53	53	0.31
2B						
3A	no	3	no	49	51	0.34
3B				54		0.33
4A	yes	0	no	54	54	0.35
4B				55		0.35
5A	yes	1	no	50	51	0.35
5B				52		0.33
6A	yes	2	no	55	53	0.32
6B				50		0.35
7A	yes	3	no	52	53	0.34
7B				54		0.32
8A	no	1	yes	52	51	0.34
8B				50		0.34
9A	yes	1	yes	52	53	0.34
9B				53		0.32
CA	no	0	no	48	45	0.31
CB				42		0.35

The VS removal in the samples was calculated according to Equation 4.10 (replace tCOD by VS). The average VS removal in the control samples was 52%. The average VS removal of the pretreated samples was 63%, which represents an 11-percentage point improvement. Because similar biogas production, VS removal and COD removal values were obtained for all pretreated samples, the simplest pretreatment option is the most desirable. This would consist of a single microwave irradiation cycle of SBR sludge to 85°C.

Table 4.11: VS removal in BMP assay #2.

Sample	Pretreatment Conditions			VS Removal (%)	VS Removal (%)
	NaOH?	# of Cycles	Keep Warm?		
1A	no	1	no	62	60
1B				58	
2A	no	2	no	63	63
2B					
3A	no	3	no	62	63
3B				63	
4A	yes	0	no	65	64
4B				63	
5A	yes	1	no	68	67
5B				66	
6A	yes	2	no	68	64
6B				61	
7A	yes	3	no	64	61
7B				57	
8A	no	1	yes	61	61
8B				60	
9A	yes	1	yes	64	62
9B				61	
CA	no	0	no	50	52
CB				54	

The capillary suction time of the sludge samples was measured in duplicate at the end of the assay. Figure 4.36 presents the relationship between CST and biogas production for untreated and NaOH-pretreated samples. The control samples are represented by red markers. Because the best pretreatment conditions yielded the most biogas, this plot permits the indirect analysis of the effect of microwave irradiation on the CST. Overall, the plot displays the worse dewaterability conditions at the pretreatment settings that produced the most biogas. The results obtained in this assay are similar to the ones obtained in BMP assay #1.

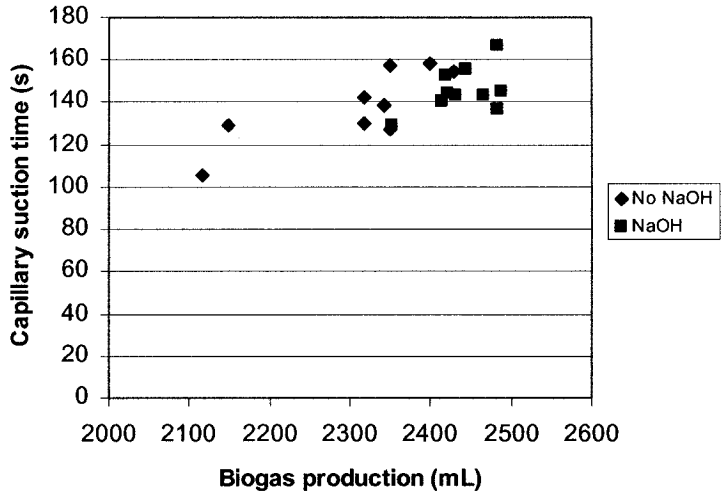


Figure 4.36: Capillary suction time versus biogas production in BMP assay #2.

Three generous volunteers were recruited at the end of BMP assay #2 to participate in a digested sludge odor comparison test. Digested sludge samples CA, 1A and 5A (refer to Table 4.11) were placed in stainless steel centrifuge tubes and presented to the volunteers. The volunteers were asked to rank the three samples from least to most offensive. They were not informed of the pretreatment conditions of each sample and were not shown the labels. Table 4.12 summarizes the results. All three volunteers agreed that the control sample was the least offensive. Two of the three volunteers ranked the sludge sample that had been contacted with NaOH the most offensive, although all three volunteers hesitated when deciding the ranking of samples 1A and 5A. While not completely scientific, this experiment indicates that odor control may be a future issue of concern.

Table 4.12: Results of the digested sludge odor comparison test.

Volunteer	CA	1A	5A
A	Least offensive	Middle	Most offensive
B	Least offensive	Most offensive	Middle
C	Least offensive	Middle	Most offensive

4.6 Effects of Microwave Irradiation on Viscosity and Surface Tension

As discussed in section 2.3.2 and 2.3.4, there are reasons to believe that irradiating sludge samples to a temperature of 85°C could alter the rheological characteristics and surface

tension. To test the effects of microwave irradiation on the viscosity of sludge, 3% TS sludge samples were irradiated to 45, 65 and 85°C. Before measurements were made, the accuracy of the Brookfield viscometer was tested using a fluid of known viscosity. Glycerin was employed for this purpose. The results of the test are shown in Table 4.13. Because glycerin is a Newtonian fluid, the viscosity did not vary when the rotor speed was set at 6, 12 and 30 revolutions per minute (rpm). The instrument was found to be properly calibrated. The factors used to convert between the instrument reading and the viscosity depend on the spindle used and the rotor speed. They were obtained from the user manual of the viscometer.

Table 4.13: Check on the calibration of the Brookfield viscometer using glycerin (viscosity = 12,980 cp).

Spindle	Speed	Factor	Reading	Viscosity (cp)
1	6	1000	13	13000
	12	500	26	13000
	30	200	63.5	12700
				12900 ± 170 cp

The viscosity values obtained at shear rates varying between 1 and 13 s^{-1} are shown in Figure 4.37. For each of the four pretreatment schemes, single 850-mL samples were prepared and a measurement was made on two portions. The data in Figure 4.37 represent the average of the two readings. As expected, Rockland SBR sludge is a pseudo-plastic fluid because higher shear rates are associated with lower viscosity values. All samples except the one irradiated to 65°C behaved very similarly at all shear rates investigated. The two sub-samples obtained from the 850-mL of sludge irradiated to 65°C behaved very differently, as can be seen in Table B.62 of Appendix B. The second sub-sample yielded viscosity measurements comparable to the ones obtained from the control, 45°C and 85°C samples. The sub-sample that yielded the contrasting results was probably less concentrated than the other ones and this would indicate that the container containing the sludge irradiated to 65°C was not properly homogenized prior to sampling.

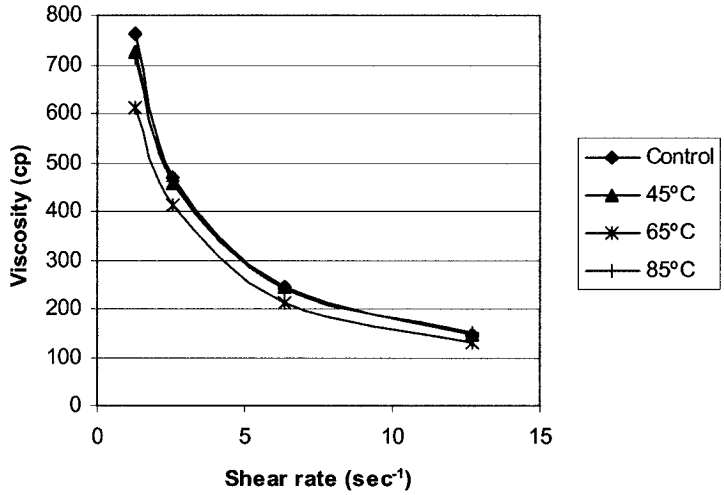


Figure 4.37: Plot of viscosity versus shear rate for control and irradiated samples.

Because pseudo-plastic fluids can be modeled according to Equation 2.2, a plot of the log of the viscosity versus the log of the shear rate was made for each sample to obtain the consistency index and the flow index. The results of these regression analyses are shown in Table 4.14. The consistency index, which is the viscosity of the fluid when the shearing rate is 1 s^{-1}, ranges between 743 and 910 for the four groups of samples. The flow index of the four groups of samples is approximately 0.3 which indicates that the sludge strongly differs from Newtonian behavior as it is much smaller than 1. Overall, these results indicate that microwave irradiation of sludge to temperatures lesser than 85°C does not affect its dynamic viscosity. A more powerful pretreatment method is likely to be required to alter the shape and size of particles enough to change the viscosity of sludge.

Table 4.14: Consistency index and flow index of the control and pretreated sludge samples.

Sample	Consistency index $(cp/(s^{-1})^{n-1})$	Flow index	R^2
Control	910	0.29	0.9999
45°C	866	0.31	0.9995
65°C	743	0.32	0.9979
85°C	851	0.32	0.9994

The surface tension of control and pretreated sludge (85°C) was compared. Triplicate measurements were made on each sample. The control sample exerted a surface tension of 55.10 ± 0.69 dynes/cm while the pretreated sample exerted a surface tension of 55.02 ± 0.46 dynes/cm. This clearly shows that the two samples exerted the same surface tension. The surface tension of the sludge is 23.5% lower than for distilled water. That the control and pretreated samples exerted the same surface tension suggests that the sludge had small quantities of filamentous organisms (or none at all). It would be interesting to repeat this test on WAS plagued with filamentous bacteria.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Microwave irradiation was found to have a positive effect on the anaerobic biodegradability of Rockland SBR sludge. Specifically, the main conclusions of this research project are:

- Microwave irradiation of the sludge to 85°C resulted in the reorganization of the particle size distribution. A fraction of the particles larger than 100 μm were broken into smaller particles.
- The maximum achievable sCOD/tCOD ratio of the sludge was found to be approximately 57% using a severe NaOH pretreatment.
- Microwave intensity and sludge concentration were found not to have an effect on the solubilization of COD. The temperature of microwave treatment had a positive effect on the solubilization of COD. The maximum sCOD/tCOD ratio achieved using microwave irradiation was approximately 7%.
- Partial treatment of SBR sludge did not enhance the anaerobic degradability of the sludge.
- Treatment of 100% of the sludge to 85°C yielded 16.2% and 10.6% improvements in biogas production (in BMP assays #1 and #2, respectively).
- Multiple microwave irradiation cycles did not improve the anaerobic degradability of the sludge as compared to a single irradiation cycle.
- Maintaining the sludge at a temperature of 85°C for 10 minutes after a single irradiation cycle did not improve the anaerobic degradability of the sludge as compared to a single irradiation cycle.
- Contacting sludge with 2 g/L of NaOH yielded higher sCOD/tCOD ratios but did not produce more biogas than the sludge irradiated with microwave.
- Improvements in biogas production and tCOD and VS removal were accompanied by a worsening of the dewaterability of the sludge as determined by the CST test.
- Pretreated digested sludge was found to be more offensive than control digested sludge in an odor comparison test.
- Microwave irradiation of Rockland SBR sludge to 85°C did not change the dynamic viscosity and surface tension of the sludge.

5.2 Recommendations for Further Research

More studies are required before microwave pretreatment can be used by municipalities to enhance the anaerobic digestion of sequencing batch reactor sludge. Suggestions include:

- Employ semi-continuous flow reactors to quantify the effects of different HRTs on the anaerobic digestion of SBR sludge.
- Obtain sludge plagued with filamentous bacteria and compare the surface tension of the sludge before and after microwave irradiation to 85°C. Control and pretreated sludge could be digested anaerobically in semi-continuous flow reactors to observe whether the foaming potential can be reduced.
- Use a more sophisticated microwave oven that can pretreat sludge at greater pressures and temperatures, perform BMP assays and compare the viscosity of control and pretreated sludge.
- Work with a microwave engineer to design a continuous microwave pretreatment process, evaluate the energy requirements and compare with other pretreatment technologies.

REFERENCES

Abraham K. and Kepp U., "Commissioning and Re-Design of a Class A Thermal Hydrolysis Facility for Pre-Treatment of Primary and Secondary Sludge Prior to Anaerobic Digestion," *Proc. 76th Water Environment Federation Technical Exhibition and Conference*, Water Environment Federation, 2003, unknown page numbers.

APHA, AWWA and WEF, *Standard Methods for the Examination of Water and Wastewater*, Baltimore: United Book Press, 1995.

Baier U. and Schmidheiny P., "Enhanced Anaerobic Degradation of Mechanically Disintegrated Sludge," *Water Science and Technology*, vol. 36, no. 11, 1997, pp. 137-143.

Barber W.P., "*The Effects of Ultrasound on Anaerobic Digestion of Sludge*," tech. report, Dirk European Holding, date unknown.

Barber, W.P., "*Anaerobic Digester Foaming: Causes and Solutions*," Water 21, February, 2005, pp. 45-49.

Barnard, J.L., Coleman P. and Weston P., "Thermal Hydrolysis of a Sludge Prior to Anaerobic Digestion," *Proc. 16th Residuals and Biosolids Management Conference*, Water Environment Federation, 2002, unknown page numbers.

Berthouex, P.M. and Brown L.C., *Statistics for Environmental Engineers*, Boca-Raton: Lewis Publishers, 2002.

Brown J.P., Clark P. and Hogan F., "*Ultrasonic Sludge Treatment for Enhanced Anaerobic Digestion at Orange County Sanitation District*," tech. report, 2003.

Chiu Y.C. *et al.*, "Alkaline and Ultrasonic Pretreatment of Sludge before Anaerobic Digestion," *Water Science and Technology*, vol. 36, no. 11, 1997, pp. 155-162.

Choi H.B., Hwang K.Y. and Shin E.B., "Effects on Anaerobic Digestion of Sewage Sludge Pretreatment," *Water Science and Technology*, vol. 35, no. 10, 1997, pp. 207-211.

CPS Instruments, "*Comparison of Particle Sizing Methods*," [Online] unknown date; www.cpsinstruments.com/TechLibrary/CompareMethods.PDF (Accessed: 10 May 2005).

Dohanyos M., Zabranska J. and Jenicek P., "Innovative Technology for the Improvement of the Anaerobic Methane Fermentation," *Water Science and Technology*, vol. 36, no. 6-7, 1997, pp. 333-340.

Dereix M., Kennedy K.J. and Parker, W.J., "*Steam-Explosion Pretreatment of Municipal Sludge to Enhance Anaerobic Digestion*," master's thesis, Dept. Civil and Environmental Engineering, Carleton University, 2005.

