ENZYMATIC HYDROLYSIS OF CELLULOSE FROM VARIOUS
WASTE SOURCES AND THEIR FEASIBILITY AS FEEDSTOCKS
FOR ETHANOL PRODUCTION

By

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fulfilment of the requirements for the degree of

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EXECUTIVE SUMMARY

This study focused on the optimization of the enzymatic hydrolysis of three feedstocks: crop residues, poultry manure, and sewage sludge. The crop residues investigated include corn stalk and bagasse, they were pre-treated with KOH prior to enzymatic hydrolysis. At 40 °C, with an enzyme loading of 800 units/g substrate, the glucose yields from enzymatic hydrolysis of corn stalk and bagasse were $65.4 \pm 2.3\%$ and $51.1 \pm 2.3\%$, respectively. The enzymatic hydrolysis of corn stalk was further optimized by using wet substrate, which was ground before KOH treatment, and by adding half of the enzyme dosage at the beginning and half in the middle of the hydrolysis instead of adding the entire dosage at the beginning.

For poultry manure, the highest glucose conversion was achieved when it was treated with KOH and then dried and ground prior to enzymatic hydrolysis. At 40°C, enzyme loading 400 units/g substrate, the conversion was $27.6 \pm 1.2\%$. Under the same optimum conditions as crop residues, $31.1 \pm 2.7\%$ of wet raw sludge was converted to glucose, and it was increased to $54.2 \pm 4.0\%$ when HCl and KOH pre-treatments were employed.

An integrated ethanol production process was proposed and cost analysis was performed for each feedstock. The operational cost to produce one litre of ethanol was estimated to be \$4.97, \$5.26 and \$4.27 for corn stalk, poultry manure, and raw sludge respectively.

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Chapter 1 Introduction

Alternatives to fossil fuels have been of serious interest in the last decade due to the limited availability and the soaring price of crude oil. Ethanol is of particular interest because of its advantages in reducing unwanted emissions, especially greenhouse gases, which are believed to be associated with global warming. Currently, annual ethanol production in Canada is about 238 million litres (Canadian Renewable Fuels Association, 2001), over 90% of which is made from grain materials such as corn, wheat, and canola. According to the Action Plan 2000, the Canadian government aims to increase its annual ethanol production to 750 million litres by 2005 (CRFA, 2001). This would result in tremendous increase in the demand of raw materials; therefore, new feedstocks, in addition to the grains, would be required.

Cellulose is the most abundant organic compound on earth (Fan et al, 1987). It is continually replenished by photosynthetic reduction of carbon dioxide catalyzed by sunlight. It can be converted into glucose through hydrolysis, and the glucose be further fermented into ethanol. The first step is critical because cellulose has a highly rigid structure which is very difficult to decompose. The research on the ethanol production from cellulose has been carried out for many years. Basically, there are two approaches to degrade cellulose: acid hydrolysis and enzymatic hydrolysis. Acid hydrolysis has been utilized in some cellulose to ethanol projects, but they were unsuccessful due to the associated high operational cost. On the other hand, interest in enzymatic hydrolysis is rapidly growing thanks to the

recent progress in biochemistry, which has greatly reduced the price of enzymes. It is expected that the cost of enzymes will continue to decrease; this supports the development of an economically viable process for the production ethanol through enzymatic hydrolysis of cellulose.

Aside from enzymes, another factor that determines the viability of a bio-ethanol project is the feedstock. The desired feedstock should have high glucose conversion rate, low cost, and be abundant in quantity. Once the costs of enzymes are sufficiently decreased, the choice of feedstock will be critical in the development of cellulose to ethanol projects.

1.1 Problem identification

There are a number of cellulosic materials available for ethanol production; natural biomass materials are the most common examples. Generally in natural plant materials, the cellulose is bounded with lignin and hemicellulose, and forms a rigid structure, which is highly resistant to hydrolysis. To extract the cellulose from the raw materials, chemical pre-treatment is required prior to enzymatic hydrolysis. However, in some cellulose-containing materials such as paper products, the cellulose content is separated from lignin and hemicellulose, thus chemical pre-treatment may not be necessary.

For the purpose of researching low cost feedstock, three types of materials from waste streams were investigated in this study: crop residues, poultry manure, and sewage sludge. Crop residues usually have high cellulose contents, but pre-treatment is required to extract the cellulose from raw material. Poultry manure

was selected because cellulose is expected in the manure and litter. Since sewage sludge contains toilet tissue, which is mainly composed of cellulose, it was also chosen as a feedstock in this study.

Through investigation of their properties in enzymatic hydrolysis for the three feedstocks, the optimum conditions for enzymatic hydrolysis could be identified, and the ethanol production process could further be proposed based on the experimental results.

1.2 Project objectives

There were two main objectives for this study. The first was to investigate the enzymatic hydrolysis of the crop residues, poultry manure, and sewage sludge under different conditions, and identify the optimum for each material by measuring their glucose yields. The conditions investigated included reaction time, temperature, enzyme loading, and pre-treatment method.

The second objective was to propose an integrated ethanol production process for each feedstock and estimate its operational costs, through which its perspective as a feedstock for ethanol production may be suggested.

1.3 Thesis topics

The following are the subsequent topics involved in this thesis:

- Literature review of the research of ethanol production and cellulose hydrolysis.
- Interpretation of the experimental procedures performed in this study and the

analytical methods.

- Determination of the optimum reaction time, temperature, and enzyme loading in enzymatic hydrolysis of crop residues.
- Process optimization of the enzymatic hydrolysis on corn stalk.
- Examination of the effect of chemical and physical pre-treatment on enzymatic hydrolysis of poultry manure, and determination of the best preparation.
- Examination of the effect of chemical and physical pre-treatment on enzymatic hydrolysis of sewage sludge, and determination of the best preparation.
- Fourier Transform Infrared Spectroscopy analysis of the substrate and residue from enzymatically hydrolysis.
- Development of the integrated ethanol production process for each feedstock based on the experimental results.
- Cost analysis of each process.
- Conclusions and recommendations derived from this study.

Chapter 2 Literature review

2.1 Introduction

Ethanol is usually produced from the fermentation of soluble sugar and starch. Corn and other starch containing crops are the most common materials for ethanol production. In Canada, 238 million litres of ethanol are produced each year (CRFA, 2001), about 73% of which is made from corn, 20% from wheat, the rest from forestry product. However, ethanol from starch materials is a mature technology, and there is little prospect to decrease the process cost. Once the demand for ethanol increases substantially, the price of such crops will increase accordingly. The Canadian government proposes to increase its annual ethanol production to 750 million litres by 2010 (CRFA, 2001), this implies that a three-fold increase in feedstock supply will be required. Alternative feedstocks will thus be essential to meet these needs, among which cellulosic material is considered the most promising supply. Although the cost of cellulose-based ethanol is higher than that of corn, progress is being made in research and a substantial decrease in process cost is expected in the near future.

2.2 Ethanol as a fuel

The use of ethanol as automobile fuel has drawn much attention in recent years. As a type of renewable fuel, ethanol is considered a potential alternative fuel for dwindling gasoline. Currently ethanol is used as an automotive fuel by itself or mixed with gasoline to form what has been termed "gasohol", the most common

blends are E10 (10% ethanol, 90% gasoline) and E85 (85% ethanol and 15% gasoline). E10 can be used in most vehicles without any modification, while the use of E85 requires small changes in the engine. There are also some vehicles designed to use pure ethanol. Demonstration fleets of heavy duty buses and trucks using pure ethanol have been operated in the U.S.

2.2.1 Ethanol fuel and the environment

Ethanol is considered a “clean” fuel and poses less environmental impact than combusted gasoline. Not only is air pollution mitigated, but also are emissions of greenhouse gas (GHG) reduced dramatically by using ethanol instead of gasoline.

2.2.1.1 Ethanol and air pollution

Ethanol can be used as an oxygenate in gasoline because it contains oxygen in the molecular structure. The addition of ethanol substantially reduces the pollution generated under oxygen deficit condition. For instance, studies have shown that up to 30% reduction in carbon monoxide emission can be achieved by using E10 (CRFA, 2001).

More emission reduction was found in higher ethanol blended fuels. A study conducted in U.S. Department of Energy showed that compared to reformulated gasoline, the sulfur oxide emissions in E95 was 60-80% lower, and the volatile organic compounds were reduced by 13-15% (Sheehan, 2001). In addition, the reduction in hydrocarbon and carbon monoxide emissions result in lower ground

level ozone formation. However, there are also some negative effects of ethanol fuel: the Reid vapor pressure is increased, and aldehyde emissions are substantially higher from spark-ignited engine using ethanol fuel. Fortunately, the concentration of aldehydes is very low and it can be effectively reduced by the three-way catalytic converters in the exhaust system.

Another improvement brought by ethanol is the octane number, which is related to the anti-knocking performance of the gasoline. Lead is a good antiknock agent, but is very harmful from a human health perspective. Thus, it has been banned in most countries. Currently, there are two types of octane boosters used in Canada to replace the lead: benzene and Methyl-cyclopentadienyl-Manganese-Tricarbonyl (MMT). Both are toxic when accumulated in confined spaces (CRFA, 2001). If ethanol is used to enhance the octane rate, such impact could be alleviated.

2.2.1.2 Ethanol and greenhouse gases

Global climate change has been an international concern in recent years. Although a consensus has not been reached by scientists on the tendency of global warming, it is widely agreed that the emission of greenhouse gas (GHG) must be controlled to prevent the climate change. The Kyoto protocol signed by many countries including Canada in 1997 called for the committed nations to reduce their greenhouse gas emissions within specified time frames. Canada's commitment to the Kyoto Protocol was to reduce its GHG emissions to 6% below that of 1990, before the year 2010.

The transportation sector is the largest single contributor of GHG emissions in Canada. It accounted for 27% of the total GHG emissions in Canada in 1995 (Environmental Canada, 1999). Such a contributor is obviously the main target for Canada to achieve its goal in GHG emission control.

Currently, most of the ethanol is produced from grain corn and wheat in Canada. The growth of crops consumes carbon dioxide through photosynthesis, and reduces the net carbon dioxide emission during the life cycle. If ethanol is produced from biomass feedstocks, lower GHG emission can be achieved.

A fuel cycle model was developed by Wang at Argonne National Laboratories (Wang, 1999) to analysis the GHG emissions from alternative transportation fuels. From this model, the GHG emissions for a 2000 model year car in the near-future (2005) using E85 would be 218 grams per kilometer traveled, compared to 292 grams for gasoline. However, if ethanol used is made from cellulosic material, the reduction rate would be 102% (The more than 100% reduction results from the electric power generated in biomass to ethanol process, which displaces the power in the grid and reduces GHG emission).

The bio-ethanol industry also benefits the environment by disposing of problematic waste materials. The traditional way of disposing of large quantities of agricultural and forestry residues is open-field burning, which generates air pollution such as particulate matter (PM) and CO. The conversion of biomass

residues into ethanol reduces such pollution. In addition, if the cellulosic materials in the municipal solid waste are used for ethanol production, the amount of waste to landfill will be reduced, thus extending the operating life of the landfill.

2.2.2 Ethanol production in Canada and other countries

The ethanol industry in Canada is on the rise. The annual capacity of ethanol production is about 238 million litres (CRFA, 2001). Table 2-1 lists the producers of ethanol in Canada (<http://www.greenfuels.org/ethaprod.html>, 2004).

Table 2-1 Ethanol production facilities in Canada

Producer	Location	Capacity, million litres	Feedstock
Commercial Alcohols, Inc.	Chatham, Ontario	150	Corn-based
Commercial Alcohols, Inc.	Tiverton, Ontario	23	Corn-based
API Grain Processors	Red Deer, Alberta	26	Wheat-based
Mohawk Oil, Canada, Ltd.	Minnedosa, Manitoba	10	Wheat-based
Tembec	Temiscaming, Québec	17	Forestry product-based
Pound-Maker Agventures, Ltd.	Lanigan, Sask.	12	Wheat-based, partnered with a cattle feedlot

A new increase could be possible in biomass-based ethanol. Iogen Corporation in Ottawa, Ontario has built a demonstration plant to produce ethanol using agricultural residues. This new technology is expected to encourage the growth of the ethanol industry by using new types of feedstocks without impacting the grain export.

Approximately 7% of the total volume of gasoline sold in Canada contains a low-level ethanol blend of up to 10%. By 2010, the government would require 35% of gasoline on the market to contain 10% ethanol. However, to promote the use of ethanol fuel, public awareness of the benefits of ethanol needs to be intensified. In the U.S., over 6.8 billion litres of ethanol is produced each year. There are more than 50 ethanol plants in the country, and expansions to existing facilities could be easily obtained if the demand increases. It is estimated that the total production capacity could rise to 23 billion litres by 2010. The consumption of ethanol makes up about 1% of the motor vehicle market, mostly in the form of E10.

Brazil is the world leader in the utilization of ethanol as an automobile fuel. Early in 1975, the Brazilian government set up the National Alcohol Program (NAP) in order to substitute petroleum oil imports and maintain the growth of the economy (Rosillo-Calle and Cortez, 1997). Approximately 12 billion litres of ethanol are produced annually with sugarcane as the exclusive feedstock (www.climatechangesask.ca/downloads/Ethanol.may9.pdf, 2002). About 15% of spark-ignition vehicles use pure ethanol while others use ethanol blended gasoline which is called anhydrous ethanol, the ethanol content is usually about 22% (Rosillo-Calle and Cortez, 1997). However, there has been a declining tendency in the ethanol industry since the 1990s due to lower support from the government.

2.3 Ethanol from cellulose

2.3.1 Introduction

The production of ethanol from cellulose has been of interest since the early 20th century. The process is relatively simple: cellulose, which is the main component of biomass material, is first hydrolyzed to glucose, then the glucose is subsequently fermented and ethanol is refined by distillation. Because of the vast resource in biomass feedstock, such technology possesses considerable potential to reduce the dependency on crude oil. However, as a type of supplemental fuel, the ethanol market is subjected to the change in the supply of crude oil. The first large scale production of ethanol from biomass appeared in the late 1940s, an acid hydrolysis process. But due to the low yield in the hydrolysis stage, as well as corrosion problems caused by the acid catalysts, the process was not deemed competitive by the petroleum industry. During the oil crisis in the 1970s, alternative fuels including ethanol came into picture again and were considered economically viable. When the price of crude oil fell back to normal, the interest in biomass-derived ethanol diminished. In the past decade, the price of gasoline has risen dramatically. On the other hand, the developments of cellulose hydrolysis technology made it possible to lower the cost of the biomass-to-ethanol processes. Thus the economics of producing substitutive fuel has changed, and biomass-based fuels have gained increasing attention once again. More importantly, as a renewable fuel, biomass-derived ethanol holds great advantages for the environment.

It was estimated that if ethanol is made from biomass, the net carbon dioxide emissions may be reduced to zero (Wang, 1999). This is because the conversion of cellulosic material to ethanol does not require energy input from fossil fuels, which is a major source of carbon dioxide. Lignin extracted in the process can be burned to provide the energy required. The carbon dioxide released during the production and use of ethanol can be consumed by the biomass during its growth. Such a closed cycle results in no net contribution to the accumulation of carbon dioxide in the atmosphere.

2.3.2 Potential of biomass to ethanol in Canada

Canada has a vast forest resource and a large agricultural capability, but only a very small portion of these are used for production of bioenergy. Due to the problems associated with the conversion of cellulosic material to fermentable sugars, ethanol plants rely on grains (corn, wheat, and barley) as the feedstock, while biomass-based ethanol projects remain at the pilot stage. However, biomass feedstocks, including forestry residue, agricultural residue, and energy crops, are abundant and relatively inexpensive and, provided that an efficient biomass conversion process is developed, the ethanol costs could be reduced substantially.

To encourage the development of environmentally friendly fuels, incentive policies and funding programs have been established in Canada. At this point, the federal government grants a 10-cent per litre excise tax exemption on fuel ethanol produced for sale and use in Canada. In addition, the National Biomass

Ethanol Program (NBEP) has been established to eliminate the cash flow impact in case of a policy change in such tax treatment. Funded by Agriculture and Agri-Food Canada (AAFC), the NBEP aims to encourage investments in the Canadian ethanol industry and promote the application of renewable fuels that are environmentally friendly and economically viable.

2.3.3 Current status on biomass to ethanol

To date, biomass to ethanol technology has not proven to be economically viable. Besides the potential for the development of low cost enzyme, there are some strategic ways to reduce the production cost of ethanol. If an ethanol production system was co-located with a biomass power plant, certain synergies could be explored. For example, lignin from the ethanol plant could be used as fuel for the power plant, while steam and electricity from the power plant could be utilized by the ethanol facility. In addition, some utilities including wastewater handling and transportation systems could also be shared by the two plants.

In North America, there are six facilities currently in various stages of planning and construction (Table 2-2).

Table 2-2 Biomass-to-ethanol projects in North America (Zhang, 2003)

Site Location	Developer	Stage	Feedstock	Process
Ottawa, Canada	IOGEN	Demonstration	Wheat straw	Enzymatic hydrolysis
Gridley, CA	BCI		Rice waste Straw/Wood	Dilute acid
Jennings, LA	Collins Pine, BCI	Planning	Wood waste	Dilute acid
Mission Viejo, CA	Arkenol	Pilot	Cellulosic biomass	Concentrated acid hydrolysis
Middletown, NY	Masada	Environmental assess	MSW	Concentrated acid
Sacramento, CA	Arkenol	Planning	Agricultural residue	

In Ottawa, Iogen Corporation is operating a demonstration plant to produce ethanol from biomass materials including wheat, oat, and barley straw. The capacity of Iogen's demonstration plant is to process 40 tonnes of feedstock per day, thereby producing 3 to 4 million litres of ethanol each year. In the Iogen process, the biomass is pre-treated by steam explosion, then the cellulose is degraded into glucose by enzymatic hydrolysis, and a mixture of yeast and microbes is used to ferment the different sugars. Microbes that will allow for simultaneous saccharification and fermentation (SSF) are at the research stage, but are not currently in operation.

2.3.4 Cellulose Feedstocks

Theoretically, all biomass materials with cellulose content are possible feedstocks for ethanol production. Due to the low energy density of cellulosic materials, preparation of feedstock, including collection, transportation and handling of materials, is very critical for the entire process and accounts for a large portion of the total cost. Consequently, potential feedstock for ethanol production should have little alternate value and high cellulose content. Such materials being considered can be categorized as agricultural waste, forest residue, municipal solid waste, and energy crops.

2.3.4.1 Agricultural Residue

Agricultural wastes suitable for ethanol conversion include crop residues such as corn stalk, wheat straw, bagasse, and rice straw. Crop residues are enormous in

quantities and are considered to be the long-term feedstock of biomass derived ethanol. Agricultural residues can be used as feed and bedding materials for livestock, as well as raw materials for strawboard production. Such alternative uses may affect the availability as feedstock for ethanol production. But in most cases, agricultural residues have to be disposed of at a cost to the farmers. If they were used as feedstock for ethanol production, economical benefits could be created for the farmers. To ethanol producers, agricultural residues can be obtained at relatively low cost. Such residues are usually located in the crop processing belt, collection infrastructure and transportation facilities have already been established, thus the collection cost of feedstock could be quite low.

The quantities of agricultural residues such as wheat straw and other crop residues generated each year in Canada are more than sufficient for the production of ethanol. For instances, the annual production of straw is about 40 million tonnes in the Western provinces of Manitoba, Saskatchewan, and Alberta (<http://www.iogen.ca/2600.html>, 2004). If 1/3 of such biomass was used as feedstock for ethanol production, 10% of the gasoline demand in Canada would be met.

2.3.4.2 Forestry waste

The use of forestry material as feedstock for ethanol production is affected by its availability, transportation cost, and the accessibility. The supply of wood comes from two main sources: forestation and wood residue. The availability of wood from forestation is constrained by the land area and its productivity, as well as

the species planted. To minimize the feedstock cost, fast-growing species with high yields would be preferred.

Wood wastes that are available for ethanol production are the residues from wood handling processes, such as the sawmill and pulp mill residues. Currently, the wood industry uses much of its own residues. Secondary wood residue processing plants, such as medium-density fiberboard producers, consume a large amount of wood residue and affect the potential feedstock supply for ethanol production. But there is still a substantial amount of residue available, which is currently incinerated or disposed of at landfills. This fraction of wood waste could be offered to ethanol producers free of charge. In this case, the feedstock cost of ethanol production would lie primarily in transportation. If sufficient wood residues could be supplied within economical distance, an ethanol plant would be a positive alternative to the disposal of these residues rather than incineration.

In addition to the secondary wood processing industries, technology improvements in wood handling have also contributed to a reduction in residue generation. As a result, the availability of wood residues is expected to decrease in the long term.

2.3.4.3 Municipal Solid Waste (MSW)

Currently, almost all of the municipal solid waste (90-95%) in Canada is disposed of in landfills, while there are a few incineration and composting plants. Landfills use vast areas of land, and pose sanitary and health threats to the residents

living nearby. This often complicates site selection. Landfills also generate large amounts of gas due to the decomposition of the organic component. Landfill gas consists mainly of methane and carbon dioxide, which are both greenhouse gas. With the capacity of existing landfills being reached quickly, alternative treatment processes such as incineration and composting have been developed to reduce the amount of waste entering landfills and prolong their operational life. But they are either too expensive or not effective in waste volume reduction, thus their application is limited. MSW to ethanol offers a good potential in waste reduction, as well as ethanol production. Paper is the main cellulosic material in MSW that can be converted to ethanol. Since the pulping process in paper making removes most of the lignin and hemicellulose, paper has very high cellulose content, and does not need pre-treatment as is required for crop and wood materials. Although a fraction of the paper is diverted through recycling systems, there is still a fair amount of paper in the waste stream. A study conducted by Stanley Industrial Consultings, R. W. Beck and Associates, and DSM Environmental Services in 1992 showed that paper accounted for 29.7% of the solid waste collected in the Ottawa-Carleton region. To reduce the cost of transportation, ethanol plants could be co-located with material recycling facility (MRF) or transfer stations, where waste paper is separated from the MSW.

2.3.4.4 Energy crops

Energy crops are crops planted for the production of biofuels, electrical and thermal energy. These crops are expected to be fast-growing, drought and pest resistant, and readily harvested to allow competitive prices when used for energy

production. Typical energy crops include hybrid poplars, willows, switchgrass and reed canary grass. Currently, they are mainly used for power generation. However, as a biomass with high cellulose content, energy crops are considered to be well suited for ethanol production. Studies have been conducted on the potential use of poplars and willows for ethanol production (Negro, 2003). In Canada, switchgrass was studied as a feedstock (Samson and Omielan, 1992). As a perennial grass grown in the southern Canadian prairies and Eastern Canada, switchgrass is well adapted to marginal soils, high drought and pest resistance, for a low cost in production. Such characteristics make switchgrass a promising potential feedstock for the biomass-to-ethanol industry in Canada.

To reduce the feedstock cost, energy crops can be grown on marginal agricultural lands that are not adequate for food, feed, or fiber. They need less fertilizer and pesticide treatment. In addition to their fuel value, energy crops can also be planted for erosion control, soil remediation, and as nutrient filters that prevent nutrient run-off from land into waterways. In the U.S., it is estimated that 77 billion hectares of land could be used to grow energy crops dedicated to biofuel production, and 154 billion tons of lignocellulosic biomass could be produced each year (Wyman, 1994).

Although energy crops are easier to plant than traditional crops, the cost of growing, harvesting, and transportation of energy crops is generally higher compared to biomass from other waste stream. Subsidies are needed for the use of such feedstock in ethanol production in the short term.

2.4 Hydrolysis of cellulose

2.4.1 Glucose and ethanol

2.4.1.1 Glucose

Glucose is the basic unit of which starch, cellulose, and glycogen are made up. It is probably the most common organic group in nature, and it is one of the main energy sources for living organisms, both in plants and animals.

Glucose has the molecular formula of $C_6H_{12}O_6$ (Figure 2-1). It can be thought of as a derivative of hexane (a 6-carbon chain) with -OH groups attached to every carbon except the endmost one, which exists as an aldehyde carbonyl. However, because the chain is flexible it can wrap around until the two ends react together to form a ring structure. Thus a solution of glucose can be thought of as a rapidly changing mixture of rings and chains, continually interconverting between the two forms.

Figure 2-1 The structural formula of glucose (May, 1997)

Glucose is a monosaccharide, since it is made up of only one unit. Individual sugar units can be joined together to form a chain, in much the same way as

monomer units are linked up to form a long polymer. If two units are joined together they become a disaccharide; likewise, three glucose units can be linked to make trisaccharide, and a very large number of units can be linked to form a polysaccharide. Cellulose is a glucose polymer that exists in the cell walls of plants; it consists of up to 1 million D-glucose units.

Glucose is a ready source of energy, since its carbon atoms are easily oxidized to form carbon dioxide, and energy is released in the process. Unlike other hydrocarbon fuels, which are insoluble in water, the numerous hydroxyl groups in glucose allow it to readily form hydrogen bonds with water molecules, making it highly soluble in water. This allows the glucose to be transported easily within biological systems, including the bloodstream of animals or the sap of plants (May, 1997).

Glucose has two structural isomers, which are also monosaccharides, with the same molecular formula as glucose: galactose, a sugar in milk and yogurt, and fructose, a sugar found in honey and fruits. The differences among these sugars are the arrangement of atoms. Glucose and galactose have six-membered ring while fructose forms a five-membered ring. Upon consumption, both fructose and galactose are converted into glucose so that they can be absorbed and used to provide energy.

2.4.1.2 Ethanol

Ethanol is a member of the group of organic molecules called alcohols. Just as alkanes are identified as saturated hydrocarbons, and alkenes are identified by

their double bond, so are alcohols identified by having an O–H group in the molecule. Ethanol has two carbons, and the molecular formula is C₂H₅OH.

Ethanol can be produced from the fermentation of glucose using appropriately selected micro-organisms in a process called fermentation (Figure 2-2). One molecule of glucose produces two molecules of ethanol and two molecules of carbon dioxide.

Figure 2-2 Glucose fermentation equation (Shleser, 1994)

2.4.2 Structure of cellulosic material

Different biomass materials such as woods and grasses are varied in appearances and differ in properties. But they all have a relatively similar composition, which consists mainly of cellulose, hemicellulose and lignin. Cellulose is generally the major component, it accounts for 40-50% of the material by weight, hemicellulose constitutes about 20-40% of the material, and the remaining components are mainly lignin, together with a little amount of ash and miscellaneous substances. The two carbohydrate polymers, cellulose and hemicellulose, form the main structure of biomass materials, whereas lignin acts as a joining material and binds the fibers together. Although extraneous compounds exist in small quantities, they play a very important role in structural

stabilization, they make cellulose resistant to decay and insect attack, but on the other hand, they also prevent cellulosic materials from decomposition.

Generally speaking, wood has higher cellulose content but less hemicellulose than grass; hardwood has more cellulose than softwood, and the lignin content in hardwood is lower than that of softwood, thereby resulting in different properties between species.

