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The Amenability Of Pre-Treated Source Separated Organic (SSO) Waste For Ethanol Production

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THE AMENABILITY OF PRE-TREATED SOURCE SEPARATED ORGANIC (SSO) WASTE FOR ETHANOL PRODUCTION

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THE AMENABILITY OF PRE-TREATED SOURCE SEPARATED ORGANIC (SSO) WASTE FOR ETHANOL PRODUCTION

Mina Mirzajani, Master of Applied Science, 2009
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Abstract

Every year, millions of tonnes of municipal solid waste are generated in the city of Toronto from residential and non-residential sources. A large fraction of the municipal solid waste is composed of organic materials. This valuable resource has traditionally been disposed of in landfills, which in turn contributes to the pollution of the environment and the generation of green house gases. This places a great emphasis on the need for the design and implementation of more sustainable waste management practices and the adequate supportive infrastructures in order to achieve sustainability.

The city of Toronto has been experiencing a huge challenge over the past few years regarding its waste problem, and having inadequate infrastructure for effective waste management practices. In the year 2000, the City of Toronto established a goal of 100% waste diversion by the year 2010 (Task Force, 2001). In the year 2005, the City of Toronto collected approximately 100,000 tonnes of source separated organic waste (SSO) from single-family households (Butts, 2005). SSO is an excellent source of fermentable carbohydrates including free sugars, starch, cellulose, hemicellulose and other degradable organic materials. However, the main obstacle is the release of some of its carbohydrates, such as cellulose and hemicellulose, from their bondage to lignin before conversion to

fermentable sugars. Cellulose and hemicellulose in SSO are bonded to lignin and are not easily separated and fermented to ethanol. Therefore, for utilizing SSO as a feedstock for ethanol production, a deep understanding of the nature of lignocellulosic materials is essential in order to overcome the challenges in the biological conversion to ethanol. As an initial part of a multi-staged project, this thesis is to examine the potential of SSO for utilization as a feedstock for ethanol production. A set of experiments were conducted on SSO in order to determine the amenability of SSO to ethanol production. The experimental results show a relatively high amount of carbohydrates in the SSO samples, indicating potential of SSO to be utilized as an ethanol production feedstock. Comparing result of the characteristics study with other cellulosic feedstocks, indicates that SSO has a reasonable amount of fermentable sugars and can be utilized for ethanol production instead of using other cellulosic feedstocks such as herbaceous energy crops. A technology for the biological conversion of SSO to ethanol was proposed based on the current techniques and the results from the characterization study on SSO.

It is foreseen that the finding of this study will enhance the overall understanding of the nature of SSO and the possibility of using it for ethanol production, and provide technical data and information for the decision makers in the assessment of the potential of SSO for ethanol production.

Acknowledgment

I would like to express my sincere thanks to Dr. Grace Luk for her guidance and supervision during the research and experiments. Many thanks, as well, to the project's industrial sponsors: Mr. Jamie Bakos and Dr. Henry Miyamoto of Clean 16 Environmental Technology Corp. for the opportunity to work on this exciting project. My appreciation also extends to Ms. Balinder Rai of Ontario Center of Excellence (OCE) for her support and coordination for the project. I would also like to thank Dr. Mostafa Warith, and Dr. James Li for their supports and advices during my graduate study.

I have to acknowledge that the completion of this successful research project is a result of a team effort. Special thanks to my team mates: Benjamin Percy, Robin Luong, Mandana Ehsanipour, Michael Faye, Grace Lin, and Valeriy Bekmuradov for their support, time, and priceless help during the completion of this research project. Special thanks to Benjamin, Mandana, and Robin for their supports and help in the laboratory and to Valeriy and Michael for their advices regarding my report.

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Chapter 1 Introduction

1.1 Background

In recent decades, with rapid growth in the population of metropolitan cities, the development of adequate urban infrastructures has become significantly important. The design and implementation of sustainable infrastructures is one way to reduce the unpleasant environmental impacts of human activities and urban development. Waste management is one of the vital infrastructures for protecting the environment, enhancing basic services to the citizens, and improving public health (Sakai et al., 1996).

There is an unavoidable need for sustainable waste management systems in the urban communities. This is to provide better services for citizens and to protect the environment. This mission is not easily reachable unless a deep consideration is devoted to the social, economic, and environmental aspects of waste management systems.

Every year, millions of tonnes of municipal solid waste are generated in the city of Toronto from residential and non-residential sources. A large fraction of the municipal solid waste is composed of organic materials. This valuable resource has traditionally been disposed of in landfills, which in turn contributes to the pollution of the environment and the generation of green house gases, such as methane and carbon dioxide (Lin, 2007). This places a great emphasis on the need for the design and implementation of more

sustainable waste management practices and the adequate supportive infrastructures in order to achieve sustainability. The city of Toronto, as a metropolitan city, has been experiencing a huge challenge over the past few years regarding its waste problem, and having inadequate infrastructure for effective waste management practices.

Toronto's waste problem became more critical in 1998 with the anticipation of the closure of Keele Valley landfill, located north of Toronto, on Major Mackenzie Blvd and Keele Street, which was the city's major landfill site (Faye and Percy, 2006). In 1998, with the anticipation of the closure of this landfill site, Toronto began to send its waste to Michigan for disposal, for the average cost of US $63 per tonne (City of Toronto, 2001). Despite recent developments, such as the opening of the Green Lane landfill site in London, Ontario, or the construction of the anaerobic digestion plant in Portland, Ontario, the city still requires more efforts in developing integrated strategies and new technologies for dealing with its wastes.

In the year 2000, the City of Toronto established a goal of 100% waste diversion by the year 2010 (Task Force, 2001). The organic fraction of municipal solid waste (OFMSW) is a valuable resource and has a great potential for the production of value added chemicals and energy products such as ethanol. In 2005, the City of Toronto collected approximately 100,000 tonnes of source separated organic waste (SSO) from single-family households (Butts, 2005). This costly collection of such a valuable resource demands a large contribution of the new technologies and innovative waste diversion plans in order to achieve the City's goal of 100% diversion by the year 2010.

SSO has the potential to be converted to value-added energy products through the use of new technologies. Ethanol, hydrogen, butanol, methane and acetone can be produced from a proper organic resource such as SSO upon the design of a suitable conversion technology.

On the other hand, with the scientific proof of energy crises and depletion of the world's oil resources, the need for an alternative renewable energy production has recently become more prominent. The high price of oil is continuously forcing countries to be less dependent on oil imports, and to seek novel solutions for renewable energy production. One of these renewable energy alternatives is ethanol from biomass resources, such as energy crops and agricultural residue. Ethanol is a renewable fuel which is used as an octane enhancer to reduce the incomplete combustion of gasoline. The blends of ethanol and gasoline can significantly improve the performance of engines and reduce the emissions. The Government of Canada has proposed the Ethanol Expansion Program to set a target to have 35 percent of all gasoline in Canada containing a blend of 10-percent ethanol by the year 2010 (Government of Canada, 2005).

Ethanol is currently produced from chemical or biological conversion of crops such as corn, wheat, rice and other food-based resources. However, the recent debates about the food crisis, because of fuel production from human food resources, has put emphasis on a need for alternative renewable feedstock other than human food for ethanol production. Therefore, lignocellulosic feedstocks, such as agricultural residue, herbaceous energy

crops, wood waste, and organic waste, have become more popular for the production of ethanol.

Lignocellulosic biomass is one of the most abundant resources on the Earth and utilizing such valuable resource for ethanol production is significantly beneficial. Lignocellulosic biomass is composed of cellulose, hemicellulose, and lignin. Cellulose is a crystalline bio-polymer which is mainly composed of glucose; hemicellulose is also a carbohydrate which is mainly composed of xylose, although traces of arabinose, mannose, and galactose exist; and lignin is a complex and three-dimensional non-crystalline polymer composed of linked six-carbon phenolic rings with other carbon chains and chemical components. Lignin binds the cellulose and hemicellulose fibres and protects them from hydrolysis of their monomeric sugars and fermentation to ethanol. Therefore, unlike starch and sugar in starchy biomass, lignocellulosic biomass is difficult to degrade to ethanol and requires a large amount of pre-treatment and processing in order to separate its carbohydrates from their bondage to lignin and ferment them to ethanol (Guffey and Wingerson, 2002).

SSO is an excellent source of fermentable carbohydrates including free sugars, starch, cellulose, hemicellulose and other degradable organic materials. However, the main obstacle is the release of some of its carbohydrates, such as cellulose and hemicellulose, from their bondage to lignin, before conversion to fermentable sugars. Cellulose and hemicellulose in SSO are bonded to lignin and are not easily separated and fermented to ethanol. Therefore, for utilizing SSO as a feedstock for ethanol production, a deep

understanding of the nature and chemistry of lignocellulosic materials is essential in order to overcome the challenges in the biological conversion to ethanol.

A research project, of which the current thesis forms a part, has been approved by the Ontario Centre of Excellence (OCE) with support from an industrial partner, the Clean 16 Environmental Technologies Corporation. The proposed research project will prove that SSO is a suitable feedstock for ethanol production through fractionation and bacterial fermentation. The research will lead to the development of a low-cost method utilizing waste biomass to produce ethanol. The outcome of this research project will benefit both the fields of waste management and the renewable energy sector in overcoming the current waste problem and energy crisis in the city of Toronto. The research project is phased into the following five stages:

Stage 1. Characterization of pre-treated SSO as a biomass feedstock in order to examine the potential of utilizing SSO for ethanol production along with the introduction of a novel technology for biological conversion of SSO to ethanol;

Stage 2. Investigation of the feasibility of converting pre-treated SSO in one-stage consolidated bio-processing fermentation by employing the bacterium *Clostridium phytofermentans;*

Stage 3. Investigation of the feasibility of converting pre-treated SSO to ethanol in two stages of enzymatic hydrolysis and fermentation utilizing the cellulase enzyme and the bacterium *Zymomonas mobilis.*

Stage 4. Production of ethanol and acetate in a continuous-culture fermentor; and

Stage 5. Design and operation of a bench-scale ethanol plant.

The Characterization Study (Stage 1) began in spring 2007. It will confirm that SSO is amenable to biological fermentation and production of ethanol under the optimal condition and employment of novel technologies. The investigation presented in this report is Stage 1 of the research project.

1.2 Scope and Study Objectives

Production of value-added energy products such as biogas from organic waste has been the subject of many studies over the past few years (Leschine, 2007; Lynd, 1996; Riggle, 1998; Wyman, 1996). However, ethanol production from such a resource has not been deeply explored, and the available literature on ethanol from organic waste is limited. SSO is a potential feedstock for ethanol production that can be utilized instead of energy crops or starchy biomass due to its high carbohydrate content.

As explained in the previous section, the objective of this study is to examine the potential of SSO for utilization as a feedstock for ethanol production. Therefore, this study should provide answers to the following questions:

1. What are the alternative waste management practices that can utilize SSO and prevent the disposal of such a valuable resource in landfills?
2. Does SSO have the potential to be utilized as a feedstock for the production of value-added energy products such as ethanol?
3. What are the possible challenges and obstacles for biological conversion of SSO to ethanol?
4. What is the proposed technology to produce ethanol from SSO?
5. What are the benefits of utilizing SSO for ethanol production instead of dedicated energy crops?

Therefore, the main objectives of this research can be summarized as follow:

- Conduct a literature review on: the nature of organic waste, developing analytical techniques for lignocellulosic biomass characteristics analysis, alternative waste management practices, and obstacles for the conversion of lignocellulosic biomass to ethanol.
- Carry out a set of experiments for the complete characterization of SSO, provide enough statistical analyses in order to make final conclusion about the nature of SSO, and provide the required data to help municipal decision makers in the assessment of the technical potential of SSO for anaerobic digestion.
- Design a comprehensive treatment technology for the conversion of SSO to ethanol based on its characteristics and using available techniques.

1.3 Project Team

The project team is composed of the following three parties:

- Ontario Center of Excellence (OCE-a Government organization for promoting research by making the connections, building strong industry and academic relationships, and providing the opportunity for commercialization);
- Industrial partner, Clean 16 Environmental Technologies Corp.; and
- Academic partner, Ryerson University, Department of Civil Engineering.

The project is supervised by Dr. G. Luk, a tenured professor and graduate program director of the Department of Civil Engineering at Ryerson University. Clean 16, Environmental Technology Corp. provides the ideas for environmental designs and implementations along with partial funding for this project. The Ontario Centre of Excellence also provides funding for this project in order to build industry and academic relationships, and facilitates the commercialization of the project in the long term.

Under Dr. Luk's guidance and mentorship, six Master students, Benjamin Percy, Robin Luong, Michael Faye, Mandana Ehsanipour, Grace Lin, and I, and one PhD's student, Valeriy Bekmuradov have been working on this project. The project includes different aspects from the feasibility study to the level of commercialization and pilot plant implementation. Most of the team members participated in all tasks in the study with different degrees of involvement, including literature review, experimental work, data analysis, model development, technical reporting, and presentation to the industry partner. My contribution was to conduct a complete characterization study on the SSO sample, propose the technology for the biological conversion of SSO to ethanol, prepare the technical reports, and present the results to the industry partner.

Chapter 2 Literature Review

In order to better understand the research findings of this study, the literature review focuses on a) cellulosic biomass characterization, b) amenability of organic waste as a cellulosic biomass feedstock for production of biofuels, c) limitation and pre-treatment of lignocellulosic biomass, and d) overview of the technologies for ethanol production from cellulosic biomass feedstock. All of these concepts will be explicitly covered throughout the following sections.

2.1 Biomass as a Feedstock

Biomass is a broad description of a group of highly diverse materials which are all organic. Biomass can be classified in a number of ways; for instance, by source, by chemical characteristics, or by the market demands. The definition of biomass, as a renewable energy source, refers to the living and recently dead biological materials that can be used for direct combustion to energy, or for industrial production of liquid fuel or biogas (NRC, 2008).

Biomass can be converted into renewable energy through a number of technological options. Conversion technologies may produce the energy directly from the biomass by chemical or biological methods and release the energy in the form of liquid fuel or biogas.

The processes, in which heat is the prevailing mechanism for the conversion of biomass into energy, are combustion, gasification, and pyrolysis. The processes which involve biochemical conversion of biomass into liquid fuel, biogas, and other value added bio-products are anaerobic digestion, fermentation, and composting (Biomass Energy Center, 2008).

Due to the renewable nature of biomass-derived end products, such as biofuel and biogas, biomass is progressively attracting attention as a potential feedstock. In Canada, approximately 6% of the total energy consumed is currently provided from biomass resources and this could be tripled in future because of Canadian biomass resource availability (Tampier et al., 2004).

Canada has abundant sources of biomass. For instance, the approximate thermal energy content of the annual biomass harvest in Canada is 5.1 exajoules which is equal to 62% of the thermal energy derived from fossil fuel combustion (Biocap, 2002).