Droste, R.L., "*Theory and Practice of Water and Wastewater Treatment*," New York: John Wiley & Sons, 1997.

Eco Equipment Systems Inc., *Process Systems and Equipment for Water and Wastewater Treatment: Rockland Sequential Batch Reactor Revision 1-A*, 1997.

EOLI, "*EOLI Project: Efficient Operation of Urban Wastewater Treatment Plants*," [Online] unknown date; www.auto.ucl.ac.be/EOLI (Accessed: 6 May 2005).

Epstein, E., *Land Application of Sewage Sludge and Biosolids*, Baca-Raton: Lewis Publishers, 2003.

Eskicioglu C., Kennedy K.J. and Droste R.L., "Effect of Microwave Dose on Biogas Production from Batch Anaerobic Digesters Treating WAS," *Proc. 20th Eastern Regional Conference of the Canadian Association on Water Quality*, Carleton University, 2004, pp.14.

Gavala, H.N. *et al.*, "Mesophilic and Thermophilic Anaerobic Digestion of Primary and Secondary Sludge. Effect of Pre-Treatment at Elevated Temperature," *Water Research*, vol. 37, no. 19, 2003, pp. 4561-4572.

Hasegawa S. *et al.*, "Solubilization of Organic Sludge by Thermophilic Aerobic Bacteria as a Pretreatment for Anaerobic Digestion," *Water Science and Technology*, vol. 41, no. 3, 2000, pp. 163-169.

Haug R.T. and Stuckey D.C., "Effect of Thermal Pretreatment on Digestibility and Dewaterability of Organic Sludges," *Journal of Water Pollution Control Federation*, vol. 40, 1978, pp. 73-85.

Hong S.M., "*Enhancement of Pathogen Destruction and Anaerobic Digestibility Using Microwaves*," Ph.D. thesis, Department of Civil and Environmental Engineering, University of Wisconsin-Madison, 2002.

Huskey P., "*336_Lectures*," [Online] Date unknown; http://andromeda.rutgers.edu/~huskey/336_lec. (Accessed: 13 May 2005).

Hwang K.Y., Shin E.B. and Choi H.B., "A Mechanical Pretreatment of Waste Activated Sludge for Improvement of Anaerobic Digestion System," *Water Science and Technology*, vol. 36, no. 12, 1997, pp. 111-116.

Imperial Petroleum Recovery Corporation (IPRC), "Emulsion Separation Rate Enhancement with High-frequency Energy," [Online] Date unknown; http://www.iprc.com/our_technology/our_technology_index.htm (Accessed: 25 May 2005).

Kim J. *et al.*, "Effects of Various Pretreatments for Enhanced Anaerobic Digestion with Waste Activated Sludge," *Journal of Bioscience and Bioengineering*, vol. 95, no. 3, 2003, pp. 271-275.

Kingston H.M. and Jassie L.B., *Introduction to Microwave Sample Preparation: Theory and Practice*, Washington: American Chemical Society, 1988.

Kopp J. *et al.*, "Anaerobic Digestion and Dewatering Characteristics of Mechanically Disintegrated Excess Sludge," *Water Science and Technology*, vol. 36, no. 11, 1997, pp. 129-136.

Kruss, "*Measuring Principles of KRUSS Tensiometers*," [Online] date unknown; http://www.kruss.info/ (Accessed: 19 May 2005).

Lin J.G., Chang C.N. and Chang S.C., "Enhancement of Anaerobic Digestion of Waste Activated Sludge by Alkaline Solubilization," *Bioresource Technology*, vol. 62, 1997, pp. 85-90.

Metcalf and Eddy, *Wastewater Engineering: Treatment and Reuse*, New York: McGraw-Hill, 2003.

Ministry of Environment and Ministry of Agriculture, Food and Rural Affairs, "*Guidelines for the Utilization of Biosolids and Other Wastes on Agricultural Land*," [Online] March 1996; www.ene.gov.on.ca/envision/gp/3425e.pdf (Accessed: 6 October 2004).

Muller J. *et al.*, "Disintegration of Sewage Sludges and Influence on Anaerobic Digestion," *Water Science and Technology*, vol. 38, no. 8-9, 1998, pp. 425-433.

Muller J., Winter A. and Strunkmann G., "Investigation and Assessment of Sludge Pre-Treatment Processes," *Water Science and Technology*, vol. 49, no. 10, 2004, pp. 97-104.

Nah I.W. *et al.*, "Mechanical Pretreatment of Waste Activated Sludge for Anaerobic Digestion Process," *Water Research*, vol. 34, no. 8, 2000, pp. 2362-2368.

National Materials Advisory Board Commission on Engineering and Technical Systems, *Microwave Processing of Materials*, Washington: National Academy Press, 1994.

Pagilla K.R., Sood A. and Kim H., "*Gordonia* (nocardia) *amarae* Foaming due to Biosurfactant Production," *Water Science and Technology*, vol. 46, no. 1-2, pp. 519-524.

Palmowski L.M. and Muller J.A., "Anaerobic Degradation of Organic Materials – Significance of the Substrate Surface Area," *Water Science and Technology*, vol. 47, no. 12, 2003, pp. 231-238.

Paradigm Environmental Technologies Inc., "*Microsludge*," [Online] 2004 ; http://www.microsludge.com/microsludge/in_detail.php (Accessed 5 May 2005).

Park B. *et al.*, "Use of Microwave Pretreatment for Enhanced Anaerobiosis of Secondary Sludge," *Water Science and Technology*, vol. 50, no. 9, pp. 17-23.

Parker W.J. and Béland M., "Alternatives for Enhancement of Methane Production in Anaerobic Digestion of Municipal Sludges," *Proc. 2nd Canadian Organic Residuals Recycling Conference*, unknown press, 2003, pp. 1-17.

Parkin G.F. and Owen W.F., "*Fundamentals of Anaerobic Digestion of Wastewater Sludges*," Journal of Environmental Engineering, vol. 112, no. 5, 1986, pp. 867-920.

Penaud V., Delgenès J.P. and Moletta R., "Thermo-Chemical Pretreatment of a Microbial Biomass: Influence of Sodium Hydroxide Addition on Solubilization and Anaerobic Biodegradibility," *Enzyme and Microbial Technology*, vol. 25, 1999, pp. 258-263.

Sanin F.D., "Effect of Solution Physical Chemistry on the Rheological Properties of Activated Sludge," *Water SA*, vol. 28, no. 2, 2002, pp. 207-211.

Shaw D.J., *Introduction to Colloid and Surface Chemistry*, Boston: Butterworth-Heinemann, 1992.

Shin K-S and Kang H., "Electron Beam Pretreatment of Sewage Sludge before Anaerobic Digestion," *Applied Biochemistry and Biotechnology*, vol. 109, 2003, pp. 227-239.

Smith G. and Goransson J., "Generation of an Effective Internal Carbon Source for Denitrification through Thermal Hydrolysis of Pre-Precipitated Sludge," *Water Science and Technology*, vol. 25, no. 4-5, 1992, pp. 211-218.

Speece, R.E., *Anaerobic Biotechnology for Industrial Wastewaters*, Nashville: Archae Press, 1996.

Stephenson R.J., Laliberté S. and Elson P., "Use of a High-Pressure Homogenizer to Pre-Treat Municipal Biosolids: Introducing the MicroSludge™ Process," *Proc. 10th World Congress on Anaerobic Digestion*, National Research Council, 2004, pp. 1010-1015.

Stuckey D.C. and McCarty P.L., "The Effect of Thermal Pretreatment on the Anaerobic Biodegradability and Toxicity of Waste Activated Sludge," *Water Research*, vol. 18, no. 11, 1984, pp. 1343-1353.

Tanaka S. *et al.*, "Effects of Thermochemical Pretreatment on the Anaerobic Digestion of Waste Activated Sludge," *Water Science and Technology*, vol. 35, no. 8, 1997, pp. 209-215.

Tanner, R.I., "*Engineering Rheology*," New York: Oxford University Press, 2000.

Tiehm A. *et al.*, "Ultrasonic Waste Activated Sludge Disintegration for Improving Anaerobic Stabilization," *Water Research*, vol. 35, no. 8, 2001, pp. 2003-2009.

Tiehm A., Nickel K. and Neis U., "The Use of Ultrasound to Accelerate the Anaerobic Digestion of Sewage Sludge," *Water Science and Technology*, vol. 36, no. 11, 1997, pp. 121-128.

Wang Q. *et al.*, "Studies on Anaerobic Digestion Mechanism: Influence of Pretreatment Temperature on Biodegradation of Waste Activated Sludge," *Environmental Technology*, vol. 18, 1997, pp. 999-1008.

Wang Q. *et al.*, "Upgrading of Anaerobic Digestion of Waste Activated Sludge by Ultrasonic Pretreatment," *Bioresource Technology*, vol. 68, 1999, pp. 309-313.

Weemaes M. *et al.*, "Anaerobic Digestion of Ozonized Biosolids," *Water Research*, vol. 34, no. 8, 2000, pp. 2330-2336.

Woodward S.E. and Wukasch R.F., "A Hydrolysis/Thickening/Filtration Process for the Treatment of Waste Activated Sludge," *Water Science and Technology*, vol. 30, no. 3, 1994, pp. 29-38.

Yeom I.T. *et al.*, "Effects of Ozone Treatment on the Biodegradability of Sludge from Municipal Wastewater Treatment Plants," *Water Science and Technology*, vol. 46, no. 4-5, 2002, pp. 421-425.

APPENDIX A:

COMPARISON OF PARTICLE SIZE ANALYSIS METHODS

Table A-1: Comparison of particle size analysis methods (CPS Instruments, date unknown).

Method	Advantages	Disadvantages	Size Range
LALLS	-wide size range -very fast -simple -non-destructive	-low resolving power -optical characteristics required -not accurate for non-spherical particles -not applicable to strongly absorbing particles	<0.1 μm to mm
PCS	-tiny sample needed -fast -simple -non-destructive	-low resolution	<4 μm
BSS	-can measure concentrated samples -simple -non-destructive	-low resolution -optical characteristics required -not applicable to strongly absorbing particles -shape of particles must be known	unknown
EC	-wide size range -fast -simple -applicable to non-spherical particles	-particles must be electrical insulators -samples must be suspended in a conductive fluid	0.5 - >300 μm
LC	-wide size range -fast -simple	-not accurate for non-spherical particles	0.5 - >2,000 μm
TFC	-wide size range -fast -fair resolution	-liquid suspensions of particles difficult or impossible to measure -not accurate for non-spherical particles	0.2 - >700 μm
MC	-wide size range -can assess particle shapes	-time consuming -small number of particles are measured	nm – mm
Sieving	-inexpensive -simple	-labor intensive -distribution depends on the duration of test and shape of particles -low resolution	> ~20 μm
GS	-relatively inexpensive -simple -good resolution	-slow analysis for small particles -large particles may not obey Stokes' law -not accurate for non-spherical particles	1 - >500 μm
DC	-relatively wide size range -fast -high resolution	-not accurate for non-spherical particles -optical and shape parameters required	<0.01 - >40 μm
CHDF	-fast	-only for aqueous emulsifier medium -poor resolution -not accurate for non-spherical particles	0.01 – 2 μm
SFFF	-not dependent on particle geometry for small particles	-complicated -frequent operational problems -slow	0.02 – 30 μm

APPENDIX B:

RAW DATA

This appendix presents all raw data obtained experimentally, except for the biogas production data because this would have required too many pages.

B.1 Microwave Calibration

B.1.1 850-mL Samples

Table B.1: Total solids test on centrifuged sludge.

Replicate	W (g)	X (g)	Y (g)	TS (%)
A	62.2092	94.3093	63.5144	4.07
B	71.7304	109.9530	73.4184	4.42
C	79.9479	124.8591	81.9658	4.49
				4.3 ± 0.2%

Density of the sludge = 1,009.3 g/L

Temperature of distilled water = 22°C

Density of distilled water at 22°C = 997.77 g/L

Table B.2: Microwave calibration for 850-mL samples in 2-L polypropylene containers.

Mass of Sludge (g)	Volume of Conc. Sludge (mL)	Mass of Distilled Water (g)	Solids Concentration (%)	Irradiation Time (s)	Temperature Reached (°C)
857.9	850.0	0.0	4.3	60	41.0
700.5	694.0	155.6	3.5	180	61.0
350.0	346.8	502.1	1.8	240	75.5
600.0	594.5	255.0	3.0	260	84.5
485.5	481.0	368.2	2.4	255	83.0
299.3	296.5	552.2	1.5	80	40.0
666.2	660.1	189.5	3.3	100	50.0
857.9	850.0	0.0	4.3	170	62.0
229.8	227.7	620.9	1.2	180	64.0
400.0	396.3	452.7	2.0	80	39.5
661.3	655.2	194.4	3.3	140	53.5
554.0	548.9	300.4	2.8	270	86.0
299.3	296.5	552.2	1.5	120	53
485.5	481.0	368.2	2.4	150	57
671.7	665.5	184.1	3.4	45	33.5

B.1.2 400-mL Samples

Table B.3: Microwave calibration for 400-mL samples in 1-L polypropylene containers.

Microwave Intensity	Time in Microwave (s)	Temperature Reached (°C)
6.0	120	57.0
6.0	180	81.5
6.0	60	37.5
6.0	150	73.5
6.0	90	51.0
8.0	60	42.0
8.0	120	71.0
8.0	150	88.0
8.0	75	53.0
10.0	60	51.0
10.0	90	65.0
10.0	120	78.0
10.0	140	88.0
10.0	30	32.5

B.2 Particle Size Distribution Analysis

Table B.4: Results obtained from Accutest Laboratories Ltd.

Parameter	Units	Sample	
		Control	85°C
Total Suspended Solids	mg/L	28,100	28,100
100 μm < x	mg/L	9,640	6,270
60 μm < x < 100 μm	mg/L	740	1,270
30 μm < x < 100 μm	mg/L	1,820	2,070
11 μm < x < 30 μm	mg/L	12,000	14,400
1 μm < x < 11 μm	mg/L	3,900	4,070

B.3 Determination of the Maximum Soluble COD of Sludge

Table B.5: Total solids determination of sludge used in this experiment.