2.4.3 Cellulose

Cellulose is the major component of cell walls of cellulosic fiber, and it is a linear polymer of D-glucose with a high molecular weight. Individual glucose molecules are linked together by β -1,4-glucosidic bonds to create a highly crystalline structure that is resistant to decomposition. The degree of polymerization (DP) which refers to the number of chain units, differs according to the type of material, Table 2-3 shows the DP and molecular weight of some cellulose materials. The hydrogen bonds within molecular chains hold the chains together tightly, and prevent the structure from being broken by water, thus cellulose is completely insoluble in water.

Table 2-3 Molecular weight and degree of polymerization of cellulosic materials (Fan *et al.*, 1987)

Cellulosic materials	Molecular weight	Degree of polymerization
Native cellulose	600,000-1,500,000	3,500-10,000
Cottons	80,000-500,000	500-3,000
Wood pulps	80,000-340,000	500-2,100
Rayon filament	57,000-73,000	350-500

In native cellulose, up to 10,000 β -anhydroglucose residues are linked to the long chain molecule (Fan *et al.*, 1987). Each anhydroglucose unit has a configuration where the hydroxyl groups are in the equatorial position and the hydrogen atoms are in the axial position. Figure 2-3 shows the conformational formula of cellulose, it can be seen that alternate chain units are rotated 180° around the main axis. Thus, an unstrained linear configuration is formed, and the steric hindrance between the chain structures is minimized.

Figure 2-3 The conformational form of cellulose (Hangarter, 2002)

The glucosidic linkage and the hydroxyl groups, mainly, determine the chemical properties of cellulose. Usually all chemical reactions take place at these locations, e.g. hydrolysis of cellulose occurs when the glucosidic linkage is broken down.

Cellulose polymers associate through hydrogen bonds. A large number of cellulose molecules that are linked by hydrogen bonds and van der Waals forces form micro fibers, which are further accumulated to form fibers. Cellulose fibers can grow very long in some cells such as cotton ovules. The high tensile strength

of cellulose is also a function of the hydrogen bonds (Hangarter, 2002).

Cellulose fibers usually consist of over 500,000 cellulose molecules. If each cellulose molecule consists of 5,000 glucose residues, the number of hydrogen bonds in the fiber would be approximately 2.5 billion. Although the hydrogen bond has much less strength than the covalent bond, the cumulative bonding energy of 2.5 billion of them is enormous. Therefore, native cellulose materials exist in quite a stable state and offer a high resistance to decomposition. But the structure of acid and alkali swollen cellulose is open and can be split relatively easily by enzyme molecules.

2.4.4 Hemicellulose

Hemicellulose is the non-cellulosic fraction of polysaccharides in plant cell walls; it is abundant in primary walls but is also found in secondary walls. Like cellulose, its main chains are also linked by β -1 $\rightarrow$ 4 glucosidic linkages, but the chains are shorter than cellulose, and they exist in amorphous form. This makes hemicellulose soluble in water. But in plant cell walls, hemicelluloses are linked to cellulose and lignin by hydrogen bonds and van der Waals forces (Robyt, 1998). There are three main groups of hemicelluloses: xylans, mannans, and galactans. These groups can exist separately as single components or collectively, and they are heterogeneous polysaccharides which vary for different species of plants. Hardwood hemicelluloses are rich in xylan polymers with small amounts of mannan, whereas softwood hemicelluloses are rich in mannan polymers and contain significant quantities of xylans.

Xylan is a polymer of xylopyranose which is β -1 $\rightarrow$ 4 linked. The main chain units are 4-O-methylglucuronoxylan and 4-O-methyl-gluc-uronoarabinoxylan. The β -1 $\rightarrow$ 4 linkages are similar to the glucose units in cellulose. The difference is that the hydroxyl groups in cellulose chain units are substituted with 4-O-methylglucuronic acid, arabinose and acetyl groups (Fan *et al.*, 1987).

The mannan groups are composed of D-mannose. In some mannans such as palm seeds, the D-mannose units are also linked by β -1 $\rightarrow$ 4 linkages and form microfibrils similar to cellulose. Not all of these polymers have the same structure. In deciduous wood, the glucomannans have a linear structure and there is no branching at either of the two carbon positions C-2 and C-3. While in coniferous wood, there are many short side-chains of galactose units in the glucomannans. In black spruce, it is proposed that the configuration of the glucomannan has a linear polysaccharide structure, it is cross linked with lignin by a large number of lignin-carbohydrate bonds that exist in the fiber.

The D-galactan hemicellulose, an arabinogalactan, mainly exists in softwood, such as larch, black spruce and pine (Robyt, 1998). The main chain of galactan is 1, 6 linked galactose with the position 3 substituted by a secondary chain of 1 - 3 linked D-galactose residues.

Figure 2-4 Some monomers of hemicellulose (Bungay, 1996)

Figure 2-4 shows some monomers of hemicellulose. In contrast to cellulose that is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. It can be easily hydrolyzed by dilute acid or base, but there are a number of hemicellulase enzymes for its hydrolysis in nature. These enzymes are commercially important because they open the structure of wood for easier bleaching, while older bleaching processes consume larger quantities of chemicals such as chlorine that adversely impact the environment.

2.4.5 Lignin

Lignin is unusual compared to other abundant natural polymers, due to the low degree of order and high degree of heterogeneity in its structure. It is generally defined as a complex hydrophobic network of phenyl-propane units held together

by ether and carbon-carbon bonds. Lignin has a high molecular weight and is amorphous in nature. The amount of lignin may account for 20-35% of the cellulose structure (Ralph *et al.*, 1999).

Lignin is vital for structural integrity of land-based plants, for defense against pathogens, and to facilitate various functions such as water transport. In cellulosic materials, lignin is interwoven with cellulose and hemicellulose, such a structure is highly resistant to decomposition. Therefore the lignin content in biomass must be extracted first in order to obtain cellulose. Such pre-treatment is essential to the cellulose hydrolysis processes. The separated lignin could be combusted to provide energy for the process requirements.

The lignin from grasses, softwoods, and hardwoods differs in composition, mainly in methoxyl substitution and the degree of linkage between phenyl groups. However, most of them have common structural features.

2.4.6 Hydrolysis of cellulose

Cellulose is a high molecular weight linear polymer. Each cellulose molecule is an unbranched chain consisting of 1000 to 1 million D-glucose units, linked together with β -1,4 glycosidic bonds. In order to utilize cellulose as a feedstock for the production of energy, food and chemicals, it is essential to break it down to glucose. Cellulose is resistant to degradation since a cellulose molecule serves largely as a structural molecule rather than a molecule to store energy. Celluloses from various biomass sources are generally the same at the molecular level, though they differ in their crystalline structures and in their

binding to other components. The cellulose present in lignocelluloses is composed of crystalline and amorphous components. The amorphous component is usually more active than the crystalline component, thus, increasing the amorphous content will enhance the hydrolysis rate. The presence of lignin forms a physical barrier for attack by either enzyme or acid molecules. As a result, treatments leading to the disruption of the lignin seal will increase the accessibility of cellulose molecules to enzymes or acid, and thus, increase the degradation rate.

2.4.6.1 Enzymatic hydrolysis method

Insoluble cellulose molecules consist of a long chain of β -1 $\rightarrow$ 4 glucosidic linkages, which forms a crystalline structure held by hydrogen bonds and Van der Waals forces. Such a configuration can not be broken by water, but exogluconase, which is a subgroup of cellulase enzymes that attack the terminal glucosidic bond, can degrade it effectively. On the other hand, amorphous cellulose such as carboxymethyl cellulose (CMC) is subject to the attack of endogluconases, another subgroup of cellulase enzymes that catalyze the degradation of internal bonds. Once broken down, a water molecule must be present to render each unit inactive. The natural consequence of the difference in the crystalline structure makes the hydrolysis rate much faster for amorphous cellulose than crystalline cellulose. In addition to the structural characteristics, the lignin compounds that surround the cellulose in cell walls also pose an obstacle to enzyme access, thus greatly affecting the hydrolysis rate. To improve the susceptibility of cellulose to enzymes, specific pre-treatment processes which are

efficient in removing lignin and hemicellulose as well as increasing the accessibility of the crystalline structure, are essential prior to hydrolysis.

2.4.6.1.1 Pre-treatment processes

There are three main types of pre-treatment processes: physical, chemical and biological pre-treatments. Depending on the biomass material, one or a combination of these methods may be used to improve the accessibility of enzyme to cellulose molecule.

Physical methods include high temperature and pressure, milling, radiation, or freezing, all of which are energy intensive. The effect of physical pre-treatment is to subdivide lignocellulosic material into fine particles which are highly susceptible to acid or enzymatic hydrolysis, and the crystalline degree of cellulose may also be reduced by the mechanical breaking up.

Chemical methods use a solvent to break apart and dissolve the crystalline structure. Alkalis, acids, and other cellulose solvents can be employed in pre-treatment. Alkaline treatment causes structure swelling which increases the internal surface area, and decreases the degree of polymerization; it can also separate the structural linkages between lignins and carbohydrates, and disrupt the lignin structure (Fan *et al.*, 1987). Acids mainly act as catalysts for the hydrolysis of cellulose rather than as a reagent for pre-treatment. Cellulose solvents such as cadoxen, can swell and transform solid cellulose into a soluble state, thus enhancing hydrolysis. Such solvents are usually expensive and toxic to the environment.

Biological pre-treatment uses micro -organisms to solubilize lignin and soften cellulose material, thereby providing easier access for the enzymes. Such a process needs less energy, but proceeds very slowly, and the growth of micro-organisms also consumes cellulose (Varga *et al.*, 2003), therefore it is rarely adopted.

The effect of pre-treatment in cellulose hydrolysis has been widely studied. Hydrothermal processes are favoured in some research. Wet oxidation, a process using oxygen and water at elevated temperature and pressure, are found to be efficient for some cellulosic materials, and it also generates few unfavorable byproducts such as furfural. Varga *et al.* (2003) investigated several conditions of wet oxidation pre-treatment on corn stover, and found that the best condition was 195 °C, 12 bar oxygen pressure, under which 85% of cellulose was converted into glucose. Another well studied hydrothermal process is steam explosion. It causes lignin transformation and hemicellulose degradation at high temperatures. Negro *et al.* (2003) used steam explosion in the hydrolysis of poplar, and obtained a conversion of 60% at 210 °C after 4 minutes. Although high conversion rates can be achieved through wet oxidation and steam explosion, they are usually only effective for certain types of biomass material, and the severe conditions may cause degradation of some cellulose fractions and the subsequent formation of sugars, which ultimately result in less yield.

Dilute acid pre-treatment is shown to be reliable in increasing the cellulose pore volume and enzyme accessibility (Esteghlalian *et al.*, 2001). Schell *et al.* (2003)

studied the hydrolysis of corn stover with dilute sulfuric acid pre-treatment. The substrate was pre-treated with 0.5-1.4% (w/w) sulfuric acid at 165-195°C for 3-12 minutes. 80-87% cellulose conversion was obtained during simultaneous saccharification and fermentation process. The disadvantages of acid pre-treatment are the costs associate with facility construction, as well as the handling and disposal of neutralization chemicals (Ward and Singh, 2002).

A number of other pre-treatment processes, such as ammonia and alkaline peroxide treatment, were shown to be very effective under certain conditions (Saha *et al.*, 1998). For a certain biomass material, the preferred pre-treatment should meet the following criteria: achieve high yield of sugars in the following enzymatic hydrolysis, minimize the degradation of carbohydrates and the formation of inhibitory byproducts, and be cost-effective (Negro *et al.*, 2003).

2.4.6.1.2 Enzymatic hydrolysis of cellulose

There are a number of cellulose enzymes that are capable of degrading cellulose, of which the most intensively studied is the enzyme complex derived from filamentous fungus *Trichoderma Reesei*. This enzyme complex contains three components: endo- β -glucanase (EG), exo- β -glucanase (CBH), and β -glucosidase, which are all needed to hydrolyze cellulose efficiently and completely. As shown in Figure 2-5, the enzymatic hydrolysis of cellulose consists of two major steps: cellulose is first hydrolyzed into cellobiose by the synergistic effects of endo- β -glucanases and exo-glucanases, then the cellobiose is converted into glucose by β -glucosidase.

Figure 2-5 Steps of cellulose hydrolysis

Although intensive research has been conducted in recent years, the mechanisms that allow the enzymes to act in an efficient manner to hydrolyze cellulose are still not sufficiently understood. Esteghlalian *et al.* (2001) proposed a simple model, which suggested that endoglucanases act at easily accessible sites, and create new sites for the attack of the crystalline structure by exo-acting cellobiohydrolases. There is an independent cellulase-binding domain (CBD) connected to a catalytic domain in enzyme components which mediate the adsorption to the substrate.

Figure 2-6 represents the suggested process of hydrolysis. First the cellulase bonds to cellulose and forms a multiple, non-covalent and thermodynamically favorable complex that stabilizes the protein on the cellulose, and mediates its penetration into the intermicrofibrillar space. Then, an increase in mechanical pressure caused by the presence of large enzyme molecules in relatively narrow spaces is exerted on the cavity walls. Thus the cellulose structure swells and more water molecules can be accommodated between the microfibrils. Finally, the hydrogen bonds are broken by the water molecules and the microfibrils are disassociated (Esteghlalian *et al.* 2001).

Figure 2-6. Schematic representation of enzyme dispersion (Esteghlalian *et al.*, 2001)

The kinetics of cellulose hydrolysis have been widely studied, and Michaelis-Menten types of rate expressions with substrate or product inhibition terms have been proposed to describe the observed reaction kinetics (Cheetham, 1995). Based on Michaelis-Menten-type kinetics and the combination of enzyme adsorption, some kinetic models have been proposed (Fan *et al.*, 1987). They generally assume that the enzyme is adsorbed to the surface of the substrate and the rate of degradation is proportional to the concentration of the adsorbed enzyme.

The rate and extent of cellulose hydrolysis by cellulase enzymes is influenced by many substrate and enzyme factors. Because of the heterogeneous nature of the process, the overall reaction rate could be affected by mass transfer resistance, this includes the surface film resistance around cellulose particles, the bulk phase resistance, and the resistance through the capillary pores of the cellulose particles. Consequently, the reaction conditions such as the size of cellulose particles, the substrate concentration, enzyme concentration, pH of the buffer, and agitation intensity will all influence the hydrolysis rate. Hang and Woodams (1998) have shown that at pH 5.0, the sugar yields from corn husk were higher than that of pH 4.0 and pH 7.0. They also found that at 40°C, 50°C and 60°C, the total sugar yields were slightly different. However, significantly more glucose and xylose were converted at higher temperature. In addition, very high substrate concentrations will slow down the hydrolysis due to the inhibition from the end product. Finally, the high viscosity of slurry may also limit the mass transfer within the reaction mixture (Ingesson *et al.*, 2001). Moreover, excessive mixing speeds (>200 rpm) will decrease the extent of hydrolysis because the enzyme activity is lowered. The lignin content appears as a barrier to the enzymes, but it is not always undesirable. Kaya *et al.* (1998) found that the addition of dissolved lignin improved the enzymatic reaction, because it was thought to bind to the enzymes in solution, then the bound enzymes were sustained and hydrolyzed the adjacent cellulose easily.

It should also be noted that the optimal condition for cellulose originated from one feedstock may not be ideal for another feedstock, and the effectiveness of

enzymes with different sources of cellulose will also vary according to the type of biomass.

Currently, the cost of enzymes is still very high due to a general lack of commercially viable cellulase production technologies, and research is continuing to bring down the cost of enzymes. However, if less expensive enzymes can be developed, enzymatic processes hold several advantages. First, the efficiency of enzymatic hydrolysis is quite high and they do not generally produce undesirable by-products. Secondly, their mild process conditions do not require expensive materials of construction and corrosion proof equipment. Furthermore, their process energy requirements are relatively low, and thereby pose less impact to the environment.

Compared to acid hydrolysis, the enzymatic reaction processes are much slower. In addition, the reaction rate decreases rapidly with the progress of the reaction due to the accumulation of end-products. The introduction of simultaneous saccharification and fermentation (SSF) provides a solution which could greatly improve the reaction rate. In SSF processes, the biomass material is first pre-treated to degrade the hemicellulose content and loosens the entangled cellulose and lignin. A fraction of the pre-treated substrate is then used for the growth of fungi to produce the cellulase enzymes. Then the cellulase enzymes and the substrate are placed into a vessel together with yeast which ferments sugar into ethanol. Thus, in the reaction vessel, the cellulose is first hydrolyzed to sugar by the enzyme, then the sugar is fermented to ethanol by the yeast, finally the

ethanol is distilled and separated from the system.

The simultaneous saccharification and fermentation system presents large advantages over separate saccharification and fermentation systems. Because the glucose is converted into ethanol as they are formed, the end-product inhibition caused by the accumulation of glucose in the hydrolysis process is greatly reduced. Consequently, the hydrolysis rate is increased and cellulase enzyme consumption is reduced significantly. In addition, the reaction time is greatly reduced, and only one reactor is required for the entire conversion process. The SSF system represents a viable approach to reduce the operation cost, thus it is being tested in many places. The disadvantage of SSF is that the cellulase enzymes and fermentation organism must be operated under the same conditions, thus, the performance of enzyme and yeast may be affected.

2.4.6.2 Acid hydrolysis method

Acid hydrolysis is a chemical process that uses acid to modify the cellulose molecular structure and decreases the degree of polymerization (DP), resulting in cellulose degradation.

The major soluble components of acid hydrolysis include sugars (such as xylose, glucose and cellobiose), furfurals (such as furfuraldehyde, hydroxymethyl furfural), and organic acids (such as levulinic acid, formic acid, and acetic acid). When natural sources of cellulose are hydrolyzed by acid, many by-products may result, mainly because of the hemicellulose contents. This makes it difficult to produce a relatively pure sugar product and limits the viability of acid hydrolysis.

There are two acid hydrolysis processes: diluted acid and concentrated acid hydrolysis. They may be operated separately, or may be combined in sequence to increase the purity of the product and reduce contamination.

As shown in Figure 2-7, the diluted acid process involves contact with dilute acid, which proceeds through the formation of hydrocellulose to soluble polysaccharides and then to simple sugars.

Figure 2-7 Diluted acid hydrolysis process (Fan *et al.*, 1987)

Dilute acid processes are conducted under relatively high temperature and pressure, but can be completed within minutes. As an example, pure cellulose is hydrolyzed by 1% sulfuric acid in about 0.22 minutes at a temperature of 237°C, with pure cellulose yielding over 50% sugars (Badger, 2002). The combination of acid and high temperature and pressure requires special reactor materials, which can make the reactors expensive.

Most dilute acid processes are limited to a sugar recovery efficiency of around 50%. The reason for this is that at least two reactions are involved in the process. With the exception of the reactions that convert the cellulosic materials to sugars, there is another process that converts sugars to other chemicals such as furfural, which is an inhibitor to fermentation micro-organisms.

Since 5-carbon sugars degrade more rapidly than 6-carbon sugars, it is possible to reduce sugar degradation by a two-stage process. The 5-carbon sugars can be recovered in the first stage under mild condition, while the 6-carbon sugars are produced under harsher conditions. However, sugar degradation is still unavoidable and yields are ultimately limited.

The biggest advantage of dilute acid processes is their rapid rate of reaction, which facilitates continuous processing. Their main disadvantage is their low sugar yield. For rapid continuous processes, in order to allow adequate acid penetration, feedstocks must be physically reduced in size to ensure the maximum particle dimension is in the range of a few millimeters.

The other process, concentrated acid process, uses relatively mild temperatures and lower pressures. As shown in Figure 2-8, this process proceeds through the formation of cellulose acid complexes, which occur only after the crystalline structure of cellulose is destroyed by its dissolution or swelling in acid, then the acid complexes are converted to oligosaccharides and further degraded to glucose.

Figure 2-8 Concentrated acid hydrolysis process

The primary advantage of the concentrated process is the high sugar recovery efficiency, which can be over 90% of both hemicellulose and cellulose sugars.

The low temperatures and pressures employed allow for the use of relatively low-cost materials such as fiberglass tanks and piping. Unfortunately, it is a relatively slow process and the cost of acid recovery systems is quite high. Without acid recovery, large quantities of lime must be used to neutralize the acid in the sugar solution. This neutralization forms large quantities of calcium sulfate, which need to be disposed of and creates an additional expense.

Dilute acid hydrolysis of biomass is the oldest technology for converting biomass to ethanol. Early in 1898, the first process for ethanol from wood appeared in Germany. During the World War II, the U.S. built an ethanol plant which used dilute acid to hydrolyze the raw wood materials. However the dilute acid process was considered to be unpromising in the 1980's.

The concentrated sulfuric acid process had been industrialized in the past, especially during the energy crisis. But because of its high cost, it could not compete with conventional ethanol production processes. Recently, improvements in acid sugar separation and recovery have made it possible to bring down the cost. Now studies aimed at the commercialization of concentrated acid hydrolysis are carried out in DOE and NREL (Neufeld, 2000).

2.4.7 Fermentation of glucose to ethanol

Ethanol has been produced since ancient times through the fermentation of sugars. Simple sugars are the raw materials. Zymase, an enzyme from yeast, converts the simple sugars to ethanol and carbon dioxide. The fermentation reaction, represented by this simple equation,

is actually very complex, and impure cultures of yeast produce varying amounts of other substances, including glycerins and various organic acids.

Essentially, fermentation is a means for micro-organisms to extract energy from their chemical environment. First the pyruvic acid is converted to acetaldehyde, and then to ethanol. The hydrogen required for the conversion of acetaldehyde to ethanol is provided by nicotinamide adenine dinucleotide (NADH). The resulting hydrogen-depleted NAD^+ is necessary to drive D-glyceraldehyde-3-phosphate to pyruvic acid, ensuring the cycle is complete and continuous.

Ethanol produced by fermentation ranges in concentration from a few percent up to about 14%. Above about 14%, ethanol destroys the zymase enzyme and fermentation stops. Ethanol is normally concentrated by distillation, but the composition of the vapor from aqueous ethanol is 96% ethanol and 4% water. Therefore, pure ethanol cannot be obtained by distillation. Commercial ethanol contains 95% ethanol and 5% of water by volume. Dehydrating agents can be used to remove the remaining water and produce pure ethanol.

Chapter 3 Methodology

3.1 Introduction

The hydrolysis of cellulose to glucose is the crucial process, which accounts for the main cost in the production of ethanol. Once the cellulose is converted into glucose, it can be easily fermented to produce ethanol. This research focuses on the hydrolysis of cellulose-containing materials from the waste stream, for the purpose of investigating low-cost feedstocks for ethanol production, and more importantly, providing environmentally-friendly alternatives to the disposal of solid waste.

The materials investigated in this study include crop residues, poultry manure, and sewage sludge. Compared to acid hydrolysis, enzymatic conversions generally proceed under milder conditions and can yield much purer products. It has been predicted that the cost of enzymes will decrease continuously over the next decade (Lynd *et al.*, 1996).

3.1.1 Feedstock characteristics

3.1.1.1 Crop residues

There are a number of biomass materials available for potential ethanol production. Among them, agricultural residues are of great interest because of their high cellulose content. Two crop residues, corn stalk and bagasse were used as substrates for enzymatic hydrolysis in this study. The typical composition

of corn stalk and bagasse is shown in Table 3-1 (Soest, 1994; Ladisch, 1983), the moisture content of the dry corn stalk and bagasse is approximately 3.0%.

Table 3-1 The main composition of corn stalk and bagasse (% weight on dry matter basis)

Ingredient	Cellulose (%)	Hemicellulose (%)	Lignin %	Ash %	Other %
Cornstalk	38	32	17	3	10
Bagasse	33	30	19	3	15

The two types of crop residues have similar composition, which mainly consists of cellulose, hemicellulose, and lignin. Depending on the growing area and plant species, there may be minor differences in the composition of crop residues, but cellulose is the most important component in all of them.

3.1.1.2 Poultry manure

Currently, poultry manure is mostly applied on farm land. However, the production of poultry manure exceeds the nutritional needs of crops. Over application of poultry manure to farm land can result in environmental problems such as surface and ground-water pollution, contamination with pathogens, odors and insects. The nutrient content in soils can also become unbalanced if excess manure is utilized. Manure components such as organic materials, nitrates and phosphates may be harmful if not managed properly, but they are also a valuable source that can be extracted and utilized.

Poultry manure generally consists of decomposed residues from the original feed, living and dead organisms, and metabolic products such as urea and uric acid. The composition of poultry manure varies between farms because of many factors, such as the type of bird, the feed, bedding, collection and storage systems. Table 3-2 shows the typical ingredients in laying hen's manure (Müller, 1980):

Table 3-2 Composition of laying hen's manure

Ingredients	Cellulose	Hemicellulose	Lignin	Ash	Other
% of dry matter	15	17	3	28	37

3.1.1.3 Sewage sludge

The disposal of sewage sludge has become an issue of great concern in many municipalities. Because there is cellulosic content in sludge, it could be used as a feedstock for hydrolysis to produce glucose and ethanol. Three types of sludge were used in this study: primary sludge, thickened activated sludge, and digested sludge. Primary sludge is produced through the mechanical wastewater treatment process including screening, gritting, and primary clarification. The sludge amassing at the bottom of the primary sedimentation basin is watery, with 3-4% solid. It consists of a wide range of organic matters, metals and other residues. The origin of sludge includes faecal materials, scraps of toilet paper, and food residues, so cellulose content is presented in sludge solids. Activated sludge results from the biological treatment step which removes the dissolved organic matter and nutrients from the wastewater. This is accomplished by

different types of bacteria and other micro-organisms, some species requiring oxygen to live, grow and multiply in order to consume the organic matter. The thickened activated sludge has a solids content of about 10%. With the dissolved organic matter and nutrients removed by bacteria, the activated sludge does not have as much odor as primary sludge. Digested sludge is the product of anaerobic digestion. It has a black color and smells earthy. The moisture content of digested sludge is approximately 70%, and the organic fraction of the solid varies from 45 to 60%. The volatile solids of all three types of sludge were determined prior to treatment. Table 3-3 shows typical composition of the three types of sludge (Governo *et al.*, 1999), composition may vary by region and treatment process.

Table 3-3 Composition of different types of sludge (% Total solids)

SludgeType	Grease & fats, %	Protein, %	Cellulose, %	Volatile solids, %	Ash, %
Primary sludge	6~30	20~30	8~15	60~80	20~40
Activated sludge	-	-	-	65~79	21~35
Digested sludge	5~20	15~20	8~15	30~60	40~70

3.2 Experimental Design

3.2.1 Hydrolysis of crop residues

The general process employed in this study for the enzymatic hydrolysis of crop residues is shown in Figure 3-1. This includes the overall process from pre-treatment to glucose measurement.