Canada's forestry sector harvests more than 190 mega tonne (Mt) of round wood and non-stem biomass annually, which together have an energy content of 36% of the current Canadian energy consumption from fossil fuels (Tampier et al., 2004). In fact, Canada's forests alone currently contain the equivalent of 69 years of fossil fuel consumption and it is a renewable resource (Canadian Bioenergy Association, 2008).

The agricultural biomass is also of great importance in Canada. Total harvestable agricultural biomass is 124 Mt/year, from which about 45% is residue and available for energy production (Tampier et al., 2004).

Municipal solid waste (MSW) is another abundant source of biomass in Canada. Each year 25 Mt of municipal solid waste is generated in Canada from which 16 Mt is disposed of in landfills. The MSW contains approximately 85% of combustible materials. The Organic Fraction of Municipal Solid Waste (OFMSW) is one of the best feedstock for biogas and biofuel production. The carbohydrates and other carbon sources present in organic waste can be fermented to ethanol or methane, depending on the pre-treatment techniques and process design configuration (Tampier et al., 2004). **Table 2.1** is summary of information for various biomass resources in Canada.

Table 2.1 Availability of different biomass resources in Canada (Tampier et al., 2004)

Biomass from ...	Specific Type	Availability [Mt/year]	Appr. Energy Content in Dry Biomass [PJ]
Forestry Residues	Non-stem wood	92	1440
	Mill residues	<5.7	<117
	Tall oil	0.18	7
	Black Liquor	24	282
Agricultural Residues	Straw & Stover	18-25	277-385
	Livestock manure	58-79	65-88
Landfill Gas	(in tonnes of CO_2e)	21.9	
Municipal and Industrial Residues	MSW (50% of total)	8	132
	Municipal water purification sludge	0.4	7
	Yellow grease*	0.16	6
	Beef tallow*	0.20	8
	Pork Lard*	0.07	3
	Canola oil* (from low-quality canola only)	0.01	0.4
Energy Crops (Potential)	Switchgrass and other grass	6.8[1]	107
	Canola and soy beans (10% of harvest)	0.23	9
	Grains (wheat and corn; enough for a national E10 standard)	>4.6	89

2.1.1 Lignocellulosic Biomass

Lignocellulosic biomass refers to plant biomass that is mostly composed of cellulose, hemicellulose, and lignin. Strands of carbohydrate polymers (cellulose and hemicelluloses) are tightly bound together with the association of lignin. Lignocellulosic biomass can be obtained from different sources including: wood resources, wastepaper resources, agricultural residue, and municipal solid waste. Lignocellulosic biomass is of a great interest for the cellulosic ethanol production. However, converting lignocellulosic biomass to ethanol is a more complex process in comparison with corn or other starchy feedstock.

2.1.2 Organic Waste as a Biomass Resource

Annually, millions of tonnes of organic waste are generated in Canada from municipal, commercial, and agricultural sources, which are often lost or underutilized by being left in place as residue or disposed of in landfill. These contribute to the release of greenhouse gases such as methane and carbon dioxide and cause climate change. A proper integrated waste management approach must be undertaken in order to not only reduce the harsh environmental impacts such as green house gas emission but also produce value-added end products such as biofuel and other biochemicals.

Therefore, more sustainable and cost-competitive alternatives should be considered for utilizing organic waste as a renewable resource. Accordingly, organic waste can be used

as a cellulosic biomass feedstock for biogas and liquid fuel production, such as ethanol, methanol, acetone, hydrogen, and methane. Waste has become an attractive feedstock for energy purposes over the past few decades. The OFMSW is of a particular interest regarding this matter.

Toronto has also a big source of organic waste which is being underutilized by disposing in landfills. In 1998, in anticipation of the closure of the Keele Valley Landfill located north of Toronto; the City of Toronto started to ship its landfill waste to Michigan at an average cost of $63 per tonne. However, due to the high cost of shipping and also in anticipation of the termination of the agreement between Toronto and Michigan in 2010, the City of Toronto has been forced to seek novel solutions for its waste disposed crisis. The City of Toronto has been performing a separate collection stream for organic wastes, since 2002. The source separated organic waste (SSO) is collected on a bi-weekly basis in Green Bins. Collection began first in Etobicoke in September 2002 and has been extended across the city. From 150,000 tonnes of household organic wastes which are produced each year in Toronto, about 100,000 tonnes are collected through the Green Bin program (Butts, 2005).

Moreover, in the year 2000, the City of Toronto created the waste diversion Task Force, with the goal of 30% residential waste diversion by 2003, 60% by 2006, and 100% by 2010 (City of Toronto, 2001). Recently, the City of Toronto has extended its goal to reach 100% diversion by the year of 2012 (Butts, 2005).

SSO is a lignocellulosic biomass feedstock with high potential for energy production due to its availability at low or even negative price and its high carbohydrate content (Wyman, 1999). A range of 30% to 50% of cellulose in municipal solid waste has been reported in a study by (Wyman, 1999). Wyman and Goodman (1993) have also reported 45% cellulose in SSO, which is similar to the cellulose content of dedicated herbaceous energy crops. In fact, cellulose-based waste is preferred for bioethanol production over the dedicated energy crops due to its satisfactorily high level of cellulose and other fermentable carbohydrate contents and the lower or even negative price in comparison with dedicated energy crops (Wyman et al., 1992).

2.1.3 Anaerobic Digestion vs. Aerobic Decomposition

Anaerobic digestion of organic waste has several advantages over the traditional aerobic treatments such as land spreading and composting. The time required for processing the organic waste in an anaerobic digester is significantly less than that of aerobic composting (Shin and Yoon, 2005; Owen et al., 1979). Also there are fewer odour problems. Finally, there are limitations and regulations for composting in Canada: a) the concentration of heavy metals should not exceed a certain amount based on the category of compost, b) the compost should be a mature and stable product at the time of marketing, and c) human pathogen and other organic contamination should not be presented in the compost at the marketing stage (CCME, 2005).These regulations make the process of composting a not-so-easy treatment for a feedstock such as organic waste in comparison with the anaerobic digestion. Moreover, the emission of greenhouse gases

is much less in anaerobic digestion in contrast with aerobic methods (Meta-Alvarez et al., 2000).

Anaerobic digestion has been frequently cited as the most cost-effective treatment for organic waste due to the high energy recovery linked to the process and its limited environmental impact. In anaerobic digestion, a considerable amount of energy is recovered while composting on the contrary is an energy consuming treatment (Meta-Alvarez et al., 2000). Also, aerobic treatment of organic waste emits a huge amount of volatile compounds such as ketones, ammonia, aldehydes, and methane (De Baere, 1999). Generally, in terms of global warming, anaerobic treatment scores much higher than other treatment technologies for organic waste (Baldasano and Soriano, 2000); and finally from the industrial perspective, anaerobic digestion of organic waste is considered as an advanced technology (Riggle, 1998).

Traditionally, organic wastes such as OFMSW have been utilized in land spreading and composting or disposed of in landfills. Landfilling of the biodegradable components of organic waste contributes to greenhouse gas generation by the emission of methane and carbon dioxide and also causes considerable environmental pollution due to leachate generation. Land spreading of organic waste has been used as an alternative to conventional fertilizers and soil conditioner. However, land spreading also introduces contaminants to the environment and causes the emission of greenhouse gases and water pollution.

Composting of organic waste as another alternative is environmentally acceptable. However, compost must be a mature and stable product which is free of human pathogens and heavy metals at the time of marketing in order to be accepted as a commercial product (CCME, 2005). The process of compost production is time consuming taking three to six month, and is an equipment and labour intensive process in which the final product has only a local market and generates little revenue for the owner (Blades, 2006).

2.1.4 Biofuel Production from Organic Waste

Value added energy products from organic waste have been a subject of many studies over the past few decades. Methane, however, has attracted a significant attention over the others because of its faster production and more convenient utilization in the current energy market. The methane recovered from the anaerobic digestion is used as a biogas for transportation as well as electricity generation.

Producing ethanol and hydrogen from organic waste is a practical solution to the existing obstacles in the current waste management issues. Organic waste is an abundant source of cellulose and other carbohydrates which are amenable to degradation for ethanol production. Utilizing organic waste as a cellulosic feedstock is not only beneficial for reducing the capital cost of the process, but is also a practical and environmentally viable waste management solution in comparison with other aerobic treatments such as composting (Baldasano and Soriano, 2000). However, producing ethanol from organic

waste as lignocellulosic materials requires a deep understanding on the chemistry of lignocellulosic biomass feedstock due to the complexity of the conversion process.

2.2 Chemistry of Lignocellulosic Biomass

In order to utilize a cellulosic biomass such as SSO as a feedstock for the purpose of ethanol production, it is requisite to truly understand the nature of lignocellulosic materials. Lignocellulosic material is composed of three major components: cellulose, hemicellulos, and lignin. Cellulose is a polymer composed of monomeric sugars in six carbon chains which are mostly glucose. These chains are bundled together forming strong fibres which have a crystalline structure. Lignin is a complex and three-dimensional polymer which contains six-carbon phenolic rings along with other carbon chains and chemical functionalities. Unlike cellulose, the structure of lignin is non-crystalline. Lignin serves to bind the cellulose fibres. Hemicellulose is a complex polymer composed of various branches of five- and six-carbon sugars which are primarily xylose. It bonds weakly to both cellulose and lignin and fills the spaces (Guffey and Wingerson, 2002). **Figure 2.1** is a schematic diagram of the structure of a plant cell wall.

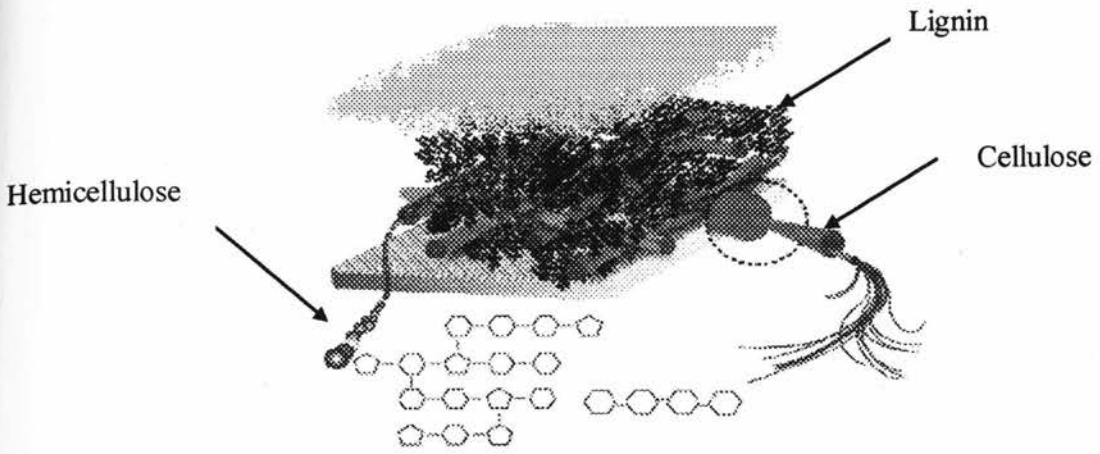


Figure 2.1 Structure of lignocellulosic materials (Ceres Inc., 2007)

Each component of lignocellulosic material is explained separately in the following sections.

2.2.1 Cellulose Component

Cellulose is a linear insoluble biopolymer composed of the repeated union of β-D-glucopyranose linked by β-1, 4 glycosidic bonds, as shown in **Figure 2.2**. In comparison with other glucan polymers such as starch, the repeating structural unit in cellulose is not glucose but the disaccharide cellobiose. With a degree of polymerisation (DP-which is the number of repeating sugar units) ranging from 2 to 7, the β-1, 4 glucose oligomers, are water soluble (Pereira et al., 1988). In cellulose, the glucan chain can reach a length of approximately 25,000 glucose residues (Brown et al., 1996). The association of cellulose macromolecules leads to the formation of a microfibril containing 15 to 45 chains in a regular crystalline arrangement, as shown in **Figure 2.2**. Moreover, cellulose

fibres contain various types of irregularities such as twists, which increase their surface area (Desvaux, 2005).

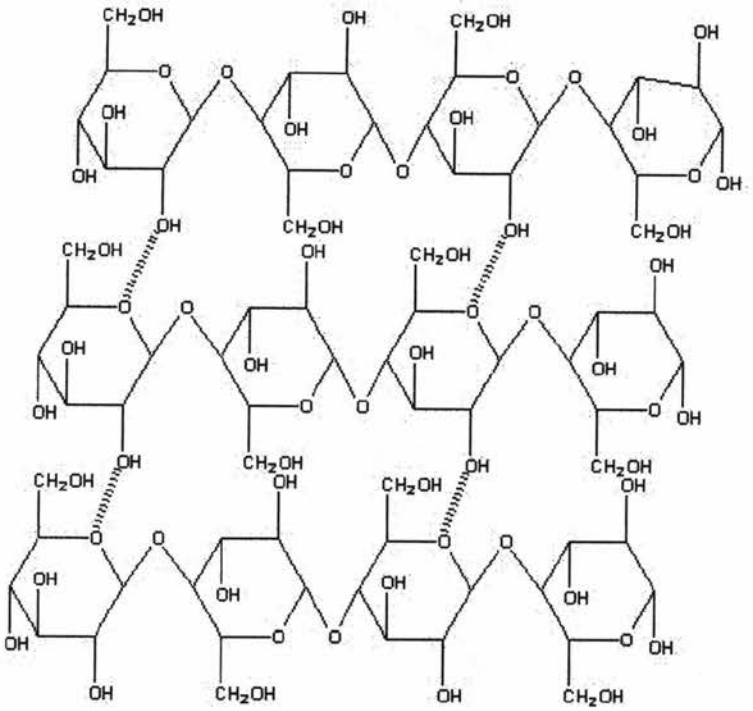


Figure 2.2 The structure of Crystalline Cellulose (Science College, 2009)

2.2.2 Hemicellulose Component

Hemicellulose, which is composed of five carbon sugars, is a heteropolymer (matrix polysaccharides) present in plant cell walls composed of five carbon sugars. Most of these five carbon sugars are xylose; however, there are also traces of mannose, galactose, and arabinose. While cellulose is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength in contrast with

cellulose which is crystalline. It is easily hydrolyzed by dilute acid or base as well as countless hemicellulase enzymes (Ruffel, 2006).

2.2.3 Lignin Component

Lignin, another structural component of lignocellulosic plants, is a complex, three-dimensional polymer composed of linked six-carbon phenolic rings with a variety of carbon chains and other chemicals. Lignin is non-crystalline and its structure is similar to a gel or foam. Lignin serves to bind the cellulose fibres and fill the spaces between cellulose and hemicellulose in the plant cell wall (Guffey and Wingerson, 2002).