Replicate	W (g)	X (g)	Y (g)	TS (%)
A	65.1768	106.1982	65.5900	1.007
B	83.9280	116.6799	84.2820	1.081
				1.04 ± 0.05%

Table B.6: Sampling order and scheduling of this experiment.

Bottle Label	Time Bottle was Capped	Contact Time (hrs)	Time Bottle was Opened
12A	10:57 AM	6	4:57 PM
12B	11:00 AM	6	5:00 PM
6B	11:03 AM	2	1:03 PM
18A	11:06 AM	12	11:06 PM
15B	11:09 AM	9	8:09 PM
21B	11:12 AM	15	2:12 AM
24B	11:15 AM	24	11:15 AM
15A	11:22 AM	9	8:22 PM
24A	11:25 AM	24	11:25 AM
9A	11:28 AM	3	2:28 PM
6A	11:31 AM	2	1:31 PM
3A	11:34 AM	1	12:34 PM
9B	11:37 AM	3	2:37 PM
3B	11:39 AM	1	12:39 PM
18B	11:43 AM	12	11:43 PM
21A	11:45 AM	15	2:45 AM

Table B.7: COD calibration data for this experiment.

COD (mg/L)	Absorbance
0	0.005
0	0.005
0	0.000
200	0.140
300	0.215
300	0.215
300	0.215
400	0.290
500	0.370
500	0.360
500	0.360
700	0.500

$$Abs = 0.0007COD + 0.002$$

$$R^2 = 0.9994$$

Table B.8: Results of the sCOD determination.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
9B	0.355	504	10.0	5043	5045
3A	0.355	504	10.0	5043	5045
24B	0.485	690	10.0	6900	6900
24A	0.425	604	10.0	6043	6045
21A	0.470	669	10.0	6686	6685
CB	0.140	197	1.0	197	195
6B	0.370	526	10.0	5257	5255
21B	0.475	676	10.0	6757	6755
15A	0.440	626	10.0	6257	6255
12A	0.410	583	10.0	5829	5830
CA	0.145	204	1.0	204	205
12B	0.425	604	10.0	6043	6045
15B	0.420	597	10.0	5971	5970
3B	0.340	483	10.0	4829	4830
18A	0.460	654	10.0	6543	6545
9A	0.415	590	10.0	5900	5900
18B	0.450	640	10.0	6400	6400
6A	0.335	476	10.0	4757	4755

Table B.9: Results of the tCOD determination.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
24A	0.165	233	50.0	11643	11645
CA	0.155	219	50.0	10929	10930
CB	0.170	240	50.0	12000	12000
18A	0.160	226	50.0	11286	11285
18B	0.170	240	50.0	12000	12000
24B	0.170	240	50.0	12000	12000

B.4 Determination of Factors Affecting Solubilization of COD

Table B.10: Total solids determination of sludge used in this experiment.

Replicate	W (g)	X (g)	Y (g)	TS (%)
A	67.3936	113.5518	69.5040	4.572
B	62.2081	106.1236	64.2189	4.579
C	110.5149	150.8857	112.1796	4.124
				4.42 ± 0.26%

Table B.11: Experimental conditions and mass of 4.42% TS sludge and distilled water required to prepare each sample. A sludge density of 1,010 g/L was assumed.

Sample	Temperature (°C)	Sludge Concentration (% TS)	Microwave Intensity (%)	Mass of 4.42% TS Sludge (g)	Mass of Distilled Water (g)
1A	45	1.5	60	137.1	263.8
1B	45	1.5	60	137.1	263.8
2A	45	1.5	100	137.1	263.8
2B	45	1.5	100	137.1	263.8
3A	45	4.0	60	365.6	37.9
3B	45	4.0	60	365.6	37.9
4A	45	4.0	100	365.6	37.9
4B	45	4.0	100	365.6	37.9
5A	65	2.8	80	251.4	150.9
5B	65	2.8	80	251.4	150.9
6A	85	1.5	60	137.1	263.8
6B	85	1.5	60	137.1	263.8
7A	85	1.5	100	137.1	263.8
7B	85	1.5	100	137.1	263.8
8A	85	4.0	60	365.6	37.9
8B	85	4.0	60	365.6	37.9
9A	85	4.0	100	365.6	37.9
9B	85	4.0	100	365.6	37.9

Table B.12: COD calibration data for this experiment.

COD (mg/L)	Absorbance
0	0.000
0	0.000
0	0.000
200	0.140
300	0.210
300	0.210
300	0.215
400	0.260
500	0.360
500	0.345
500	0.370
700	0.480

$Abs = 0.0007COD + 0.0005$

$R^2 = 0.9962$

Table B.13: Results of the sCOD determination.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
2B	0.240	342	1.0	342	340
5B	0.380	542	2.0	1084	1085
8A	0.270	385	5.0	1925	1925
3B	0.305	435	2.0	870	870
6B	0.230	328	2.0	656	655
4B	0.340	485	2.0	970	970
9A	0.120	171	10.0	1707	1705
7B	0.190	271	2.5	677	675
7A	0.445	635	1.0	635	635
5A	0.415	592	2.0	1184	1185
4A	0.195	278	3.3	926	925
1B	0.190	271	1.3	338	340
CA	0.330	471	1.0	471	470
3A	0.300	428	2.0	856	855
CC	0.370	528	1.0	528	530
CB	0.290	414	1.0	414	415
2A	0.235	335	1.0	335	335
6A	0.260	371	2.0	741	740
9B	0.130	185	10.0	1850	1850
1A	0.230	328	1.0	328	330
8B	0.130	185	10.0	1850	1850

Table B.14: Results of the tCOD determination.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
6B	0.310	442	24.7	10913	10915
CA	0.250	356	96.6	34446	34445
9B	0.215	306	96.6	29614	29615
8A	0.215	306	96.6	29614	29615
4A	0.265	378	96.6	36516	36515
4B	0.205	292	96.6	28233	28230
8B	0.190	271	96.6	26162	26160
9A	0.215	306	96.6	29614	29615
3A	0.210	299	96.6	28923	28925
5A	0.290	414	48.8	20171	20170
3B	0.205	292	96.6	28233	28230
CC	0.270	385	96.6	37207	37205
7B	0.330	471	24.7	11618	11620
5B	0.260	371	48.8	18080	18080
1B	0.295	421	24.7	10384	10385
CB	0.240	342	96.6	33065	33065
2A	0.300	428	24.7	10560	10560
7A	0.315	449	24.7	11089	11090
1A	0.310	442	24.7	10913	10910
2B	0.410	585	19.8	11559	11560
6A	0.310	442	24.7	10913	10910

B.5 Biochemical Methane Potential Assay #1

Table B.15: Sample preparation during BMP assay #1. Assumption: density of sludge = 1,010 g/L.

Sample	Fraction Treated (%)	Mass of Sludge Treated (g)	Temp. Reached by Fraction(°C)	Time in Microwave from Calibration (s)	Mass of Sludge not Treated (g)
3A	20	171.7	85	271	686.8
6A	60	515.1	85		343.4
8A	100	858.5	55	138	0.0
CB	0	0.0	-	-	858.5
5B	60	515.1	65	182	343.4
2B	20	171.7	65		686.8
8B	100	858.5	55	138	0.0
9A	100	858.5	65	182	0.0
1B	20	171.7	45	94	686.8
4A	60	515.1	45		343.4
11B	100	858.5	85	271	0.0
5A	60	515.1	65	182	343.4
2A	20	171.7	65		686.8
CA	0	0.0	-	-	858.5
7A	100	858.5	45	94	0.0
11A	100	858.5	85	271	0.0
1A	20	171.7	45	94	686.8
4B	60	515.1	45		343.4
10B	100	858.5	75	227	0.0
7B	100	858.5	45	94	0.0
3B	20	171.7	85	271	686.8
6B	60	515.1	85		343.4
9B	100	858.5	65	182	0.0
10A	100	858.5	75	227	0.0

Table B.16: Sample pH at the beginning of BMP assay #1.

Sample	pH	Sample	pH	Sample	pH
3A	6.15	4A	6.18	10B	6.05
6A	6.08	11B	5.97	7B	6.19
8A	5.94	5A	6.07	3B	6.26
CB	6.17	2A	6.20	6B	6.28
5B	5.94	CA	6.11	9B	6.08
2B	6.12	7A	6.05	10A	6.00
8B	5.98	11A	6.03	InA	6.90
9A	5.91	1A	6.20	InB	6.92
1B	6.20	4B	6.19		

Table B.17: Alkalinity of samples at the beginning of BMP assay #1. These values do no reflect the 1 g/L each of $KHCO_3$ and $NaHCO_3$ that were added directly to the batch bottles.

Sample	P (mL)	T (mL)	Normality Used (N)	$[HCO_3^-]$ (mg/L)
10B	0	25.5	0.02	510
1A	0	24.4	0.02	488
8A	0	20.8	0.02	416
9A	0	18.8	0.02	376
6A	0	22.9	0.02	458
4A	0	21.4	0.02	428
5A	0	25.2	0.02	504
3A	0	20.6	0.02	412
11B	0	20.0	0.02	400
6B	0	26.0	0.02	520
5B	0	21.5	0.02	430
1B	0	18.2	0.02	364
10A	0	19.4	0.02	388
4B	0	27.5	0.02	550
9B	0	22.0	0.02	440
7A	0	25.8	0.02	516
2A	0	32.6	0.02	652
2B	0	24.0	0.02	480
3B	0	20.4	0.02	408
CB	0	16.5	0.02	330
11A	0	23.3	0.02	466
8B	0	20.2	0.02	404
CA	0	17.9	0.02	358
7B	0	44.7	0.02	894
InA	0	27.3	0.10	2730
InB	0	28.1	0.10	2810

Table B.18: COD calibration data used to measure sCOD at the beginning of BMP assay #1.

COD (mg/L)	Absorbance
0	0.000
0	0.005
0	0.010
200	0.145
300	0.225
300	0.215
300	0.215
400	0.290
500	0.360
500	0.360
500	0.360
700	0.485

$$Abs = 0.0007COD + 0.007$$

$$R^2 = 0.9989$$

Table B.19: sCOD of the samples at the beginning of BMP assay #1.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
9A	0.185	254	5.0	1271	1270
8B	0.335	469	2.0	937	935
10A	0.205	283	5.0	1414	1415
6A	0.340	476	2.0	951	950
5A	0.365	511	2.0	1023	1025
10B	0.365	511	3.3	1705	1705
5B	0.360	504	2.0	1009	1010
1B	0.115	154	2.0	309	310
7A	0.285	397	2.0	794	795
11A	0.325	454	3.3	1514	1515
11B	0.270	376	3.3	1252	1250
2B	0.195	269	1.4	384	385
7B	0.280	390	2.0	780	780
CB	0.180	247	1.0	247	245
InA	0.150	204	1.0	204	205
9B	0.250	347	5.0	1736	1735
1A	0.140	190	2.0	380	380
InB	0.155	211	1.0	211	210
CA	0.190	261	1.0	261	260
2A	0.300	419	1.3	523	525
3B	0.340	476	1.3	595	595
3A	0.295	411	1.0	411	410
6B	0.410	576	2.0	1151	1150
4B	0.295	411	1.3	514	515
4A	0.290	404	1.0	404	405
8A	0.370	519	2.0	1037	1035

Table B.20: COD calibration data used to measure tCOD at the beginning of BMP assay #1.

COD (mg/L)	Absorbance
0	0.000
0	0.005
0	0.010
200	0.150
300	0.230
300	0.215
300	0.215
400	0.295
500	0.360
500	0.360
500	0.360
700	0.490

$Abs = 0.0007COD + 0.0077$

$R^2 = 0.9987$

Table B.21: tCOD of the samples at the beginning of BMP assay #1.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
CA	0.340	475	48.77	23153	23155
3B	0.355	496	48.77	24198	24200
8B	0.280	389	48.77	18972	18970
11B	0.290	403	48.77	19669	19670
10B	0.330	460	48.77	22456	22455
6A	0.285	396	48.77	19321	19320
7B	0.350	489	48.77	23850	23850
4A	0.270	375	48.77	18276	18275
7A	0.325	453	48.77	22108	22110
9B	0.345	482	48.77	23501	23500
5A	0.340	475	48.77	23153	23155
1A	0.310	432	48.77	21063	21065
10A	0.290	403	48.77	19669	19670
4B	0.330	460	48.77	22456	22455
InB	0.235	325	32.66	10606	10605
6B	0.330	460	48.77	22456	22455
3A	0.260	360	48.77	17579	17580
5B	0.265	368	48.77	17927	17925
2B	0.260	360	48.77	17579	17580
9A	0.280	389	48.77	18972	18970
2A	0.330	460	48.77	22456	22455
1B	0.270	375	48.77	18276	18275
CB	0.290	403	48.77	19668	19670
InA	0.230	318	32.66	10373	10375
11A	0.355	496	48.77	24198	24200
8A	0.270	375	48.77	18276	18275

Table B.22: TS and VS of the samples at the beginning of BMP assay #1.

Sample	W (g)	X (g)	Y (g)	Z (g)	TS (%)	VS (%)
10B	68.8090	94.5321	69.3330	68.9924	2.04	1.32
10A	70.3690	97.7437	70.8180	70.5288	1.64	1.06
3B	64.2195	91.6060	64.7445	64.4140	1.92	1.21
2A	63.9902	83.5976	64.3646	64.1239	1.91	1.23
11B	70.9412	99.3613	71.5267	71.1480	2.06	1.33
8B	73.1786	98.2386	73.6186	73.3368	1.76	1.12
6B	64.6035	84.1020	65.0550	64.7595	2.32	1.52
7B	66.6838	82.2240	67.0048	66.8004	2.07	1.32
6A	74.2291	101.7581	74.7466	74.4141	1.88	1.21
CB	63.6461	84.5162	63.9936	63.7739	1.67	1.05
CA	71.7027	86.0943	72.0603	71.8331	2.48	1.58
9B	75.2511	98.3227	75.7665	75.4318	2.23	1.45
InB	70.5941	95.2420	70.8890	70.7299	1.20	0.65
4A	72.4445	101.3696	72.9691	72.6300	1.81	1.17
3A	78.5232	106.6496	79.0266	78.6997	1.79	1.16
InA	76.6850	102.3893	76.9417	76.7939	1.00	0.58
2B	70.1721	98.7900	70.6832	70.3515	1.79	1.16
7A	65.8129	90.4375	66.3451	66.0007	2.16	1.40
11A	77.7195	109.7651	78.4553	77.9759	2.30	1.50
9A	77.4149	109.1155	77.9982	77.6255	1.84	1.18
1B	71.7248	101.5327	72.2567	71.9154	1.78	1.14
5B	64.8150	94.1049	65.3588	65.0065	1.86	1.20
1A	67.6835	92.6197	68.2172	67.8744	2.14	1.37
4B	66.8805	89.0610	67.3715	67.0527	2.21	1.44
8A	75.5457	103.9015	76.1115	75.7433	2.00	1.30
5A	111.7522	139.3638	112.3716	111.9705	2.24	1.45

Table B.23: Calibration data used to measure ammonia at the beginning of BMP assay #1.

