FEASIBILITY OF GLUCOSE RECOVERY FROM MUNICIPAL SEWAGE SLUDGES AS FEEDSTOCKS USING ACID HYDROLYSIS

By

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ABSTRACT

In light of rising costs in fossil fuels and petroleum, as well as the strain on this largely non-renewable resource, the conversion of biomass, in this case waste biomass, to value-added products is becoming more attractive. In this study, municipal sewage sludge and biosolids were used to determine their potential for glucose recovery. This research focused on three pretreatment processes including drying/grinding, as well as acid and alkaline pretreatments, followed by acid hydrolysis on primary sludge, activated sludge and biosolids. After each pretreatment under specified conditions, the residues remaining from the sludges and biosolids underwent a 2 % H_2SO_4 acid hydrolysis at 120^oC for 1 hr. Compared with activated sludge and biosolids, primary sludge was found to demonstrate the highest potential for glucose recovery in this study. Primary sludge with 1.0 N HCl pretreatment over a 24 hour contact period yielded the highest glucose conversion result as 5.67±0.24%. The best KOH pretreatment condition for primary sludge was a 0.5 N KOH concentration for a 0.5 hour contact period. However, no consistent glucose recovery trend as a function of reagent concentration or contact time was identified for any of the sludges or biosolids in this study. Drying and grinding were also found to efficiently improve the acid hydrolysis results.

Fibre content analysis was also performed on the sludge and biomass feedstocks and their residues following pretreatment and acid hydrolysis during this study, to better understand the conversion of these waste biomass feedstock. The Van

Soest methods for neutral-detergent, acid-detergent and acid insoluble lignin analysis and the Weende crude fibre analysis were applied to the sewage sludge and biosolids samples prior to and after acid hydrolysis to determine the fibre content including cellulose, hemicellulose and lignin. A modification to the Weende crude fibre analysis was introduced, where a centrifuge step was added prior to the second filtration after the alkaline digestion of the procedure to reduce filter clogging problems. The centrifuge modification effectively reduced the filtering time from one day to 30 minutes; however, there was an average loss of 46% in crude fibre with the addition of this centrifugation step. It was found that most of cellulose content in the feedstock samples was hydrolyzed to glucose after the acid hydrolysis process and most hemicellulose content was likely to have been solubilized and washed away during acid and alkaline pretreatments and acid hydrolysis. The lignin content did not appear to be affected by the pretreatments applied nor the acid hydrolysis.

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SYMBOLS AND NOMENCLATURE

ABE	Acetone, butanol and ethanol
ADF	Acid-detergent fiber
ADL	Acid-detergent lignin
AFEX	Ammonia fibre explosion
ANOVA	An analysis of variance
AOAC	Association of Official Agricultural Chemists
AS	Ash solid
CF	Crude fibre
CMC	Carboxymethylcellulose
CO	Carbon oxide
CO_2	Carbon dioxide
CH_4	Methane
DNS	3,5-dinitrosalicylic acid
E10	10% (V/V) Ethanol and 90% gasoline blended gasoline
E85	85% (V/V) Ethanol and 15% gasoline blended gasoline
ETBE	Ethyl tertiary butyl ether
FPU/g	filter paper units (FPU) per gram
GHGs	Greenhouse gases
GY	Glucose yield
H_2SO_4	Sulphuric acid

HCl	Hydrochloride acid
HP	Hydrolyzed portion
KOH	Potassium hydroxide
MC	Moisture content
MMT	Methylcyclopentadienyl manganese tricarbonyl
MSW	Municipal solid waste
MTBE	Methyl tertiary butyl ether
NaOH	Sodium hydroxide
NDF	Neutral-detergent fiber
NIRS	Near-infrared spectroscopy
NHP	Non-hydrolyzed portion
PC	Percentage of conversion
RPM	Round per minute
SHF	Hydrolysis-fermentation process
SSF	Simultaneous saccharification fermentation process
TS	Total solid
VS	Volatile solid
MSW	Municipal solid waste

FORMULAE

3.1 $$\%\,\mathrm{MC} = \frac{(\text{initial weight, g - dry weight, g})}{\text{initial weight, g}} \times 100$$

3.2 $$\%\,\mathrm{VS} = \frac{(\text{dry weight, g - ash weight, g})}{\text{dry weight, g}} \times 100$$

3.3 $$\%\,\mathrm{AS} = \frac{\text{ash weight, g}}{\text{dry weight, g}} \times 100$$

3.4 $$\%\mathrm{TS} = \%\mathrm{VS} + \%\mathrm{AS}$$

3.5 $$\mathrm{GY} = \text{Glucose concentration} \times \mathrm{V}$$

3.6 $$\%\mathrm{PC} = \frac{\text{Glucose yield, mg}}{\text{dry weight of initial substrates added, mg}} \times 100$$

A-1 $$w_{\mathrm{f}} = \frac{m2 - m3}{m1}$$

B-1 $$\frac{(Wo - Wt) \times 100}{S}$$

B-2 $$\frac{(Wo - Wt) \times 100}{S}$$

B-3 $$\frac{L \times 100}{S}$$

Chapter 1

INTRODUCTION

1.1 Introduction

As the world population and industry have grown rapidly, large quantities of waste materials have been generated. Meanwhile, with the increased energy consumption caused by rapid industrialization, the exhaustion of fossil fuels has become a serious concern. Ethanol has been used as liquid fuel for several decades, and has a higher octane rating (able to resist compression) than gasoline, enabling combustion engines to run at a higher compression ratio, thus, yielding a superior net performance (Wyman, 1999). By using biomass-derived ethanol, a net reduction in the levels of carbon dioxide (the primary greenhouse gas of concern) as low as 60-90% could be achieved relative to gasoline-consuming vehicles (Brown et al, 1998). The Government of Canada has committed to reducing its greenhouse gas emission by 6% from 1990 levels, between 2008 and 2012, to achieve the goal of the Kyoto Protocol (Champagne, 2007). Thus, ethanol-blended gasoline has the potential to contribute significantly to the reduction of greenhouse gas emissions. To date, the conversion of cellulosic biomass to fuels and other chemicals has been shown to have potential to improve energy security, reduce the trade deficit, dramatically reduce greenhouse gas emission, and improve fuel price stability (Wyman, 1999).

Biomass, the most common form of natural and renewable carbon resource can be employed for energy production. Biomass includes all land- and water- based organisms, and vegetation that have been produced through photosynthesis, as well as all organic wastes. Large amounts of biomass are generated through forestry and agricultural practices, paper-pulp industries, timber industries, agro-industries and municipalities, including waste materials such as crop residues, livestock manures, as well as sludges and biosolids, which contain large quantities of lignocelluloses, polysaccharides, proteins and other organic materials (Champagne, 2007). These organic constituents and their chemical properties (being able to be converted to ethanol and other higher value organic species) provide biomass with a potentially enormous biotechnological value. The conversion of these materials to value-added products has been recognized as an attractive waste management approach. Large amounts of residual plant biomass can potentially be converted into a number of different value-added products, including biofuels and other chemicals, and used as a relatively inexpensive energy source (Willke and Vorlop, 2004). While feedstock costs associated with the utilization of waste biomass are extremely low, the environmental benefits are very important as the process consumes wastes. In addition, the recovery of raw materials from biomass and their conversion to value-added products has the potential to reduce the microbiological production of carbon dioxide (CO_2) and methane (CH_4), while minimizing the potential environmental impacts of greenhouse gas emissions and the strain on non-renewable resource reserves.

The cost of bioethanol as an energy source in the current market, however, is

relatively high compared to that of fossil fuels. Efficient ethanol production processes and inexpensive starting substrates are needed for this approach to be economically viable. Current ethanol production processes using crops such as sugarcane bagasse and corn as starting materials are well established and industrialized (Zaldivar et al., 2001). The increased ethanol production using the current cornstarch-based technology however, may not be possible, because the corn-based production of ethanol increases the stress on and competes with agricultural lands used in food and feed production. The use of less expensive starting substrates such as lignocellulosic waste including crop residues, grasses, sawdust, wood chips and solid waste and livestock manure, therefore, could make bioethanol more competitive with fossil fuels. Recent efforts have concentrated on the utilization of lignocellulose biomass, although the feasibility of using these materials as a feedstock is often limited by the cost of the production process based on currently available technologies. The bottleneck in the conversion process is the low yield and high cost of the pretreatment and hydrolytic processes. The complex physical and chemical composition of organic wastes, which resists being broken down, often makes it difficult to utilize this biomass as a feedstock using processes established for starch-based feedstocks. The big challenge for researchers is to optimize biotechnological processes to lower the processing cost, which will make the use of waste materials for the production of bioproducts more competitive. With the abundance of biomass in Canada, considerable bioproduct production opportunities are expected to emerge in the near future, particularly in the physical, chemical and biological conversion of primary and residual biomass to

bio-based energy and industrial products (Industry Canada, 2004). Extensive research has been focused on the conversion of biomass to bioproducts over the past few decades.

It is very noteworthy that, among all these readily available biomass feedstocks, municipal sewage sludge typically has one of the higher cellulose, but lower lignin content based on its higher paper component, as well as being one of the most inexpensive raw materials. As such, it represents a promising biomass material for bioproduct recovery. The primary research aim of this study will be (1) to investigate the conditions leading to a higher glucose conversion percentage, by comparing physical drying/grinding, as well as acid and alkaline pretreatment processes of municipal sewage sludges and biosolids; (2) to analyze the fibre composition, cellulose, hemicellulose and lignin content using Van Soest's neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin (ADL) analysis (Goering et al.,1970); and (3) to compare the grinding/drying, acid and alkaline treatments and acid hydrolysis effects on different types of sludges and biosolids.

1.2 Objectives

The first purpose of this investigation is to examine the acid hydrolysis of three types of municipal sewage sludges: namely, primary sludge, waste activated sludge and biosolids, under different physical and chemical pretreatment conditions,

expanding upon experimental procedures developed in the previous studies (Li and Champagne, 2005; Henderson et al., 2003; Levy et al., 2003). These pretreatments include: drying/grinding, a range of concentrations of acid and alkaline agents and different catalytic pretreatment contact periods, to determine the more efficient pretreatment conditions for future process optimization, as well as to target the most promising sludge material as a potential lignocellulosic feedstock for conversion to glucose. Specifically, drying and grinding, acid (HCl), and alkaline (KOH) pretreatments were employed on the three types of sewage sludges and biosolids used in this study.

Fibre analysis methodologies have primarily been developed for their application in the characterization of feed materials, such as cereal, livestock feeds, and forage analysis. No specific method has been developed or adapted for the fibre analysis of waste biomass feedstocks, such as sewage sludge. The secondary objective of this study was to analyze the fibre components, cellulose, hemicellulose and lignin, of the sewage sludge using the Van Soest's neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin (ADL) analysis (Goering et al., 1970) by employing methods that have traditionally been applied to the fibre content analysis of animal feed or cell walls in plants (Van Soest, 1963a,b). As well, modifications to the Weende's crude fibre analysis (International Standard) was investigated in order to apply the crude fibre analysis methodology more efficiently to waste biomass and to reduce some procedural issues, including clogging, during the filtration process.

The third objective of this study was to investigate the effects of acid and alkaline pretreatments and acid hydrolysis on the fibre content of different types of sludges and biosolids.

1.3 Organization of Thesis

In Chapter 2 Literature Review, the background and literature review of the feasibility of producing bioproducts from lignocellulosic materials and their conversion processes are presented.

In Chapter 3 Methodology, experimental approaches including physical drying/grinding and acid (HCl) and alkaline (KOH) pretreatments on different municipal sewage sludge samples (primary sludge, activated sludge and biosolids) followed by acid hydrolysis and glucose recovery are presented. The fibre content characterization procedures for cellulose, hemicellulose and lignin analysis using Van Soest's neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin (ADL) analyses are described. Finally, modifications to the Weende's crude fibre analysis (International Standard) are proposed.

In Chapter 4 Results and Discussions, the results of the effects of pretreatment conditions such as physical drying/grinding, acid (HCl) concentration and alkaline (KOH) concentration and duration, of the sludge and biosolid feedstocks on the feasibility of sugar recovery are presented and discussed. The fibre content characterization results of cellulose, hemicellulose and lignin content, for the untreated and pretreated sludges and biosolids and their significance are also

demonstrated. A modification to the crude fibre analysis methodology for waste material is introduced and its advantages and limitations compared to the traditional crude fibre analysis methodology are discussed.

In Chapter 5 Conclusions and Recommendations, the research conclusions are drawn, and recommendations for future research derived from the thesis study are outlined.

Chapter 2

LITERATURE REVIEW

2.1 Introduction

As the world population and industries continue to grow rapidly, large quantities of waste material are being generated, from which the need to establish better waste management approaches has arisen. With the coincident increase in energy consumption, the exhaustion of fossil fuels has become a serious concern and a great deal interest has emerged in exploring alternative energy sources, including bioenergy derived from waste biomass. Biomass is the most common form of natural and renewable carbon resource which can be employed for energy production. The energy from the sunlight is stored in the chemical bonds of the organic biomass (McKendry, 2002a). Biomass includes all land- and water-based organisms, vegetation which has been produced through photosynthesis, as well as all organic wastes. Large amounts of biomass are generated through forestry and agricultural practices, pulp and paper industries, timber industries, agro-industries and municipalities, in addition to waste materials such as crop residues, livestock manures, sludges and biosolids, which contain large quantities of lignocellulose, polysaccharides, proteins and other organic materials (Champagne, 2007). The chemical

properties of various components of the lignocellulosic biomass give them an enormous biotechnological value. Hence, the conversion of these materials to value-added products has been recognized as an attractive waste management approach. Large amounts of residual plant biomass can potentially be converted into a number of different value-added products including biofuels and chemicals; and its use as a relatively inexpensive energy source makes biotechnological conversion processes replacing conventional chemical techniques attractive (Willke and Vorlop, 2004). In addition, the recovery of raw materials from biomass and their conversion to value-added products has the potential to reduce the microbiological production of carbon dioxide (CO_2) and methane (CH_4), while reducing potential environmental impacts and the strain on non-renewable resource reserves.

With the abundance of biomass in Canada, there is a large potential for bioproduct recovery, particularly resulting from primary and residual biomass conversion to bio-based energy and chemical products by physical, chemical and biological processes (Industry Canada, 2004). Extensive research has been focused on the conversion of biomass to bioproducts, including bioethanol, over the past few decades. However, the cost of bioethanol as an energy source remains relatively high compared to that of fossil fuels. Efficient ethanol production processes and inexpensive substrates are needed for this approach to be economically viable. Current ethanol production processes using crops such as sugarcane bagasse and corn as substrates are well established (Zaldivar et al., 2001). However, increased ethanol production may not be possible using the current

cornstarch-based technology, because crop-based production for ethanol could increase the stress on agricultural lands traditionally used for food and feed production. The use of less expensive substrates such as lignocellulosic waste including crop residues, grasses, sawdust, wood chips, solid wastes and livestock manures could make bioethanol more competitive with fossil fuels. Recent efforts have concentrated on utilizing lignocellulosic biomass. Recent investigations for bioethanol production from lignocellulosic waste materials were presented in a review by Champagne (2007), including: crop residues (Kim and Dale, 2004; Cozens and Miller, 1997; Zayed and Meyer, 1996; Rivers and Emert, 1988), municipal solid waste (Mtui and Nakamura, 2005; Lark et al., 1997; Green and Shelef, 1989; Green et al., 1988), forest product industry waste (Kadar et al., 2004; Fan et al., 2003; Duff and Murray, 1996), leaf and yard waste (Lissen et al., 2004), municipal sludge (Cheung and Anderson, 1997), as well as dairy and cattle manures (Chen et al., 2004; Wen et al., 2004; Chen et al., 2003). However, the complex physical and chemical composition of organic wastes, which often resists degradation, often make them difficult to utilize. The bottleneck in the biochemical conversion process is the low yield and the potentially high costs of the pretreatment and hydrolysis processes. The primary challenge for researchers is to optimize biotechnological processes to lower the cost, which will make the use of waste materials for the production of bioproducts more competitive.

This Chapter will present an overview of some of the biotechnologies used in deriving higher-value bioproducts. The main focus will be to summarize the current status

of bioproducts; the processes involved in the production of bioethanol from biomass; the potential for existing and developing approaches which could be employed to reduce overall process costs; and to explore the feasibility of using municipal waste biomass for bioproduct recovery.

2.2 Biomass

2.2.1 Fibre Characterization

Biomass is considered to be the mass of organic material originating from any biological material, and by extension, any large mass of biological matter (Howard et al., 2003). The chemical properties of the components of lignocellulose make it a substrate of enormous biotechnological value (Malherbe and Cloete, 2003).

Biomass is a complex substrate mostly made up of three fractions: cellulose, hemicelluloses and lignin. Cellulose is generally the largest fraction, representing about 40–50% of the biomass by dry-weight, while the hemicellulose fraction represents 20–40% of the material by dry-weight. Approximately 25% of the material is lignin and the remaining 5% accounts for other extractives (Wyman, 1994).

Cellulose is the major component of the cell wall fibre. The structure of cellulose is presented in Figures 2.1. Figure 2.1 shows that cellulose, a high molecular weight molecule, is a linear polymer of D-glucose linked together by β-1,4-glycosidic

bonds to form a highly crystalline material.

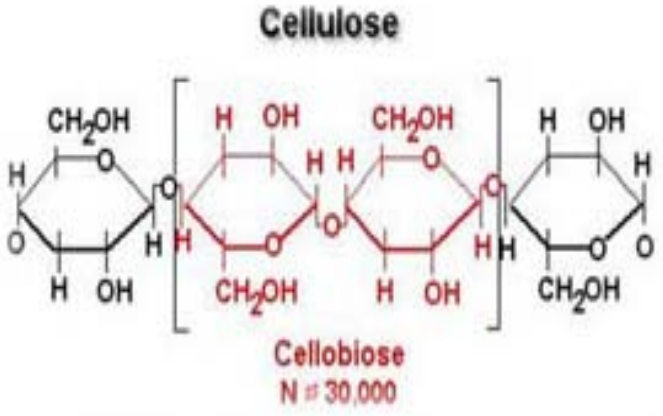

Figure 2.1 Structure of Cellulose (Oregon State University, 2006)

The number of chain units, which is also referred as the degree of polymerization, varies for different cellulosic materials. The glucosidic bonds in chain molecules, along with the hydroxyl groups, mainly determine its chemical properties. The extensive hydrogen bonding, holding the chains together, occurs between linear molecules resulting in a strong microcrystalline structure, which makes cellulose completely insoluble in water. However, cellulose can be swelled or hydrolyzed enzymatically or chemically using diluted or concentrated acid (Fan et al., 1987).

Hemicellulose is composed of shorter chained polysaccharides and has a much lower molecular weight than cellulose. It surrounds the cellulose fibres and is interwoven through the cellulose pores. This component provides a linkage between lignin and cellulose. It exists in an amorphous form in nature and can be divided into three groups, xylans, mannans and galactans, which can exist separately as single components or

collectively (Fan et al., 1987). Hemicellulose differs from cellulose in consisting primarily of xylose and other five-carbon monosaccharides. Hemicellulose is more easily hydrolyzed than cellulose (Brigham et al., 1996). The composition of hemicellulose differs depending on the source of raw material, as well as varies between vegetation species, particularly between soft- and hardwoods (Wiselogel et al., 1996).

Lignin is a complex, high molecular-weight compound in nature. It is essentially a three dimensional phenylpropane polymer with phenylpropane units held together by ether and carbon-carbon bonds. Lignin surrounds and strengthens the cellulose-hemicellulose framework, which provides structural rigidity by holding the fibres of polysaccharide together (Fan et al., 1987).

Different biomass materials contain varying quantities of cellulose, hemicellulose, lignin and a small amount of other extractives. The relative proportions of cellulose and lignin is one of the determining factors in identifying the suitability of plant species for subsequent processing.

2.2.2 Methodology of Fibre Characterization

The utilization of waste biomass such as sewage sludge and biosolids for the recovery of value-added products requires chemical characterization to provide a better understanding of the recovery process and insights into improving process efficiencies. Information about the detailed chemical composition of waste biomass, particularly fibre

content, including cellulose, hemicellulose, and lignin, is critical for its future utilization as a feedstock for the extraction of value-added product.

There are a number of methods used in forage fibre analysis, but each method has its own advantages and limitations, which are summarized in Table 2.1. The choice of fibre characterization approach depends on the objective of the research.

Table 2.1 Uses and limitations of fibre analysis methodologies (Jung, 1997)

Method of analysis	Forage fraction measured	Limitation of method
Crude fibre	Portion of plant cell wall, complete cellulose recovery	Most noncellulosic polysaccharides and lignin removed
Neutral detergent fibre (NDF)	Incompletely digestible feed fraction, almost complete recovery of grass cell walls	Pectin almost completely removed, protein and starch removal can be a problem
Acid detergent fibre (ADF)	Portion of plant cell wall, complete cellulose recovery	A significant portion of the lignin is solubilized
Acid detergent lignin (ADL)	Lignin	Lignin solubilization at ADF step, especially in grasses
Dietary fibre	Complete recovery of cell wall polymers	Protein and starch removal can be difficult
Uppsala dietary fibre	Total cell wall recovery and composition of the cell wall	Complexity of method
Crampton-Maynard	Cellulose	Small amount of xylan contamination
ADF minus ADL	Cellulose	Suffers from limitations of ADF and ADL methods
NDF minus ADF	Hemicellulose	Suffers from limitations of NDF and ADF methods
Klason lignin	Lignin	Possible protein and carbohydrate contamination

In forage fibre analysis, the fibre analysis is mostly focused on the determination of the concentration of fibre in a particular feed. Crude fibre (CF) determination is a

gravimetric methodology in the proximate or Weende system of analysis, which is the oldest methodology still in use today (Henneberg and Stohmann, 1859). Crude fibre is composed primarily of cellulose and variable proportions of noncellulosic polysaccharides and lignin in the samples (Hindrichsen et al., 2006). However, because CF method only recovers a portion of cell wall polysaccharides and lignin, it underestimates the total plant cell wall content of a feedstock (Van Soest, 1994). It continues being used today because it is recognized as an official Association of Official Agricultural Chemists (AOAC) method for feed analysis, a large database has been accumulated for a wide variety of feedstocks and it is a relatively easy method of analysis. Many modifications on crude fibre analysis have been studied in last century. The use of an Oklahoma filter screen or a California Büchner filter was proposed as alternative filters to limit filtration problems (Holt, 1962). Holst and Gehrke (1975) developed a new method for the analysis of crude fibre using Holst filtration apparatus which eliminated the use of asbestos and thereby the possible inhalation of asbestos fibre (Holst and Gehrke, 1975).

In ruminant nutrition, the neutral detergent fibre (NDF) method developed by Van Soest has largely replaced CF analysis (Van Soest, 1963b). It provides a more satisfactory alternative to better characterize the carbohydrates in the plant cell wall (Van Soest et al., 1991). The neutral detergent fibre (NDF) method is also a gravimetric method, which employs a chemical extraction with a neutral detergent solution under reflux followed by

the determination of the fibre residue. Neutral-detergent fibre is considered to represent the entire fibre fraction of the feedstock, however, it has been reported that water-extractable and pectinous polysaccharides are soluble in neutral detergent which can lead to the underestimation of cell wall concentration (Carre and Brillouet, 1986; Reichert, 1981; Bailey and Ulyatt, 1970). As well, starch and protein may contaminate the NDF residue (Theander and Åman, 1980). Theander et al. (Theander et al., 1990) developed methods by incubation with thermostable α-amylase to gelatinize and hydrolyze starch to effectively eliminate the starch fraction (Van Soest et al., 1991). Heat-damaged proteins in processed feedstocks are also retained in the NDF fraction, which can, therefore, overestimate fibre content. These limitations of the NDF methodology in determining cell wall concentration are a concern if one is interested in the plant cell wall as the incompletely digestible fraction of feedstocks (Jung, 1997). Acid detergent fibre (ADF) represents a portion of the plant fibre including the cellulose and lignin from cell walls and variable amounts of xylans and other constituents (Van Soest, 1963a). A common variation of the ADF method is to use NDF as a pretreatment (Van Soest and Robertson, 1980). In this approach, the fractions of the fibre that are insoluble either in neutral detergents or in acid detergent are measured, and the residue after treatment of the ADF fraction with 12 mol/L sulphuric acid is considered to be acid detergent lignin (ADL). The fibre fraction differences obtained with each of these fibre analyses are used to determine the hemicellulose (NDF-ADF) and cellulose (ADF-ADL) contents.

Some semi-automatic instruments (Ankom 200 Fibre Analyzer, Ankom Technology Corp., Fairport, NY, and Fibretec I, Perstop Analytical, Silver Spring, MD) and analyses have been developed for NDF and ADF concentration analysis methodologies to increase the analytical capacity (Jung, 1997). As well, gas chromatography and near-infrared spectroscopy (NIRS) methodologies have been developed to determine composition (Martin et al., 1985).

2.3 Bioproducts from the Recovery of Sugars from Biomass

2.3.1 Xylitol

Xylitol is a five-carbon sugar alcohol which can be produced by the chemical reduction of xylose, derived from the hydrolysis of hemicellulose, under alkaline conditions. Xylitol has drawn much attention because the bulk production of xylitol can be consumed in food products as a natural food sweetener, a dental cavity reducer and as a sugar substitute for diabetics. The bioconversion of hemicellulose to produce xylitol has been studied using microorganisms and their enzymes, because its chemical hydrolyzation production has introduced a number of concerns including high pressure and temperature requirements, the use of expensive catalysts and the need for extensive separation steps to remove by-products (Meinander et al., 1994). Various bioconversion methods have been studied for the production of xylitol from hemicellulose by

microorganisms, including: fermentation processing by yeasts, bacteria or fungi, and enzymatic processes (Nigam and Singh, 1995). Lavarack et al. (2002) investigated the acid catalyzed hydrolysis (diluted sulphuric acid or hydrochloric acid) of bagasse and similar materials to break down the constituent hemicellulose to produce xylose and other sugars. *Candida peltata* NRRL Y-6888 was studied by Saha and Bothast (1999) to determine its ability to ferment xylose to xylitol under different fermentation conditions such as pH, temperature, aeration, substrate concentration and in the presence of glucose, arabinose, ethanol, methanol and organic acids. Various xylose-rich hemicellulosic materials can be used as inexpensive feedstocks for xylitol production by fermentation, because of the large requirement for xylitol in the food industry as an alternative sweetener, which drives the development of biotechnological applications and reduces the cost of the xylitol production process.

2.3.2 Higher Value Bioproducts

Aside from bioethanol production, a variety of products such as organic acids, amino acids, vitamins and a number of bacterial and fungal polysaccharides such as xanthan can be produced through the fermentation of lignocellulosic residues. Hemicellulose is an available source of xylose from which xylitol and furtural can be derived, which has drawn much interest from the food production industry (Howard et al., 2003).

The biotechnological production of acetone, butanol and ethanol through the ABE-process (Acetone, Butanol, and Ethanol process) is one option for utilizing hydrolyzed starch or cellulose. Butanol can be used as a fuel additive because of its relatively high calorific value, low vapour pressure and low miscibility with water. In 2001, its volume increased by 3% on the US market (mainly as chemical feedstock and solvent) (TIG, 2002). ABE production is generally derived from cornstarch. The classical "Weizmann process" developed in the early 20th century has been recently modified to significantly reduce processing costs (Willke and Vorlop, 2004). A mixture of biodiesel with approximately 18% ABE can be produced, and can also be used as a No.2 diesel substitute, which does not require further purification (Crabbe et al., 2001). Several years ago, research involving the development of a hyper-amylolytic culture (*Clostridium beijerinckii* BA101) to hydrolyze starch and starch-based peanuts and agricultural wastes, and use them for the production of butanol was conducted by University of Illinois (Jesse et al., 2002), to reduce the overall cost associated with the production of butanol. The advantage of this system was a reduction in the inhibition of fermentation by the acid hydrolysis.

2.4 Ethanol Production from Biomass

There is a significant worldwide market for bioethanol, where ethanol is either used as a chemical feedstock, liquid fuel, octane enhancer or petroleum additive. The

technology development focus for the production of bioethanol has shifted towards the utilization of residual lignocellulosic materials in an attempt to lower production costs.

Ethanol is considered to be an excellent automotive fuel that can be used directly or mixed with gasoline in different ratios to form what has been termed gasohol. The most common blends are E10 (10% ethanol, 90% gasoline) and E85 (85% ethanol and 15% gasoline). Instead of ethyl tertiary butyl ether (ETBE), or methyl tertiary butyl ether (MTBE), ethanol can provide oxygen when blended with gasoline, which can reduce tailpipe emissions of carbon monoxide and unburned hydrocarbons through a more complete combustion, and its lower vapor pressure than gasoline can reduce smog formation by decreasing evaporative emissions (Wyman, 1999). Furthermore, ethanol can also be used as an antiknocking agent instead of lead, benzene or methylcyclopentadienyl manganese tricarbonyl (MMT), to reduce the health impacts from these harmful antiknocking agents (Li, 2004).

The potential global warming effects of major greenhouse gases (GHGs) have drawn people's attention to the need to reduce GHG emissions worldwide. A study carried out by Wang et al., (1999) at the US Center for Transportation Research, Energy Systems Division indicated that by 2010, the use of E10 could achieve a 7% reduction in petroleum use, an 8–10% reduction in GHG emissions, and an 8–9% reduction in fossil energy use if derived from cellulosic feedstocks by around 2010. Similarly, the use of E85 could achieve a 71–73% reduction in petroleum use, 68–91% reduction in GHG emissions, and a 71–75% reduction in fossil energy use by around 2010, while the use of

E95 could achieve an 83–85% reduction in petroleum use, a 79–105% reduction in GHG emissions (the more than 100% reduction for GHG emissions results from GHG emissions offsets in electrical power generation), and an 82–86% reduction in fossil energy use by around 2010 (Wang et al., 1999). Thus, using ethanol as a gasoline additive could significantly reduce GHG emissions.

The bioethanol industry also solves the problem of disposing large quantities of waste materials. Agricultural and forestry residues have traditionally been burned, which generates air pollution such as particulate matter and carbon monoxide. The use of biomass residues for ethanol production has the potential to significantly reduce air pollution. In addition, the amount of waste to landfill can be reduced which can extend the operational life of the landfill (Li, 2004) and decrease the burden on communities with limited land availability for this type of land use.

Established technologies for ethanol production are based on the recovery of crop materials, including cornstarch or sugarcane juice. As the demand for ethanol increases, the price of crops which have traditionally been used in ethanol production will also likely increase accordingly. The demand for ethanol production is expected to increase to 1.4 billion liters by 2010, as proposed by the Canadian government (IEA Bioenergy Task 40, 2006). A large demand for substrates for ethanol production could potentially limit land use for agricultural and feed production. Thus, alternative feedstocks will be needed to meet this anticipated demand (Sun and Cheng, 2001). Research efforts have shifted to the use of lignocellulosic biomass and waste materials as feedstocks as a promising

resource for bioethanol production due to their abundance and renewability.

2.4.1 Characterization of Biomass Feedstocks for Bioethanol Production

Biomass which contains a large lignocellulosic fraction presents a feasible low-cost resource for ethanol production. Biomass materials can generally be categorized as energy crops, agricultural residues, municipal solid waste and forestry residues. The content of cellulose, hemicellulose and lignin generally differs for each feedstock.

2.4.1.1 Energy Crops

Energy crops are fast-growing, drought and pest resistant crops grown specifically for the purpose of producing energy, such as biofuels, electrical and thermal energy. They are typically selected for their advantageous environmental qualities such as erosion control, soil organic matter build-up and reduced fertilizer and pesticide requirements. There are many plant species which could be used as energy crops, including eucalyptus, willows and poplars, sorghum, sugarcane and artichokes, soya beans, sunflowers, cotton and rapeseeds (Demirbaş, 2001). Due to their high cellulose content, energy crops are useful for bioethanol production. In Canada, switchgrass, well adapted to marginal soils, as well as highly drought and pest resistant, has been investigated as a potential feedstock for the bioethanol industry (Samson and Omielan, 1992).

2.4.1.2 Agricultural Residues

Agricultural residues can be used as feed and bedding materials for livestock, as well as raw materials for strawboard production. In many areas, agricultural residues have been harvested and returned back to the field to nourish the land in order to protect the soil from wind and water erosion and maintain the organic matter content. Agricultural residues are also available as feedstock for ethanol production, which also creates economical benefits for farmers because in many cases, agricultural residues need to be disposed of at a cost to farmers. To ethanol producers, agricultural residues could be obtained at relatively low costs. In addition, such residues are usually located in a crop processing region, where collection infrastructure and transportation facilities have generally already been established, thus the collection costs associated with these feedstock materials could be quite low (Li, 2004). Chen et al. (2004) conducted research on the extraction of value-added products from dairy and cattle manures and developed a process for hydrolyzing lignocellulosic materials from these manure feedstocks into ethanol.

2.4.1.3 Municipal Solid Waste (MSW)

Municipal solid waste (MSW) is generated by household and commercial activities and wastewater treatment residues such as sewage sludge and biosolids. Most

MSW is currently disposed of in landfill sites. There are other disposal methods such as incineration or composting to produce a fertilizer. The disposal of this waste is a growing problem worldwide. Landfills use large areas of land, and pose sanitary and health threats to surrounding neighborhoods. Landfills also generate large quantities of gas known as biogas, largely composed of methane and carbon dioxide (considered GHGs), resulting from the decomposition of the organic component of the waste (Li, 2004). These issues have led to the development of other uses for MSW. Much of the waste could be used for energy production through incineration, composting or other processes to divert a fraction of the waste materials going into landfills and to prolong their operational life. The utilization of MSW for ethanol production is a good alternative for waste reduction. Paper and food residues are the main cellulosic materials in MSW, and this constituent could be converted to ethanol. Since paper pulping processes remove most of the lignin and hemicellulose, paper typically has a relatively high cellulose content, and does not need extensive pretreatment as is generally required for crops and wood feedstocks (Li, 2004).

2.4.1.4 Wastewater Sewage Sludges

Wastewater sewage sludges or biosolids, are generated from municipal and industrial wastewater treatment processes. Primary sludge, with 3-5% solids, is generated through primary sedimentation, which contains a large amount of organic matter, metals and other residues. The organic matter and nutrients are dissolved and reduced through

biological processes using different types of microorganisms. Activated sludge is accumulated in secondary clarifiers following biological treatment processes. Digested sludge or biosolids is the product of anaerobic digestion. The most common disposal method for these residuals is land application. Viable alternatives for the use and disposal of biosolids need to be considered. One option is the possible recovery of beneficial constituents from the biosolids for use as marketable commodities. Since paper is the main cellulosic material in sewage sludge, sewage sludge can be used as a feedstock for hydrolysis to produce glucose and ethanol.

2.4.1.5 Forestry Residues

Wood residues are another source of lignocellulosic feedstocks for ethanol production which are residues from manufacturing sites such as sawmills and pulp mills. Other wood residues are also produced including small branches which are not suitable for wood or pulp production (Duff and Murray, 1996). In some regions, forests are being actively thinned and fallen branches removed in order to reduce the risk of forest fires. Both of these practices would lead to a significant increase in the quantity of wood residues which could be used as feedstocks for fuel ethanol production in Canada (CRFA, 2004). However, the technology for ethanol production from wood residues is not as advanced as it is for the conversion of agricultural residues. As well, the collection, processing and transportation of the wood residue are comparatively costly (CRFA,

2004).

2.4.2 Biomass to Ethanol Conversion Processes

Ethanol can be produced through the fermentation of glucose which can be obtained by breaking the long chains of glucose from cellulosic materials using specific bacteria, fungi, or yeast. The conversion includes two steps: hydrolysis to fermentable reducing sugars and sugar fermentation to ethanol. The hydrolysis can be catalyzed using acid or cellulose enzymes, and fermentation carried out by yeast or bacteria. The presence of lignin plays a role as a physical barrier which blocks the access of the cellulase enzymes to the cellulose. Thus, pretreatment is generally required in the conversion process in order to increase the yield of ethanol produced.

2.4.2.1 Pretreatment Processes for Lignocellulosic Biomass

The benefits of pretreatment of lignocellulosic materials in ethanol production have long been recognized (McMillan, 1994). The factors that have been noted to affect the hydrolysis of cellulose include porosity, accessible surface area of the lignocellulosic materials, cellulose fibre crystallinity, as well as lignin and hemicellulose content (McMillan, 1994). Thus, to utilize lignocellulosic biomass, it must first be pretreated to increase its surface area, bulk density and decrease the crystallinity of the cellulose, to

make it more accessible for further hydrolysis.

Pretreatment is required to change the structure of cellulosic materials to remove impediments to hydrolysis, such as lignin, which consequently improves the rate of further hydrolysis and increases the yield of fermentable sugars from feedstock materials.

The main objectives of pretreatment include: (1) improving the formation of sugars or the ability to subsequently form sugars through the hydrolysis process; (2) avoiding the loss of carbohydrate; (3) avoiding the formation of inhibitory by-products to subsequent hydrolysis and fermentation processes; and (4) making the overall conversion process cost effective (Sun and Cheng, 2002). The primary pretreatment methods include mechanical size reduction, alkali swelling, acid hydrolysis, steam and other fibre explosion techniques, as well as biological methods. These approaches can be categorized as physical, chemical, physico-chemical and biological. Depending on the type of biomass material, one or a combination of these methods in varying sequence can be used to improve the conversion of cellulose molecules.