Figure 3-1 Scheme of crop residues hydrolysis process

3.2.1.1 Pre-treatment

The research on pre-treatment was conducted in previous studies (Levy, 2003; Henderson, 2003), the best conditions identified in both studies were to treat the feedstock with 0.5N KOH at 70°C for 1 hour, then neutralize the solution and separate the solid fraction which consists mainly of cellulose. In order to increase

the accessibility for the enzyme, the substrate was ground prior to hydrolysis. There were two types of substrate preparations used for enzymatic hydrolysis: dry and wet, they were prepared in different pre-treatment sequences. For the dry substrate, the raw crop residue was first treated with KOH, then neutralized and filtered to separate the solid and liquid fraction. The solid fraction was dried in the oven at 70°C and then ground to desired particle size for enzymatic hydrolysis. To prepare the wet substrate, the first process was to grind the raw material, followed by KOH treatment, then neutralization and filtration of the solution. The wet solid fraction was used as the substrate for enzymatic hydrolysis. Because the substrate was not dried before the hydrolysis, less energy was consumed in the wet substrate preparation.

3.2.1.2 Enzymatic hydrolysis

The enzymatic hydrolysis was carried out in a pH 4.8 sodium citrate buffer. The glucose yield from cellulose is affected by many factors including pre-treatment method, reaction time, temperature, and enzyme load. Figure 3-2 shows the trials to determine the optimum conditions to achieve the highest conversion rate per enzyme unit for cellulose hydrolysis. Dry substrates were used in these trials because of its convenience in handling. After the optimum condition was obtained, it was used on wet substrate to determine the effect of the pre-treatment sequence on the reaction rate of enzymatic hydrolysis.

Note: t- Reaction time E- Enzyme loading T- Temperature

Figure 3-2 Trials sequence to optimize the conditions for enzymatic hydrolysis

3.2.2 Hydrolysis of poultry manure

Since there is a recoverable lignocellulosic content in poultry manure, it can be hydrolyzed to produce glucose. Figure 3-3 represents the process of manure hydrolysis. Poultry manure was first washed with distilled water, and sand was removed through sedimentation, then the solid fraction was separated. Because of the complex composition of poultry manure, the effect of pre-treatment processes on the enzymatic conversion rate may be different than that of crop residues. To determine the most effective pre-treatment process for poultry manure, enzymatic hydrolysis was conducted on poultry manure at four different treated stages: un-treated poultry manure, KOH treated wet poultry manure,

KOH treated and dried poultry manure, and KOH treated then dried and ground poultry manure.

Figure 3-3 Schematic representation of poultry manure hydrolysis

The KOH treatment was the same as that used for the crop residues. All enzymatic hydrolyses were conducted under the same experimental conditions. Because poultry manure had a much lower cellulose content than alkaline treated crop residues, the substrate loading in the hydrolysis of poultry manure

was higher than that of crop residues. The optimal reaction time and temperature determined from trials on crop residues were adopted for poultry manure. Finally the glucose yields of the four trials were measured, and the one with the highest conversion rate indicated the best treatment.

3.2.3 Hydrolysis of municipal sewage sludge

Figure 3-4 shows the process of sludge conversion conducted in this research. The substrates used for enzymatic hydrolysis were from different pre-treatment stages. It was suspected that the metal content in sewage sludge may have an impact on the activity of the enzyme; therefore HCl treatment was used to reduce the metal content in the substrate. The enzymatic hydrolysis was conducted on sludge from four pre-treatment stages: un-treated sludge, HCl-treated sludge, KOH-treated sludge, and HCl- followed by KOH-treated sludge. Both wet and dried substrates were tried in each stage, the sludge sample formed large agglomerates after drying, so all the dry substrate was ground before enzymatic hydrolysis. The glucose yield of each trial was measured, and the one with the highest conversion rate indicated the best preparation.

EH- Enzymatic hydrolysis

Figure 3-4 Schematic representation of sewage sludge hydrolysis

3.3 Experimental implementation

3.3.1 Feedstock collection and storage

Corn stalk was obtained from the Agriculture Canada Experimental Farm (Ottawa, Ontario). The samples were dried and stored in paper bags. Dry bagasse (the

sugarcane residue after squeeze) was obtained from South America, and stored in a closed plastic container.

Poultry manure was collected from Conrad Longtin poultry farm in St. Isidore (Ontario, Canada), no bedding material is used in the bird cage. The solid manure sample was transported in a sealed plastic bucket and then stored frozen in Ziploc plastic bags. Prior to treatment, manure samples were thawed in a refrigerator at 4°C for 24 hours and then mixed adequately. The collected poultry manure was in solid state with moisture content of about 73.0%. The raw manure is generally composed of fine particle, thus grinding is not deemed necessary during the pre-treatment. It was noted that there was some sand in the manure, which is most probably from the feed and collection system.

Sewage sludge was collected from Robert O. Pickard Environmental Centre (ROPEC, the wastewater treatment plant for the Regional Municipality of Ottawa-Carleton). Three types of sludge were collected: primary sludge, thickened activated sludge, and digested sludge. The fresh primary sludge and activated sludge samples were transported in sealed plastic bucket and used directly for treatment; biosolids were stored refrigerated at 4°C in sealed plastic bag.

3.3.2 Feedstock characterization

The moisture content (MC) of the wet substrate was determined before each enzymatic hydrolysis. The pre-treated wet sample was weighted and dried in oven at 70°C for 48 hours, then it was cooled to room temperature in a

dessicator. The samples were then weighed and the moisture content was determined using equation 3 – 1.

$$(3 - 1) \quad \%MC = (\text{Wet Mass} - \text{Dry Mass}) / (\text{Wet Mass}) \times 100 \%$$

%MC = moisture content

Wet Mass = mass of wet substrate prior to drying

Dry Mass = mass of dried solids

Compared to crop residues, poultry manure and sewage sludge have much higher inorganic contents such as phosphate and potassic salts. Therefore the volatile solids (VS) were determined for poultry manure. The measured mass of dried manure samples was incinerated at 550° C for 16 hours, then it was cooled to room temperature in a dessicator. The cooled ash residue was weighed and the volatile solids (VS) were determined using equation 3 – 2.

$$(3 - 2) \quad \%VS = (\text{Dry Mass} - \text{Ash}) / (\text{Dry Mass}) \times 100\%$$

%VS = Percent VS in poultry manure

Dry Mass = Mass of dried poultry manure

Ash = Mass of ash residue after incineration

3.3.3 Experimental procedure

3.3.3.1 Pre-treatment of crop residues

Alkaline treatment was used for both corn stalk and bagasse. Slightly different pre-treatment sequences were employed to yield dry and wet substrates (Figure 3-1). It should be noted that wet or dry refers to the state of crop residues prior to the enzymatic hydrolysis. The following is the pre-treatment procedure for dry corn stalk and dry bagasse.

500 mL of 0.5 N KOH was prepared in a 1000 mL beaker and heated to 70°C on a Mirak Thermolyne hot plate. Then, 40 g dry corn stalk was added and stirred using a magnetic stirring bar. The treatment was stopped after 1 hour, and the mixture was filtered. The solid fraction was washed with distilled water, then neutralized with 1.0 N HCl until the pH reached 7.0. The mixture was filtered again and the solid was dried in an oven at 70°C for 48 hours. Once dried, the corn stalk was ground using a Wiley Intermediate grinder with a mesh size of 40. The ground corn stalk was stored in sealed plastic bottles at room temperature until the start of enzymatic hydrolysis.

In the preparation of wet substrate, 15g of crop residue was first milled with a 20 mesh, then the grind was treated with 0.5 N KOH at 70°C for 1 hour. The mixture was transferred into 750 mL Nalgene bottles and centrifuged for 10 minutes at 4700 rpm using a Centra GP8 Thermo IEC centrifuge. The supernatant was discarded and the solid was washed with distilled water. After being neutralized with 1.0 N HCl to pH 7.0, the mixture was centrifuged again. The solid fraction was kept refrigerated at 4°C in sealed plastic bottles until the start of enzymatic hydrolysis.

3.3.3.2 Enzymatic hydrolysis process for crop residues

The experimental procedure for enzymatic hydrolysis of crop residue is as follows.

50.0 mg of substrate (corn residue or bagasse) was weighted and placed into

capped test tube (for wet substrate, the moisture content was determined first, then the amount equivalent to 50.0 mg dry weight was added into the tube). Then, the desired amount of enzyme was added into the tube. The enzyme used in this research was cellulase from *Trichoderma Reesei*, made by Sigma Alderich with an activity of 8.3 Units/mg (1 unit enzyme will liberate 1 μ mol of glucose in 1 hour at 40 °C). For the trial with an enzyme loading of 800 unit/g substrate, 4.8 mg enzyme was needed. Likewise, 2.4 mg and 9.6 mg were needed for enzyme loadings of 400 and 1600 unit/g substrates respectively. Next, 2.5 mL of pH 4.8 sodium citrate buffer was added using a pipette, then the tube with all the reactants was capped and put it into a Lab-line Microprocessor Shake Bath at a shaking speed 160 rpm. The temperature remained constant throughout each trial, 35°C, 40°C, and 50°C were tried in this study. The reaction was stopped after desired time period (24 hours or 48 hours). This was accomplished by taking out the tubes from the water bath and filtering with glass filters. The filtrate was then transferred into a 50 mL capped plastic bottle for glucose measurement, and the residue was dried in an oven at 70°C for 48 hours. The dried residue was cooled in a dessicator and kept in capped plastic bottles for analysis. All the trials were performed in triplicate, together with a control sample that contained only the substrate in buffer.

3.3.3.3 Hydrolysis of poultry manure

1. Substrate preparation Because of the complex composition of poultry manure, the easily separable materials were first removed. 200g of raw poultry manure was washed with distilled water and stirred adequately in a 1000 mL

beaker, then the settled sand was removed by transferring the liquid manure between two beakers. The mixture was transferred into a 750 mL Nalgene bottle and centrifuged for 10 minutes at 4700 rpm, the supernatant, which contains dissolved organic matters and other soluble substances, was discarded. The solid fraction was washed again and its pH was measured to be approximately 8.3. It was then neutralized with 1.0 N HCl until the pH reached 7.0. Then the mixture was centrifuged to remove the liquid. After the moisture content of solid fraction was determined, it was used for subsequent treatment and enzymatic hydrolysis.

2. Hydrolysis of wet raw poultry manure 100 mg dry mass of wet poultry manure prepared from step 1 was added into a test tube, then 4.8 mg cellulase enzyme was added into the same tube. 2.5 mL sodium citrate buffer (pH 4.8) was added using a pipette the test tube was capped. Then the tube was placed into the water shaker bath which was set at 40°C, shaking speed of 160 rpm. After 24 hours of enzymatic hydrolysis, the sample was taken out of the bath and filtered with a glass filter. The filtrate was transferred into a 50 mL capped plastic bottle for glucose measurement, and the residue was dried in an oven at 70°C for 48 hours. The dried residue was cooled in a dessicator and kept in capped plastic bottles for analysis. The trials were performed in triplicate, together with a control sample that had only substrate and buffer.

3. Hydrolysis of KOH-treated wet poultry manure 500 mL of 0.5 N KOH was prepared in a 1000 mL beaker and heated at 70°C on a hot plate. An amount

equivalent to 50 g dry mass of wet poultry manure from step 1 was added into the KOH solution. The same pre-treatment procedure as specified for crop residue was followed. Then the pre-treated solid fraction was kept refrigerated at 4°C in sealed plastic bottles. The same procedure for enzymatic hydrolysis as outlined in step 2 was performed on KOH-treated poultry manure. Finally the yielded glucose solution was kept in 50 mL plastic bottles for measurement, and the hydrolyzed residue was kept in capped plastic bottles for analysis.

4. Hydrolysis of KOH-treated dry poultry manure With the exception of the fraction that was hydrolyzed wet, the remainder of KOH treated poultry manure prepared in step 3 was dried in the oven at 70°C. Then, 100 mg dried substrate was enzymatically hydrolyzed using the same procedure as performed in step 2. The yielded glucose solution was kept in 50 mL plastic bottles for measurement, and the hydrolyzed residue was kept in capped plastic bottles for analysis.

5. Hydrolysis of KOH-treated and ground poultry manure About 10g of the dried poultry manure prepared in step 3 was ground with a 20 mesh. Then, the same enzymatic hydrolysis procedure as described in step 2 was performed on 100 mg of the ground substrate. The yielded glucose solution was kept in 50 mL plastic bottles for measurement, and the hydrolyzed residue was kept in capped plastic bottles for analysis.

3.3.3.4 Hydrolysis of sewage sludge

The following is the hydrolysis procedure for primary sludge, it was also applied

to the activated sludge and the digested sludge.

1. Substrate preparation Because of the high water content in primary sludge, it was first centrifuged to remove the liquid. About 2000 mL of primary sludge was placed into four 750 mL nalgene bottles and centrifuged for 10 minutes at 4700 rpm. The supernatant was discarded, and the solid fraction was washed with distilled water and its pH was brought to 7.0 using 1.0N HCl. Then, the mixture was centrifuged again to remove the liquid. After the moisture content of solid fraction was determined, it was used for subsequent treatment and enzymatic hydrolysis.

2. Hydrolysis of un-treated primary sludge Equivalent to 50mg dry mass of the wet primary sludge prepared in step1 was added into a test tube, together with 4.8 mg cellulose enzyme and 2.5 mL sodium citrate buffer (pH 4.8). Then the reactant was placed into a 40°C water bath at shaking speed 160 rpm. After 24 hours of hydrolysis, the sample was filtered, and the filtrate was transferred into 50 mL plastic bottles for glucose measurement.

Approximately 5g (dry weight) of material from step1 was dried in the oven at 70°C for 48 hours, then it was cooled in a dessicator. After that, the cooled feedstock was ground with a 20 mesh. 50mg of the ground substrate was hydrolyzed under the same conditions as the wet substrate, and the yielded glucose solution was also kept in 50 mL plastic bottles for measurement.

3. Hydrolysis of KOH-treated primary sludge About 10g (dry weight) of the

material prepared in step1 was added into 250mL 0.5N KOH solution. It was then treated at 70°C for 1 hour. After that, it was centrifuged for 10 minutes at 4700 rpm, then the solid fraction was washed with distilled water and neutralized with 1.0N HCl to bring the pH down to 7.0. The neutralized mixture was centrifuged again and the solid fraction was kept refrigerated at 4°C in plastic bottles.

50mg (dry mass) of wet KOH-treated primary sludge was hydrolyzed under the same conditions as described in step2 (40°C, enzyme loading 800 units/g substrate for 24 hours). The yielded glucose solution was also kept in 50 mL plastic bottles for measurement.

The same procedure for the hydrolysis of dried substrate outlined in step2 was performed on the KOH-treated primary sludge, and the yielded glucose sample was kept in 50 mL plastic bottles.

4. Hydrolysis of HCl-treated primary sludge About 10g (dry weight) of the material prepared in step1 was added into 250mL of 1.0N HCl solution in a 500mL beaker. It was then stirred using a magnetic stirring bar on a hot plate. After being treated for 24 hours, the mixture was centrifuged for 10 minutes at 4700 rpm. The solid fraction was washed with distilled water and neutralized with 0.5N KOH. Then the neutralized sample was centrifuged again, and the solid fraction was kept refrigerated at 4°C in sealed plastic bottles.

Once again, the procedure for the hydrolysis of wet and dry substrate outlined in step2 was followed for the HCl-treated primary sludge.

5. Hydrolysis of HCl followed by KOH-treated primary sludge About 10g (dry weight) of the HCl-treated wet primary sludge, which was prepared in step4, was treated with KOH at 70°C for 1 hour. Then the substrate was kept refrigerated at 4°C in wet state.

The same procedure for hydrolysis of the prepared wet and dry substrates was followed for the hydrolysis of both acid and alkaline treated sludges.

3.3.3.5 Summary of experimental design

The details of all the trials performed in this study including the substrate type, enzyme loading, temperature, and reaction time are summarized and listed in Table 3-4.

Table 3-4 Summary of experimental trials

Trial	Substrate	Enzyme loading, units/g substrate	Temp. , °C	Time, hrs	Trial	Substrate		Enzyme loading, units/g substrate	Temp. °C	Time, hrs
RC8	Raw corn stalk	800	40	8	CP	Twice KOH-treated corn stalk		800	40	24
RC16				16	CD	KOH-treated corn stalk	dry	800	40	24
RC24				24	CW		wet			
RC32				32	CED1	KOH-treated corn stalk		800	40	24
RC40				40	CED2			400+400	40	12+12
RC48				48	CC1	KOH-treated corn stalk		800	40	24
KC8	KOH-treated corn stalk	800	40	8	CC2			800, with cellobiase		
KC16				16	BC1	KOH-treated bagasse		800	40	24
KC24				24	BC2	Raw poultry manure, wet		800, with cellobiase		
KC32				32	RP1			800	40	24
KC40				40	RP2	400				
KC48				48	RP3	267				
KB8	KOH-treated bagasse	800	40	8	KP1	KOH-treated poultry manure	wet	400	40	24
KB16				16	KP2			267		
KB24				24	WP			400		
KB32				32	DP		Dry, un-ground			
KB40				40	UP					
KB48				48	GP1			400		
					GP2	Dry, ground	267			
CT1	KOH-treated corn stalk	800	35	24						
CT2			40		PS1	Raw primary sludge	Wet			
CT3			50		PS2		dry			
BT1	KOH-treated bagasse	800	35	24	PS3	KOH-treated primary sludge	wet			
BT2			40		PS4		dry			
BT3			50		PS5	wet				
CE1	KOH-treated corn stalk	400	40	24	PS6	HCl-treated primary sludge	dry			
CE2		800			PS7		wet			
CE3		1600			PS8	dry				
BE1	KOH-treated bagasse	400	40	24	AS1	Activated sludge	wet			
BE2		800			AS2		dry			
BE3		1600			AS3	KOH-treated activated sludge	wet			
					DS1	Digested sludge	dry			
					DS2		wet			
					DS3	KOH-treated digested sludge	dry			
					DS4		wet			

3.4 Product analysis

3.4.1 Glucose measurement

At the end of the enzymatic hydrolysis, the filtrate which contains the converted glucose was transferred to a 50 mL plastic bottle, distilled water was added to top-up the liquid level to 50 mL. Thus, the mass of converted glucose could be calculated from its concentration in the solution.

The concentration of glucose was determined by DNS assay. Figure 3-5 shows the reaction: the reducing end (aldehyde group) of glucose converts the oxidized form of DNS (3,5-dinitrosalicylic acid) to its reduced form (3-amino-5-nitrosalicylic acid). The amount of reduced DNS is proportional to the amount of glucose.

Figure 3-5 The reaction of glucose with DNS (Brown, 2003)

The reduction of DNS results in a change in the absorption spectrum from red/orange to red/brown. Measured at 575nm using spectrophotometer, the absorbance is directly proportional to the amount of reducing sugar. Therefore, a calibration curve of Absorbance Vs. Concentration can be developed by

measuring the absorbance of known solutions, and the equation of the calibration curve can be used to calculate the unknown concentrations corresponding to their absorbance. The procedure employed in this study was as follows:

500mL of 1% DNS solution was prepared by weighting 5.0g of dinitrosalicylic acid, 0.25g of sodium sulfite, and 5.0g sodium hydroxide, and placing all these reactants in a 1000mL beaker. Then 400mL distilled water was added, and the solution was agitated. After thorough mixing, it was transferred to a 500mL flask and topped up with distilled water. The next step was to prepare 100mL 40% (w/v) potassium sodium tartrate by adding 40g potassium sodium tartrate into 50mL distilled water and topping the solution to 100mL. To make 1 g/l standard glucose solution, 50mg glucose was added into 40mL distilled water, it was then topped to 50mL and mixed adequately. Four other standard solutions, with the concentration of 0.8g/l, 0.6g/l, 0.4g/l, 0.2g/l, were made by diluting the 1g/l solution with distilled water. When all the solutions (including the glucose solution to be measured and the standard glucose solution) were ready, 3mL was taken from every glucose solution and each of them was placed in a test tube, then 3mL of DNS was added into each tube and capped. The samples were heated in 90°C hot water for 5 minutes, afterward, they were taken out and 1mL of 40% potassium sodium tartrate was added into each tube to stabilize the color. After the samples were cooled to room temperature with tap water, their absorbance at 575 μm was measured with a Spectronic 20D spectrophotometer. The absorbance value of the five standard glucose solutions were used to develop the calibration curve, thus the equation of Absorbance Vs. Concentration was

determined. Finally the unknown glucose concentrations were calculated using the developed equation and the measured absorbance.

The glucose yield (GY) was determined using equation 3-3

$$(3 - 3) \quad GY = \text{Glucose concentration mg/mL} \times 50 \text{ mL}$$

GY = mass of glucose yield in each trial, mg

Glucose concentration = the calculated glucose concentration, mg/mL

The percentage of conversion (PC) was determined using equation 3-4

$$(3 - 4) \quad \%PC = (\text{Glucose yield mg}) / (\text{Substrate Added mg}) \times 100\%$$

%PC = the percentage of substrate converted

Glucose yield = the glucose yield determined from equation 3-3

Substrate Added = the dry mass of substrate entering each enzymatic hydrolysis

3.4.2 Fourier Transform Infrared Spectroscopy

To further examine the effect of enzymatic hydrolysis on the substrate components, Fourier Transform Infrared Spectroscopy (FTIR) was used to analyze the functional groups in the substrates and the hydrolyzed residues for crop residues and poultry manure. In addition, it was also performed on the three types of sludge to examine their differences on composition. FTIR was conducted in Carleton University Chemistry Instrument Laboratory. Samples to be analyzed were dried and ground prior to scanning. About 300mg of dry KBr was taken to a mortar and milled with a pestle, once fine enough the powder was placed in a mould and compressed with a force 10 tons for about 1 minute to make a pellet. Then the pellet was placed in a holder and scanned using a

Bomem Michelson 120 FTIR analyzer, the background spectra were generated using Bomem Grams 386 software. To obtain the spectra for each sample, about 5mg of sample was mixed with 300mg KBr in the mortar and then milled sufficiently. The same procedure as described for the background was applied to every sample, each one was scanned 5 times between the wavelengths 4500 and 650 cm^{-1} . The generated spectra were compared to published FTIR spectra of other organic materials to analyze the function groups.

Chapter 4 Results and discussion

4.1 Enzymatic hydrolysis of crop residues

4.1.1 Screening of appropriate reaction conditions for corn stalk and bagasse hydrolysis

4.1.1.1 Conversion percentage by time

Depending on the affinity of the enzyme used and the pre-treatment employed, enzymatic hydrolysis often proceeds at slow rate. It may take a few days to achieve the desired conversion percentage. As a catalytic reaction, the reaction rate decreases due to the inhibition caused by end product. However, the hydrolysis is an energy-consuming process because it is conducted at constant mixing and a higher than room temperature; therefore, it is not economical to prolong the reaction beyond a certain period of time. To determine the appropriate reaction time, a series experiments were conducted on pre-treated corn stalk, raw corn stalk, and pre-treated bagasse. There were six trials for each material, which lasted for 8, 16, 24, 32, 40, and 48 hours respectively, all other operational conditions of these trials remained constant: 40°C, pH 4.8, enzyme loading 800 units/g substrate, mixing speed 160 rpm. The glucose yields were measured using DNS assay and the percentages of conversion were calculated using equation 3-4. The conversion percentages of corn stalk and bagasse with time is shown in Figure 4-1, and the glucose conversion rates is shown in Table 4-1.

Figure 4-1 Conversion percentage of corn stalk and bagasse with time

Table 4-1 Glucose conversion rates with time (mg glucose/hr)

Time, hrs	Raw corn stalk	KOH-treated corn stalk	KOH-treated bagasse
8	0.37±0.005	2.32±0.03	2.08±0.03
16	0.19±0.004	1.61±0.01	1.33±0.02
24	0.14±0.021	1.28±0.02	1.03±0.04
32	0.11±0.001	1.03±0.01	0.81±0.01
40	0.09±0.002	0.85±0.01	0.72±0.01
48	0.07±0.003	0.75±0.03	0.64±0.04

It can be seen from Figure 4-1 and Table 4-1 that although the glucose yields for KOH pre-treated corn stalk and bagasse increased continually with time, there was a tendency that the hydrolysis rates decreased with time. After 24 hours periods, the conversion percentages for corn stalk and bagasse were $61.2 \pm 0.7\%$ and $49.5 \pm 2.2\%$, respectively. When the hydrolysis time was prolonged to

48 hours, $72.2 \pm 2.9\%$ and $61.2 \pm 3.4\%$ of corn stalk and bagasse were converted into glucose. From 24 hours to 48 hours, the conversion of corn stalk and bagasse only increased $11.0 \pm 3.7\%$ and $11.7 \pm 2.4\%$ respectively. This indicated that the conversion rates in this period were considerably slower than that in the first 24 hours. As a 24-hour reaction time period would be considered more acceptable from an industry perspective, the reaction times of the remaining experiments were 24 hours. It can also be noted that for raw corn stalk, there was no significant change on the conversion rate after 8 hours.

Corn stalk and bagasse feedstocks were treated under the same conditions both in pre-treatment and enzymatic hydrolysis, but a higher glucose yield was achieved from corn stalk. This can probably be attributed to their difference in cell wall structure; it is shown in Table 3-1 that bagasse generally has higher lignin but lower cellulose content than corn stalk. This made it more difficult to remove lignin and hemicellulose from the raw material during the pre-treatment, and in turn resulted in lower glucose yield.

Figure 4-1 also showed that alkaline pre-treatment contributed to greater glucose yields during enzymatic hydrolysis, the effect of pre-treatment is discussed in section 4.2.1.

4.1.1.2 Temperature

The preferred temperature for enzymes in hydrolysis is generally between 35-50°C (Jakobsson, 2004). However, higher operational temperatures mean higher

energy consumption. This can be of particularly concern for operational process cost because enzymatic processes require long time periods to proceed. To determine the effect of temperature on hydrolysis, three experiments were conducted on both pre-treated corn stalk and bagasse. The trials were carried out in 35°C, 40°C, and 50°C water baths. All other experimental conditions including enzyme loading, pH, mixing speed, and reaction times remained constant. The glucose yields obtained after 24 hours are shown in Figure 4-2,

Figure 4-2 Conversion percentage of corn stalk and bagasse by temperature

Table 4-2 Glucose conversion rate with temperatures (mg glucose/hr)

Temperature, °C	KOH-treated corn stalk	KOH-treated bagasse
35	1.00±0.07	0.96±0.04
40	1.36±0.06	1.06±0.05
50	1.05±0.03	0.96±0.07

Figure 4-2 shows that the glucose yield from corn stalk was the highest at 40°C, it was markedly lower at both 35°C and 50°C, with the difference of $17.3 \pm 0.05\%$ and $15.0 \pm 0.03\%$ respectively. Table 4-2 also shows that the glucose conversion rate for corn stalk was the highest at 40°C. Bagasse had the same character as corn stalk as well, but its conversion differences between 40°C and the other two temperatures appeared to be less significant. These results suggested that the enzyme is most active at approximately 40°C, higher or lower temperatures appeared to depress its activity. Therefore, the appropriate hydrolysis temperature was determined to be 40°C. It can also be noted from Figure 4-2 that if taking the standard deviation into account, the conversion rates of the two feedstocks were fairly close at 35°C and 50°C.