NH_3-N (mg/L)	Electrode Potential (mV)
10	58.6
100	-1.3
1000	-57.3

$$EP = -25.167LN[NH_3 - N] + 115.9$$

$$R^2 = 0.9996$$

Table B.24: Ammonia of the samples at the beginning of BMP assay #1.

Sample	Electrode Potential (mV)	NH_3-N (mg/L)
1A	30.7	30
11A	40.9	20
4A	33.7	26
1B	38.9	21
7A	22.8	40
10B	34.7	25
8B	30.6	30
10A	33.8	26
CB	44.4	17
9B	25.9	36
InB	-33.4	377
4B	23.6	39
2B	32.6	27
8A	28.8	32
InA	-36.1	420
3A	31.2	29
CA	35.7	24
7B	14.4	56
9A	23.4	39
2A	20.2	45
5A	14.6	56
6B	10.6	66
11B	35.5	24
6A	18.6	48
5B	17.2	50
3B	13.7	58

Table B.25: Concentration of VFA in the samples at the beginning of BMP assay #1.

Sample	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)
Calibration	1999	2000	2004
1B	103	68	0
3B	83	99	0
11B	39	39	0
5B	62	80	0
2B	34	0	0
9B	65	83	0
6A	85	0	0
10A	55	73	0
InA	0	0	0
6B	130	103	0
CB	40	52	0
7B	113	101	0
10B	60	75	0
7A	110	103	0
11A	53	68	0
8B	56	0	0
2A	79	0	0
9A	63	0	0
3A	39	58	0
4A	67	0	0
4B	91	84	0
1A	79	77	0
InB	0	0	0
5A	80	99	0
8A	0	0	0
CA	34	52	0

Table B.26: Biogas composition during BMP assay #1.

Bottle 1A			Bottle 1B			Bottle 2A			Bottle 2B		
Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2
11/28/04	66.7	33.3	11/28/04	62.9	37.1	11/29/04	68.1	31.9	11/29/04	70.5	29.5
12/3/04	72.8	27.2	12/3/04	73.9	26.1	12/3/04	72.9	27.1	12/3/04	74.6	25.4
12/7/04	73.8	26.2	12/7/04	72.9	27.1	12/7/04	73.7	26.3	12/7/04	74.2	25.8
12/11/04	70.3	29.7	12/11/04	70.5	29.5	12/11/04	70.8	29.2	12/11/04	70.9	29.1
Bottle 3A			**Bottle 3B**			**Bottle 4A**			**Bottle 4B**		
Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2
11/28/04	60.8	39.2	11/29/04	69.7	30.3	11/28/04	62.8	37.2	11/28/04	62.5	37.5
12/3/04	74.8	25.2	12/3/04	73.8	26.2	12/3/04	74.5	25.5	12/3/04	79.1	20.9
12/7/04	74.7	25.3	12/7/04	74.6	25.4	12/7/04	74.7	25.3	12/7/04	73.7	26.3
12/11/04	71.3	28.7	12/11/04	70.6	29.4	12/11/04	71.1	28.9	12/11/04	70.3	29.7
Bottle 5A			**Bottle 5B**			**Bottle 6A**			**Bottle 6B**		
Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2
11/28/04	57.2	42.8	11/28/04	57.7	42.3	11/29/04	70.7	29.3	11/28/04	59.2	40.8
12/3/04	74.1	25.9	12/3/04	74.8	25.2	12/3/04	74.7	25.3	12/3/04	73.0	27.0
12/7/04	75.8	24.2	12/7/04	75.0	25.0	12/7/04	75.3	24.7	12/7/04	74.4	25.6
12/11/04	72.1	27.9	12/11/04	71.9	28.1	12/11/04	71.8	28.2	12/11/04	71.0	29.0
Bottle 7A			**Bottle 7B**			**Bottle 8A**			**Bottle 8B**		
Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2
11/28/04	58.3	41.7	11/28/04	59.1	40.9	11/29/04	69.3	30.7	11/29/04	69.9	30.1
12/3/04	73.6	26.4	12/3/04	73.1	26.9	12/3/04	74.5	25.5	12/3/04	75.0	25.0
12/7/04	75.0	25.0	12/7/04	74.8	25.2	12/7/04	76.5	23.5	12/7/04	75.1	24.9
12/11/04	71.0	29.0	12/11/04	71.3	28.7	12/11/04	72.1	27.9	12/11/04	71.8	28.2
Bottle 9A			**Bottle 9B**			**Bottle 10A**			**Bottle 10B**		
Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2
11/28/04	62.3	37.7	11/28/04	53.5	46.5	11/28/04	62.1	37.9	11/29/04	65.0	35.0
12/3/04	74.2	25.8	12/3/04	74.1	25.9	12/3/04	74.7	25.3	12/3/04	73.8	26.2
12/7/04	74.4	25.6	12/7/04	77.6	22.4	12/7/04	75.0	25.0	12/7/04	72.5	27.5
12/11/04	71.1	28.9	12/11/04	72.8	27.2	12/11/04	71.7	28.3	12/11/04	71.6	28.4
Bottle 11A			**Bottle 11B**			**Bottle CA**			**Bottle CB**		
Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2
11/29/04	69.8	30.2	11/28/04	61.4	38.6	11/28/04	58.3	41.7	11/29/04	70.7	29.3
12/3/04	71.3	28.7	12/3/04	76.0	24.0	12/3/04	77.6	22.4	12/3/04	74.1	25.9
12/7/04	73.4	26.6	12/7/04	75.5	24.5	12/7/04	74.8	25.2	12/7/04	75.4	24.6
12/11/04	69.5	30.5	12/11/04	71.8	28.2	12/11/04	70.9	29.1	12/11/04	71.5	28.5
Bottle InA			**Bottle InB**								
Date	CH_4	CO_2	Date	CH_4	CO_2						
11/29/04	72.4	27.6	11/29/04	74.6	25.4						
12/3/04	70.5	29.5	12/3/04	71.4	28.6						
12/7/04	71.4	28.6	12/7/04	68.5	31.5						
12/11/04	69.9	30.1	12/11/04	65.9	34.1						

Table B.27: Total biogas generated by the samples in BMP assay #1.

Sample	Biogas Production (mL)	Sample	Biogas Production (mL)	Sample	Biogas Production (mL)
1A	2168.7	5B	1853.0	10A	1977.6
1B	1676.5	6A	1841.0	10B	2389.7
2A	2137.1	6B	2393.4	11A	2505.4
2B	1739.8	7A	2156.4	11B	1956.1
3A	1753.0	7B	2158.3	CA	2132.0
3B	2250.7	8A	1784.0	CB	1703.0
4A	1727.8	8B	1799.4	InA	262.8
4B	2149.6	9A	1884.0	InB	267.4
5A	2127.6	9B	2366.1		

Table B.28: Sample pH at the end of BMP assay #1.

Sample	pH	Sample	pH	Sample	pH
8A	7.25	CA	7.18	10A	7.24
9A	7.16	CB	7.17	3A	7.19
4A	7.16	11A	7.29	9B	7.26
6B	7.23	6A	7.22	10B	7.26
4B	7.21	2A	7.19	11B	7.23
8B	7.19	InB	7.44	5B	7.20
3B	7.21	1A	7.20	2B	7.19
InA	7.52	7A	7.20	5A	7.23
1B	7.16	7B	7.21		

Table B.29: Alkalinity of samples at the end of BMP assay #1.

Sample	P (mL)	T (mL)	Normality Used (N)	$[HCO_3^-]$ (mg/L)
InB	0	39.9	0.10	3990
6B	0	40.5	0.10	4050
9A	0	32.9	0.10	3290
4B	0	35.9	0.10	3590
7A	0	35.6	0.10	3560
9B	0	38.5	0.10	3850
CB	0	31.9	0.10	3190
3B	0	36.7	0.10	3670
11B	0	35.9	0.10	3590
4A	0	32.2	0.10	3220
8A	0	34.0	0.10	3400
8B	0	34.2	0.10	3420
10B	0	39.2	0.10	3920
3A	0	32.6	0.10	3260
6A	0	33.8	0.10	3380
2B	0	32.4	0.10	3240
1B	0	32.4	0.10	3240
CA	0	35.1	0.10	3510
2A	0	34.2	0.10	3420
7B	0	34.4	0.10	3440
InA	0	38.8	0.10	3880
1A	0	34.5	0.10	3450
10A	0	34.2	0.10	3420
5A	0	34.3	0.10	3430
5B	0	33.6	0.10	3360
11A	0	40.8	0.10	4080

Table B.30: COD calibration data used to measure sCOD at the end of BMP assay #1.

COD (mg/L)	Absorbance
0	0.000
0	0.000
0	0.000
200	0.140
300	0.205
300	0.205
300	0.200
400	0.280
500	0.360
500	0.360
500	0.360
700	0.455

$Abs = 0.0007COD + 0.0021$

$R^2 = 0.9944$

Table B.31: sCOD of the samples at the end of BMP assay #1.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
3B	0.260	368	1.00	368	370
6B	0.290	411	1.00	411	410
10B	0.295	418	1.00	418	420
5A	0.240	340	1.00	340	340
9A	0.210	297	1.00	297	295
2A	0.260	368	1.00	368	370
11B	0.230	326	1.00	326	325
7B	0.225	318	1.00	318	320
InA	0.190	268	1.00	268	270
11A	0.320	454	1.00	454	455
3A	0.185	261	1.00	261	260
7A	0.250	354	1.00	354	355
1B	0.190	268	1.00	268	270
InB	0.180	254	1.00	254	255
8A	0.205	290	1.00	290	290
2B	0.190	268	1.00	268	270
CB	0.180	254	1.00	254	255
6A	0.210	297	1.00	297	295
CA	0.230	326	1.00	326	325
1A	0.220	311	1.00	311	310
5B	0.205	290	1.00	290	290
8B	0.200	283	1.00	283	285
9B	0.305	433	1.00	433	435
10A	0.230	326	1.00	326	325
4A	0.190	268	1.00	268	270
4B	0.230	326	1.00	326	325

Table B.32: COD calibration data used to measure tCOD at the end of BMP assay #1.

COD (mg/L)	Absorbance
0	0.000
0	0.000
0	0.000
200	0.135
300	0.210
300	0.205
300	0.200
400	0.265
500	0.360
500	0.350
500	0.360
700	0.480

$$Abs = 0.0007COD - 0.0017$$

$$R^2 = 0.9978$$

Table B.33: tCOD of the samples at the end of BMP assay #1.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
1B	0.210	302	32.66	9878	9880
3A	0.205	295	32.66	9645	9645
CB	0.205	295	32.66	9645	9645
3B	0.250	360	32.66	11745	11745
4B	0.245	352	32.66	11512	11510
10B	0.235	338	32.66	11045	11045
9B	0.240	345	32.66	11278	11280
CA	0.255	367	32.66	11978	11980
4A	0.215	310	32.66	10112	10110
2A	0.250	360	32.66	11745	11745
6A	0.205	295	32.66	9645	9645
5A	0.250	360	32.66	11745	11745
8A	0.195	281	32.66	9179	9180
5B	0.200	288	32.66	9412	9410
6B	0.230	331	32.66	10812	10810
1A	0.240	345	32.66	11278	11280
7A	0.250	360	32.66	11745	11745
9A	0.200	288	32.66	9412	9410
11A	0.235	338	32.66	11045	11045
2B	0.290	417	24.68	10285	10285
InB	0.185	267	32.66	8712	8710
10A	0.210	302	32.66	9878	9880
7B	0.240	345	32.66	11278	11280
11B	0.255	367	24.68	9051	9050
InA	0.180	260	32.66	8479	8480
8B	0.195	281	32.66	9179	9180

Table B.34: TS and VS of the samples at the end of BMP assay #1.

Sample	W (g)	X (g)	Y (g)	Z (g)	TS (%)	VS (%)
InB	71.7210	114.1119	72.2341	71.9971	1.21	0.56
11A	83.9246	135.8177	84.5629	84.2524	1.23	0.60
11B	63.6428	112.5987	64.1708	63.9176	1.08	0.52
10A	78.5145	128.0581	79.0247	78.7723	1.03	0.51
9A	66.6820	115.7429	67.2423	66.9654	1.14	0.56
1A	78.2892	139.9534	79.1111	78.6887	1.33	0.69
6B	74.2265	123.1154	74.8379	74.5233	1.25	0.64
CB	73.3549	126.6825	73.9531	73.6446	1.12	0.58
4A	64.2256	110.5209	64.7679	64.4986	1.17	0.58
4B	77.7154	127.5873	78.3681	78.0403	1.31	0.66
5A	77.4205	137.7449	78.1665	77.7946	1.24	0.62
6A	65.1766	115.0086	65.7162	65.4344	1.08	0.57
1B	76.6808	126.0032	77.2243	76.9477	1.10	0.56
3A	70.1674	109.7123	70.6094	70.3923	1.12	0.55
7A	67.6831	103.1809	68.1214	67.8989	1.23	0.63
InA	74.1070	123.3658	74.6406	74.3904	1.08	0.51
5B	72.4373	112.7127	72.9049	72.6738	1.16	0.57
3B	111.7463	153.5389	112.2958	112.0165	1.31	0.67
2B	66.0586	115.1173	66.5888	66.3178	1.08	0.55
CA	75.2455	114.8130	75.7676	75.5013	1.32	0.67
10B	70.0075	111.1367	70.5234	70.2728	1.25	0.61
9B	63.9917	104.3232	64.4850	64.2295	1.22	0.63
8B	110.5106	164.1788	111.1101	110.8109	1.12	0.56
7B	62.2061	99.1715	62.6759	62.4317	1.27	0.66
8A	70.9412	122.4618	71.5069	71.2206	1.10	0.56
2A	67.3907	107.6037	67.9432	67.6562	1.37	0.71

Table B.35: Calibration data used to measure ammonia at the end of BMP assay #1.