Physical Pretreatment

Waste materials can be comminuted by chipping, grinding and milling to reduce cellulose crystallinity. Various kinds of mills have been evaluated including ball, hammer, vibratory (Millet et al., 1976) and two-roll mills (Ramos, 2003). Comminution can effectively reduce the particle size of the substrate, increase the available surface area and decrease the cellulose crystallinity and degree of polymerization. However, the major

disadvantage is the potentially high energy requirement (Sun and Cheng, 2002).

Physico-Chemical Pretreatment

Steam explosion (autohydrolysis) is the most commonly used physico-chemical pretreatment. Biomass is treated in a batch reactor at high temperature and high pressure, followed by mechanical disruption of the pretreated material either by violent discharge into a collection tank (explosive) or by mild blending after bleeding the steam pressure down to atmospheric (non-explosive) (Ramos, 2003). Typically, the initial temperature of steam is around 160-260^{o}C and the corresponding pressure is 0.69-4.83 MPa. Factors that affect steam explosion pretreatment are residence time, temperature, material size and moisture content (Duff and Murray, 1996).

Steam explosion can also be enhanced by adding sulphuric acid (H_2SO_4) (or sulphur dioxide SO_2) or carbon dioxide (CO_2), which can effectively improve enzymatic hydrolysis, by decreasing the inhibition to hydrolysis, and enhancing the removal of hemicellulose (Morjanoff and Gray, 1987). The advantages of steam explosion pretreatment include the low recovery costs and low energy requirement compared to mechanical comminution (Holtzapple et al., 1989). It also has limitations, including destruction of a portion of the xylan fraction, disruption of the lignin–carbohydrate matrix and generation of compounds that may be inhibitory to microorganisms used in downstream processes (Mackie et al., 1985). However, steam explosion is not suitable for all types of biomass. It can be highly effective for hardwoods and agricultural residues,

but less effective for softwoods (Clark and Mackie, 1987).

Ammonia fibre explosion (AFEX) is another physico-chemical pretreatment which has been used for many types of lignocellulosic materials including alfalfa, wheat straw, wheat chaff (Mes-Hartree et al., 1988), barley straw, rice straw, corn stover (Vlasenko et al., 1997), municipal solid waste, softwood newspaper (Holtzapple et al., 1992a), coastal Burmuda grass, switch grass (Reshamwala et al., 1995), aspen chips (Tengerdy and Nagy, 1988) and bagasse (Holtzapple et al., 1991). In this pretreatment, biomass materials are exposed to liquid ammonia at moderated temperature (60^{o}C to 100^{o}C) under high pressure (250-300psi) for a period of time and then the pressure is reduced (Mosier et al., 2004). The AFEX pretreatment does not produce inhibitory products for the downstream biological processes, hence, wash water is not required (Mes-Hartree et al., 1988; Dale et al., 1984). Also, AFEX pretreatment efficacy does not depend on small particle sizes prior to pretreatment (Holtzapple et al., 1990), hence, a physical pretreatment such comminution is not necessarily required. The AFEX pretreatment does not significantly solubilize hemicellulose compared to other pretreatment approaches such as acid pretreatment and acid catalyzed steam explosion (Mes-Hartree et al., 1988; Vlasenko et al., 1997). However, the AFEX process is not very effective for biomass with a high lignin content (McMillan, 1994).

Carbon dioxide (CO_2) explosion is also a physico-chemical pretreatment process. Carbon dioxide forms carbonic acid and increases the rate of hydrolysis following this pretreatment. The yield is relatively low compared to steam or AFEX pretreatments, but

high compared to the enzymatic hydrolysis without pretreatment (Dale and Moreira, 1982). Zheng et al. (1998) compared CO_2 explosion with steam explosion and AFEX in the pretreatment of a recycled paper mixture, sugarcane bagasse and recycled paper repulping waste. It was found that CO_2 explosion was more cost effective than AFEX and did not result in the formation of the inhibitory compounds that typically occurred in steam explosion.

Chemical Pretreatment

Several chemicals can be used to break and dissolve the crystalline structure of lignocellulose, including alkaline, acids and other cellulose solvents. However, the use of certain cellulose solvents, such as organic acid is a less desirable pretreatment method due to its high cost, as well as its corrosive, toxic and hazardous properties.

Traditionally, concentrated acid hydrolysis with strong acids such as concentrated sulphuric and hydrochloric acids, has been the most commonly used pretreatment method for lignocellulosic materials. However, concentrated acids are toxic, corrosive and hazardous and require corrosion-resistant reactors. In addition, concentrated acids must be recovered after hydrolysis to make the pretreatment process economically viable (Sivers and Zacchi, 1995). Dilute sulfuric acid pretreatment has been shown to achieve high reaction rates and significantly improve cellulose hydrolysis (Esteghlalian et al., 1997). According to a review written by Sun and Cheng (2002), two types of pretreatment processes which mainly employ dilute acid have been utilized: a continuous flow process

for low solids loadings (5–10%) at high temperatures (typically > 160^{o}C) (Converse et al., 1989; Brenna et al., 1986) and a batch process for high solids loadings (10–40%) at low temperatures (typically <160^{o}C) (Esteghlalian et al., 1997; Cahela et al., 1983). Although dilute acid pretreatment can significantly improve cellulose hydrolysis, its cost is usually higher than some physico-chemical pretreatment processes such as steam explosion or AFEX. A neutralization of pH is necessary for downstream enzymatic hydrolysis or fermentation processes (Sun and Cheng, 2002).

Alkaline pretreatment can also be used for the pretreatment of lignocellulosic materials. The lignin content of the materials is the one factor affecting the efficiency of the pretreatment (McMillan, 1994; Fan et al., 1987). The primary mechanism of alkaline hydrolysis is the saponification of intermolecular ester bonds crosslinking xylan hemicellulose and other components, in order to increase the porosity of lignocellulosic materials (Tarkow and Feist, 1969). Dilute NaOH treatment of lignocellulosic materials causes swelling, leading to an increase in internal surface area, a decrease in crystallinity, the separation of structural linkages between lignin and carbohydrates, and the disruption of the lignin structure (Fan et al., 1987). Pretreatment with ammonia has more recently been shown to be effective in improving cellulose digestion with the advantage that ammonia can be recovered and recycled due to its volatility (Wyman et al., 2005a). Ammonia decrystallizes crystalline cellulose and deacetylates acetyl linkages (Gollapalli et al., 2002; Mitchell et al., 1990). Both of these effects increase the enzymatic hydrolysis of cellulose.

Biological Pretreatment

In biological pretreatment processes, microorganisms such as brown, white and soft rot fungi are used to degrade lignin and hemicellulose in organic materials (Schurz and Ghose, 1978). Brown rots mainly affect cellulose, while white and soft rots attack both cellulose and lignin (Sun and Cheng, 2002). The advantages of biological pretreatments include their low energy requirements and relatively mild operational conditions. However, the rate of hydrolysis in most biological processes is very low compared to physical and chemical pretreatment processes (Sun and Cheng, 2002).

2.4.2.2 Hydrolysis Processes of Biomass

In order to break down the complex structure of lignocellulose for the conversion of biomass to its corresponding mono-sugars, which fermenting bacteria can then employ for ethanol production, there are two major hydrolytic processes that can be successfully employed to produce a variety of reducing sugars: acid hydrolysis and enzymatic hydrolysis.

Acid hydrolysis

Acid hydrolysis is widely used to treat lignocellulosic materials. The β-1,4-glucosidic bonds of the cellulose chain are split with the addition of water molecules in the acid medium; the addition yields fragments of shorter chain lengths

while preserving the basic structure (Fan et al.,1987). Acid first cleaves the matrix structure of the fibre into cellulose, hemicellulose, and lignin, and then further reduces these polysaccharides to mono-sugars. There are generally two types of acid hydrolyses which differ depending on their concentration, temperature and reaction time. Concentrated acid is used under conditions of low temperature and short reaction times and dilute acid under conditions of high temperature and longer reaction times. The main advantages of using dilute acid in the hydrolysis process are that acid recovery may not be required, and it is less corrosive and hazardous in terms of operational process environment. However, the yields of glucose are relatively poor in the hydrolysis step, which results in lower ethanol yields. Compared to the dilute acid hydrolysis process, concentrated acid hydrolysis produces higher sugar yields, at lower temperatures and shorter reaction times are required. However, specialized vessels to prevent excessive corrosion and good acid recovery processes are typically required for the process to be economically viable. With the present requirements for increasingly stringent environmental controls, the waste treatment problems posed by byproduct formation and highly acidic off-streams conspire to limit the likelihood of future implementation of acid hydrolysis technologies.

Enzymatic hydrolysis

Enzymatic hydrolysis of cellulose is highly specific, and must be carried out by cellulase enzymes. Compared to acid hydrolysis, enzymatic hydrolysis is usually

conducted under mild conditions (pH 4.8 and temperature 45-50^{o}C) and does not generally cause environmental concerns (Duff and Murray, 1996).

Cellulases, the enzymes that can degrade cellulose, perform a crucial task by catalyzing the hydrolysis of cellulose to soluble, fermentable carbohydrates. These enzymes are synthesized by fungi, bacteria and plants, but recent research has focused primarily on fungal and bacterial cellulases which can be produced both aerobically and anaerobically, under mesophilic or thermophilic conditions.

The enzymatic degradation of cellulose to glucose, represents the most desirable fermentation feedstock. It is generally accomplished by the synergistic action of three distinct classes of enzymes:

(1) The endo-1,4-β-glucanases or 1,4-β-D-glucan-4-glucanohydrolases (EC3.2.1.4), act randomly on soluble and insoluble 1,4-β-glucan substrates and are commonly measured by detecting the reducing groups released from carboxymethylcellulose (CMC)

(2) The exo-1,4-β-D-glucanases, including both the 1,4-β-D-glucan glucohydrolases (EC 3.2.1.74), liberate D-glucose from 1,4-β-D-glucans and hydrolyze D-cellobiose slowly, and 1,4-β-D-glucan cellobiohydrolase (EC3.2.1.91), which liberates D-cellobiose from 1,4-βglucans

(3) The β-D-glucosidases or β-D-glucoside glucohydrolases (EC3.2.1.21), acts to release D-glucose units from cellobiose and soluble cellodextrins, as well as an array of glycosides (Coughlan and Ljungdahl, 1988).

In addition to the three major groups of cellulose enzymes, there are also a number of ancillary enzymes that attack hemicellulose, such as glucuronidase, acetylesterase, xylanase, β-xylosidase, galactomannanase and glucomannanase (Duff and Murray, 1996).

There are several factors that may affect the enzymatic hydrolysis of cellulose, including substrate feedstock, cellulase activity and reaction conditions (Sun and Cheng, 2002). Substrate concentration is one of the main factors that affect the yield and the initial rate of enzymatic hydrolysis of cellulose. An increase in substrate concentration was found to result in an increase in the yield and reaction rate of the hydrolysis at low substrate levels (Cheung and Anderson, 1997). However, high substrate concentrations can cause substrate inhibition, which substantially lowers the rate of hydrolysis and the extent of substrate inhibition depends upon the ratio of total substrate to total enzyme (Penner and Liaw, 1994; Huang and Penner, 1991). Increasing the dosage of cellulase to a certain extent can enhance the yield and rate of hydrolysis, but consequently significantly increases the cost of the process. A cellulase dosage of 10 FPU/g cellulose is often used in laboratory studies because it provides a hydrolysis profile with high levels of glucose yield within a reasonable time (48–72 h) at a reasonable enzyme cost (Gregg and Saddler, 1996).

The enzymatic hydrolysis of cellulose consists of three steps: the adsorption of cellulase enzymes onto the surface of cellulose, the biodegradation of cellulose to fermentable sugars and the desorption of cellulase. The irreversible adsorption of

cellulase onto cellulose was found to be partially responsible for the decrease in enzyme activity during hydrolysis in a study by Converse et al. (1988). The addition of surfactants during hydrolysis can modify the cellulose surface property and reduce the irreversible binding of cellulase to cellulose. And the cellulases can be recovered from the liquid supernatant. These have included non-ionic Tween 20, 80 (Wu and Ju, 1998), polyoxyethylene glycol (Park et al., 1992), Tween 81, Emulgen 147, amphoteric Anhitole 20BS, cationic Q-86W (Ooshima et al., 1986), sophorolipid, rhamnolipid and bacitracin (Helle et al., 1993). Enzyme recycling can effectively increase the rate and yield of hydrolysis and lower the enzyme cost (Mes-Hartree et al., 1987). Cellobiose and, to a lesser extent, glucose in the hydrolysis system can inhibit cellulase activity. High concentrations of enzymes, the supplementation of β-glucosidases during hydrolysis and the removal of sugars during hydrolysis by ultrafiltration or stimultaneous saccharification and fermentation (SSF) process have been developed to decrease the inhibition. The SSF process has been extensively studied and has been demonstrated to reduce the inhibition effects of end products during the hydrolysis process (Zheng et al., 1998; Saxena et al., 1992).

2.4.2.3 Sugar Fermentation

Yeast fermentation has been considered to be a mature technology for bioethanol production for many years, where future scientific improvements will only result in lower

fermentation costs (Foody and Foody, 1991). However, traditional yeast fermentation is not ideally suitable for the unique fermentation requirements of cellulose hydrolysis. The basic problems are (1) that glucose concentration yields from recent developments in hydrolysis technologies are relatively low; (2) the large pentosan fraction is not fermented by traditional brewing yeasts and (3) inhibitory compounds may be generated by the pretreatment and hydrolytic processes. Research is progressing to overcome these potential technology development barriers.

The simultaneous saccharification and fermentation (SSF) process has been extensively studied to reduce process inhibition due to the build-up of the end products of hydrolysis (Sun and Cheng, 2002). The SSF process combines cellulose hydrolysis and fermentation into one step. Because the glucose produced by the hydrolysis process is immediately fermented to ethanol, only very low levels of cellobiose and glucose are observed in the system. This reduces cellulase inhibition, which in turn increases sugar production rates, concentrations, and yields, and decreases enzyme loading requirements. The number of vessels required for SSF is reduced compared to two-step hydrolysis-fermentation process (SHF), because hydrolysis and fermentation are performed in the same bioreactor, resulting in capital cost savings. Furthermore, the presence of ethanol during hydrolysis reduces the likelihood of contamination, especially in continuous operations of commercial interest. The optimal temperature for SSF, 37-38^{o}C, is a compromise between the best temperature for hydrolysis (45-50^{o}C) and the best temperature for yeast performance (30^{o}C). The development of thermotolerant yeast

strains that perform well above 40^{o}C with high ethanol tolerance is expected to significantly improve SSF performance (Philippidis, 1996). The SSF has many advantages, such as increasing the hydrolysis rate by immediate conversion of the inhibitor of cellulase activity, lower enzyme requirements, higher product yields, lower requirements for sterile conditions, shorter process time and less reactor volume (Sun and Cheng, 2002).

2.5 Conclusion

The utilization of waste biomass as a renewable resource for energy has the potential to contribute to a cleaner environment. It also has the potential to contribute solutions to the current energy crisis, while reducing the environmental pressure from waste material disposal. Research on technologies, particularly biotechnologies, will contribute to a better use of these abundant waste materials as a feedstock in order to decrease the cost of processing.

Biomass ethanol as a fuel and fuel additive provides environmental and economical benefits of global proportions. Bioethanol can reduce global GHG emissions, and also reduce the pressure of international requirements for fossil fuels. Furthermore, the conversion of waste biomass to ethanol is an alternative disposal option, and also reduces the potential for water, air and soil contamination, as well as contributes to the minimization of greenhouse gas emissions.

Although the benefits of bioethanol are obvious, there are limitations to the utilization of waste materials due to their complexity and lignocellulosic structure. The big challenge for further research is reducing the cost of the conversion processes. The key issue in reducing the cost of bioethanol production and the development of economically viable large-scale applications for waste materials, is the optimization of pretreatment methods, enzymatic cellulose hydrolysis conditions and sugar fermentation approaches.

Chapter 3

METHODOLOGY

3.1 Introduction

In this chapter, the experimental procedures for the different lignocellulosic feedstock pretreatments, fibre analyses and acid hydrolysis are described. Physical and chemical pretreatments were applied for a range of pretreatment conditions using primary sludge, waste activated sludge and biosolids, as waste biomass feedstocks. The pretreatment was then followed by acid hydrolysis to convert the cellulose fraction of the feedstock to glucose. Finally, Van Soest fibre analyses (Goering and Van Soest, 1970) including: neutral-detergent fiber (NDF), acid-detergent fiber (ADF) and acid-detergent lignin (ADL) were employed to determine the fraction of cellulose, hemicellulose and lignin remaining in the samples following the different pretreatment applications and acid hydrolysis. A modification that was introduced to the Weende crude fibre analysis (International Standard) is also presented.

3.2 Experimental Design

The potential use of three types of municipal sludge residuals as

lignocellulosic feedstocks for the recovery of glucose was examined in this study: primary sludge, waste activated sludge and biosolids. For comparative purposes, four pretreatment processes were applied to each of the lignocellulosic feedstocks, designated as (1) unpretreated, (2) physical, (3) acid, and (4) alkaline pretreatments. The unpretreated condition, where the wet solid lignocellulosic feedstock was employed directly "as is" in acid hydrolysis without physical or chemical pretreatment was considered the control for this experiment. Physical pretreatment examined the effect of drying and grinding of the lignocellulosic feedstock. The effects of acid and alkaline chemical pretreatments were investigated in which the wet substrate was treated with hydrochloric acid (HCl) or potassium hydroxide (KOH). A more detailed description of each pretreatment application is presented in the following sections.

Each pretreatment application was followed by a glucose recovery procedure via acid hydrolysis catalyzed with 2% sulphuric acid (H_2SO_4) (w/v) at 120 ^oC ($\pm 1^oC$) for 1 hour ($\pm$1 minute). This acid hydrolysis procedure was employed by Chen et al. (2004) using animal manures as waste biomass feedstocks. The glucose product was collected for glucose yield measurements and the hydrolyzed residues were collected for further fiber analyses. The experimental design and testing plans of the investigation are outlined in Figure 3.1 and summarized in Table 3.1. All experiments were conducted in triplicate.

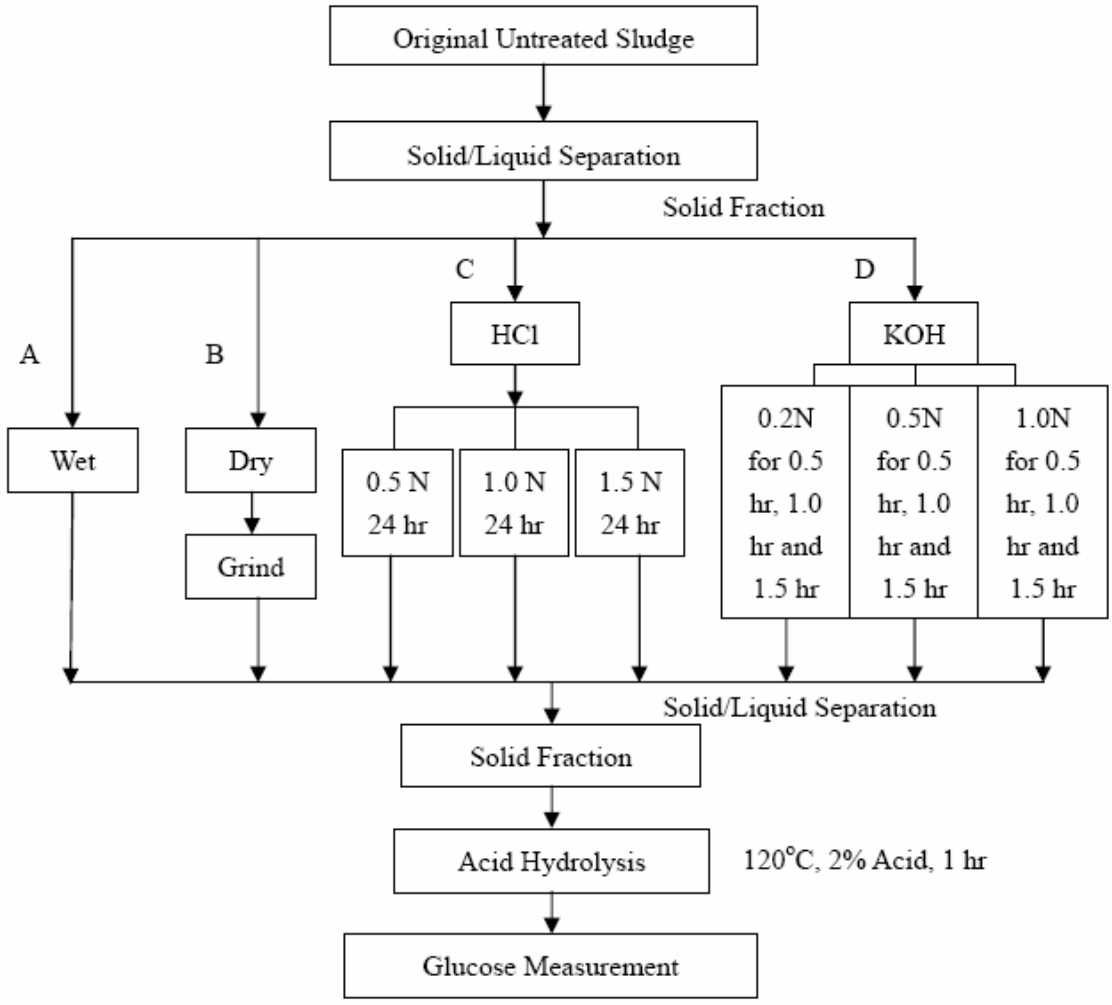

Figure 3.1 Experimental flow diagram for the investigation of pretreatment applications to municipal sludge residuals as lignocellulosic feedstocks for sugar recovery (A=Pretreatment A, control, B=Pretreatment B, drying and grinding, C=Pretreatment C, HCl , and D=Pretreatment D, KOH)

Table 3.1 Summary of the experimental plan for the investigation of pretreatment applications to municipal sludge residuals as lignocellulosic feedstocks for sugar recovery (to be continued to the next page)

Trials	Substrate	Conc.,%	Temp.,^{o}C	Time,hrs	Days
APS	Primary sludge	2	120	1	1 days
AAS	Waste activated sludge				
ABS	Biosolids				
BPS	Primary sludge				
BAS	Waste activated sludge				
BBS	Biosolids				

Trials	Substrate	Conc.,N	Temp.,^{o}C	Time,hrs	Days	Trials	Substrate	Conc.,N	Temp.,^{o}C	Time,hrs	Days
CPS1	HCl Treated Primary Sludge	0.5N	50	24	5days	DPS1	KOH treated Primary Sludge	0.2N	70	0.5	1day
CPS2		1N				DPS2		0.5N			
CPS3		1.5N				DPS3		1.0N			
CBS1	HCl treated Biosolids	0.5N			5days	DPS4		0.2N		1	1day
CBS2		1.0N				DPS5		0.5N			
CBS3		1.5N				DPS6		1.0N			
CAS1	HCl treated Activated Sludge	0.5N			5days	DPS7		0.2N		1.5	2days
CAS2		1N				DPS8		0.5N			
CAS3		1.5N				DPS9		1.0N			
DBS1	KOH treated Biosolids	0.2N	70	0.5	1day	DAS1	KOH treated Activated Sludge	0.2N	70	0.5	1day
DBS2		0.5N				DAS2		0.5N			
DBS3		1.0N				DAS3		1.0N			
DBS4		0.2N		1	1day	DAS4		0.2N		1	1day
DBS5		0.5N				DAS5		0.5N			
DBS6		1.0N				DAS6		1.0N			

Table 3.1 Summary of the experimental plan for the investigation of pretreatment applications to municipal sludge residuals as lignocellulosic feedstocks for sugar recovery (continued from previous page)

DBS7	KOH treated Biosolids	0.2N	70	1.5	2days	DAS7	KOH treated Activated Sludge	0.2N	70	1.5	2days
DBS8		0.5N				DAS8		0.5N			
DBS9		1.0N				DAS9		1.0N			

3.3 Materials Collection and Preparation

3.3.1 Materials Collection

The sludge samples were collected from the Kingston West Wastewater Treatment Plant (KWWTP) located in Kingston, Ontario, Canada, on September 15th 2006, April 26th 2007, and July 22th 2007

The KWWTP provides conventional activated sludge treatment of raw wastewater from residential, commercial and Industrial areas in the western portion of the City of Kingston, west of the Little Cataraqui Creek. The annual average plant constituents presented in the table 3.2

Table 3.2 Annual average plant performances (KWWTP, 2007)

	Biochemical Oxygen Demand, BOD	Suspended Solids, SS	Phosphorus
Average Raw Wastewater	367.8mg/L	542.8mg/L	7.8mg/L
Average Final Effluent	15.36mg/L	17mg/L	0.73mg/L
Percent Removal	95.8%	96.4%	89.6%
Certificate of Approval criteria	25mg/L	25mg/L	1.0mg/L

Municipal sludges are composed primarily of fecal materials, scraps of toilet paper, and food residues, which would indicate the potential for a relatively high cellulose content in the sludge residuals (Li, 2004). The primary sludge, which is accumulated as a result of the mechanical wastewater treatment processes including screening, gritting, and primary clarification (sedimentation), was collected from the primary clarifier, in which the solid fraction is approximately 4%. Primary sludge

typically consists of a wide range of organic species, metals and other constituents (Li, 2004). Waste activated sludge was collected from the return pipe to the aeration tank after the activated sludge process, where the biological treatment of the wastewater takes place to remove dissolved organic species and nutrients, of which the solid fraction is approximately 1%. The digested sludge, also referred to as biosolids, is the product remaining after anaerobic digestion, which was collected from the outflow pipe of the secondary sedimentation tank, in which the solid fraction is approximately 1%.

These sludge samples were collected from their respective sources and then transferred in a 4 L sealed plastic buckets for temporary storage and transportation. The collected materials were then stored in a dark cold room at 4^{o}C (±2^{o}C) to make sure the organic composition would not change until subsequent sample preparation.

3.3.2 Materials Preparation

Because of the high water content in the freshly collected municipal sludge residuals, samples were first centrifuged to remove a large portion of the liquid fraction. Approximately 1000 ml (±50 mL) of each municipal sludge residual mixture was transferred into four 250 mL (±5 mL) graduate Nalgene bottles and centrifuged for 10 minutes (±1 minute) at 5500 RPM (±55 RPM). The supernatant (mainly water) was discarded. The solid fraction of the municipal sludge residual samples was then used as the lignocellulosic feedstock in the subsequent pretreatment

and acid hydrolysis investigation. The physical characterization of the three types of sludges was conducted; including moisture content (MC) which was performed on the centrifuged as well as non-centrifuged samples, total solids (TS), and volatile solids (VS) to determine organic and ash content of the feedstock materials.

To determine the MC, TS and VS, crucibles were first placed into a muffle furnace at 550^{o}C ($\pm$5 ^{o}C) for 30 minutes ($\pm$1 minute) to remove any organic residues. The crucibles were then transferred to a desiccator for 24 hours to cool down, and thereafter, were weighed (to establish the dry weight of the crucibles). Approximately 5.00 g ($\pm$0.01 g) of each sample was placed into three separate crucibles and weighed to record the wet weight. The samples were then dried in a preheated oven at 70 °C ($\pm$1°C) for 48 hours, placed in a desiccator overnight and then reweighed to obtain the respective dry weights of the samples. The percent moisture content (%MC) was calculated using Equation (3.1). The relative error related to the instrumental error of the moisture content determination was taken to be the sum of the relative errors of the every value in the formula. The relative error is the measurement error divided by the value of the measurement.

$$\%\,\mathrm{MC} = \frac{(\text{initial weight, g - dry weight, g})}{\text{initial weight, g}} \times 100 \qquad (3.1)$$

Next, the samples (together with crucibles) were placed in a muffle furnace at 550°C ($\pm$5°C) for 2 hours ($\pm$1 minutes). The ashed samples were cooled briefly on the benchtop and then placed in a desiccator for a minimum of 24 hours. Once cooled,

the ashed samples were weighed to obtain their ashed weight. The fraction of volatile solids (%VS) was calculated using Equation (3.2). The relative error related to the instrumental error of the volatile solids determination was taken to be the sum of the relative errors of the every value in the formula. The relative error is the measurement error divided by the value of the measurement.

$$\%\,\mathrm{VS} = \frac{(\text{dry weight, g} - \text{ash weight, g})}{\text{dry weight, g}} \times 100 \tag{3.2}$$

The fraction of ashed solids (%AS) based on dry weight was calculated using Equation (3.3). The relative error related to the instrumental error of the ashed solids was taken to be the sum of the relative errors of the every value in the formula. The relative error is the measurement error divided by the value of the measurement.

$$\%\,\mathrm{AS} = \frac{\text{ash weight, g}}{\text{dry weight, g}} \times 100 \tag{3.3}$$

The total solids (%TS), on dry mass basis, were computed using Equation 3.4. The relative error related to the instrumental error of the total solids was taken to be the sum of the relative errors of the every value in the formula. The relative error is the measurement error divided by the value of the measurement.

$$\%\mathrm{TS} = \%\mathrm{VS} + \%\mathrm{AS} \tag{3.4}$$

3.4 Methodology

It is worth noting that each of the four pretreatment applications described in the following sections was individually applied to each of the three different types of municipal sludge residual lignocellulosic feedstocks. Each of the pretreatment experimental procedures was conducted in triplicate, followed by acid hydrolysis, and analyzed to obtain the glucose recovery and remaining fibre content.

3.4.1 Pretreatments

Unpretreated Unpretreated municipal sludge residual samples were used as controls to provide a comparison for glucose recovery and fibre analysis to the pretreatment applications. After initial feedstock dewatering through centrifugation at 5500 RPM (±55 RPM) for 10 minutes (±1 minute), the wet sludge and biosolid residues were employed directly in acid hydrolysis for glucose recovery. Approximately 5.00 g (±0.01g) of each of the lignocellulosic feedstocks, on a dry-mass basis, was used in the subsequent acid hydrolysis. Each experimental procedure was conducted in triplicate.

Physical Pretreatment After initial dewatering through centrifugation at 5500 (±55 RPM) for 10 minutes (±1 minute), each of the lignocellulosic feedstocks

was dried in the oven at 70^{o}C (±1^{o}C) for 48 hours, placed in a desiccator at room temperature overnight, and then weighed to obtain the dry mass. The samples were ground into a fine power using a Wiley Mill with a 40 mesh. Approximately 5.00 g (± 0.01g) (dry mass) of pretreated lignocellulosic feedstock was used in the subsequent acid hydrolysis. Each experimental procedure was conducted in triplicate.

Acid Pretreatment Acid pretreatment was previously studied for animal manures, and it was found that dilute acid pretreatment could effectively improve enzymatic hydrolysis (Li and Champange, 2004; Henderson et al., 2003; Levy et al., 2003). In this study, different acid concentrations, 0.5 N, 1.0 N and 1.5 N (±0.005 N), for a 24 hour reaction time and 5% substrate load were studied to identify the acid pretreatment concentrations which would result in the higher acid-hydrolyzed glucose conversion yields using the sludge residuals as feedstocks. As the acid concentration of Van Soest method for acid-detergent fibre analysis is 1N H_2SO_4 to determine the cellulose and lignin portion in the cell wall and the acid concentration for acid-detergent lignin analysis is 72% H_2SO_4 to determine the insoluble lignin portion, the acid pretreatment concentrations used in this study were moderate and could be act as pretreatment reagent to improve the glucose recovery. Approximately 5.00 g (± 0.01 g) (dry mass) each of the prepared lignocellulosic feedstocks, dewatered through centrifugation at 5500 (±55 RPM) for 10 minutes (±1 minute), was added to 250 (± 5 mL) Erlenmeyer flasks filled with 100 (±1 mL) mL of 0.5 N, 1.0 N or 1.5 N (±

0.001 N) HCl solution. The mixture was stirred using a magnetic stirring bar on a hot plate at 50^{o}C (±1^{o}C) for 24 hours and then centrifuged for 10 minutes (±1 minute) at 5500 (±55 RPM). The solid fraction was washed with distilled water and neutralized with 0.5 N KOH to a pH of 7.0 (±0.1). The neutralized sample was then centrifuged again. The liquid supernatant was removed and the solid fraction was collected and kept refrigerated at 4^{o}C (±2^{o}C) in sealed plastic bottles for subsequent acid hydrolysis. Each experimental procedure was conducted in triplicate.

Alkaline Pretreatment In previous studies conducted by Levy and Champagne (2003), alkaline pretreatment was performed on the hydrolyzable (HP) and non-hydrolyzable (NHP) fractions recovered from hog manure, and it was found that alkaline pretreatment improved HP and NHP recovery from manure. In this study, not only were different alkaline solution concentrations, 0.2 N, 0.5 N and 1.0 N (± 0.005 N), tested and investigated to seek the better alkaline pretreatment conditions for higher acid-hydrolyzed glucose conversion yields, but different contact periods, 0.5 hour, 1.0 hour and 1.5 hours (±1 minute), were investigated as well. Approximately 5.00 g (±0.01 g) (dry mass) each of the prepared lignocellulosic feedstocks, dewatered through centrifugation at 5500 (±55 RPM) for 10 minutes (±1 minute), was added to 250 mL (±5 mL) Erlenmeyer flasks filled with 100 mL (±1 mL) of 0.2 N, 0.5 N or 1.0 N (±0.005 N) KOH solution. The mixture was then stirred using a magnetic stir bar on a hot plate at 100 ^{o}C (±1^{o}C) for a period of 0.5 hour, 1

hour or 1.5 hours (±1 minute) and then centrifuged for 10 minutes (±1 minute) at 5500 RPM (±55 RPM). The solid fraction was washed with distilled water and neutralized with 1.0 N (±0.005 N) HCl to a pH of 7.0 (±0.1). The neutralized mixture was then centrifuged at 5500 RPM (±55 RPM) for 10 minutes (±1 minute) once again, and the solid fraction was collected and kept refrigerated at 4^{o}C (±2^{o}C) in sealed plastic bottles for subsequent acid hydrolysis. Each experimental procedure was conducted in triplicate.

3.4.2 Acid Hydrolysis

After each pretreatment application, the remaining solid fraction of the lignocellulosic feedstock was subjected to acid hydrolysis for glucose recovery. The sludge residue after pretreatment was added to a 250 mL (±5 mL) Erlenmeyer flask with 100 mL (±1 mL) of 2% (W/V) (±0.005) of H_2SO_4 solution. The mixture was heated and mixed with a magnetic stir bar on a hot plate at 120^{o}C (±1^{o}C) for 1 hour (±1 minute). The mixture was cooled to room temperature and then centrifuged at 5500 RPM (±55 RPM) for 10 minutes (±1 minute). The liquid fraction was transferred to a sealed plastic bottle, the volume measured and the sample stored at 4^{o}C (±2^{o}C) until the glucose yield measurement. The solid fraction was transferred to a sealed plastic bottle and refrigerated at 4^{o}C (±2^{o}C) for subsequent fibre content analyses.

3.4.3 Glucose Content Measurement (Miller, 1959)

The glucose concentration was determined by DNS glucose assay. Figure 3.2 demonstrates the mechanism of the DNS assay. Reducing sugars (aldehyde group C=O) react with 3,5-dinitrosalicylic acid (DNS) to convert it to its reduced amine form, 3-amino-5-nitrosalicylic acid. In theory, one mole of sugar will react with one mole of DNS. This reaction results in a yellow DNS color change to red-brown. The production of the red-brown 3-amino-5-nitrosalicylic acid can then be recorded by measuring the absorption intensity at 575 nm using a spectrophotometer. The absorbance at 575 nm is directly proportional to the amount of the red-brown product, where the molar ratio of glucose to the red-brown 3-amino-5-nitrosalicylic acid is expected to be 1:1.

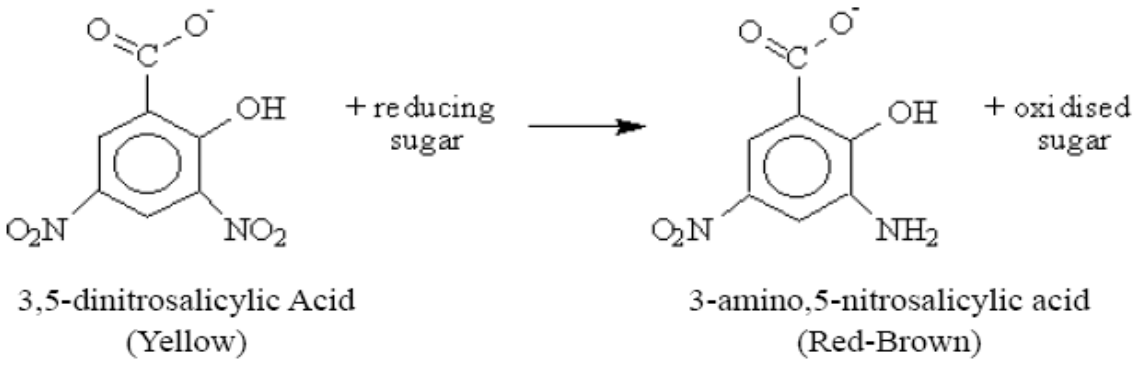

Figure 3.2 The reaction of DNS assay (Miller, 1959).