The complex structure of lignin defends against enzymatic and hydrolysis attack, by anchoring cellulose and hemicellulose fibres (Guffey and Wingerson, 2002; Howard et al., 2003). Even by removing the lignin from the biomass with a proper pre-treatment technology, the formation of intermediate toxic substances from lignin may inhibit the further process of fermentation by microorganisms (Palonen, 2004). Therefore, choosing the right pre-treatment techniques to remove lignin is of particular importance for the conversion of cellulosic biomass to ethanol.

2.2.4 Extractives

Extractives are organic components present in cellulosic biomass that have lower molecular weight than carbohydrate and lignin. Resins, fats, waxes, fatty acids, alcohols,

terpentines, tannins and flavonoids are examples of extractives. Extractives typically comprise 4-10% of the total dry mass of cellulosic biomass; however, the amount and the variety of extractives highly depend on the quality and grade of the biomass samples. For instance, there are high percentage of extractives in such feedstock as municipal solid waste and woody feedstock than other (Fengel at al., 1989).

The presence of extractives in biomass samples causes interference with the quantitative measurement of carbohydrate and lignin. Generally, extractives in biomass result in an inflated measurement of lignin and a lower measurement of carbohydrate than the actual amount in the samples (Thammasouk et al., 1997).

According to Ibrahim (1998), the amount of cellulose and hemicellulose in the biomass are considered as carbohydrate components and the amount of lignin and extractives as non-carbohydrate components of the biomass. In fact, the isolation and analysis of structural components such as carbohydrates and lignin is more important than extractives and other components because the amount of carbohydrates can provide an estimation of the theoretical ethanol production yield from microbial fermentation.

2.2.5 Compositional Analysis

Lignocellulosic biomass has a more complex structure in comparison with starch in corn and sugarcane. As explained before, the complexity of the lignocellulosic biomass is because of the bondage between the carbohydrates and lignin. The main dilemma in the

biological conversion of cellulosic biomass to ethanol is the liberating of carbohydrates from their bondage and releasing their monomeric sugars for the further fermentation and ethanol production (Lynd, 1996). Understanding the chemistry of the structure of the plant cell wall along with the accurate compositional analysis of lignocellulosic biomass are requisites for the design of a successful conversion process of the cellulosic feedstock to ethanol.

Table 2.2 is a comparison between cellulose, hemicellulose, and lignin content of different cellulosic biomasses (Howard et al., 2003). Additional compounds found in cellulosic biomass samples are extractives such as resin, grease, fatty acids, and etc.

Table 2.2 Comparison of composition of different lignocellulosic materials (Howard et al., 2003)

Lignocellulosic feedstock	Cellulose (%)	Hemicellulose(%)	Lignin (%)
Hardwood stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Corn cobs	45	35	15
Corn stover	38	32	15
Paper	85-99	0	0-15
Wheat straw	30	50	15
Rice straw	32.1	24	18
Leaves	15-20	80-85	0
Newspaper	40-55	25-40	18-30
Chemicals pulps	60-70	10-20	5-10
Primary wastewater solids	8-15	NA	24-29
Fresh bagasse	33.4	30	18.9
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12
Grasses	25-40	25-50	10-30

The widespread interest in the utilization of cellulosic biomass feedstock for ethanol and hydrogen production has increased the demand for more accurate and practical analytical techniques for the compositional analysis of these types of feedstock. Ideally, the methods used for the compositional analysis should be precise, robust, fast, and applicable to all types of biomass (Thammasouk et al., 1997).

A precise compositional analysis of the biomass feedstock is crucial to advancing and commercializing the technology for converting biomass to energy. The needs for promoting standards and reliable methods for analysis of biomass samples are growing.

The three major parameters of interest in the analysis of cellulosic biomass feedstock are cellulose, hemicellulose, and lignin, which are the main components of the plant cell wall (Zhang et al., 1995). Cellulose and hemicellulose are the primary carbon source in the process of fermentation of biomass to ethanol. Cellulose represents the majority of degradable glucan available and hemicellulose represents the majority of degradable xylan available in the samples (Thammasouk et al., 1997). Lignin is the recalcitrance component of the feedstock, and it is very important to measure it because it is negatively correlated with the cellulose and hemicellulose degradability (Chandler et al., 1980).

There are also other parameters of interest in biomass such as total volatile organic compounds, moisture content, ash, extractives, trace elements, crude fat, and crude protein. Although these parameters are not the main structural components of interest, they should be measured for the purpose of mass balance of the biomass feedstock.

Compositional analysis of cellulosic biomass feedstock is generally conducted by standard analytical approaches among which the National Renewable Energy Laboratory (NREL), American Society for Testing and Materials (ASTM), and the Technical Association of the Pulp and Paper Industry (TAPPI) are the most well known.

NREL has been particularly involved in the research and development of analytical techniques and procedures for characterization of biomass samples. These series of procedures are referred to as NREL Lab protocol for compositional analysis of cellulosic biomass (NREL, 2008).

A scientifically accurate compositional analysis of biomass samples should be conducted in certain sequences. A recommended sequence for the analysis of cellulosic biomass by Kim et al. (2007) is shown in **Figure 2.3**. Based on this diagram, the moisture content and ash are the two parameters that should be analysed prior to any experiment on the *as received* biomass samples. Carbohydrate measurement is the most crucial part of the analysis, extractive and protein content should also be determined in order to minimize the errors in the measurement of carbohydrate and lignin.

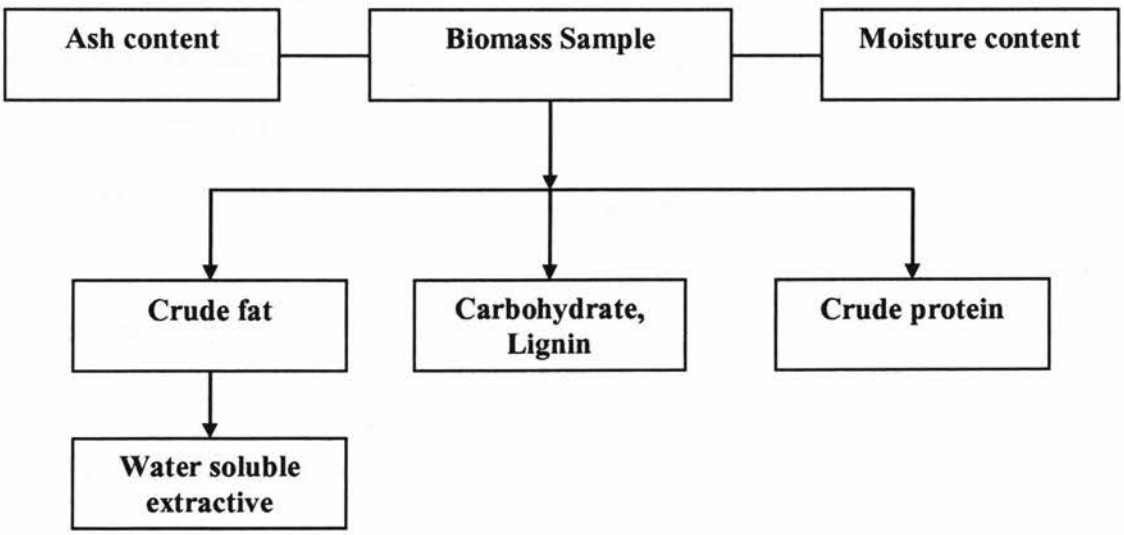


Figure 2.3 Flow diagram of cellulosic biomass compositional analysis (Kim et al., 2007)

Determination of all the above characteristics of biomass samples is not feasible unless a comprehensive understanding of the chemistry of cellulosic biomass structure is achieved and modern analytical techniques and instruments are developed.

As elucidated above, the main part of a compositional analysis of cellulosic samples is the carbohydrate analysis. Cellulose, hemicellulose, and any other carbohydrate present in the samples are indicators of the biodegradability of biomass. Hence, a robust and accurate method for the analysis of carbohydrates should be developed in order to minimize the measurement errors.

There are two distinct approaches for the measurement of cellulose and hemicellulose; one is the gravimetric method of Van Soest (1991), and the other is the hydrolysis and indirect quantification of carbohydrates by liquid chromatography and measuring the total monomeric sugars as a representative of the cellulose, hemicelllulose. The quantitative saccharification method also measures monomeric sugars from the other carbohydrates such as starch.

The gravimetric method of Van Soest is a relatively long and time consuming procedure (Wyman, 1996) and is not suitable for heterogeneous biomass samples such as organic waste due to the interferences of other compounds. Hence, the indirect method of quantitative saccharification looks a more promising alternative in comparison to the gravimetric method of Van Soest.

The indirect method of carbohydrate analysis by liquid chromatograph has been developed and standardized by several procedures, among which NREL and American Society for Testing and Material (ASTM) are the most well known.

The indirect analysis of carbohydrate is based on acid hydrolysis of biomass in order to break the chains of carbohydrates and release the monomeric sugars. The monomers are then analysed by employing a high performance liquid chromatograph apparatus (HPLC).

2.3 Limitation of Lignocellulosic Decomposition

Notwithstanding that lignocellulosic biomass is the most abundant resource on the earth, there are several characteristics of biomass samples which hinder the process of fermentation. These characteristics make the cellulosic ethanol production a less economically-favorable approach. The current challenge to making the cellulosic ethanol production more economic mostly focuses on developing a proper pretreatment technique. The following are the most common limitations in the cellulosic biomass feedstock for the purpose of biological ethanol production.

2.3.1 Presence of Lignin

Lignin is the main deterrent to enzyme attack on cellulose. The detrimental effect of lignin on the process of cellulose fermentation has frequently been cited. For instance, Chandler et al. (1980) formulated a mathematical relation between the bioavailability of

an organic substrate and lignin content. The data used for their study have been collected from the anaerobic degradation of a range of lignocellulosic materials (with 40 day retention time); they developed a linear connection between biodegradability and the lignin content as expressed in Equation 2.1:

$$BF = 0.83 - (0.028 \times L_{\%vs}) \quad (2.1)$$

Where:

BF= biodegradable fraction

$$L_{\%vs} = \frac{L\%}{VS_{\%}/100}$$

L%= the lignin content as a percent of total solids

VS%= volatile solid as a percent of total solids

There are many more mathematical relationships describing the negative effects of lignin on biodegradability in an anaerobic environment. In general, the higher is the amount of lignin that exists in a biomass; the lower is the bioavailability of the substrate, because lignin physically restricts the process of enzymatic penetration and hydrolysis of cellulose (Haug, 1993).

In order to enhance digestibility, high lignin content biomass samples should undergo pretreatment to remove or alter the lignin (Lynd, 1996). However, lignin should not be reduced to less than 12%, because more lignin removal beyond this level does not significantly increase digestibility (Chang and Holtzapple, 2000).

2.3.2 Presence of Hemicellulose

Hemicellulose in biomass physically restrains the penetration and contact of the cellulase enzymes with cellulose (Yoon, 1998). Although it is a source of xylan in the biomass which can increase the ethanol yield in the process of fermentation, it also has a detrimental effect on the cellulose degradation.

The removal of hemicellulose can increase the efficiency of enzymatic hydrolysis as hemicellulose and lignin are covalently linked together, and removal of hemicellulose can open the structure of biomass and increase the enzyme penetration (Mosier et al., 2005). Hemicellulose removal can substantially increase cellulose digestion even with high lignin content (Grohmann et al., 1985).

2.3.3 Acetyl Content

Approximately 70% of xylan backbone in lignocellulosic biomass is acetylated by linking to an acetyl group (CH_3CO), which in turn hinders the enzymatic hydrolysis of cellulosic biomass (Browning, 1967). Removing the acetyl content of biomass samples can increase digestibility (Chang and Holtzapple, 2000).

2.3.4 Cellulose Crystalinity

The crystalline structure of cellulose protects it from the enzyme attack and inhibits the process of hydrolysis (Zhu et al., 2007). Many studies (Chang and Holtzapple, 2000; Fan et al., 1981; Koullas et al., 1990) have shown the positive relation of reducing crystallinity and digestibility. Therefore, crystallinity negatively affects the efficiency of enzyme attack and digestibility. A common method for reducing crystallinity is ball milling, a process in which the biomass material is become in contact with the moving balls inside the machine and transferred to a very powdery form. The process decreases the particle size and increases the surface area of biomass available for enzymatic hydrolysis (Caulfield and Moore, 1974).

2.3.5 Accessible Surface Area

Accessible surface area is an important factor that positively affects the digestibility of biomass (Grethlein, 1985; Sinitsyn et al., 1991; Thompson et al., 1992). However, some studies have shown that there is no relationship between the accessible surface area and digestibility (Fan et al,. 1981). Studies have also shown a positive relationship between size reduction and increase in digestability in cellulosic biomass samples (Palmowsky and Muller, 1999).

The above parameters are the major impediments in the process of lignocellulosic biomass degradation. As described, some disagreement has been reported in the literature

on the relationships between each parameter and cellulosic biomass digestibility. **Table 2.3** is an abridged of these relationships with regard to their source and reference.

Table 2.3 Relationship between different components of lignocellulosic biomass with digestibility (Zhu et al., 2007)

Characteristics	**Relationship with digestibility**	**Reference**
Lignin	Negative	Draude et al. (2001), Mooney et al. (1998)
Hemicellulose	Negative	Grohmann et al. (1989), Kim et al. (2007)
Acetyl content	Negative	Grohmann et al. (1989), Kong et al. (1992)
Particle size	Not related	Draude et al. (2001)
	Positive	Sinitsyn et al. (1991)
Crystallinity of cellulose	Negative	Caulfield and Moore (1974), Fan at al. (1981), Grethlein (1985), Puri (1984)
Pore volume	Positive	Grethlein (1985),Weimer and Weston (1985)
Degree of polymerization (DP)	Negative	Puri (1984)
	Not related	Sinitsyn et al. (1991)
Surface area	Positive	Grethlein (1985) Sinitsyn et al. (1991)

2.4 Pre-treatment of Lignocellulosic Biomass

The structural complexity of lignocellulosic biomass makes it a less convenient feedstock in terms of digestibility and fermentability. Hence, a set of pre-treatment technologies is required to overcome the lignocellulosic biomass recalcitrance. The ultimate objective of the pre-treatment methods is to alter the structural composition of biomass in order to increase digestibility (Sun and Cheng, 2002).

Since the understanding of the actual mechanisms of pre-treatment is still incomplete, the design of pre-treatment processes is mostly empirical (Lynd, 1996). However, the primary function of pre-treatment is to open up the bondages between cellulose, hemicellulose, and lignin and liberate them for further hydrolysis.

Pre-treatment is an expensive process in the cellulosic ethanol pathway, and can account up to 20% of total processing costs (NREL, 2002). However, it greatly enhances the performance of the process and increases the hydrolysis and production yield.

Figure 2.4 illustrates the change in lignocellulosic structure by an effective pre-treatment technique (Mosier et al., 2005).