4.1.1.3 Enzyme loading

The enzyme used in this study is a commercial cellulase enzyme from *Trichoderma reesei*, which has an activity of 8.3 unit/mg. There are a number of enzymes available and some may have higher activity than others. However, the purpose of this study was to investigate the degradability of various cellulosic feedstocks, and to compare their conversion rates under different conditions, thus, the selection of the enzyme was not the focus of this study. It is obvious that higher enzyme loading should generate higher cellulose conversion rates. However, currently enzyme is an expensive material that accounts for the main cost of the entire process (Ward and Singh, 2002). From an economic point of view, it is important to produce glucose at relatively low enzyme loading. Figure 4-3 presents the conversion percentages of corn stalk and bagasse at three

different enzyme loadings: 400, 800, and 1600 units per gram substrate. Each reactions was carried out at 40°C and for a 24-hour period.

Figure 4-3 Conversion of corn stalk and bagasse at various enzyme loadings

Table 4-3 Glucose conversion rate with enzyme loading (mg glucose/hr)

Enzyme loading	KOH-treated corn stalk	KOH-treated bagasse
400 unit/g	0.87±0.05	0.84±0.05
800 unit/g	1.36±0.06	1.06±0.05
1600 unit/g	1.46±0.05	1.23±0.04

It can be seen from Figure 4-3 that increasing the enzyme loading from 400 units/g to 800 units/g increased the conversion of corn stalk from 41.8±2.3% to 65.4 ± 2.3%, the corresponding conversion rate (Table 4-3) was increased from 0.87 ± 0.05 to 1.36 ± 0.06 mg/hr. However, doubling the enzyme loading to 1600 units/g only achieved 4.8% increase in conversion and 0.11mg/hr in rate. Taking

into account the standard deviation, the differences were actually not significant. The experiments on bagasse also showed similar trends, but the conversion differences for the three enzyme loadings were less than that of corn stalk. $40.2 \pm 2.3\%$ was converted at the enzyme loading of 400 units/g substrate, it was increased by 10.9% and 18.8% when the enzyme loading was 800 and 1600 units/g respectively. It can also be seen from Table 4-3 that the glucose conversion rate was not proportional to the enzyme loading. This is probably because in the cellulose molecule, the accessible site to enzyme was limited. Therefore the percentage of enzyme which had access to the cellulose molecule would decrease at higher enzyme loading, and the conversion rate per unit enzyme would then decrease accordingly.

Table 4-4 Glucose yield per unit substrate and enzyme

Enzyme loading	KOH-treated corn stalk		KOH-treated bagasse	
	Glucose yield, g/g substrate	Glucose yield per unit enzyme, mg/enzyme unit	Glucose yield, g/g substrate	Glucose yield per unit enzyme, mg/enzyme unit
400 unit/g	0.42 ± 0.02	1.05 ± 0.06	0.40 ± 0.02	1.01 ± 0.06
800 unit/g	0.65 ± 0.02	0.82 ± 0.06	0.51 ± 0.03	0.64 ± 0.06
1600 unit/g	0.70 ± 0.02	0.44 ± 0.05	0.59 ± 0.02	0.37 ± 0.04

Table 4-4 shows the glucose yield per enzyme unit at different enzyme loadings. It can be found that among the three trials on KOH-treated corn stalk, the one with enzyme loading 400 unit/g had the highest glucose yield per unit enzyme, but its yield per gram substrate was substantially lower than the other two. Through the above comparison, the appropriate enzyme loading for later experiments was determined to be 800 units/g substrate.

4.1.1.4 Enzyme activity test

The enzyme activity was tested using whatman (filter paper) as the substrate. Two enzyme loadings were tested: 400 units/g and 800 units/g, their conversion percentages and glucose conversion rates are listed in Table 4-5.

Table 4-5 Result of enzyme activity test

Substrate	Enzyme loading, unit/g	Glucose yield, g/g substrate	Glucose conversion rate per unit enzyme, mg/hr/unit
Whatman	400	0.554 ± 0.017	0.058 ± 0.001
	800	0.736 ± 0.018	0.039 ± 0.001

It can be seen from Table 4-5 that when the enzyme loading increased from 400 units/g to 800 units/g substrate, the glucose conversion rate per unit enzyme decreased, but the glucose yield increased from $55.4 \pm 1.7\%$ to $73.6 \pm 1.8\%$. This suggested that although the reaction rate was not proportional to the enzyme loading, to achieve high glucose yield in 24 hours, a relatively high enzyme loading is required.

4.1.2 Optimization of hydrolysis process

4.1.2.1 Pre-treatment

4.1.2.1.1 Time of pre-treatment

The pre-treatment processes used for crop residues include size reduction through grinding and chemical modification via alkaline treatment. Grinding increases the surface area and reduces the degree of polymerization, thus providing better substrate accessibility to the enzyme. It is recommended for crop

residues not only to improve enzymatic hydrolysis, but also for easier handling of materials during the experiments. Alkaline pre-treatment breaks the structural linkages between cellulose, lignin, and hemicellulose; thus, extracting the cellulose from the raw cellulosic materials. Moreover, it can also cause swelling in the crystalline structure which increases the internal surface area thereby improving enzyme accessibility. To determine the effect of pre-treatment, three types of pre-treatment methods were applied to corn stalk residues, and the enzymatic hydrolysis was carried out under the same experimental conditions. The three pre-treatments were: 1. The first pre-treatment simply involved the grinding of raw corn stalk. 2. The second was to treat the feedstock with KOH and then grind it. 3. For the third pre-treatment, corn stalk was first treated with KOH, then the mixture was filtered. The solid fraction was washed and treated with KOH again, it was then dried and ground before hydrolysis. Figure 4-4 shows the conversion percentage resulting from the use of these three substrate preparations and Table 4-6 lists the conversion rates of the enzymatic hydrolysis.

Figure 4-4 Hydrolysis of different pre-treated corn stalk

Table 4-6 Glucose conversion rate for different pre-treated corn stalk (mg glucose/hr)

Substrate type	Raw corn stalk	One KOH treatment	Two KOH treatment
Conversion rate, mg/hr	0.14±0.02	1.36±0.06	1.38±0.08

Figure 4-4 shows that for corn stalk without alkaline treatment, only $6.6 \pm 1.0\%$ was converted into glucose, but this was increased to $65.4 \pm 2.3\%$ for KOH-treated substrate. The glucose conversion rate shown in Table 4-6 also indicated ten-fold increase due to the KOH treatment. This demonstrated the importance of chemical pre-treatment on the enzymatic hydrolysis. Although the substrate was

ground, the cellulose was still intertwined with lignin and hemicellulose, structures which are highly resistant to enzyme attack. Another reason for the low conversion percentage might be that since the lignin and hemicellulose were not physically removed from the raw corn stalk feedstock, the cellulose content percentage of substrate was lower than that in the chemically pre-treated feedstock. This would result in lower conversion percentage of raw corn stalk, although it is not the main cause.

Figure 4-4 also shows that when corn stalk was treated twice with KOH, little improvement was obtained compared to pre-treating once with KOH. Taking into account the standard errors, the difference was not significant. This would suggest that one KOH treatment was sufficient, extra pre-treatment is not necessary.

4.1.2.1.2 Sequence of pre-treatment

Thus far, all the feedstocks employed in the experiments were dried and ground prior to being subjected to the enzymatic hydrolysis process. This allows for easier storage and handling, but consumes energy during the drying process. If the crop residues were ground first, then the fine residue could be conveyed to the enzymatic hydrolysis immediately following the KOH treatment. Without drying, the overall process time and cost could be reduced. To verify this, the corn stalk was ground, then subjected to alkaline pre-treatment followed by the enzymatic hydrolysis. A trial on KOH-pre-treated dry corn stalk was conducted at the same time, the dry mass of wet substrate that entered the hydrolysis was the

same as the dry one. The moisture content of wet substrate was determined prior to the enzymatic process in order to determine the quantity to be added. The conversion percentages of these two substrates are shown in Figure 4-5, and the corresponding conversion rates were listed in Table 4-7.

Figure 4-5 Hydrolysis of dry and wet corn stalk

Table 4-7 Glucose conversion rate for dry vs. wet substrate (mg glucose/hr)

Substrate type	KOH-treated dry corn stalk	KOH-treated wet corn stalk
Conversion rate, mg/hr	1.27±0.03	1.42±0.01

As can be seen from Figure 4-5, wet substrate had 68.2±0.4% conversion, while the dry substrate only had 60.7±1.6%. Table 4-7 also shows that the glucose

conversion rate of the wet substrate was also higher than that of dry substrate. Thus, there appeared to be some increase in the enzymatic hydrolysis when the substrate was used wet. The difference could be attributed to the pre-treatment sequence. It could be argued that when the corn stalk was ground into small size particles before alkaline treatment, its surface area was greatly increased, thus the residue had better contact with KOH solution, and it became easier to break down the interaction forces among cellulose, hemicellulose and lignin. Therefore more lignin and hemicellulose could be removed from the residue, and the crystalline structure of cellulose was swelled to a greater extent. As a result, the enzyme had better accessibility to the wet substrate and more β -glucosidic linkages in the cellulose molecular structure were broken down and converted into glucose.

4.1.2.2 Time of enzyme addition

In the experiments presented to date, all of the enzyme dosage for each experimental run was added at the beginning of the hydrolysis. Generally, in enzymatic reactions, the highest reaction rate occurs at the beginning, as the substrate concentration decreases and product concentration increases, the reaction rate decreases gradually. It was also suspected that the activity of enzyme may not remain constant throughout the entire process. Thus, two experiments using KOH-treated corn stalk were carried out at the same time. In the first trial the entire enzyme dosage was added at the beginning of the experiment and the reaction was allowed to process for 24 hours. In the second trial, only half of the enzyme dosage was added at the beginning of the

experiment, the other half was added after 12 hours, the reaction was also stopped after a 24-hour period. The conversion percentages for these are trials was shown in Figure 4-6, and the glucose conversion rates were listed in Table 4-8.

Figure 4-6 Effect of enzyme addition time on the hydrolysis

Table 4-8 Glucose conversion rate for dry vs. wet substrate
(mg glucose/hr)

Enzyme addition time	All at beginning	Half beginning/ half middle
Conversion rate, mg/hr	1.32±0.04	1.40±0.03

When the enzyme was added in its entirety at the beginning of the process, a conversion of $63.3 \pm 1.6\%$ was observed, while when half was added at the

beginning and half was added halfway through the process, a slight increase to $66.9 \pm 1.2\%$ was obtained. This implied that splitting the amount of enzyme and adding it at different times during the enzymatic hydrolysis process could yield more glucose than adding it all at the beginning. The main reason for this observation was probably because the activity of the enzymes typically decreases with time. When the second half of the enzyme dosage was added in the middle of the hydrolysis, the fresh enzyme boosted the reaction rate. This would suggest that in an industrial operation, one possible way to increase the productivity is to split the enzyme dosage and add them at even time intervals instead of adding the entire dosage at one time. In addition, the accumulation of some by-products may also have impact on the enzyme activity, so it might be worthwhile to withdraw the by-products during the hydrolysis in order to increase the reaction rate.

4.1.2.3 Influence of cellobiase

As stated in the chapter 2, the hydrolysis of cellulose consists of two steps: cellulose is first converted to cellobiose by the synergy of endo-glucanases and exo-glucanases, then the cellobiose is converted to glucose by β -glucosidase (cellobiase). Cellobiose is a strong inhibitor to cellulase enzyme, thus, it is important that the enzyme selected have enough β -glucosidase activity. However, most commercial enzymes are deficient in β -glucosidase, thus, their overall activity are affected. Therefore adding additional cellobiase into the reactants may help to improve the hydrolysis rate. The following two experiments were

designed to determine the effect of cellobiase on the hydrolysis of both KOH pre-treated corn stalk and bagasse: the first one was an enzymatic hydrolysis without cellobiase, the second included 10% (v/v) cellobiase into the enzyme mixture. All the trials were carried out at 40°C and with an enzyme loading of 800 units/g substrate, and they were performed over a 24 hour period. For the one with cellobiase, there were two blank samples acting as controls, one had cellobiase and substrate, the other one had only cellobiase. The glucose yields were measured using DNS assay. Table 4-9 lists the absorbance values of the glucose samples from the above trials.

Table 4-9 Measured and adjusted absorbance values of glucose samples from hydrolysis with cellobiase

Substrate	Enzyme	Cellobiase	Absorbance	Adjusted Absorbance
Corn stalk-1	Yes	Yes	0.587	0.420
Corn stalk-2	Yes	Yes	0.602	0.435
Corn stalk-3	Yes	Yes	0.599	0.432
Corn stalk/control-A	No	Yes	0.170	0.003
-/control-B	No	Yes	0.167	0.000
Bagasse-1	Yes	Yes	0.610	0.439
Bagasse-2	Yes	Yes	0.621	0.450
Bagasse-3	Yes	Yes	0.605	0.434
Bagasse/control-A	No	Yes	0.181	0.010
-/control-B	No	Yes	0.171	0.000

It can be seen that the measured absorbance of control samples were all greater than zero. The two controls of both corn stalk and bagasse had similar absorbance value. The results imply that cellobiase may have react with DNS which would have resulted in color change in the control samples. Thus, for the trials with cellobiase, the measured absorbance of glucose solutions had two

sources: glucose and cellobiose. To calculate the net glucose yield from the measured absorbance, the contribution of cellobiose was subtracted. Because the absorbance was proportional to the amount of reacted DNS, the adjusted absorbance was calculated by subtracting the absorbance value of the cellobiose control from the measured value. The glucose yields were then calculated using the adjusted values. Figure 4-7 shows the conversion of the trials with cellobiose, as well as the trials without cellobiose that were conducted at the same time. Their corresponding glucose conversion rates were presented in Table 4-10.

Figure 4-7 Effect of cellobiose on the hydrolysis of corn stalk and bagasse

Table 4-10 Glucose conversion rate for hydrolysis with/without cellobiose (mg glucose/hr)

Substrate type	Corn stalk		Bagasse	
	No	Yes	No	Yes
conversion rate, mg/hr	1.37±0.04	1.40±0.02	1.06±0.05	1.12±0.02

As can be seen from Figure 4-7 and Table 4-10, comparing to the trials without cellobiase, the ones with additional cellobiase did not significantly increase glucose yield. Taking into account the standard errors, the improvement of cellobiase on the enzymatic hydrolysis was negligible. This also demonstrated that the cellulase enzyme used in this study had sufficient β -glucosidase activity, and that additional cellobiase was not necessary to enhance the reaction.

4.1.3 Mass balance

Due to the difficulty in quantification of some substances, such as the enzyme in the filtrate after hydrolysis, performance of a complete mass balance was not possible. However, the mass flow of the studied material during the process was recorded. Figure 4-8 is the mass flow of corn stalk during the regular treatment process.

Figure 4-8 Mass flow of corn stalk throughout the process

The pre-treatment was conducted in relatively large quantity, 20.0 g raw corn stalk yielded 12.8 g after 1 hour treatment. Thus, 50.0 mg of substrate would require 78.1 mg of raw material. After 24 hours of enzymatic hydrolysis at 40°C, 32.7 mg of glucose was converted and 17.3 mg of residue remained.

4.2 Enzymatic hydrolysis of poultry manure

4.2.1 Hydrolysis of raw poultry manure

The poultry manure samples had an average moisture content of $73.2 \pm 0.5\%$. Due to the fine particle texture of the manure, hydrolysis without physical treatments such as drying and grinding was selected. About 100g of poultry manure was added into 500mL distilled water and yielded a pH of 8.3. Because the pH is a critical factor that influences the enzyme activity, it is important to neutralize the substrate before enzymatic hydrolysis. Therefore the raw poultry manure was washed with distilled water and then neutralized with 1.0N HCl to lower the pH to 7.0. The neutralized manure was separated by centrifugation at 4700 rpm for 10 minutes. The supernatant containing dissolved organic matter was discarded, and the solid fraction, which had a moisture content of $88.1 \pm 0.6\%$, was used for enzymatic hydrolysis. The amount of substrate entering the enzymatic hydrolysis was calculated on dry mass basis. As expected, the cellulose content in poultry manure was much lower than in the pre-treated crop residues. Thus, it was thought that a higher substrate loading should be employed to provide comparative amount of cellulose for enzymatic hydrolysis. In the hydrolysis of crop residues, the optimum enzyme loading was determined to

be 800 units/g substrate, which meant that 40 units of enzyme was added with 50mg (dry mass) substrate and 2.5mL buffer. When the substrate loading was doubled (i.e. the enzyme loading was decreased to 400 units/g substrate), the conversion percentage decreased substantially (Section 4.1.1.3). In the hydrolysis of raw poultry manure, three different substrate loadings were tried simultaneously: respectively 50mg, 100mg and 150mg (dry mass) of wet poultry manure were hydrolyzed with 40 units enzyme and 2.5 mL, the temperature was maintained at 40°C and the reaction period was 24 hour. The glucose yields, conversion percentages and conversion rates are presented in Table 4-11.

Table 4-11 Hydrolysis results of raw poultry manure at different substrate loading

Substrate mass, mg	Enzyme amount, unit	Glucose yield, mg	Conversion rate, mg glucose/hr	Conversion percentage %
50	40	3.58±0.19	0.149±0.008	7.2±0.40
100	40	7.15±0.30	0.298±0.013	7.1±0.30
150	40	10.17±0.91	0.424±0.038	6.8±0.60

The results in Table 4-11 show that for the same quantity of enzyme, the glucose yield was proportional to substrate loading. But the conversion percentages were not significantly different. This indicated that for raw poultry manure, only about 7% of the total mass could be enzymatically hydrolyzed. Because of the low cellulose content in poultry manure and less end-product inhibition, higher substrate mass than that of crop residues could be used without affecting the conversion percentage. However, if more cellulose could be extracted from the

raw poultry manure, the conversion might decrease at high substrate loading (as was observed for crop residues).

4.2.2 Hydrolysis of alkaline treated poultry manure

Since KOH treatment was demonstrated to be an effective pre-treatment in extracting cellulose from crop residues, it was thought that alkaline treatment could also be effective in improving enzymatic hydrolysis with poultry manure because a portion of the cellulose content in the poultry manure is also from crop materials such as grain hulls. To verify this assumption, the same KOH pre-treatment was performed on poultry manure, the procedure described in section 3.3.3.3 was employed. The enzymatic hydrolysis of pre-treated wet manure was conducted at two different substrate loadings: 100mg and 150mg, the quantity of enzyme and buffer was the same as employed in the previous trials (40 units, 2.5mL). The temperature was maintained at 40°C and the reaction period was 24 hour. Figure 4-9 shows the conversion of these two trials and the trials on raw poultry manure.

Figure 4-9 Conversion of wet KOH-treated poultry manure

Table 4-12 Glucose conversion rates of KOH-treated poultry manure (mg glucose/hr)

Substrate loading, mg	Raw poultry manure	KOH-treated poultry manure
100	0.30±0.01	0.36±0.12
150	0.42±0.04	0.56±0.01

The results in Figure 4-9 shows that the conversion of KOH-treated poultry manure were $9.5 \pm 3.1\%$ and $8.9 \pm 0.8\%$ at the substrate loading of 100mg and 150mg respectively. Table 4-12 also shows that the glucose conversion rate generally increased with the substrate loading.

Compared to raw poultry manure, the KOH treated substrate had 2.4% higher conversion at the substrate loading of 100mg. Taking into account the standard deviation, the difference did not appear to be statistically significant. It is quite

different to that of crop residues, which achieved 10-fold increase in the conversion percentage due to the KOH treatment. This would suggest that in the poultry manure, most of the cellulose was not as easily extracted from other organic matters. Although the integrated structure of cellulosic material might be broken up during the KOH treatment, most of the cellulose was still not readily extractable, thus, the enzymes would not gain sufficient access to the cellulose molecules. As a result, only little improvement to the hydrolysis could be achieved through the KOH pre-treatment. In attempting to extract more cellulose from other components in poultry manure, physical pre-treatment was performed and the results are discussed in the following section.

4.2.3 Effect of physical treatment on the hydrolysis of poultry manure

4.2.3.1 Effect of drying

The first physical treatment involved was drying. The KOH-treated wet poultry manure was dried at 40°C for 48 hours, then 100mg dried substrate was hydrolyzed with 40 units enzyme and 2.5mL buffer. The temperature and reaction time were the same as for the other trials. A trial on wet KOH-treated substrate was carried out simultaneously under the same conditions. Their conversion percentages are presented in Figure 4-10 and the conversion rates are listed in Table 4-13.

Figure 4-10 Effect of drying on the hydrolysis of poultry manure

Table 4-13 Glucose conversion rate of KOH-treated poultry manure (mg glucose/hr)

Substrate loading, mg	KOH-treated wet poultry manure	KOH-treated dry poultry manure
100	0.43±0.07	0.59±0.03

For the KOH-treated wet poultry manure, $10.2 \pm 1.5\%$ was converted into glucose from the enzymatic hydrolysis, and it had a conversion rate of 0.43 ± 0.07 mg glucose/hr. In case of the KOH-treated manure which was dried before enzymatic hydrolysis, the conversion percentage increased to $13.9 \pm 0.7\%$ and the conversion rate increased to 0.59 ± 0.03 mg glucose/hr. These results show that drying the KOH-treated manure did improve the glucose yield from enzymatic hydrolysis. It can be inferred that some of the organic matter might be degraded at high temperature (up to 70°C), thus more cellulose could be uncovered during

the drying process; or the physical structure of substrate became more accessible to the enzyme due to the drying.

4.2.3.2 Effect of grinding

Initially, because the raw poultry manure sample was already in small particles, it was not deemed necessary to grind the feedstock prior to enzymatic hydrolysis. However, thus far the highest glucose yield obtained was $13.9 \pm 0.7\%$ from the KOH treated and dried poultry manure, this was still substantially lower than the potential estimated cellulose content in poultry manure. The results to date suggested that although the poultry manure was pre-treated with KOH, which had proven effective on crop residues, the cellulase enzyme still had insufficient access to cellulose molecules.

To clear the obstacles that block the enzyme, size reduction was attempted on the KOH treated poultry manure. The dried manure was ground with a 20 mesh, then the fine substrate was enzymatically hydrolyzed at two different loadings: 100mg and 150mg. 100mg of un-ground sample was also hydrolyzed simultaneously; the temperature, enzyme loading and reaction time were the same as in the prior trials. The glucose yield, conversion percentage and conversion rate of these three trials are listed in Table 4-14.

As can be seen in Table 4-14, when 100mg substrate was added, $27.6 \pm 1.2\%$ ground poultry manure was converted into glucose, $13.3 \pm 1.6\%$ higher than that of the un-ground manure. These results would suggest that by grinding the

poultry manure, the substances that covered the cellulose was broken up, thus exposing the surface of the cellulose. In addition, reducing the particle size of substrate also created a larger surface area for reaction. Thus, the substrate became much more accessible to enzyme. As a result, the glucose yield increased significantly. This observation demonstrated that the accessibility of cellulose in enzymatic hydrolysis is a limit factor to the reaction rate.

Table 4-14 Hydrolysis results of ground vs. not ground poultry manure

Substrate type	Substrate mass, mg	Enzyme, unit	Glucose yield, mg	Glucose conversion rate, mg/hr	Conversion, %
KOH treated, un-ground	100	40	14.34±0.85	0.60±0.04	14.3±0.9
KOH treated, ground	100	40	27.83±1.38	1.16±0.06	27.6±1.2
KOH treated, ground	150	40	39.64±1.34	1.65±0.06	26.3±1.0

It can also be seen that when the substrate loading was increased to 150mg, the glucose yield increased to 39.64±1.34 mg. Table 4-15 presents the comparison of glucose yield from poultry manure and corn stalk. It shows that with the same enzyme and buffer additions, when the substrate loading of poultry manure was three times of corn stalk, its glucose yield was slightly higher than that of corn stalk. This could be important in the fermentation of glucose and distillation, because the concentration of ethanol is proportional to that of the glucose, the higher glucose concentration will ultimately result in lower cost in the distillation of ethanol.

Table 4-15 Glucose yield from poultry manure vs. corn stalk

Substrate	Substrate mass, mg	Enzyme, unit	Buffer, mL	Glucose yield, mg	Glucose concentration, g/l
KOH pre-treated dry poultry manure (Ground)	150	40	2.5	39.64±1.34	15.86±0.54
corn stalk (Pre-treated)	50	40	2.5	33.21±0.75	13.29±0.30

4.2.4 Mass flow of poultry manure hydrolysis

The highest conversion percentage from the enzymatic hydrolysis of poultry manure was achieved using the KOH-pre-treated, then dried and ground substrate. The mass flow of the entire process is shown in Figure4-11:

Figure 4-11 Mass flow of poultry manure hydrolysis

4.3 Enzymatic hydrolysis of sewage sludge

4.3.1 Hydrolysis of primary sludge and activated sludge

Unlike the crop residues and poultry manure, the cellulose content in the primary sludge is mainly from waste paper, which has a high cellulose content and very low lignin content. As with poultry manure, the collected primary sludge and activated sludge samples were also composed of fine particles. Thus, after being neutralized and centrifuged, the primary sludge and activated sludge were enzymatically hydrolyzed without any other pre-treatment using the procedure described in section 3.3.3.4. Both wet and dry substrates were employed with these two materials, and the other experimental conditions of these trials were the same as those employed for the crop residues (40°C, with enzyme loading 800 units/g substrate and reaction period 24 hours). The conversion percentages on dry mass basis and glucose yield rates are shown in Figure 4-12 and Table 4-16.

Figure 4-12 Enzymatic hydrolysis of primary sludge and activated sludge

Table 4-16 Glucose conversion rates from primary sludge and activated sludge (on dry mass basis), mg glucose/hr

Substrate type	Primary sludge	Activated sludge
Wet	0.647 ± 0.056	0.270 ± 0.025
Dry	0.520 ± 0.016	0.116 ± 0.024

Figure 4-12 shows that for primary sludge, $31.1 \pm 2.7\%$ and $25.0 \pm 0.8\%$ were converted into glucose when the substrate added was wet and dry respectively. Higher conversion percentage and glucose conversion rate were observed when the substrate was used wet. This is contrary to what was noted with poultry manure, which had higher conversion when dry substrate was used. It would suggest that the cellulose in primary sludge was readily accessible to the enzyme, and thus drying and grinding might not improve the enzymatic hydrolysis. It can also be seen from Figure 4-12 that primary sludge yielded more glucose than activated sludge. Such a difference might result for two reasons: the first was that some cellulose might be degraded during the activated sludge treatment; the other possible reason is that the chemicals added during the activated sludge process, or the micro-organisms grown in this process, might suppress or compete with the activity of enzyme. The conversion rate for activated sludge also showed large differences between wet and dry substrates, suggesting that the cellulose in the activated sludge might become less accessible to enzymes during drying and grinding.