For this investigation, a 1% DNS solution was prepared by mixing 5.00 g (± 0.01 g) of 3,5-dinitrosalicylic acid with 0.25 g (±0.01 g) of sodium sulphite and 5.0 g (±0.01 g) sodium hydroxide in 400 mL of distilled water. The mixture was agitated and the volume of the solution was toped to 500 mL (±0.5 mL) with distilled water

using volumetric flask and mixing well. A 40% (W/V) potassium sodium tartrate solution was prepared by adding 40 g (±0.01 g) potassium sodium tartrate to 50 mL of distilled water and topping the volume to 100 mL (±0.1 mL) using a volumetric flask and mixing well.

A calibration curve of absorbance as a function of known concentration was required to calculate the glucose concentration of the samples. As such, a glucose standard solution (1 g/L) was diluted to 0.2 g/L, 0.4 g/L, 0.6 g/L and 0.8 g/L (±0.001 g/L), respectively, with distilled water to generate the calibration curve. Three milliliters (±0.015mL) was aliquoted from each of the standard glucose solutions to a measurement cuvette, and 3 mL (±0.015) mL of DNS was added to each cuvette respectively, after which the cuvette was capped and swirled. The cuvettes were then heated at 90^{o}C (±1^{o}C) in a hot water bath for 5 minutes (±1 minute). The samples were thereafter taken out from the water bath and 1 mL of 40% (±0.001)% potassium sodium tartrate was added to each cuvette to stabilize the product color. After the samples were cooled to room temperature, their absorbance was measured using a Spectronic 20 D spectrophotometer at 575 nm. The absorbance values of the five standard glucose solutions were used to develop the calibration curve, thus the equation of absorbance as a function of concentration was determined.

The glucose yield (GY) was determined by using

$$GY = \text{Glucose concentration} \times V \tag{3.5}$$

The relative error of instrument error of the glucose concentration was taken to be the sum of the relative errors of the every value in the Equation 3.5.

GY represents the mass of glucose yield in the sample, mg; V the volume of the supernatant, mL; and Glucose concentration the computed glucose concentration, mg/ mL.

The percentage of conversion (%PC) was determined as

$$\%PC = \frac{\text{Glucose yield, mg}}{\text{dry weight of initial substrates added, mg}} \times 100 \qquad (3.6)$$

The relative error of instrument error of the glucose conversion percentage was taken to be the sum of the relative errors of the every value in the Equation 3.6. The relative error is the measurement error divided by the value of the measurement.

Using this method, the glucose yield for each sludge sample after pretreatment was measured and the effects of the different pretreatment applications were evaluated and compared to identify the more effective pretreatment applications to recover glucose from municipal sludge residuals.

3.5 Statistical Analyses

Statistical analyses were performed on the data using MINITAB V15 software to provide a comparison of the glucose conversion yields obtained for the three

different sludges as a result of the various pretreatment applications. An analysis of variance (one-way ANOVA), was used to test the variances between the groups of data and T-tests were performed to determine if the results between selected groups were significantly different. Non-parametric statistical tests were also applied to the results, as these may not have been normally distributed in which case the ANOVA and T-tests would not be applicable as the results would not be parametric. The Kruskal-Wallis Test was applied among the groups and Mann-Whitney Test was applied between two selected groups. The results of the statistical analyses are presented in Appendices C, D, E and F.

3.6 Fibre Content Analyses Methods

3.6.1 Crude Fibre Analysis and Modification Procedure (ISO 6865:2000E)

The standard method, ISO 6865:2000(E), for CF analysis was modified and used on the three types of municipal sludge residues, primary sludge, without chemical pretreatment or acid hydrolysis. The samples were collected and stored in sealed plastic bottles at 4^{o}C ($\pm$2^{o}C). All of the samples were prepared as noted in Section 3.3.2, dried in the dry oven at 70^{o}C ($\pm$1 ^{o}C) for 48 hours and then cooled in a desiccator to room temperature.

For each of the feedstocks, approximately 1.00 ($\pm$0.01)g of dried sample was treated with boiling dilute sulfuric acid (150 mL $\pm$1 mL, 0.13 mol/L $\pm$0.005 mol/L)

H_2SO_4 for 30 minutes (±1 minute) in a conical flask. The residues were separated by filtration through Gooch-type filtering crucibles with Celite® 545 as a filter aid, washed with hot water (90 °C ±1°C) and then treated with boiling potassium hydroxide solution (150 mL ±1 mL, 0.23 mol/L ±0.005 mol/L KOH for 30 minutes (±1 minute). Next, the residues were separated by a second filtration through a Gooch-type filtering crucibles, washed with hot water (90 °C ±1°C), dried at 130 °C (±1°C) for at least 2 hours (±1 minute). The residues were then cooled in a desiccator, weighed and then ashed at 500±5°C for 2 hours (±1 minute).. The loss in mass resulting from ashing corresponded to the mass of crude fibre content in the sample. The detailed procedure is outlined in the Appendix A.

A modification to the ISO 6865:200(E) methodology was introduced during the secondary filtration step. Samples can contain a significant fraction of starch, protein or other mucilaginous substances which would be expected in the digestive tract of animals or humans, as well as in biological or chemical breakdown processes. These are often difficult to filter after the alkaline digestion because of the formation of a gelatinous material. Centrifugation was added prior to the second filtration to minimize excessive clogging of the filter crucible and to reduce the filtration time. After alkaline digestion, the mixture was centrifuged at 6000 RPM (±55.RPM) for 30 minutes (±1 minute) and the supernatant was discharged. The solid fraction was washed with hot water (90 °C ±1°C) and the mixture was subsequently filtered using the procedure outlined in the Appendix A.

3.6.2 Neutral-Detergent Fibre and Acid-Detergent Fibre and Acid-Detergent Lignin Analysis

The fibre content characterization was applied to the unpretreated and pretreated feedstock samples. The previously prepared unpretreated sludge and biosolid samples, as well as the solid residue remaining after the acid hydrolysis of the pretreated samples were dried at 70^{o}C ($\pm$1^{o}C) for 48 hours ($\pm$1 minute), and then cooled in a desiccator to room temperature.

The cellulose, hemicellulose and lignin content of the municipal sludge residual feedstocks were characterized using the standard Van Soest methods (Van Soest 1963a; 1963b; and 1967), which employs NDF, ADF and ADL procedures. Cellulose is considered to be represented by the difference between ADF and ADL (ADF-ADL), and hemicellulose the difference between NDF and ADF (NDF-ADF). The detailed apparatus, reagents and digestion procedures are outlined in Appendix B.

The residue remaining after digestion in the neutral-detergent solution was the NDF fibre which is predominantly composed of hemicellulose, cellulose and lignin. The ADF fibre, the residue remaining after acid detergent digestion, consists of cellulose, lignin, cutin and acid-insoluble ash (silica). The acid-detergent digestion was a preparatory step for the determination of lignin, as acid-insoluble lignin. Ashing the residue obtained from 72% ($\pm$0.001%) H_2SO_4 treated ADF represents the crude lignin fraction.

Chapter 4

RESULTS AND DISCUSSIONS

4.1 Glucose Yield of Acid Hydrolysis on Sludges

Due to the presence of undigested toilet paper, food residues and other organic constituents in municipal wastewaters, sludges and biosolids are generally expected to have a relatively high cellulose content. The aim of this study was to examine and compare the glucose conversion yields from municipal sludges and biosolids collected at three different stages along a municipal wastewater treatment process, and subjected to different pretreatment conditions.

The hydrolyzed sludge-to-glucose conversion yields based on the dry mass of the original substrate for each of the sludge and biosolids samples are described and discussed in the following sections which are separated based on the pretreatment application. The glucose conversion yields are compared to those obtained for the control conditions, for which no pretreatment was applied to the lignocellulosic material prior to acid hydrolysis.

4.1.1 Glucose Yield from Physically Pretreated Sludges and Biosolids

The acid hydrolyzed sludge-to-glucose conversion percentages of the three types of sludge and biosolids samples, employed as lignocellulosic feedstocks, after physical pretreatment and no pretreatment, and the standard deviations obtained from the triplicate results are presented in Table 4.1. The acid hydrolysis of the unpretreated primary sludge (control) yielded 4.30% glucose based on the dry mass of original substrate, while the physically dried and ground pretreated primary sludge gave 4.66% glucose conversion. On the other hand, the acid-hydrolyzed sludge-to-glucose conversion yields of the physically pretreated activated sludge samples and unpretreated activated sludge were found to be 4.48% and 2.61% based on the dry mass of original substrate, respectively, indicating that physical pretreatment enhanced glucose conversion. In the case of the biosolids samples, neither the physically pretreated nor the unpretreated samples yielded detectable levels of glucose following acid hydrolysis. One of the reasons for these results might be that some of the more readily available cellulose content may be digested during the activated sludge and anaerobic digestion processes. Based on the results of the glucose conversion yields noted for the primary sludge and activated sludge samples, the drying and grinding pretreatment appeared to lead to better glucose conversion yields. This was likely due to the fact that grinding can effectively reduce the cellulose crystallinity and increase the surface area of the particles resulting in a more efficient acid-catalyzed hydrolysis treatment.

Table 4.1 The glucose conversion percentages of physical pretreated and unpretreated sludges and biosolids and the standard deviations from triplicate results

	Primary sludge, PC%	Activated sludge, PC%	Biosolids, PC%
Unpretreated	4.30±0.11	2.61±0.19	1.56±0.058
physical pretreatment	4.66±0.15	4.48±0.18	1.15±0.042

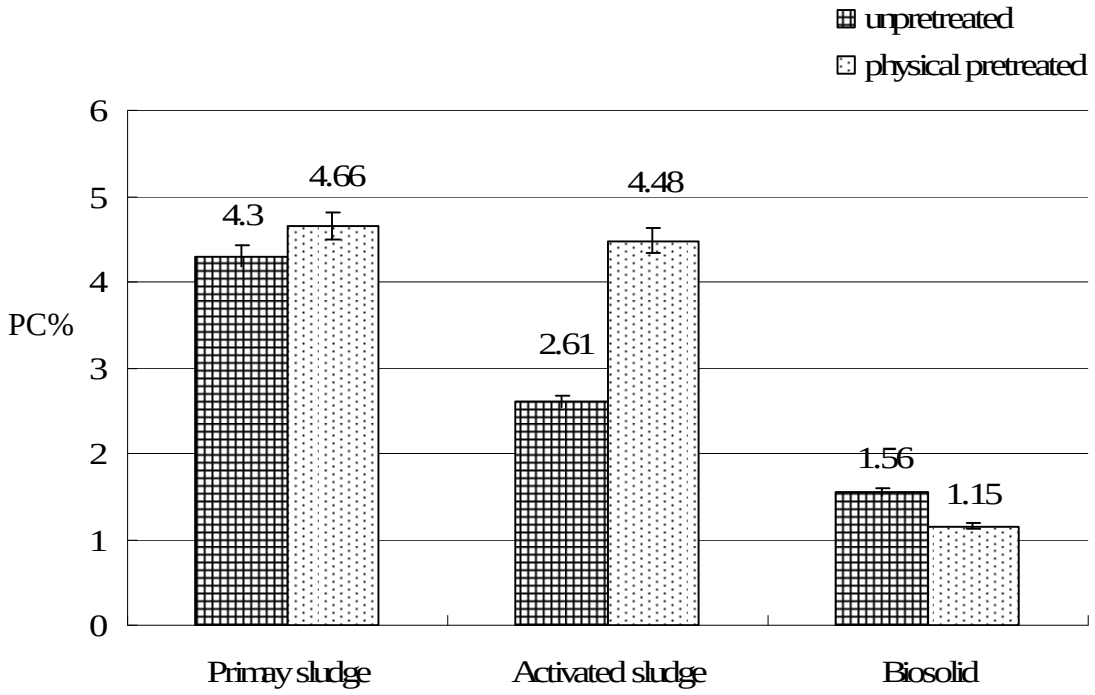

Figure 4.1 Acid hydrolysis with physical pretreatment of three types of sludges and biosolids, error bars indicated the analytical error

The results obtained in this study were different from those obtained in a similar study conducted by Li and Champagne (2005), where unpretreated samples yielded better results than the dried and ground samples using enzymatic hydrolysis. One of the factors which could account for this difference in the results is related to the structure of the fibres of the cellulose-containing material in the untreated samples, which could have been more readily accessible to the enzymes during enzymatic hydrolysis, while the available surface area of the cellulose may have been the more critical when acid hydrolysis was employed for glucose recovery in this study.

In this experiment, the drying and grinding pretreatments were found to be

more effective for glucose recovery following acid hydrolysis. However, drying and grinding requires a larger energy input. More research is needed for studies on a larger scale in order to reduce the energy consumption required in conversion processes.

4.1.2 Glucose Yield from Acid-pretreated Sludges and Biosolids

Prior to the acid hydrolysis of the sludge samples, different HCl pretreatments were applied to the sludges and biosolids used as lignocellulosic feedstocks, as previously described in Section 3.4.2. The glucose conversion yields obtained following the subsequent acid hydrolysis and the standard deviation computed from the triplicate results are presented in Table 4.2 and Figure 4.2. Statistical analyses were conducted using MINITAB V15 and the results from these, the one-way ANOVA, t-test, and non-parametric (NP) tests are presented in Appendix C.

Table 4.2 The acid-hydrolyzed sludge-to-glucose conversion percentages of three types of sludges and biosolids pretreated with various acid pretreatments and standard deviations from triplicate results

Sludge type	0.5 N, PC%	1.0 N, PC%	1.5 N, PC%
Primary sludge	2.41±0.41	5.67±0.24	2.46±0.15
Activated sludge	4.84±0.47	3.52±0.63	4.44±0.23
Biosolids	2.82±0.40	4.67±0.25	4.81±0.18

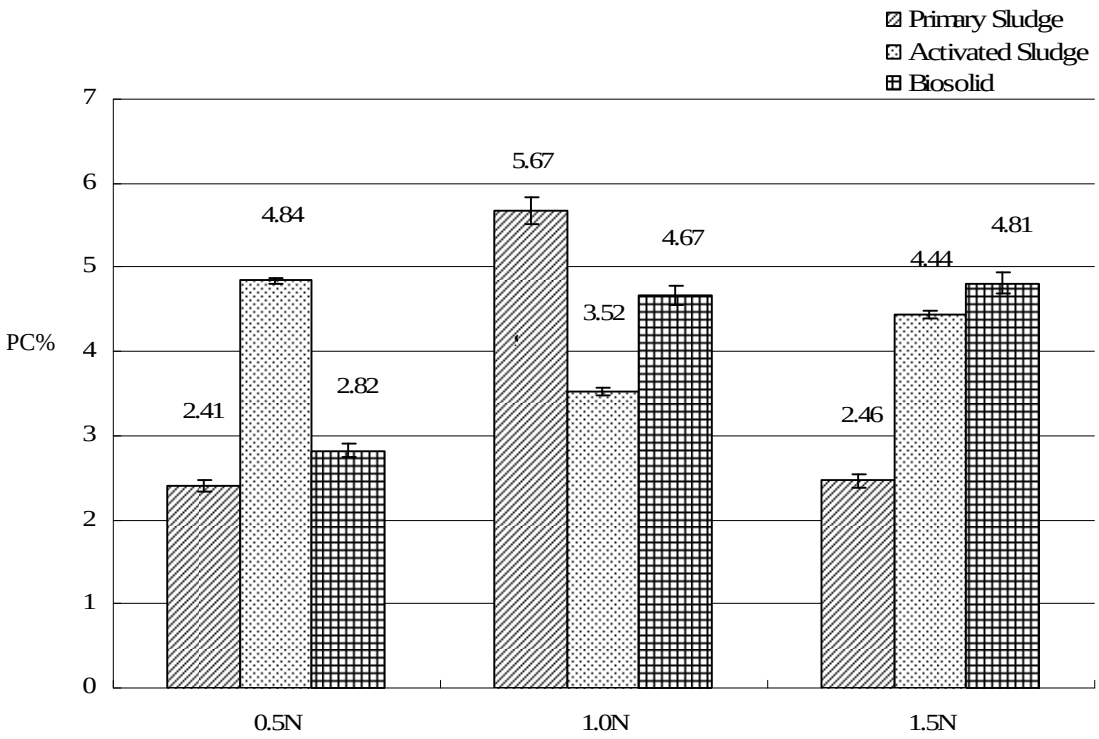

Figure 4.2 Acid hydrolysis with HCl pretreatment of three types of sludges and biosolids, error bars indicated the analytical error

As can be seen from Table 4.2 and Figure 4.2, the highest acid-hydrolyzed glucose conversion yield was noted for primary sludge pretreated with 1.0 N HCl at 5.67%, which was significantly higher than the 2.41% and 2.46% conversion yields obtained with the 0.5 N HCl and 1.5 N HCl pretreatments. This glucose conversion yield was also found to be higher than the 4.30% yield obtained for the unpretreated primary sludge. HCl was found to be effective in the removal of metals which can inhibit enzymatic hydrolysis, as was demonstrated in a study by Li and Champagne (2005), where HCl pretreatments at lower concentrations (0.5 N to 1.0 N) were applied to primary sludge prior to enzymatic hydrolysis. Using HCl for metal removal was so studied by Levy et al. (2003) and Champagne et al. (2005), where the application of HCl at 0.1 N to 1.0 N concentrations showed good metal removal

efficiencies for hog manure.

In this study, using HCl at 1.0 N concentrations was also found to be an effective pretreatment for primary sludge prior to acid hydrolysis. It should be noted that the acid-hydrolyzed sludge-to-glucose conversion of the primary sludge pretreated with higher (1.5 N) HCl concentrations appeared to reduce the glucose conversion yield in the subsequent acid hydrolysis when compared to the glucose conversion yield obtained with the unpretreated primary sludge (Table 4.1). This would suggest that the primary sludge cellulose content may be converted to glucose during the pretreatment stage at higher HCl pretreatment concentrations (1.5 N), which could therefore be lost during the separation stage, since the acid can act as a catalyst for hydrolysis, as well as for pretreatment.

The one-way ANOVA performed on the results obtained for the primary sludge indicated that the glucose yields resulting from the 0.5 N, 1.0 N and 1.5 N HCl pretreatments were different. The statistical results are presented in Appendix C. The NP statistics using the Kruskal-Wallis test showed that the results obtained from 0.5 N, 1.0 N and 1.5 N HCl were not significantly different, but the results from the 1.0 N HCl pretreatment (5.67%) were found to be higher. The inconsistency between the results of the parametric (ANOVA) and NP tests might be due to the limitation of groups of test results or the variability of the natural substrates. The t-test (p-value=0.881) and NP Mann-Whitney test, both indicated that the glucose conversion yields obtained from the 0.5 N and 1.5 N HCl pretreatments were not

significantly different from each other, however, the glucose yields from the 1.0 N HCl pretreatment were significantly different from the 0.5 N and 1.5 N pretreatments. Hence, the statistical results would suggest that for the range of HCl pretreatment conditions tested, the 1.0 N HCl pretreatment of the primary sludge yielded the highest increase in glucose recovery.

In the case of the activated sludge, the highest glucose conversion yield was found to be 4.84% for the sludge pretreated with 0.5 N HCl, which was a significant increase compared to the sludge-to-glucose conversion obtained for the unpretreated activated sludge (2.61% in Table 4.1). The one-way ANOVA test performed on the results obtained for the activated sludge indicated that the glucose yields from the 1.0 N HCl pretreatment were significantly lower than the yields from the 0.5 N and 1.5 N HCl pretreatments (p-value=0.036), while the NP Kruskal-Wallis test showed the yields resulting from 0.5 N, 1.0 N and 1.5 N pretreatments were not significantly different (p-value=0.051). The inconsistency between the results of the parametric (ANOVA) and NP tests might be due to the limitation of groups of test results or the variability of the natural substrates. The t-test (p-value=0.270) and Mann-Whitney test (NP test) both indicated that the glucose yields from acid hydrolysis obtained following the 0.5 N and 1.5 N HCl pretreatments were not significantly different from each other. The statistical results indicated that activated sludge showed better yields after HCl pretreatment at the lower or higher concentrations (0.5 N and 1.5 N), compared with primary sludge which exhibited higher glucose yields with the HCl pretreatment at a concentration of 1.0 N, however, the statistical results showed that

the glucose yields from 0.5 N and 1.5 N HCl pretreated activated sludge were not significantly different, which indicated that the lower HCl concentration (0.5 N) might be a better HCl pretreatment concentration for activated sludge considering the lower cost and milder pretreatment condition.

In the case of the biosolids, the acid-hydrolyzed glucose conversion yield was 4.81% for the sludge pretreated with 1.5 N HCl, which was significantly higher than the glucose conversion yields obtained for the unpretreated (1.56%) and physically pretreated (1.15%) biosolids (Table 4.1). The acid pretreatment appeared to enhance the acid-hydrolyzed sludge-to-glucose conversion of the biosolids. Increases in acid pretreatment concentrations from 0.5 N to 1.5 N further increased the yield from 2.82% to 4.82%, where the one-way ANOVA test indicated that the results from 0.5 N, 1.0 N and 1.5 N HCl pretreatments were significantly different, while the NP Kruskal-Wallis test showed that these results were not significantly different (p-value=0.061). Conversely, the t-test (p-value=0.465) and Mann-Whitney test, showed similar results indicating that the glucose conversion yield for the 1.0 N and 1.5 N HCl pretreatments were not significantly different. Although confounding results were obtained statistically, overall these results could be interpreted as suggesting that a maximum glucose yield from the HCl pretreatment application could be obtained with the 1.0 N HCl pretreatment.

It is worth noting that the acid pretreatment of each of the three sludge feedstocks resulted in higher acid-hydrolyzed sludge-to-glucose conversion yields.

However, it was found that an increase in HCl concentration in the pretreatment application did not necessarily increase the glucose conversion yields, particularly in the glucose conversion experiments of both the primary sludge and the activated sludge. From the results, it would appear that each sludge lignocellulosic feedstock would have its own optimal acid-pretreatment condition. The optimal HCl pretreatment for primary sludge and biosolids would likely be around a 1.0 N HCl concentration, while that of activated sludge should be at lower (0.5 N) HCl concentrations. This might be due to an early cellulose to sugar conversion catalyzed at higher HCl concentrations, which could be lost during the solids-liquid separation process following pretreatment. However, there was no specific trend found in this study. Statistically, using the t-test to compare the results of the HCl and physical pretreatments, it was found that the acid-hydrolyzed glucose-conversion results of primary sludge and biosolids with HCl pretreatment were significantly better than the one with the physical pretreatment (p-value=0.080 and 0.002 respectively, presented in Appendix F), which would indicate that the HCl pretreatment of primary sludge and biosolids was more effective than physical pretreatment. However, the HCl pretreatment conversion results did not appear to improve the glucose yields from activated sludge compared to the yield obtained following physical pretreatment.

4.1.3 Glucose Yield from Alkaline-Pretreated Sludge and Biosolids

The benefit of an alkaline (KOH) pretreatment prior to the acid-catalyzed

hydrolysis of the three sludge and biosolids lignocellulosic feedstock samples was also investigated. The KOH pretreatment was applied at three different KOH concentrations for various reaction periods, using the procedure described in Section 3.4.1.

4.1.3.1 Alkaline Pretreatment of Sludges and Biosolids at Different Concentrations for 0.5 hours, 1.0 hour and 1.5 hours.

The acid hydrolyzed glucose conversion yields of the three sludges used as lignocellulosic feedstocks pretreated with different concentrations of KOH for various pretreatment contact times (0.5 hours, 1.0 hour and 1.5 hours), and the standard deviation conducted from triplicate results are presented in Tables 4.3 to 4.5. The difference in the hydrolysis of three types of sludges and biosolids with different KOH pretreatment is presented in Figures 4.3 to 4.5. The statistical analysis was conducted using MINITAB V15 and the results of the statistical analyses are presented in Appendix D, E and F.

Table 4.3 The acid-hydrolyzed sludge-to-glucose conversion percentages of three types of sludges and biosolids pretreated with three KOH concentration for 0.5 hour and the standard deviations from triplicate results

Sludge type	0.2 N, PC%	0.5 N, PC%	1.0 N, PC%
Primary sludge	3.00±0.33	3.46±0.19	2.20±0.12
Activated Sludge	1.80±0.19	2.25±0.25	1.61±0.27
Biosolids	1.75±0.18	0.90±0.08	1.09±0.11

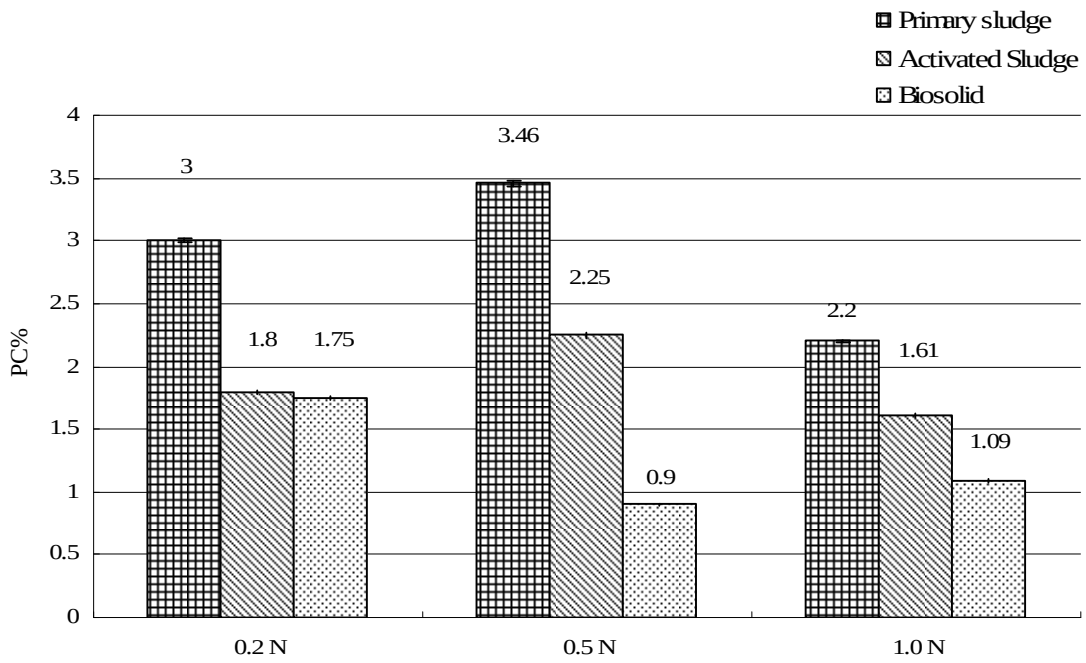

Figure 4.3 Acid hydrolysis of three types of sludges and biosolids pretreated with various concentration of KOH for 0.5 hour, error bars indicated the analytical error

Figure 4.3 illustrates that primary sludge demonstrated a higher glucose conversion yield (3.46%) when pretreated with 0.5 N KOH for a 0.5 hour reaction time, compared to 3.00% when pretreated with 0.2 N KOH and 2.20% when pretreated with 1.0 N KOH. The one-way ANOVA test (p-value=0.002) showed that the glucose conversion yields for primary sludge pretreated with 0.5 N KOH were significantly higher than those obtained for the 0.2 N and 1.0 N KOH concentrations, while the NP Kruskal-Wallis test also showed similar results (p-value=0.027). The t-test (p-value=0.058) demonstrated that the glucose yields obtained for the 0.2 N and 1.0 N KOH concentrations were not significantly different from each other, which also indicated that the higher glucose yields were obtained with the 0.5 N KOH pretreatment.

In the case of activated sludge, the highest glucose conversion percentage was

found to be 2.25% for the 0.5 hour 0.5 N KOH pretreatment, followed by 1.80% and 1.61% for the 0.2 N and 1.0 N KOH concentrations, respectively. The one-way ANOVA test showed that the glucose yield from the 0.5 N KOH pretreatment was significantly higher than yields obtained from the 0.2 N and 1.0 N KOH pretreatments (p-value=0.039), while the NP Kruskal-Wallis test showed that the data for the three pretreatments were not significantly different (p-value=0.061). The differences noted between the results of the statistical tests might be due to the limitation of groups of test results or the variability of the natural substrates. The t-test indicated that the 0.2 N and 1.0 N KOH pretreatments did not yield significantly different results (p-value=0.393). However, the Mann-Whitney test showed that the data from the 0.5 N pretreatment were significantly different from the 0.2 N and 1.0 N pretreatments, which appeared to indicate that activated sludge showed better glucose conversion yields with the 0.5 N pretreatment.

In the case of the biosolids, the highest glucose conversion yield was 1.75 % with the 0.2 N KOH pretreatment for 0.5 hour. The one-way ANOVA test showed that the glucose conversion yield from the 0.2 N KOH pretreatment was significantly higher than the yields obtained from the 0.5 N and 1.0 N KOH pretreatments (p-value=0.001), and the t-test indicated that the 0.5 N and 1.0 N KOH pretreatments did not yield significantly different results (p-value=0.088). The NP Kruskal-Wallis test showed consistent results with the one-way ANOVA, but the Mann-Whitney test showed that the 0.5 N and 1.0 KOH pretreatments obtained significantly different results, however, they both indicated that the 0.2 N pretreatment showed higher results

than the 0.5 N and 1.0 N pretreatments.

Compared with the results of the activated sludge and biosolids, the primary sludge gave the highest glucose conversion results with the 0.5 hour pretreatment duration time, which indicated that primary sludge might contain a more readily convertible cellulose fraction than the activated sludge or biosolids.

Table 4.4 The acid-hydrolyzed sludge-to-glucose conversion percentages of three types of sludges and biosolids pretreated with three KOH concentration for 1.0 hour and the standard deviations from triplicate results

Sludge Type	0.2 N, PC%	0.5 N, PC%	1.0 N, PC%
Primary sludge	1.09±0.11	2.16±0.08	1.35±0.12
Activated	2.28±0.07	1.58±0.16	1.23±0.09
Biosolids	1.88±0.22	4.24±0.23	1.59±0.26

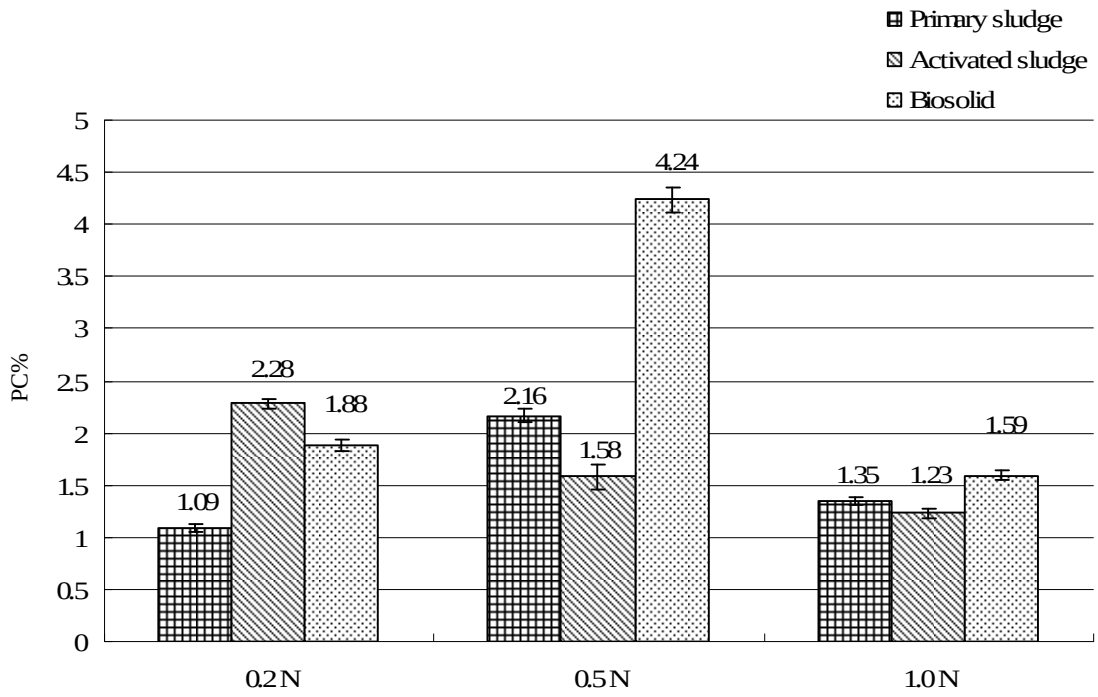

Figure 4.4 Acid hydrolysis of three types of sludges and biosolids pretreated with various concentration of KOH for 1.0 hour, error bars indicated the analytical error

For the 1.0 hour pretreatment contact time (Figure 4.4), the primary sludge showed higher glucose conversion yields (2.16%) with the 0.5 N KOH concentration,

while with activated sludge, higher glucose conversion yields (2.28%) were obtained with the 0.2 N KOH. On the other hand, in this case, the glucose conversion yields were found to be highest for the biosolids (4.24%) with 0.5 N KOH. The one-way ANOVA test results (p-value=0.000) showed that the acid-hydrolyzed glucose conversion yields of primary sludge with the 0.5 N KOH pretreatment was significantly higher than the 0.2 N and 1.0 N KOH pretreatments, respectively, the NP Kruskal-Wallis test also showed that the results were statistically different (p-value=0.027). The t-test (p-value=0.067) showed that data from the 0.2 N and 1.0 N pretreatments were not significantly different from each other, but the Mann-Whitney test showed conflicting results. These confounding results between statistical tests are likely due to the limitation of groups of test results or the variability of the natural substrates. However, the Mann-Whitney test showed that data from the 0.5 N pretreatment were significantly higher than the yields from the 0.2 N and 1.0 N KOH pretreatments. The activated sludge had significantly higher glucose yields (2.28%) with the 0.2 N KOH pretreatment, which was shown from the one-way ANOVA and Kruskal-Wallis tests (p-value=0.000 and p-value=0.027, respectively). The t-test (p-value=0.050) and Mann-Whitney test both showed that data from the 0.5 N and 1.0 N KOH pretreatments were significantly different from each other.

In the case of biosolids, the one-way ANOVA test showed that the biosolids pretreated with 0.5 N KOH yielded a significantly higher glucose conversion than the 0.2 N and 1.0 N KOH pretreatments (p-value=0.000), and the t-test showed that the glucose conversion results obtained from the 0.2 N and 1.0 N KOH pretreatments

were not significantly different (p-value=0.246). Although the p-value (0.051) of NP Kruskal-Wallis test showed that the data from the 0.2 N, 0.5 N and 1.0 N pretreatments were not significantly different. Again, the differences in the results of the statistical analyses could be attributed to the limitation of groups of test results or the variability of the natural substrates. The Mann-Whitney test showed that data from the 0.2 N and 0.5 N pretreatments were significantly different from each other and the data from the 1.0 N and 0.5 N pretreatments were also significantly different, which would suggest that the yields from the 0.5 N pretreatment were significantly higher than those obtained with the 0.2 N and 1.0 N pretreatments. In the other pretreatment applications, the biosolids feedstock generally showed lower glucose conversion yields compared to the primary and activated sludges, which was attributed to the fact that most of the readily accessible cellulose content might have been digested during the aerobic and anaerobic digestion processes in the wastewater treatment facility. In this case, the less readily accessible materials might be broken down with 0.5 N KOH pretreatment for 1 hour, which obtained higher glucose conversion yields.