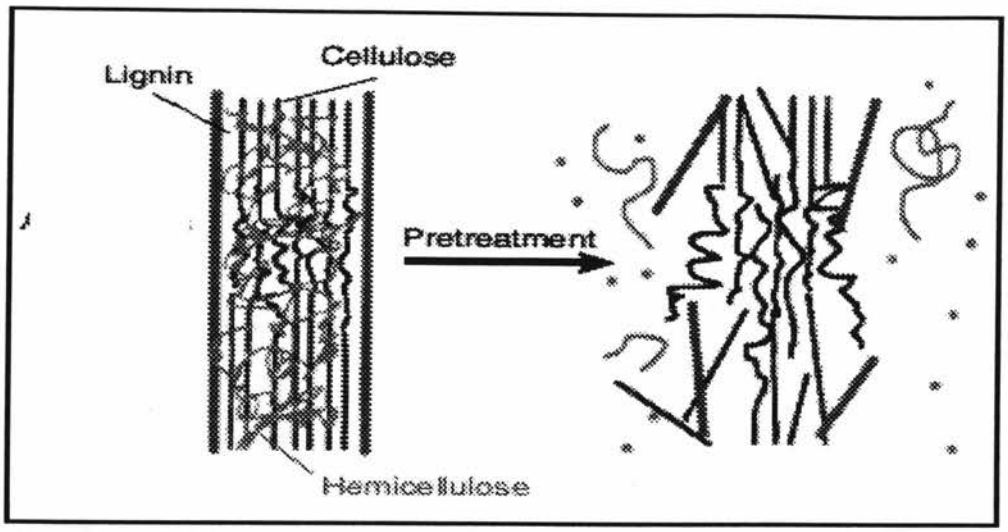


Figure 2.4 Change in the structure of lignocellulosic material by pre-treatment (Mosier et al., 2005)

The goals of a successful pre-treatment is removing or changing the structure of lignin, removing hemicellulose, removing acetyl content, reducing the crystallinity and degree of polymerization, reducing the particle size. However, a pre-treatment capable of accomplishing all of these tasks will be very expensive and infeasible. Hence, the goal of most of the pre-treatment techniques is to accomplish a few of the above tasks, maintain the cellulose and hemicellulose fraction, minimize the formation of inhibitory products during the pre-treatment process, consume a minimum amount of energy, and minimize capital and operating costs (Sierra et al., 2008).

Within the context of production of ethanol from cellulosic biomass, pre-treatment plays an important role by which the feedstock becomes amenable to the process of enzymatic hydrolysis. Almost all lignocellulosic biomass materials need pre-treatment. Without pre-treatment the hydrolysis yield can barely exceed 20% of theoretical yield whereas yields

after pre-treatment can reach up to 90% (Lynd, 1996). Pre-treatment techniques are generally classified as physical, chemical, or biological pre-treatment and vary from substrate to substrate (Ruffle, 2006).

2.4.1 Physical Pre-treatment

Physical pre-treatment techniques are those that rely on mechanical processes and generally do not involve the use of any chemicals. These pre-treatments intend to reduce particle size and cellulose crystallinity. Examples of physical pre-treatment are the milling processes, such as dry and wet ball milling, which do not significantly increase digestibility (Rivers and Emert, 1987). Communition, compression, milling, and radiation are also examples of physical pre-treatment which are not economically viable technologies.

2.4.2 Chemical Pre-treatment

Chemical techniques involve the addition of chemicals for the purpose of pre-treatment of biomass and delignification. These methods have received a remarkable amount of attention among all other pre-treatment techniques. The examples of chemical pre-treatment include autohydrolysis, dilute acid, alkaline, solvent ammonia, SO2, CO2, organosolve, lime and other chemical treatments (Ruffle, 2006). Some of the more popular pre-treatment methods are explained in the following.

2.4.2.1 Steam Explosion

The Steam Explosion (STEX) method involves high temperature steam (185° C-260 °C) pre-treatment with the presence of a subsequent explosive pressure resulting in fibre separation (Mabee et al., 2006). The moisture combines with the organic compound in the biomass at high temperature and forms an organic acid which catalyzes the process of hydrolyzing. The effect of STEX resembles the effect of acid hydrolysis while causing far less corrosion to the processing equipment (Wyman, 1996). However, in STEX there is still a significant formation of unfavourable substances that inhibit a possible subsequent fermentation process. Furthermore, decomposition of lignin is not satisfying with STEX, and lignin is still able to inhibit a possible enzymatic hydrolysis (Ahring et al., 2006). The process of STEX is sometime supplemented by a chemical such as sulphuric acid or sulphur dioxide (Torget et al., 1990; Clark and Mackie, 1987). STEX is a methods that has one of the highest commercialization potentials among all the methods of pre-treatment due to the availability of the equipment, less formation of inhibitory products during the process, and its effects on the structure of lignin (Sierra et al., 2008) .

2.4.2.2 Acid Hydrolysis

One of the common chemical pre-treatment processes is the acid hydrolysis of lignocellulosic biomass, and consists of two types of treatment: dilute acid and concentrated acid hydrolysis. In dilute acid pre-treatment, a dilute acid (e.g. sulphuric, nitric, hydrochloric) in the concentration of 0.5 to 10 % is added to the biomass slurry, in

the temperature of 140° C to 190° C in order to hydrolyse cellulose (Brenna et al., 1986; Esteghlalian et al., 1997). Concentrated acid pre-treatment solubilises the cellulose in biomass at room temperature. Generally, concentrated phosphoric acid (85% H_3PO_4) is used at ambient temperature and atmospheric pressure for this purpose (Sierra et al., 2008).

However, the conditions required for acid pre-treatment can result in the formation of undesired components, which inhibit the process of fermentation or decrease the yield of production. For example, in dilute acid pre-treatment, the harsh condition (temperature, pressure, and the presence of acid) may result in degradation of glucose into hydroxymethyl furfural (HMF), which in turn degrades to tars and other co-products (Wyman, 1996). These degraded co-products must be sold to reach favourable economics; however, the current market size for these co-products is too small to support the large scale production (Wyman, 1996). Therefore, the low yield of sugar and ethanol through the formation of undesirable co-products make the dilute acid pre-treatment a less favourable technology (Wright, 1983).

Another similar method to acid hydrolysis is CO_2 explosion, which is also referred to as the supercritical carbon dioxide method in some studies. High pressure and high temperature cause the reaction of CO_2 with water to form carbonic acid, which fractionates and hydrolyzes the biomass feedstock (Walsum and Shi, 2004) and decreases the crystallinity of cellulosic biomass. This method is contemplated as an inexpensive and environmentally viable pre-treatment (Sierra et al., 2008).

2.4.2.3 Ammonia Fibre Explosion

Aqueous ammonia is capable of lignin removal and cellulose decrystalination when it comes in contact with lignocellulosic material (Sierra et al., 2008). The process of Ammonia Fibre Explosion (AFEX) combines high temperature with sudden explosive release of the pressure in the presence of liquid ammonia. The combined chemical and physical effect of this technique results in a simultaneous decrystallization and increases the available surface area (Holtzapple et al., 1992). Typical process conditions for AFEX pre-treatment are 60°C to 160°C, 5 mins residence time, pressure of 20 atm, and the ammonia loading of 1 to 2 g NH_3/g biomass (Sun and Cheng, 2002). However, AFEX is not an effective pre-treatment technique for samples with high lignin content such as softwood (Holtzapple et al., 1992).

Another alkali pre-treatment is lime pre-treatment. Lime is an inexpensive chemical (Miller, 2001) for pre-treatment of lignocellulosic biomass, which is capable of solubilising lignin and some hemicellulose and can be simply recovered (Chang et al., 1998). In low lignin content biomass samples, a simple boiling in saturated lime water can significantly remove the lignin and increase digestibility (Sierra et al., 2008). Lime pre-treatment technology has been frequently cited as a promising method of treatment for lignocellulosic biomass (Chang et al., 1998; Kaar et al., 2000).

Sodium hydroxide is also a strong base which is capable of catalyzing the removal of lignin and decreasing the degree of polymerization (DP) of cellulose (Fan et al., 1981). A

pre-treatment of using sodium hydroxide can remove the lignin and increase the susceptibility of lignocellulosic biomass to digestion. Typical operational conditions for sodium hydroxide pre-treatment are 24-96 hrs, temperature of 25 °C, and pressure of 1 atm (Sierra et al., 2008).

2.4.2.4 Organic Solvent

The method of organic solvent (organosolv) pre-treatment uses organic solvent such as methanol and ethanol, to provide fractionation of cellulosic biomass. An inorganic acid like sulphuric acid or hydrochloric acid is used as a catalyst. Organic solvent solubilises the lignin with the presence of heat and catalyst. Once the solvent is removed, lignin is precipitated and can be separated from the biomass (Sarkanen, 1980).

2.4.3 Biological Pre-treatment

The biological pre-treatment technique comprises of applying lignin-solubilising microorganisms such as white and soft-rot fungi to render lignocellulosic biomass more digestible. In limited nitrogen or carbon source, P. chrysosporium, a white-rot Fungus, produces enzymes (lignin peroxidases and manganese-dependent peroxidases) that can degrade lignin (Boominathan and Reddy, 1992). The process of biological pre-treatment has several advantages over the other pre-treatment methods as it requires no chemicals and it consumes less energy input. However, biological delignification is a relatively slow process, and most of the lignin degradation microorganisms also solubilise and

consume hemicellulose and cellulose, which decreases the production yield of ethanol and is therefore undesirable for commercialization (Wyman, 1999).

In general, the fractionation and pre-treatment of cellulosic material is necessary for the process of ethanol production. Choosing the right pre-treatment technique is highly dependant on the feedstock characteristics. Therefore, a deep understanding and estimation of the feedstock characteristics can greatly improve the success of the design of the pre-treatment process.

2.5 Cellulosic Ethanol Production Technologies

There are two different approaches for producing ethanol from lignocellulosic materials: a) gasification and b) biological conversion processes. Distillation and recovery of ethanol at the final step is required for both approaches. Each of these technologies is described in the following sections.

2.5.1 Gasification

Gasification is a distinct approach of converting carbonaceous materials such as cellulosic biomass to ethanol, in which biomass is first converted to carbon monoxide and hydrogen under high temperature and presence of steam or oxygen. The ensuing gas mixture is called synthetic gas or syngas, which is also a fuel. Syngas may also be fermented in further steps with particular strains of microorganism including Clostridium

Ljungdahlii, or similar strains to produce ethanol (US Patent 5807722, 1992). The main disadvantage of gasification technology is the high cost of syngas generation.

In spite of the above facts, the gasification approach is promising because of its competitive cost with biological conversion of lignocellulosic materials. Also, this approach does not require the pre-treatment of cellulosic material which is one of the main challenges in the conversion process of lignocellulosic biomass to ethanol (Lynd, 1996).

2.5.2 Biological Approach

The biological technologies of producing ethanol consist of five major steps, as described in the followings (Lynd, 1996; Grethlein and Dill, 1993):

1- Pre-treatment to make the lignocellulosic material susceptible to hydrolysis and fermentation

2- Hydrolysis process in order to saccharify the carbohydrate which can be chemical or enzymatic hydrolysis

3- Fermentation of carbohydrate to ethanol and hydrogen mostly by employing microorganisms.

4- Distillation and recovery of ethanol

5- Utilities and waste treatment

The process of pre-treatment is essential prior to hydrolysis and fermentation and is a common operational unit among the ethanol plants.

The hydrolysis process is generally categorized into two different approaches: one uses mineral acids and the other uses the enzyme of a particular microorganism to hydrolyse the carbohydrate present in sugars. However, the processes of hydrolysis using acids are considered to have more environmental disadvantages than the enzymatic processes due to harsh nature of the chemicals during the process. Moreover, enzymatic hydrolysis processes have approximately equal costs with acid hydrolysis processes. The cost of enzymatic hydrolysis can be even lower than chemical hydrolysis upon the development of this technology in future (Lynd et al., 1991).

Microbial fermentation is the common operational unit among all of the biological conversion plants for converting cellulosic biomass to ethanol. A wide range of microorganisms, such as bacteria, yeast, and fungi, are capable of fermenting carbohydrate to ethanol and other co-products in an anaerobic environment (Lynd, 1996). The general equation for ethanol production can simply be expressed as follows:

Carbohydrate + microorganisms $\longrightarrow$ more microorganisms + CO_2 + ethanol + others

The selection of the ideal organism for fermentation is crucial to the process of ethanol production. An ideal organism should actively be capable of synthesis of enzymes at a high level and of growth on carbohydrate, and finally producing ethanol or other end

products at a high yield. In fact, no such microorganism has been introduced in the literature heretofore (Lynd et al., 2005). The process of biological conversion of feedstock to ethanol after the pre-treatment can be performed in four different configurations. Each of these process configurations is explained as follow.

1) Separate Hydrolysis and Fermentation (SHF), which conducts cellulose production, cellulose hydrolysis, fermentation of hexose and fermentation of pentose separately in different steps. In **Figure 2.5,** the main process train is a SHF configuration in which the hydrolysis and fermentation perform separately in different reactors.

2) Simultaneous Saccharification and Fermentation (SSF), in which cellulose hydrolysis and the fermentation of hexose sugar are accomplished simultaneously in one reactor by employing the microorganisms capable of simultaneous hydrolysis and fermentation of cellulose, and pentose fermentation is carried out in a separate reactor. In **Figure 2.5**, the SSF is indicated by combining the two steps in one.

3) Simultaneous Saccharification and Co-fermentation (SSCF) is a process similar to SSF, in which the fermentation of hexose sugar and pentose sugar is also carried out in a same reactor by employing a genetically modified organism capable of co-fermentation or by inoculation of recombinant pentose and hexose utilizing microorganisms. The process of SSCF is indicated in **Figure 2.5**.

4) Consolidated Bio-processing (CBP) which simultaneously accomplishes cellulase production, cellulose hydrolysis, and fermentation of hexose sugars and fermentation of pentose sugars in one step by employing genetically modified microorganisms capable of doing so (Lynd, 1996; Wyman, 1999).

CBP, which is also known as Direct Microbial Fermentation (DMF), is a promising technology for the conversion of cellulosic biomass to ethanol and other co-products, which offers the lowest possible cost for the project if the limitations can be overcome and if the project matures through more research and development. The key difference between CBP and other technologies is that in CBP a single cellulolitic microorganism is employed, which is capable of synchronized cellulase production and fermentation. This process has several advantages over the SSF; for instance, there is no capital cost for a separate enzyme production. However, this system of conversion suffers from some technological immaturities. For example, the formation of other by-products such as acetic acid and formic acid during the fermentation processes by some cellulolitic bacteria may result in lower ethanol yield (Wyman, 1999). Moreover, these cellulolitic bacteria need some genetic modification in order to increase ethanol tolerance, pentose fermentation, and production yield (Warnick et al., 2002; Lynd et al., 2005).