4.3.2 Hydrolysis of alkaline treated primary and activated sludge

Besides waste paper, other sources of cellulose in primary sludge might include plant residues such as grass, vegetables and wood. For these materials, alkaline treatment may be useful to extract the cellulose and thus improve enzymatic hydrolysis. Therefore KOH pre-treatment was performed on both primary sludge and activated sludge using the procedure described in section 3.3.3.4. The enzymatic hydrolysis was conducted under the same conditions as the prior trials. The conversion percentages of these two pre-treated materials on dry mass basis are presented in Figure 4-13 and the glucose yield rates are listed in Table 4-17.

Figure 4-13 Enzymatic conversion of KOH treated primary and activated sludge

Table 4-17 Glucose conversion rates from KOH-treated primary sludge and activated sludge (mg glucose/hr)

Substrate type	Primary sludge	Activated sludge
Wet	0.739±0.035	0.235±0.068
Dry	0.612±0.024	N/A

The results in Figure 4-13 show that when KOH pre-treatment was performed on primary sludge, the conversion for wet substrate increased from $31.1 \pm 2.7\%$ to $35.4 \pm 1.2\%$, which was 4.3% increase due to the pre-treatment. The glucose conversion rate also increased from 0.647 ± 0.056 to 0.739 ± 0.035 mg/hr. This demonstrated that the KOH pre-treatment helped to extract some of the cellulose from the plant derived materials in the primary sludge. However, compared to that of crop residues, which achieved almost ten-fold increase in the conversion percentages, the KOH pre-treatment was not as effective on primary sludge. This suggested that most of the cellulose content in the primary sludge might be paper based. Alkaline pre-treatment was not necessary for the enzymatic hydrolysis of such materials because they are highly purified cellulose. Once again, the dried substrate yielded less glucose than the wet one.

For activated sludge, the conversion rate was $11.3 \pm 3.3\%$ for wet substrate; no improvement was achieved through the KOH pre-treatment. This is probably because the easily biodegradable plant materials in the activated sludge were decomposed by the micro-organisms present in the activated sludge treatment process. No conversion data was available for the dried substrate because precipitation was observed in the DNS solutions which were used to measure the glucose converted from the enzymatic hydrolysis. When glucose strips were used to test the filtrate after enzymatic hydrolysis, the glucose was also not detectible.

4.3.3 Effect of acid treatment on the hydrolysis of primary sludge

The metals contained in the sewage sludge, in particular heavy metals such as copper, zinc, and nickel, may have adverse effects on the enzyme activity. To reduce the impact of metals, primary sludge was subjected to HCl treatment, through which the metals were dissolved and removed from the solid fraction. In addition, part of the HCl treated sludge was applied a KOH treatment before enzymatic hydrolysis. The conversion percentages on dry mass basis are presented in Figure 4-14, and the glucose conversion rates are listed in Table 4-18. The hydrolysis of HCl treated activated sludge was not conducted because of its low glucose yield in the prior experiments.

Figure 4-14 Enzymatic conversion of HCl treated primary sludge

Table 4-18 Glucose conversion rate of HCl-treated primary sludge (mg glucose/hr)

Substrate type	HCl-treated primary sludge	HCl and KOH treated primary sludge
Wet	0.888 ± 0.025	1.128 ± 0.083
Dry	0.712 ± 0.016	0.771 ± 0.020

Figure 4-14 shows that when treated with HCl, the conversion of primary sludge achieved $42.6 \pm 1.2\%$ for wet substrate, which was 11.5% higher than that of the primary sludge without acid and alkaline treatment (shown in Figure 4-12), and the conversion rate also increased from 0.647 ± 0.056 mg/hr to 0.888 ± 0.025 mg/hr. This indicated that through the HCl treatment, the enzymes inhibition due to metals may have been reduced, and the activity of enzyme was better exerted, resulting in higher glucose yield from the hydrolysis. The dried substrate yielded $34.2 \pm 1.2\%$ conversion, which was also 9.2% higher than that of the dried primary sludge without acid and alkaline treatment.

It is also shown in Figure 4-14 that when both acid and alkaline pre-treatment were performed on the primary sludge, the enzymatic conversion reached $54.2 \pm 4.0\%$ and $37.0 \pm 4.0\%$, and glucose conversion rate was 1.128 ± 0.083 mg/hr and 0.771 ± 0.020 mg/hr for wet and dry substrates respectively. Such results proved the value of acid and alkaline pre-treatment on the glucose conversion from enzymatic hydrolysis once again. Moreover, it implied that considerable cellulose is contained in the primary sludge on a dry mass basis, and it might be worthwhile to explore recovery for energy use.

4.3.4 Enzymatic hydrolysis of digested sewage

Enzymatic hydrolysis was conducted on raw and KOH-treated digested sludge (biosolids). With the exception of the substrate loadings, which were 150 mg instead of 50mg, all other experimental conditions of KOH treatment and enzymatic hydrolysis were the same as in the prior trials. The conversion percentages and glucose conversion rates are listed in Table 4-19.

As can be seen from Table 4-19, only about 1% or less of digested sludge was converted in all four trials, and the conversion rates were also very low even though high substrate loadings were used. This suggested that the usable cellulose in the sludge was consumed first during the activated sludge process and then the anaerobic digestion. Thus, in order to be viable as a feedstock, primary sludge would need to be employed for the enzymatic hydrolysis to yield substantial glucose and, subsequently, ethanol.

Table 4-19 Enzymatic hydrolysis of raw and KOH-treated biosolids

Substrate type		Conversion, %	Glucose conversion rate, mg glucose/hr
Biosolids without pre-treatment	dry	1.1±0.1	0.067±0.009
	wet	0.5±0.2	0.033±0.012
KOH-treated biosolids	dry	1.0±0.3	0.065±0.021
	wet	0.9±0.5	0.057±0.028

4.3.5 Mass flow of primary sludge treatment

The results from the previous experiments show that the wet primary sludge with HCl treatment followed by KOH treatment had the highest glucose yield. To have

a better understanding of the entire process, the mass flow throughout the process was recorded and is presented below:

Figure 4-15 Mass flow of primary sludge treatment

Figure 4-15 demonstrates that after HCl treatment, 100g (dry weight) primary sludge yielded 78.8g solid, and it was reduced to 59.7g after KOH treatment. This is consistent with the high enzymatic conversion rate of HCl and KOH treated sludge because 40.3g non-cellulosic content was separated during the pre-treatment. Overall 32.4g glucose was produced from 100g primary sludge.

4.4 Volatile solids content in poultry manure and sludge

Unlike the crop residue, poultry manure and sewage sludge have a high ash content. Thus, examination of the volatile solids (VS) in these two materials may assist in interpreting their respective behavior during enzymatic hydrolysis. The percentage VS of poultry manure measured at each stage of the process is listed in Table 4-20. The enzymatically hydrolyzed residue is from the trial which used the KOH-treated and ground poultry manure as a substrate (see section 4.2.3.2).

Table 4-20 Volatile solids of poultry manure in different treatment stages

Material type	Raw poultry manure	KOH treated poultry manure	Enzymatically hydrolyzed residue
Volatile solids, %	66.8±2.4	78.4±1.0	75.1±0.8

Table 4-20 demonstrates that the volatile solids in raw poultry manure were lower ($66.8 \pm 2.4\%$) compared to crop residues, this was attributed to the sand contained in the manure. The high ash content in poultry manure also contributed to its lower conversion rate during enzymatic hydrolysis. Table 4-19 also shows that the volatile solids in enzymatically hydrolyzed residue were lower than that of the substrates entering the hydrolysis. This supports the conclusion that the cellulose content in the substrate was converted into glucose during enzymatic hydrolysis.

Table 4-21 shows the volatile solids in primary sludge, activated sludge and digested sludge. It can be seen that the primary sludge has much higher volatile solids than both activated sludge and digested sludge. This is consistent with the distinct differences in the enzymatic conversion rates that were observed for these feedstocks, and also suggests that the cellulose content in the primary sludge should be much higher than in the other two which are further along the wastewater treatment process at ROPEC.

Table 4-21 Volatile solids of sewage sludge

Sludge type	Primary sludge	Activated sludge	Digested sludge
Volatile solids, %	83.6 ± 0.8	65.7 ± 1.6	57.2 ± 1.3

4.5 Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is widely used to identify the functional groups in complex organic mixtures and compare the similarities

between substances. To further examine the component changes brought on by the enzymatic hydrolysis, FTIR was performed to analyze both the feedstocks and the hydrolyzed residues for crop residues and poultry manure. Their spectra were compared with published literature to identify the functional groups observed for each of these materials (Socrates, 1994; Coates, 2000). FTIR was also performed on the three types of sewage sludge- primary sludge, activated sludge and digested sludge, to compare their difference in functional groups on a dry mass basis.

4.5.1 FTIR of crop residues

The FTIR spectra of corn stalk presented in Figure 4-16 include the KOH treated substrate prior to entering the enzymatic hydrolysis and one typical enzymatically hydrolyzed residue. The spectra of hydrolyzed residues of all the trials on corn stalk and bagasse can be found in Appendix D. Upon examination of these spectra, it was found that they all possessed fairly similar peaks, which would imply that for the same substrate, the residues from different enzymatic hydrolysis processes would yield similar functional groups. Thus, the residue of a typical trial (40° C, enzyme 800 units/g, 24 hours) was selected to compare with the feedstock entering the enzymatic hydrolysis process. The functional groups assignment is summarized in Table 4-22.

Figure 4-16 FTIR spectra for corn stalk

Table 4-22 Functional group assignment for the spectra of corn stalk

Observed Peaks (cm ⁻¹)	Functional group assignment	Comment
3400	Alcoholic OH or N-H stretch of aliphatic primary amine	PR
2900~2880	CH stretching vibration	PR
1660	Alkenyl or aromatic C=C stretching	PR
1600	Conjugated C=C vibration	R
1510	Symmetric aromatic ring stretch or NH deformation	R
1470	Methyl C-H asymmetric/symmetric bending	R
1380	CH ₃ asymmetric/symmetric bend, aliphatic nitro compounds	PR
1240	Aromatic ethers or aromatic phosphates	PR
1170	Secondary amine CN stretching or C-O-C vibration in ethers	PR
1055	Primary alcohol stretch	PR
900	Aromatic CH stretch out-of-plane bending	PR
850	Aromatic C-H bending	R

Note: P- Observed in the spectrum of pre-treated corn stalk

R- Observed in the spectrum of enzymatically hydrolyzed residue

PR- Observed in both spectra

It can be seen in Figure 4-16 that beyond the wavenumber 1700 cm^{-1} , the two spectra have almost identical peaks. The broad band centered at 3400 cm^{-1} can be assigned to alcoholic OH or the N-H stretch of aliphatic primary amine. A peak at $2900\text{--}2880\text{ cm}^{-1}$ represents the vibration of CH stretching. The absorption at 1660 cm^{-1} for both spectra indicates alkenyl or aromatic C=C stretching. The spectra of hydrolyzed residue shows a peak at 1600 cm^{-1} , which can be attributed to conjugated C=C. There is also a unique peak for the hydrolyzed residue at 1510 cm^{-1} , which can be assigned to symmetric aromatic ring stretching or NH deformation. In addition, the absorption at 1470 cm^{-1} for the hydrolyzed residue indicates the presence of methyl C-H asymmetric/symmetric bending. Both spectra show analogous weak peaks between 1400 and 900 cm^{-1} . The band at 1380 cm^{-1} can be attributed to CH_3 asymmetric/symmetric bending or aliphatic nitro compounds, or organic sulphates; and the absorption at 1320 cm^{-1} shows aromatic amino CN stretching. The weak band at 1240 cm^{-1} is assigned to aromatic ethers or aromatic phosphates, and the moderate absorption at 1170 cm^{-1} is related to secondary amine CN stretching or C-O-C vibration in ethers. There is a broad and strong absorption centred at 1055 on both spectra, which can be attributed to primary alcohol stretching. The peak at 900 cm^{-1} is assigned to aromatic CH stretching or out-of-plane bending. A sharp band appears at 850 cm^{-1} only on the spectrum of hydrolyzed residue would indicate the aromatic C-H bending.

The two spectra were tentatively compared to published literature of cellulose (Cao & Tan, 2002) and lignin (Ghosh, 1998). As can be seen from Figure 4-17

and 4-18, the spectra from these materials also share many common elements. However, in comparing the spectra for corn stalk, it can be seen that the spectrum for pre-treated corn stalk is quite analogous to that of cellulose, which implies that the dominant component in the pre-treated substrate was cellulose. Similarly, the spectrum of the residue from enzymatic hydrolysis shared common elements with that of lignin, especially in the region of 1700 cm^{-1} to 800 cm^{-1} , which contained most of the differences between the two spectra presented in Figure 4-16. The three peaks which are located at 1600 , 1510 and 850 cm^{-1} respectively, were observed on the spectra of hydrolyzed residue but not in the pre-treated substrate. These can be found on the spectrum for lignin shown in Figure 4-18. These peaks can be assigned to benzene ring stretching, which is a common functional group in lignin compounds. This comparison might suggest that in the pre-treated substrate, the main compound presented was cellulose and lignin was not conspicuous. After the hydrolysis, most of the cellulose having been converted to glucose, the lignin became more prominent. Therefore the spectrum of residue from enzymatic hydrolysis shows similar peaks as lignin. It should be noted that because the lignin itself is composed of many complex compounds, it is difficult to be quantified from the FTIR spectra, however it is a useful supplement in the analysis of material component and lends support to some of the results observed in these experiments which have been included in these discussions.

Figure 4-17 Published FTIR spectrum for cellulose (Cao and Tan, 2002)

Figure 4-18 FTIR spectrum of lignin (Ghosh, 1998)

4.5.2 FTIR of poultry manure

FTIR was also performed on both the pre-treated poultry manure and the residue from enzymatic hydrolysis. Figure 4-19 shows the spectra for these two samples

from the KOH-treated and ground poultry manure trial. The other spectra are presented in Appendix D. Interpretation of the two spectra is listed in Table 4-23.

Examination of the functional groups presented in the spectra helps to identify the difference in composition between the pre-treated poultry manure and residue from enzymatic hydrolysis, thus the effect of enzymatic hydrolysis is better illustrated. Table 4-23 shows that the peak at 1050 cm^{-1} which represents primary alcohol C-O stretching only appeared on the spectrum of pre-treated poultry manure. Because the alcohol group exists in the glucose molecule which is the basic unit of the cellulose chain structure, this would lend support to the argument that the cellulose in the substrate was enzymatically hydrolyzed and removed after the hydrolysis, and was not retained in the residue. In addition, a peak at 1095 cm^{-1} , which stands for the organic siloxane or silicone, was only observed on the spectrum of residue from enzymatic hydrolysis. This is consistent with the fact that a fair amount of white ash was found in the residue. Although the mechanism of the formation of this ash is unknown and beyond the scope of this study, the composition change during the hydrolysis is reflected by the spectra.

Figure 4-19 FTIR spectra for poultry manure

Table 4-23 Functional group assignment for the spectra for poultry manure

Observed Peaks (cm ⁻¹)	Functional group assignment	Comment
3440	O-H stretching of peroxides or secondary aromatic amines	PH
2930	Asymmetric CH ₂ stretching	PH
2850	Symmetric CH ₂ stretching	PH
2550	Free SH stretching vibration	PH
1725	Carboxylic acid or ketone	H
1640	Organic nitrates	P
1620~1550	Carboxylate or conjugated C=C vibration	H
1470~1430	Methyl C-H in plane bending	H
1420	Organic sulfates	P
1310~1290	Vinylidene C-H in plane bending	H
1150	Tertiary amine CN stretching	H
1095	Organic siloxane or silicone	H
1050	Primary alcohol C-O stretching	P
895	CH out-of-plane bending in substituted ethylenic systems	PH
840	Two neighbouring aromatic C-H	H

Note: P- Observed in the spectrum of pre-treated poultry manure
H- Observed in the spectrum of hydrolyzed manure residue
PH- Observed in both spectra

4.5.3 FTIR of municipal sewage sludge

It was shown in section 4.3 that there were considerable differences in the glucose yield from primary sludge, activated sludge and digested sludge, which suggested that the cellulose content in these materials varied from one another. To further examine their differences in content, FTIR was performed on the three pre-treated (HCl followed by KOH) feedstocks, their corresponding spectra are shown in Figure 4-20 and the interpretation of functional groups is presented in Table 4-24.

Figure 4-20 FTIR spectra of pre-treated sewage sludge

Table 4-24 Functional group assignment for the spectra of poultry manure

Observed Peaks (cm ⁻¹)	Functional group assignment	Comment
3400~3350	Aliphatic primary amine NH stretching	RAD
2915	Methylene C-H asymmetric/symmetric stretching	RAD
2850	Methylene C-H asymmetric/symmetric stretching	RAD
1650	Primary amides in solid state	RAD
1590	α -amino carboxylates $R_2N.CH_2.COO^-$	R
1540	Secondary amides	RAD
1450~1430	Carboxylic acids, or aliphatic nitro compounds	RAD
1370	Aryl aldehydes, or organic sulphates	R
1320	Alkyl ketones	R
1250	CH deformation of CH_3 or CH_2 bending	A
1170	Ester C-O-C stretching vibration	R
1120	Ester asymmetric vibration	R
1060~1040	Organic siloxane or silicone	AD
900~880	Aromatic CH out of plane bending	RAD

Note: R- Observed in the spectrum primary sludge

A- Observed in the spectrum of activated sludge

D- Observed in the spectrum of digested sludge

The activated sludge and digested sludge had similar spectra, with the exception that the former had a peak at 1250 cm⁻¹, which can be assigned to aliphatic compounds. This might indicated the presence of carbohydrates in the activated sludge. The spectrum for primary sludge was also similar to the other two; the main difference that was observed was the presence of peaks between 1400 cm⁻¹ and 1000 cm⁻¹ which was not apparent on the activated sludge and digested sludge. This region is associated to the carbonyl groups including ketones, esters and aldehydes which are all cellulose related groups. To sum up, examination of the FTIR spectra suggested that the cellulose content in the primary sludge might be much more prominent than in the activated sludge and digested sludge. This

is consistent with the high glucose yield that was observed in the primary sludge compared to the other two feedstocks.

4.6 Process design and cost analysis

4.6.1 Ethanol production from crop residues

4.6.1.1 Proposed integrated process of ethanol production

The process following the hydrolysis of cellulose would be the fermentation of glucose to produce ethanol. This is a mature process and there are a number of organisms available for fermentation, among them the yeast *saccharomyces cerevisiae* is most widely used due to its high ethanol productivity, ability to function under both aerobic and anaerobic conditions, and high tolerance for ethanol. However, separate hydrolysis and fermentation is generally not favoured because of the high process cost. Instead Simultaneous Saccharification and Fermentation (SSF), which combines the enzymatic hydrolysis and fermentation in one vessel, is a preferred alternative. Combining the two processes in one reactor reduces both the capital and operational cost. Moreover, because the glucose converted from hydrolysis is fermented to ethanol by yeast, the end-product inhibition of enzymatic hydrolysis can be prevented, resulting in a higher reaction rate. Based on the process adopted in this study, also referring to some recent researches in this area (Wooley *et al.*, 1999; McAloon *et al.*, 2000), an integrated process for ethanol production from corn stalk is proposed, the scheme is presented in Figure 4-21. Based on process optimization conducted in this study, wet substrate is employed for enzymatic hydrolysis.

The collected raw corn stalk would first be ground into desired particle size, and then treated with 0.5N KOH at 70°C. After being treated for one hour, the mixture would be filtered. The solid fraction would then be washed and neutralized with 1.0N HCl, and the liquid which contains lignin and alkaline be sent to an evaporator to enhance the solid concentration. The condensed liquor would be burned in a recovery boiler, the heat generated used in other heat consuming processes, and the residue sent to chemical recovery system which recovers the KOH for pre-treatment use.

The pre-treated solid substrate would be sent to the main SSF reactor where both hydrolysis and fermentation would take place. To ensure that the conversion of glucose to ethanol will not be affected, further study would be required on the pre-treatment process. The temperature in the SSF reactor would be maintained at 40°C with cellulose enzyme and yeast added at certain time intervals. The reactions would be conducted continuously with a retention time of 24 hours. Ethanol would be produced at low concentrations after the fermentation; to prevent the yeast from being killed, the concentration should be kept lower than 14%. Enzyme in the discharged ethanol solution could be recycled through ultrafiltration, it would then be treated to enhance activity before reuse. Ethanol would be led to the distillatory, and purified to about 95% (w/w). The solid residues from the SSF contain mainly of lignin; it could then be burned in the recovery boiler to recover its heat energy.

Figure 4-21 Scheme of ethanol production process

4.6.1.2 Cost analysis

The operational cost of ethanol production can be broken down into process blocks which are: feedstock, pre-treatment, SSF, distillation and solids recovery, and accessories. The following is the description of each part for the process using corn stalk:

1. Feedstock

This section includes the cost of raw material and its collection and transportation. Because corn stalk is usually treated as waste on farms, it can be obtained at low price. Thus, the main cost would be associated with the transportation of materials. This could be reduced by optimization in site selection, so that the distance from the plant to all feedstock supplying farms would be minimized. The feedstock cost used in analysis is \$25 (CAD) per dry ton (Gallagher *et al.*, 2003).

2. Pre-treatment

Pre-treatment includes material grinding, KOH treatment, filtration and neutralization. The primary costs of this portion of the process would come from use of chemicals, energy and water consumption. The chemicals employed in pre-treatment include KOH for treatment and HCl for neutralization. Most of the KOH could be recovered and recycled through the chemical recovery furnace, HCl would also be needed in small quantity. The energy consumption includes electricity required in grinding and materials handling, and the heat energy necessary to maintain the desired treatment

temperature. However, the latter could be supplied from the incineration of lignin in the recovery boiler. Therefore the main cost for pre-treatment would consist of electricity and water. Based on the experimental data from this study, the pre-treatment cost is estimated to be \$0.81/litre ethanol, the detailed calculations are presented in Appendix E.

3. SSF

The cost of saccharification and fermentation consists primary of energy, enzymes and yeast. Enzyme cost are the greatest consideration in this portion of the process due to their high prices. It represents the critical factor that that would establish the economical viability of the entire production process. It is believed that the future of cost effective cellulose derived ethanol production will depend on the development of low price and high activity cellulase enzyme. Fortunately, recent progresses in the genetic and biochemistry areas have brought down the cost of enzyme considerably. Twenty-fold reduction in cellulase enzyme cost has been achieved by Novozymes in February 2004

(http://www.biospace.com/news_story.cfm?StoryID=15882620&full=1, 2004).

Because enzyme is not consumed during the hydrolysis, it can be recycled and reused in SSF. Another approach to reduce the enzyme cost is to produce it from the pre-treated cellulose. Based on the experimental data from this study and reference information (McMillan, 2000), the cost in this portion is estimated to be \$3.97/litre ethanol.

4. Distillation and solids recovery

Distillation is necessary to purify the ethanol from the effluence by vaporization and condensation. The heat energy required to vaporize ethanol would be supplied by the heat from recovery boiler, so the main cost is the electricity consumed in fluid handling. The bottoms from distillation usually contains unconverted solids, they can be sent to the recovery boiler and burned to recover the heat energy. Another important function of the recovery system is to recover the chemicals used in the pre-treatment process, which would minimize the chemical usage and thus reduce the operational cost. This process would be energy consuming because of the operation of a furnace to recover the KOH. The estimated operational cost in distillation and chemical recovery would be \$0.063/litre ethanol (Wooley *et al.*, 1999).

5. Accessories

The main accessories cost includes wastewater treatment and product storage. The wastewater treatment would include anaerobic digestion followed by aerobic lagoon, then the water would be clarified and filtered. The methane generated from the digestion and the sludge after filtration can be burned in the furnace, and the treated water can be reused. The operational cost of wastewater treatment and product storage would be the energy consumed in the materials handling, it is estimated to be \$0.04/litre ethanol (Wooley *et al.*, 1999).

Based on experiment data and the above analysis, the cost of producing ethanol from corn stalk was estimated and summarized in Table 4-25.

Table 4-25 Cost estimate for ethanol production from corn stalk

Section	Cost to produce 10000 litres of ethanol, \$	Unit cost, \$/litre	Percentage, %
Feedstock	960.4	0.10	1.93
Pre-treatment	8057.33	0.81	16.20
SSF	39684.9	3.97	79.80
Enzyme	38821.3	3.88	78.06
Distillation and solids recovery	630.0	0.06	1.27
Accessories	400.0	0.04	0.80
Total	49732.6	4.97	100

The cost estimation in Table 4-25 shows that the unit cost of ethanol produced from corn stalk is \$4.70/litre, which is about six times of the retail fuel price. The enzyme cost accounts for about 80%, and the pre-treatment accounts for 16% of the total cost. Therefore to achieve economical viability in producing ethanol from crop residues, substantial cost reduction in both enzyme and pre-treatment is required.

4.6.2 Ethanol production from poultry manure

4.6.2.1 Proposed process interpretation

Similar to that of corn stalk, an integrated ethanol production process from poultry manure is proposed, and the scheme is presented in Figure 4-22. The feedstock would first be gritted to remove the sand, and then treated with 0.5N KOH at 70°C. After being treated for one hour, the mixture would be filtered. The

solid fraction would be washed and neutralized with 1.0N HCl, and then it would be dried and ground. The liquid would be condensed in an evaporator then burned in a recovery boiler to provide heat energy for other processes. The heat generated could then be used in other heat consuming processes, and the residue would be sent to chemical recovery system which recovers the KOH for pre-treatment use.

The ground solid substrate would be sent to the main SSF reactor, in which cellulose enzyme and yeast would be added and the temperature maintained at 40°C. The reactions would be conducted continuously with a retention time of 24 hours. Ethanol would be produced at low concentrations in the fermentation, it would then be distilled and purified to about 95% (w/w). The cellulase enzyme contained in the effluent could be recycled, and the final residue would then be burned in the recovery boiler to recover its heat energy.

Figure 4-22 Scheme of ethanol production from poultry manure

4.6.2.2 Cost estimation for poultry manure process

Based on the experimental results obtained in this study, as well as the cost analysis of corn stalk process, the operational cost of ethanol production from poultry manure is estimated. Similar to that of corn stalk, it can also be divided into five portions: feedstock, pre-treatment, SSF, distillation and solid recovery, and accessories. The detailed calculation is shown in Appendix E.

The primary feedstock cost would be the transportation of poultry manure, which is decided by the hauling distance. This could be minimized by optimization in site selection. The feedstock cost was estimated to be CAD\$15/ton (Timmenga & Associates Inc., 2003), it would be \$55.6 per dry ton considering a moisture content of 73%.

The pre-treatment would include gritting, KOH treatment, filtration, drying and grinding. The operational cost involved would include the energy required in material handling and grinding, the water consumed in the process, and the chemicals required in the treatment. The heat energy needed in drying could be supplied from the recovery furnace. Based on the experimental data from this study, the pre-treatment cost was estimated to be \$85.4/dry ton poultry manure.