Table 4.5 The acid-hydrolyzed sludge-to-glucose conversion percentages of three types of sludges and biosolids pretreated with three KOH concentration for 1.5 hour and the standard deviations from triplicate results

Sludge Type	0.2 N, PC%	0.5 N, PC%	1.0 N, PC%
Primary sludge	2.04±0.09	1.70±0.13	1.67±0.14
Activated sludge	1.75±0.16	0.74±0.03	1.34±0.23
Biosolids	2.21±0.17	0.86±0.09	0.69±0.05

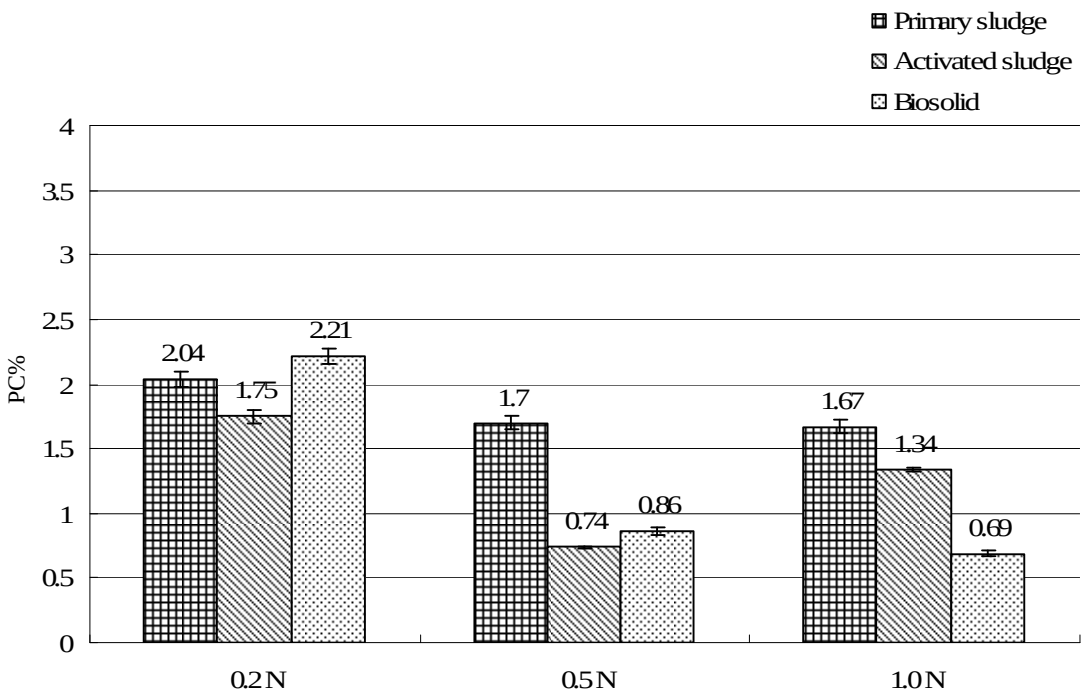

Figure 4.5 Acid hydrolysis of three types of sludges and biosolids pretreated with various concentration of KOH for 1.5 hour, error bars indicated the analytical error

For the KOH pretreatment with a contact time of 1.5 hours (Figure 4.5), the highest glucose conversion yield for primary sludge was 2.04% obtained with the 0.2 N KOH pretreatment. The 0.5 N and 1.0 N KOH concentrations resulted in glucose conversion yields of 1.70% and 1.67%, respectively, which based on the t-test (p-value=0.787) and NP Mann-Whitney tests were not considered to be significantly different. The one-way ANOVA (p-value=0.017) showed that the glucose yields obtained from 0.2 N, 0.5 N and 1.0 N KOH pretreatments were significant different, however, the NP Kruskal-Wallis tests (p-value=0.061) did not show consistent results, which might be due to the limitation of groups of test results or the variability of the natural substrates as previously stated. The one-way ANOVA test indicated that primary sludge might produce higher glucose yields with lower KOH pretreatment concentrations (0.2 N) for longer contact periods (1.5 hr).

In the case of activated sludge, the one-way ANOVA test (p-value=0.001) showed that activated sludge with the 0.5 N KOH pretreatment resulted in significantly lower (0.74%) glucose conversion yields than 0.2 N (1.75%) and 1.0 N (1.34%) KOH pretreatments, and the t-test (p-value=0.087) showed that the glucose conversion results from the 0.2 N and 1.0 N KOH pretreatments were not significantly different. The NP Kruskal-Wallis test (p-value=0.027) showed that data for the three different pretreatments were significantly different and the Mann-Whitney test indicated that conversion yields from the 0.2 N pretreatment were significantly higher than the other two KOH concentration pretreatments. The statistical test results indicated that activated sludge might produce higher glucose yields with lower (0.2 N) KOH concentration pretreatments with longer (1.5 hr) contact periods.

In the case of biosolids, the one-way ANOVA test (p-value=0.000) showed that the conversion results with the 0.2 N KOH pretreatment (2.21%) were significantly higher than the 0.5 N (0.86%) and 1.0 N (0.69%) KOH pretreatments, however, the NP Kruskal-Wallis test (p-value=0.067) did not show the same results, which might be due to the limitation of groups of test results or the variability of the natural substrates. According to the t-test (p-value=1.000) and Mann-Whitney test, the results from the 0.5 N and 1.0 N KOH pretreatments were not significantly different, however, the Mann-Whitney test showed that the conversion yields from the 0.2 KOH pretreatment were significantly higher than pretreatment using the other two concentrations.

The results showed that for the 1.5 hr contact period, primary sludge, activated sludge and biosolids obtained higher glucose yields with the 0.2 N KOH pretreatment, which indicated that longer pretreatment periods might cause the more resistant matters in the sludges to break down in order to allow the cellulose content to be converted to more glucose.

4.1.3.2 Alkaline Pretreatment of Sludges and Biosolids for Different Reaction Periods at 0.2 N, 0.5 N and 1.0 N

The acid hydrolyzed glucose conversion percentage of the three sludge and biosolid feedstocks pretreated for different reaction periods at 0.2 N, 0.5 N and 1.0 N KOH concentrations are presented in Figures 4.6 to 4.8, respectively. Results of the statistical analyses are presented in Appendix E and F.

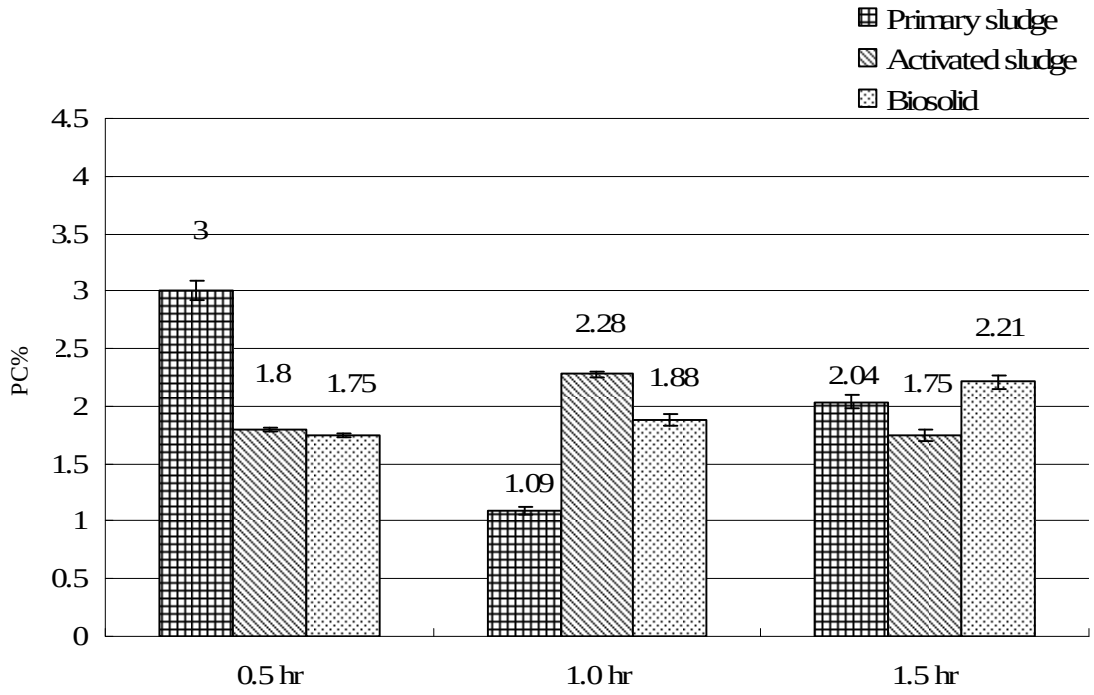

Figure 4.6 Acid hydrolysis of three types of sludges and biosolids pretreated with a 0.2 N concentration of KOH for different reaction periods, error bars indicated the analytical error

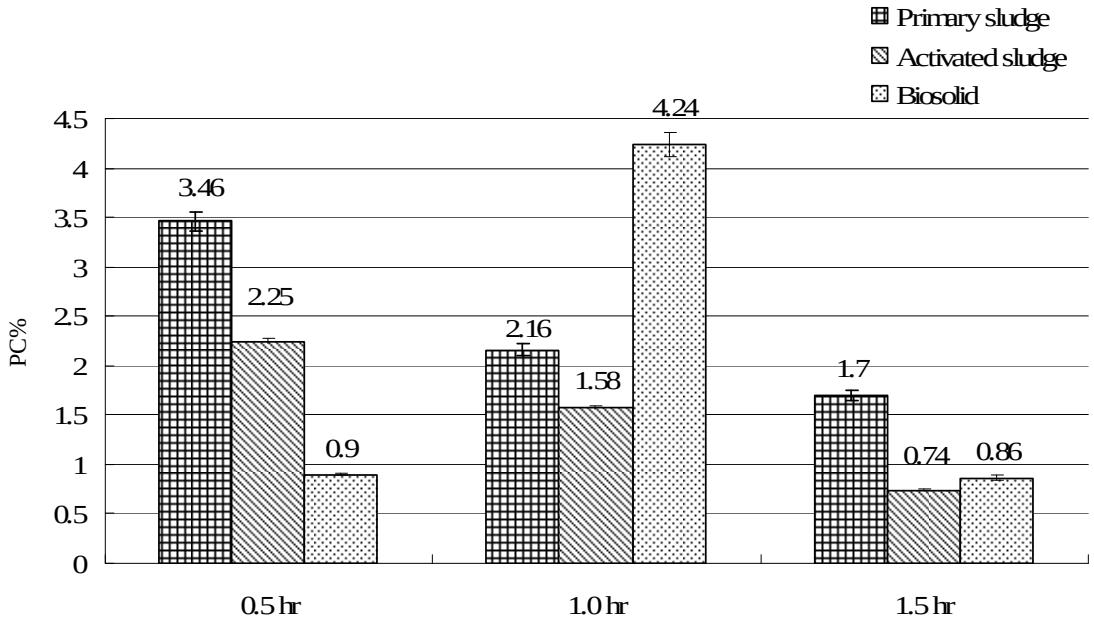

Figure 4.7 Acid hydrolysis of three types of sludges and biosolids pretreated with a 0.5 N concentration of KOH for different reaction periods, error bars indicated the analytical error

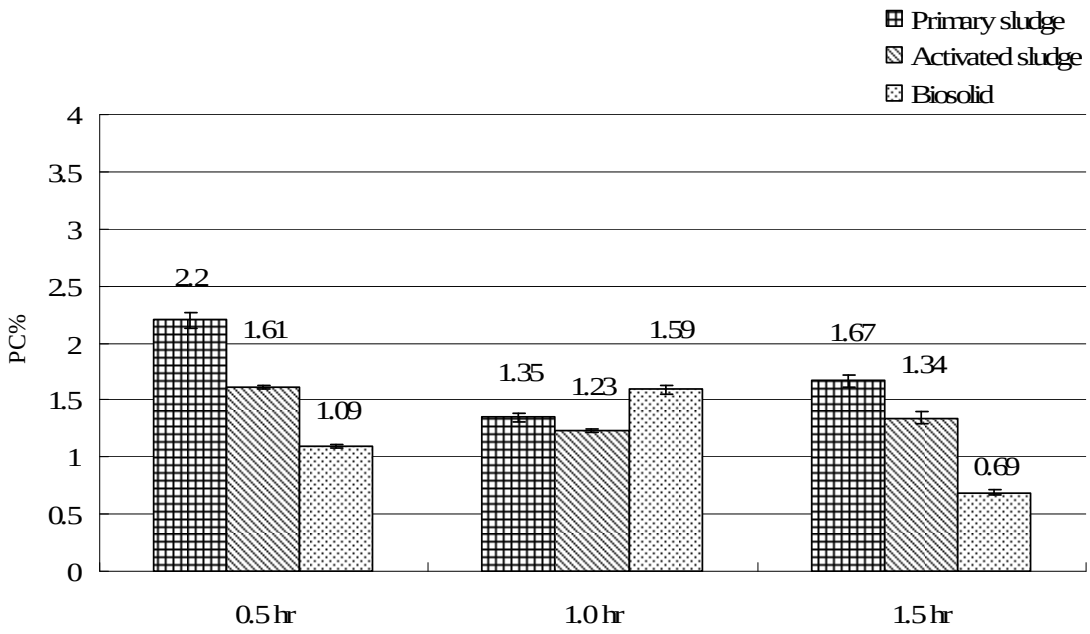

Figure 4.8 Acid hydrolysis of three types of sludges and biosolids pretreated with a 1.0 N concentration of KOH for different reaction periods, error bars indicated the analytical error

As can be seen from Figure 4.6, which illustrates the glucose conversion percentage of primary sludge, activated sludge and biosolids with a 0.2 N KOH pretreatment for different reaction periods, the highest glucose conversion yield (3.00%) for primary sludge was observed for the shorter KOH pretreatment time (0.5 hour) at a concentration of 0.2 N. The same trend was also noted in Figures 4.7 and 4.8, where the primary sludge obtained noticeably higher yields with 0.5 N (3.46%) and 1.0 N (2.20%) for the 0.5 hour pretreatment reaction period. The one-way ANOVA and NP Kruskal-Wallis tests both demonstrated that primary sludge showed significantly higher results for the 0.5 hour reaction period with the 0.2 N, 0.5 N and 1.0 N KOH pretreatments. A similar behavior was not noted with activated sludge and biosolids. This observation might suggest that the cellulose structure of the fibres contained in the primary sludge was more readily accessible for further hydrolysis and

required a shorter contact time (0.5 hour) than the other more digested activated sludge and biosolid feedstocks, and that 0.5 N may be a more beneficial KOH concentration for primary sludge. The activated sludge was found to show higher conversion results (2.28%) for the 1.0 hours pretreatment with 0.2 N KOH.

The one-way ANOVA test showed that significantly higher conversion yields were obtained from 0.2 N KOH pretreated activated sludge for a 1.0 hour contact period. Although the Kruskal-Wallis test did not show that conversion yields for 0.5 hr, 1.0 hr and 1.5 hr pretreated activated sludge were significantly different at 0.2 N KOH (p-value=0.061), the t-test and Mann-Whitney test both showed that conversion yields from 1.0 hour pretreatment were significantly higher. In Figure 4.7, the activated sludge was found to have significantly higher results (2.25%) with 0.5 N KOH pretreatment for 0.5 hour based on the one-way ANOVA and NP Kruskal-Wallis test. In Figure 4.8, the different pretreatment duration periods were not found to demonstrate significantly different results in the conversion yields of activated sludge with the 1.0 N pretreatment. These results appeared to indicate that activated sludge might have higher yields with higher alkaline pretreatment concentrations over shorter contact periods or, alternatively, at lower concentrations for longer contact times.

In the case of biosolids, the one-way ANOVA test (p-value=0.065) and Kruskal-Wallis test (p-value=0.099) both showed that the conversion results obtained for three different duration times (0.5 hrs, 1.0 hr and 1.5 hrs) were not significantly

different for the 0.2 N KOH pretreatment, which indicated that 0.5 hour (Figure 4.6) was an adequate contact time for the pretreatment of biosolids with 0.2 N KOH, in consideration of the lower energy cost. From Figure 4.7 and 4.8, the biosolids both showed significantly higher conversion yields for a 1.0 hour contact time at the 0.5 N (4.24%) and 1.0 N (1.59%) pretreatment concentrations, which indicated that 1.0 hour might be a better pretreatment time for biosolids with 0.5 N and 1.0 N pretreatments. The statistical analyses showed that the results of the 1.0 hour contact time with the 0.5 N and 1.0 N pretreatments were significantly higher than for other pretreatment reaction times. This could be attributed to the fact that the less readily accessible materials were broken down by the alkaline solution over a longer (1.0 hour) pretreatment time, which would allow the cellulose to be separated after a longer KOH pretreatment, whereas the cellulose might not be readily accessible for conversion after shorter pretreatment times. However, the 1.5 hour pretreatment time did not appear to have any effects on glucose conversion yields, which might be because the longer contact time caused the cellulosic content to become hydrolyzed during the alkaline pretreatment process, and washed out in the separation step.

Comparing the different alkaline pretreatment concentrations, primary sludge showed better glucose conversion yields at lower (0.2 N and 0.5 N) KOH pretreatment concentrations and higher glucose conversion yields than activated sludge and biosolids, which indicated that primary sludge may contain a higher or more readily accessible cellulosic fraction than activated sludge and biosolids. Based on a comparison of the pretreatment contact times for alkaline pretreatment, the primary

sludge with the 0.5 N KOH pretreatment produced the highest conversion results for the shortest (0.5 hour) pretreatment contact time, as well as higher conversion yields than those obtained from activated sludge and biosolids. This would suggest that the primary sludge may have a higher, more readily available, cellulose content that could be more readily converted using a 0.5 N KOH pretreatment. It can, therefore, be concluded that a 0.5 N KOH concentration for a 0.5 hour pretreatment could provide better KOH pretreatment conditions for primary sludge. In studies by Henderson et al. (2003), Levy et al. (2003) and Champagne (2005), an alkaline delignification technique was elaborated for extracting and fractionating using different types of waste materials, such as corn stovers and bagasse. The alkaline delignification was effective in separating cellulose, and the most efficient separations were obtained using two alkaline cycles with 0.5 N KOH pretreatment (Henderson et al. 2003). It can be concluded in this study that the 0.5 N KOH pretreatment concentration also gave better acid-hydrolyzed glucose conversion yields with primary sludge. Li and Champagne (2004) also examined different pretreatments including physical, acid and alkaline pretreatments followed by enzymatic hydrolysis on crop residues, poultry manure and municipal sludges. KOH pretreatment was effective in providing higher conversion yields on wet primary sludges; however, it was not found to be as effective as HCl pretreatment and HCl followed by KOH delignification. HCl pretreatment showed a better metal removal efficiency, which reduced the potentially detrimental effect of metals on the enzymes during the hydrolytic process. In this study, KOH pretreatment was found to have an effect on the acid-hydrolyzed glucose conversion

yields of primary sludge and activated sludge. However, it was not as effective as HCl pretreatment, which might suggest that most of the cellulose content in the sludge was more easily been broken down by acid. In the case of activated sludge, higher yields were obtained with either higher KOH concentrations over shorter contact times, or lower concentrations over longer contact times. This study showed that 0.5 N KOH concentrations for 0.5 hour was the most favorable KOH pretreatment condition for activated sludge as well.

4.2 Fibre Content Analysis and Analysis Method Modification

4.2.1 Fibre Content Analysis on Sludges

The fibre analysis of cellulose, hemicellulose and lignin was conducted on the residues after acid hydrolysis as well as on the raw samples without pretreatment/acid hydrolysis. The cellulose contents were determined from the results of the ADF anlysis minus those of the ADL analysis, and hemicellulose as the results of the NDF analysis minus those of the ADF analysis, and the detailed definitions and methodologies are presented in Appendix B and Section 3.6. The results were reported on a dry mass basis of untreated sludge (g/g). And the analytical error from instrument error are presented in Appendix G. The results of the fibre content of the different sludges and biosolids after different pretreatments are compared and discussed in this section. More specifically, a comparison of the results for the untreated and

pretreated/acid hydrolyzed samples used in the fibre analysis of NDF, ADF and ADL is provided.

Table 4.6 summarizes the different pretreatment processes applied to the primary sludge, activated sludge and biosolids.

Table 4.6 Different pretreatments applied to primary sludge, activated sludge and biosolids

	Sludge Type	Pretreatment	
DP1	Primary sludge	0.5 hr, KOH	0.2 N followed with acid hydrolysis
DP2			0.5 N followed with acid hydrolysis
DP3			1.0 N followed with acid hydrolysis
DP4		1.0 hr, KOH	0.2 N followed with acid hydrolysis
DP5			0.5 N followed with acid hydrolysis
DP6			1.0 N followed with acid hydrolysis
DP7		1.5 hr, KOH	0.2 N followed with acid hydrolysis
DP8			0.5 N followed with acid hydrolysis
DP9			1.0 N followed with acid hydrolysis
DA1	Activated sludge	0.5 hr, KOH	0.2 N followed with acid hydrolysis
DA2			0.5 N followed with acid hydrolysis
DA3			1.0 N followed with acid hydrolysis
DA4		1.0 hr, KOH	0.2 N followed with acid hydrolysis
DA5			0.5 N followed with acid hydrolysis
DA6			1.0 N followed with acid hydrolysis
DA7		1.5 hr, KOH	0.2 N followed with acid hydrolysis
DA8			0.5 N followed with acid hydrolysis
DA9			1.0 N followed with acid hydrolysis
DA1	Biosolids	0.5 hr, KOH	0.2 N followed with acid hydrolysis
DA2			0.5 N followed with acid hydrolysis
DA3			1.0 N followed with acid hydrolysis
DA4		1.0 hr, KOH	0.2 N followed with acid hydrolysis
DA5			0.5 N followed with acid hydrolysis
DA6			1.0 N followed with acid hydrolysis
DA7		1.5 hr, KOH	0.2 N followed with acid hydrolysis
DA8			0.5 N followed with acid hydrolysis
DA9			1.0 N followed with acid hydrolysis
CP1	Primary sludge	0.5N HCl followed with acid hydrolysis	
CP2		1.0 N HCl followed with acid hydrolysis	
CP3		1.5 N HCl followed with acid hydrolysis	

CA1	Activated sludge	0.5N HCl followed with acid hydrolysis
CA2		1.0 N HCl followed with acid hydrolysis
CA3		1.5 N HCl followed with acid hydrolysis
CB1	Biosolids	0.5N HCl followed with acid hydrolysis
CB2		1.0 N HCl followed with acid hydrolysis
CB3		1.5 N HCl followed with acid hydrolysis
AP	Primary sludge	Wet sample followed with acid hydrolysis
AA	Activated sludge	
AB	Biosolids	
BP	Primary sludge	Dried, ground sample followed with acid hydrolysis
BA	Activated sludge	
BB	Biosolids	
Untreated	without pretreatment/acid hydrolysis	

The fibre content, cellulose, hemicellulose and lignin of pretreated/acid hydrolyzed primary sludge and untreated primary sludge samples are presented in Table 4.7.

Table 4.7 The fibre content of primary sludge with different pretreatment applications and the fibre content of untreated primary sludge. (Based on the dry mass of untreated primary sludge)

	Cellulose, g/g ADF-ADL	Hemicellulose, g/g NDF-ADF	Lignin, g/g ADL
DP1	0.24	ND	0.21
DP2	0.07	ND	0.04
DP3	0.20	ND	0.24
DP4	0.27	ND	0.18
DP5	0.05	ND	0.48
DP6	0.22	ND	0.18
DP7	0.19	ND	0.27
DP8	0.20	ND	0.29
DP9	0.29	ND	0.13
CP1	ND	0.07	0.25
CP2	0.03	0.05	0.09
CP3	ND	ND	0.14
AP	ND	0.10	0.08
BP	ND	ND	0.49
Untreated	0.17	2.50	0.09

In Table 4.7, the remaining cellulose and hemicellulose contents of primary sludge after HCl pretreatment (CP1, 2 and 3, respectively) were generally found to be low. The remaining cellulose content of the primary sludge following the 0.5 N and 1.5 N HCl pretreatments, and remaining hemicellulose of the primary sludge after 1.5 N HCl pretreatment were not detectable. The remaining cellulose content was lower for the HCl pretreated primary sludge, coinciding with glucose conversion yields for primary sludge that were relatively high with HCl pretreatment (Section 4.1.2), which would suggest that most of the cellulose was hydrolyzed during acid hydrolysis. HCl pretreatment was effective in improving the hydrolysis of the cellulose in the primary sludge, which also indicated that HCl pretreatment was an effective pretreatment for glucose recovery, where the cellulose was more readily hydrolyzed to glucose after HCl pretreatment. In section 4.1.2, KOH pretreatment on the primary sludge for glucose recovery was not found to be as effective as HCl pretreatment. This was supported by the fibre analysis which demonstrated that the decrease in the cellulose content of the primary sludge following KOH pretreatment was less than that observed after the HCl pretreatment.

From the Table 4.7, a hemicellulose content was not detected in the samples after KOH pretreatment indicating that the hemicellulose content could have been hydrolyzed and washed away after the acid hydrolysis processes, which would also indicate that KOH was effective in separating the hemicellulose from the raw materials. The lignin content of primary sludge did not appear to be affected by either the acid or alkaline pretreatments.

From Table 4.7, the remaining cellulose content was not detectable in the samples of the wet primary sludge (AP) and dried/ground primary sludge (BP) followed by acid hydrolysis. The remaining hemicellulose content of the dried/ground and pretreated primary sludge followed by acid hydrolysis (BP) was also undetectable, and the hemicellulose content of the acid hydrolyzed wet primary sludge (AP) was much lower than that of the untreated primary sludge. Compared with the dried/ground pretreated primary sludge, the remaining cellulose and hemicellulose content of the untreated samples were relatively high (0.17 and 2.50, respectively), while the dried/ground pretreated samples gave better glucose conversion yields. These results indicated that drying and grinding were an effective way to improve the acid hydrolysis yield of primary sludge and that most of the cellulose and hemicellulose was hydrolyzed during the acid hydrolysis process. The fibre content results indicated that HCl and physical drying/grinding pretreatments showed a higher increase in glucose recovery from the cellulose of primary sludge than KOH pretreatment. The undetectable or low remaining hemicellulose content of the primary sludge with acid, alkaline or physical pretreatment indicated that acid, alkaline and physical pretreatments improved hemicellulose separation from the raw materials, which could lead to higher C_5 sugar recoveries from the hemicellulose in primary sludge using acid, alkaline or physical pretreatments.

The fibre, cellulose, hemicellulose and lignin content of the pretreated and acid hydrolyzed activated sludge and untreated activated sludge samples are presented in Table 4.8.

Table 4.8 The fibre content of activated sludge with different pretreatments applications and the fibre content of untreated activated sludge. (Based on the dry mass of untreated activated sludge)

	Cellulose, g/g ADF-ADL	Hemicellulose, g/g NDF-ADF	Lignin, g/g ADL
DA1	ND	ND	0.34
DA2	0.25	ND	0.19
DA3	0.19	ND	0.32
DA4	0.21	ND	0.15
DA5	0.10	0.02	0.12
DA6	0.09	ND	0.07
DA7	0.23	ND	0.22
DA8	0.19	ND	0.11
DA9	0.16	0.05	0.07
CA1	ND	0.02	0.15
CA2	ND	0.06	0.12
CA3	0.09	ND	0.07
AA	0.06	0.01	0.12
BA	ND	ND	0.06
Untreated	0.09	0.16	0.13

The remaining hemicellulose content of the activated sludge following KOH pretreatment, presented in Table 4.8, was found to be undetectable with the exception of the samples with a 0.5 N KOH pretreatment applied for 1.0 hr followed by acid hydrolysis (DA5), as well as the 1.0 N KOH pretreatment applied for 1.5 hr followed by acid hydrolysis (DA9), although the remaining hemicellulose content of these samples was relatively low. The remaining hemicellulose content of the activated sludge samples following HCl pretreatment was also low and the remaining hemicellulose content of the samples with 1.5 N HCl pretreatment followed by acid hydrolysis was undetectable. These low remaining hemicellulose contents could have resulted from one of two factors: the hemicellulose was washed in the liquid fraction during the acid or alkaline pretreatment or the hemicellulose content was hydrolyzed during the acid hydrolysis process. The undetectable or low remaining hemicellulose

content indicated that acid or alkaline pretreatments could provide a relatively good hemicellulose separation from raw materials, particularly if C_5 sugar recovery from hemicelluloses was sought. The acid and alkaline pretreatment did not have much of an effect on the lignin content of activated sludge.

The remaining cellulose content of activated sludge with the 0.5 N and 1.0 N HCl (CA1 and CA2) pretreatments was not detectable, and the cellulose content remaining after the 1.5 N HCl pretreatment was relatively low, which indicated that most of cellulose content in the activated sludge was hydrolyzed, and that the HCl pretreatment improved the hydrolysis of the activated sludge for the recovery of glucose as C_6 sugar. These results are also consistent with the conclusions drawn from Section 4.1.2, where higher glucose conversion yields were obtained from 0.5 N and 1.0 N HCl pretreated activated sludge, and it was demonstrated that the HCl pretreatment was the most effective pretreatment for glucose recovery from cellulose.

From Table 4.8, the remaining cellulose and hemicellulose contents of activated sludge following physical drying/grinding pretreatments were undetectable and that of the wet activated sludge was relatively low, which indicated that physical pretreatment could effectively improve cellulose and hemicellulose hydrolysis.

The fibre content, cellulose, hemicellulose and lignin of pretreated/acid hydrolyzed biosolids and untreated biosolids samples are presented in Table 4.9.

Table 4.9 The fibre content of biosolids with different pretreatments applications and the fibre content of untreated biosolids. (Based on the dry mass of untreated biosolids samples)

	Cellulose, g/g ADF-ADL	Hemicellulose, g/g NDF-ADF	Lignin, g/g ADL
DB1	0.05	0.19	0.27
DB2	0.19	ND	0.17
DB3	0.21	ND	0.15
DB4	0.09	0.08	0.18
DB5	0.17	ND	0.21
DB6	0.11	ND	0.14
DB7	0.08	ND	0.14
DB8	0.03	ND	0.22
DB9	0.07	ND	0.17
CB1	0.52	ND	0.21
CB2	0.12	ND	0.17
CB3	ND	ND	0.11
AB	0.01	0.04	0.06
BB	0.16	ND	0.15
Untreated	0.14	0.19	0.08

In Section 4.1.2 and 4.1.3 it was noted that the glucose recovery yields from biosolids were low compared to those of primary sludge and activated sludge, which indicated that biosolids contained a potentially lower readily accessible cellulose content than primary and activated sludge, and that neither acid nor alkaline pretreatments were effective in enhancing the hydrolysis of cellulose from biosolids. The remaining hemicellulose content of the biosolids following KOH and HCl pretreatments were relatively low (0.19 and 0.08) to undetectable levels in some samples, which indicated that hemicellulose was being washed away during the separation step or hydrolyzed during the acid hydrolysis process. The remaining cellulose contents of physically pretreated biosolids were not very different from that of the untreated biosolids, which indicated that physical pretreatment did not improve the hydrolysis of cellulose in biosolids to glucose significantly. This was consistent

with the conclusions from section 4.1.1 which demonstrated that physical pretreatment was not an effective pretreatment to enhance the hydrolysis of the cellulose in biosolids to glucose because the glucose conversion yields from the physically pretreated biosolids were lower than of the wet biosolids. The acid and alkaline pretreatments did not appear to have an effect on the lignin content of the biosolids, which was consistent with the results obtained for primary and activated sludges, indicating that neither acid, alkaline nor physical pretreatment was effective in removing the lignin content, and that the lignin content should be separated from the raw materials in order to improve the hydrolysis.

The remaining hemicellulose was found to be low or undetectable in the three types of sludges and biosolids under physical, acid or alkaline pretreatment, which might suggest that pretreatment did have effect on the separation of hemicellulose from the raw materials, if the goal is to seek for C_5 sugar recovery from hemicellulose, acid, alkaline or physical pretreatments were all effective, particularly the acid pretreatment.

4.2.2 Modification of Crude Fibre Analysis on Untreated Primary Sludge

A modification in procedure was attempted for the crude fibre analysis which involved the centrifugation of the digestion mixture and discharge of the supernatant prior to the second filtration, after alkaline digestion (Appendix A). The starch content

is generally released during the alkaline digestion, forming a gelatinous solid which would readily clog the glass filter resulting in an extended filtration time requirement (one day). Centrifugation was introduced to improved filtration and decreased the time for crude fibre analysis. The results of crude fibre analysis with and without modification are presented in Appendix G and the analytical error from instrumentation are also presented in Appendix G. The crude fibre content with/without modification are presented and compared in Table 4.10.

Table 4.10 The comparison of crude fibre with/without modified (g/g, based on the dry mass of untreated primary sludge)

Raw	CF, g/g	CF(modified), g/g
1	0.42	0.22
2	0.44	0.23
3	0.49	0.26

However, as can be seen from Table 4.10, the modified crude fibre content results were lower than the crude fibre results without centrifugation. This was likely due to the lost (average 46%) of some soluble crude fibre material under this experiment condition during centrifugation, which would have been discarded with the supernatant. Hence, modifications to the crude fibre analysis should be examined in further studies.

4.3 Summary

In this study, different pretreatment conditions were performed on primary

sludge, activated sludge and biosolids. In general, primary sludge showed higher acid-hydrolyzed glucose conversion yields than activated sludge and biosolids which would indicate that primary sludge has the most potential as feedstock for glucose recovery which could be used in the subsequent ethanol fermentation. Physical pretreatment was effective in enhancing glucose recovery and acid pretreatment was found to be the most effective imn improving glucose recovery compared to the other pretreatment applications applied to the sludges and biosolids. The better acid pretreatment condition for primary sludge was found to be the 1.0 N HCl pretreatment applied for 24 hours. Alkaline pretreatment was also found to improve glucose recovery and the better alkaline pretreatment condition was found to be a 0.5 N KOH concentration for a 0.5 hour contact period.

The sludge and biosolids materials were collected at different times of the year. Hence, it should be noted that the composition of the sludges and biosolids may not be necessarily spatially or temporally consistent. There could also be differences in the sludge and biosolids composition depending on the municipality or season for example. However the form of pretreatment best suited for a particular feedstock should remain consistent.

The amount of cellulose content was found decreased in the hydrolyzed sludge residue which would indicate that cellulose was hydrolyzed by acid hydrolysis. And the decrease in the hemicellulose content would suggest that hemicellulose might be hydrolyzed or washed away during the pretreatment or separation steps.

The advantage of centrifugation introduced in the modification of the crude fibre analysis approach was for the purpose of time efficiency and process economics. However, a loss of crude fibre was also noted as a result of this additional centrifugation. More studies on modifications to the crude fibre analysis methodology are needed in the future, especially for the removal of the starch or protein content which might lead to clogging difficulties during filtration translatin to long experimental and process time requirements.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The utilization of waste biomass as renewable resource for energy has the potential to contribute to a cleaner environment. It can reduce the need for fossil fuels and petroleum-based products, while reducing the environmental pressures associated with the disposal of waste materials. Research on biomass conversion technologies, particularly biotechnologies, has the potential to contribute to the better utilization of the Canada's abundant waste biomass materials in order to decrease processing costs.

Biomass ethanol as a fuel and fuel additive could provide environmental and economic benefits of global proportions. The use of bioethanol can assist in meeting global reduction objectives in greenhouse gas emission, and also reduce the pressures of the international dependency on fossil fuels.

Municipal sewage sludges and biosolids contain large quantities of lignocellulosic constituents which could be converted to value-added products. Primary sludge, waste activated sludge, and biosolids were employed as lignocellulosic feedstocks for the recovery of glucose. These feedstocks were

hydrolyzed via acid hydrolysis at 120^{o}C for 1hr, following the application of a physical, acid or alkaline pretreatment. It was found that a higher glucose conversion yield could be obtained from the acid hydrolysis of the primary sludge than the activated sludge or biosolids, which might suggest that primary sludge contained a higher more readily available cellulosic content, which could otherwise have been consumed in the aeration tank during the activated sludge process or digested in the anaerobic digestion process in the wastewater treatment plant. Generally, physically pretreated sludges and biosolids yielded a higher glucose concentration than the unpretreated materials, which suggested that physical treatment could improve the glucose conversion yield from acid hydrolysis, because grinding could reduce the particle size of the materials, thereby increasing the surface area of the material and allowing for greater contact with the acid catalysis. The acid (HCl) pretreatment was generally found to be more effective in yielding higher glucose conversion from the subsequent acid hydrolysis than the alkaline pretreatment. From the results of this study, the most favorable HCl pretreatment conditions were found to be for the primary sludge at a 1.0 N HCl concentration. The KOH pretreatment condition resulting in the highest glucose conversion yields was observed to be the 0.5 N KOH concentration with a contact period of 0.5 hour.

Forage fibre analysis is generally applied on materials to determine the amount and type of crude fibre in ruminant nutrition, diet and feedstuff. In this study, fibre analysis methodologies were applied to determine the fibre content in the waste biomass, including the neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and

acid-detergent lignin (ADL) analysis of untreated and treated primary sludge, activated sludge and biosolids, and the crude fibre analysis of untreated primary sludge. However, the application of the Van Soest methods for NDF, ADF and ADL analyses and the Weende system for crude fibre analysis may have limitations in their application with waste biomass. For instance, the application of different pretreatments on materials could result in an overestimation or underestimation of the remaining NDF, ADF and ADL fractions. As well, starch, protein and other mucilaginous substances may form gelatinous solids during alkaline digestion which often cause the clogging of the glass filter system. In this study, centrifugation was introduced prior to the secondary filtration for the crude fibre analysis. Centrifugation between the alkaline digestion and the second filtration effectively reduced the filtration time required for crude fibre analysis from one day to 30 minutes. However, an average of 46% soluble crude fibre material loss was estimated under this experiment after added centrifugation step.

The cellulose and hemicellulose concentrations were estimated as the difference between ADF minus ADL, and NDF minus ADF, respectively. The remaining hemicellulose contents were generally very low to undetectable in samples which were pretreated followed by acid hydrolysis, suggesting that the hemicellulose was either hydrolyzed during acid hydrolysis process or washed away during the pretreatment process. As hemicellulose is soluble in acid or could be hydrolyzed to C_5 sugar, the results also indicated that acid pretreatment was effective in separating the hemicellulose content from the raw materials which could be used for C_5 sugar

recovery. Remaining cellulose contents were generally found to be very low to undetectable in the samples which exhibited higher glucose conversion yields, which would suggest that the cellulose contents were hydrolyzed to glucose during the acid hydrolysis processes. Acid and alkaline pretreatments did not appear to have an effect on lignin contents. The cellulose and hemicellulose contents of the primary sludge were higher than in the activated sludge and biosolids, which indicated that the primary sludge had the most potential as feedstocks for sugar recovery.

5.2 Recommendations

This study was conducted on a laboratory-scale and should be further investigated on a larger scale for its potential industrialization. The most important challenges will be to reduce the costs of pretreatment and conversion processes.