Figure 2.5 is an illustration of the process flow diagram for biological conversion of lignocellulosic material to ethanol. As can be seen from **Figure 2.5**, distinctive steps are involved for cellullase production, cellulose hydrolysis, and fermentation in the SHF. In SSF, the hydrolysis of cellulose and fermentation of hexose are performed in one step. In

SSCF, the hydrolysis of cellulose, fermentation of hexose, and fermentation of pentose sugars are performed in one step. It is obvious that the hydrolysis of hemicellulose is performed during the pre-treatment step. In CBP, a single step of enzyme production, hydrolysis and fermentation is conducted by employing a certain type of cellulosic microorganism (Wyman, 1994).

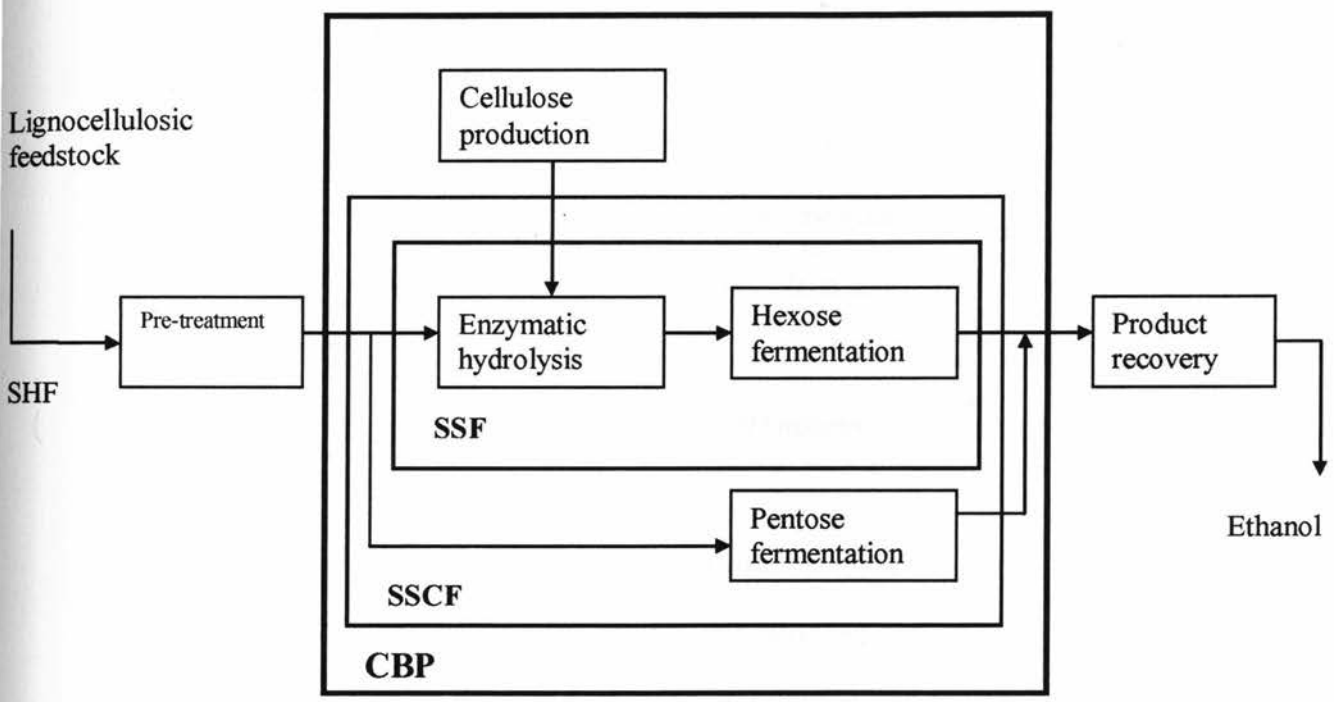


Figure 2.5 Steps in different conversion configurations for lignocellulosic feedstock to ethanol (Wyman et al., 1992).

There are some cellulolityc microorganisms capable of synergistically hydrolysing and fermentation of cellulose, among which the group Clostridia are more popular. These bacteria are capable of the direct hydrolysis and fermentation of cellulose with the use of an extracellular enzymatic complex called the cellulosome (Schwarz, 2001). These

bacteria are very promising for the direct production of ethanol from cellulosic biomass (Lynd, 1996). However, the metabolism pathway and functioning of the cellulolytic microorganisms on cellulose contains a set of substantial fundamental phenomena beyond those involved with enzymatic hydrolysis (Lynd et al., 2005); hence, the pathway of cellulose digestion in CBP is poorly understood and much research and investigation is required in order for it to be industrially commercialized. Moreover, these microorganisms need to be genetically modified to perform cellulose hydrolysis and sugar fermentation simultaneously and effectively (Desvaux, 2005). Clostridium cellulolyticum, Clostridium acetobutylicum, Clostridium populeti, Clostridium Cellobioparum, Clostridium thermochellum, and Clostridium phytofementant are all examples from clostridia family which are reported to be capable of direct hydrolysis and fermentation of cellulose (Ren et al., 2007). Among these, Clostridium thermocellum is the most common and well studied species for simultaneous enzyme production, hydrolysis, and glucose fermentation (Wyman, 1996).

However, the above and many more existing cellulolytic microorganisms should undergo a set of genetic modifications in order to be utilized for industrial purposes. For example, the low ethanol tolerance and production of by-products such as acetic acid and formic acid by these organisms results in low ethanol yield. This limitation can be overcome upon more progress in genetic modification of these microorganisms (Lynd et al., 2005).

In general, CBP has the potential to reduce the overall cost of bio-ethanol production from cellulosic material by fourfold in comparison with SSF (Lynd et al., 2005).

However, this is dependant on the development of a genetically modified microorganism capable of performing cellulose hydrolysis, fermentation of hexose and pentose sugars, and production of ethanol at high yield.

Currently the most practical pathway for the conversion of lignocellulosic materials to ethanol is SSCF process which is composed of two stages of hydrolysis and co-fermentation of pentose and hexose sugars (i.e. glucose and xylose). More detail on the biological conversion technology for the conversion of SSO to ethanol is explained in **Chapter 5**.

Chapter 3 Experimental Investigation

3.1 Experimental Plan

The primary objective of this research was to conduct a rigorous characteristics analysis of the SSO samples after the pre-treatment by the Aufberestungs Technology and System (ATS) thermal screw machine from Vartec Waste Management Corp. The secondary objective of this research was to study the time fluctuation of these characteristics and examine the potential of utilizing SSO as a feedstock for bio-ethanol production. The third objective of this research was to propose a sustainable technology for biological conversion of SSO to ethanol and other value-added end-products based on the results from these experiments. In order to accomplish these objectives, an experimental plan was established to measure the characteristics of SSO over a time span of six months.

A set of parameters of SSO were identified over the period of study and these parameters were measured to gain an understanding of the suitability of SSO for composting, methane generation, and ethanol production. **Figure 3.1** shows the grouping of parameters needed to assess these three end uses.

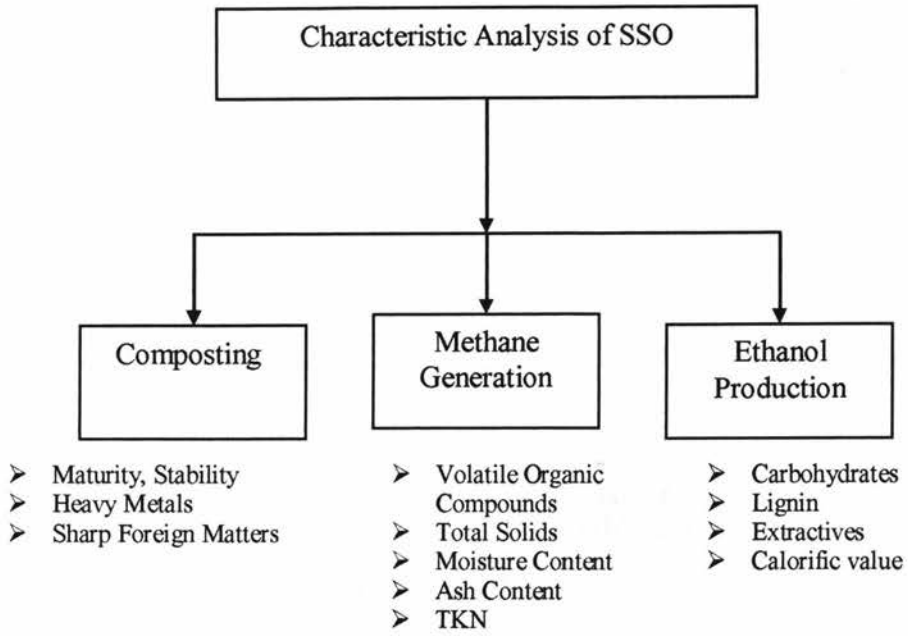


Figure 3.1 Experimental plan for measurement of SSO characteristics; each parameter of interest listed under its purpose for measuring.

As illustrated in **Figure 3.1**, the parameters under each end use will address the suitability of SSO for that particular application. For example, the maturity level, the stability, heavy metal content, and sharp foreign matter, were measured in order to examine the potential of utilizing SSO as a compost material.

As discussed in **Chapter 2**, in the analysis of organic waste, there are myriad of interferences from different compounds which may cause inaccuracy in the results. A scientific and reliable characteristics analysis of biomass feedstocks such as organic waste should be conducted in certain sequences in order to minimize the interferences and to provide more accurate results (Kim et al., 2007). **Figure 3.2** shows the sequences

that have been followed for the characteristics analysis of SSO, in this set of experiments, in order to seek more precision and reduce interference. The procedure is divided into two stages:

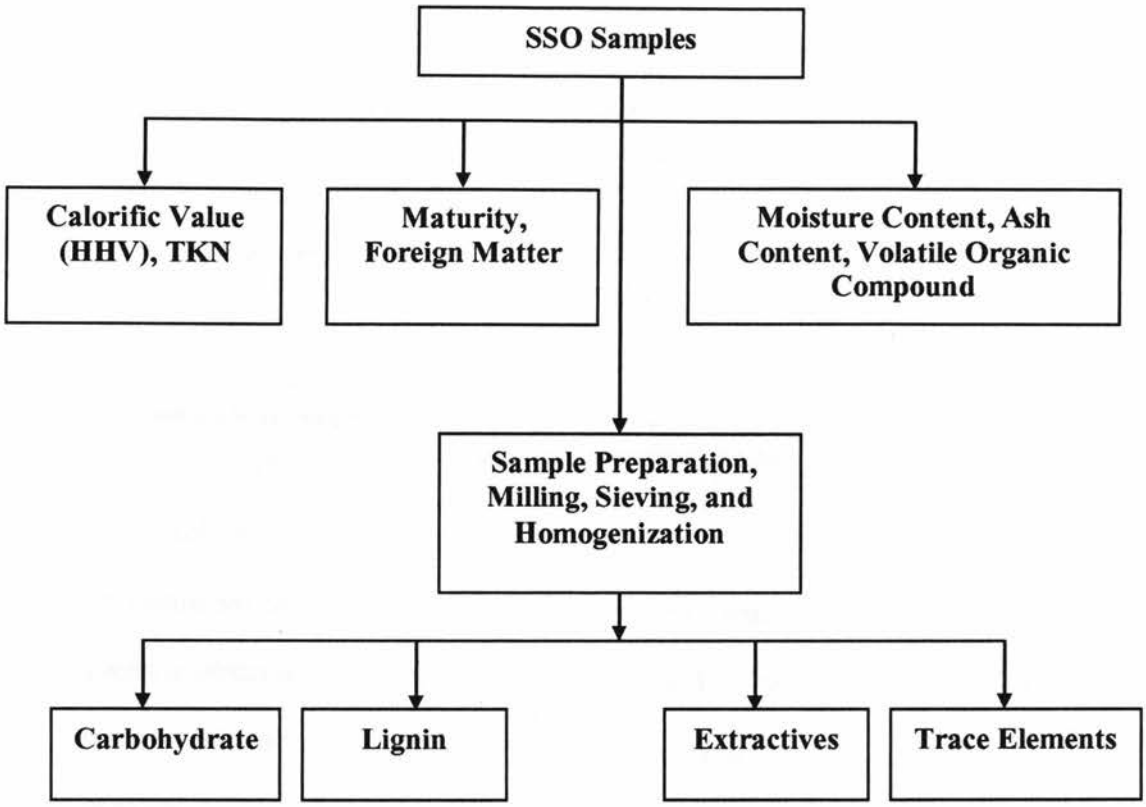


Figure 3.2 Sequences for SSO characteristics analysis

The details of the materials, methods, and the experimental procedures are presented in the following sections.

3.1.1 Feedstock Material

SSO samples were obtained from the output of the ATS machine. A blend of construction and demolition (CD) wood wastes in the form of wood chips was added to SSO prior to pre-treatment in the ATS machine in order to enhance the performance of the machine , stabilize the SSO, and extend the shelf life of the final product. The term SSO henceforth in this dissertation will be used to refer to the source separated organic waste and the blend of wood chips which has undergone pre-treatment in the ATS machine. The pre-treatment in the ATS machine is a physical pre-treatment which is comprised of heating, pulverising, and reducing the size of SSO materials. In the ATS machine, the friction caused by the plates of the screws increases the temperature of the process to 150 °C and the pressure to approximately 50 bars (M. Crupi, personal communication, March 26, 2009). The combination of high temperature and high pressure causes the transformation of SSO to a more homogenous, stable, and fibrous product. **Figure 3.3** shows the ATS machine for pre-treatment of SSO.