NH_3-N (mg/L)	Electrode Potential (mV)
10	46.3
100	-10.6
1000	-70.2

$$EP = -25.298LN[NH_3 - N] + 105$$

$$R^2 = 0.9998$$

Table B.36: Ammonia of the samples at the end of BMP assay #1.

Sample	Electrode Potential (mV)	NH_3-N (mg/L)
4B	-48.5	432
6B	-53.0	516
10B	-51.6	488
InA	-47.7	418
11A	-53.1	518
6A	-46.5	399
1A	-48.1	425
2A	-47.6	417
7A	-47.5	415
11B	-47.5	415
3B	-48.1	425
8B	-43.3	351
3A	-42.4	339
7B	-46.0	391
9A	-44.0	361
1B	-41.6	329
4A	-42.0	334
9B	-48.2	427
5A	-46.0	391
8A	-43.1	349
CB	-41.3	325
InB	-45.8	388
CA	-45.0	376
10A	-45.2	379
2B	-41.6	329
5B	-43.0	347

Table B.37: Concentration of VFA in the samples at the end of BMP assay #1.

Sample	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)
Calibration	2024	2008	2003
All Samples	0	0	0

Table B.38: CST of sludge samples at the end of BMP assay #1.

Sample	Capillary Suction Time (s)		
	Trial 1	Trial 2	Average
4B	110	110	110
11A	136	149	142
11B	88	111	99
7A	110	122	116
9B	153	158	155
9A	89	104	96
6B	153	144	149
10B	150	158	154
CB	76	72	74
6A	105	110	107
5A	111	128	119
2A	104	122	113
CA	130	136	133
2B	77	85	81
4A	90	87	89
1A	134	129	132
8B	81	102	92
8A	91	101	96
10A	134	136	135
3A	130	121	126
1B	98	93	96
7B	159	152	155
3B	153	143	148
5B	123	113	118

B.6 Biochemical Methane Potential Assay #2

Table B.39: Sample pH at the beginning of BMP assay #2.

Sample	pH	Sample	pH	Sample	pH
1A	6.08	5A	6.28	8A	6.72
1B	6.06	5B	6.37	8B	6.61
CB	6.60	4B	6.37	9B	6.31
CA	6.64	4A	6.36	9A	6.27
7B	7.03	3B	6.28	InA	7.16
7A	6.95	3A	6.17	InB	7.28
2A	6.32	6B	6.48		
2B	6.39	6A	6.37		

Table B.40: Alkalinity of samples at the beginning of BMP assay #2. These values do no reflect the 1 g/L each of $KHCO_3$ and $NaHCO_3$ that were added directly to the batch bottles.

Sample	P (mL)	T (mL)	Normality Used (N)	$[HCO_3^-]$ (mg/L)
8B	0	20.3	0.02	406
7A	0	25.3	0.02	506
6B	0	25.2	0.02	504
3B	0	22.7	0.02	454
CA	0	27.0	0.02	540
4A	0	39.6	0.02	792
CB	0	26.0	0.02	520
1B	0	22.8	0.02	456
9A	0	23.4	0.02	468
9B	0	25.1	0.02	502
6A	0	26.2	0.02	524
1A	0	22.2	0.02	444
7B	0	26.0	0.02	520
3A	0	24.0	0.02	480
2A	0	22.4	0.02	448
8A	0	21.5	0.02	430
4B	0	33.1	0.02	662
2B	0	23.2	0.02	464
5B	0	24.3	0.02	486
5A	0	26.5	0.02	530
InA	0	27.5	0.10	2750
InB	0	27.0	0.10	2700

Table B.41: COD calibration data used to measure sCOD and tCOD at the beginning of BMP assay #2.

COD (mg/L)	Absorbance
0	0.000
0	0.000
0	0.005
200	0.145
300	0.205
300	0.205
300	0.210
400	0.280
500	0.355
500	0.360
500	0.365
700	0.490

$$Abs = 0.0007COD + 0.0005$$

$$R^2 = 0.9988$$

Table B.42: sCOD of the samples at the beginning of BMP assay #2.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
3A	0.290	414	3.33	1377	1375
2A	0.230	328	3.33	1092	1090
1A	0.230	328	3.33	1092	1090
CA	0.235	335	1.00	335	335
CB	0.240	342	1.00	342	340
9A	0.225	321	10.00	3207	3205
6A	0.240	342	10.00	3421	3420
InB	0.235	335	1.00	335	335
5B	0.220	314	10.00	3136	3135
5A	0.215	306	10.00	3064	3065
4B	0.190	271	10.00	2707	2705
7A	0.250	356	10.00	3564	3565
2B	0.240	342	3.33	1139	1140
7B	0.250	356	10.00	3564	3565
InA	0.200	285	1.00	285	285
8B	0.245	349	3.33	1163	1165
1B	0.230	328	3.33	1092	1090
6B	0.235	335	10.00	3350	3350
8A	0.240	342	3.33	1139	1140
4A	0.185	264	10.00	2636	2635
3B	0.280	399	3.33	1330	1330
9B	Broke Tube				

Table B.43: tCOD of the samples at the beginning of BMP assay #2.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
InB	0.260	371	32.66	12109	12110
7B	0.230	328	48.77	15990	15990
6B	0.230	328	48.77	15990	15990
8B	0.230	328	48.77	15990	15990
4A	0.245	349	48.77	17035	17035
6A	0.230	328	48.77	15990	15990
4B	0.240	342	48.77	16687	16685
CB	0.230	328	48.77	15990	15990
InA	0.265	378	32.66	12342	12340
2B	0.235	335	48.77	16339	16340
CA	0.255	364	48.77	17732	17730
2A	0.240	342	48.77	16687	16685
9A	0.245	349	48.77	17035	17035
5B	0.230	328	48.77	15990	15990
9B	0.245	349	48.77	17035	17035
3B	0.235	335	48.77	16339	16340
7A	0.245	349	48.77	17035	17035
3A	0.235	335	48.77	16339	16340
1A	0.220	314	48.77	15294	15295
5A	0.250	356	48.77	17384	17385
8A	0.235	335	48.77	16339	16340
1B	0.230	328	48.77	15990	15990

Table B.44: TS and VS of the samples at the beginning of BMP assay #2.

Sample	W (g)	X (g)	Y (g)	Z (g)	TS (%)	VS (%)
CB	111.7497	151.3468	112.3001	111.9212	1.390	0.957
3B	70.1701	113.3586	70.8499	70.3826	1.574	1.082
8B	77.4193	115.0504	78.0277	77.6115	1.617	1.106
3A	71.7253	118.8251	72.4489	71.9487	1.536	1.062
9B	75.2461	110.9974	75.9607	75.5610	1.999	1.118
6B	70.0080	122.2460	70.9858	70.4326	1.872	1.059
InB	78.2859	122.6802	78.9733	78.5995	1.548	0.842
2A	70.5908	128.3793	71.5483	70.8924	1.657	1.135
7A	83.9310	131.8617	84.7754	84.3052	1.762	0.981
8A	62.2071	105.5963	62.8488	62.4084	1.479	1.015
CA	70.9389	108.0661	71.5401	71.1343	1.619	1.093
5B	67.3893	110.1312	68.1399	67.7159	1.756	0.992
InA	66.0553	104.9253	66.6404	66.3205	1.505	0.823
1B	110.5123	153.4085	111.0769	110.6844	1.316	0.915
4B	107.8118	150.9810	108.5615	108.1406	1.737	0.975
6A	70.3586	103.7785	71.0157	70.6424	1.966	1.117
7B	74.2243	117.9529	74.9923	74.5572	1.756	0.995
1A	72.4440	115.3167	73.0181	72.6241	1.339	0.919
5A	77.7150	114.4945	78.4301	78.0281	1.944	1.093
2B	78.5146	118.3698	79.1198	78.7069	1.518	1.036
4A	65.1736	102.4916	65.8715	65.4819	1.870	1.044
9A	66.6826	111.5534	67.4759	67.0245	1.768	1.006

Table B.45: Calibration data used to measure ammonia at the beginning of BMP assay #2.

NH_3-N (mg/L)	Electrode Potential (mV)
10	63.5
100	3.8
1000	-55.3

$$EP = -25.797 LN[NH_3 - N] + 122.8$$

$$R^2 = 1$$

Table B.46: Ammonia of the samples at the beginning of BMP assay #2.

Sample	Electrode Potential (mV)	NH_3-N (mg/L)
9A	20.9	52
7B	28.0	39
8A	58.0	12
2A	60.1	11
6A	27.6	40
4A	24.0	46
6B	26.2	42
2B	56.2	13
3A	53.8	15
5A	22.9	48
3B	50.9	16
CB	51.4	16
8B	48.7	18
7A	17.8	59
1B	49.6	17
InB	-40.5	561
CA	46.8	19
5B	15.9	63
9B	13.5	69
4B	12.6	72
1A	45.9	20
InA	-43.4	628

Table B.47: Concentration of VFA in the samples at the beginning of BMP assay #2.

Sample	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)
Calibration	1991	1997	2023
2A	16	92	0
7B	40	0	0
CA	22	90	0
1A	31	79	0
4B	107	0	0
9A	62	0	0
3B	29	106	0
InB	0	0	0
6B	107	0	0
3A	11	112	0
5A	15	35	0
5B	98	0	0
9B	51	63	0
8A	10	108	0
6A	44	59	0
8B	84	109	0
InA	0	0	0
CB	10	85	0
7A	104	49	0
4A	13	70	0
2B	47	37	0
1B	Forgot to put this vial in the GC		

Table B.48: Biogas composition during BMP assay #2.

Bottle 1A			Bottle 1B			Bottle 2A			Bottle 2B		
Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2
2/19/05	62.7	37.3	2/19/05	63.1	36.9	2/19/05	63.8	36.2	Bottle broke		
2/24/05	61.4	38.6	2/24/05	62.2	37.8	2/24/05	62.5	37.5			
Bottle 3A			**Bottle 3B**			**Bottle 4A**			**Bottle 4B**		
Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2
2/19/05	63.4	36.6	2/20/05	65.4	34.6	2/20/05	65.9	34.1	2/19/05	65.1	34.9
2/24/05	62.6	37.4	2/24/05	62.8	37.2	2/24/05	64.0	36.0	2/24/05	64.3	35.7
Bottle 5A			**Bottle 5B**			**Bottle 6A**			**Bottle 6B**		
Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2
2/19/05	64.5	35.5	2/19/05	64.0	36.0	2/19/05	64.4	35.6	2/20/05	67.3	32.7
2/24/05	63.5	36.5	2/24/05	62.9	37.1	2/24/05	63.5	36.5	2/24/05	63.3	36.7
Bottle 7A			**Bottle 7B**			**Bottle 8A**			**Bottle 8B**		
Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2
2/19/05	64.3	35.7	2/19/05	62.2	37.8	2/19/05	65.9	34.1	2/19/05	63.7	36.3
2/24/05	64.3	35.7	2/24/05	63.4	36.6	2/24/05	64.1	35.9	2/24/05	62.4	37.6
Bottle 9A			**Bottle 9B**			**Bottle CA**			**Bottle CB**		
Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2	Date	CH_4	CO_2
2/19/05	63.5	36.5	2/19/05	64.6	35.4	2/20/05	63.9	36.1	2/19/05	62.6	37.4
2/24/05	63.8	36.2	2/24/05	63.5	36.5	2/24/05	61.7	38.3	2/24/05	61.7	38.3
Bottle InA			**Bottle InB**								
Date	CH_4	CO_2	Date	CH_4	CO_2						
2/19/05	62.0	38.0	2/19/05	58.1	41.9						

Table B.49: Total biogas generated by the samples in BMP assay #2.

Sample	Biogas Production (mL)	Sample	Biogas Production (mL)	Sample	Biogas Production (mL)
1A	2318.4	5A	2484.5	9A	2444.6
1B	2399.1	5B	2431.7	9B	2465.8
2A	2350.2	6A	2483.9	CA	2116.3
2B	253.6	6B	2351.8	CB	2148.4
3A	2341.0	7A	2422.6	InA	229.0
3B	2429.0	7B	2489.6	InB	256.3
4A	2419.4	8A	2348.5		
4B	2414.2	8B	2316.7		

Table B.50: Sample pH at the end of BMP assay #2.

Sample	pH	Sample	pH	Sample	pH
InB	7.63	5A	7.51	9B	7.45
4B	7.45	3B	7.48	6B	7.47
1B	7.44	8B	7.43	InA	7.73
CB	7.41	4A	7.45	6A	7.47
7B	7.48	1A	7.46	9A	7.50
7A	7.49	8A	7.49		
3A	7.48	5B	7.45		
2A	*	CA	7.43		

* Forgot to measure this sample

Table B.51: Alkalinity of samples at the end of BMP assay #2.

Sample	P (mL)	T (mL)	Normality Used (N)	$[HCO_3^-]$ (mg/L)
4B	0	34.9	0.10	3490
1A	0	34.2	0.10	3420
8B	0	35.2	0.10	3520
InB	0	40.5	0.10	4050
CA	0	33.7	0.10	3370
5B	0	35.2	0.10	3520
6B	0	36.4	0.10	3640
1B	0	34.6	0.10	3460
6A	0	35.4	0.10	3540
4A	0	34.8	0.10	3480
2A	0	36.5	0.10	3650
7A	0	37.4	0.10	3740
CB	0	34.5	0.10	3450
3B	0	37.8	0.10	3780
InA	0	41.0	0.10	4100
7B	0	37.1	0.10	3710
8A	0	35.1	0.10	3510
9B	0	35.3	0.10	3530
9A	0	35.4	0.10	3540
3A	0	37.9	0.10	3790
5A	0	34.9	0.10	3490

Table B.52: COD calibration data used to measure sCOD and tCOD at the end of BMP assay #2.