In future studies, the different types of sugar (e.g. glucose, xylose) should be separated and the quantity of sugar recovered as a function of acid or alkaline pretreatment quantified using high-performance liquid chromatography (HPLC), UV/VIS and RI detector. This would allow for a better determination of the nature of the sugars converted during the pretreatment processes and provide information for further study on the recovery of sugars which could be solubilized and lost between the pretreatment and separation steps. In this study, only the glucose content was tested in the liquid phase after the acid hydrolysis. The decrease in the amount of

hemicellulose content in samples which were pretreated followed by acid hydrolysis indicated that C_5 sugar recovery such as (xylose) from hemicellulose should be included in further studies and subsequent xylitol fermentation can also be studied in the further studies.

More research focusing on modifications to the crude fibre analysis for application to waste biomass characterization should be examined. In particular, approaches to reduce clogging and the time required for filtration would need to be developed such as using thermal amylase to remove starch which may cause the clogging problems resulting in long time requirements during the filtration stage.

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APPENDIX A

PROCEDURE FOR CRUDE FIBER ANALYSIS

(ISO 6865 MODIFIED)

Crude fiber (CF) refers to the indigestible portion of a biomass feedstock, which is composed primarily of cellulose and a fraction of non-cellulosic polysaccharides and lignin. The crude fiber content is expressed as a dry mass basis fraction in percentage.

Reagents

(1) Hydrochloric acid, HCl = 0.5 ± 0.005mol/L.

(2) Sulfuric acid, H_2SO_4= (0.13 ± 0.005) mol/L.

(3) Potassium hydroxide solution, KOH = (0.23 ± 0.005) mol/l.

(4) Acetone.

(5) Filter aid, Celite® 545.

(6) Antifoaming agent, 1-octanol.

(7) Light petroleum, boiling range 40°C to 60°C.

Apparatus

(1) Grinding device, Wiley Mill with 40 mesh.

(2) Analytical balance, with accuracy of at least 0.1 mg.

(3) Filter crucibles, Corning* Pyrex* Gooch-type Filtering Crucibles, coarse

size (40-60 μm), high form 50 mL, Fisher.

(4) Cold-extraction device, provided with

_ support for the filter crucible;

_ discharge pipe with a tap to the vacuum and liquid outlet; and

_ connecting rings for connecting the filter crucible.

(5) Incineration dishes.

(6) Conical flasks, of 250 ml capacity, provided with a refluxing condenser.

(7) Drying oven.

(8) Desiccator.

(9) Muffle furnace.

Procedure

(1) Sample Testing

Air-dried samples were grinded into fine particles using a Wiley Mill with a mesh size of 40. Approximately 1.00±0.01 g of prepared test sample was weighed. If the fat content of the sample exceeded 100g/kg, the samples were transferred to a crucible for defatting according to the defatting procedure.

(2) Preliminary defatting

Approximately 1.00±0.01 g of prepared sample was placed into a filter crucible with a thin layer of filter aid and washed with 30±1 mL of light petroleum three times under vacuum. The residue was dried using filtration suction after each washing.

If the carbonate content, expressed as calcium carbonate, exceeded 50 g/kg, the sample the carbonate was removed using the following procedure, otherwise the sample underwent acid digestion.

(3) Carbonate Removal

One hundred milliliter of HCl was added over the sample and stirred continuously for 5±1 minutes. Then the sample was decanted twice with 100±1 mL of water each time, ensuring that minimal residue remained on the filter. Next, the contents of the crucible were transferred carefully to the original conical flask and acid digestion of the sample was performed.

(4) Acid digestion

The sample was boiled with 150±1 mL of H_2SO_4 for 30±1 min and swirled a few times at the beginning of boiling. If foaming occurred, a few drops of antifoaming agent were added. During boiling, a constant volume was maintained using a refluxing condenser.

(5) First filtration

After the acid digestion, the mixture was transferred to a filter crucible containing a filter aid layer with a thickness of approximately one-fifth of the height of the filter crucible. The mixture was washed with distilled water five times with 10 ±1 mL of hot water (90±1°C) and suctioned dry by using a weak vacuum at first and increasing it as necessary. The filter plate of the crucible remained covered by the filter aid to ensure that the crude fiber would not reach the filter plate. The residue was then washed with sufficient quantity of acetone without suction for 2±1 minutes, and then dried with a slight suction. If the filter was blocked, the crude fiber covering the filter aid was carefully stirred with a stirring rod.

(6) Alkaline digestion

The remaining residue was transferred back to the conical flask used for the acid digestion and boiled with 150±1 ml of KOH solution for 30±1 minutes. During boiling, a constant volume was maintained using a refluxing condenser.

(7) Centrifuge (Modified step)

After alkaline digestion, the mixture was transferred into a 250±5 mL Nalgene bottle and then centrifuged at 7000±55 rpm for 30±1 minutes and the supernatant was discarded. The solid fraction was washed with 30 mL of hot water (90±1°C) and the mixture was subsequently filtered through the original filter crucible.

(8) Second filtration

The same procedure as that outlined in **(5) First filtration** was followed for the second filtration.

(9) Drying

The filter crucible with digested residue was placed in a ceramic dish and dried for at least 2 hours (±1 minute) in the drying oven set at a temperature of 130 ±1°C, then cooled to room temperature in a desiccator. The filter crucible and the ceramic dish were weighed immediately after removal from the desiccator and recorded as m2.

(10) Ashing

The dried filter crucible and ceramic dish were ashed in the muffle furnace at a temperature of 500±25°C until the difference between two consecutive weighings after cooling did not exceed 2 mg.

After each ashing cycle, the filter crucible and ceramic dish were cooled partly and, while still warm, placed in a desiccator to cool completely. Once cooled the sample, filter crucible and ceramic dish were weigh and recorded as m3.

(11) Blank determination

A blank determination was conducted by using only the filter aid and following the procedure described in **4** to **11.**

Calculation

The crude fiber content of the sample was calculated using Equation (A-1):

$$w_{\mathrm{f}} = \frac{m2 - m3}{m1} \qquad \text{(A-1)}$$

where wf was the crude fiber content, in grams per kilogram, of the test sample; m1 was the mass, in grams, of the test portion1; m2 was the mass, in milligrams, of the ceramic dish with the filter crucible and the residue remaining after drying at 130°C; m3 was the mass, in milligrams, of the incineration dish with the filter crucible with the residue remaining after ashing at 500±25°C.

APPENDIX B

PROCEDURE FOR NEUTRAL-DETERGENT, ACID-DETERGENT AND ACID-DETERGENT LIGNIN ANALYSIS (GOERING AND VAN SOEST, 1970)

NDF—Neutral-detergent fibre (total lignocellulosic content)

NDF fibre is the residue remaining after digestion in a detergent solution. The fibre residues were predominantly hemicellulose, cellulose and lignin. The NDF content is expressed as a dry mass basis fraction in percentage.

Reagents

(1) Neutral-detergent solution. 30.00±0.01 g sodium lauryl sulphate, USP; 18.61±0.01 g disodium dihydrogen ethylene-diamine tetra acetic dehydrate, reagent grade; 4.56±0.01 g disodium hydrogen phosphate, anhydrous, reagent grade; and 10 ±0.01 ml 2-ethoxy-ethanol (ethylene glycol, monoethyl ether), purified grade, were added into distilled water to make up a 1 L±0.3mL solution. The mixture was agitated to dissolve the chemicals and a pH range of 6.7-7.1 was obtained.

(2) Decahydronaphthalene. Technical grade.

(3) Acetone. Grade free from color and leaving no residue upon evaporation.

(4) Sodium sulfite. Anhydrous, reagent grade.

(5) Filter aid, Celite® 545.

Apparatus

(1) Grinding device, WileyMill with 40 mesh.

(2) Analytical balance, with accuracy of at least 0.1 mg.

(3) Refluxing apparatus. Any conventional apparatus suitable for crude fibre determination. Conical flasks of 250 ml capacity provided with a refluxing condenser.

(4) Filtering crucibles, Corning* Pyrex* Gooch Type Filtering Crucibles, coarse size (40-60 μm), high form 50 mL, Fisher.

(5) Drying oven.

(6) Desiccator.

(7) Muffle furnace.

Procedure

Approximately 0.50-1.00±0.01 g of air-dried sample grinded using a Wiley Mill with a 40 mesh was put into the conical flasks. Reagents were added in the following order: 100±1 mL room temperature neutral-detergent solution, 2±0.015 mL decahydronaphthalene, and 0.50±0.01 g sodium sulfite. The mixture was then heated to boiling and refluxed for 60±1 minutes, timed from the onset of boiling. After boiling, the mixture was transfer into a previously tared filter crucible (with filter aid) and a slight vacuum suction was applied, using a low vacuum at first, increasing it only as needed. The residue in the filter crucible was washed with 50±1 mL of hot water (80-90^{o}C) and then the liquid was filtered. The washing procedure was repeated 3 times. Next, the sample was washed and filtered dry twice with acetone in same manner. Finally, the crucible was dried at 100±1^{o}C for 8 hours(±1 minute) or overnight in the drying oven and cooled in a desiccator and then weighed. The yield of recovered neutral-detergent fibre was reported as the lignocellulosic content. The dried crucible was ashed for 3 hours(±1 minute) at 500-550±5^{o}C in the muffle furnace, cooled in a desiccator and weighed. The ash content was reported as

the ash insoluble in neutral-detergent.

Calculations

The neutral-detergent fibre fraction was computed using Equation (B-1):

$$\frac{(Wo - Wt) \times 100}{S} \quad \text{(B-1)}$$

Where W_o was weight of dry crucible including fibre, g; W_t was tared weight of dry crucible, g; S was oven-dry sample weight, g.

ADF— Acid-detergent fibre (lignin and cellulose)

The acid-detergent fibre procedure provided a rapid method for lignocellulose determination. The fibre residues are primarily cellulose and lignin, but can also include silica. The difference between the neutral-detergent and acid-detergent fibre is an estimate of hemicellulose; however, this difference includes some protein attached to the cell walls. The acid detergent fibre was used as a preparatory step for lignin determination. The ADF content is expressed as a dry mass basis fraction in percentage.

Reagents

(1) Acid-detergent solution. 20.00±0.01 g cetyltrimethylammonium bromide (CTAB), technical grade, was added to 1 L±0.3 mL of 1±0.001 N H_2SO_4 previously standardized and mixed.

(2) Decalin. Reagent grade decahydronaphthalene.

(3) Acetone. A grade of acetone free of color was employed which would leave no residue upon evaporation. Ethanol (95%) could be substituted but would result in a less rapid filtration.

(4) Filter aid, Celite® 545.

Apparatus

(1) Grinding device, Wiley Mill with 40 mesh.

(2) Analytical balance, with accuracy of at least 0.1 mg.

(3) Refluxing apparatus. A conventional apparatus suitable for crude fibre determinations was employed, as well as conical flasks, of 250 ml capacity, provided with a refluxing condenser.

(4) Sintered glass crucibles. Corning* Pyrex* Gooch-Type Filtering Crucibles, coarse size (40-60 μm), high form 50 mL, Fisher.

(5) Drying Oven.

(6) Desiccator.

(7) Muffle furnace.

Procedure

Approximately 0.50-1.00±0.01 g air-dry sample grinded to pass through a 1 mm screen was placed in a conical flask, to which 100±1 mL of room temperature acid-detergent solution (1) was added. Two milliliters of decalin was added in the mixture and the mixture was heated to constant boiling and refluxed for 60±1

minutes, timed from the onset of boiling. After boiling, the mixture was transfer into a previously tared filter crucible (with filter aid) and a slight vacuum suction was applied, using a low vacuum at first, increasing it only as needed. Next, the residue in the filter crucible was washed with 50±1 mL of hot water (80-90±1°C) and the liquid was filtered. The washing procedure was repeated 3 times, then the sample was washed and filtered dry with acetone in same manner until color removal was no longer apparent. The crucible was dried at 100±1°C for 8 hours (±1 minute) or overnight in the drying oven and cooled in a desiccator and then weighed. Finally, the crucible was dried at 100±1°C for 8 hours(±1 minute) or overnight in the drying oven and cooled in a desiccator and weighed once completely cooled.

Calculations

The acid-detergent fibre was computed as per Equation (B-2):

$$\frac{(Wo - Wt) \times 100}{S} \qquad \text{(B-2)}$$

Where W_o was weight of dry crucible including fibre, g; W_t was tared weight of dry crucible, g; S was oven-dry sample weight, g.

Acid-detergent Lignin

In the acid-detergent lignin procedure, the acid-detergent fibre (ADF) procedure is used as a preparatory step. The detergent removes the protein and other acid-soluble material that would interfere with the lignin determination. The ADF

residue consists of cellulose, lignin, cutin and acid-insoluble ash (mainly silica). Treatment with 72 % H_2SO_4 dissolves cellulose. Ashing of the residue will determine the crude lignin fraction including cutin. The ADL content is expressed as a dry mass basis fraction in percentage.

Reagent

Sulfuric acid (72±0.001%). Standardize reagent grade H_2SO_4 to specific gravity of 1.634 at 20°C.

Procedure

The filter crucible containing the acid-detergent fibre was placed in a ceramic dish for support. About 50±1 mL cooled (15±1°C) 72% H_2SO_4 (4) was added to the residue and stirred with a glass rod to a smooth paste, breaking all lumps, and allowing the excess acid to gravity drain. Another 50 mL of 72% H_2SO_4 was added and stirred at hourly intervals and the excess acid allowed to gravity drain away. After the acid-detergent fibre was treated with 72% H_2SO_4 for 3 hours(±1 minute), it was filtered under vacuum to remove as much acid as possible, and washed with hot water (80-90±1°C) until it was free from acid (the pH of 7 from the filter crucible outflow). The filter crucible was then placed in a drying oven at 100±1°C for 2 hours(±1 minute) and weighed after cooling in a desiccator overnight. The remaining residue was ashed in a muffle furnace at 500±5°C for 2 hours(±1 minute). Then the filter crucible was cooled in a desiccator and weighed once completely cool.

Calculations

The acid-detergent lignin was determined from Equation (B-3):

$$\frac{L \times 100}{S} \qquad \text{(B-3)}$$

Where L was loss upon ignition after 72% H_2SO_4 treatment, g; S was oven-dry sample weight.

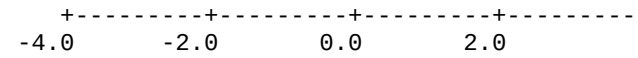
APPENDIX C

STATISTIC TESTS OF DIFFERENT TYPES OF SLUDGE AND BIOSOLIDS WITH DIFFERENT HCL CONCENTRATION PRETREATMENTS (MINITAB V15)

One-way ANOVA test of three types sludge with different HCl concentration pretreatment. Conversion yields Vs. Concentration.

One-way ANOVA: Primary sludge versus Cat

```
Source  DF       SS       MS       F      P
Cat      2  20.8763  10.4382  127.48  0.000
Error    6   0.4913   0.0819
Total    8  21.3676

S = 0.2861   R-Sq = 97.70%   R-Sq(adj) = 96.93%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ---+---------+---------+---------+------
0.5    3  2.4150  0.4081  (--*--)
1.0    3  5.6657  0.2355                           (--*---)
1.5    3  2.4552  0.1537  (--*---)
                          ---+---------+---------+---------+------
                           2.4       3.6       4.8       6.0

Pooled StDev = 0.2861


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper  ---------+---------+---------+---------+
0.5    -3.7967  -3.2507  0.0000  (--*---------------)
1.0     0.0000   3.2105  3.7565                    (---------------*--)
1.5    -3.7565  -3.2105  0.0000  (--*---------------)
                                 ---------+---------+---------+---------+
                                       -2.0       0.0       2.0       4.0


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.5 subtracted from:

Cat    Lower  Center   Upper     +---------+---------+---------+---------
1.0   2.5337  3.2507  3.9677                                      (--*---)
1.5  -0.6768  0.0402  0.7572                        (--*---)
                               +---------+---------+---------+---------
                             -4.0      -2.0       0.0       2.0

Cat = 1.0 subtracted from:

Cat    Lower   Center    Upper     +---------+---------+---------+---------
1.5  -3.9275  -3.2105  -2.4936     (---*---)
```



Two-Sample T-Test and CI: Primary sludge -1, Cat-1 (0.5N and 1.5N)

```
Two-sample T for Raw-1

Cat-1  N   Mean  StDev  SE Mean
0.5    3  2.415  0.408     0.24
1.5    3  2.455  0.154    0.089


Difference = mu (0.5) - mu (1.5)
Estimate for difference:  -0.040174
95% CI for difference:  (-0.739206, 0.658858)
T-Test of difference = 0 (vs not =): T-Value = -0.16  P-Value = 0.881  DF = 4
Both use Pooled StDev = 0.3084
```

Data for 1N are statistically different and higher than the other two groups. The 0.5N and1.5N groups are not statistically different.

One-way ANOVA: Activated sludge versus Cat

```
Source  DF     SS     MS     F      P
Cat      2  2.731  1.366  6.09  0.036
Error    6  1.346  0.224
Total    8  4.077

S = 0.4736   R-Sq = 66.99%   R-Sq(adj) = 55.99%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ---------+---------+---------+---------+
0.5    3  4.8354  0.4706                    (--------*---------)
1.0    3  3.5225  0.6310  (--------*---------)
1.5    3  4.4487  0.2308              (---------*--------)
                          ---------+---------+---------+---------+
                                 3.50      4.20      4.90      5.60

Pooled StDev = 0.4736


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper   --+---------+---------+---------+-------
0.5    -0.5170   0.3867  1.2904                    (--------*--------)
1.0    -2.2167  -1.3130  0.0000   (--------*------------)
1.5    -1.2904  -0.3867  0.5170            (--------*--------)
                                --+---------+---------+---------+-------
                               -2.0      -1.0       0.0       1.0


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.5 subtracted from:

Cat    Lower   Center    Upper    -+---------+---------+---------+--------
1.0  -2.4997  -1.3130  -0.1262    (---------*---------)
1.5  -1.5735  -0.3867   0.8001             (---------*---------)
                                -+---------+---------+---------+--------
                              -2.4      -1.2       0.0       1.2


Cat = 1.0 subtracted from:

Cat    Lower  Center   Upper    -+---------+---------+---------+--------
1.5  -0.2605  0.9263  2.1130                      (---------*---------)
                              -+---------+---------+---------+--------
                            -2.4      -1.2       0.0       1.2
```

Two-Sample T-Test and CI: Act-1, Cat-1 (0.5N and 1.5N)

```
Two-sample T for ACT-1

Cat-1  N   Mean  StDev  SE Mean
0.5    3  4.835  0.471     0.27
1.5    3  4.449  0.231     0.13


Difference = mu (0.5) - mu (1.5)
Estimate for difference:  0.386697
95% CI for difference:  (-0.453595, 1.226989)
T-Test of difference = 0 (vs not =): T-Value = 1.28  P-Value = 0.270  DF = 4
Both use Pooled StDev = 0.3707
```

Data for 1N are statistically different and lower than the other two groups. The 0.5N and1.5N groups are not statistically different.

One-way ANOVA: Biosolids versus Cat

```
Source  DF      SS      MS      F      P
Cat      2  7.3694  3.6847  44.52  0.000
Error    6  0.4966  0.0828
Total    8  7.8660

S = 0.2877   R-Sq = 93.69%   R-Sq(adj) = 91.58%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ------+---------+---------+---------+---
0.5    3  2.8196  0.3968  (-----*-----)
1.0    3  4.6651  0.2452                           (-----*----)
1.5    3  4.8054  0.1752                             (-----*----)
                          ------+---------+---------+---------+---
                              2.80      3.50      4.20      4.90

Pooled StDev = 0.2877


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper  -----+---------+---------+---------+----
0.5    -2.5348  -1.9859  0.0000  (----*-------------------)
1.0    -0.6893  -0.1404  0.4086                   (-----*----)
1.5    -0.4086   0.1404  0.6893                      (----*-----)
                               -----+---------+---------+---------+----
                                 -2.0      -1.0       0.0       1.0


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.5 subtracted from:

Cat   Lower  Center   Upper  ---------+---------+---------+---------+
1.0  1.1246  1.8455  2.5664                     (------*-------)
1.5  1.2650  1.9859  2.7068                       (------*------)
                            ---------+---------+---------+---------+
                                   0.0       1.0       2.0       3.0


Cat = 1.0 subtracted from:

Cat    Lower  Center   Upper  ---------+---------+---------+---------+
1.5  -0.5805  0.1404  0.8612     (------*-------)
                            ---------+---------+---------+---------+
                                   0.0       1.0       2.0       3.0
```

Two-Sample T-Test and CI: Bio-1, Cat-1 (1.0 N and 1.5 N)

```
Two-sample T for Pri-1

Cat-2  N   Mean  StDev  SE Mean
1.0    3  4.665  0.245     0.14
1.5    3  4.805  0.175     0.10


Difference = mu (1.0) - mu (1.5)
Estimate for difference:  -0.140368
95% CI for difference:  (-0.623488, 0.342752)
T-Test of difference = 0 (vs not =): T-Value = -0.81  P-Value = 0.465  DF = 4
Both use Pooled StDev = 0.2131
```

Data for 0.5N are statistically different and lower than the other two groups. The 1N and1.5N groups are not statistically different.

Non-parametric test of three types of sludge with different HCl concentration pretreatment. Conversion yields Vs. Concentration.

Kruskal-Wallis Test: Primary sludge versus Cat

```
Kruskal-Wallis Test on Primary sludge

                     Ave
Cat      N  Median  Rank      Z
0.5      3   2.314   3.3  -1.29
1.0      3   5.691   8.0   2.32
1.5      3   2.437   3.7  -1.03
Overall  9           5.0

H = 5.42  DF = 2  P = 0.066

* NOTE * One or more small samples
```

Data for three groups are not significantly different, but 1.0 N group is higher than 0.5 N and 1.5 N groups.

Mann-Whitney Test and CI: Primary sludge_1, Primary sludge_2 (0.5 N and 1.5 N)

```
                  N  Median
Primary sludge_1  3  2.3143
Primary sludge_2  3  2.4366

Point estimate for ETA1-ETA2 is -0.1223
91.9 Percent CI for ETA1-ETA2 is (-0.5508,0.5524)
W = 10.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 1.0000
```

0.5 N and 1.5 N groups are not significantly different.

Kruskal-Wallis Test: Activated sludge versus Cat

```
Kruskal-Wallis Test on Activated sludge

                     Ave
Cat      N  Median  Rank      Z
0.5      3   4.650   7.3   1.81
1.0      3   3.824   2.0  -2.32
1.5      3   4.347   5.7   0.52
Overall  9           5.0

H = 5.96  DF = 2  P = 0.051

* NOTE * One or more small samples
```

Data for three groups are not significantly different. But 1.0 N groups are lower than 0.5 N and 1.5 N groups.

Mann-Whitney Test and CI: Activated sludge_1, Activated sludge_2 (0.5 N and 1.5N)

```
                    N  Median
Activated sludge_1  3  4.6501
```

```
Activated sludge_2  3  4.3466


Point estimate for ETA1-ETA2 is 0.3035
91.9 Percent CI for ETA1-ETA2 is (-0.2271,1.0839)
W = 13.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3827
```

0.5 N and 1.5 N groups are not significantly different.

Kruskal-Wallis Test: Biosolids versus Cat

```
Kruskal-Wallis Test on Biosolids

                    Ave
Cat      N  Median  Rank      Z
0.5      3   2.899   2.0  -2.32
1.0      3   4.655   6.0   0.77
1.5      3   4.764   7.0   1.55
Overall  9           5.0

H = 5.60  DF = 2  P = 0.061

* NOTE * One or more small samples
```

Data for three groups are not significantly different. But 0.5 N are lower than 1.0 N and 1.5 N groups.

Mann-Whitney Test and CI: Biosolids_1, Biosolids_2 (1.0 N and 1.5 N)

```
             N  Median
Biosolids_1  3  4.6548
Biosolids_2  3  4.7643


Point estimate for ETA1-ETA2 is -0.1095
91.9 Percent CI for ETA1-ETA2 is (-0.5725,0.2607)
W = 9.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6625
```

1.0 N and 1.5 N groups are not significantly different.

APPENDIX D

STATISTIC TESTS OF DIFFERENT TYPES OF SLUDGES AND BIOSOLIDS WITH DIFFERENT KOH CONCENTRATION PRETREATMENTS FOR THREE CONTACT PERIODS

(MINITAB V15)

One-way ANOVA test of three types of sludge with different KOH concentration pretreatment for three duration time. Conversion yields Vs. Concentration.

Three sludges were pretreated for 0.5 hr

One-way ANOVA: Primary sludge-0.5hr versus Cat

```
Source  DF     SS     MS     F     P
Cat      2  2.4298  1.2149  22.83  0.002
Error    6  0.3193  0.0532
Total    8  2.7491

S = 0.2307   R-Sq = 88.39%   R-Sq(adj) = 84.51%


                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Level  N    Mean   StDev  --+---------+---------+---------+-------
0.2    3  3.0035  0.3294               (-----*------)
0.5    3  3.4609  0.1938                      (-----*------)
1.0    3  2.2036  0.1166  (-----*------)
                     --+---------+---------+---------+-------
                     2.00     2.50     3.00     3.50

Pooled StDev = 0.2307


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper  ----+---------+---------+---------+-----
0.2    -0.8976  -0.4574  0.0000           (-----*------)
0.5     0.0000   0.4574  0.8976                     (------*-----)
1.0    -1.6975  -1.2573  0.0000  (-----*-----------------)
                          ----+---------+---------+---------+-----
                           -1.40    -0.70     0.00     0.70


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.2 subtracted from:

Cat    Lower   Center    Upper  --------+---------+---------+---------+-
0.5  -0.1206   0.4574   1.0354               (-----*----)
1.0  -1.3779  -0.7999  -0.2219     (-----*-----)
                          --------+---------+---------+---------+-
                              -1.0      0.0      1.0      2.0


Cat = 0.5 subtracted from:
```

```
Cat   Lower   Center   Upper  --------+---------+---------+---------+-
1.0  -1.8353  -1.2573  -0.6793  (----*-----)
                          --------+---------+---------+---------+-
                              -1.0      0.0      1.0      2.0
```

Two-Sample T-Test and CI: Pri-1, Cat-1 (0.2 N and 1.0 N)

```
Two-sample T for Pri-1

Cat-1  N   Mean  StDev  SE Mean
0.2    3  3.004  0.329    0.19
1.0    3  2.204  0.117   0.067


Difference = mu (0.2) - mu (1.0)
Estimate for difference:  0.800
95% CI for difference:  (-0.068, 1.668)
T-Test of difference = 0 (vs not =): T-Value = 3.96  P-Value = 0.058  DF = 2
```

Data for 0.5 N group are higher than the other, 0.2 N and 1.0 N are not significantly different.

One-way ANOVA: Activated sludge-0.5hr versus Cat

```
Source  DF     SS     MS    F     P
Cat      2  0.6670  0.3335  5.86  0.039
Error    6  0.3415  0.0569
Total    8  1.0085

S = 0.2386   R-Sq = 66.14%   R-Sq(adj) = 54.85%


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level  N    Mean   StDev  ----+---------+---------+---------+-----
0.2    3  1.7958  0.1885       (--------*---------)
0.5    3  2.2543  0.2472                 (--------*---------)
1.0    3  1.6057  0.2722  (---------*---------)
                      ----+---------+---------+---------+-----
                        1.40     1.75     2.10     2.45

Pooled StDev = 0.2386


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper  --------+---------+---------+---------+-
0.2    -0.9137  -0.4585  0.0000    (------*-------)
0.5     0.0000   0.4585  0.9137               (-------*------)
1.0    -1.1038  -0.6486  0.0000  (------*----------)
                          --------+---------+---------+---------+-
```

```
                              -0.60     0.00     0.60     1.20


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.2 subtracted from:

Cat   Lower   Center   Upper  --------+---------+---------+---------+-
0.5  -0.1393   0.4585  1.0563              (--------*-------)
1.0  -0.7879  -0.1901  0.4077       (-------*--------)
                         --------+---------+---------+---------+-
                             -0.70     0.00     0.70     1.40


Cat = 0.5 subtracted from:

Cat   Lower   Center   Upper  --------+---------+---------+---------+-
1.0  -1.2464  -0.6486  -0.0508  (--------*-------)
                          --------+---------+---------+---------+-
                              -0.70     0.00     0.70     1.40
```

Two-Sample T-Test and CI: Act-0.5hr-1, Cat-1

```
Two-sample T for Act-0.5hr-1

Cat-1  N   Mean  StDev  SE Mean
0.2    3  1.796  0.189    0.11
1.0    3  1.606  0.272    0.16


Difference = mu (0.2) - mu (1.0)
Estimate for difference:  0.190
95% CI for difference:  (-0.418, 0.798)
T-Test of difference = 0 (vs not =): T-Value = 0.99  P-Value = 0.393  DF = 3
```

Data for 0.5 N group are higher than 0.2 N and 1.0 N groups. 0.2 N and 1.0 N are not significantly different.

One-way ANOVA: Biosolids-0.5h versus Cat

```
Source  DF     SS     MS     F     P
Cat      2  1.1922  0.5961  34.78  0.001
Error    6  0.1028  0.0171
Total    8  1.2950

S = 0.1309   R-Sq = 92.06%   R-Sq(adj) = 89.41%


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level  N    Mean   StDev  ---------+---------+---------+---------+
```

```
0.2   3  1.7531  0.1838                      (----*----)
0.5   3  0.9034  0.0812  (----*----)
1.0   3  1.0948  0.1051      (----*-----)
                     ---------+---------+---------+---------+
                           1.05     1.40     1.75     2.10

Pooled StDev = 0.1309


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level   Lower   Center   Upper  --------+---------+---------+---------+-
0.2    0.0000   0.6584  0.9082                (----------*---)
0.5   -1.0995  -0.8498  0.0000  (---*-------------)
1.0   -0.9082  -0.6584  0.0000    (---*----------)
                          --------+---------+---------+---------+-
                              -0.60     0.00     0.60     1.20


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.2 subtracted from:

Cat   Lower   Center   Upper  ----+---------+---------+---------+-----
0.5  -1.1778  -0.8498  -0.5217  (------*------)
1.0  -0.9864  -0.6584  -0.3304     (------*-----)
                         ----+---------+---------+---------+-----
                          -1.00    -0.50     0.00     0.50


Cat = 0.5 subtracted from:

Cat   Lower  Center   Upper  ----+---------+---------+---------+-----
1.0  -0.1366  0.1914  0.5194                   (------*-----)
                        ----+---------+---------+---------+-----
                         -1.00    -0.50     0.00     0.50
```

Two-Sample T-Test and CI: Bio-0.5-1, Cat-1 (0.2 N and 1.0 N)

```
Two-sample T for Bio-0.5-1

Cat-1  N   Mean  StDev  SE Mean
0.2    3  1.753  0.184    0.11
1.0    3  1.095  0.105   0.061


Difference = mu (0.2) - mu (1.0)
Estimate for difference:  0.658
95% CI for difference:  (0.269, 1.047)
T-Test of difference = 0 (vs not =): T-Value = 5.39  P-Value = 0.013  DF = 3
```

Two-Sample T-Test and CI: Bio-0.5h_1, Cat-2 (0.5 N and 1.0 N)

```
Two-sample T for Bio-0.5h_1

Cat-2  N    Mean   StDev  SE Mean
0.5    3  0.9034  0.0812    0.047
1.0    3   1.095   0.105    0.061


Difference = mu (0.5) - mu (1.0)
Estimate for difference:  -0.1914
95% CI for difference:  (-0.4354, 0.0526)
T-Test of difference = 0 (vs not =): T-Value = -2.50  P-Value = 0.088  DF = 3
```

Data for 0.2N group are the highest, 0.5 N and 1.0 N are not significantly different.

Three types of sludges were pretreated for 1.0 hr.

One-way ANOVA: Primary sludge-1.0hr versus Cat

```
Source  DF     SS     MS     F     P
Cat      2  1.8665  0.9332  84.75  0.000
Error    6  0.0661  0.0110
Total    8  1.9326

S = 0.1049   R-Sq = 96.58%   R-Sq(adj) = 95.44%


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level  N   Mean   StDev  ---+---------+---------+---------+------
0.2    3  1.0888  0.1060  (---*---)
0.5    3  2.1591  0.0832                          (----*---)
1.0    3  1.3516  0.1220       (----*---)
                      ---+---------+---------+---------+------
                       1.05     1.40     1.75     2.10

Pooled StDev = 0.1049


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level   Lower   Center   Upper    -+---------+---------+---------+--------
0.2    -1.2705  -1.0703  0.0000   (--*-----------------)
0.5     0.0000   0.8074  1.0077                    (------------*---)
1.0    -1.0077  -0.8074  0.0000       (---*------------)
                           -+---------+---------+---------+--------
                         -1.20     -0.60     0.00     0.60


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.2 subtracted from:

Cat   Lower  Center  Upper  -----+---------+---------+---------+----
0.5  0.8073  1.0703  1.3332                        (--*---)
1.0 -0.0001  0.2628  0.5258              (---*---)
                         -----+---------+---------+---------+----
                           -0.70     0.00     0.70     1.40


Cat = 0.5 subtracted from:

Cat   Lower   Center   Upper  -----+---------+---------+---------+----
1.0 -1.0704  -0.8074  -0.5445  (--*---)
                           -----+---------+---------+---------+----
                             -0.70     0.00     0.70     1.40
```

Two-Sample T-Test and CI: Pri-1.0-1, Cat-1 (0.2 N and 1.0 N)

```
Two-sample T for Pri-1.0-1

Cat-1  N   Mean  StDev  SE Mean
0.2    3  1.089  0.106   0.061
1.0    3  1.352  0.122   0.070


Difference = mu (0.2) - mu (1.0)
Estimate for difference:  -0.2628
95% CI for difference:  (-0.5598, 0.0341)
T-Test of difference = 0 (vs not =): T-Value = -2.82  P-Value = 0.067  DF = 3
```

Data for 0.5 N group are higher than the other, 0.2 and 1.0 are not significantly different.

One-way ANOVA: Activated sludge-1.0h versus Cat

```
Source  DF     SS     MS     F     P
Cat      2  1.7211  0.8605  63.08  0.000
Error    6  0.0818  0.0136
Total    8  1.8029

S = 0.1168   R-Sq = 95.46%   R-Sq(adj) = 93.95%


                      Individual 95% CIs For Mean Based on Pooled StDev
Level  N    Mean   StDev   +---------+---------+---------+---------
0.2    3  2.2830  0.0745                          (---*----)
0.5    3  1.5781  0.1644            (----*----)
1.0    3  1.2321  0.0913   (----*----)
                       +---------+---------+---------+---------
                      1.05     1.40     1.75     2.10

Pooled StDev = 0.1168


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level   Lower   Center   Upper    -+---------+---------+---------+--------
0.2    0.0000   0.7049  0.9278                     (-----------*--)
0.5   -0.9278  -0.7049  0.0000         (--*-----------)
1.0   -1.2738  -1.0509  0.0000    (--*-----------------)
                           -+---------+---------+---------+--------
                         -1.20     -0.60     0.00     0.60


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.2 subtracted from:
```

```
Cat   Lower   Center   Upper  -------+---------+---------+---------+--
0.5  -0.9976  -0.7049  -0.4123        (-----*-----)
1.0  -1.3436  -1.0509  -0.7583  (-----*-----)
                          -------+---------+---------+---------+--
                             -1.00    -0.50     0.00     0.50


Cat = 0.5 subtracted from:

Cat   Lower   Center   Upper  -------+---------+---------+---------+--
1.0  -0.6386  -0.3460  -0.0533             (-----*-----)
                          -------+---------+---------+---------+--
                             -1.00    -0.50     0.00     0.50
```

Two-Sample T-Test and CI: Act-1.0h-1, Cat-2 (0.5 N and 1.0 N)

```
Two-sample T for Act-1.0N-1

Cat-2  N    Mean   StDev  SE Mean
0.5    3   1.578   0.164   0.095
1.0    3  1.2321  0.0913   0.053


Difference = mu (0.5) - mu (1.0)
Estimate for difference:  0.346
95% CI for difference:  (0.000, 0.692)
T-Test of difference = 0 (vs not =): T-Value = 3.19  P-Value = 0.050  DF = 3
```

Data for 0.2 N group are higher than the other two groups, data for 1.0 N groups are the lowest.