As to that of corn stalk, different substrate quantity would be used to produce certain amount of ethanol from poultry manure. Therefore the operational cost of energy and water consumption for poultry manure would be different to that of corn stalk, while the enzyme and yeast cost would be the same for the two

feedstocks. The SSF cost for poultry manure is estimated to be \$3.96/litre ethanol based on the experimental and reference data.

The cost in distillation and solids recovery, as well as the accessories, would be the same as that of corn stalk. The entire process cost is summarized in Table 4-26.

Table 4-26 Cost estimate for ethanol production from poultry manure

Section	Cost to produce 10000 litres of ethanol, CAD \$	Unit cost, \$/litre	Percentage, %
Feedstock	4738.5	0.47	9.00
Pre-treatment	7285.3	0.73	13.84
SSF	39583.8	3.96	75.20
Distillation and solids recovery	630.0	0.06	1.20
Accessories	400.0	0.04	0.76
Total	52637.6	5.26	100.00

It is shown in Table 4-26 that the operational cost in producing one litre of ethanol from poultry manure would be \$5.26. The cost in SSF portion, which would primary be the cost of enzyme, accounts for about 75% of the entire cost. The other two main portions of the cost would be feedstock and pre-treatment, they account for about 9% and 14% of the entire cost, respectively. In order to make the ethanol from poultry manure process economically viable, not only the enzyme price should be lowered greatly, but also the cost in feedstock and pre-treatment would need to be reduced.

4.6.3 Ethanol production from primary sludge

4.6.3.1 Proposed process interpretation

The results in section 4.3 show that for the highest conversion percentage from primary sludge, both HCl and KOH pre-treatment were needed. However, the prior cost analysis shows that the pre-treatment is expensive. The KOH treatment would cost over \$0.70 for producing one litre ethanol, this would be at least doubled if HCl treatment is involved due to the high cost in acid recovery. In addition, the increase in conversion percentage achieved by the pre-treatment for primary sludge was not as tremendous as for crop residues. Therefore, chemical pre-treatment is not employed in the proposed process. Figure 4-23 represents the scheme of the process.

Without the chemical pre-treatment, the overall process is much simplified. The primary sludge would first be conditioned to bring the pH to about 7.0, then it would be dewatered through gravity thickening to a solid content of about 10%. The wet sludge would then be sent to the SSF reactor in which enzymatic hydrolysis and fermentation take place. The retention time in the SSF reactor would be 24 hours and ethanol would be converted in low concentration, it is then purified through distillation. The solid bottoms from distillation could be burned in the recovery boiler to recover the heat energy.

Figure 4-23 Scheme of ethanol production from primary sludge

4.6.3.2 Cost estimation for primary sludge process

Based on the experimental results obtained in this study and the prior cost analysis for corn stalk and poultry manure process, the operational cost of ethanol production from primary sludge is estimated. Same as that of the other two feedstocks, the operational cost of ethanol production from primary sludge can be divided into five portions: feedstock, pre-treatment, SSF, distillation and solid recovery, and accessories.

To minimize the feedstock cost, the ethanol from the primary sludge plant can be co-located with the wastewater treatment plant. Thus the primary clarified raw sludge could be pumped directly to the ethanol plant, and the feedstock cost would be incurred from the energy consumed in delivery of primary sludge. This portion of cost is estimated to be \$0.06/litre ethanol (calculations are presented in Appendix E).

The pre-treatment would include the neutralization and thickening of raw sludge. Therefore the primary cost of this portion would consist of the chemicals required to neutralize the sludge, and the energy consumption in the material conveyance and equipment operation. The operational cost in pre-treatment is estimated to be \$0.18/litre ethanol (calculations are listed in Appendix E).

The cost in SSF would be similar to that of corn stalk and poultry manure, which is mainly consist of cost in enzyme, yeast, energy and water. Because different quantity of substrate would be used to produce certain amount of ethanol, the cost in energy and water would vary by the type of feedstock. While the cost of enzyme and yeast would remain constant. The cost in SSF to produce one litre ethanol is estimated to be \$3.98 (detailed calculations are shown in Appendix E).

Since chemical pre-treatment would not be employed in the primary sludge process, chemical recovery would not be required. In addition, the wastewater generated during the process could be treated in the adjacent sewage plant. Therefore, the cost in solids recovery and accessories would be substantially reduced. The cost is estimated to be \$0.03/litre ethanol for distillation and solids

recovery and \$0.02/litre ethanol for accessories, which would be half of that of corn stalk. The entire process cost is summarized in Table 4-27.

Table 4-27 Cost estimate for ethanol production from primary sludge

Section	Cost to produce 10000 litres of ethanol, CAD \$	Unit cost, \$/litre	Percentage, %
Feedstock	561.6	0.06	1.32
Pre-treatment	1841.2	0.18	4.31
SSF	39774.5	3.98	93.17
<i>Enzyme</i>	38821.3	3.88	90.93
Distillation and solids recovery	315.0	0.03	0.74
Accessories	200.0	0.02	0.47
Total	42692.4	4.27	100.00

It is shown in Table 4-27 that the operational cost to produce one litre of ethanol from primary sludge would be \$4.27, about 91% of which is from the cost of enzyme. To achieve economically viable in ethanol production from primary sludge, a substantial decrease in enzyme cost would be required. It can also be seen from Table 4-27 that because of the lower cost in feedstock and pre-treatment, the overall operational cost of primary sludge process would be less than that of the other two feedstocks. This would suggest that once the price of cellulase enzyme is reduced sufficiently, primary sludge would be a potential feedstock for ethanol production.

Chapter 5 Conclusions and Recommendations

5.1 Conclusions

The enzymatic hydrolysis of three potential feedstocks for ethanol production was studied. For alkaline pre-treated corn stalk, the glucose yield from enzymatic hydrolysis increased with time. However, the hydrolysis rate was found to be the highest in the beginning, and decreased with time due to the accumulation of end-products. Through the comparison of enzymatic conversion rates at different reaction times, 24 hours was determined to be the appropriate reaction time. In addition, increasing the enzyme loading resulted in higher conversion rate, but the glucose yield per unit enzyme decreased as the enzyme loading increased. 800 units/g substrate was selected as the optimum enzyme loading by comparing the conversion rate of hydrolysis to that of 400 units/g substrate and 1600 units/g substrate. It was also demonstrated in experiments that the enzyme activity was subject to change at different temperatures. The optimum temperature for the enzyme used in this study was found to be 40°C. Either increasing the temperature to 50°C or decreasing it to 35°C resulted in lower glucose yields.

The enzymatic hydrolysis of alkaline pre-treated bagasse showed the same characteristics as corn stalk, except that its conversion rate was lower than that of corn stalk under the same experimental conditions. After 24 hours enzymatic hydrolysis at 40°C, the glucose yields for KOH treated corn stalk and bagasse were 65.4% and 51.1% respectively.

The pre-treatment processes used in this study include alkaline treatment drying, and grinding. The sequence of these processes affected the enzymatic hydrolysis rate. When the crop residue was ground before alkaline treatment and the substrate was used wet, higher conversion rates were achieved compared to the trial using dry substrate which was first treated with an alkaline solution and then ground. This suggested that the alkaline treatment was more effective in cellulose extraction when the crop residue was ground first.

The hydrolysis rate was also improved by adding enzyme gradually rather than all in the beginning, suggesting that fresh enzyme has higher activity.

At 40°C, with an enzyme loading 400 units/g substrate, the hydrolysis of raw poultry manure resulted in $7.1 \pm 0.3\%$ conversion in 24 hours. When the poultry manure was pre-treated with 0.5N KOH and hydrolyzed wet, no significant increase in enzymatic conversion rate was obtained. On the other hand, physical pre-treatment was effective for enzymatic hydrolysis. When the KOH treated poultry manure was dried at 70°C, the glucose yield of enzymatic hydrolysis doubled to $13.9 \pm 0.7\%$. Moreover, when the KOH treated and dried substrate was ground before enzymatic hydrolysis, the glucose yield was increased to $27.6 \pm 1.7\%$. Such results suggested that the main barrier for the enzyme to access the cellulose in poultry manure was the organic substances that stick to the cellulose.

Although the enzymatic conversion rates of poultry manure was much lower than that of crop residues, by increasing the substrate loading to three times as that of corn stalk, approximately the same glucose concentration could be obtained using the same quantity of enzyme and buffer.

When wet primary sludge was enzymatically hydrolyzed at 40°C with an enzyme loading of 800 units/g substrate, $31.1 \pm 2.7\%$ of it was converted into glucose in 24 hours. Under the same conditions, the conversion rate for dried primary sludge was $25.0 \pm 0.8\%$. Unlike the crop residues, the main cellulose in sewage sludge is from waste paper. Thus, the KOH pre-treatment on primary sludge had little effect on the hydrolysis, the enzymatic conversion rate for KOH treated wet primary sludge was $35.4 \pm 1.2\%$. On the other hand, HCl treatment was more effective to remove the non-cellulose content in primary sludge. The enzymatic conversion of HCl treated wet primary sludge increased to $42.6 \pm 1.2\%$. When the combination of HCl and KOH treatment was performed, $54.2 \pm 4.0\%$ conversion was achieved in the enzymatic hydrolysis of wet primary sludge. Dry substrates generally yielded less glucose than wet ones.

Because the cellulose in sewage sludge may be consumed in the activated sludge process and further degraded in the anaerobic digestion process, enzymatic hydrolysis of activated sludge yielded much less glucose than primary sludge, and almost no glucose was converted from digested sludge.

FTIR analysis showed that the content of KOH treated crop residues was dominated by cellulose, and the enzymatic hydrolyzed residue mainly consisted

of lignin. The peaks associated to the functional groups in cellulose were also observed on the spectrum of KOH treated poultry manure. In addition, comparing the spectra of three types of sludge indicated that cellulose was contained in primary sludge but not activated sludge and digested sludge.

The cost of producing ethanol from corn stalk through simultaneous hydrolysis and fermentation was estimated to be \$4.97/litre, the primary portion of which was from cellulase enzyme. Substantial reduction in enzyme cost would be required in order to make the ethanol from cellulose process economically viable.

For ethanol production from the poultry manure process, the operational cost was estimated to be \$5.26/litre ethanol. In addition to the expensive enzyme, it also had high costs in feedstock and pre-treatment, which would make it difficult to achieve economical viability for such a process.

Although HCl and KOH treatment are demonstrated to be effective for the enzymatic hydrolysis, chemical pre-treatment was not used in the proposed ethanol from primary sludge process due to the high operational cost. The overall cost for this process was estimated to be \$4.27/litre ethanol, lower than that of corn stalk and poultry manure. Once the price of enzyme is lowered, primary sludge might be a possible feedstock for ethanol production.

5.2 Recommendations

Recommendations and suggestions of future work on this study are as follows:

- Detailed characterization of substrates and products of enzymatic hydrolysis to show the whole picture and obtain better control of the reactions, useful analysis methods include HPLC, NMR and IR, etc.
- Test of Simultaneous Saccharification and Fermentation to integrate the ethanol producing process and achieve higher reaction rates.
- Further research on pre-treatment to ensure that the subsequent enzymatic hydrolysis and fermentation would not be hindered.
- Further investigation in enzyme recycling to reduce the process cost.
- Examination in the use of enzymes that convert hemicellulose into sugars that can be further fermented into ethanol. Thus the ethanol yield from certain quantity of raw materials could be substantially increased.
- Further examination of approaches to remove non-cellulose content in poultry manure in order to achieve higher conversion rate and reduce the quantity of substrate entering the hydrolysis.
- Examination into the recycle of nutritional content in poultry manure.
- Examination into the process on a continuous basis and retain the enzyme in process so as to maximize its activity.
- Further examination into other potential feedstocks, as well as pre-treatment processes and enzymatic hydrolysis conditions that may increase the glucose yield and reduce the operational cost.

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APPENDIX A

Experimental Results

A.1 Enzymatic hydrolysis of corn stalk and bagasse with time

Raw corn stalk

	Substrate, mg	Enzyme, mg	Reaction time, hr	Hydrolyzed residue, mg		Substrate, mg	Enzyme, mg	Reaction time, hr	Hydrolyzed residue, mg
RC8-1	50.10	4.70	8.00	48.30	RC16-1	49.90	4.90	16.00	48.60
RC8-2	50.20	4.80	8.00	48.60	RC16-2	50.30	4.80	16.00	48.70
RC8-3	50.00	4.70	8.00	48.50	RC16-3	50.00	4.80	16.00	48.50
RC8-C	49.80	0.00	8.00	49.80	RC16-C	49.90	0.00	16.00	49.90
RC24-1	50.00	4.90	24.00	48.30	RC32-1	50.20	4.70	32.00	48.30
RC24-2	49.70	4.90	24.00	48.10	RC32-2	50.00	4.80	32.00	48.10
RC24-3	50.00	5.10	24.00	48.60	RC32-3	50.00	4.60	32.00	48.00
RC24-C	50.00	0.00	24.00	50.00	RC32-C	49.70	0.00	32.00	49.70
RC40-1	49.80	4.60	40.00	47.20	RC48-1	50.30	4.80	48.00	47.00
RC40-2	49.60	4.70	40.00	47.30	RC48-2	50.00	4.90	48.00	47.80
RC40-3	50.00	4.90	40.00	47.90	RC48-3	50.00	4.60	48.00	47.70
RC40-C	50.20	0.00	40.00	50.00	RC48-C	49.90	0.00	48.00	49.90

KOH-treated corn stalk

KC8-1	49.70	4.80	8.00	32.50	KC16-1	50.30	4.90	16.00	26.10
KC8-2	50.20	4.60	8.00	32.90	KC16-2	50.10	4.80	16.00	25.80
KC8-3	50.00	4.60	8.00	32.70	KC16-3	50.00	4.70	16.00	25.60
KC8-C	49.90	0.00	8.00	49.60	KC16-C	50.30	0.00	16.00	50.30

Temperature = 40 °C, enzyme loading = 800 units/g substrate, buffer = 2.5ml

	Substrate, mg	Enzyme, mg	Reaction time, hr	Hydrolyzed residue, mg		Substrate, mg	Enzyme, mg	Reaction time, hr	Hydrolyzed residue, mg
KC24-1	50.10	4.70	24.00	20.90		KC32-1	50.30	4.90	21.50
KC24-2	50.20	4.60	24.00	20.60		KC32-2	49.80	4.70	21.10
KC24-3	50.00	4.90	24.00	21.00		KC32-3	50.00	4.80	21.30
KC24-C	50.00	0.00	24.00	50.00		KC32-C	50.20	0.00	50.00
KC40-1	50.10	4.80	40.00	17.50		KC48-1	49.70	4.80	15.20
KC40-2	50.00	4.70	40.00	17.10		KC48-2	49.90	4.70	15.50
KC40-3	50.00	4.90	40.00	16.70		KC48-3	50.10	4.60	15.80
KC40-C	49.80	0.00	40.00	49.70		KC48-C	50.20	0.00	49.80

KOH-treated bagasse

KB8-1	50.10	4.80	8.00	35.00		KB16-1	50.30	4.80	16.00	30.20
KB8-2	50.40	4.70	8.00	34.80		KB16-2	49.80	4.70	16.00	30.10
KB8-3	50.00	4.60	8.00	34.50		KB16-3	50.00	4.70	16.00	30.30
KB8-C	50.20	0.00	8.00	49.90		KB16-C	50.10	0.00	16.00	50.00
KB24-1	49.70	4.60	24.00	26.30		KB32-1	50.20	4.60	32.00	25.20
KB24-2	49.80	4.80	24.00	26.80		KB32-2	49.90	4.70	32.00	25.60
KB24-3	50.00	4.60	24.00	26.20		KB32-3	50.00	4.70	32.00	24.60
KB24-C	49.80	0.00	24.00	49.50		KB32-4	49.80	0.00	32.00	49.70
KB40-1	50.10	4.60	40.00	22.80		KB48-1	49.70	4.90	48.00	20.60
KB40-2	50.30	4.70	40.00	22.00		KB48-2	50.30	4.60	48.00	20.90
KB40-3	50.00	4.70	40.00	21.60		KB48-3	50.20	4.70	48.00	20.80
KB40-C	50.20	0.00	40.00	49.80		KB48-C	49.80	0.00	48.00	49.80

Temperature = 40 °C, enzyme loading = 800 units/g substrate, buffer = 2.5ml

A.2 Enzymatic hydrolysis of corn stalk and bagasse at various temperatures

KOH-treated corn stalk

	Substrate, mg	Enzyme, mg	Temperature, °C	Hydrolyzed residue, mg		Substrate, mg	Enzyme, mg	Temperature, °C	Hydrolyzed residue, mg
CT1-1	50.00	4.90	35	27.60		BT1-1	50.50	4.80	29.10
CT1-2	49.80	5.00	35	27.40		BT1-2	49.80	4.70	28.60
CT1-3	49.70	4.80	35	27.20		BT1-3	50.20	4.70	28.50
CT1-C	50.10	0.00	35	49.90		BT1-C	49.90	0.00	49.90
CT2-1	49.90	4.90	40	19.20		BT2-1	50.00	4.90	26.10
CT2-2	50.40	4.80	40	18.90		BT2-2	49.80	4.90	26.60
CT2-3	49.60	4.80	40	18.60		BT2-3	49.90	4.70	26.00
CT2-C	49.90	0.00	40	49.60		BT2-C	49.80	0.00	49.60
CT3-1	50.50	4.80	50	26.70		BT3-1	50.10	4.80	28.60
CT3-2	49.50	4.70	50	26.10		BT3-2	49.90	4.70	28.50
CT3-3	50.20	4.60	50	25.90		BT3-3	49.80	4.60	28.00
CT3-C	49.80	0.00	50	49.80		BT3-C	50.30	0.00	50.00

KOH-treated bagasse

Reaction time = 24 hours, buffer = 2.5ml for all trials

A.3 Enzymatic hydrolysis of corn stalk and bagasse at various enzyme loadings

KOH-treated corn stalk					KOH-treated bagasse				
	Substrate, mg	Enzyme, mg	Enzyme loading, units/g	Hydrolyzed residue, mg		Substrate, mg	Enzyme, mg	Temperature, °C	Hydrolyzed residue, mg
CE1-1	50.20	2.40	396.81	31.00	BE1-1	50.10	2.40	397.60	31.60
CE1-2	49.60	2.50	418.35	30.50	BE1-2	50.20	2.50	413.35	31.90
CE1-3	49.70	2.40	400.80	30.60	BE1-3	49.80	2.40	400.00	31.50
CE1-C	50.30	0.00	0.00	50.10	BE1-C	50.40	0.00	0.00	50.20
CE2-1	49.90	4.90	815.03	19.20	BE2-1	50.00	4.90	813.40	26.10
CE2-2	50.40	4.80	790.48	18.90	BE2-2	49.80	4.90	816.67	26.60
CE2-3	49.60	4.80	803.23	18.80	BE2-3	49.90	4.70	781.76	26.00
CE2-C	49.90	0.00	0.00	49.60	BE2-C	49.80	0.00	0.00	49.60
CE3-1	50.30	9.80	1617.10	21.60	BE3-1	49.60	9.70	1623.19	26.60
CE3-2	49.80	9.70	1616.67	21.30	BE3-2	50.30	9.80	1617.10	27.10
CE3-3	50.00	9.70	1610.20	21.60	BE3-3	49.70	9.60	1603.22	26.80
CE3-C	50.10	0.00	0.00	50.00	BE3-C	49.70	0.00	0.00	49.80

Reaction time = 24 hours, temperature = 40 °C, buffer = 2.5ml for all trials

A.4 Enzymatic hydrolysis of twice KOH-treated corn stalk

	Substrate, mg	Enzyme, mg	Temperature, °C	Buffer, ml	Reaction time, hours	Hydrolyzed residue, mg
CP-1	50.100	4.80	40	2.50	24	18.60
CP-2	50.100	4.70	40	2.50	24	18.70
CP-3	49.700	4.70	40	2.50	24	18.20
CP-C	50.500	0.00	40	2.50	24	50.10

A.5 Enzymatic hydrolysis of dry vs. wet corn stalk

	Substrate wet weight, mg	Substrate dry mass, mg	Enzyme, mg	Hydrolyzed residue, mg
CD-1		50.30	4.90	20.90
CD-2		49.80	4.80	20.60
CD-3		50.20	4.80	21.20
CD-C		49.80	0.00	49.70
CW-1	517.200	46.03	4.70	15.30
CW-2	520.300	46.31	4.80	15.90
CW-3	518.800	46.17	4.70	15.20
CW-C	520.100	46.29	0.00	46.10

Reaction time = 24 hours, temperature = 40 °C, buffer = 2.5ml

Moisture content of wet substrate

Sample #	Crucible weight, mg	Crucible+wet substrate, mg	Substrate wet weight, mg	Crucible+dry mass, mg	Dry mass, mg	Moisture content, %	Average, %	SD, %
1	11598.2	13754.6	2156.4	11789.0	190.8	91.2	91.07	0.08
2	12157.6	15226.1	3068.5	12434.1	276.5	91.0		
3	10258.5	12906.8	2648.3	10494.7	236.2	91.1		

A.6 Enzymatic hydrolysis of corn stalk with different enzyme addition time

	Substrate, mg	Enzyme added in the beginning, mg	Enzyme added after 12 hours, mg	Hydrolyzed residue, mg
CED1-1	50.20	4.80	0.00	19.10
CED1-2	49.80	4.70	0.00	18.20
CED1-3	50.10	4.90	0.00	19.60
CED1-C	49.60	0.00	0.00	49.50
CED2-1	50.40	2.30	2.40	15.10
CED2-2	49.80	2.50	2.40	14.20
CED2-3	50.40	2.50	2.50	15.30
CED2-C	49.60	0.00	0.00	49.30

Reaction time = 24 hours, temperature = 40 °C, buffer = 2.5ml

A.7 Enzymatic hydrolysis of crop residues with addition of cellobiase

Corn stalk

	Substrate, mg	Enzyme , mg	Cellobiase, ml	Hydrolyzed residue, mg
CC1-1	49.90	4.70	0.00	17.70
CC1-2	50.40	4.60	0.00	17.60
CC1-3	49.60	4.60	0.00	18.10
CC1-C	49.90	0.00	0.00	49.80
CC2-1	50.00	4.80	0.20	17.60
CC2-2	50.30	4.60	0.20	17.20
CC2-3	50.40	4.80	0.20	18.00
CC2-C1	50.10	0.00	0.20	49.90
CC2-C2	0.00	0.00	0.20	0.00

Bagasse

BC1-1	50.00	4.80	0.00	25.50
BC1-2	49.00	4.60	0.00	24.90
BC1-3	49.90	4.60	0.00	25.30
BC1-C	49.80	0.00	0.00	49.90
BC2-1	50.20	4.80	0.20	24.10
BC2-2	50.10	4.70	0.20	23.20
BC2-3	49.90	4.60	0.20	23.60
BC2-C1	50.20	0.00	0.20	49.70
BC2-C2	0.00	0.00	0.20	0.00

Reaction time = 24 hours, temperature = 40 °C, buffer = 2.5ml

A.8 Enzymatic hydrolysis of poultry manure

Raw poultry manure

	Substrate wet weight, mg	Substrate dry mass, mg	Enzyme, mg	Hydrolyzed residue, mg
RP1-1	419.3	49.9	4.6	46.8
RP1-2	421.0	50.1	4.7	47.2
RP1-3	419.3	49.9	4.6	45.6
RP1-C	421.0	50.1	0.0	50.2
RP2-1	838.7	99.8	4.7	94.1
RP2-2	841.2	100.1	4.8	93.5
RP2-3	842.0	100.2	4.8	93.1
RP2-C	844.5	100.5	0.0	100.2
RP3-1	1260.5	150.0	4.9	139.1
RP3-2	1256.3	149.5	4.8	137.9
RP3-3	1251.3	148.9	4.7	135.5
RP3-C	1261.3	150.1	0.0	146.7

KOH-treated poultry manure

KP1-1	918.2	90.9	4.6	80.7
KP1-2	920.6	91.1	4.6	85.2
KP1-3	930.9	92.2	4.8	85.9
KP1-C	940.6	93.1	0.0	92.1
KP2-1	1524.2	150.9	4.7	137.5
KP2-2	1518.8	150.4	4.7	139.1
KP2-3	1516.4	150.1	4.8	135.5
KP2-C	1526.1	151.1	0.0	145.8

Reaction time = 24 hours, temperature = 40 °C, buffer = 2.5ml

Moisture content

Sample #	Crucible weight, mg	Crucible+ wet substrate, mg	Substrate wet weight, mg	Crucible+ dry mass, mg	Dry mass, mg	Moisture content, %	Average, %	SD, %
RP1-1	9462.5	11109.3	1646.8	10915.0	1452.5	0.882	0.881	0.12
RP1-2	12589.4	14874.7	2285.3	14591.3	2001.9	0.876		
RP1-3	11259.8	13206.9	1947.1	12982.9	1723.2	0.885		
KP1-1	9638.6	11745.4	2106.8	11545.3	1906.7	0.905	0.901	0.10
KP1-2	10124.8	11693.2	1568.4	11530.3	1405.3	0.896		
KP1-3	12154.8	14018.6	1863.8	13835.9	1681.1	0.902		

Wet vs. dry poultry manure

	Substrate wet weight, mg	Substrate dry mass, mg	Enzyme, mg	Hydrolyzed residue, mg
WP-1	1022.2	101.2	4.8	92.7
WP-2	1015.2	100.5	4.9	91.6
WP-3	1008.1	99.8	4.7	90.1
WP-C	1030.3	102.0	0.0	99.8
DP-1		100.5	4.8	87.2
DP-2		102.6	4.9	89.6
DP-3		101.3	5.0	88.9
DP-C		102.1	0.0	100.3

Un-grinded vs. grinded poultry manure

Un-grinded	Substrate dry mass, mg	Enzyme, mg	Hydrolyzed residue, mg
UP-1	100.6	4.8	86.1
UP-2	100.1	4.9	85.6
UP-3	101.7	4.8	87.2
UP-C	100.7	0.0	99.5
Grinded			
GP1-1	100.6	4.9	85.6
GP1-2	100.1	5.0	86.5
GP1-3	101.7	4.8	87.3
GP1-C	100.7	0.0	99.8
GP2-1	150.1	4.8	129.5
GP2-2	151.0	4.8	128.6
GP2-3	150.6	4.7	130.9
GP2-4	149.5	0.0	148.9

Reaction time = 24 hours, temperature = 40 °C, buffer = 2.5ml

A.9 Enzymatic hydrolysis of sewage sludge

Primary sludge

Primary sludge	Substrate wet weight, mg	Substrate dry mass, mg	Enzyme, mg	Hydrolyzed residue, mg
PS1-1	366.3	63.4	5.9	45.5
PS1-2	368.8	63.9	6.0	45.9
PS1-3	368.8	63.9	5.9	44.6
PS1-C	374.5	64.9	0.0	61.2
PS2-1		48.7	4.7	35.7
PS2-2		49.8	4.8	36.5
PS2-3		50.5	4.7	37.2
PS2-C		48.7	0.0	48.3