COD (mg/L)	Absorbance
0	0.000
0	0.000
0	0.000
200	0.145
300	0.210
300	0.210
300	0.210
400	0.280
500	0.355
500	0.360
500	0.365
700	0.490

$Abs = 0.0007COD + 1E - 04$

$R^2 = 0.9992$

Table B.53: sCOD of the samples at the end of BMP assay #2.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
3A	0.262	374	1.00	374	375
4B	0.148	211	2.00	423	425
5A	0.260	371	1.00	371	371
7A	0.330	471	1.00	471	471
4A	0.251	358	1.00	358	360
6B	0.271	387	1.00	387	385
1A	0.239	341	1.00	341	340
CA	0.202	288	1.00	288	290
8A	0.264	377	1.00	377	375
8B	0.264	377	1.00	377	375
InA	0.248	354	1.00	354	355
InB	0.247	353	1.00	353	355
7B	0.305	436	1.00	436	435
CB	0.209	298	1.00	298	300
3B	0.276	394	1.00	394	395
1B	0.259	370	1.00	370	370
6A	0.297	424	1.00	424	425
5B	0.262	374	1.00	374	375
9B	0.282	403	1.00	403	405
2A	0.242	346	1.00	346	345
9A	0.308	440	1.00	440	440

Table B.54: tCOD of the samples at the end of BMP assay #2.

Sample	Absorbance	Curve COD (mg/L)	Dilution Factor	Actual COD (mg/L)	Rounded COD (mg/L)
3A	0.250	357	24.68	8819	8820
2A	0.233	333	24.68	8219	8220
5B	0.237	339	24.68	8360	8360
3B	0.232	332	24.68	8184	8185
1B	0.240	343	24.68	8466	8465
9B	0.233	333	24.68	8219	8220
8B	0.244	349	24.68	8607	8605
InB	0.275	393	24.68	9700	9700
5A	0.244	349	24.68	8607	8605
7A	0.238	340	24.68	8395	8395
InA	0.291	416	24.68	10264	10265
CA	0.253	362	24.68	8924	8925
6B	0.247	353	24.68	8713	8715
4A	0.232	332	24.68	8184	8185
9A	0.240	343	24.68	8466	8465
CB	0.272	389	24.68	9594	9595
1A	0.250	357	24.68	8819	8820
7B	0.230	329	24.68	8113	8115
6A	0.226	323	24.68	7972	7970
4B	0.228	326	24.68	8043	8045
8A	0.181	259	32.66	8451	8450

Table B.55: TS and VS of the samples at the end of BMP assay #2.

Sample	W (g)	X (g)	Y (g)	Z (g)	TS (%)	VS (%)
7A	75.2448	140.2779	75.9894	75.6948	1.145	0.453
4A	74.2236	128.9229	74.8230	74.5774	1.096	0.449
1A	73.3511	128.5007	73.8809	73.6228	0.961	0.468
3A	78.5149	138.6642	79.0855	78.8034	0.949	0.469
InB	66.0582	111.5388	66.7075	66.3905	1.428	0.697
CB	83.9293	132.2407	84.4394	84.1819	1.056	0.533
6B	62.2050	114.2840	62.8291	62.5786	1.198	0.481
8B	77.7148	127.8588	78.2069	77.9632	0.981	0.486
5A	77.4201	130.7218	77.9726	77.7482	1.037	0.421
9B	66.6826	127.6012	67.4003	67.1085	1.178	0.479
8A	63.6428	119.4744	64.1803	63.9151	0.963	0.475
4B	70.5881	131.2158	71.2853	71.0052	1.150	0.462
CA	70.0084	116.8169	70.5233	70.2593	1.100	0.564
InA	78.2874	109.5271	78.7438	78.5170	1.461	0.726
7B	67.3923	116.8992	68.0090	67.7580	1.246	0.507
9A	70.9395	132.7197	71.6267	71.3456	1.112	0.455
1B	70.3591	125.8902	70.9091	70.6320	0.990	0.499
2A	65.1774	121.8083	65.6985	65.4346	0.920	0.466
5B	72.4440	127.5810	73.0416	72.8001	1.084	0.438
6A	111.7491	159.8060	112.2464	112.0436	1.035	0.422
3B	110.5163	159.1801	110.9624	110.7366	0.917	0.464

Table B.56: Calibration data used to measure ammonia at the end of BMP assay #2.

NH_3-N (mg/L)	Electrode Potential (mV)
10	53.5
100	-3.2
1000	-60.1

$$EP = -24.668LN[NH_3 - N] + 110.33$$

$$R^2 = 1$$

Table B.57: Ammonia of the samples at the end of BMP assay #2.

Sample	Electrode Potential (mV)	NH_3-N (mg/L)
4A	-42.1	483
InA	-45.7	558
1A	-42.0	481
9B	-40.9	460
8A	-42.7	495
7B	-43.1	503
CB	-37.5	401
2A	-41.0	462
3B	-43.1	503
8B	-41.5	471
5B	-43.1	503
5A	-43.4	509
InB	-48.1	616
9A	-42.5	491
4B	-42.2	485
1B	-42.5	491
6A	-42.5	491
7A	-41.5	471
3A	-43.2	505
CA	-39.0	426
6B	-40.1	445

Table B.58: Concentration of VFA in the samples at the end of BMP assay #2.

Sample	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)
Calibration	1969	1966	1948
All Samples	0	0	0

Table B.59: CST of sludge samples at the end of BMP assay #2.

Sample	Capilary Suction Time (s)		
	Trial 1	Trial 2	Average
9A	158	154	156
4A	147	159	153
6A	158	175	167
CA	104	107	106
9B	151	136	144
8B	148	137	142
3A	137	139	138
6B	127	130	129
7B	151	139	145
1A	121	139	130
7A	138	151	145
8A	129	125	127
2A	162	153	157
3B	159	151	155
5B	139	147	143
1B	151	165	158
5A	145	129	137
CB	139	120	130
4B	147	134	140

Table B.60: Comparison of offensiveness of sludge samples upon anaerobic digestion.

Volunteer	Control B	1B	5B
A	Least offensive	Middle	Most offensive
B	Least offensive	Most offensive	Middle
C	Least offensive	Middle	Most offensive

B.7 Effects of Microwave Irradiation on the Viscosity of the Sludge

Table B.61: Total solids assay on sludge used to test the viscosity and the surface tension.

Replicate	W (g)	X (g)	Y (g)	TS (%)
A	1.2624	16.9020	1.7375	3.038
B	1.2591	18.0167	1.7677	3.035
C	1.2815	14.1373	1.6761	3.069
				3.05 ± 0.02

Table B.62: Results of the viscosity determination.

Sample	Spindle	Speed (rpm)	Factor	Shear Rate (s^{-1})	Readings		Viscosity (cp)		
					1	2	1	2	Average
Control	2	60	5	12.72	26	33	130.0	165.0	147.5
		30	10	6.36	22	27	220.0	270.0	245.0
		12	25	2.544	17	20.5	425.0	512.5	468.8
		6	50	1.272	13.5	17	675.0	850.0	762.5
45°C	2	60	5	12.72	25.5	33	127.5	165.0	146.3
		30	10	6.36	21	28	210.0	280.0	245.0
		12	25	2.544	15	21.5	375.0	537.5	456.3
		6	50	1.272	12.5	16.5	625.0	825.0	725.0
65°C	2	60	5	12.72	20.5	32	102.5	160.0	131.3
		30	10	6.36	16.5	26	165.0	260.0	212.5
		12	25	2.544	12.5	20.5	312.5	512.5	412.5
		6	50	1.272	9	15.5	450.0	775.0	612.5
85°C	2	60	5	12.72	29.5	31	147.5	155.0	151.3
		30	10	6.36	23	25	230.0	250.0	240.0
		12	25	2.544	17	20	425.0	500.0	462.5
		6	50	1.272	12	16.5	600.0	825.0	712.5

B.8 Effects of Microwave Irradiation on the Surface Tension of the Sludge

Table B.63: Results of the surface tension determination.

Sample	Sample T (°C)	Surface Tension (dynes/cm)				Standard Deviation (dynes/cm)
		Reading 1	Reading 2	Reading 3	Average	
Control	23.9	56.23	54.62	54.45	55.10	0.69
85°C	24	55.02	55.67	54.36	55.02	0.46

APPENDIX C:

ANALYSIS OF THE MICROWAVE CALIBRATION RESULTS FOR 850-ML SAMPLES

As discussed in section 4.1.1, the simplest model to predict the temperature reached by microwave irradiated 850-mL samples is the one shown in Figure C.1. The residuals from the model are presented in Table C.1. The residuals are plotted in Figures C.2 and C.3.

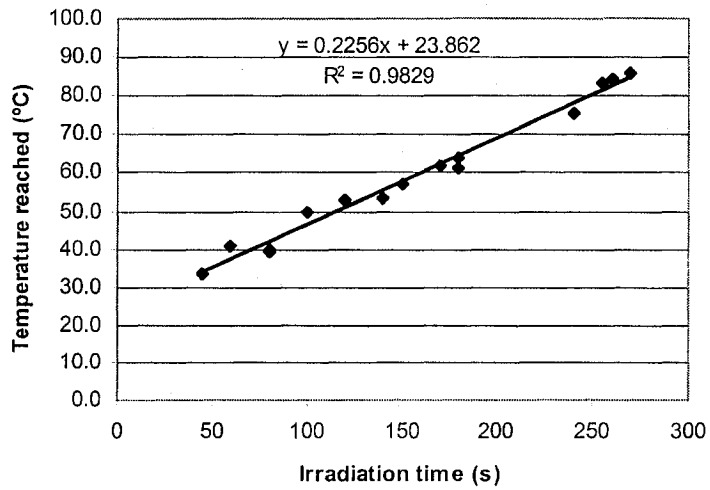


Figure C.1: Model relating irradiation time and temperature reached.

$$\text{Model: } T = (0.2256 * MWt) + 23.862 \qquad \text{(Eq. C.1)}$$

Table C.1: Calibration data and residuals from the model.

Total Solids Concentration (%TS)	Irradiation Time (s)	Temperature Reached (°C)	Temperature Estimate by Model 1 (°C)	Residuals (°C)
4.30	60	41.0	37.4	3.6
3.52	180	61.0	64.5	-3.5
1.77	240	75.5	78.0	-2.5
3.02	260	84.5	82.5	2.0
2.45	255	83.0	81.4	1.6
1.51	80	40.0	41.9	-1.9
3.35	100	50.0	46.4	3.6
4.30	170	62.0	62.2	-0.2
1.16	180	64.0	64.5	-0.5
2.02	80	39.5	41.9	-2.4
3.32	140	53.5	55.4	-1.9
2.79	270	86.0	84.8	1.2
1.51	120	53.0	50.9	2.1
2.45	150	57.0	57.7	-0.7
3.38	45	33.5	34.0	-0.5

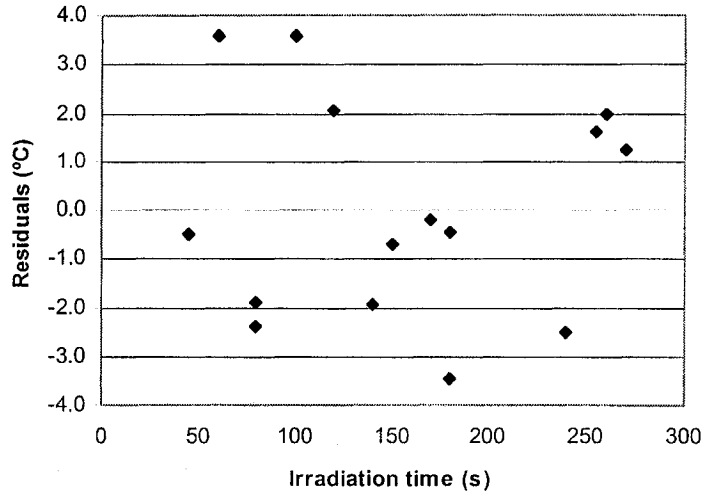


Figure C.2: Residuals of Model 1 versus irradiation time.

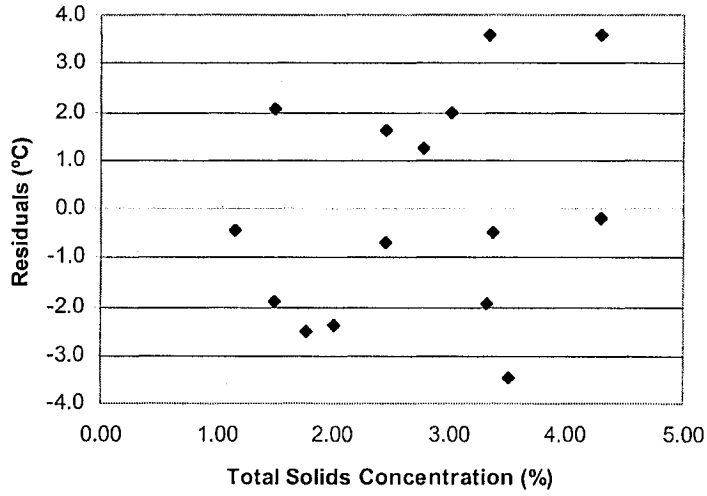


Figure C.3: Residuals of Model 1 versus sludge concentration.

The residuals in Figures C.2 and C.3 seem to be randomly distributed. Because the residuals seem to be randomly distributed, a second order model using the irradiation time variable is not necessary and sludge concentration does not seem to have an effect on the temperature reached by the sludge. Another way to check for this last observation is to plot temperature reached versus irradiation time for the low concentration and high concentration samples. The samples were classified into two groups based on sludge concentration: low (1.2-2.8% TS) and high (3.0-4.3% TS). The relationship between irradiation time and the temperature reached by the samples is plotted for these two groups in Figure C.4. Again, sludge

concentration is a variable that does not seem to affect the temperature of the sludge samples in the 1.2-4.3% TS range.