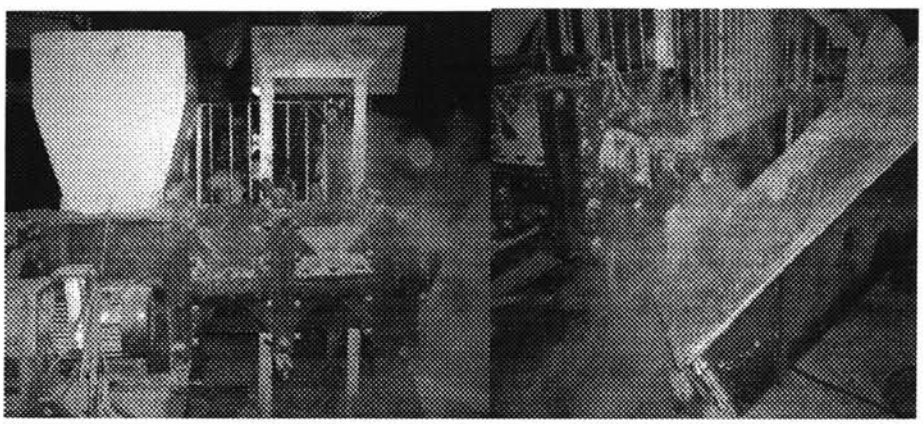
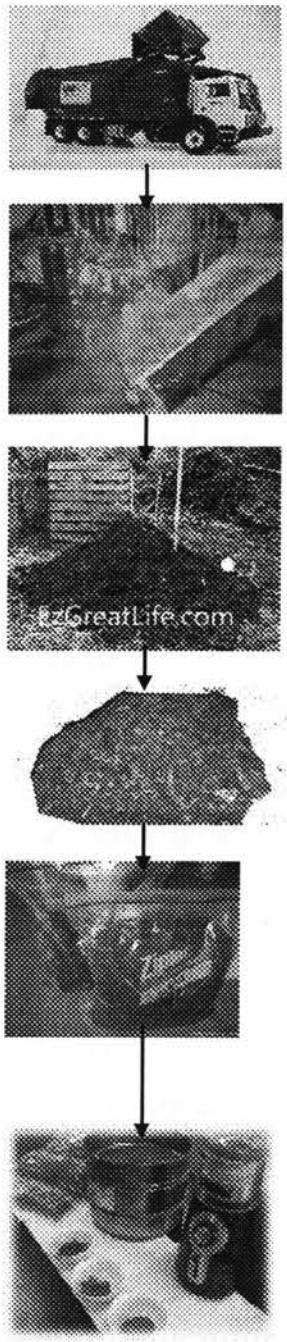


Figure 3.3 Aufbereitungs Technology and System (ATS) Thermal Screw machine

3.1.2 Sampling Procedure

In the characteristics analysis of the organic wastes like SSO, a major problem is obtaining a representative sample due to the heterogeneity of the materials. The sampling procedure is often costly and tedious if a representative sample is needed (Jansen et al., 2004). In order to minimize the uncertainty in the analysis of SSO, a reliable sampling procedure was followed which included techniques of field sampling, laboratory sampling, homogenization, and chemical analysis. As explained in the previous section, the ATS machine is capable of partially homogenizing the SSO samples, because of the high pressure, high temperature, the shredding, and the screwing involved in its operation. Therefore, it is reasonable to assume that the homogeneity of the samples from the outflow of the ATS machine is satisfactory for the purpose of this research. However, in order to produce more reliable data from the analysis, a sampling procedure was designed based on the method of Jansen et al. (2004). Some modifications were made in order to minimize any possible uncertainty involved in the analysis. The sampling procedure steps are shown in **Figure 3.4** on the following page.



Truck of organic waste

Mixing with wood waste and fed the ATS machine (**8 tons input capacity**)

Processing in the ATS machine (**5 tons dried SSO output**)

Mixing in pile (**100 kg**)

2% of the mixed pile material transferred to the bags and sent to the laboratory for analysis

Sample collection in field (**2 kg**)

Sampling in lab (**1 kg**)

After the physical sorting to remove plastics and sharp foreign matter, 50% was separated for drying, milling, sieving, and final chemical analysis

Drying, milling, sieving (**1 kg**)

Spreading the material remains on sieve No 80 in a tray and dividing into 10 subsamples. Analyse each subsample for the parameter of interest

Figure 3.4 Sampling procedures for compositional analysis

As illustrated in **Figure 3.4**, the final step of the sampling procedure includes spreading of the material in a tray, dividing the material into ten parts, and taking samples from the tray to analyse one parameter. Therefore, each parameter, except: total Kjeldahl nitrogen (TKN), calorific value, trace metals, and extractives, was measured up to 10 times and the mean value of the data considered as a representative measurement. The reasons behind the above exceptions are the complexity, lack of enough equipment, and the cost of the procedures for measuring these parameters. The uncertainty associated with the method of Jansen et al. is satisfactorily low (3% to 10%) and the analysis is representative of the characteristics of SSO (Jansen et al., 2004). Therefore, it can be predicted that the uncertainty of the sampling procedure in this study will be less than 10%.

3.1.3 Periodical Fluctuation of SSO Characteristics

SSO samples were taken from the ATS machine on a monthly basis for a time span of six months and analysed in order to find the monthly fluctuations in characteristics. The reason for obtaining the monthly fluctuations in characteristics was to minimize the uncertainty in process design caused by the heterogeneous nature of SSO. The extent of the possibilities for conversion of SSO to other value-added products like ethanol could not be guaranteed unless the fluctuations in its characteristics and minimum expected values could be predicted. Therefore, the same set of experiments on the SSO samples was repeated six times for a period of ten months from which samples collected in: September 2008, December 2008, March 2009, April 2009, May 2009, and June 2009.

3.1.4 Statistical Analysis of Data

Statistical analyses of the data were conducted in Microsoft Excel. The analyses divided into two categories as follow:

1. Sample range and tendency including maximum, minimum, range, median, and mean
2. Sample variation including: standard deviation, standard error, 95% confidence intervals, and sample variance

The spreadsheets and details of statistical analysis for each parameter are reported in appendix A.

3.2 General Characteristics Analysis

As explained above, the general characteristic analysis includes determination of moisture, ash, total and volatile solids, TKN, and calorific value in the SSO sample. Each of these analyses is explained in the following sections.

3.2.1 Moisture, Volatile Organic Compounds

The most widely accepted method of determining the moisture content involves drying in a conventional oven at 105° C to achieve a constant weight. There is an unavoidable error associated with this method, which is caused by the loss of some volatile solids in heat.

Volatile organic compounds (VOC) of the SSO samples were determined by the standard method of NREL (NREL, 2008), which includes an ignition at 575°C until a constant weight is achieved and a recording of the portion lost during ignition. The portion lost is volatile organic compounds (VOC). Ash is the percentage of residues that remains after ignition at 575 °C. The percentage of ash in the biomass samples is also an approximation of mineral contents (NREL, 2008).

3.2.2 Calorific Value

The calorific value of the SSO samples was determined in order to have an indication of the samples energy content. The calorific value indicates the amount of energy that could be released in the form of heat, during complete combustion of the samples. The calorific value of the SSO samples was determined in AMEC Earth and Environmental laboratory by following method E 870 from ASTM for "Analysis of wood fuel".

3.2.3 Total Kjeldahl Nitrogen

Total Kjeldahl Nitrogen (TKN) is the sum of organic nitrogen, ammonia (NH_3), and ammonium (NH_4^+) in a substance. The TKN content of the SSO samples was determined in order to have a better understanding of the possibilities for utilizing SSO in different applications. For instance, the amount of nitrogen in the organic waste is essential to microorganisms in compost for protein synthesis and microbial growth. If it is

inadequate, the composting process can not proceed. Determination of TKN was performed in AMEC Earth and Environmental analytical laboratory, Mississauga, Ontario, by following method 351.2 from U.S. Environmental protection Agency (EPA) for "determination of total Kjeldahl Nitrogen by semi-automated colorimetry". The procedure began by heating the samples in the presence of sulfuric acid for 2.5 hrs. The residue is then cooled, diluted to 25 ml, and analysed for ammonia by colorimetric method. Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen which are converted to ammonium sulfate, under the conditions during the digestion by sulfuric acid (U.S. EPA, 1993).

3.2.4 Extractives

As explained in chapter 2, the samples should be extractive free at the time of analysis for carbohydrate and lignin in order to minimize interference in the results. Extractives in the biomass samples are tannins, chlorophyll, waxes, nitrogenous materials, fats, etc. Extractives are soluble in some solvents, such as 95% ethanol, benzene, and hot water. The amount of extractives can be determined by refluxing in boiling ethanol or water and weighing the total dissolved solid results from the extraction.

The extractives in the SSO samples were determined by the ethanol extraction procedure from the Technical Association of Pulp and Paper Industry (TAPPI, 1988). Based on this procedure, 5 g of SSO samples were extracted in a 500 ml flask with 250 ml of 95% ethanol under the refluxing conditions. The samples and solvent were then cooled and

vacuum filtered through the medium porosity sintered glass crucible filter. The residue after filtering was dried at 45°C to a constant weight, and the filtrate evaporated and dried at 45°C for 24 hrs. **Figure 3.5** shows the extraction apparatus during refluxing in the laboratory.

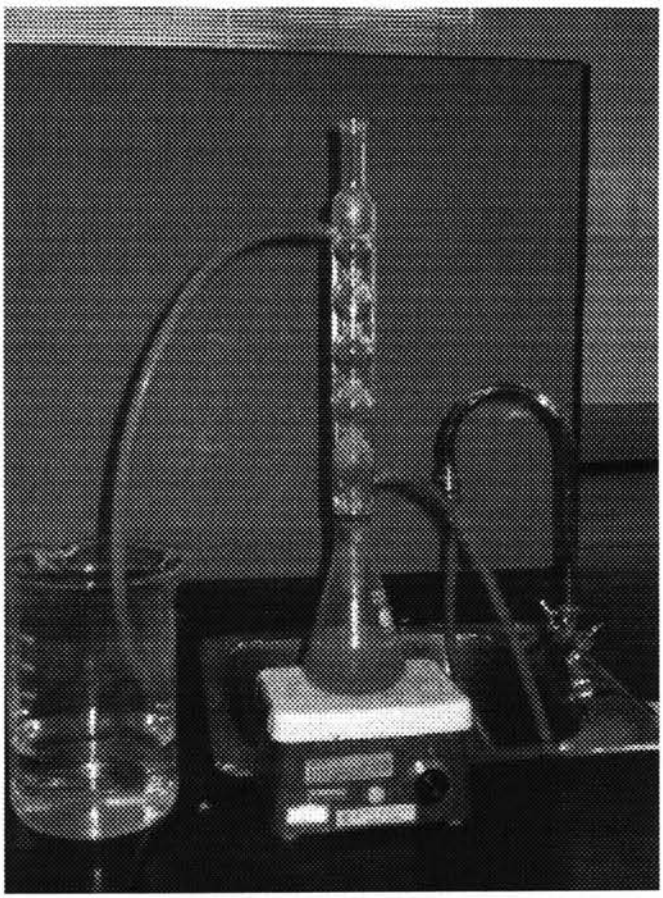


Figure 3.5 Apparatus for measuring extractives during refluxing

The amount of extractives was then calculated using **Equation 3.1** below:

$$\text{Extractives}(\%\text{ of total dry mass}) = \frac{\text{weight of dried dissolved solids from filtrate}}{\text{weight of solids loaded in extracting flask}} \times 100 \quad (3.1)$$

3.3 Compost Characteristics

The SSO samples obtained from the ATS machine were examined for their potential utilization as compost. In order to examine the feasibility of using SSO as compost, a set of characteristics was measured according to the "Guidelines for Compost Quality" from the Canadian Council of Ministers of the Environment (CCME, 2005). These parameters are explained in the following sections.

3.3.1 Compost Maturity and Respiration Rate

Based on the criteria in the CCME Guidelines for Compost Quality from the Canadian Council of Ministers of the Environment, the carbon dioxide evaluation rate should be less than, or equal to, 4 mg of carbon in the form of carbon dioxide per gram of organic matter per day (CCME, 2005). The ratio of carbon dioxide emission per oxygen depletion and the maturity of compost were determined by using the Solvita standard kits from Wood End Laboratories, Inc. The kits contain ammonia and carbon dioxide indicators as well as colour index. The rates of carbon dioxide and ammonia emission were determined by these indicators and interpreted by the colour index to indicate the compost condition.

Instead of reporting the carbon dioxide per weight of dry solid, the ratio of the volume of carbon dioxide emitted to volume of oxygen depleted was reported because the Solvita kit has been calibrated for this purpose. Based on the ratio of carbon dioxide to oxygen, the condition of compost was defined as Very Active, Active, Raw, or Mature.

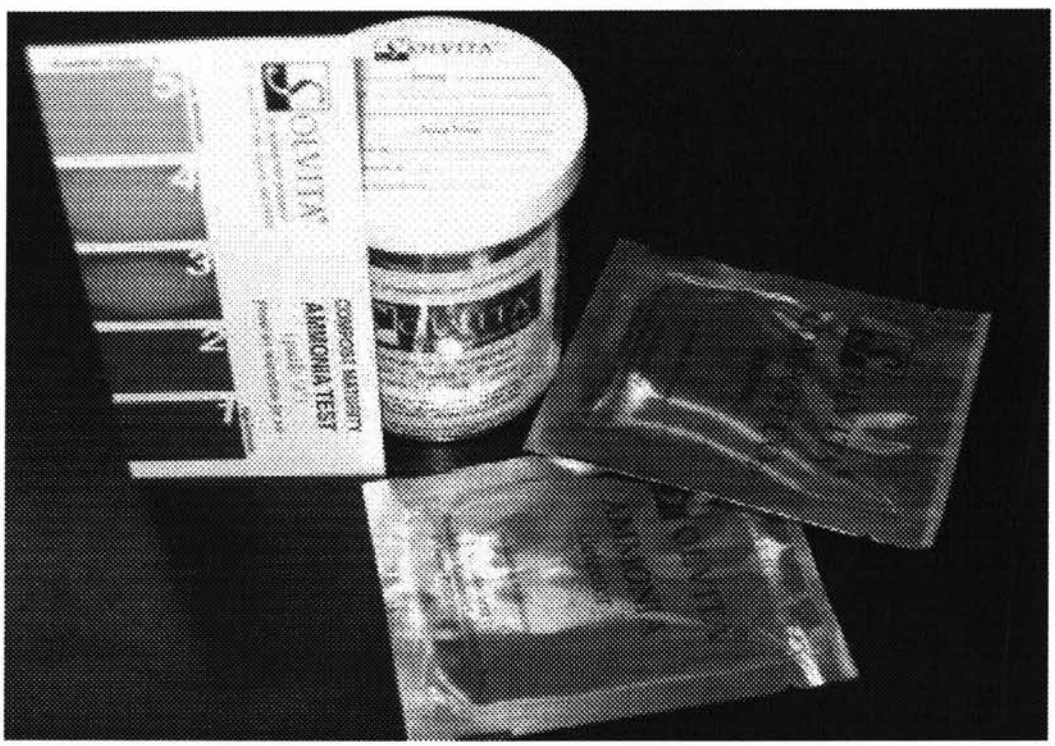


Figure 3.6 Solvita Test kit from Woods End laboratory Inc., ME, USA

3.3.2 Sharp Foreign Matter

The amount of sharp foreign matter in the SSO samples was measured in order to examine the potential of utilizing the SSO for composting. According to Canadian Council of the Minister of Environment (CCME) guidelines for composting, compost

shall not contain any sharp foreign matter of dimension greater than 3 mm per 500 ml of compost, to be considered as Category A (Unrestricted use), or shall not contain more than three pieces of sharp foreign matter of maximum 12.5 mm per 500 ml of compost, to be considered as category B (Restricted use) (CCME, 2005). The determination of sharp foreign matter was conducted by placing the sample in 500 ml container and separating the sharp foreign matter manually.

3.4 Preparation and Homogenization of SSO Samples

The nature of SSO is heterogeneous. Therefore, the results of analysis are not usually consistent for one batch of samples even after the pre-treatment in the ATS machine. In order to minimize uncertainty in the results, samples must be prepared and homogenized prior to compositional analysis. As illustrated in **Figure 3.2**, after determination of moisture, volatile solids, ash, maturity, TKN, calorific value, and sharp foreign matter, samples were homogenized for the compositional analysis by following the standard procedure from NREL, which includes oven drying, milling, and sieving. The portion which remained on sieve No 80 was used for compositional analysis (NREL, 2008).