Activated sludge

AS1-1	497.8	47.7	4.6	41.2
AS1-2	501.5	48.1	4.7	44.1
AS1-3	503.2	48.2	4.6	40.6
AS1-C	501.1	48.0	0.0	47.5
AS2-1		100.2	4.6	94.5
AS2-2		99.6	4.8	93.2
AS2-3		99.8	4.8	95.0
AS2-C		101.2	0.0	99.8

KOH-treated sludge

Primary sludge	Substrate wet weight, mg	Substrate dry mass, mg	Enzyme, mg	Hydrolyzed residue, mg
PS3-1	401.2	72.0	6.6	47.5
PS3-2	402.6	72.2	6.7	46.6
PS3-3	398.6	71.5	6.7	48.1
PS3-C	405.5	72.8	0.0	70.5
PS4-1		50.3	4.7	36.5
PS4-2		49.7	4.6	35.2
PS4-3		50.1	4.6	34.5
PS4-C		50.7	0.0	50.6

Activated sludge

AS3-1	500.5	46.5	4.5	42.6
AS3-2	501.5	46.6	4.5	41.3
AS3-3	502.6	46.7	4.6	41.1
AS3-C	498.5	46.4	0.0	46.1

Reaction time = 24 hours, temperature = 40 °C, buffer = 2.5ml

HCl treated primary sludge

Primary sludge	Substrate wet weight, mg	Substrate dry mass, mg	Enzyme, mg	Hydrolyzed residue, mg
PS5-1	301.5	33.9	3.4	18.5
PS5-2	302.1	34.0	3.3	18.9
PS5-3	303.4	34.2	3.3	19.3
PS5-C	302.2	34.0	0.0	33.2
PS6-1		49.5	4.6	33.2
PS6-2		49.8	4.7	33.1
PS6-3		50.1	4.7	33.9
PS6-C		50.5	0.0	48.9

HCl and KOH treated primary sludge

PS7-1	451.5	34.5	3.5	15.5
PS7-2	456.2	34.9	3.4	16.1
PS7-3	450.5	34.4	3.4	14.6
PS7-C	453.2	34.6	0.0	34.2
PS8-1		49.8	4.6	31.6
PS8-2		50.1	4.7	32.1
PS8-3		50.0	4.7	33.1
PS8-C		49.2	0.0	48.6

Digested sludge**No pre-treatment**

DS1-1		152.0	4.8	150.2
DS1-2		150.3	4.8	148.5
DS1-3		149.0	5.0	147.1
DS1-C		152.9	0.0	152.4
DS2-1	550.2	149.1	4.8	148.9
DS2-2	549.4	148.9	4.9	147.2
DS2-3	549.2	148.8	4.9	148.6
DS2-C	550.1	149.1	0.0	149.5

KOH-treated digested sludge

DS3-1		153.9	4.8	152.6
DS3-2		151.5	4.6	150.1
DS3-3		151.6	4.6	149.1
DS3-C		149.2	0.0	149.1
DS4-1	558.9	149.8	4.6	148.6
DS4-2	558.8	149.8	4.8	149.4
DS4-3	560.2	150.1	4.9	147.3
DS4-C	559.5	149.9	0.0	148.7

Reaction time = 24 hours, temperature = 40 °C, buffer = 2.5ml

Moisture content

Sample #	Crucible weight, mg	Crucible+wet substrate, mg	Substrate wet weight, mg	Crucible+dry mass, mg	Dry mass, mg	Moisture content, %	Average, %	SD, %
PS1-1	10258.6	20784.7	10526.1	12090.1	1831.5	0.83	0.83	0.002
PS1-2	11786.3	21349.1	9562.8	13421.5	1635.2	0.83		
PS1-3	9584.2	18340.3	8756.1	11107.8	1523.6	0.83		
PS3-1	11547.3	26182.5	14635.2	14167.0	2619.7	0.82	0.82	0.004
PS3-2	11698.5	28521.5	16823.0	14642.5	2944.0	0.83		
PS3-3	10547.6	26034.8	15487.2	13381.8	2834.2	0.82		
PS5-1	9634.1	26531.4	16897.3	11543.5	1909.4	0.89	0.89	0.003
PS5-2	11546.7	27789.8	16243.1	13430.9	1884.2	0.88		
PS5-3	10682.9	26465.2	15782.3	12419.0	1736.1	0.89		
PS7-1	11643.2	23226.8	11583.6	12535.1	891.9	0.92	0.92	0.005
PS7-2	10647.5	23345.8	12698.3	11663.4	1015.9	0.92		
PS7-3	11684.2	22311.3	10627.1	12438.7	754.5	0.93		
AS1-1	11458.1	27145.3	15687.2	13058.2	1600.1	0.90	0.90	0.007
AS1-2	9865.2	22234.6	12369.4	11077.4	1212.2	0.90		
AS1-3	1084.3	15070.0	13985.7	2329.0	1244.7	0.91		
AS3-1	11648.1	31234.4	19586.3	13469.6	1821.5	0.91	0.91	0.003
AS3-2	10947.3	31510.5	20563.2	12921.4	1974.1	0.90		
AS3-3	11634.2	30399.4	18765.2	13323.1	1688.9	0.91		
DS2-1	10485.3	29442.9	18957.6	15509.1	5023.8	0.74	0.73	0.006
DS2-2	11564.2	33212.5	21648.3	17474.2	5910.0	0.73		
DS2-3	11485.1	33850.0	22364.9	17657.8	6172.7	0.72		
DS4-1	10486.2	36379.7	25893.5	17296.2	6810.0	0.74	0.73	0.005
DS4-2	11562.1	36199.9	24637.8	18263.6	6701.5	0.73		
DS4-3	11648.2	33260.7	21612.5	17483.6	5835.4	0.73		

APPENDIX B

Glucose yields calculation

B.1 Glucose yields from enzymatic hydrolysis of crop residues

Enzymatic hydrolysis of corn stalk and bagasse with time Glucose calibration curve

Glucose concentration, g/l	Absorbance
0.00	0.00
0.20	0.10
0.40	0.25
0.60	0.41
0.80	0.61
1.00	0.77
X	Y
$X=1.265*Y+0.048$

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average conversion rate, mg/hr	SD, mg/hr
RC8-1	0.009	0.059	2.969	50.100	5.927	0.371	5.884	0.060	0.369	0.005
RC8-2	0.009	0.059	2.969	50.200	5.915	0.371				
RC8-3	0.008	0.058	2.906	50.000	5.812	0.363				
RC8-C	0.000	0.048	2.400	49.800	4.819	0.300				
RC16-1	0.010	0.061	3.033	49.900	6.077	0.190	6.183	0.102	0.193	0.004
RC16-2	0.012	0.063	3.159	50.300	6.280	0.197				
RC16-3	0.011	0.062	3.096	50.000	6.192	0.193				
RC16-C	0.000	0.048	2.400	49.900	4.810	0.150				

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average conversion rate, mg/hr	SD, mg/hr
RC24-1	0.009	0.059	2.969	50.000	5.939	0.124	6.629	0.984	0.138	0.021
RC24-2	0.023	0.077	3.855	49.700	7.756	0.161				
RC24-3	0.011	0.062	3.096	50.000	6.192	0.129				
RC24-C	0.000	0.048	2.400	50.000	4.800	0.100				
RC32-1	0.016	0.068	3.412	50.200	6.797	0.107	6.857	0.082	0.107	0.001
RC32-2	0.017	0.070	3.475	50.000	6.951	0.109				
RC32-3	0.016	0.068	3.412	50.000	6.824	0.107				
RC32-C	0.000	0.048	2.400	49.700	4.829	0.075				
RC40-1	0.022	0.076	3.792	49.800	7.613	0.095	7.486	0.115	0.093	0.002
RC40-2	0.020	0.073	3.665	49.600	7.389	0.092				
RC40-3	0.021	0.075	3.728	50.000	7.457	0.093				
RC40-C	0.001	0.049	2.463	50.200	4.907	0.062				
RC48-1	0.022	0.076	3.792	50.300	7.538	0.079	7.188	0.309	0.075	0.003
RC48-2	0.017	0.070	3.475	50.000	6.951	0.072				
RC48-3	0.018	0.071	3.539	50.000	7.077	0.074				
RC48-C	0.001	0.049	2.463	49.900	4.936	0.051				
KC8-1	0.251	0.366	18.276	49.700	36.772	2.284	37.081	0.322	2.316	0.032
KC8-2	0.259	0.376	18.782	50.200	37.414	2.348				
KC8-3	0.255	0.371	18.529	50.000	37.058	2.316				
KC8-C	0.000	0.048	2.400	49.900	4.810	0.300				

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average conversion rate, mg/hr	SD, mg/hr
KC16-1	0.368	0.514	25.676	50.300	51.046	1.605	51.510	0.481	1.614	0.013
KC16-2	0.374	0.521	26.056	50.100	52.007	1.628				
KC16-3	0.369	0.515	25.739	50.000	51.479	1.609				
KC16-C	0.000	0.048	2.400	50.300	4.771	0.150				
KC24-1	0.441	0.606	30.293	50.100	60.466	1.262	61.182	0.839	1.277	0.016
KC24-2	0.446	0.612	30.610	50.200	60.975	1.275				
KC24-3	0.453	0.621	31.052	50.000	62.105	1.294				
KC24-C	0.002	0.051	2.527	50.000	5.053	0.105				
KC32-1	0.485	0.662	33.076	50.300	65.758	1.034	66.152	0.670	1.034	0.007
KC32-2	0.489	0.667	33.329	49.800	66.926	1.042				
KC32-3	0.482	0.658	32.887	50.000	65.773	1.028				
KC32-C	0.000	0.048	2.400	50.200	4.781	0.075				
KC40-1	0.505	0.687	34.341	50.100	68.545	0.859	68.131	0.439	0.852	0.006
KC40-2	0.497	0.677	33.835	50.000	67.671	0.846				
KC40-3	0.501	0.682	34.088	50.000	68.177	0.852				
KC40-C	0.002	0.051	2.527	49.800	5.073	0.063				
KC48-1	0.553	0.748	37.377	49.700	75.206	0.779	72.208	2.895	0.751	0.027
KC48-2	0.530	0.718	35.923	49.900	71.989	0.748				
KC48-3	0.512	0.696	34.784	50.100	69.429	0.725				
KC48-C	0.003	0.052	2.590	50.200	5.159	0.054				

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average conversion rate, mg/hr	SD, mg/hr
KB8-1	0.223	0.330	16.505	50.100	32.944	2.063	33.193	0.283	2.082	0.025
KB8-2	0.229	0.338	16.884	50.400	33.500	2.111				
KB8-3	0.224	0.331	16.568	50.000	33.136	2.071				
KB8-C	0.000	0.048	2.400	50.200	4.781	0.300				
KB16-1	0.295	0.421	21.059	50.300	41.866	1.316	42.555	0.720	1.331	0.016
KB16-2	0.303	0.431	21.565	49.800	43.303	1.348				
KB16-3	0.298	0.425	21.249	50.000	42.497	1.328				
KB16-C	0.001	0.049	2.463	50.100	4.917	0.154				
KB24-1	0.339	0.477	23.842	49.700	47.971	0.993	49.493	1.854	1.028	0.039
KB24-2	0.368	0.514	25.676	49.800	51.558	1.070				
KB24-3	0.349	0.489	24.474	50.000	48.949	1.020				
KB24-C	0.000	0.048	2.400	49.800	4.819	0.100				
KB32-1	0.366	0.511	25.550	50.200	50.895	0.798	51.699	0.787	0.808	0.010
KB32-2	0.376	0.524	26.182	49.900	52.469	0.818				
KB32-3	0.371	0.517	25.866	50.000	51.732	0.808				
KB32-C	0.001	0.049	2.463	49.800	4.946	0.077				
KB40-1	0.412	0.569	28.459	50.100	56.804	0.711	57.060	0.568	0.715	0.009
KB40-2	0.421	0.581	29.028	50.300	57.710	0.726				
KB40-3	0.410	0.567	28.333	50.000	56.665	0.708				
KB40-C	0.000	0.048	2.400	50.200	4.781	0.060				
KB48-1	0.442	0.607	30.357	49.700	61.079	0.632	61.177	3.402	0.638	0.037
KB48-2	0.476	0.650	32.507	50.300	64.626	0.677				
KB48-3	0.421	0.581	29.028	50.200	57.825	0.605				
KB48-C	0.000	0.048	2.400	49.800	4.819	0.050				

Enzymatic hydrolysis of corn stalk at various enzymes loading
Glucose calibration curve

Glucose concentration, g/l	Absorbance
0.000	0.000
0.200	0.134
0.400	0.237
0.600	0.378
0.800	0.526
1.000	0.640
X	Y
X=1.55*Y+0.005

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
CE1-1	0.265	0.416	20.788	50.20	41.409	0.87	41.818	2.27	0.868	0.049
CE1-2	0.252	0.396	19.780	49.60	39.879	0.82				
CE1-3	0.280	0.439	21.950	49.70	44.165	0.91				
CE1-C	0.000	0.005	0.250	50.30	0.497	0.01				
CE2-1	0.412	0.644	32.180	49.90	64.489	1.341	65.425	2.258	1.362	0.058
CE2-2	0.439	0.685	34.273	50.40	68.001	1.428				
CE2-3	0.405	0.633	31.638	49.60	63.785	1.318				
CE2-C	0.000	0.005	0.250	49.90	0.501	0.010				
CE3-1	0.460	0.718	35.900	50.30	71.372	1.496	70.203	2.007	1.464	0.045
CE3-2	0.456	0.712	35.590	49.80	71.466	1.483				
CE3-3	0.434	0.678	33.885	50.00	67.770	1.412				
CE3-C	0.000	0.005	0.250	50.10	0.499	0.010				

Enzymatic hydrolysis of bagasse at various enzyme loading
Glucose calibration curve

Glucose concentration, g/l	Absorbance
0.000	0.000
0.200	0.128
0.400	0.245
0.600	0.412
0.800	0.590
1.000	0.770
X	Y
X=1.296*Y+0.037

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
BE1-1	0.282	0.402	20.124	50.10	40.167	0.838	40.214	2.302	0.838	0.051
BE1-2	0.301	0.427	21.355	50.20	42.539	0.890	51.105	2.533	1.063	0.053
BE1-3	0.263	0.378	18.892	49.80	37.937	0.787				
BE1-C	0.000	0.037	1.850	50.40	3.671	0.077				
BE2-1	0.381	0.531	26.539	50.00	53.078	1.106	58.973	1.499	1.225	0.040
BE2-2	0.371	0.518	25.891	49.80	51.990	1.079				
BE2-3	0.343	0.482	24.076	49.90	48.249	1.003				
BE2-C	0.000	0.037	1.850	49.80	3.715	0.077				
BE3-1	0.413	0.572	28.612	49.60	57.686	1.192	58.973	1.499	1.225	0.040
BE3-2	0.442	0.610	30.492	50.30	60.619	1.270				
BE3-3	0.421	0.583	29.131	49.70	58.613	1.214				
BE3-C	0.000	0.037	1.850	49.70	3.722	0.077				

Enzymatic hydrolysis of crop residues at various temperatures

Glucose calibration curve

Glucose concentration, g/l	Absorbance
0.000	0.000
0.200	0.134
0.400	0.237
0.600	0.378
0.800	0.526
1.000	0.640
X=1.55*Y+0.005

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
CT1-1	0.327	0.512	25.593	50.00	51.185	1.066	48.086	3.253	0.999	0.069
CT1-2	0.284	0.445	22.260	49.80	44.699	0.928				
CT1-3	0.307	0.481	24.043	49.70	48.375	1.002				
CT1-C	0.000	0.005	0.250	50.10	0.499	0.010				
CT2-1	0.412	0.644	32.180	49.90	64.489	1.341	65.425	2.258	1.362	0.058
CT2-2	0.439	0.685	34.273	50.40	68.001	1.428				
CT2-3	0.405	0.633	31.638	49.60	63.785	1.318				
CT2-C	0.000	0.005	0.250	49.90	0.501	0.010				

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
CT3-1	0.329	0.515	25.748	50.50	50.985	1.073	50.397	1.167	1.051	0.025
CT3-2	0.324	0.507	25.360	49.50	51.232	1.057				
CT3-3	0.314	0.492	24.585	50.20	48.974	1.024				
CT3-C	0.002	0.008	0.405	49.80	0.813	0.017				
BT1-1	0.305	0.478	23.888	50.50	47.302	0.995				
BT1-2	0.294	0.461	23.035	49.80	46.255	0.960	45.709	1.925	0.955	0.042
BT1-3	0.279	0.437	21.873	50.20	43.571	0.911				
BT1-C	0.000	0.005	0.250	49.90	0.501	0.010				
BT2	same as BE2									
BT3-1	0.315	0.493	24.663	50.10	49.227	1.028				
BT3-2	0.295	0.462	23.113	49.90	46.318	0.963	46.125	3.202	0.960	0.069
BT3-3	0.272	0.427	21.330	49.80	42.831	0.889				
BT3-4	0.000	0.005	0.250	50.30	0.497	0.010				

Enzyme activity test using whatman (performed on Oct. 17, 2003)

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Enzyme, units	Enzyme loading	Conversion, %	Conversion rate, mg/hr/unit	Average conversion, %	SD, %	Average rate, mg/hr/unit	SD, mg/hr/unit
WM1-1	0.462	0.721	36.055	50.10	39.800	794.411	71.966	0.038				
WM1-2	0.469	0.732	36.598	50.00	39.000	780.000	73.195	0.039	73.56	1.81	0.039	0.001
WM1-3	0.486	0.758	37.915	50.20	39.800	792.829	75.528	0.040				
WM1-C	0.000	0.005	0.250	49.80	0.000	0.000	0.502					
WM2-1	0.356	0.557	27.840	49.90	19.900	398.798	55.792	0.058				
WM2-2	0.342	0.535	26.755	50.00	20.350	407.000	53.510	0.055	55.38	1.71	0.058	0.001
WM2-3	0.365	0.571	28.538	50.20	19.900	396.414	56.848	0.060				
WM2-C	0.000	0.005	0.250	49.90	0.000	0.000	0.501					

Effect of pre-treatment sequence, enzyme addition time, and cellobiase on enzymatic hydrolysis of crop residues

Glucose calibration curve

Glucose concentration, g/l	Absorbance
0.000	0.000
0.200	0.108
0.400	0.256
0.600	0.384
0.800	0.532
1.000	0.658
X	Y
X=1.493+0.018

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
CP-1	0.455	0.697	34.866	50.100	69.592	1.45	66.084	3.41	1.376	0.076
CP-2	0.430	0.660	33.000	50.100	65.867	1.37				
CP-3	0.406	0.624	31.208	49.700	62.793	1.30				
CP-C	0.004	0.024	1.199	50.500	2.373	0.05				
CD-1	0.395	0.608	30.387	50.300	60.411	1.27	60.657	1.60	1.266	0.028
CD-2	0.404	0.621	31.059	49.800	62.367	1.29				
CD-3	0.386	0.594	29.715	50.200	59.193	1.24				
CD-C	0.004	0.024	1.199	49.800	2.407	0.05				

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
CW-1	0.407	0.626	31.283	46.031	67.960	1.30	68.240	0.42	1.313 1.43 if substrate mass adjusted to 50mg	0.009
CW-2	0.410	0.630	31.507	46.307	68.039	1.31				
CW-3	0.413	0.635	31.730	46.173	68.720	1.32				
CW-4	0.000	0.018	0.900	46.289	1.944	0.04				
CED1-1	0.416	0.639	31.954	50.200	63.654	1.33	63.266	1.64	1.319	0.039
CED1-2	0.398	0.612	30.611	49.800	61.467	1.28				
CED1-3	0.422	0.648	32.402	50.100	64.675	1.35				
CED1-C	0.000	0.018	0.900	49.600	1.815	0.04				
CED2-1	0.433	0.664	33.223	50.400	65.920	1.38	66.924	1.23	1.400	0.009
CED2-2	0.432	0.663	33.149	49.800	66.564	1.38				
CED2-3	0.449	0.688	34.418	50.400	68.289	1.43				
CED2-C	0.000	0.018	0.900	49.600	1.815	0.04				
CC1-1	0.417	0.641	32.029	49.900	64.186	1.33	65.998	1.94	1.374	0.036
CC1-2	0.432	0.663	33.149	50.400	65.771	1.38				
CC1-3	0.440	0.675	33.746	49.600	68.036	1.41				
CC1-C	0.000	0.018	0.900	49.900	1.804	0.04				
CC2-1	0.431	0.661	33.074	50.000	66.148	1.38	66.732	0.97	1.397	0.022
CC2-2	0.445	0.682	34.119	50.300	67.832	1.42				
CC2-3	0.435	0.667	33.373	50.400	66.216	1.39				
CC2-C1	0.002	0.021	1.049	50.100	2.094	0.04				
CC2-C2	0.000	0.018	0.900	0.000						

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
BC1-1	0.314	0.487	24.340	50.000	48.680	1.01	51.125	2.50	1.063	0.050
BC1-2	0.346	0.535	26.729	49.800	53.672	1.11				
BC1-3	0.329	0.509	25.460	49.900	51.022	1.06				
BC1-C	0.000	0.018	0.900	49.800	1.807	0.04				
BC2-1	0.350	0.541	27.028	50.200	53.840	1.13	53.686	0.86	1.120	0.016
BC2-2	0.342	0.529	26.430	50.100	52.755	1.10				
BC2-3	0.352	0.544	27.177	49.900	54.463	1.13				
BC2-C1	0.000	0.018	0.900	50.200	1.793	0.04				
BC2-C2	0.000	0.018	0.900	0.000	0.000	0.000				

B.2 Glucose yields from enzymatic hydrolysis of poultry manure

Enzymatic hydrolysis of raw poultry manure Glucose calibration curve

Concentration, g/l	Absorbance
0.000	0.000
0.200	0.098
0.400	0.243
0.600	0.382
0.800	0.546
1.000	0.645
X	Y
X=1.487*Y+0.026

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
RP1-1	0.028	0.068	3.382	49.900	6.777	0.14	7.164	0.40	0.149	0.008
RP1-2	0.033	0.075	3.754	50.100	7.492	0.16				
RP1-3	0.031	0.072	3.605	49.900	7.224	0.15				
RP1-C	0.000	0.026	1.300	50.100	2.595	0.05	7.146	0.30	0.298	0.013
RP2-1	0.075	0.138	6.876	99.800	6.890	0.29				
RP2-2	0.078	0.142	7.099	100.100	7.092	0.30				
RP2-3	0.083	0.149	7.471	100.200	7.456	0.31				
RP2-C	0.000	0.026	1.300	100.500	1.294	0.05				
RP3-1	0.122	0.207	10.371	150.000	6.914	0.43				
RP3-2	0.130	0.219	10.966	149.500	7.335	0.46	6.805	0.60	0.424	0.038
RP3-3	0.106	0.184	9.181	148.900	6.166	0.38				
RP3-C	0.000	0.026	1.300	150.100	0.866	0.05				

Enzymatic hydrolysis of KOH-treated poultry manure

Glucose calibration curve

Concentration, g/l	Absorbance
0.000	0.000
0.200	0.122
0.400	0.297
0.600	0.455
0.800	0.612
1.000	0.725
X	Y
$X=1.3281*Y+0.0106$

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
KP1-1	0.169	0.235	11.752	90.900	12.929	0.49	9.475	3.10	0.361	0.115
KP1-2	0.090	0.130	6.506	91.140	7.139	0.27				
KP1-3	0.108	0.154	7.702	92.160	8.357	0.32				
KP1-C	0.000	0.011	0.530	93.120	0.569	0.02				
KP2-1	0.195	0.270	13.479	150.900	8.932	0.56	8.914	0.80	0.559	0.013
KP2-2	0.212	0.292	14.608	150.360	9.715	0.61				
KP2-3	0.175	0.243	12.151	150.120	8.094	0.51				
KP2-C	0.000	0.011	0.530	151.080	0.351	0.02				

**Enzymatic hydrolysis of wet vs. dried poultry manure
Glucose calibration curve**

Concentration, g/l	Absorbance
0.000	0.000
0.200	0.122
0.400	0.297
0.600	0.455
0.800	0.612
1.000	0.725
X	Y
X=1.333*Y+0.009

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
WP-1	0.156	0.217	10.847	101.200	10.719	0.45	10.214	1.50	0.428	0.066
WP-2	0.165	0.229	11.447	100.500	11.390	0.48				
WP-3	0.121	0.170	8.515	99.800	8.532	0.35				
WP-C	0.068	0.100	4.982	102.000	4.885	0.21				
DP-1	0.192	0.265	13.247	100.500	13.181	0.55	13.883	0.65	0.587	0.033
DP-2	0.216	0.297	14.846	102.600	14.470	0.62				
DP-3	0.206	0.284	14.180	101.300	13.998	0.59				
DP-C	0.000	0.009	0.450	102.100	0.441	0.02				

Enzymatic hydrolysis of un-grinded vs. grinded poultry manure

Glucose calibration curve

Concentration, g/l	Absorbance
0.000	0.000
0.200	0.112
0.400	0.246
0.600	0.398
0.800	0.563
1.000	0.715
X	Y
X=1.378+0.033

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
UP-1	0.199	0.307	15.361	100.600	15.269	0.64	14.308	0.90	0.601	0.035
UP-2	0.182	0.284	14.190	100.100	14.176	0.59				
UP-3	0.175	0.274	13.708	101.700	13.478	0.57				
UP-C	0.000	0.033	1.650	100.700	1.639	0.07	27.605	1.22	1.160	0.058
GP1-1	0.385	0.564	28.177	100.600	28.008	1.17				
GP1-2	0.357	0.525	26.247	100.100	26.221	1.09				
GP1-3	0.398	0.581	29.072	101.700	28.586	1.21	26.327	1.02	1.652	0.056
GP1-C	0.000	0.033	1.650	100.700	1.639	0.07				
GP2-1	0.575	0.825	41.268	150.100	27.493	1.72				
GP2-2	0.543	0.781	39.063	151.000	25.869	1.63	26.327	1.02	1.652	0.056
GP2-3	0.536	0.772	38.580	150.600	25.618	1.61				
GP2-C	0.000	0.033	1.650	149.500	1.104	0.07				

B.3 Glucose yields from enzymatic hydrolysis of sewage sludge

Primary sludge and activated sludge

Glucose calibration curve

Concentration, g/l	Absorbance
0.000	0.000
0.200	0.097
0.400	0.269
0.600	0.468
0.800	0.628
1.000	0.772
X	Y
$X=1.239*Y+0.039$

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
PS1-1	0.281	0.387	19.358	63.434	30.517	0.81	31.058	2.71	0.825	0.071 0.06(50mg)
PS1-2	0.264	0.366	18.305	63.867	28.661	0.76			0.647	
PS1-3	0.319	0.434	21.712	63.867	33.996	0.90			(adjusted to 50mg)	
PS1-C	0.000	0.039	1.950	64.854	3.007	0.08				
PS2-1	0.164	0.242	12.110	48.700	24.866	0.50	25.042	0.80	0.518	0.016
PS2-2	0.165	0.243	12.172	49.800	24.441	0.51				
PS2-3	0.179	0.261	13.039	50.500	25.820	0.54				
PS2-C	0.000	0.039	1.950	48.700	4.004	0.08				