One-way ANOVA: Biosolids-1.0h versus Cat

```
Source  DF      SS     MS      F     P
Cat      2  12.6460  6.3230  110.52  0.000
Error    6   0.3433  0.0572
Total    8  12.9892

S = 0.2392   R-Sq = 97.36%   R-Sq(adj) = 96.48%


                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Level  N    Mean   StDev  -------+---------+---------+---------+--
0.2    3  1.8798  0.2227    (---*--)
0.5    3  4.2383  0.2284                       (--*---)
1.0    3  1.5924  0.2644  (--*--)
                     -------+---------+---------+---------+--
                         2.0      3.0      4.0      5.0

Pooled StDev = 0.2392


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means
```

```
Level   Lower   Center   Upper    -+---------+---------+---------+--------
0.2   -2.8149  -2.3585  0.0000     (--*---------------)
0.5    0.0000   2.3585  2.8149                   (---------------*--)
1.0   -3.1023  -2.6459  0.0000   (--*-----------------)
                           -+---------+---------+---------+--------
                         -3.0     -1.5      0.0      1.5


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.2 subtracted from:

Cat   Lower   Center   Upper  ------+---------+---------+---------+---
0.5  1.7592   2.3585  2.9579                       (--*--)
1.0 -0.8867  -0.2873  0.3120            (--*--)
                        ------+---------+---------+---------+---
                           -2.0      0.0      2.0      4.0


Cat = 0.5 subtracted from:

Cat   Lower   Center   Upper  ------+---------+---------+---------+---
1.0 -3.2452  -2.6459  -2.0466  (--*--)
                          ------+---------+---------+---------+---
                             -2.0      0.0      2.0      4.0
```

Two-Sample T-Test and CI: Bio-1.0h_1, Cat-1 (0.2 N and 1.0 N)

```
Two-sample T for Bio-1.0h_1

Cat-1  N   Mean  StDev  SE Mean
0.2    3  1.880  0.223    0.13
1.0    3  1.592  0.264    0.15


Difference = mu (0.2) - mu (1.0)
Estimate for difference:  0.287
95% CI for difference:  (-0.348, 0.922)
T-Test of difference = 0 (vs not =): T-Value = 1.44  P-Value = 0.246  DF = 3
```

Data for 0.5 N group are highest, 0.2 N and 1.0 N groups are not significantly different

Three types of sludges were pretreated for 1.5 hr.

One-way ANOVA: Primary sludge-1.5h versus Cat

```
Source  DF     SS     MS    F     P
Cat      2  0.2611  0.1306  8.57  0.017
Error    6  0.0914  0.0152
Total    8  0.3525

S = 0.1234   R-Sq = 74.08%   R-Sq(adj) = 65.43%


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level  N   Mean   StDev  -----+---------+---------+---------+----
0.2    3  2.0439  0.0913                (--------*--------)
0.5    3  1.7002  0.1329   (--------*--------)
1.0    3  1.6673  0.1403  (-------*--------)
                      -----+---------+---------+---------+----
                        1.60     1.80     2.00     2.20

Pooled StDev = 0.1234


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper    +---------+---------+---------+---------
0.2     0.0000   0.3438  0.5792                     (----------*-------)
0.5    -0.5792  -0.3438  0.0000     (-------*----------)
1.0    -0.6121  -0.3766  0.0000    (------*------------)
                            +---------+---------+---------+---------
                          -0.60     -0.30     0.00      0.30


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.2 subtracted from:

Cat    Lower   Center    Upper  ---+---------+---------+---------+------
0.5  -0.6530  -0.3438  -0.0345   (----------*---------)
1.0  -0.6859  -0.3766  -0.0674  (---------*----------)
                          ---+---------+---------+---------+------
                          -0.60     -0.30     0.00      0.30


Cat = 0.5 subtracted from:

Cat    Lower   Center   Upper  ---+---------+---------+---------+------
1.0  -0.3421  -0.0329  0.2763            (---------*---------)
                         ---+---------+---------+---------+------
                         -0.60     -0.30     0.00      0.30
```

Two-Sample T-Test and CI: Pri-1.5-1, Cat-2 (0.5 N and 1.0 N)

```
Two-sample T for Pri-1.5-1

Cat-2  N   Mean  StDev  SE Mean
0.5    3  1.700  0.133   0.077
1.0    3  1.667  0.140   0.081


Difference = mu (0.5) - mu (1.0)
Estimate for difference:  0.033
95% CI for difference:  (-0.322, 0.388)
T-Test of difference = 0 (vs not =): T-Value = 0.29  P-Value = 0.787  DF = 3
```

Data for 0.2 N group are higher than the other two groups, 0.5 N and 1.0 N are not significantly different.

One-way ANOVA: Activated sludge-1.5h versus Cat

```
Source  DF     SS     MS     F     P
Cat      2  1.5444  0.7722  28.54  0.001
Error    6  0.1624  0.0271
Total    8  1.7068

S = 0.1645   R-Sq = 90.49%   R-Sq(adj) = 87.32%


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level  N    Mean   StDev  -------+---------+---------+---------+--
0.2    3  1.7464  0.1670                       (-----*----)
0.5    3  0.7376  0.0281  (----*-----)
1.0    3  1.3366  0.2291             (----*-----)
                      -------+---------+---------+---------+--
                          0.80     1.20     1.60     2.00

Pooled StDev = 0.1645


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper   --+---------+---------+---------+-------
0.2     0.0000   0.4098  0.7237                     (------*----)
0.5    -1.3227  -1.0088  0.0000   (----*----------------)
1.0    -0.7237  -0.4098  0.0000           (----*------)
                           --+---------+---------+---------+-------
                          -1.20    -0.60     0.00     0.60


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat
```

```
Individual confidence level = 97.80%


Cat = 0.2 subtracted from:

Cat   Lower   Center   Upper    +---------+---------+---------+---------
0.5  -1.4210  -1.0088  -0.5966    (-----*----)
1.0  -0.8220  -0.4098   0.0024          (-----*-----)
                          +---------+---------+---------+---------
                       -1.40     -0.70     0.00     0.70


Cat = 0.5 subtracted from:

Cat   Lower  Center   Upper    +---------+---------+---------+---------
1.0  0.1868  0.5990  1.0112                      (-----*----)
                         +---------+---------+---------+---------
                      -1.40     -0.70     0.00     0.70
```

Two-Sample T-Test and CI: Act-1.5h-1, Cat-1 (0.2 N and 1.0 N)

```
Two-sample T for Act-1.5h-1

Cat-1  N   Mean  StDev  SE Mean
0.2    3  1.746  0.167   0.096
1.0    3  1.337  0.229    0.13


Difference = mu (0.2) - mu (1.0)
Estimate for difference:  0.410
95% CI for difference:  (-0.111, 0.931)
T-Test of difference = 0 (vs not =): T-Value = 2.50  P-Value = 0.087  DF = 3
```

Data for 0.5 N group are lower than the other two groups, 0.2 and 1.0 N are not significantly different.

One-way ANOVA: Biosolids-1.5h versus Cat

```
Source  DF     SS     MS      F     P
Cat      2  4.6290  2.3145  198.77  0.000
Error    6  0.0699  0.0116
Total    8  4.6989

S = 0.1079   R-Sq = 98.51%   R-Sq(adj) = 98.02%


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level  N    Mean   StDev  ---------+---------+---------+---------+
0.2    3  2.2110  0.1706                           (--*--)
0.5    3  0.6897  0.0540  (--*--)
1.0    3  0.6897  0.0540  (--*--)
                      ---------+---------+---------+---------+
                           1.00     1.50     2.00     2.50
```

```
Pooled StDev = 0.1079


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level   Lower   Center   Upper  -------+---------+---------+---------+--
0.2    0.0000   1.5213  1.7272               (--------------*-)
0.5   -1.7272  -1.5213  0.0000  (-*--------------)
1.0   -1.7272  -1.5213  0.0000  (-*--------------)
                           -------+---------+---------+---------+--
                              -1.0      0.0      1.0      2.0


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.2 subtracted from:

Cat   Lower   Center   Upper    +---------+---------+---------+---------
0.5  -1.7917  -1.5213  -1.2510    (----*---)
1.0  -1.7917  -1.5213  -1.2510    (----*---)
                            +---------+---------+---------+---------
                         -1.80    -1.20    -0.60     0.00


Cat = 0.5 subtracted from:

Cat   Lower  Center   Upper    +---------+---------+---------+---------
1.0  -0.2704  0.0000  0.2704                        (----*----)
                          +---------+---------+---------+---------
                       -1.80    -1.20    -0.60     0.00
```

Two-Sample T-Test and CI: Bio-1.5h_1, Cat-2 (0.5 N and 1.0 N)

```
Two-sample T for Bio-1.5h_1

Cat-2  N   Mean   StDev  SE Mean
0.5    3  0.6897  0.0540   0.031
1.0    3  0.6897  0.0540   0.031


Difference = mu (0.5) - mu (1.0)
Estimate for difference:  0.0000
95% CI for difference:  (-0.1224, 0.1224)
T-Test of difference = 0 (vs not =): T-Value = 0.00  P-Value = 1.000  DF = 4
```

Data for 0.2 N group are highest, 0.5N and 1.0 N groups are not significantly different

Non-parametric test of Primary Sludge with different KOH concentration for three duration time. Conversion yields Vs. Concentration.

Kruskal-Wallis Test: Pri-0.5hr versus Cat

```
Kruskal-Wallis Test on Pri-0.5hr

                  Ave
Cat     N  Median  Rank     Z
0.2     3   3.095   5.0   0.00
0.5     3   3.418   8.0   2.32
1.0     3   2.194   2.0  -2.32
Overall  9         5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Three groups are significantly different. And the 0.5 N is the higher group.

Mann-Whitney Test and CI: Pri-0.5hr_0.2, Pri-0.5hr_1.0

```
             N  Median
Pri-0.5hr_0.2  3  3.0954
Pri-0.5hr_1.0  3  2.1938

Point estimate for ETA1-ETA2 is 0.9016
91.9 Percent CI for ETA1-ETA2 is (0.3130,1.1849)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.2 N and 1.0 N are significantly different.

Mann-Whitney Test and CI: Pri-0.5hr_0.2, Pri-0.5hr_0.5

```
             N  Median
Pri-0.5hr_0.2  3  3.0954
Pri-0.5hr_0.5  3  3.4176


Point estimate for ETA1-ETA2 is -0.3954
91.9 Percent CI for ETA1-ETA2 is (-1.0349,-0.0150)
W = 6.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.2 N and 0.5 N are significantly different.

Kruskal-Wallis Test: Pri-1.0hr versus Cat

```
Kruskal-Wallis Test on Pri-1.0hr

                  Ave
Cat     N  Median  Rank     Z
0.2     3   1.062   2.0  -2.32
0.5     3   2.168   8.0   2.32
1.0     3   1.316   5.0   0.00
Overall  9         5.0
```

```
H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Three groups are significantly different. And the 0.5 N is the higher group.

Mann-Whitney Test and CI: Pri-1.0h_1.0, Pri-1.0h_0.2

```
            N  Median
Pri-1.0h_1.0  3  1.3160
Pri-1.0h_0.2  3  1.0623


Point estimate for ETA1-ETA2 is 0.2537
91.9 Percent CI for ETA1-ETA2 is (0.0459,0.4889)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.2 N and 1.0 N are significantly different.

Mann-Whitney Test and CI: Pri-1.0h_1.0, Pri-1.0h_0.5

```
            N  Median
Pri-1.0h_1.0  3  1.3160
Pri-1.0h_0.5  3  2.1678


Point estimate for ETA1-ETA2 is -0.8205
91.9 Percent CI for ETA1-ETA2 is (-0.9861,-0.5845)
W = 6.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.5 N and 1.0 N are significantly different.

Kruskal-Wallis Test: Pri-1.5h versus Cat

```
Kruskal-Wallis Test on Pri-1.5h

                  Ave
Cat      N  Median  Rank     Z
0.2      3   2.074   8.0  2.32
0.5      3   1.707   4.0 -0.77
1.0      3   1.654   3.0 -1.55
Overall  9         5.0

H = 5.60  DF = 2  P = 0.061

* NOTE * One or more small samples
```

Three groups are not significantly different. 0.2 N group is higher than 0.5 N and 1.0 N groups.

Mann-Whitney Test and CI: Pri-1.5h-0.5, Pri-1.5h-1.0

```
            N  Median
Pri-1.5h-0.5  3  1.7069
Pri-1.5h-1.0  3  1.6536
```

```
Point estimate for ETA1-ETA2 is 0.0297
91.9 Percent CI for ETA1-ETA2 is (-0.2499,0.2953)
W = 12.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6625
```

0.5 N and 1.0 N groups are not significant different.

Mann-Whitney Test and CI: Pri-1.5h-0.2, Pri-1.5h-0.5

```
            N  Median
Pri-1.5h-0.2  3  2.0737
Pri-1.5h-0.5  3  1.7069


Point estimate for ETA1-ETA2 is 0.3668
91.9 Percent CI for ETA1-ETA2 is (0.1119,0.5526)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.2 N and 0.5 N groups are significantly different.

Non-parametric test of Activated Sludge with different KOH concentration for three duration time. Conversion yields Vs. Concentration.

Kruskal-Wallis Test: Act-0.5hr versus Cat

```
Kruskal-Wallis Test on Act-0.5hr

                Ave
Cat     N  Median  Rank     Z
0.2     3   1.745   4.0  -0.77
0.5     3   2.125   8.0   2.32
1.0     3   1.659   3.0  -1.55
Overall 9           5.0

H = 5.60  DF = 2  P = 0.061

* NOTE * One or more small samples
```

Three groups are not significantly different, but 0.5 N group is higher than 0.2 N and 1.0 N groups.

Mann-Whitney Test and CI: Act-0.5hr_0.2, Act-0.5hr_1

```
             N  Median
Act-0.5hr_0.2  3  1.7453
Act-0.5hr_1    3  1.6593


Point estimate for ETA1-ETA2 is 0.1574
91.9 Percent CI for ETA1-ETA2 is (-0.2094,0.6937)
W = 12.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6625
```

0.2 N and 1.0 N groups are not significantly different.

Mann-Whitney Test and CI: Act-0.5hr_0.2, Act-0.5hr_0.5

```
             N  Median
Act-0.5hr_0.2  3  1.7453
Act-0.5hr_0.5  3  2.1247


Point estimate for ETA1-ETA2 is -0.4611
91.9 Percent CI for ETA1-ETA2 is (-0.9018,-0.0944)
W = 6.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.2 N and 0.5 N groups are significantly different.

Mann-Whitney Test and CI: Act-0.5hr_1, Act-0.5hr_0.5

```
             N  Median
Act-0.5hr_1    3  1.6593
Act-0.5hr_0.5  3  2.1247


Point estimate for ETA1-ETA2 is -0.6923
91.9 Percent CI for ETA1-ETA2 is (-1.2289,-0.2517)
W = 6.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.5 N and 1.0 N groups are significantly different.

Kruskal-Wallis Test: Act-1.0h versus Cat

```
Kruskal-Wallis Test on Act-1.0h

                Ave
Cat     N  Median  Rank     Z
0.2     3   2.281   8.0   2.32
0.5     3   1.673   5.0   0.00
1.0     3   1.190   2.0  -2.32
Overall  9          5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Three groups are significantly different, and 0.2 N group is higher than 0.5 N and 1.0 N groups.

Mann-Whitney Test and CI: Act-1.0h_0.5, Act-1.0h_1.0

```
           N  Median
Act-1.0h_0.5  3  1.6729
Act-1.0h_1.0  3  1.1899


Point estimate for ETA1-ETA2 is 0.3362
91.9 Percent CI for ETA1-ETA2 is (0.0513,0.5036)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.5 N and 1.0 N groups are significantly different.

Mann-Whitney Test and CI: Act-1.0h_0.2, Act-1.0h_0.5

```
           N  Median
Act-1.0h_0.2  3  2.2807
Act-1.0h_0.5  3  1.6729


Point estimate for ETA1-ETA2 is 0.6855
91.9 Percent CI for ETA1-ETA2 is (0.5366,0.9704)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.2 N and 0.5 N are significantly different.

Kruskal-Wallis Test: Act-1.5H versus Cat

```
Kruskal-Wallis Test on Act-1.5H

                Ave
Cat     N  Median  Rank     Z
0.2     3  1.7170   8.0   2.32
0.5     3  0.7506   2.0  -2.32
1.0     3  1.4447   5.0   0.00
Overall  9          5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Three groups are significantly different. And 1.0 N group is higher than 0.2 N and 0.5 N groups.

Mann-Whitney Test and CI: Act-1.5h-_0.2, Act-1.5h-_1.0

```
            N  Median
Act-1.5h-_0.2  3  1.7170
Act-1.5h-_1.0  3  1.4447


Point estimate for ETA1-ETA2 is 0.4345
91.9 Percent CI for ETA1-ETA2 is (0.1042,0.8528)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.2 N and 1.0 N are significantly different.

Non-parametric test of Biosolids with different KOH concentration for three duration time. Conversion yields Vs. Concentration.

Kruskal-Wallis Test: Bio-0.5h versus Cat

```
Kruskal-Wallis Test on Bio-0.5h

                Ave
Cat     N  Median  Rank     Z
0.2     3  1.7988   8.0  2.32
0.5     3  0.9032   2.0 -2.32
1.0     3  1.0373   5.0  0.00
Overall 9          5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. 0.2 N group is higher than 0.5 N and 1.0 N groups.

Mann-Whitney Test and CI: Bio-0.5h_0.2, Bio-0.5h_1.0

```
            N  Median
Bio-0.5h_0.2  3  1.7988
Bio-0.5h_1.0  3  1.0373


Point estimate for ETA1-ETA2 is 0.6938
91.9 Percent CI for ETA1-ETA2 is (0.3347,0.8788)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for 0.2 N and 1.0 N groups are significantly different.

Mann-Whitney Test and CI: Bio-0.5h_0.2, Bio-0.5h_0.5

```
            N  Median
Bio-0.5h_0.2  3  1.7988
Bio-0.5h_0.5  3  0.9032


Point estimate for ETA1-ETA2 is 0.8956
91.9 Percent CI for ETA1-ETA2 is (0.5662,1.0875)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for 0.2 N and 0.5 N groups are significantly different.

Mann-Whitney Test and CI: Bio-0.5h_0.5, Bio-0.5h_1.0

```
            N  Median
Bio-0.5h_0.5  3  0.9032
Bio-0.5h_1.0  3  1.0373


Point estimate for ETA1-ETA2 is -0.2087
91.9 Percent CI for ETA1-ETA2 is (-0.3938,-0.0463)
W = 6.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for 0.5 N and 1.0 N groups are significantly different.

Kruskal-Wallis Test: Bio-1.0h versus Cat

```
Kruskal-Wallis Test on Bio-1.0h

                Ave
Cat     N  Median  Rank     Z
0.2     3   1.825   4.3  -0.52
0.5     3   4.175   8.0   2.32
1.0     3   1.701   2.7  -1.81
Overall 9           5.0

H = 5.96  DF = 2  P = 0.051

* NOTE * One or more small samples
```

Data for three groups are not significantly different, but 0.5 N group is higher than 0.2 N and 1.0 N groups.

Mann-Whitney Test and CI: Bio-1.0h_0.2, Bio-1.0h_1.0

```
           N  Median
Bio-1.0h_0.2  3  1.8251
Bio-1.0h_1.0  3  1.7012


Point estimate for ETA1-ETA2 is 0.3396
91.9 Percent CI for ETA1-ETA2 is (-0.0955,0.8335)
W = 13.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3827
```

Data for 0.2 N and 1.0 N groups are not significantly different.

Mann-Whitney Test and CI: Bio-1.0h_0.2, Bio-1.0h0.5

```
           N  Median
Bio-1.0h_0.2  3  1.8251
Bio-1.0h0.5   3  4.1752


Point estimate for ETA1-ETA2 is -2.3586
91.9 Percent CI for ETA1-ETA2 is (-2.8020,-1.9233)
W = 6.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for 0.2 N and 0.5 N groups are significantly different.

Mann-Whitney Test and CI: Bio-1.0h_1.0, Bio-1.0h_0.5

```
           N  Median
Bio-1.0h_1.0  3  1.7012
Bio-1.0h_0.5  3  4.1752


Point estimate for ETA1-ETA2 is -2.7066
91.9 Percent CI for ETA1-ETA2 is (-3.2007,-2.2631)
W = 6.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for 0.5 N and 1.0 N are significantly different.

Kruskal-Wallis Test: Bio-1.5h versus Cat

```
Kruskal-Wallis Test on Bio-1.5h

                Ave
Cat     N  Median  Rank     Z
0.2     3  2.2146   8.0  2.32
0.5     3  0.6809   3.5 -1.16
1.0     3  0.6809   3.5 -1.16
Overall 9          5.0

H = 5.40  DF = 2  P = 0.067
H = 5.54  DF = 2  P = 0.063  (adjusted for ties)

* NOTE * One or more small samples
```

Data for 0.2 N groups are higher than 0.5 N and 1.0 N groups. Three groups are not significantly different.

Mann-Whitney Test and CI: Bio-1.5h_0.5, Bio-1.5h_1.0

```
           N  Median
Bio-1.5h_0.5  3  0.6809
Bio-1.5h_1.0  3  0.6809


Point estimate for ETA1-ETA2 is -0.0000
91.9 Percent CI for ETA1-ETA2 is (-0.1069,0.1069)
W = 10.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 1.0000
The test is significant at 1.0000 (adjusted for ties)
```

Data for 0.5N and 1.0 N groups are not significantly different.

Mann-Whitney Test and CI: Bio-1.5h_0.2, Bio-1.5h_1.0

```
           N  Median
Bio-1.5h_0.2  3  2.2146
Bio-1.5h_1.0  3  0.6809


Point estimate for ETA1-ETA2 is 1.5337
91.9 Percent CI for ETA1-ETA2 is (1.2912,1.7393)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for 0.2 N and 1.0 N are significantly different.

APPENDIX E

STATISTIC TESTS OF DIFFERENT TYPES OF SLUDGES AND BIOSOLIDS FOR DIFFERENT KOH PRETREATMENT CONTACT PERIODS WITH THREE KOH CONCENTRATIONS

(MINITAB V15)

One-way ANOVA test of three types of sludge with different KOH concentration pretreatment for three duration time. Conversion yields Vs. Time.

One-way ANOVA: Primary sludge-0.2N versus Cat

```
Source  DF     SS     MS     F     P
Cat      2  5.4991  2.7495  64.40  0.000
Error    6  0.2562  0.0427
Total    8  5.7552

S = 0.2066   R-Sq = 95.55%   R-Sq(adj) = 94.07%


                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Level  N    Mean   StDev  ---------+---------+---------+---------+
0.5    3  3.0035  0.3294                        (---*---)
1.0    3  1.0888  0.1060  (----*---)
1.5    3  2.0439  0.0913             (---*---)
                     ---------+---------+---------+---------+
                           1.40     2.10     2.80     3.50

Pooled StDev = 0.2066


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper  ---+---------+---------+---------+------
0.5     0.0000   0.9596  1.3539                    (---------*---)
1.0    -2.3090  -1.9147  0.0000  (---*------------------)
1.5    -1.3539  -0.9596  0.0000         (---*---------)
                          ---+---------+---------+---------+------
                          -2.0     -1.0      0.0      1.0


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.5 subtracted from:

Cat    Lower   Center    Upper  ----+---------+---------+---------+-----
1.0  -2.4324  -1.9147  -1.3969  (----*----)
1.5  -1.4773  -0.9596  -0.4418         (----*-----)
                         ----+---------+---------+---------+-----
                          -2.0     -1.0      0.0      1.0


Cat = 1.0 subtracted from:

Cat   Lower  Center   Upper  ----+---------+---------+---------+-----
1.5  0.4374  0.9551  1.4728                       (-----*----)
                       ----+---------+---------+---------+-----
```

```
                        -2.0     -1.0      0.0      1.0
```

Two-Sample T-Test and CI: Pri-0.2N_1, Cat_1 (0.5 h and 1.5 h)

```
Two-sample T for Pri-0.2N_1

Cat_1  N    Mean   StDev  SE Mean
0.5    3   3.004   0.329    0.19
1.5    3  2.0439  0.0913   0.053


Difference = mu (0.5) - mu (1.5)
Estimate for difference:  0.960
95% CI for difference:  (0.110, 1.809)
T-Test of difference = 0 (vs not =): T-Value = 4.86  P-Value = 0.040  DF = 2
```

Data for 0.5 hr group are the highest, 1.0 hr group are the lowest

One-way ANOVA: Primary sludge-0.5N versus Cat

```
Source  DF     SS     MS      F     P
Cat      2  5.0056  2.5028  120.88  0.000
Error    6  0.1242  0.0207
Total    8  5.1298

S = 0.1439   R-Sq = 97.58%   R-Sq(adj) = 96.77%


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level  N    Mean   StDev  -----+---------+---------+---------+----
0.5    3  3.4609  0.1938                          (---*--)
1.0    3  2.1591  0.0832         (--*--)
1.5    3  1.7002  0.1329  (--*---)
                      -----+---------+---------+---------+----
                        1.80     2.40     3.00     3.60

Pooled StDev = 0.1439


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper    +---------+---------+---------+---------
0.5     0.0000   1.3018  1.5764                     (------------*--)
1.0    -1.5764  -1.3018  0.0000        (--*------------)
1.5    -2.0353  -1.7607  0.0000    (-*-----------------)
                            +---------+---------+---------+---------
                         -2.0     -1.0      0.0      1.0


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%
```

```
Cat = 0.5 subtracted from:

Cat   Lower   Center   Upper  -------+---------+---------+---------+--
1.0  -1.6624  -1.3018  -0.9413       (----*---)
1.5  -2.1213  -1.7607  -1.4002  (----*---)
                          -------+---------+---------+---------+--
                             -1.60    -0.80    -0.00     0.80


Cat = 1.0 subtracted from:

Cat   Lower   Center   Upper  -------+---------+---------+---------+--
1.5  -0.8195  -0.4589  -0.0984               (---*----)
                          -------+---------+---------+---------+--
                             -1.60    -0.80    -0.00     0.80
```

Two-Sample T-Test and CI: Pri-0.5N_1, Cat-2 (1.0 h and 1.5 h)

```
Two-sample T for Pri-0.5N_1

Cat-2  N    Mean   StDev  SE Mean
1.0    3  2.1591  0.0832   0.048
1.5    3   1.700   0.133   0.077


Difference = mu (1.0) - mu (1.5)
Estimate for difference:  0.4589
95% CI for difference:  (0.1709, 0.7469)
T-Test of difference = 0 (vs not =): T-Value = 5.07  P-Value = 0.015  DF = 3
```

Data for 0.5 hr group are highest, 1.5 hr group is the lowest.

One-way ANOVA: Primary sludge-1.0N versus Cat

```
Source  DF     SS     MS     F     P
Cat      2  1.1131  0.5566  34.66  0.001
Error    6  0.0963  0.0161
Total    8  1.2095

S = 0.1267   R-Sq = 92.03%   R-Sq(adj) = 89.38%


                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Level  N    Mean   StDev  ------+---------+---------+---------+---
0.5    3  2.2036  0.1166                     (----*----)
1.0    3  1.3516  0.1220  (----*----)
1.5    3  1.6673  0.1403         (----*----)
                     ------+---------+---------+---------+---
                        1.40     1.75     2.10     2.45

Pooled StDev = 0.1267


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means
```

```
Level   Lower   Center   Upper   --+---------+---------+---------+-------
0.5    0.0000   0.5363  0.7781                    (----------*----)
1.0   -1.0938  -0.8520  0.0000   (----*----------------)
1.5   -0.7781  -0.5363  0.0000        (----*----------)
                           --+---------+---------+---------+-------
                          -1.00     -0.50     0.00     0.50


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.5 subtracted from:

Cat   Lower   Center   Upper  ---+---------+---------+---------+------
1.0  -1.1695  -0.8520  -0.5345  (------*------)
1.5  -0.8539  -0.5363  -0.2188       (------*------)
                          ---+---------+---------+---------+------
                          -1.00     -0.50     0.00     0.50


Cat = 1.0 subtracted from:

Cat   Lower  Center   Upper  ---+---------+---------+---------+------
1.5  -0.0019  0.3156  0.6332                     (------*------)
                         ---+---------+---------+---------+------
                         -1.00     -0.50     0.00     0.50
```

Two-Sample T-Test and CI: Pri-1.0N_1, Cat-2 (1.0 h and 1.5 h)

```
Two-sample T for Pri-1.0N_1

Cat-2  N   Mean  StDev  SE Mean
1.0    3  1.352  0.122   0.070
1.5    3  1.667  0.140   0.081


Difference = mu (1.0) - mu (1.5)
Estimate for difference:  -0.316
95% CI for difference:  (-0.657, 0.026)
T-Test of difference = 0 (vs not =): T-Value = -2.94  P-Value = 0.061  DF = 3
```

Data for 0.5 hr group is higher than 1.0 h and 1.5 h groups, 1.0 h and 1.5 h groups are not significant different.

One-way ANOVA: Activated sludge-0.2N versus Cat

```
Source  DF     SS     MS     F     P
Cat      2  0.5278  0.2639  11.48  0.009
Error    6  0.1380  0.0230
Total    8  0.6658

S = 0.1517   R-Sq = 79.28%   R-Sq(adj) = 72.37%


                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Level  N    Mean   StDev  ---------+---------+---------+---------+
0.5    3  1.7958  0.1885    (--------*-------)
1.0    3  2.2830  0.0745                     (-------*--------)
1.5    3  1.7464  0.1670  (--------*-------)
                     ---------+---------+---------+---------+
                           1.75     2.00     2.25     2.50

Pooled StDev = 0.1517


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper  -------+---------+---------+---------+--
0.5    -0.7766  -0.4873  0.0000   (-----*---------)
1.0     0.0000   0.4873  0.7766                (---------*-----)
1.5    -0.8260  -0.5366  0.0000  (-----*----------)
                         -------+---------+---------+---------+--
                            -0.50     0.00     0.50     1.00


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.5 subtracted from:

Cat    Lower   Center   Upper  --------+---------+---------+---------+-
1.0   0.1073   0.4873  0.8673                   (-------*------)
1.5  -0.4294  -0.0494  0.3306          (-------*-------)
                        --------+---------+---------+---------+-
                            -0.50     0.00     0.50     1.00


Cat = 1.0 subtracted from:

Cat    Lower   Center    Upper  --------+---------+---------+---------+-
1.5  -0.9166  -0.5366  -0.1566  (------*-------)
                         --------+---------+---------+---------+-
                             -0.50     0.00     0.50     1.00
```

Two-Sample T-Test and CI: Act-0.2N_1, Cat_1 (0.5 h and 1.5 h)

```
Two-sample T for Act-0.2N_1

Cat_1  N   Mean  StDev  SE Mean
0.5    3  1.796  0.189    0.11
1.5    3  1.746  0.167   0.096


Difference = mu (0.5) - mu (1.5)
Estimate for difference:  0.049
95% CI for difference:  (-0.413, 0.512)
T-Test of difference = 0 (vs not =): T-Value = 0.34  P-Value = 0.757  DF = 3
```

Data for 1.0 hr groups is higher 0.5 h and 1.5 h groups, 0.5 and 1.5 hr groups are not significantly different.

One-way ANOVA: Activated sludge-0.5N versus Cat

```
Source  DF     SS     MS     F     P
Cat      2  3.4640  1.7320  58.42  0.000
Error    6  0.1779  0.0296
Total    8  3.6419

S = 0.1722   R-Sq = 95.12%   R-Sq(adj) = 93.49%


                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Level  N   Mean   StDev  --+---------+---------+---------+-------
0.5    3  2.2543  0.2472                      (---*---)
1.0    3  1.5781  0.1644             (---*---)
1.5    3  0.7376  0.0281  (---*---)
                     --+---------+---------+---------+-------
                     0.60     1.20     1.80     2.40

Pooled StDev = 0.1722


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level   Lower   Center   Upper  ---+---------+---------+---------+------
0.5    0.0000   0.6762  1.0047                    (-------*----)
1.0   -1.0047  -0.6762  0.0000          (----*-------)
1.5   -1.8452  -1.5167  0.0000  (---*------------------)
                          ---+---------+---------+---------+------
                          -1.60    -0.80    -0.00     0.80


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.5 subtracted from:

Cat    Lower   Center   Upper  ---------+---------+---------+---------+
```

```
1.0  -1.1076  -0.6762  -0.2447        (---*----)
1.5  -1.9481  -1.5167  -1.0852  (---*---)
                          ---------+---------+---------+---------+
                               -1.0      0.0      1.0      2.0


Cat = 1.0 subtracted from:

Cat   Lower   Center   Upper  ---------+---------+---------+---------+
1.5  -1.2720  -0.8405  -0.4091       (----*---)
                          ---------+---------+---------+---------+
                               -1.0      0.0      1.0      2.0
```

Two-Sample T-Test and CI: Act-0.5N_1, Cat-2 (1.0 h and 1.5 h)

```
Two-sample T for Act-0.5N_1

Cat-2  N    Mean   StDev  SE Mean
1.0    3   1.578   0.164    0.095
1.5    3  0.7376  0.0281    0.016


Difference = mu (1.0) - mu (1.5)
Estimate for difference:  0.8405
95% CI for difference:  (0.4262, 1.2549)
T-Test of difference = 0 (vs not =): T-Value = 8.73  P-Value = 0.013  DF = 2
```

Data for 0.5 h group are highest, and 1.5 h group are lowest.

One-way ANOVA: Activated sludge-1.0N versus Cat

```
Source  DF     SS     MS    F     P
Cat      2  0.2229  0.1114  2.48  0.164
Error    6  0.2698  0.0450
Total    8  0.4927

S = 0.2121   R-Sq = 45.24%   R-Sq(adj) = 26.98%


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level  N   Mean   StDev  ---+---------+---------+---------+------
0.5    3  1.6057  0.2722              (-----------*-----------)
1.0    3  1.2321  0.0913  (-----------*-----------)
1.5    3  1.3366  0.2291     (-----------*-----------)
                      ---+---------+---------+---------+------
                       1.00     1.25     1.50     1.75

Pooled StDev = 0.2121


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level   Lower   Center   Upper  ---------+---------+---------+---------+
0.5   -0.1355   0.2691  0.6737               (---------*---------)
1.0   -0.7782  -0.3736  0.0311  (---------*---------)
```

```
1.5   -0.6737  -0.2691  0.1355   (---------*---------)
                           ---------+---------+---------+---------+
                                -0.40     0.00     0.40     0.80


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.5 subtracted from:

Cat   Lower   Center   Upper  ---+---------+---------+---------+------
1.0  -0.9049  -0.3736  0.1578  (-------------*------------)
1.5  -0.8005  -0.2691  0.2623    (------------*-------------)
                         ---+---------+---------+---------+------
                          -0.80    -0.40    -0.00     0.40


Cat = 1.0 subtracted from:

Cat   Lower  Center   Upper  ---+---------+---------+---------+------
1.5  -0.4269  0.1045  0.6358           (-------------*------------)
                        ---+---------+---------+---------+------
                        -0.80    -0.40    -0.00     0.40
```

Data for three groups are not significantly different.

One-way ANOVA: Biosolids-0.2N versus Cat

```
Source  DF     SS     MS    F     P
Cat      2  0.3354  0.1677  4.47  0.065
Error    6  0.2249  0.0375
Total    8  0.5604

S = 0.1936   R-Sq = 59.86%   R-Sq(adj) = 46.48%


                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Level  N    Mean   StDev   -+---------+---------+---------+--------
0.5    3  1.7531  0.1838   (--------*---------)
1.0    3  1.8798  0.2227       (--------*--------)
1.5    3  2.2110  0.1706                (--------*--------)
                     -+---------+---------+---------+--------
                     1.50     1.80     2.10     2.40

Pooled StDev = 0.1936


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper    -+---------+---------+---------+--------
0.5    -0.8273  -0.4579  0.0000    (---------*----------)
1.0    -0.7007  -0.3313  0.0382      (---------*--------)
1.5    -0.0382   0.3313  0.7007                (--------*---------)
                          -+---------+---------+---------+--------
                        -0.80     -0.40     -0.00      0.40


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.5 subtracted from:

Cat    Lower  Center   Upper  ------+---------+---------+---------+---
1.0  -0.3585  0.1266  0.6118         (---------*--------)
1.5  -0.0272  0.4579  0.9430              (---------*---------)
                        ------+---------+---------+---------+---
                           -0.50     0.00     0.50     1.00


Cat = 1.0 subtracted from:

Cat    Lower  Center   Upper  ------+---------+---------+---------+---
1.5  -0.1539  0.3313  0.8164            (---------*--------)
                        ------+---------+---------+---------+---
                           -0.50     0.00     0.50     1.00
```

Two-Sample T-Test and CI: Bio-0.2N_1, Cat-2 (1.0 h and 1.5 h)

```
Two-sample T for Bio-0.2N_1

Cat-2  N   Mean  StDev  SE Mean
1.0    3  1.880  0.223    0.13
1.5    3  2.211  0.171   0.098


Difference = mu (1.0) - mu (1.5)
Estimate for difference:  -0.331
95% CI for difference:  (-0.847, 0.184)
T-Test of difference = 0 (vs not =): T-Value = -2.05  P-Value = 0.133  DF = 3
```

Data for three groups are not significantly different

One-way ANOVA: Biosolids-0.5N versus Cat

```
Source  DF      SS      MS      F     P
Cat      2  23.7601  11.8800  577.77  0.000
Error    6   0.1234   0.0206
Total    8  23.8835

S = 0.1434   R-Sq = 99.48%   R-Sq(adj) = 99.31%


                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Level  N    Mean   StDev  -----+---------+---------+---------+----
0.5    3  0.9034  0.0812    (-*-)
1.0    3  4.2383  0.2284                              (-*-)
1.5    3  0.6897  0.0540  (-*-)
                     -----+---------+---------+---------+----
                        1.0      2.0      3.0      4.0

Pooled StDev = 0.1434


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper  ---------+---------+---------+---------+
0.5    -3.6085  -3.3349  0.0000   (*----------------)
1.0     0.0000   3.3349  3.6085                 (----------------*)
1.5    -3.8222  -3.5486  0.0000  (*-----------------)
                           ---------+---------+---------+---------+
                                -2.0      0.0      2.0      4.0


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.5 subtracted from:

Cat    Lower   Center   Upper    +---------+---------+---------+---------
1.0   2.9756   3.3349  3.6942                                (-*)
```

```
1.5  -0.5730  -0.2137  0.1456                  (-*-)
                                +---------+---------+---------+---------
                              -4.0      -2.0      0.0       2.0


Cat = 1.0 subtracted from:

Cat   Lower   Center   Upper    +---------+---------+---------+---------
1.5  -3.9079  -3.5486  -3.1893    (-*-)
                               +---------+---------+---------+---------
                             -4.0      -2.0      0.0       2.0
```

Two-Sample T-Test and CI: Bio-0.5N_1, Cat_1 (0.5 h and 1.5 h)

```
Two-sample T for Bio-0.5N_1

Cat_1  N    Mean   StDev  SE Mean
0.5    3  0.9034  0.0812   0.047
1.5    3  0.6897  0.0540   0.031


Difference = mu (0.5) - mu (1.5)
Estimate for difference:  0.2137
95% CI for difference:  (0.0345, 0.3929)
T-Test of difference = 0 (vs not =): T-Value = 3.79  P-Value = 0.032  DF = 3
```

Data for 1.0 h group are significantly different, 0.5 h and 1.5 h groups are not significantly different.

One-way ANOVA: Biosolids-1.0N versus Cat

```
Source  DF     SS     MS     F     P
Cat      2  1.2267  0.6133  21.95  0.002
Error    6  0.1677  0.0279
Total    8  1.3944

S = 0.1672   R-Sq = 87.98%   R-Sq(adj) = 83.97%

                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level  N    Mean   StDev  -------+---------+---------+---------+--
0.5    3  1.0948  0.1051           (-----*------)
1.0    3  1.5924  0.2644                      (-----*------)
1.5    3  0.6897  0.0540  (------*-----)
                      -------+---------+---------+---------+--
                          0.70      1.05      1.40      1.75

Pooled StDev = 0.1672


Hsu's MCB (Multiple Comparisons with the Best)

Family error rate = 0.05
Critical value = 2.34

Intervals for level mean minus largest of other level means

Level    Lower   Center   Upper    +---------+---------+---------+---------
0.5    -0.8166  -0.4977  0.0000         (-----*-------)
1.0     0.0000   0.4977  0.8166                    (-------*-----)
1.5    -1.2217  -0.9027  0.0000    (----*--------------)
```

```
                              +---------+---------+---------+---------
                            -1.20     -0.60      0.00      0.60


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Cat

Individual confidence level = 97.80%


Cat = 0.5 subtracted from:

Cat   Lower   Center   Upper  ---------+---------+---------+---------+
1.0   0.0788   0.4977  0.9165                   (-----*-----)
1.5  -0.8239  -0.4051  0.0138        (-----*-----)
                          ---------+---------+---------+---------+
                               -0.70     0.00     0.70     1.40


Cat = 1.0 subtracted from:

Cat   Lower   Center   Upper  ---------+---------+---------+---------+
1.5  -1.3216  -0.9027  -0.4839  (-----*-----)
                           ---------+---------+---------+---------+
                                -0.70     0.00     0.70     1.40
```

Two-Sample T-Test and CI: Bio-1.0N_1, Cat_1 (0.5 h and 1.5 h)

```
Two-sample T for Bio-1.0N_1

Cat_1  N    Mean   StDev  SE Mean
0.5    3   1.095   0.105    0.061
1.5    3  0.6897  0.0540    0.031


Difference = mu (0.5) - mu (1.5)
Estimate for difference:  0.4051
95% CI for difference:  (0.1117, 0.6985)
T-Test of difference = 0 (vs not =): T-Value = 5.94  P-Value = 0.027  DF = 2
```

Data for 1.0 h group are higher than 0.5 h and 1.5 h groups. 0.5 h and 1.5 h groups are significantly different.

Non-parametric test of primary sludge for three duration time with different KOH pretreatment condition. Conversion yields Vs. Time.

Kruskal-Wallis Test: Primary sludge-0.2N versus Cat

```
Kruskal-Wallis Test on Pri-0.2N

                Ave
Cat     N  Median  Rank     Z
0.5     3   3.095   8.0  2.32
1.0     3   1.062   2.0 -2.32
1.5     3   2.074   5.0  0.00
Overall 9           5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. And 0.5 hr group is higher than 1.0 and 1.5 hr.