Homogenization of samples not only improves the consistency of data for the compositional analysis, but also enhances the process of fermentation to ethanol by reducing the time required for digestion and increasing the fermentability of the samples (Palmowski and Muller, 1999). **Figure 3.7** shows some images of the steps in the

homogenization process which were performed on the SSO samples prior to the compositional analysis.

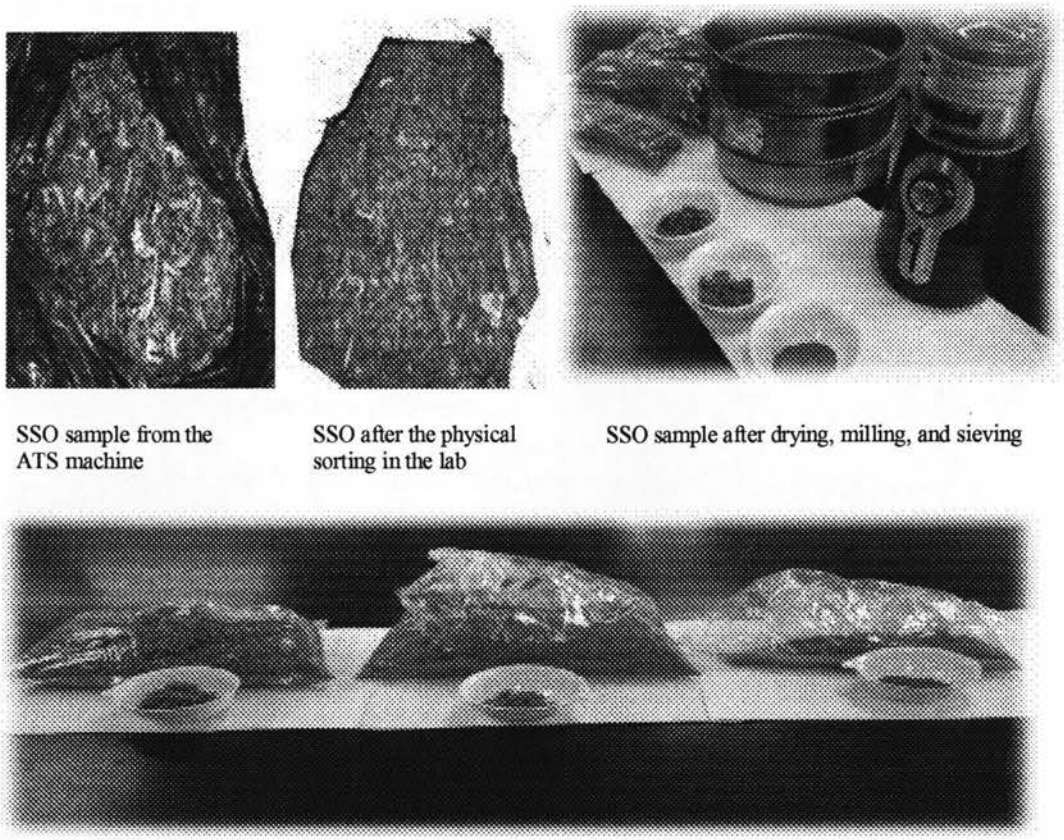


Figure 3.7 Homogenization of the SSO samples for compositional analysis

3.5 Compositional Analysis of SSO

After preparation and homogenization of the samples, the compositional analysis of SSO was continued in order to determine the amount of carbohydrates, lignin, heavy metals, and other components. The analytical instruments that were used are: High Performance Liquid Chromatograph (HPLC), Flame Ionization Atomic Absorption machine (FLAA), and Spectrophotometer from Perkin Elmer. Determination of each parameter is explained in the following sections.

3.5.1 Heavy Metals

The Canadian Council of the Minister of Environment developed two categories of compost (A and B) in order to safeguard against inappropriate land applications. The appropriate applications of compost materials are as follow:

1. **Category A** (Unrestricted Use) is compost that can be used in any application.
2. **Category B** (Restricted Use) is compost that can be used in restricted applications because of higher concentration of trace metals or the presence of sharp foreign matter.

Table 3.1 shows the maximum concentration of trace elements in compost (categories A and B) from the guideline for compost quality (CCME, 2005).

Table 3.1 Maximum concentration of trace element in compost (CCME, 2005)

Trace Elements	Category A	Category B
	Maximum Concentration within Product ($mg/kg_{dry\ weight}$)	Maximum Concentration within Product ($mg/kg_{dry\ weight}$)
Arsenic (As)	13	75
Cobalt (Co)	34	150
Chromium (Cr)	210	1060
Copper (Cu)	400	757
Molybdenum (Mo)	5	20
Nickel (Ni)	62	180
Selenium (Se)	2	14
Zinc (Zn)	700	1850
Cadmium (Cd)	3	20
Mercury (Hg)	0.8	5
Lead (Pb)	150	500

Metals present in the SSO samples can be analyzed by the technique of atomic absorption, once the organic fraction has been destroyed. Method 3050 B from the U.S. Environmental Protection Agency (EPA) was used for digestion and determination of heavy metals in the SSO sample (EPA, 1986). A set of five homogenized SSO samples was digested by nitric acid in the presence of an oxidizing agent (hydrogen peroxide), and filtered through No. 41 Wattman filter paper. The filtrate was then analyzed by the Flame Ionization Atomic Absorption (FLAA) apparatus from Perkin Elmer. The elements that were measured are: arsenic, cobalt, chromium, copper, nickel, selenium, zinc, cadmium,

mercury, and lead. The FLAA machine was calibrated by a five-point calibration curve for each metal within the proper detection limit suggested for organic waste samples.

3.5.2 Structural Components

One of the objectives of this research is to examine the amenability of the SSO to ethanol production. In order to achieve this goal, some particular characteristics in SSO should be targeted for measurement. The main components of interest in SSO as a feedstock are cellulose, hemicellulose, starch, free sugars, and lignin. The procedure for determination of the quantity of each of these components is described in the following sections.

3.5.2.1 Carbohydrates Content

In recent decades, techniques for determination of carbohydrates in lignocellulosic materials have been rapidly developed and chromatographic techniques have been replaced by the old gravimetric techniques (Van Soest and Georing, 1977). The carbohydrates present in the biomass samples can be determined by hydrolysing and converting them to their monomeric sugars and quantifying the resultant sugars using chromatographic techniques (Wyman, 1996). The procedure that was used to determine the carbohydrates in SSO was based on the NREL Laboratory Analytical Procedure (LAP) for "determination of structural carbohydrate in biomass" (NREL, 2008).

The procedure began by weighing 0.3 g of prepared SSO samples and hydrolysing with 3 ml of concentrated 72% sulphuric acid in a water bath for two hours. Samples were then

diluted with distilled water to a total volume of 87 ml. A set of standards and four-point calibration curves were prepared for glucose, xylose, arabinose, galactose, and mannose. The samples were then autoclaved for 1 hour at 121° C. After completion of autoclaving, the samples were vacuum filtered, neutralized by calcium carbonate, centrifuged, and re-filtered through a 0.2 μm filter syringe. The samples were then analysed by HPLC with Refractive Index (RI) detector and Aminex HPX-87P carbohydrate column. The results were corrected by R (%) factor which stands for the loss of sugar during the hydrolysation by acids. The concentration of each sugar can be obtained via **Equations 3.2 to 3.5** (NREL, 2008), respectively. This method measures all carbohydrates by quantifying the monomeric sugars in the samples.

$$\%\,R_{\,sugar} = \frac{\text{Conc. detected by HPLC, mg/ml}}{\text{Known Conc. of sugar before hydrolysis, mg/ml}} \times 100 \qquad (3.2)$$

$$C_x = \frac{C_{HPLC} \times \text{dilution factor}}{\%\,R_{sugar}/100} \qquad (3.3)$$

Where: C_{HPLC} = Conc. detected by HPLC

$\%\,R_{\,sugar}$ = from the equation 3.2

$$\%\,\text{Sugars}_{\text{ext free}} = \frac{C_{anhydro} \times V_{filtrate} \times \frac{1g}{1000mg}}{ODW_{sample}} \times 100 \qquad (3.4)$$

Where: $V_{\,filtrate}$ is volume of filtrate, 87.00 ml

$C_{anhydrous} = C_x$* Anhydrous correction

% Sugar $_{ext\ free}$ = the amount of sugars in the dry weight of SSO after extraction

$$\%\ \text{Sugar}_{as\ received} = (\%\ \text{Sugar}_{ext\ free}) \times \frac{(100 - \%\ \text{Extractive})}{100} \qquad (3.5)$$

% Sugar $_{as\ received}$ = the amount of sugar in the dry weight of SSO before extraction

3.5.2.2 Acid Insoluble Lignin

In order to determine the potential biodegradability of SSO, it is essential to measure the amount of lignin in the samples. The determination of lignin in the SSO samples can lead to a better understanding of the process of biological degradation, as lignin usually inhibits the carbohydrate degradation in an anaerobic environment.

The analysis of lignin was performed based on the NREL Laboratory Analytical Procedure (LAP) for "determination of structural carbohydrates and lignin in biomass". The procedure began with hydrolyzing of 0.3 g of the SSO samples with 72% sulphuric acid in two stages of concentrated and dilute acid hydrolysis, then autoclaved at 121°C for 1 hour in order to fractionate the lignocellulosic structure of the sample. Lignin was then separated by vacuum filtering through a medium size Gooch crucible filter. The lignin content was then measured as loss in ignition at 575°C for 4 hours.

However, as mentioned in chapter 2, the interference of extractives and crude protein with lignin measurement may lead to a reporting of higher lignin content than the actual value. Therefore, the samples should be extractive free, or the amount of extractives deducted from the results. The crude protein content should also be subtracted from the results in order to represent more accurate values for lignin. However, due to the limitations in our analytical instruments, the interference of crude protein with lignin content of the samples was neglected, and the amount of lignin in SSO was reported as the highest possible value. The amount of acid insoluble lignin can be calculated based on the Equation 3.6 below (NREL, 2008).

$$\%AIL = \frac{\text{Residue after105 °C} - \text{Residue after 575°C} - \text{Weight of protein}}{\text{Oven Dried Weight of Samples}} \times 100 \qquad (3.6)$$

3.5.2.3 Acid Soluble Lignin

In the procedure of determining lignin by acid hydrolysis, a portion of lignin is dissolved in acid (Wyman, 1996). In samples like SSO, this portion is about 0.2% of the total lignin content (Browning, 1967). The method that is more commonly used to determine acid soluble lignin is Ultraviolet (UV) Spectroscopy. However, due to the small amount of acid soluble lignin in comparison with acid insoluble lignin, the acid soluble lignin content was ignored in this experiment, and the total lignin content of the samples was assumed to be equal to acid insoluble lignin.

Chapter 4 Results and Discussions

4.1 Characteristics of SSO

As discussed in the previous chapter, the primary objective of this research was to conduct a reliable characteristics analysis of the SSO samples. This analysis was divided into three parts: a) general characteristics, b) compost characteristics, and c) compositional characteristics. Each analysis was conducted six times over a period of ten months from which samples were collected in: September 2008, November 2008, March 2009, April 2009, May 2009, and June 2009, in order to achieve reliable data, and the conclusions. The emphasis of this dissertation is to introduce SSO as a potential feedstock for conversion to ethanol and other value-added products.

4.1.1 General Characteristics Analysis

The general characteristics analysis of the SSO samples included determination of moisture, total solids (TS), volatile organic compound (VOC), total Kjeldahl nitrogen (TKN), and calorific value. These parameters were measured six times and each measurement was repeated up to 10 times per month except for TKN, calorific value, extractives, and heavy metals. The details of data for replications and statistical analyses can be found in Appendices A, B, C, and D; and the summary of the results are presented

in **Table 4.1**. The values presented in **Table 4.1** for each month represent the mean and deviation of the replications with 95% confidence.

Table 4.1 General characteristics of the SSO samples

Parameter	Sep 2008	Nov 2008	March 2009	April 2009	May 2009	June 2009
TS	48% ± 1.5%	48% ± 0.7%	39% ± 1.8%	43% ± 1.2%	44% ± 1%	44% ± 0.6%
Moisture	52% ± 1.5%	52% ± 0.7%	61% ± 1.8%	57% ± 1.2%	56% ± 1%	56% ± 0.6%
VOC $_{\text{per dry mass}}$	83% ± 1.7%	90% ± 0.4%	65% ± 1.6%	92% ± 0.9%	96% ± 1.5%	92% ± 0.7%
Ash $_{\text{per dry mass}}$	17% ± 1.7%	10% ± 0.4%	35% ± 1.6%	8% ± 0.9%	4 % ± 1.5%	8% ± 0.7%
TKN	13200 *	4300	12500	8650	10300	6240
Calorific Value	-	-	13232 **	-	20691.2	-

*: Unit for the measurement of TKN is ug/g of SSO
**: Unit for the calorific value is kj/kg of dry SSO

As shown in **Table 4.1**, the average moisture content of the samples was high at the time of the measurement even after the reduction of moisture during the pre-treatment in the ATS machine. However, the high amount of water in the SSO samples indicates a potential for biodegradability as water is essential for the microbial growth.

The amount of VOC in the SSO samples is sufficiently high, from 65-96% per dry mass, making it an excellent feedstock for anaerobic digestion and subsequent methane generation. As is to be expected from the nature of the samples, there are some variations in the results; however, the general amount of VOC seems to be sufficiently high.

The calorific value of the SSO samples indicates a significant potential for heat or steam generation of this feedstock via combustion. The majority of heat generated from the combustion of SSO is generated from the lignin content. There is a significant correlation between the lignin content and the calorific value of a substance (Demirbas, 2006). As

explained in Chapter 2, the lignin content of the post-fermentation sludge is very high. Therefore, in the design of the ethanol plant, the post fermentation sludge can be combusted for heat, electricity generation, and steam production.

4.1.2 Compost Characteristics Analysis

In order to investigate the amenability of SSO after pre-treatment by the ATS machine for composting, a set of experiments was conducted each month using the same sampling procedure. As explained in the previous chapter, the parameters for measurement were taken from the "Guidelines for Compost Quality" from the Canadian Council of Minister of the Environment (CCME, 2005). **Table 4.2** is a summary of the results for the measured parameters. Each value is the average of a set of replicated measurements for each month. The details of the replications and statistical analysis can be found in Appendix A.