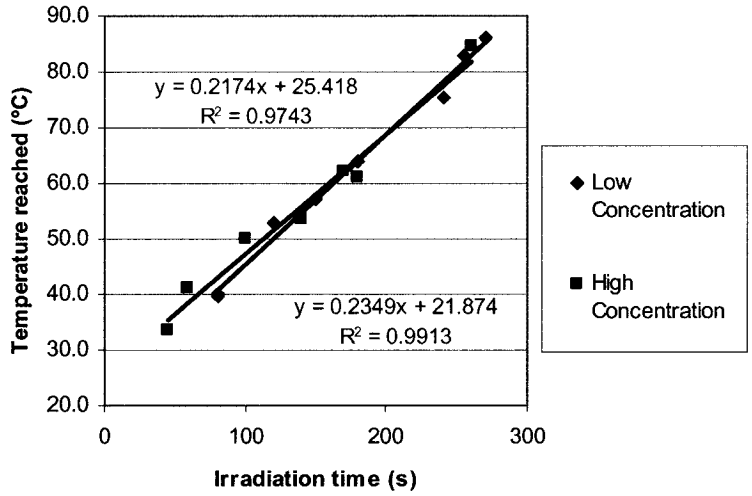


Figure C.4: Linear regression on low and high concentration samples.

APPENDIX D:

MICROSCOPIC PICTURES

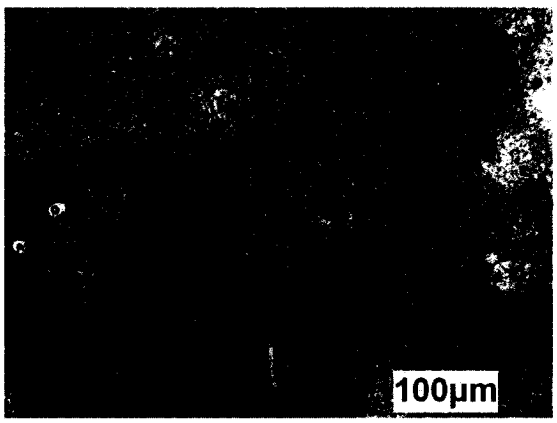


Figure D.1: Control, no treatment, 20x.

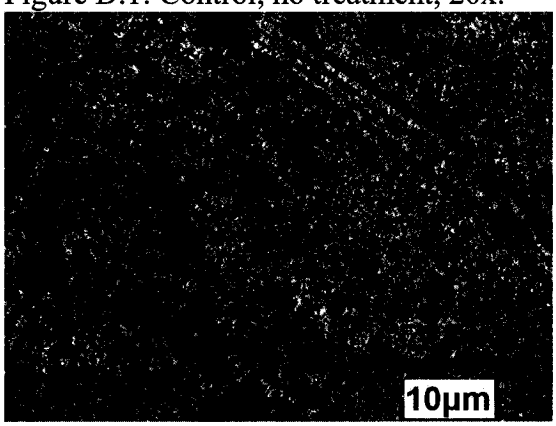


Figure D.2: Control, no treatment, 200x.

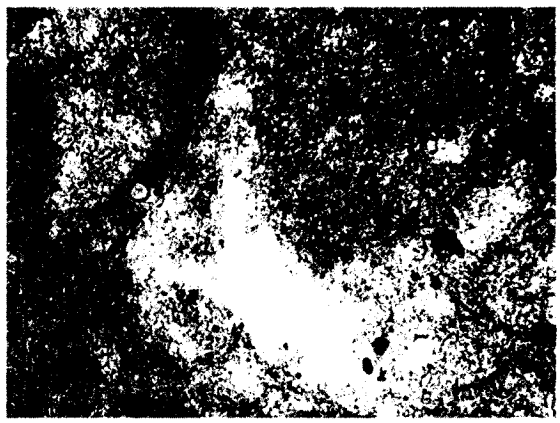


Figure D.3: 85°C MW, no treatment, 20x.

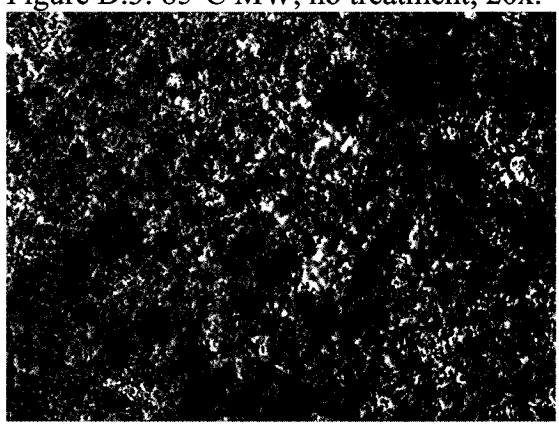


Figure D.4: 85°C MW, no treatment, 200x.

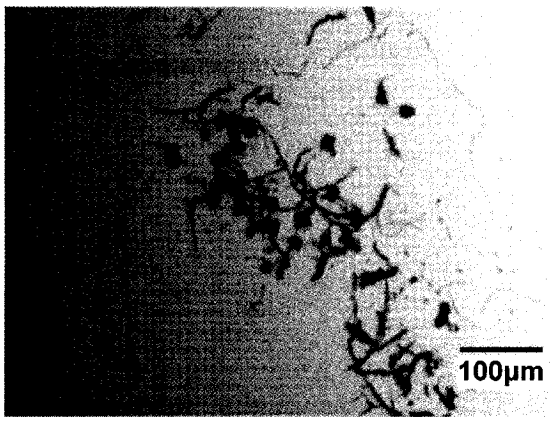


Figure D.5: Control A, methyl blue staining, 74 µm<particles<177 µm, 20x.

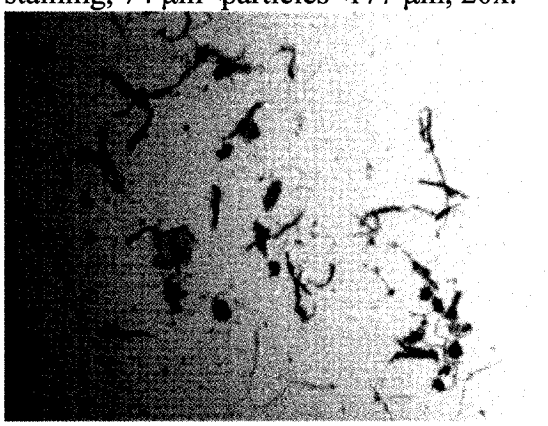


Figure D.6: Control B, methyl blue staining, 74 µm<particles<177 µm, 20x.

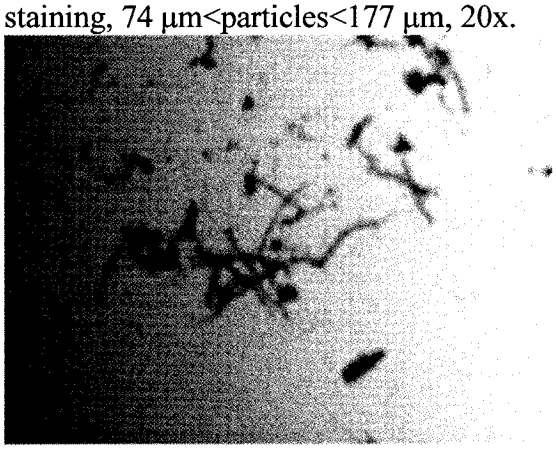


Figure D.7: Control C, methyl blue staining, 74 µm<particles<177 µm, 20x.

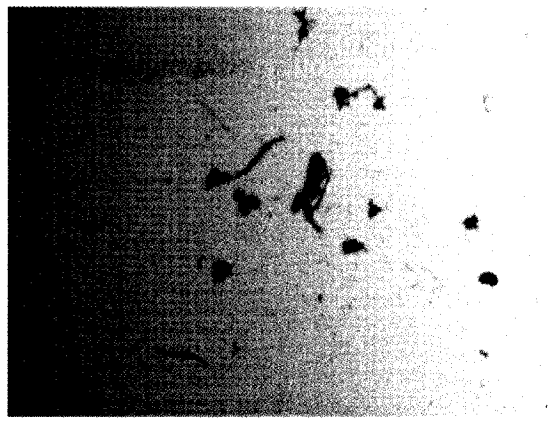


Figure D.8: 85°C MW A, methyl blue staining, 74 µm<particles<177 µm, 20x.

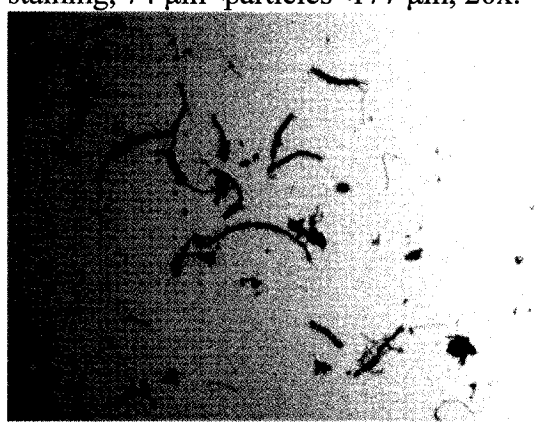


Figure D.9: 85°C MW B, methyl blue staining, 74 µm<particles<177 µm, 20x.

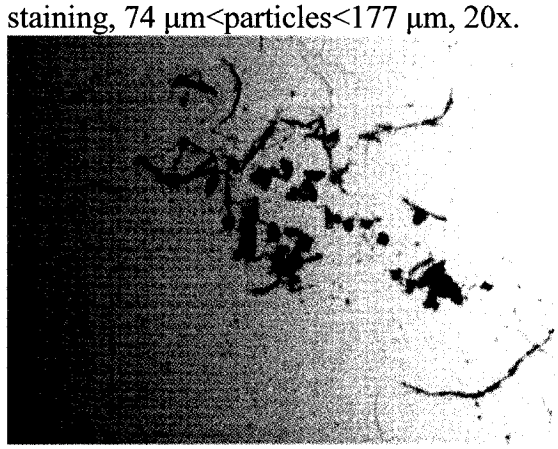


Figure D.10: 85°C MW C, methyl blue staining, 74 µm<particles<177 µm, 20x.

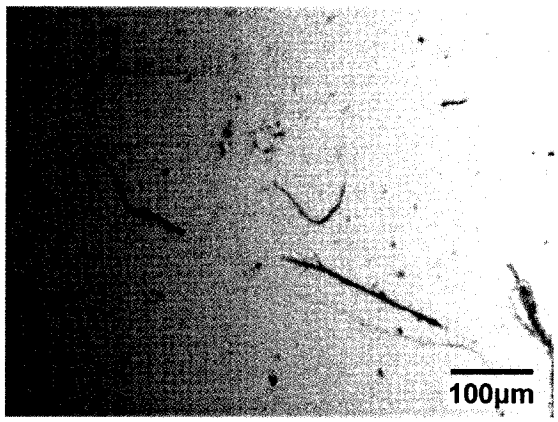


Figure D.11: Control A, methyl blue staining, 177 µm<particles<420 µm, 20x.

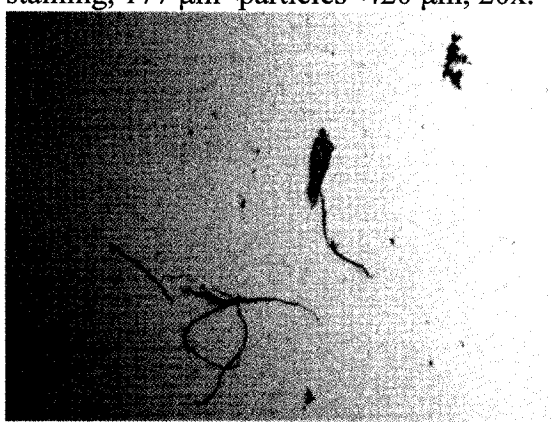


Figure D.12: Control B, methyl blue staining, 177 µm<particles<420 µm, 20x.

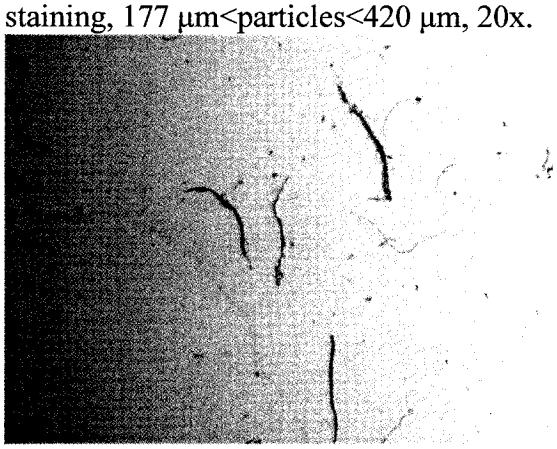


Figure D.13: Control C, methyl blue staining, 177 µm<particles<420 µm, 20x.

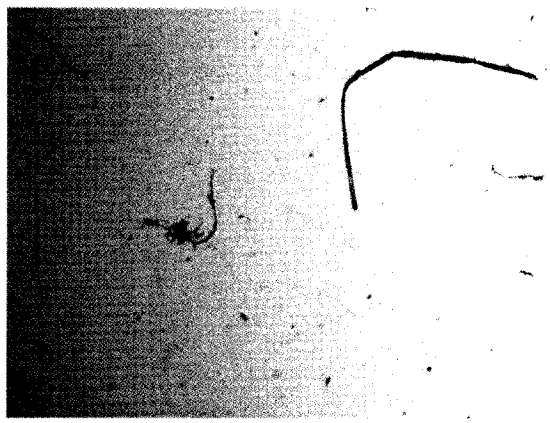


Figure D.14: 85°C MW A, methyl blue staining, 177 µm<particles<420 µm, 20x.