Mann-Whitney Test and CI: Pri-0.2N_0.5, Pri-0.2N_1.5

```
           N  Median
Pri-0.2N_0.5  3  3.0954
Pri-0.2N_1.5  3  2.0737


Point estimate for ETA1-ETA2 is 1.0216
91.9 Percent CI for ETA1-ETA2 is (0.5212,1.3357)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for the 0.5 hr and 1.5 hr groups are significantly different.

Kruskal-Wallis Test: Pri-0.5N versus Cat

```
Kruskal-Wallis Test on Pri-0.5N

                Ave
Cat     N  Median  Rank     Z
0.5     3   3.418   8.0  2.32
1.0     3   2.168   5.0  0.00
1.5     3   1.707   2.0 -2.32
Overall 9           5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. And the 0.5 hr group are higher than the other two groups.

Mann-Whitney Test and CI: Pri-0.5N_0.5, Pri-0.5N_1.0

```
           N  Median
Pri-0.5N_0.5  3  3.4176
Pri-0.5N_1.0  3  2.1678
```

```
Point estimate for ETA1-ETA2 is 1.2498
91.9 Percent CI for ETA1-ETA2 is (1.0550,1.6008)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for 0.5 hr and 1.0 hr groups are significantly different.

Kruskal-Wallis Test: Pri-1.0N versus Cat

```
Kruskal-Wallis Test on Pri-1.0N

                 Ave
Cat     N  Median  Rank     Z
0.5     3   2.194   8.0  2.32
1.0     3   1.316   2.0 -2.32
1.5     3   1.654   5.0  0.00
Overall 9           5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. And 0.5 hr group is higher than the other two groups.

Mann-Whitney Test and CI: Pri-1.0N_1.0, Pri-1.0N_1.5

```
           N  Median
Pri-1.0N_1.0  6  1.5109
Pri-1.0N_1.5  3  1.6536


Point estimate for ETA1-ETA2 is -0.1632
97.2 Percent CI for ETA1-ETA2 is (-0.5625,0.2797)
W = 25.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3017
The test is significant at 0.2956 (adjusted for ties)
```

1.0 hr and 1.5 hr groups are not significantly different.

Non-parametric test of activated sludge for three duration time with different KOH pretreatment condition. Conversion yields Vs. Time.

Kruskal-Wallis Test: Act-0.2N versus Cat

```
Kruskal-Wallis Test on Act-0.2N

                 Ave
Cat     N  Median  Rank     Z
0.5     3   1.745   4.0  -0.77
1.0     3   2.281   8.0   2.32
1.5     3   1.717   3.0  -1.55
Overall 9           5.0

H = 5.60  DF = 2  P = 0.061

* NOTE * One or more small samples
```

Data for three groups are not significantly different, but 1.0 hr group is higher than 0.5 hr and 1.5 hr groups.

Mann-Whitney Test and CI: Act-0.2N_0.5, Act-0.2N_1.5

```
            N  Median
Act-0.2N_0.5  3  1.7453
Act-0.2N_1.5  3  1.7170


Point estimate for ETA1-ETA2 is 0.0417
91.9 Percent CI for ETA1-ETA2 is (-0.2887,0.4083)
W = 12.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6625
```

Data for 0.5 h and 1.5 h groups are not significantly different.

Mann-Whitney Test and CI: Act-0.2N_1.0, Act-0.2N_0.5

```
            N  Median
Act-0.2N_1.0  3  2.2807
Act-0.2N_0.5  3  1.7453


Point estimate for ETA1-ETA2 is 0.5355
91.9 Percent CI for ETA1-ETA2 is (0.2052,0.7210)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for 1.0 h and 0.5 h groups are significantly different.

Kruskal-Wallis Test: Act-0.5N versus Cat

```
Kruskal-Wallis Test on Act-0.5N

                Ave
Cat     N  Median  Rank     Z
0.5     3  2.1247   8.0  2.32
1.0     3  1.6729   5.0  0.00
1.5     3  0.7506   2.0 -2.32
Overall  9         5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. 0.5 hr group is higher than 1.0 hr and 1.5 hr groups.

Mann-Whitney Test and CI: Act-0.5N_0.5, Act-0.5N_1

```
           N  Median
Act-0.5N_0.5  3  2.1247
Act-0.5N_1    3  1.6729


Point estimate for ETA1-ETA2 is 0.7105
91.9 Percent CI for ETA1-ETA2 is (0.4255,1.1510)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.5 h and 1.0 h groups are significantly different.

Kruskal-Wallis Test: Act-1.0N versus Cat

```
Kruskal-Wallis Test on Act-1.0N

                Ave
Cat     N  Median  Rank     Z
0.5     3   1.659   7.0  1.55
1.0     3   1.190   3.3 -1.29
1.5     3   1.445   4.7 -0.26
Overall  9         5.0

H = 2.76  DF = 2  P = 0.252

* NOTE * One or more small samples
```

Three groups are not significantly different. But 0.5 hr group is higher than 1.0 h and 1.5 h groups.

Mann-Whitney Test and CI: Act-1.0N_0.5, Act-1.0N_1.5

```
           N  Median
Act-1.0N_0.5  3  1.6593
Act-1.0N_1.5  3  1.4447
```

```
Point estimate for ETA1-ETA2 is 0.2372
91.9 Percent CI for ETA1-ETA2 is (-0.1810,0.7736)
W = 13.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3827
```

0.5 h and 1.5 h groups are not significantly different.

Non-parametric test of biosolids for three duration time with different KOH pretreatment condition. Conversion yields Vs. Time.

Kruskal-Wallis Test: Bio-0.2N versus Cat

```
Kruskal-Wallis Test on Bio-0.2N

                 Ave
Cat     N  Median  Rank     Z
0.5     3   1.799   3.0  -1.55
1.0     3   1.825   4.3  -0.52
1.5     3   2.215   7.7   2.07
Overall  9          5.0

H = 4.62  DF = 2  P = 0.099

* NOTE * One or more small samples
```

Data for three groups are not significantly different. But 1.5 h group are higher than the other two groups.

Mann-Whitney Test and CI: Bio-0.2N_1.0, Bio-0.2N_1.5

```
           N  Median
Bio-0.2N_1.0  3  1.8251
Bio-0.2N_1.5  3  2.2146


Point estimate for ETA1-ETA2 is -0.3491
91.9 Percent CI for ETA1-ETA2 is (-0.6901,0.0860)
W = 7.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1904
```

1.0 h and 1.5 h groups are not significantly different.

Mann-Whitney Test and CI: Bio-0.2N_1.5, Bio-0.2N_0.5

```
           N  Median
Bio-0.2N_1.5  3  2.2146
Bio-0.2N_0.5  3  1.7988


Point estimate for ETA1-ETA2 is 0.4700
91.9 Percent CI for ETA1-ETA2 is (0.1289,0.8289)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.5 h and 1.5 h groups are significantly different.

Kruskal-Wallis Test: Bio-0.5N versus Cat

```
Kruskal-Wallis Test on Bio-0.5N

                 Ave
Cat     N  Median  Rank     Z
```

```
0.5     3  0.9032   5.0   0.00
1.0     3  4.1752   8.0   2.32
1.5     3  0.6809   2.0  -2.32
Overall  9          5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. And 1.0 h group are higher than the other two groups.

Mann-Whitney Test and CI: Bio-0.5N_0.5, Bio-0.5N_1.5

```
            N  Median
Bio-0.5N_0.5  3  0.9032
Bio-0.5N_1.5  3  0.6809


Point estimate for ETA1-ETA2 is 0.2223
91.9 Percent CI for ETA1-ETA2 is (0.0747,0.3440)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.5 h and 1.5 h groups are significantly different.

Kruskal-Wallis Test: Bio-1.0N versus Cat

```
Kruskal-Wallis Test on Bio-1.0N

                 Ave
Cat     N  Median  Rank     Z
0.5     3  1.0373   5.0  0.00
1.0     3  1.7012   8.0  2.32
1.5     3  0.6809   2.0 -2.32
Overall  9          5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. And 1.0 h group are higher than the other two groups.

Mann-Whitney Test and CI: Bio-1.0N_1.5, Bio-1.0N_0.5

```
            N  Median
Bio-1.0N_1.5  3  0.6809
Bio-1.0N_0.5  3  1.0373


Point estimate for ETA1-ETA2 is -0.3903
91.9 Percent CI for ETA1-ETA2 is (-0.5754,-0.2834)
W = 6.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.5 h and 1.5 h groups are significantly different.

Non-parametric test of primary sludge for three duration time with different KOH pretreatment condition. Conversion yields Vs. Time.

Kruskal-Wallis Test: Primary sludge-0.2N versus Cat

```
Kruskal-Wallis Test on Pri-0.2N

                Ave
Cat     N  Median  Rank     Z
0.5     3   3.095   8.0  2.32
1.0     3   1.062   2.0 -2.32
1.5     3   2.074   5.0  0.00
Overall 9           5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. And 0.5 hr group is higher than 1.0 and 1.5 hr.

Mann-Whitney Test and CI: Pri-0.2N_0.5, Pri-0.2N_1.5

```
           N  Median
Pri-0.2N_0.5  3  3.0954
Pri-0.2N_1.5  3  2.0737


Point estimate for ETA1-ETA2 is 1.0216
91.9 Percent CI for ETA1-ETA2 is (0.5212,1.3357)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for the 0.5 hr and 1.5 hr groups are significantly different.

Kruskal-Wallis Test: Pri-0.5N versus Cat

```
Kruskal-Wallis Test on Pri-0.5N

                Ave
Cat     N  Median  Rank     Z
0.5     3   3.418   8.0  2.32
1.0     3   2.168   5.0  0.00
1.5     3   1.707   2.0 -2.32
Overall 9           5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. And the 0.5 hr group are higher than the

other two groups.

Mann-Whitney Test and CI: Pri-0.5N_0.5, Pri-0.5N_1.0

```
            N  Median
Pri-0.5N_0.5  3  3.4176
Pri-0.5N_1.0  3  2.1678


Point estimate for ETA1-ETA2 is 1.2498
91.9 Percent CI for ETA1-ETA2 is (1.0550,1.6008)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for 0.5 hr and 1.0 hr groups are significantly different.

Kruskal-Wallis Test: Pri-1.0N versus Cat

```
Kruskal-Wallis Test on Pri-1.0N

                  Ave
Cat     N  Median  Rank     Z
0.5     3   2.194   8.0  2.32
1.0     3   1.316   2.0 -2.32
1.5     3   1.654   5.0  0.00
Overall 9           5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. And 0.5 hr group is higher than the other two groups.

Mann-Whitney Test and CI: Pri-1.0N_1.0, Pri-1.0N_1.5

```
            N  Median
Pri-1.0N_1.0  6  1.5109
Pri-1.0N_1.5  3  1.6536


Point estimate for ETA1-ETA2 is -0.1632
97.2 Percent CI for ETA1-ETA2 is (-0.5625,0.2797)
W = 25.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3017
The test is significant at 0.2956 (adjusted for ties)
```

1.0 hr and 1.5 hr groups are not significantly different.

Non-parametric test of activated sludge for three duration time with different KOH pretreatment condition. Conversion yields Vs. Time.

Kruskal-Wallis Test: Act-0.2N versus Cat

```
Kruskal-Wallis Test on Act-0.2N

                 Ave
Cat     N  Median  Rank     Z
0.5     3   1.745   4.0  -0.77
1.0     3   2.281   8.0   2.32
1.5     3   1.717   3.0  -1.55
Overall 9           5.0

H = 5.60  DF = 2  P = 0.061

* NOTE * One or more small samples
```

Data for three groups are not significantly different, but 1.0 hr group is higher than 0.5 hr and 1.5 hr groups.

Mann-Whitney Test and CI: Act-0.2N_0.5, Act-0.2N_1.5

```
            N  Median
Act-0.2N_0.5  3  1.7453
Act-0.2N_1.5  3  1.7170


Point estimate for ETA1-ETA2 is 0.0417
91.9 Percent CI for ETA1-ETA2 is (-0.2887,0.4083)
W = 12.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6625
```

Data for 0.5 h and 1.5 h groups are not significantly different.

Mann-Whitney Test and CI: Act-0.2N_1.0, Act-0.2N_0.5

```
            N  Median
Act-0.2N_1.0  3  2.2807
Act-0.2N_0.5  3  1.7453


Point estimate for ETA1-ETA2 is 0.5355
91.9 Percent CI for ETA1-ETA2 is (0.2052,0.7210)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

Data for 1.0 h and 0.5 h groups are significantly different.

Kruskal-Wallis Test: Act-0.5N versus Cat

```
Kruskal-Wallis Test on Act-0.5N

                Ave
Cat     N  Median  Rank     Z
0.5     3  2.1247   8.0  2.32
1.0     3  1.6729   5.0  0.00
1.5     3  0.7506   2.0 -2.32
Overall  9         5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. 0.5 hr group is higher than 1.0 hr and 1.5 hr groups.

Mann-Whitney Test and CI: Act-0.5N_0.5, Act-0.5N_1

```
           N  Median
Act-0.5N_0.5  3  2.1247
Act-0.5N_1    3  1.6729


Point estimate for ETA1-ETA2 is 0.7105
91.9 Percent CI for ETA1-ETA2 is (0.4255,1.1510)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.5 h and 1.0 h groups are significantly different.

Kruskal-Wallis Test: Act-1.0N versus Cat

```
Kruskal-Wallis Test on Act-1.0N

                Ave
Cat     N  Median  Rank     Z
0.5     3   1.659   7.0  1.55
1.0     3   1.190   3.3 -1.29
1.5     3   1.445   4.7 -0.26
Overall  9         5.0

H = 2.76  DF = 2  P = 0.252

* NOTE * One or more small samples
```

Three groups are not significantly different. But 0.5 hr group is higher than 1.0 h and 1.5 h groups.

Mann-Whitney Test and CI: Act-1.0N_0.5, Act-1.0N_1.5

```
           N  Median
Act-1.0N_0.5  3  1.6593
Act-1.0N_1.5  3  1.4447
```

```
Point estimate for ETA1-ETA2 is 0.2372
91.9 Percent CI for ETA1-ETA2 is (-0.1810,0.7736)
W = 13.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3827
```

0.5 h and 1.5 h groups are not significantly different.

Non-parametric test of biosolids for three duration time with different KOH pretreatment condition. Conversion yields Vs. Time.

Kruskal-Wallis Test: Bio-0.2N versus Cat

```
Kruskal-Wallis Test on Bio-0.2N

                 Ave
Cat     N  Median  Rank     Z
0.5     3   1.799   3.0  -1.55
1.0     3   1.825   4.3  -0.52
1.5     3   2.215   7.7   2.07
Overall  9          5.0

H = 4.62  DF = 2  P = 0.099

* NOTE * One or more small samples
```

Data for three groups are not significantly different. But 1.5 h group are higher than the other two groups.

Mann-Whitney Test and CI: Bio-0.2N_1.0, Bio-0.2N_1.5

```
           N  Median
Bio-0.2N_1.0  3  1.8251
Bio-0.2N_1.5  3  2.2146


Point estimate for ETA1-ETA2 is -0.3491
91.9 Percent CI for ETA1-ETA2 is (-0.6901,0.0860)
W = 7.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1904
```

1.0 h and 1.5 h groups are not significantly different.

Mann-Whitney Test and CI: Bio-0.2N_1.5, Bio-0.2N_0.5

```
           N  Median
Bio-0.2N_1.5  3  2.2146
Bio-0.2N_0.5  3  1.7988


Point estimate for ETA1-ETA2 is 0.4700
91.9 Percent CI for ETA1-ETA2 is (0.1289,0.8289)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.5 h and 1.5 h groups are significantly different.

Kruskal-Wallis Test: Bio-0.5N versus Cat

```
Kruskal-Wallis Test on Bio-0.5N

                 Ave
Cat     N  Median  Rank     Z
```

```
0.5     3  0.9032   5.0   0.00
1.0     3  4.1752   8.0   2.32
1.5     3  0.6809   2.0  -2.32
Overall  9          5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. And 1.0 h group are higher than the other two groups.

Mann-Whitney Test and CI: Bio-0.5N_0.5, Bio-0.5N_1.5

```
            N  Median
Bio-0.5N_0.5  3  0.9032
Bio-0.5N_1.5  3  0.6809


Point estimate for ETA1-ETA2 is 0.2223
91.9 Percent CI for ETA1-ETA2 is (0.0747,0.3440)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.5 h and 1.5 h groups are significantly different.

Kruskal-Wallis Test: Bio-1.0N versus Cat

```
Kruskal-Wallis Test on Bio-1.0N

                  Ave
Cat     N  Median  Rank     Z
0.5     3  1.0373   5.0  0.00
1.0     3  1.7012   8.0  2.32
1.5     3  0.6809   2.0 -2.32
Overall  9          5.0

H = 7.20  DF = 2  P = 0.027

* NOTE * One or more small samples
```

Data for three groups are significantly different. And 1.0 h group are higher than the other two groups.

Mann-Whitney Test and CI: Bio-1.0N_1.5, Bio-1.0N_0.5

```
            N  Median
Bio-1.0N_1.5  3  0.6809
Bio-1.0N_0.5  3  1.0373


Point estimate for ETA1-ETA2 is -0.3903
91.9 Percent CI for ETA1-ETA2 is (-0.5754,-0.2834)
W = 6.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0809
```

0.5 h and 1.5 h groups are significantly different.

APPENDIX F

TWO-SAMPLE T-TEST BETWEEN PHYSICAL, HCL AND KOH PRETREATMENTS OF DIFFERENT TYPES OF SLUDGES AND BIOSOLIDS (MINITAB V15)

Two-Sample T-Test and CI: Pri 1.0 N, Pri Dry

```
Two-sample T for Pri Dry vs Pri 1.0 N

          N   Mean  StDev  SE Mean
Pri Dry   3  4.661  0.146   0.084
Pri 1.0 N  3  5.666  0.236    0.14


Difference = mu (Pri Dry) - mu (Pri 1.0 N)
Estimate for difference:  -1.005
95% CI for difference:  (-1.514, -0.496)
T-Test of difference = 0 (vs not =): T-Value = -6.28  P-Value = 0.008  DF = 3
```

Primary sludge with 1.0N HCl pretreatment was significantly higher than physical pretreatment.

Two-Sample T-Test and CI: Act 0.5 N, Act Dry

```
Two-sample T for Act 0.5 N vs Act Dry

          N   Mean  StDev  SE Mean
Act 0.5 N  3  4.835  0.471    0.27
Act Dry    3  4.483  0.178    0.10


Difference = mu (Act 0.5 N) - mu (Act Dry)
Estimate for difference:  0.353
95% CI for difference:  (-0.897, 1.603)
T-Test of difference = 0 (vs not =): T-Value = 1.21  P-Value = 0.348  DF = 2
```

Activated sludge with 0.5 N HCl pretreatment was not significantly different from physical pretreatment.

Two-Sample T-Test and CI: Bio 1.0 N, Bio Dry

```
Two-sample T for Bio 1.0 N vs Bio Dry

          N    Mean   StDev  SE Mean
Bio 1.0 N  3   4.665   0.245    0.14
Bio Dry    3  1.1472  0.0418   0.024


Difference = mu (Bio 1.0 N) - mu (Bio Dry)
Estimate for difference:  3.518
95% CI for difference:  (2.900, 4.136)
T-Test of difference = 0 (vs not =): T-Value = 24.49  P-Value = 0.002  DF = 2
```

1.0 N HCl pretreatment of Biosolid was significantly higher than physical pretreatment.

Two-Sample T-Test and CI: Pri-0.5N-0.5H, Pri 1.0 N HCl

```
Two-sample T for Pri-0.5N-0.5H vs Pri 1.0 N HCl

              N   Mean  StDev  SE Mean
Pri-0.5N-0.5H  3  3.461  0.194    0.11
Pri 1.0 N HCl  3  5.666  0.236    0.14


Difference = mu (Pri-0.5N-0.5H) - mu (Pri 1.0 N HCl)
Estimate for difference:  -2.205
95% CI for difference:  (-2.765, -1.644)
T-Test of difference = 0 (vs not =): T-Value = -12.52  P-Value = 0.001  DF = 3
```

Data for primary sludge with 0.5 N KOH pretreatment for 0.5 h were significantly different from the primary sludge with 1.0 N HCl pretreatment.

Two-Sample T-Test and CI: Act 0.5 N HCl, Act-0.5N-0.5hr

```
Two-sample T for Act 0.5 N HCl vs Act-0.5N-0.5hr

               N   Mean  StDev  SE Mean
Act 0.5 N HCl  3  4.835  0.471    0.27
Act-0.5N-0.5hr  3  2.254  0.247    0.14