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
AS1-1	0.068	0.123	6.163	47.710	12.917	0.26	12.883	2.71	0.258	0.024
AS1-2	0.078	0.136	6.782	48.064	14.110	0.28			0.27	0.03
AS1-3	0.059	0.112	5.605	48.227	11.622	0.23			(50mg)	(50mg)
AS1-C	0.090	0.151	7.526	48.026	15.670	0.31				
AS2-1	0.038	0.086	4.304	100.200	4.296	0.18	5.594	1.21	0.233	0.048
AS2-2	0.076	0.133	6.658	99.600	6.685	0.28			0.116	0.024
AS2-3	0.062	0.116	5.791	99.800	5.803	0.24			(50mg)	(50mg)
AS2-C	0.000	0.039	1.950	101.200	1.927	0.08				
PS3-1	0.365	0.491	24.562	71.979	34.123	1.02	35.449	1.19	1.062	0.050
PS3-2	0.393	0.526	26.296	72.230	36.406	1.10			0.739	0.035
PS3-3	0.382	0.512	25.615	71.513	35.819	1.07			(50mg)	(50mg)
PS3-C	0.000	0.039	1.950	72.751	2.680	0.08				
PS4-1	0.191	0.276	13.782	50.300	27.400	0.57	29.367	1.74	0.612	0.024
PS4-2	0.209	0.298	14.898	49.700	29.975	0.62				
PS4-3	0.217	0.308	15.393	50.100	30.725	0.64				
PS4-C	0.000	0.039	1.950	50.700	3.846	0.08				
AS3-1	0.052	0.103	5.171	46.547	11.110	0.22	11.262	3.31	0.219	0.063
AS3-2	0.029	0.075	3.747	46.640	8.033	0.16			0.235	0.068
AS3-3	0.079	0.137	6.844	46.742	14.642	0.29			(50mg)	(50mg)
AS3-C	0.000	0.039	1.950	46.361	4.206	0.08				

HCl treated primary sludge

Glucose calibration curve

Concentration, g/l	Absorbance
0.000	0.000
0.200	0.088
0.400	0.243
0.600	0.412
0.800	0.590
1.000	0.770
X	Y
$X=1.267*Y+0.006$

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
PS5-1	0.202	0.289	14.464	33.950	42.604	0.60	42.609	1.18	0.604 0.888 (50mg)	0.017
PS5-2	0.209	0.298	14.898	34.017	43.794	0.62				0.025
PS5-3	0.197	0.283	14.154	34.164	41.430	0.59				(50mg)
PS5-C	0.001	0.040	2.012	34.029	5.912	0.08				
PS6-5	0.224	0.317	15.827	49.500	31.973	0.66	31.700	1.19	0.658 0.660 (50mg)	0.035
PS6-6	0.228	0.321	16.075	49.800	32.278	0.67				0.035
PS6-7	0.218	0.309	15.455	50.100	30.849	0.64				(50mg)
PS6-C	0.000	0.039	1.950	50.500	3.861	0.08				
PS7-1	0.279	0.385	19.234	34.504	55.744	0.80	54.177	3.97	0.781 1.128 (50mg)	0.057
PS7-2	0.248	0.346	17.314	34.864	49.661	0.72				0.083
PS7-3	0.286	0.393	19.668	34.428	57.127	0.82				(50mg)
PS7-C	0.000	0.039	1.950	34.634	5.630	0.08				

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
PS8-1	0.272	0.376	18.800	49.800	37.752	0.78	37.008	0.96	0.770 0.771 (50mg)	0.020 0.020 (50mg)
PS8-2	0.259	0.360	17.995	50.100	35.918	0.75				
PS8-3	0.270	0.374	18.677	50.000	37.353	0.78				
PS8-C	0.000	0.039	1.950	49.200	3.963	0.08				

Digested sludge

Glucose calibration curve

Concentration, g/l	Absorbance
0.000	0.000
0.200	0.118
0.400	0.258
0.600	0.412
0.800	0.552
1.000	0.672
X	Y
$X=1.4513*Y + 0.0133$

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
DS1-1	0.016	0.037	1.826	153.200	1.192	0.08	1.060	0.14	0.067	0.009
DS1-2	0.010	0.028	1.391	151.500	0.918	0.06				
DS1-3	0.013	0.032	1.608	150.100	1.072	0.07				
DS1-C	0.000	0.013	0.665	152.400	0.436	0.03				

Sample #	Absorbance	Glucose concentration, g/l	Glucose mass, mg	Substrate, mg	Conversion, %	Conversion rate, mg/hr	Average conversion, %	SD, %	Average rate, mg/hr	SD, mg/hr
DS2-1	0.006	0.022	1.100	149.104	0.738	0.05	0.528	0.20	0.033	0.012
DS2-2	-0.002	0.010	0.520	148.887	0.349	0.02				
DS2-3	0.001	0.015	0.738	148.833	0.496	0.03				
DS2-C	0.000	0.013	0.665	149.077	0.446	0.03				
DS3-1	0.010	0.028	1.391	153.900	0.904	0.06	1.025	0.33	0.065	0.021
DS3-2	0.020	0.042	2.116	151.500	1.397	0.09				
DS3-3	0.007	0.023	1.173	151.600	0.774	0.05				
DS3-C	0.000	0.013	0.665	149.200	0.446	0.03				
DS4-1	0.002	0.016	0.810	149.785	0.541	0.03	0.912	0.45	0.057	0.028
DS4-2	0.020	0.042	2.116	149.758	1.413	0.09				
DS4-3	0.007	0.023	1.173	150.134	0.781	0.05				
DS4-C	0.000	0.013	0.665	149.946	0.443	0.03				

APPENDIX C

Volatile solids of poultry manure and sewage sludge

Volatile solids of poultry manure and sewage sludge

Sample		Crucible weight, mg	Crucible+dry residue, mg	Mass of dry solids, mg	Ash + crucible, mg	Fixed solids, mg	Volatile solids, mg	Volatile solids, %	Average, %	SD, %
Raw poultry manure	1	10658.100	11670.700	1012.600	11020.600	362.500	650.100	64.201	66.801	2.360
	2	11264.300	12479.300	1215.000	11643.400	379.100	835.900	68.798		
	3	9687.500	10873.500	1186.000	10074.100	386.600	799.400	67.403		
KOH treated poultry manure	1	11257.100	12199.600	942.500	11458.300	201.200	741.300	78.653	78.393	0.216
	2	10598.600	11491.600	893.000	10801.100	202.500	690.500	77.324		
	3	11587.100	12540.100	953.000	11785.300	198.200	754.800	79.203		
Residue from enzymatic hydrolysis	GP1-1	9685.200	9919.700	234.500	9741.500	56.300	178.200	75.991	75.108	0.800
	GP1-2	11256.100	11513.600	257.500	11321.700	65.600	191.900	74.524		
	GP1-3	10561.800	10821.400	259.600	10627.200	65.400	194.200	74.807		
Primary sludge	1	10684.200	11034.600	350.400	10741.800	57.600	292.800	83.562	83.554	0.800
	2	11258.700	11627.600	368.900	11316.617	57.917	310.983	84.300		
	3	9856.500	10198.600	342.100	9915.341	58.841	283.259	82.800		
HCl and KOH treated primary sludge	1	10615.200	10919.700	304.500	10647.400	32.200	272.300	89.425	87.173	3.760
	2	11357.100	11477.700	120.600	11377.800	20.700	99.900	82.836		
	3	11586.500	11836.000	249.500	11613.300	26.800	222.700	89.259		
Residue from enzymatic hydrolysis	PS7-1	10597.100	10607.300	10.200	10599.200	2.100	8.100	79.412	75.351	3.580
	PS7-2	11257.600	11269.900	12.300	11260.800	3.200	9.100	73.984		
	PS7-3	9684.100	9696.900	12.800	9687.600	3.500	9.300	72.656		
Activated sludge	1.000	10568.200	10837.900	269.700	10656.000	87.800	181.900	67.445	65.682	1.600
	2.000	11645.300	11921.600	276.300	11743.387	98.087	178.214	64.500		
	3.000	11251.200	11533.100	281.900	11349.583	98.383	183.517	65.100		
Digested sludge	1.000	11257.600	12611.300	1353.700	11853.700	596.100	757.600	55.965	57.229	1.300
	2.000	11586.500	14321.500	2735.000	12756.500	1170.000	1565.000	57.221		
	3.000	10597.100	12755.700	2158.600	11492.919	895.819	1262.781	58.500		

APPENDIX D

FTIR spectra

D.1 FTIR of corn stalk

D.2 Residue from enzymatic hydrolysis

D.3 FTIR of bagasse

D.4 Residue from enzymatic hydrolysis

D.5 FTIR of poultry manure

D.6 Residue from enzymatic hydrolysis

D.7 FTIR of sewage sludge

APPENDIX E

Cost analysis

D.1 Operational cost of producing 10000 litres of ethanol from corn stalk (cost in Canadian dollar)

	C	O	H	C ₆ H ₁₂ O ₆	C ₂ H ₅ OH	CO ₂	K
Molecular weight	12.01	16	1.01	180.18	46.08	44.01	39.10

Glucose fermentation: $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{C}_2\text{H}_5\text{OH} + 2\text{CO}_2$

Density of ethanol = 0.789kg/l

Volume of ethanol = 10000 litres

Mass of ethanol = 10000 litres $\times$ 0.789 kg/litre = 7890 kg

Glucose to ethanol yield = 92% (Wooley et al, 1999)

Mass of glucose needed = $7890 \times 180.18 \div (2 \times 46.08) \div 0.92 = 16766.92$ kg

Glucose yield from enzymatic hydrolysis = 68.2% (the best result achieved in this study)

Substrate needed for enzymatic hydrolysis = $16766.92 \text{ kg} \div 0.682 = 24584.93$ kg

KOH pre-treatment yield = 64.0%

Mass of raw corn stalk needed = $24584.93 \text{ kg} \div 0.64 = 38413.95 \text{ kg} = 38.414$ ton

1. Feedstock cost

Unit cost of feedstock = \$25/dry ton

Feedstock cost = \$25/dry ton $\times$ 38.414 = \$960.35

2. Pre-treatment cost

Electricity:

Grinding capacity per unit electricity = 50 kg/kwh (Guo et al, 2002)

Electricity consumption in grinding = $38413.95 \text{ kg} \div 50 \text{ kg/kwh} = 768.28$ kwh

Electricity consumption in pre-treatment = $3 \times$ electricity consumption in grinding
 $= 3 \times 768.28 \text{ kwh} = 2304.84$ kwh

Electricity cost = $2304.84 \text{ kwh} \times \$0.07/\text{kwh} = \$161.34$

Water:

In the pre-treatment process, 30.0g raw corn stalk was treated in 500ml KOH solution, thus the water loading = $500\text{ml} \div 30\text{g} = 16.67 \text{ ml/g} = 16.67 \text{ l/kg}$

$$\text{Water consumption} = 16.67 \text{ l/kg} \times 38413.95 \text{ kg} = 640232.5 \text{ l} = 640.23 \text{ m}^3$$

$$\text{Water price} = \$0.583 / \text{m}^3$$

$$\text{Cost in water} = 640.23 \text{ m}^3 \times \$0.583 / \text{m}^3 = \$373.26$$

KOH:

$$\text{KOH concentration} = 0.5 \text{ N}$$

$$\text{Mass of KOH in 500ml 0.5N KOH} = 0.5 \text{ mol/l} \times 0.5\text{l} \times 56 \text{ g/mol} = 14 \text{ g}$$

$$\text{KOH loading} = 14 \text{ g KOH} \div 40 \text{ g raw corn stalk} = 0.35 \text{ g KOH/g raw material}$$

$$\text{KOH needed} = 0.35 \text{ kg KOH/kg raw material} \times 38413.95 \text{ kg} = 13444.88\text{kg}$$

$$\text{KOH recovery rate} = 95\% \text{ (as in chemical pulping process)}$$

$$\text{KOH consumption} = 13444.88\text{kg} \times (1-95\%) = 672.24 \text{ kg}$$

$$\text{Cost of KOH} = \$10.04 / \text{kg} \times 672.24 \text{ kg} = \$6991.34$$

HCl:

$$\text{HCl concentration} = 1.0 \text{ N}$$

$$\text{HCl loading} = 0.033\text{ml/g feedstock} = 0.033 \text{ l/kg feedstock}$$

$$\text{HCl needed} = 0.033 \text{ l/kg feedstock} \times 38413.95 \text{ kg} = 1280.45 \text{ litre}$$

$$\text{Concentrated HCl needed} = 1280.45 \text{ l} \times 0.083 = 106.28 \text{ litre}$$

$$\text{Cost of HCl} = \$5.0 \times 106.28 \text{ litre} = \$531.39$$

$$\begin{aligned} \text{Total Pre-treatment cost} &= \$161.34 + \$373.26 + \$6991.34 + \$531.39 \\ &= \$8057.33 \end{aligned}$$

3. SSF**Enzyme:**

$$\text{Enzyme activity} = 1\mu\text{mol glucose per hour/unit} = 0.18 \text{ mg glucose/hr/unit}$$

$$\text{Retention time} = 24 \text{ hours}$$

Enzyme capability = $0.18 \text{ mg glucose/hr/unit} \times 24 \text{ hr} = 0.00432 \text{ g glucose/unit}$
 Enzyme needed = $16766.92 \text{ kg glucose} \times 1000 \div 0.00432 \text{ g glucose/unit}$
 $= 3,881,231,483 \text{ units}$

Enzyme recycling rate = 90% (Miyamoto, 1997)

Enzyme consumption = $3,881,231,483 \times (1-0.9) = 38812.31 \times 10^4 \text{ units}$

Enzyme price = \$10/10000 units

Consider 10-fold price reduction in enzyme price

Enzyme cost = $38812.31 \times 10^4 \text{ units} \times \$10/10000 \text{ units}/10 = \38821.31

Yeast:

Yeast cost per unit ethanol = \$0.0525 /gallon (Aden et al, 2002)
 $= \$0.0525 \text{ /gallon} \div 3.785 \text{ litre/gal}$
 $= \$0.01387/\text{litre ethanol}$

Yeast cost = $\$0.01387/\text{litre ethanol} \times 10000 \text{ litre} = \138.7

Electricity:

Electricity efficiency = 100 kg substrate/kwh

Electricity consumption = $24584.93 \text{ kg} \div 100 \text{ kg substrate/kwh} = 245.85 \text{ kwh}$

Electricity cost = $245.85 \text{ kwh} \times \$0.07/\text{kwh} = \$17.21$

Water:

Water loading = 20 kg substrate/m³

Water consumption = $24584.93 \text{ kg} \div 20 \text{ kg substrate/m}^3 = 1229.25 \text{ m}^3$

Water cost = $1229.25 \text{ m}^3 \times \$0.583/\text{m}^3 = \$716.65$

Total SSF cost = $\$38821.31 + \$138.7 + \$17.21 + \$716.65 = \$39684.87$

4. Distillation and solids recovery

Unit cost in this portion = \$0.238 /gallon ethanol = \$0.063/litre

Total cost = $\$0.063/\text{litre} \times 10000 \text{ litre} = \630

5. Accessories

Unit cost for accessories = \$0.151/gallon ethanol = \$0.04 /litre ethanol

Total accessories cost = \$0.04 /litre ethanol $\times$ 10000 litre = \$400

Overall operational cost for producing 10000 litre of ethanol

= Feedstock cost + Pre-treatment cost + SSF cost + distillation and recovery cost
+ accessories cost

= \$960.35 + \$8057.33 + \$39684.87 + \$630 + \$400

= \$49732.55

D.2 Operational cost of producing 10000 litres of ethanol from poultry manure

Mass of glucose needed = 16766.92 kg (from D.1)

Glucose yield from enzymatic hydrolysis = 27.6% (the best result achieved in this study)

Substrate needed for enzymatic hydrolysis = $16766.92 \text{ kg} \div 0.276 = 60749.7 \text{ kg}$

KOH pre-treatment yield = 71.2%

Mass of raw poultry manure needed = $60749.7 \text{ kg} \div 0.712 = 85292.59 \text{ kg} = 85.29 \text{ dry ton}$

Moisture content of raw poultry manure = 73.0%

Mass of raw poultry manure = $85.29 \text{ dry ton} \div (1-0.73) = 315.90 \text{ ton}$

1. Feedstock cost

Unit cost of raw poultry manure = \$15/ ton (Timmenga & Associates Inc., 2003)

Feedstock cost = $\$15/\text{ton} \times 315.90 = \4738.48

2. Pre-treatment cost

Electricity:

Grinding capacity per unit electricity = 50 kg/kwh (Guo et al, 2002)

Electricity consumption in grinding = $85292.59\text{kg} \div 50 \text{ kg/kwh} = 1705.85\text{kwh}$

Electricity consumption in pre-treatment = $3 \times \text{electricity consumption in grinding}$
 $= 3 \times 1705.85\text{kwh} = 5117.56\text{kwh}$

Electricity cost = $5117.56 \text{ kwh} \times \$0.07/\text{kwh} = \$358.23$

Water:

In the pre-treatment process, 60.0g dry poultry manure was treated in 300ml KOH solution, thus the water loading = $300\text{ml} \div 60\text{g} = 5 \text{ ml/g} = 5 \text{ l/kg}$

Water consumption = $5 \text{ l/kg} \times 85292.59\text{kg} = 426463 \text{ l} = 426.46 \text{ m}^3$

Water price = $\$0.583 / \text{m}^3$

Cost in water = $426.46 \text{ m}^3 \times \$0.583 / \text{m}^3 = \$248.63$

KOH:

KOH concentration = 0.5 N

Mass of KOH in 300ml 0.5N KOH = $0.5 \text{ mol/l} \times 0.3 \text{ l} \times 56 \text{ g/mol} = 8.4 \text{ g}$

KOH loading = $8.4 \text{ g KOH} \div 60 \text{ g raw corn stalk} = 0.14 \text{ g KOH/g raw material}$

KOH needed = $0.14 \text{ kg KOH/kg raw material} \times 85292.59\text{kg} = 11941\text{kg}$

KOH recovery rate = 95% (as in chemical pulping process)

KOH consumption = $11941\text{kg} \times (1-95\%) = 597.05\text{kg}$

Cost of KOH = $\$10.04 / \text{kg} \times 597.05\text{kg} = \5970.48

HCl:

HCl concentration = 1.0 N

HCl loading = $0.02\text{ml/g feedstock} = 0.033 \text{ l/kg feedstock}$

HCl needed = $0.02 \text{ l/kg feedstock} \times 85292.59\text{kg} = 1705.85\text{litre}$

Concentrated HCl needed = $1705.85\text{litre} \times 0.083 = 141.59 \text{ litre}$

Cost of HCl = $\$5.0 \times 141.59 \text{ litre} = \707.93

Total Pre-treatment cost = $\$358.23 + \$248.63 + \$5970.48 + \707.93
 $= \$7285.27$

3. SSF

Enzyme:

Enzyme activity = $1\mu\text{mol glucose per hour/unit} = 0.18\text{ mg glucose/hr/unit}$

Retention time = 24 hours

Enzyme capability = $0.18\text{ mg glucose/hr/unit} \times 24\text{ hr} = 0.00432\text{ g glucose/unit}$

Enzyme needed = $16766.92\text{ kg glucose} \times 1000 \div 0.00432\text{ g glucose/unit}$
 $= 3,881,231,483\text{ units}$

Enzyme recycling rate = 90% (Miyamoto, 1997)

Enzyme consumption = $3,881,231,483 \times (1-0.9) = 38812.31 \times 10^4\text{ units}$

Enzyme price = \$10/10000 units

Consider 10-fold price reduction in enzyme price

Enzyme cost = $38812.31 \times 10^4\text{ units} \times \$10/10000\text{ units}/10 = \38821.31

Yeast:

Yeast cost per unit ethanol = \$0.0525 /gallon (Aden et al, 2002)
 $= \$0.0525\text{ /gallon} \div 3.785\text{ litre/gal}$
 $= \$0.01387/\text{litre ethanol}$

Yeast cost = $\$0.01387/\text{litre ethanol} \times 10000\text{ litre} = \138.7

Electricity:

Electricity efficiency = 100 kg substrate/kwh

Electricity consumption = $60749.7\text{kg} \div 100\text{ kg substrate/kwh} = 607.50\text{ kwh}$

Electricity cost = $607.50\text{ kwh} \times \$0.07/\text{kwh} = \$42.52$

Water:

Water loading = 60 kg substrate/m³

Water consumption = $60749.7\text{kg} \div 60\text{ kg substrate/m}^3 = 1012.50\text{m}^3$

Water cost = $1012.50\text{m}^3 \times \$0.583/\text{m}^3 = \$590.28$

Total SSF cost = $\$38821.31 + \$138.7 + \$42.52 + \$590.28 = \$39583.8$

4. Distillation and solids recovery

Unit cost in this portion = \$0.238 /gallon ethanol = \$0.063/litre

$$\text{Total cost} = \$0.063/\text{litre} \times 10000 \text{ litre} = \$630$$

5. Accessories

$$\text{Unit cost for accessories} = \$0.151/\text{gallon ethanol} = \$0.04 / \text{litre ethanol}$$

$$\text{Total accessories cost} = \$0.04 / \text{litre ethanol} \times 10000 \text{ litre} = \$400$$

Overall operational cost for producing 10000 litre of ethanol

$$\begin{aligned} &= \text{Feedstock cost} + \text{Pre-treatment cost} + \text{SSF cost} + \text{distillation and recovery cost} \\ &\quad + \text{accessories cost} \\ &= \$4738.48 + \$7285.27 + \$39583.8 + \$630 + \$400 \\ &= \$52637.6 \end{aligned}$$

D.3 Operational cost of producing 10000 litres of ethanol from raw sewage

$$\text{Mass of glucose needed} = 16766.92 \text{ kg (from D.1)}$$

$$\text{Glucose yield from enzymatic hydrolysis} = 31.1\% \text{ (from raw sewage)}$$

$$\text{Substrate needed for enzymatic hydrolysis} = 16766.92 \text{ kg} \div 0.311 = 53912.9 \text{ kg}$$

$$\text{Moisture content of raw sewage} = 96.5\%$$

$$\text{Mass of wet raw sewage needed} = 53912.9 \text{ kg} \div (1 - 0.965) = 1540369 \text{ kg} = 1540.4 \text{ ton}$$

$$\text{Volume of raw sewage needed} = 1540.4 \text{ ton} \div 1.12 \text{ ton/m}^3 = 1375.33 \text{ m}^3$$

1. Feedstock cost

$$\text{Unit consumption of energy in conveying of raw sewage} = 150 \text{ m}^3 / \text{kwh}$$

$$\text{Feedstock cost} = 1375.33 \text{ m}^3 \div 150 \text{ m}^3 / \text{kwh} \times \$0.07 / \text{kwh} = \$641.82$$

2. Pre-treatment cost

Electricity:

$$\begin{aligned}\text{Electricity in pre-treatment} &= 1.5 \times \text{energy cost in feedstock conveying} \\ &= 1.5 \times \$ 641.82 = \$962.73\end{aligned}$$

HCl:

$$\text{HCl loading} = 2\text{ml/litre feedstock} = 2 \text{ l/m}^3 \text{ feedstock}$$

$$\text{HCl needed} = 2 \text{ l/kg feedstock} \times 1375.33 \text{ m}^3 = 2750.66 \text{ litre}$$

$$\text{Concentrated HCl needed} = 2750.66 \text{ litre} \times 0.083 = 228.3 \text{ litre}$$

$$\text{Cost of HCl} = \$5.0 \times 228.3 \text{ litre} = \$1141.52$$

$$\begin{aligned}\text{Total Pre-treatment cost} &= \$962.73 + \$1141.52 \\ &= \$2104.25\end{aligned}$$

3. SSF**Enzyme:**

$$\text{Enzyme activity} = 1\mu\text{mol glucose per hour/unit} = 0.18 \text{ mg glucose/hr/unit}$$

$$\text{Retention time} = 24 \text{ hours}$$

$$\text{Enzyme capability} = 0.18 \text{ mg glucose/hr/unit} \times 24 \text{ hr} = 0.00432 \text{ g glucose/unit}$$

$$\begin{aligned}\text{Enzyme needed} &= 16766.92 \text{ kg glucose} \times 1000 \div 0.00432 \text{ g glucose/unit} \\ &= 3,881,231,483 \text{ units}\end{aligned}$$

$$\text{Enzyme recycling rate} = 90\% \text{ (Miyamoto, 1997)}$$

$$\text{Enzyme consumption} = 3,881,231,483 \times (1-0.9) = 38812.31 \times 10^4 \text{ units}$$

$$\text{Enzyme price} = \$10/10000 \text{ units}$$

Consider 10-fold price reduction in enzyme price

$$\text{Enzyme cost} = 38812.31 \times 10^4 \text{ units} \times \$10/10000 \text{ units}/10 = \$38821.31$$

Yeast:

$$\begin{aligned}\text{Yeast cost per unit ethanol} &= \$0.0525 \text{ /gallon (Aden et al, 2002)} \\ &= \$0.0525 \text{ /gallon} \div 3.785 \text{ litre/gal} \\ &= \$ 0.01387/\text{litre ethanol}\end{aligned}$$

$$\text{Yeast cost} = \$ 0.01387/\text{litre ethanol} \times 10000 \text{ litre} = \$138.7$$

Electricity:

Electricity efficiency = 100 kg substrate/kwh

Electricity consumption = $53912.9\text{kg} \div 100 \text{ kg substrate/kwh} = 539.13 \text{ kwh}$

Electricity cost = $539.13 \text{ kwh} \times \$0.07/\text{kwh} = \$37.74$

Water:

Water loading = 40 kg substrate/m³

Water consumption = $53912.9\text{kg} \div 40 \text{ kg substrate/m}^3 = 1347.82\text{m}^3$

Water cost = $1347.82\text{m}^3 \times \$0.583/\text{m}^3 = \$785.78$

Total SSF cost = $\$38821.31 + \$138.7 + \$37.74 + \$785.78 = \$39774.5$

4. Distillation and solids recovery

Unit cost in this portion = $\$0.119 / \text{gallon ethanol} = \$0.032/\text{litre}$

Total cost = $\$0.032/\text{litre} \times 10000 \text{ litre} = \315

5. Accessories

Unit cost for accessories = $\$0.076/\text{gallon ethanol} = \$0.02 / \text{litre ethanol}$

Total accessories cost = $\$0.02 / \text{litre ethanol} \times 10000 \text{ litre} = \200

Overall operational cost for producing 10000 litre of ethanol

= Feedstock cost + Pre-treatment cost + SSF cost + distillation and recovery
cost + accessories cost

= $\$641.82 + \$2104.25 + \$39774.5 + \$315 + \$200$

= $\$43035.6$