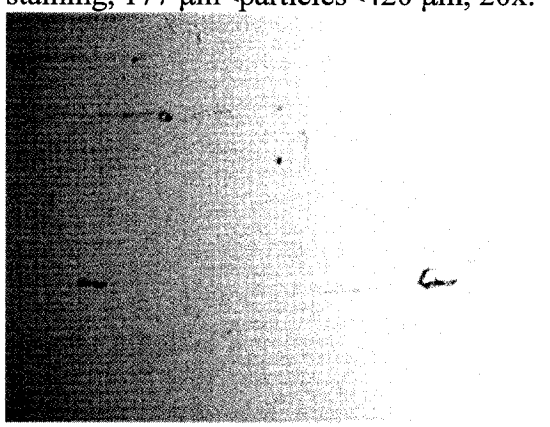


Figure D.15: 85°C MW B, methyl blue staining, 177 µm<particles<420 µm, 20x.

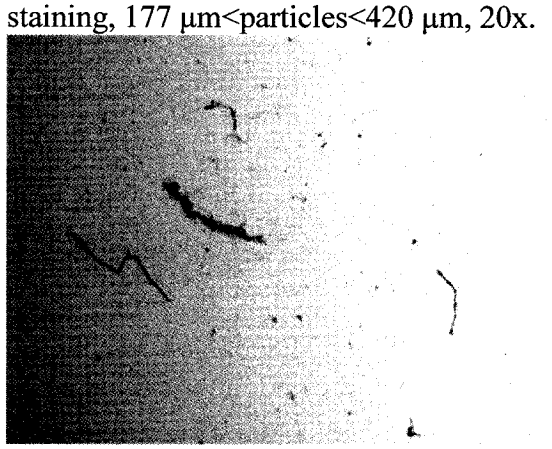


Figure D.16: 85°C MW C, methyl blue staining, 177 µm<particles<420 µm, 20x.

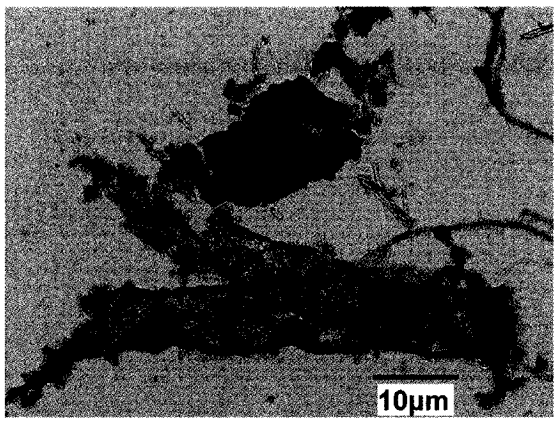


Figure D.17: Control A, methyl blue staining, 74 µm<particles<177 µm, 200x.

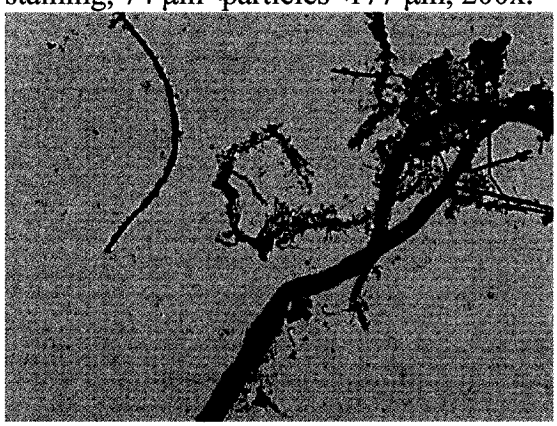


Figure D.18: Control B, methyl blue staining, 74 µm<particles<177 µm, 200x.

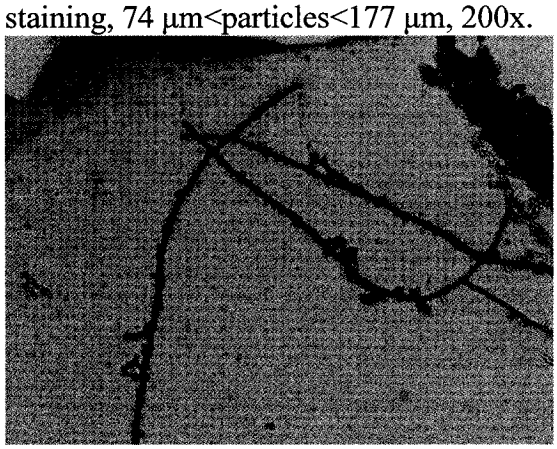


Figure D.19: Control C, methyl blue staining, 74 µm<particles<177 µm, 200x.

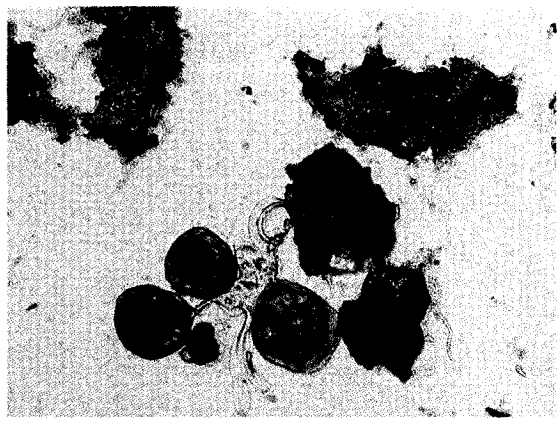


Figure D.20: 85°C MW A, methyl blue staining, 74 µm<particles<177 µm, 200x.

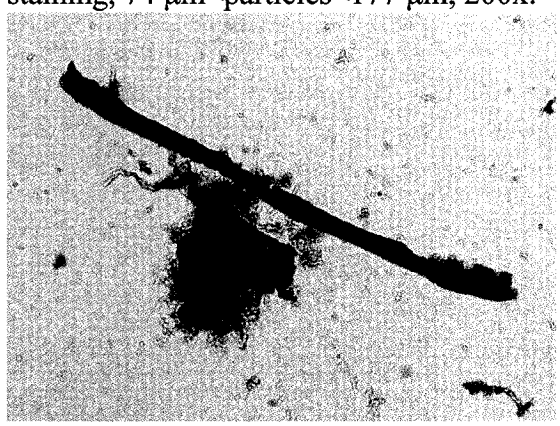


Figure D.21: 85°C MW B, methyl blue staining, 74 µm<particles<177 µm, 200x.

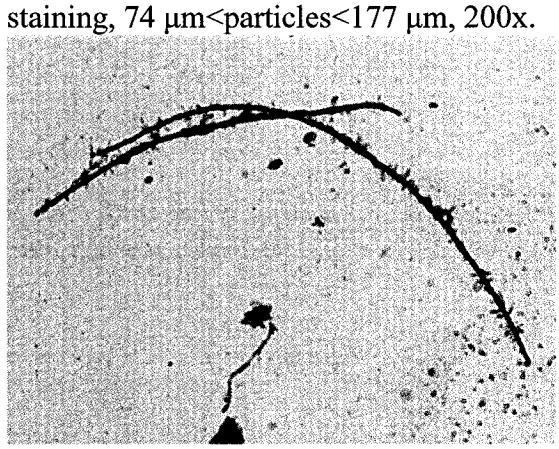


Figure D.22: 85°C MW C, methyl blue staining, 74 µm<particles<177 µm, 200x.

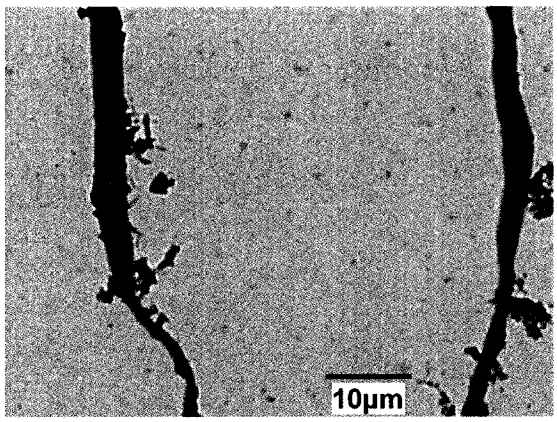


Figure D.23: Control A, methyl blue staining, 177 µm<particles<420 µm, 200x.

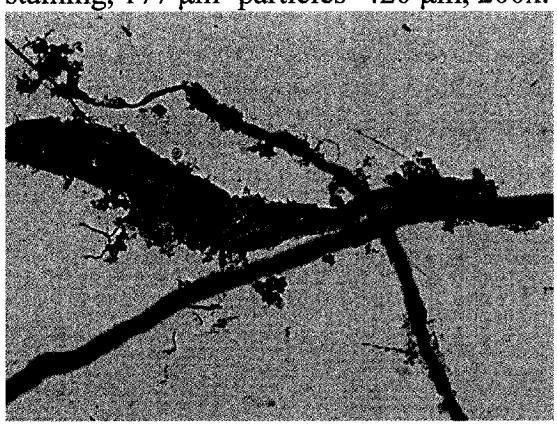


Figure D.24: Control B, methyl blue staining, 177 µm<particles<420 µm, 200x.

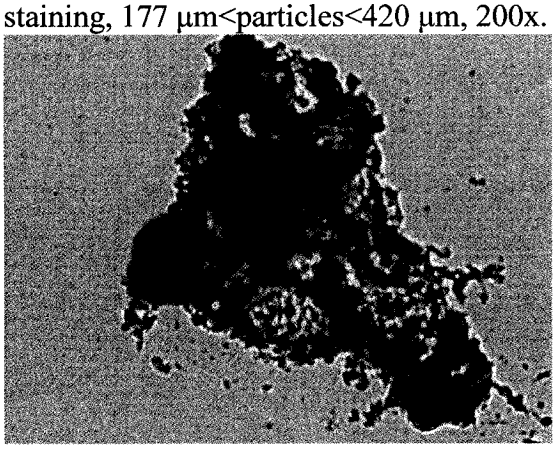


Figure D.25: Control C, methyl blue staining, 177 µm<particles<420 µm, 200x.

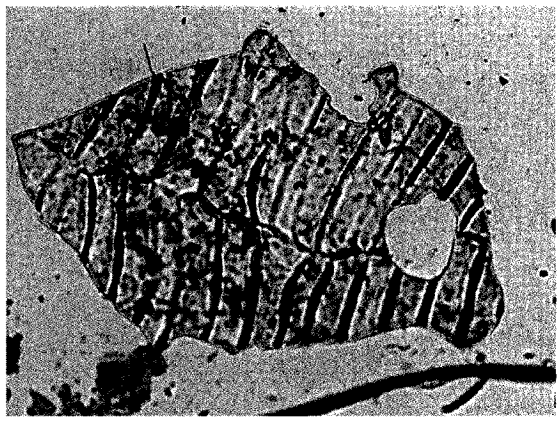


Figure D.26: 85°C MW A, methyl blue staining, 177 µm<particles<420 µm, 200x.

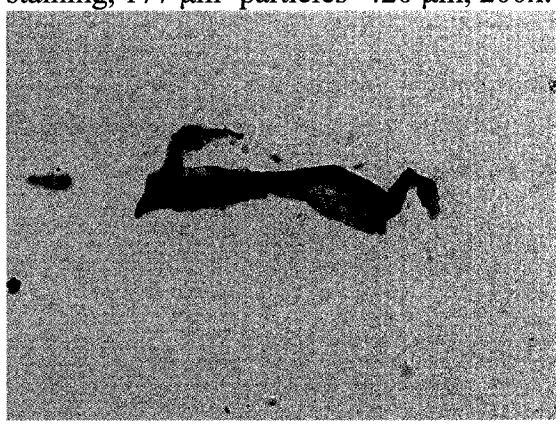


Figure D.27: 85°C MW B, methyl blue staining, 177 µm<particles<420 µm, 200x.

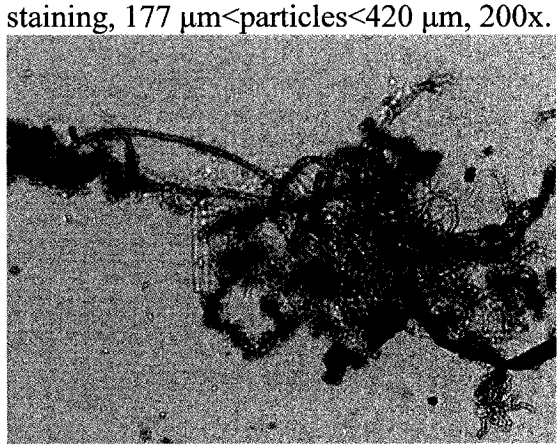


Figure D.28: 85°C MW C, methyl blue staining, 177 µm<particles<420 µm, 200x.

APPENDIX E:

RANDOM NUMBER GENERATOR

This appendix shows the QBASIC code used to randomize laboratory analyses and an example output.

QBASIC Code

```
CLS
counter = 1
'Dimensioning the tables
DIM sample(22)
DIM actualsample(24)
RANDOMIZE TIMER
'Assigning a random number between 1 and 22 to variable "presentsample"
1 presentsample = INT(RND * 22) + 1
FOR z = 1 TO 22
IF actualsample(z) = presentsample GOTO 1
NEXT z
actualsample(counter) = presentsample
counter = counter + 1
IF counter < 23 GOTO 1
'Each entry in array "actualsample" contains a unique number between 1 and 22
'Inputing the name of the samples
DIM table1$(1000)
table1$(1) = "1A"
table1$(2) = "1B"
table1$(3) = "2A"
table1$(4) = "2B"
table1$(5) = "3A"
table1$(6) = "3B"
table1$(7) = "4A"
table1$(8) = "4B"
table1$(9) = "5A"
table1$(10) = "5B"
table1$(11) = "6A"
table1$(12) = "6B"
table1$(13) = "7A"
table1$(14) = "7B"
table1$(15) = "8A"
table1$(16) = "8B"
table1$(17) = "9A"
table1$(18) = "9B"
table1$(19) = "CA"
table1$(20) = "CB"
table1$(21) = "InA"
table1$(22) = "InB"
'Printing the random sample order
PRINT "Random Number Generator"
PRINT
PRINT "Order        Sample"
PRINT
```

```
FOR a = 1 TO 22
PRINT "  "; a; "              "; table1$(actualsample(a))
NEXT a
```

Example Output

Random Number Generator

Order	Sample
1	9B
2	5B
3	7A
4	4A
5	8A
6	9A
7	3A
8	2A
9	1A
10	InA
11	CB
12	2B
13	3B
14	4B
15	5A
16	8B
17	6B
18	InB
19	7B
20	6A
21	1B
22	CA