Difference = mu (Act 0.5 N HCl) - mu (Act-0.5N-0.5hr)
Estimate for difference:  2.581
95% CI for difference:  (1.604, 3.558)
T-Test of difference = 0 (vs not =): T-Value = 8.41  P-Value = 0.004  DF = 3
```

Data for activated sludge with 0.5 N KOH pretreatment for 0.5 h were significantly different from the activated sludge with 0.5 N HCl pretreatment.

APPENDIX G

EXPERIMENTAL DATA

No.	Wo	W1	WI	W2	Wd	W3	Wa	MC%	Err, %	%TS	Err, %	VS%	Err, %
P1	45.5198	93.738	48.2182	47.2545	1.7347	45.8835	0.3637	96.4024	0.06	79.2435	5	79.03384	2
P2	45.5385	94.1534	48.6149	47.3003	1.7618	45.9057	0.3672	96.37601	0.06	79.3661	5	79.15768	2
P3	41.2968	89.6722	48.3754	43.0092	1.7124	41.6603	0.3635	96.46018	0.06	78.98476	5	78.77248	2
B1	46.2671	93.7308	47.4637	47.7895	1.5224	46.7263	0.4592	96.7925	0.06	70.13873	5	69.8371	2
B2	42.8098	90.059	47.2492	44.3264	1.5166	43.2684	0.4586	96.79021	0.06	70.0637	5	69.76131	2
B3	43.1761	90.5441	47.368	44.6977	1.5216	43.6335	0.4574	96.7877	0.06	70.24014	5	69.93954	2
AS1	46.0462	94.7537	48.7075	46.2311	0.1849	46.1036	0.0574	99.62039	0.06	69.26663	43	68.95619	21
AS2	45.937	94.4216	48.4846	46.1281	0.1911	45.9915	0.0545	99.60585	0.06	71.76609	43	71.4809	20
AS3	48.3471	97.4755	49.1284	48.5356	0.1885	48.4042	0.0571	99.61631	0.06	70.01114	43	69.70822	20

P= Primary sludge

B=Biosolids

A=Activated sludge

Wo=Weight of crucible

W1=Weight of sludge before dried in the oven with crucible

WI=W1-Wo

W2=Weight of dried sludge with crucible

Wd=W2-Wo

W3=Weight of sludge after ashing

Wa=Ashed weight

MC%=Moisture Content

TS%=Total Solid

VS%=Volatile Solid

AS%=Ash content

Err= relative error from instrument error

	S	V	V'	A	Conc. g/L	GY, mg	Sdry	PC%	Err, %	Average	STd
APS1	27.5	105	51	1.320	2.050326	219.5899	5.00	4.39	3	4.3	0.11
APS2	27.54	100	54	1.128	1.648159	178.0012	5.01	3.55	3		
APS3	27.66	74	52	1.826	2.836285	218.2805	5.03	4.33	3		
AAS1	69.47	116	53	0.732	1.136999	139.8054	5.00	2.80	3	2.61	0.19
AAS2	69.58	154	54	0.505	0.784405	130.4623	5.00	2.60	3		
AAS3	69.76	120	52	0.628	0.975458	121.7372	5.02	2.42	3		
ABS1	54.62	114	53	0.404	0.627524	75.83001	5.06	1.50	3	1.56	0.058
ABS2	54.51	122	56	0.384	0.596459	81.50009	5.05	1.61	3		
ABS3	54.58	123	52	0.397	0.616651	78.88201	5.05	1.56	3		

APS=Wet primary sludge
AAS=Wet activated sludge
ABS=Wet biosolids
S=Wet sample weight
V=Volume of hydrolyzed liquid
V’=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=Average PC%
PC%=Percentage of conversion
STd=Standard Deviation
Err= relative error from instrument error

Trails	S	V	V'	A	Conc. g/L	GY, mg	PC%	Err, %	Average	STd
BPS1	5.04	108	50	1.468	2.144944	231.654	4.59631	3	4.66	0.15
BPS2	5.04	83	51	1.967	2.87405	243.3171	4.82772	3		
BPS3	5.01	100	47	1.662	2.428404	228.27	4.556288	3		
BBS1	5.02	119	51	0.303	0.470643	57.12665	1.137981	3	1.15	0.042
BBS2	5.01	104	53	0.349	0.542094	59.76042	1.192823	3		
BBS3	5.00	118	50	0.303	0.470643	55.53588	1.110718	3		
BAS1	5.03	101	49	1.559	2.277908	225.4673	4.482451	3	4.48	0.18
BAS2	5.01	106	56	1.346	1.966686	233.485	4.660379	3		
BAS3	5.03	104	49	1.454	2.124489	216.5279	4.304729	3		

APS=Dried and ground primary sludge
AAS= Dried and ground activated sludge
ABS= Dried and ground biosolids
S= Dried and ground sample weight
V=Volume of hydrolyzed liquid
V'=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err= relative error from instrument

Trial	S	V	V'	A	Conc. mg/L	GY,mg	Sdry,g	PC%	Err, %	Average	STd
CP1-1	27.43	167	53	0.52	0.807704	142.9798	4.99226	2.86403	3	2.41	0.41
CP1-2	27.55	156.5	52	0.459	0.712954	116.0404	5.0141	2.314283	3		
CP1-3	27.64	156.5	54	0.396	0.615098	103.9638	5.03048	2.066678	3		
CP2-1	27.65	166	53	1.084	1.683753	296.2731	5.0323	5.88743	3	5.67	0.24
CP2-2	27.56	153	52	1.155	1.794035	285.4669	5.01592	5.691217	3		
CP2-3	27.48	131	49	1.359	2.110904	270.9979	5.00136	5.418483	3		
CP3-1	27.37	152	54	0.476	0.73936	121.3733	4.98134	2.43656	3	2.46	0.15
CP3-2	27.28	172	40	0.608	0.944393	129.9484	4.96496	2.617311	3		
CP3-3	27.54	126.5	54	0.546	0.848089	115.866	5.01228	2.311642	3		

CP= HCl pretreated primary sludge
S=Wet primary sludge weight
V=Volume of hydrolyzed liquid
V'=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

Trail	S	V mL	A	Conc.mg/L	GY,mg	Sdry,g	PC%	Err, %	Average	STd
CA1-1	69.6	121	1.196	1.85772	224.7841	5.0112	4.485634	1	4.84	0.47
CA1-2	69.24	152	1.134	1.761417	267.7353	4.98528	5.370517	1		
CA1-3	69.2	120	1.243	1.930724	231.6869	4.9824	4.650106	1		
CA2-1	69.6	89	1.014	1.575023	140.1771	5.0112	2.797276	1	3.52	0.63
CA2-2	69.34	74	1.714	2.662317	197.0115	4.99248	3.946165	1		
CA2-3	69.86	70	1.769	2.747748	192.3423	5.02992	3.823964	1		
CA3-1	69.35	157	0.965	1.498913	235.3293	4.9932	4.712996	1	4.45	0.23
CA3-2	69.73	69	2.008	3.118981	215.2097	5.02056	4.286567	1		
CA3-3	69.9	123	1.145	1.778503	218.7558	5.0328	4.346603	1		

CA= HCl pretreated activated sludge
S=Wet activated sludge weight
V=Volume of hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

Trail	S	V	V'	A	Conc. g/L	GY, mg	Sdry	PC%	Err, %	Average	Std
CBS1-1	54.33	158	53	0.462	0.717614	120.186	5.030958	2.388929	3	2.82	0.40
CBS1-2	54.04	146	55	0.636	0.987884	158.6542	5.004104	3.170482	3		
CBS1-3	54.64	148	53	0.602	0.935073	146.6943	5.059664	2.899288	3		
CBS2-1	54.52	137	58	0.952	1.47872	234.9982	5.048552	4.654764	3	4.67	0.25
CBS2-2	54.55	162	56	0.881	1.368437	248.2893	5.05133	4.915325	3		
CBS2-3	54.19	120	58	1.027	1.595216	222.0541	5.017994	4.425156	3		
CBS3-1	54.83	142	59	0.908	1.410376	236.3226	5.077258	4.654532	3	4.81	0.18
CBS3-2	54.59	124	60	1.042	1.618515	240.835	5.055034	4.764262	3		
CBS3-3	54.31	133	55	1.106	1.717925	251.3324	5.029106	4.997556	3		

CB= HCl pretreated biosolids
S=Wet biosolids weight
V=Volume of hydrolyzed liquid
V'=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

Trail	S	V	V'	A	Conc. g/L	GY, mg	Sdry	PC%	Err, %	Average	STd
DPS1-1	27.81	104	54	0.898	1.394843	156.6688	5.06142	3.095352	3	3.00	0.33
DPS1-2	27.52	130	55	0.739	1.147872	164.1457	5.00864	3.277251	3		
DPS1-3	27.86	115	64	0.585	0.908667	133.7558	5.07052	2.637911	3		
DPS2-1	27.73	132	57	0.793	1.231749	185.3536	5.04686	3.672652	3	3.46	0.19
DPS2-2	27.13	114	50	0.976	1.426067	162.5716	4.93766	3.292483	3		
DPS2-3	27.69	118	56	0.839	1.3032	172.2309	5.03958	3.417564	3		
DPS3-1	27.86	105	57	0.634	0.984778	117.8779	5.07052	2.32477	3	2.20	0.12
DPS3-2	27.49	109	41	0.754	1.171171	104.6793	5.00318	2.092255	3		
DPS3-3	27.71	116	51	0.602	0.935073	110.6378	5.04322	2.193794	3		

DP=KOH pretreated primary sludge (0.5 hr)
S=Wet primary sludge weight
V=Volume of hydrolyzed liquid
V’=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

Trail	S	V	V'	A	Conc. g/L	GY, mg	Sdry	PC%	Err, %	Average	STd
DPS4-1	27.64	144	51	0.249	0.363822	53.43822	5.03048	1.062289	3	1.09	0.11
DPS4-2	27.54	112	51	0.362	0.52893	60.42501	5.01228	1.205539	3		
DPS4-3	27.35	135	50	0.252	0.368206	49.70777	4.9777	0.998609	3		
DPS5-1	27.09	137	49	0.529	0.821684	110.3193	4.93038	2.237541	3	2.16	0.08
DPS5-2	27.2	132	52	0.535	0.781707	107.3127	4.9504	2.167758	3		
DPS5-3	27.07	134	49	0.532	0.777323	102.0781	4.92674	2.071919	3		
DPS6-1	27.68	141	51	0.3	0.43834	63.04208	5.03776	1.251391	3	1.35	0.12
DPS6-2	27.93	139	51	0.365	0.533314	75.61324	5.08326	1.487495	3		
DPS6-3	27.64	137	52	0.318	0.464641	66.20199	5.03048	1.316017	3		

DP=KOH pretreated primary sludge (1.0 hr)
S=Wet primary sludge weight
V=Volume of hydrolyzed liquid
V'=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

Trail	S	V	V'	A	Conc. g/L	GY, mg	Sdry	PC%	Err, %	Average	Std
DPS7-1	27.67	149	60	0.408	0.596143	106.5903	5.03594	2.116592	3	2.04	0.09
DPS7-2	27.78	118	43	0.662	0.967271	98.15862	5.05596	1.941444	3		
DPS7-3	27.13	118	49	0.606	0.885447	102.3931	4.93766	2.073717	3		
DPS8-1	27.78	74	53	0.753	1.100234	86.30234	5.05596	1.706943	3	1.70	0.13
DPS8-2	27.57	90	53	0.563	0.822618	78.47779	5.01774	1.564007	3		
DPS8-3	27.56	90	52	0.671	0.980421	91.76739	5.01592	1.829523	3		
DPS9-1	27.42	74	53	0.72	1.052016	82.52016	4.99044	1.653565	3	1.67	0.14
DPS9-2	27.54	150	51	0.344	0.50263	76.9024	5.01228	1.53428	3		
DPS9-3	27.64	73	62	0.649	1.008077	91.25113	5.03048	1.813965	3		

DP=KOH pretreated primary sludge (1.5 hrs)
S=Wet primary sludge weight
V=Volume of hydrolyzed liquid
V’=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

Trail	S	V	V'	A	Conc. g/L	GY, mg	Sdry	PC%	Err, %	Average	STd
DAS1-1	69.19	175	178	0.298	0.488445	86.94312	4.98168	1.745257	1	1.80	0.19
DAS1-2	69.29	168	169	0.361	0.591706	99.99836	4.98888	2.004425	1		
DAS1-3	69.36	126	126	0.396	0.649074	81.78331	4.99392	1.637658	1		
DAS2-1	69.7	132	132	0.589	0.965416	127.4348	5.0184	2.539352	1	2.25	0.25
DAS2-2	69.04	129.5	134	0.475	0.778561	104.3272	4.97088	2.098766	1		
DAS2-3	69.53	96	97	0.669	1.096542	106.3645	5.00616	2.124673	1		
DAS3-1	69.67	124.5	109	0.368	0.60318	65.7466	5.01624	1.310675	1	1.61	0.27
DAS3-2	69.55	135	135	0.418	0.685134	92.49303	5.0076	1.847053	1		
DAS3-3	69.64	133	135	0.376	0.616292	83.19948	5.01408	1.659317	1		

DA=KOH pretreated activated sludge (0.5 hrs)
S=Wet activated sludge weight
V=Volume of hydrolyzed liquid
V'=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

Trail	S	V	V'	A	Conc. g/L	GY, mg	Sdry	PC%	Err, %	Average	STd
DAS4-1	69.5	112	113	0.597	0.978528	110.5737	5.004	2.209706	1	2.28	0.07
DAS4-2	69.61	109	110	0.634	1.039174	114.3091	5.01192	2.280745	1		
DAS4-3	69.43	127	128	0.562	0.92116	117.9085	4.99896	2.358661	1		
DAS5-1	69.38	96	99	0.515	0.844124	83.56827	4.99536	1.672918	1	1.58	0.16
DAS5-2	69.47	96	98	0.521	0.853958	83.68792	5.00184	1.673143	1		
DAS5-3	69.51	95	97	0.437	0.716276	69.47877	5.00472	1.388265	1		
DAS6-1	69.43	93.5	95	0.382	0.626127	59.48205	4.99896	1.189889	1	1.23	0.09
DAS6-2	69.01	131	136	0.298	0.488445	66.42845	4.96872	1.336933	1		
DAS6-3	69.49	105	105	0.34	0.557286	58.515	5.00328	1.169533	1		

DA=KOH pretreated activated sludge (1.0 hrs)
S=Wet activated sludge weight
V=Volume of hydrolyzed liquid
V'=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

Trail	S	V	A	Conc. g/L	GY,mg	Sdry	PC%	Err, %	Average	Std
DAS7-1	69.96	145	0.384	0.596459	86.48649	5.03712	1.716983	3	1.75	0.16
DAS7-2	69.57	138	0.333	0.517241	79.94483	5.00904	1.596011	3		
DAS7-3	69.42	126	0.473	0.7347	96.27512	4.99824	1.92618	3		
DAS8-1	72.34	137	0.192	0.260692	35.71487	5.0638	0.705298	3	0.74	0.13
DAS8-2	70.86	144	0.192	0.260692	37.53971	4.9602	0.756819	3		
DAS8-3	72	149	0.187	0.253904	37.83164	5.04	0.750628	3		
DAS9-1	69.14	156	0.265	0.411619	71.91799	4.97808	1.444693	3	1.34	0.23
DAS9-2	69.82	131	0.255	0.396086	53.96272	5.02704	1.073449	3		
DAS9-3	69.76	110	0.457	0.667738	74.92022	5.02272	1.491626	3		

DA=KOH pretreated activated sludge (1.5 hrs)
S=Wet activated sludge weight
V=Volume of hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

Trail	S	V	V'	A	Conc g/L	GY,mg	Sdry	PC%	Err, %	Average	Std
DBS1-1	54.35	143	143	0.333	0.545812	78.05114	5.03281	1.550846	1	1.75	0.18
DBS1-2	54.52	167	171	0.344	0.563842	96.41698	5.048552	1.909795	1		
DBS1-3	54.45	145	146	0.379	0.62121	90.69661	5.04207	1.798797	1		
DBS2-1	54.18	168	176	0.143	0.234388	41.25225	5.017068	0.822238	1	0.90	0.08
DBS2-2	54.19	123	124	0.223	0.365514	45.32372	5.017994	0.903224	1		
DBS2-3	54.16	129.5	131	0.23	0.376987	49.38535	5.015216	0.98471	1		
DBS3-1	54.57	123	123	0.26	0.42616	52.41764	5.053182	1.037319	1	1.09	0.11
DBS3-2	54.5	122.5	124	0.256	0.419603	52.03081	5.0467	1.030987	1		
DBS3-3	54.54	88	89	0.421	0.690051	61.41452	5.050404	1.216032	1		

DB=KOH pretreated biosolids (0.5 hrs)
S=Wet biosolids weight
V=Volume of hydrolyzed liquid
V’=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

	S	V	V'	A	Conc. g/L	GY, mg	Sdry	PC%	Err, %	Average	Std
DBS4-1	54.62	106	59	0.588	0.859147	107.4621	5.057812	2.124675	3	1.88	0.22
DBS4-2	54.45	132	56	0.426	0.622443	92.02198	5.04207	1.825083	3		
DBS4-3	54.21	139	58	0.36	0.526008	84.81356	5.019846	1.689565	3		
DBS5-1	54.51	99	62	1.264	1.846873	226.7222	5.047626	4.491659	3	4.24	0.23
DBS5-2	54.36	105	58	1.145	1.672998	203.7712	5.033736	4.04811	3		
DBS5-3	54.56	100	56	1.289	1.883402	210.941	5.052256	4.175184	3		
DBS6-1	54.79	105	58	0.485	0.70865	86.31356	5.073554	1.701245	3	1.59	0.26
DBS6-2	54.04	87	87	0.36	0.590067	89.32437	5.004104	1.785022	3		
DBS6-3	54.47	99	61	0.369	0.539158	65.11955	5.043922	1.29105	3		

DB=KOH pretreated biosolids (1.0 hrs)
S=Wet biosolids weight
V=Volume of hydrolyzed liquid
V'=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

Trail	S	V	V'	A	Conc. g/L	GY, mg	Sdry	PC%	Err, %	Average	STd
DBS7-1	54.06	146	52	0.46	0.672122	102.0549	5.005956	2.03867	3	2.21	0.17
DBS7-2	54.27	144	57	0.464	0.677966	111.2949	5.025402	2.214647	3		
DBS7-3	54.37	143	56	0.512	0.748101	119.8158	5.034662	2.379818	3		
DBS8-1	54.14	142	57	0.194	0.28346	45.8865	5.013364	0.915284	3	0.86	0.09
DBS8-2	54.52	144	56	0.162	0.236704	38.17557	5.048552	0.756169	3		
DBS8-3	54.72	141	57	0.198	0.289305	46.50281	5.067072	0.917745	3		
DBS9-1	54.07	134	59	0.162	0.236704	37.42759	5.006882	0.747523	3	0.69	0.05
DBS9-2	54.24	140	65	0.121	0.176797	32.17709	5.022624	0.640643	3		
DBS9-3	54.11	132	61	0.145	0.211864	34.11864	5.010586	0.680931	3		

DB=KOH pretreated biosolids (1.5 hrs)
S=Wet biosolids weight
V=Volume of hydrolyzed liquid
V'=Volume of storage hydrolyzed liquid
A=Absorption
Conc=glucose concentration, g/L
GY=glucose yields, mg
Sdry=Dried sample weight
Average=PC% average
PC%=Percentage of conversion
STd=Standard Deviation
Err=relative error from instrument

Trail	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err, %	NDF,ave
DA1-1	0.25	81.72	81.85	81.77	0.247619	17.30769	0.330159
DA1-2	0.53	80.52	80.79	80.61	0.514286	9.330484	
DA1-3	0.24	83.74	83.86	83.79	0.228571	18.58974	
DA2-1	0.46	83.84	84.1	83.93	0.495238	9.615385	0.355556
DA2-2	0.24	81.76	81.89	81.81	0.247619	17.30769	
DA2-3	0.25	83.39	83.56	83.48	0.32381	13.68778	
DA3-1	0.24	82.56	82.76	82.63	0.380952	11.92308	0.342857
DA3-2	0.27	76.98	77.14	77.05	0.304762	14.42308	
DA3-3	0.29	80.5	80.68	80.58	0.342857	13.03419	
DA4-1							0.209524
DA4-2	0.3	36.75	36.94	36.81	0.361905	12.44939	
DA4-3	0.24	79.68	79.82	79.73	0.266667	16.20879	
DA5-1	0.24	37.5	37.59	37.54	0.171429	24.1453	0.24127
DA5-2	0.21	85.12	85.26	85.18	0.266667	16.20879	
DA5-3	0.24	81.4	81.55	81.48	0.285714	15.25641	
DA6-1	0.18	83.17	83.24	83.18	0.133333	30.49451	0.126984
DA6-2	0.1	77.33	77.36	77.34	0.057143	68.58974	
DA6-3	0.15	81.27	81.37	81.31	0.190476	21.92308	
DA7-1	0.25	82.7	82.82	82.75	0.228571	18.58974	0.304762
DA7-2	0.25	85.65	85.78	85.71	0.247619	17.30769	
DA7-3	0.39	83.54	83.77	83.62	0.438095	10.61873	
DA8-1	0.21	83.07	83.2	83.13	0.247619	17.30769	0.279365
DA8-2	0.25	80.88	81.04	80.95	0.304762	14.42308	
DA8-3	0.26	83.81	83.96	83.87	0.285714	15.25641	

DA9-1	0.25	83.42	83.59	83.52	0.32381	13.68778	0.273016
DA9-2	0.26	85.68	85.84	85.77	0.304762	14.42308	
DA9-3	0.17	80.48	80.58	80.52	0.190476	21.92308	

NDF for Activated sludge with KOH pretreatment

DAS4-1 sample was missing.

NDF was based on the initial dried weight of untreated activated sludge.

Trail	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err,%	NDF,ave
AA1	0.21	81.04	81.17	81.07	0.247619	17.28938	0.190476
AA2	0.2	85.17	85.27	85.19	0.190476	21.90476	
AA3	0.22	80.45	80.52	80.46	0.133333	30.47619	
BA1	0.18	84.83	84.87	84.83	0.07619	51.90476	0.057143
BA2	0.16	80.51	80.53	80.51	0.038095	101.9048	
BA3	0.13	81.72	81.75	81.72	0.057143	68.57143	

AA=hydrolyzed wet activated sludge

BA=hydrolyzed drying and ground pretreated activated sludge

	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err, %	NDF,ave
A1	0.53	85.08	85.31	85.13	0.433962	10.60041	0.383648
A2	0.51	80.93	81.1	80.98	0.333333	13.66947	

Untreated Activated Sludge NDF

A3 sample was missing.

	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err,%	NDF,ave
CA1-1	0.17	80.52	80.57	80.54	0.095238	41.90476	0.07619
CA1-2	0.17	84.59	84.62	84.6	0.057143	68.57143	
CA1-3	0.21	82.09	82.13	82.10	0.07619	51.90476	
CA2-1	0.17	83.56	83.6	83.58	0.07619	51.90476	0.07619
CA2-2	0.20	86.66	86.7	86.67	0.07619	51.90476	
CA2-3	0.17	84.54	84.58	84.55	0.07619	51.90476	
CA3-1	0.17	81.52	81.57	81.54	0.095238	41.90476	0.07619
CA3-2	0.21	84.82	84.86	84.83	0.07619	51.90476	
CA3-3	0.17	81.20	81.23	81.21	0.057143	68.57143	

NDF for HCl pretreated activated sludge

CA=hydrolyzed HCl pretreated activated sludge

	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err, %	NDF,ave
DP1-1	0.22	77.38	77.52	77.45	0.27451	16.2465	0.261438
DP1-2	0.21	84.54	84.66	84.58	0.235294	18.62745	
DP1-3	0.25	82.91	83.05	82.96	0.27451	16.2465	
DP2-1	0.18	83.56	83.61	83.56	0.098039	41.96078	0.084967
DP2-2	0.16	77.78	77.82	77.78	0.078431	51.96078	
DP2-3	0.15	84.37	84.41	84.37	0.078431	51.96078	
DP3-1	0.21	83.92	84.03	83.97	0.215686	20.1426	0.20915
DP3-2	0.21	83.31	83.42	83.35	0.215686	20.1426	
DP3-3	0.19	83.12	83.22	83.16	0.196078	21.96078	
DP4-1	0.5	83.59	83.85	83.66	0.509804	9.653092	0.385621
DP4-2	0.25	80.31	80.47	80.38	0.313725	14.46078	

DP4-3	0.24	82.24	82.41	82.31	0.333333	13.72549	
DP5-1	0.23	83.08	83.25	83.16	0.333333	13.72549	0.352941
DP5-2	0.46	82.57	82.81	82.65	0.470588	10.29412	
DP5-3	0.23	80.46	80.59	80.53	0.254902	17.3454	
DP6-1	0.23	84.46	84.6	84.52	0.27451	16.2465	0.326797
DP6-2	0.33	82.55	82.76	82.63	0.411765	11.48459	
DP6-3	0.24	80.76	80.91	80.81	0.294118	15.29412	
DP7-1	0.25	81.71	81.86	81.76	0.294118	15.29412	0.359477
DP7-2	0.25	82.26	82.39	82.31	0.254902	17.3454	
DP7-3	0.49	81.75	82.02	81.85	0.529412	9.368192	
DP8-1	0.35	80.85	81.05	80.93	0.392157	11.96078	0.326797
DP8-2	0.22	82.46	82.6	82.53	0.27451	16.2465	
DP8-3	0.25	83.57	83.73	83.65	0.313725	14.46078	
DP9-1	0.26	80.48	80.62	80.53	0.27451	16.2465	0.320261
DP9-2	0.24	79.81	79.97	79.86	0.313725	14.46078	
DP9-3	0.31	82.32	82.51	82.4	0.372549	12.4871	

NDF for KOH pretreated primary sludge

DP=hydrolyzed KOH pretreated primary sludge

NDF was based on the initial dried weight of untreated primary sludge.

	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err, %	NDF,ave
CP1-1	0.24	83.16	83.31	83.24	0.294118	15.25641	0.267974
CP1-2	0.25	80.95	81.09	81	0.27451	16.20879	
CP1-3	0.22	81.56	81.68	81.6	0.235294	18.58974	
CP2-1	0.17	84.45	84.53	84.47	0.156863	26.92308	0.150327
CP2-2	0.18	82.16	82.25	82.17	0.176471	24.1453	
CP2-3	0.18	84.17	84.23	84.18	0.117647	35.25641	
CP3-1	0.21	84.11	84.14	84.12	0.058824	68.58974	0.104575
CP3-2	0.2	83.1	83.17	83.1	0.137255	30.49451	
CP3-3	0.18	81.19	81.25	81.19	0.117647	35.25641	

NDF for HCl pretreated primary sludge

CP=hydrolyzed HCl pretreated primary sludge

	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err, %	NDF,ave
AP1	0.19	81.87	81.94	81.88	0.137255	30.49451	0.189542
AP2	0.18	84.8	84.89	84.82	0.176471	24.1453	
AP3	0.26	77.16	77.29	77.19	0.254902	17.30769	
BP1	0.37	81.6	81.75	81.64	0.294118	15.25641	0.326797
BP2	0.51	82.35	82.55	82.39	0.392157	11.92308	
BP3	0.45	76.52	76.67	76.55	0.294118	15.25641	

AP=hydrolyzed wet primary sludge

BP= hydrolyzed drying and ground pretreated primary sludge

	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err, %	NDF,ave
P1	0.52	81.43	82.69	81.96	2.423077	3.510379	2.761187
P2	0.52	83.63	85.25	84.24	3.115385	3.157645	
P3	0.51	80.7	82.1	81.53	2.745098	3.351648	

NDF for untreated primary sludge

	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err, %	NDF,ave
DB1-1	0.6	37.26	37.62	37.4	0.692308	7.478632	0.50641
DB1-2	0.35	83.43	83.63	83.51	0.384615	11.92308	
DB1-3	0.36	76.99	77.22	77.09	0.442308	10.61873	
DB2-1	0.26	82.99	83.16	83.07	0.326923	13.68778	0.307692
DB2-2	0.23	80.1	80.25	80.19	0.288462	15.25641	
DB2-3	0.27	36.38	36.54	36.45	0.307692	14.42308	
DB3-1	0.25	82.87	83.03	82.95	0.307692	14.42308	0.333333
DB3-2	0.26	82.97	83.13	83.05	0.307692	14.42308	
DB3-3	0.3	37.11	37.31	37.21	0.384615	11.92308	
DB4-1	0.16	83.71	83.83	83.74	0.230769	18.58974	0.352564
DB4-2	0.3	37.05	37.26	37.13	0.403846	11.44689	
DB4-3	0.38	82.63	82.85	82.71	0.423077	11.01399	
DB5-1	0.07	81.02	81.07	81.06	0.096154	41.92308	0.282051
DB5-2	0.24	83.51	83.67	83.63	0.307692	14.42308	
DB5-3	0.33	84.23	84.46	84.45	0.442308	10.61873	
DB6-1	0.21	81.59	81.72	81.65	0.25	17.30769	0.25641
DB6-2	0.23	37.11	37.25	37.17	0.269231	16.20879	
DB6-3	0.18	81.31	81.44	81.36	0.25	17.30769	

DB7-1	0.19	84.06	84.17	84.09	0.211538	20.1049	0.230769
DB7-2	0.22	84.61	84.75	84.64	0.269231	16.20879	
DB7-3	0.21	82.78	82.89	82.8	0.211538	20.1049	
DB8-1	0.23	83.88	84	83.94	0.230769	18.58974	0.237179
DB8-2	0.22	84.7	84.84	84.76	0.269231	16.20879	
DB8-3	0.17	85.67	85.78	85.73	0.211538	20.1049	
DB9-1	0.19	86.83	86.92	86.84	0.173077	24.1453	0.217949
DB9-2	0.21	82.95	83.09	82.97	0.269231	16.20879	
DB9-3	0.18	80.34	80.45	80.35	0.211538	20.1049	

NDF for KOH pretreated biosolids

DB=hydrolyzed KOH pretreated biosolids

NDF was based on the initial dried weight of untreated biosolids

	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err, %	NDF,ave
CB1-1	0.24	81.69	81.8	81.73	0.211538	20.1049	0.192308
CB1-2	0.24	84.19	84.29	84.23	0.192308	21.92308	
CB1-3	0.22	80.74	80.83	80.77	0.173077	24.1453	
CB2-1	0.19	83.7	83.76	83.71	0.115385	35.25641	0.134615
CB2-2	0.23	86.18	86.26	86.19	0.153846	26.92308	
CB2-3	0.23	81.57	81.64	81.58	0.134615	30.49451	
CB3-1	0.15	80.83	80.87	80.83	0.076923	51.92308	0.083333
CB3-2	0.15	80.91	80.94	80.91	0.057692	68.58974	
CB3-3	0.2	82.31	82.37	82.31	0.115385	35.25641	

CB =hydrolyzed HCl pretreated biosolids

	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err, %	NDF,ave
AB1	0.21	81.37	81.45	81.38	0.153846	26.92308	0.121795
AB2	0.17	83.69	83.74	83.7	0.096154	41.92308	
AB3	0.22	85.12	85.18	85.14	0.115385	35.25641	
BB1	0.43	83.26	83.42	83.29	0.307692	14.42308	0.314103
BB2	0.28	83.31	83.43	83.34	0.230769	18.58974	
BB3	0.47	82.52	82.73	82.58	0.403846	11.44689	

AP=hydrolyzed wet biosolids

BP= hydrolyzed drying and ground pretreated biosolids

	Wo,g	Wt,g	Wd,g	Wa,g	NDF(g/g)	Err, %	NDF,ave
B1	0.53	83.07	83.26	83.13	0.358491	12.44939	0.400779
B2	0.5	80.57	80.79	80.69	0.44	11.01399	
B3	0.52	81.72	81.93	81.86	0.403846	11.44689	

NDF for untreated biosolids

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
DA1-1	0.45	83.24	83.41	83.41	83.24	0.34	13.76471	0.34	13.76471	0.36	0.34
DA1-2	0.5	80.26	80.42	80.45	80.3	0.32	14.5	0.3	15.33333		
DA1-3	0.52	84.21	84.42	84.47	84.28	0.42	11.52381	0.38	12.52632		
DA2-1	0.52	85.06	85.36	85.3	85.18	0.6	8.666667	0.24	18.66667	0.44	0.186667
DA2-2	0.39	76.78	77.01	76.97	76.88	0.46	10.69565	0.18	24.22222		
DA2-3	0.23	83.29	83.42	83.4	83.33	0.26	17.38462	0.14	30.57143		
DA3-1	0.43	81.75	82	82	81.83	0.5	10	0.34	13.76471	0.513333	0.32
DA3-2	0.39	80.45	80.68	80.63	80.52	0.46	10.69565	0.22	20.18182		
DA3-3	0.49	82.76	83.05	83.08	82.88	0.58	8.896552	0.4	12		
DA4-1										0.36	0.15
DA4-2	0.46	81.09	81.3	81.25	81.17	0.42	11.52381	0.16	27		
DA4-3	0.31	84.84	84.99	84.97	84.9	0.3	15.33333	0.14	30.57143		
DA5-1	0.23	81.42	81.56	81.53	81.47	0.28	16.28571	0.12	35.33333	0.22	0.12
DA5-2	0.18	81.35	81.45	81.47	81.4	0.2	22	0.14	30.57143		
DA5-3	0.19	82.31	82.4	82.4	82.35	0.18	24.22222	0.1	42		
DA6-1	0.24	81.621	81.77	81.74	81.69	0.298	15.42282	0.1	42	0.166	0.073333
DA6-2	0.06	82.58	82.61	82.61	82.6	0.06	68.66667	0.02	202		
DA6-3	0.15	83.83	83.9	83.9	83.85	0.14	30.57143	0.1	42		
DA7-1	0.3	80.19	80.33	80.31	80.22	0.28	16.28571	0.18	24.22222	0.453333	0.22
DA7-2	0.51	83.94	84.2	84.13	84.01	0.52	9.692308	0.24	18.66667		
DA7-3	0.53	80.79	81.07	81.01	80.89	0.56	9.142857	0.24	18.66667		
DA8-1	0.33	82.92	83.09	83.05	82.98	0.34	13.76471	0.14	30.57143	0.3	0.106667
DA8-2	0.17	82.45	82.58	82.54	82.5	0.26	17.38462	0.08	52		
DA8-3	0.24	80.16	80.31	80.26	80.21	0.3	15.33333	0.1	42		

DA9-1	0.21	82.28	82.41	82.37	82.33	0.26	17.38462	0.08	52	0.226667	0.066667
DA9-2	0.22	79.5	79.63	79.59	79.56	0.26	17.38462	0.06	68.66667		
DA9-3	0.18	83.55	83.63	83.6	83.57	0.16	27	0.06	68.66667		

ADF and ADL for KOH pretreated activated sludge

DA=hydrolyzed KOH pretreated activated sludge

ADF was based on the initial dried weight of untreated activated sludge.

ADL was based on the initial dried weight of untreated activated sludge.

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
CA1-1	0.16	83.52	83.53	83.59	83.53	0.02	202	0.12	35.33333	0.053333	0.146667
CA1-2	0.2	80.35	80.39	80.43	80.35	0.08	52	0.16	27		
CA1-3	0.17	84.48	84.51	84.57	84.49	0.06	68.66667	0.16	27		
CA2-1	0.17	77.91	77.92	78.01	77.92	0.02	202	0.18	24.22222	0.02	0.12
CA2-2	0.14	80.83	80.84	80.86	80.79	0.02	202	0.14	30.57143		
CA2-3	0.15	83.51	83.52	83.53	83.51	0.02	202	0.04	102		
CA3-1	0.17	83.93	83.98	83.96	83.93	0.1	42	0.06	68.66667	0.08	0.06
CA3-2	0.19	81.28	81.32	81.31	81.28	0.08	52	0.06	68.66667		
CA3-3	0.23	83.58	83.61	83.61	83.58	0.06	68.66667	0.06	68.66667		

ADF and ADL for HCl pretreated activated sludge

CA =hydrolyzed HCl pretreated activated sludge

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
AA1	0.32	82.19	82.28	82.28	82.21	0.18	24.22222	0.14	30.57143	0.18	0.12
AA2	0.48	83.79	83.9	83.85	83.8	0.22	20.18182	0.1	42		
AA3	0.45	79.46	79.53	79.53	79.47	0.14	30.57143	0.12	35.33333		

BA1	0.17	83.24	83.28	83.28	83.24	0.08	52	0.08	52	0.053333	0.06
BA2	0.16	83.3	83.32	83.32	83.29	0.04	102	0.06	68.66667		
BA3	0.13	82.45	82.47	83.43	83.41	0.04	102	0.04	102		

AA=hydrolyzed wet activated sludge

BA= hydrolyzed drying and ground pretreated activated sludge

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
A1	0.51	82.64	82.75	82.74	82.68	0.215686	20.18182	0.117647	35.33333	0.220088	0.130252
A2	0.49	83.26	83.37	83.36	83.29	0.22449	20.18182	0.142857	30.57143		

ADF and ADL for Untreated Activated Sludge

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
DP1-1	0.5	84.57	84.83	84.76	84.64	0.509804	9.653092	0.235294	18.62745	0.45098	0.20915
DP1-2	0.49	84.36	84.61	84.55	84.45	0.490196	9.960784	0.196078	21.96078		
DP1-3	0.37	82.02	82.2	82.18	82.08	0.352941	13.0719	0.196078	21.96078		
DP2-1	0.13	80.98	81.02	81.02	80.99	0.078431	51.96078	0.058824	68.62745	0.104575	0.039216
DP2-2	0.19	83.48	83.55	83.49	83.47	0.137255	30.53221	0.039216	101.9608		
DP2-3	0.15	81.33	81.38	81.34	81.33	0.098039	41.96078	0.019608	201.9608		
DP3-1	0.36	83.56	83.71	83.69	83.57	0.294118	15.29412	0.235294	18.62745	0.437908	0.24183
DP3-2	0.46	79.94	80.21	80.11	79.96	0.529412	9.368192	0.294118	15.29412		
DP3-3	0.43	83.48	83.73	83.66	83.56	0.490196	9.960784	0.196078	21.96078		
DP4-1	0.51	83.3	83.54	83.48	83.38	0.470588	10.29412	0.196078	21.96078	0.45098	0.176471
DP4-2	0.43	83.05	83.26	83.18	83.1	0.411765	11.48459	0.156863	26.96078		
DP4-3	0.37	80.09	80.33	80.27	80.18	0.470588	10.29412	0.176471	24.18301		

DP5-1	0.5	83.5	83.79	83.82	83.61	0.568627	8.857336	0.411765	11.48459	0.529412	0.48366
DP5-2	0.59	83.82	84.1	84.17	83.91	0.54902	9.103641	0.509804	9.653092		
DP5-3	0.5	77.15	77.39	77.48	77.21	0.470588	10.29412	0.529412	9.368192		
DP6-1	0.36	82.51	82.71	82.69	82.57	0.392157	11.96078	0.235294	18.62745	0.398693	0.176471
DP6-2	0.4	82.02	82.27	82.19	82.11	0.490196	9.960784	0.156863	26.96078		
DP6-3	0.28	82.83	82.99	82.93	82.86	0.313725	14.46078	0.137255	30.53221		
DP7-1	0.37	82.94	83.11	83.08	82.98	0.333333	13.72549	0.196078	21.96078	0.457516	0.267974
DP7-2	0.4	80.06	80.26	80.2	80.12	0.392157	11.96078	0.156863	26.96078		
DP7-3	0.62	80.95	81.28	81.32	81.09	0.647059	8.02139	0.45098	10.65644		
DP8-1	0.5	86.61	86.89	86.89	86.71	0.54902	9.103641	0.352941	13.0719	0.48366	0.287582
DP8-2	0.35	85.77	85.98	86.01	85.85	0.411765	11.48459	0.313725	14.46078		
DP8-3	0.47	82.98	83.23	83.15	83.05	0.490196	9.960784	0.196078	21.96078		
DP9-1	0.35	83.44	83.66	83.63	83.55	0.431373	11.05169	0.156863	26.96078	0.424837	0.130719
DP9-2	0.32	83.37	83.56	83.5	83.45	0.372549	12.4871	0.098039	41.96078		
DP9-3	0.44	80.81	81.05	80.96	80.89	0.470588	10.29412	0.137255	30.53221		

ADF and ADL for KOH pretreated primary sludge

DA=hydrolyzed KOH pretreated primary sludge

ADF was based on the initial dried weight of untreated primary sludge.

ADL was based on the initial dried weight of untreated primary sludge.

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
CP1-1	0.46	79.99	80.11	80.14	80.02	0.235294	18.62745	0.235294	18.62745	0.196078	0.248366
CP1-2	0.5	82.9	82.97	83.05	82.92	0.137255	30.53221	0.254902	17.3454		
CP1-3	0.5	80.49	80.6	80.56	80.43	0.215686	20.1426	0.254902	17.3454		
CP2-1	0.14	81.68	81.72	81.7	81.68	0.078431	51.96078	0.039216	101.9608	0.104575	0.078431
CP2-2	0.17	77.04	77.09	77.09	77.04	0.098039	41.96078	0.098039	41.96078		
CP2-3	0.19	83.95	84.02	84.01	83.96	0.137255	30.53221	0.098039	41.96078		
CP3-1	0.11	84.19	84.24	84.25	84.2	0.098039	41.96078	0.098039	41.96078	0.117647	0.137255
CP3-2	0.14	83.25	83.31	83.31	83.25	0.117647	35.29412	0.117647	35.29412		
CP3-3	0.17	77.59	77.66	77.69	77.59	0.137255	30.53221	0.196078	21.96078		

ADF and ADL for HCl pretreated primary sludge

CP =hydrolyzed HCl pretreated primary sludge

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
AP1	0.13	84.67	84.71	84.73	84.67	0.078431	52	0.117647	35.33333	0.084967	0.084967
AP2	0.17	80.78	80.83	80.83	80.78	0.098039	42	0.098039	42		
AP3	0.18	84.28	84.32	84.3	84.28	0.078431	52	0.039216	102		
BP1	0.45	82.66	82.87	82.94	82.71	0.411765	11.52381	0.45098	10.69565	0.411765	0.490196
BP2	0.52	82.75	82.96	82.89	82.58	0.411765	11.52381	0.607843	8.451613		
BP3	0.49	78.92	79.13	79.14	78.93	0.411765	11.52381	0.411765	11.52381		

AP=hydrolyzed wet primary sludge

BP= hydrolyzed drying and ground pretreated primary sludge

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
P1	0.5	83.15	83.29	83.24	83.19	0.28	16.28571	0.1	42	0.261383	0.091528
P2	0.52	80.75	80.9	80.84	80.79	0.288462	15.33333	0.096154	42		
P3	0.51	83.57	83.68	83.67	83.63	0.215686	20.18182	0.078431	52		

ADF and ADL for Untreated Primary Sludge

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
DB1-1	0.5	86.43	86.68	86.78	86.54	0.480769	9.923077	0.461538	10.25641	0.320513	0.269231
DB1-2	0.32	82.39	82.5	82.48	82.4	0.211538	20.1049	0.153846	26.92308		
DB1-3	0.41	83.03	83.17	83.14	83.04	0.269231	16.20879	0.192308	21.92308		
DB2-1	0.27	83.36	83.5	83.48	83.4	0.269231	16.20879	0.153846	26.92308	0.352564	0.166667
DB2-2	0.25	79.63	79.73	79.72	79.65	0.192308	21.92308	0.134615	30.49451		
DB2-3	0.5	83.74	84.05	83.99	83.88	0.596154	8.37469	0.211538	20.1049		
DB3-1	0.27	84.52	84.67	84.63	84.57	0.288462	15.25641	0.115385	35.25641	0.365385	0.153846
DB3-2	0.32	80.93	81.11	81.08	81	0.346154	13.03419	0.153846	26.92308		
DB3-3	0.47	84.87	85.11	85.06	84.96	0.461538	10.25641	0.192308	21.92308		
DB4-1	0.14	79.6	79.65	79.65	79.6	0.096154	41.92308	0.096154	41.92308	0.269231	0.179487
DB4-2	0.44	81.07	81.28	81.25	81.09	0.403846	11.44689	0.307692	14.42308		
DB4-3	0.33	81.06	81.22	81.2	81.13	0.307692	14.42308	0.134615	30.49451		
DB5-1	0.5	84.7	85.03	84.97	84.84	0.634615	7.983683	0.25	17.30769	0.371795	0.205128
DB5-2	0.24	83.05	83.19	83.22	83.11	0.269231	16.20879	0.211538	20.1049		
DB5-3	0.24	82.86	82.97	82.96	82.88	0.211538	20.1049	0.153846	26.92308		
DB6-1	0.16	83.82	83.9	83.9	83.84	0.153846	26.92308	0.115385	35.25641	0.25	0.141026
DB6-2	0.3	83.7	83.89	83.89	83.8	0.365385	12.44939	0.173077	24.1453		

DB6-3	0.2	79.86	79.98	79.97	79.9	0.230769	18.58974	0.134615	30.49451		
DB7-1	0.14	79.83	79.88	79.88	79.84	0.096154	41.92308	0.076923	51.92308	0.224359	0.141026
DB7-2	0.34	81.82	82.02	81.99	81.89	0.384615	11.92308	0.192308	21.92308		
DB7-3	0.21	80.21	80.31	80.3	80.22	0.192308	21.92308	0.153846	26.92308		
DB8-1	0.28	85.08	85.25	85.23	85.09	0.326923	13.68778	0.269231	16.20879	0.25	0.217949
DB8-2	0.19	82.17	82.29	82.33	82.22	0.230769	18.58974	0.211538	20.1049		
DB8-3	0.16	76.5	76.6	76.62	76.53	0.192308	21.92308	0.173077	24.1453		
DB9-1	0.23	82.91	83.06	83.1	82.98	0.288462	15.25641	0.230769	18.58974	0.237179	0.166667
DB9-2	0.24	76.74	76.86	76.85	76.77	0.230769	18.58974	0.153846	26.92308		
DB9-3	0.21	80.41	80.51	80.49	80.43	0.192308	21.92308	0.115385	35.25641		

ADF and ADL for KOH pretreated biosolids

DB=hydrolyzed KOH pretreated biosolids

ADF was based on the initial dried weight of untreated biosolids

ADL was based on the initial dried weight of untreated biosolids

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
CB1-1	0.48	79.58	79.97	79.78	79.67	0.75	7.051282	0.211538	20.1049	0.730769	0.211538
CB1-2	0.49	82.8	83.21	83.01	82.89	0.788462	6.801126	0.230769	18.58974		
CB1-3	0.45	83.21	83.55	83.43	83.33	0.653846	7.80543	0.192308	21.92308		
CB2-1	0.5	83.4	83.61	83.57	83.45	0.403846	11.44689	0.230769	18.58974	0.294872	0.173077
CB2-2	0.18	85.63	85.69	85.68	85.63	0.115385	35.25641	0.096154	41.92308		
CB2-3	0.52	85.69	85.88	85.86	85.76	0.365385	12.44939	0.192308	21.92308		
CB3-1	0.21	83.77	83.82	83.82	83.77	0.096154	41.92308	0.096154	41.92308	0.096154	0.108974
CB3-2	0.21	86.54	86.6	86.59	86.54	0.115385	35.25641	0.096154	41.92308		
CB3-3	0.17	83.36	83.4	83.43	83.36	0.076923	51.92308	0.134615	30.49451		

CB =hydrolyzed HCl pretreated biosolids

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
AB1	0.2	83.69	83.72	83.73	83.69	0.057692	52	0.076923	35.33333	0.076923	0.064103
AB2	0.15	86.36	86.39	86.4	86.38	0.057692	42	0.038462	42		
AB3	0.17	84.6	84.66	84.66	84.62	0.115385	52	0.076923	62		
BB1	0.38	80.35	80.49	80.48	80.41	0.269231	16.20879	0.134615	30.49451	0.307692	0.147436
BB2	0.48	79.95	80.14	80.1	80	0.365385	12.44939	0.192308	21.92308		
BB3	0.43	79.4	79.55	79.5	79.44	0.288462	15.25641	0.115385	35.25641		

AB=hydrolyzed wet biosolids

BB= hydrolyzed drying and ground pretreated biosolids

	Wo,g	Wt,g	Wd1,g	Wd2,g	Wa,g	ADF(g/g)	Err, %	ADL(g/g)	Err, %	ADF,ave	ADL,ave
B1	0.53	80.96	81.07	81.05	81	0.207547	20.1049	0.09434	41.92308	0.212973	0.077198
B2	0.51	84.03	84.13	84.11	84.07	0.196078	21.92308	0.078431	51.92308		
B3	0.51	83.2	83.32	83.3	83.27	0.235294	18.58974	0.058824	68.58974		

ADF and ADL for Untreated Biosolids

Crude Fiber of Primary Sludge

Non-modified	Wo,g	W2,g	W3,g	CF(g/g)	Average	Err,%	Modified CF of Primary Sludge	Wo	W2,g	W3,g	CF(g/g)	Average	Err
1	1.01	86.53	86.11	0.415842	0.446997	5.752004		1.01	84.59	84.37	0.217822	0.235941	10.08101
2	1.00	86.62	86.18	0.44		5.545455		1.00	86.44	86.21	0.23		9.695652
3	1.01	87.58	87.09	0.485149		5.071732		1.00	82.39	82.13	0.26		8.692308