Table 4.2 Characteristics of the SSO samples for composting purposes

Parameter	Sep 2008	Nov 2008	Mar 2009	Apr 2009	May 2009	Jun 2009
Respiration Rate (CO_2 produced/ O_2 consumed)	3.0%	0.3%	0.2%-0.3%	10%	10%	3.0%
Compost Condition	Active*	Active	Very Active	Raw	Raw	Active
Sharp Foreign Matter	NS	NS	NS	NS	NS	NS
Arsenic (As) **	28.3± 4.3	31.7±7.03	32.50 ± 2.1	22.60 ±1.4	27.10± 0.9	35.20 ± 4.2
Cobalt (Co)	< 2	< 2	20.80 ± 2.9	2.60± 0.1	2.70 ± 0.01	<2
Chromium (Cr)	<2	<2	32.60 ± 0.6	< 2	< 2	<2
Copper (Cu)	86.12 ±1.5	70.86 ± 2.3	170.80 ±11.9	96.70±6.6	298.10±3.9	116.03 ± 4.9
Nickel (Ni)	<2	<2	<2	< 2	< 2	3.66 ± 0.01
Selenium (Se)	<2	41.31± 1.39	41.36 ± 0.10	41.63 ± 0.36	52.46 ± 18.27	72.12± 25.8
Zinc (Zn)	575.2± 85.4	20.30 ± 2.1	7.80 ± 1.1	< 2	26.30 ±1.4	16.10± 2.3
Cadmium (Cd)	< 2	< 2	< 2	< 2	< 2	< 2
Mercury (Hg)	<2	<2	<2	<2	<2	16.70 ± 1.4
Lead (Pb)	<2	<2	62.40±0.5	3.70±0.06	242.90±8.3	16.60 ± 1.2

*** The compost condition indicates the maturity of compost by four categories: 1) Raw, which indicates a very odorous and unstable compost , 2) Very Active, 3) Active which indicate less maturity, 4) Finished, which stands for inactive and highly matured compost**

**** Unit for trace metals is presented in mg/kg** dry weight of SSO

4.1.2.1 Maturity and Respiration Rate

According to the "Guideline for Compost Quality", the compost characteristics must fall within certain limits at the time of marketing, and compost shall be mature and stable at the time of sale and distribution (CCME, 2005). Plants generally utilize compost better when it is more mature. As shown in **Table 4.2**, the SSO samples were quite active and had a high carbon dioxide emission rate at the time immediately following the pre-treatment in the ATS machine, which indicates that the SSO cannot be used for composting applications unless an adequate aeration treatment is provided in order to produce a more stable product for marketing.

4.1.2.2 Heavy Metals

Trace metals may be present in raw materials from which compost products are produced. Although presence of some trace metals such as copper, cobalt, molybdenum, and zinc are beneficial for plants and humans as micronutrients, the long term accumulation of trace elements in soil may cause adverse effects on public health and the environment (CCME, 2005). Based on this fact, the Canadian Council of the Minister of Environment developed two categories of compost (A and B) in order to safeguard against inappropriate land applications. The appropriate applications of compost materials are as follow:

1. **Category A** (Unrestricted Use) is compost that can be used in any application.

2. **Category B** (Restricted Use) is compost that can be used in restricted applications because of higher concentration of trace metals or the presence of sharp foreign matter.

Table 4.3 shows the maximum concentration of trace elements in compost (categories A and B) from the guideline for compost quality (CCME, 2005), and the actual concentration of trace elements in the SSO samples.

Table 4.3 Maximum concentration of trace elements in compost (CCME, 2005)

Trace Elements	Maximum Concentration for Category A (mg/kg dry weight)	Maximum Concentration for Category B (mg/kg dry weight)	Maximum Concentration in the SSO samples (mg/kg dry weight
Arsenic (As)	13	75	35.2
Cobalt (Co)	34	150	20.8
Chromium (Cr)	210	1060	32.6
Copper (Cu)	400	757	298.1
Molybdenum (Mo)	5	20	-
Nickel (Ni)	62	180	5.11
Selenium (Se)	2	14	83.09
Zinc (Zn)	700	1850	575.2
Cadmium (Cd)	3	20	<2
Mercury (Hg)	0.8	5	16.7
Lead (Pb)	150	500	242.9

A direct comparison of the maximum allowable concentration and the sample concentration of heavy metals indicates that there is some potential for utilization of SSO as compost. However, the concentration of some elements, such as selenium, exceeds the maximum level even for category B of the guideline, and the concentration of Arsenic is above the maximum level for category A, indicating that SSO can not be used as category A compost. Moreover, the high variation in the results would make it difficult to suggest with confidence that SSO is a safe composting product, unless a proper treatment is provided in order to remove these elements. For example, the concentration of lead for the month of May is 277.9 mg/kg $_{\text{dry weight}}$, which is above the maximum level for category B, whereas in the other months, the level of lead is satisfactorily low. In general, the concentration of heavy metals in SSO is quite high, and that SSO is not a proper material to be used as category A compost. Even land application under category B would be unwise given the concentration fluctuations, unless a proper pre-treatment is provided to remove or reduce the amount of heavy metals prior to use.

4.1.2.3 Sharp Foreign Matter

According to "Canadian Guidelines for Compost Quality", compost shall not contain any sharp foreign matter of dimension greater than 3 mm per 500 ml of compost and shall contain no more than one piece of other foreign matter in any dimension greater than 25 mm per 500 ml to be considered as category A. Similarly, the sharp foreign matter content of compost shall be equal to or less than 3 pieces per 500 ml of compost with the maximum dimension of 12.5 mm; and compost shall contain no more than two pieces of

other foreign matter greater than 25 mm per 500 ml to be considered as Category B (CCME, 2005). As is indicated in **Table 4.2**, the presence of sharp foreign matter is not significant in the SSO samples based on the results for six-month analysis. The combination of pressure and heat during the pre-treatment in the ATS machine causes the foreign matter to be pulverized or melted. Hence, pre-treatment by the ATS machine seems to have a great influence on reducing the amount and dimension of sharp foreign matter and making SSO more suitable as a compostable material.

4.1.3 Compositional Analysis of SSO

As explained in chapter 3, the reason behind conducting a compositional analysis of the SSO samples was to evaluate the potential utilization of SSO as a feedstock for ethanol production. The focus of the compositional analysis of the SSO samples is the analysis of carbohydrates and lignin because of their correlations to fermentability. The results for each of these components are explained in the following sections.

4.1.3.1 Carbohydrates Content

In the process of ethanol production through fractionation and bacterial fermentation of biomass, the dominant sources of carbon for microbial growth are carbohydrates. Cellulose, hemicellulose, and starch are of the most significance because they can be converted to ethanol. Cellulose is a chain of hexose sugars, mostly comprised of glucose. Hemicellulose is a chain of pentose sugars, which is mostly consisted of xylose, although

some traces of arabinose, mannose, and galactose exist. Starch is a carbohydrate which is mainly composed of glucose. It can be assumed that 90% of cellulose is composed of glucose, and 90% of hemicellulose is composed of xylose (Guffey and Wingerson, 2004). By estimating the amount of glucose and xylose, a rough estimation of the amount of total carbohydrates in the SSO sample can be obtained. Other monomeric sugars, such as galactose, arabinose, and mannose, were also measured in order to obtain a comprehensive analysis of the carbohydrates in the SSO samples. **Table 4.4** is a summary of the results for quantitative saccharafication of the SSO samples. Each value in **Table 4.4** is the mean of a set of replications for each month of analysis and the deviation of which was done with 95% confidence. The detail of the results and statistical analysis for each replication can be found in Appendix A.

Table 4.4 Quantitative saccharification of the SSO samples

Parameter	Sep 2008	Nov 2008	March 2009	April 2009	May 2009	June 2009
Glucose	25.83%± 2%	38.19%± 2%	30.61%± 0.7%	27.30%± 2.6%	34.8%± 3.6%	27.10%± 2%
Xylose	19.77%± 2%	10.5%± 0.9%	21.97%± 0.1%	17.30% ± 2%	27.80%± 3%	17.50%± 2%
Arabinose	< 3%	< 3%	< 3%	< 3%	< 3%	< 3%
Mannose	< 3%	7.99%± 0.8%	3.03%± 0.3%	9.60%± 1.5%	4.50%± 1%	3.6%± 0.9%
Galactose	< 3%	< 3%	< 3%	< 3%	< 3%	< 3%
Total Sugars	45.6%	56.68%	55.61%	54.2%	67.1%	48.25%

As shown in **Table 4.4,** the presence of glucose is prevalent, reflecting a considerable contribution of starch and cellulosic compounds in the composition of SSO. The data also shows a prevalence of xylose, which indicates a considerable amount of hemicellulose in the SSO samples. The quantitative saccharification method measured all the sugars present in the SSO samples over six months of analysis. The amount of total fermentable

sugars is satisfactorily high for SSO to be considered as an excellent ethanol production feedstock. The amount of glucose that was quantified by HPLC could be from cellulose hydrolysis, starch hydrolysis, or the free sugars in the samples, but the amount of xylose is mainly from hemicellulose.

4.1.3.2 Acid Insoluble Lignin

In investigation of the digestibility of lignoellulosic biomass samples, determination of the percentage of lignin is essential because lignin bonds carbohydrate chains and inhibits liberation and fermentation of their monomeric sugars. The percentage of lignin was measured for the six months of study with ten sets of replications completed each month. **Table 4.5** is a summary of the lignin content for each set of analysis over six months. The details of the statistical analysis can be found in appendix A. The results show a significant amount of lignin in the samples. The deviation from mean was calculated with 95% confidence for each month of analysis. The mean values for all the replications and the deviation indicate a consistency in the presence of lignin in the SSO samples during the period of this study. As explained in chapter 2, high lignin content in the SSO is detrimental for the process of biological degradation. Therefore, for a successful biological conversion of the SSO to ethanol, particular attention must be devoted to the design of an effective delignification pre-treatment of the feedstock, in order to facilitate the process of degradation and microbial fermentation.

Table 4.5 Lignin content of the SSO samples

Sample	Sep 2008	Nov 2008	March2009	April 2009	May 2009	June 2009
1	26.03%	22.12%	18.51%	29.62%	18.51%	22.02%
2	26.87%	22.90%	18.51%	30.03%	22.22%	22.12%
3	26.70%	20.98%	22.22%	29.62%	22.22%	22.01%
4	25.70%	19.76%	18.51%	30.33%	25.92%	22.65%
5	20.65%	21.01%	18.51%	29.62%	22.22%	22.10%
6	24.30%	17.87%	18.99%	29.62%	18.51%	21.90%
7	34.20%	19.76%	16.12%	28.01%	18.70%	20.56%
8	21.20%	24.02%	21.01%	31.01%	22.32%	22.03%
9	27.01%	21.80%	19.01%	29.89%	21.38%	22.10%
10	25.90%	24.96%	18.19%	30.09%	22.12%	22.54%
Mean	25.86%	21.51%	18.96%	29.78%	21.41%	22.00%
SD	3.7%	2.1%	1.6%	0.7%	2.3%	0.5%
95 % CI	±2.6%	±1.5%	±1.1%	±0.5%	±1.6%	±0.4%

4.1.3.3 Extractives

The amount of extractives was determined for the SSO samples during the period of study. **Table 4.6** shows the percentage of extractives per dry weight of SSO samples. As is obvious from the table, the results for extractives are highly variable for each month of analysis, which could be due to the heterogeneous nature of SSO. The amount of extractives varies due to several factors, such as the plastic content of the samples, fat, grease, paint, and other materials which are all common components in SSO. The relatively high percentage of extractives in the SSO samples emphasizes the need for separate washing and detoxification units prior to fermentation in order to minimize the amount of extractives and toxic substances which are detrimental to bacterial fermentation.

Table 4.6 Hot water extractives in the SSO sample

Parameter	Sep 2008	Nov 2008	March2009	April 2009	May 2009	June 2009
Extractives	7.50%	6.80%	7.60%	7.40%	4.40%	10.40%

4.2 Mass Balance of SSO Components

Based on the above analyses of SSO characteristics, a mass balance for the SSO samples based on the average values of the sampling period can be obtained. Due to their correlation with fermentability, the main parameters of interest are carbohydrates and lignin. The remaining materials are extractives, protein, synthetic materials, or any other unknown substances. **Figure 4.1** shows the average mass balance diagram for the dry weight of the SSO samples during the six months of analysis.

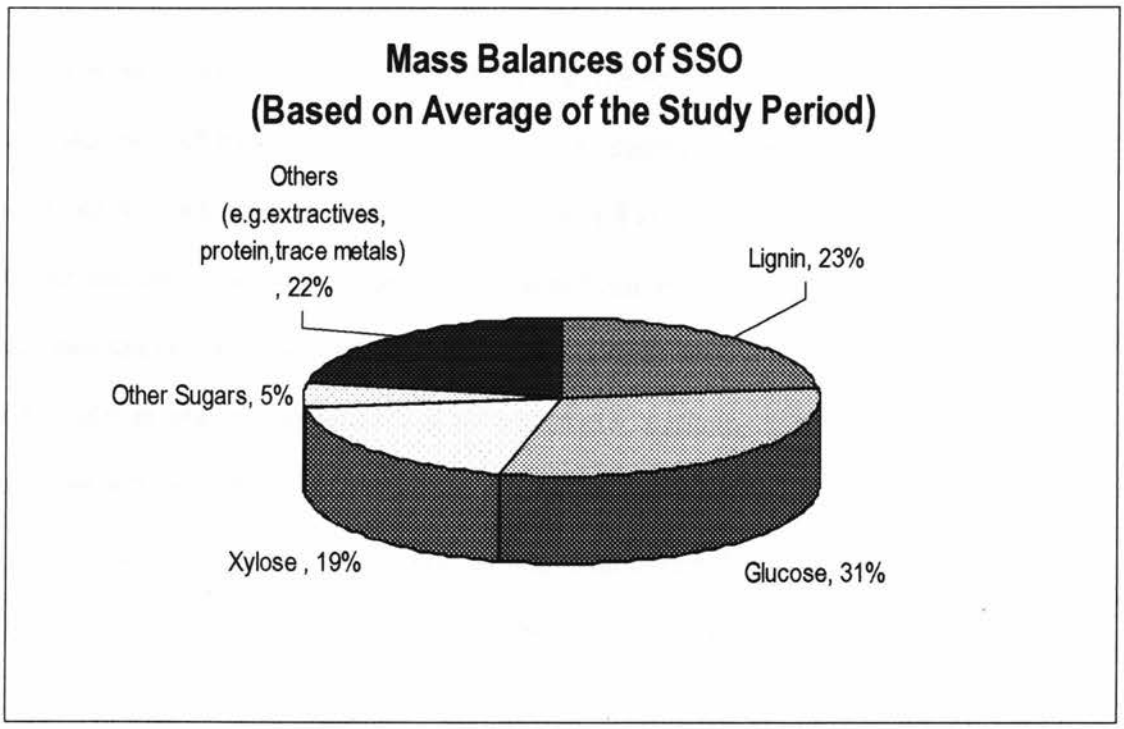


Figure 4.1 Mass balances of the structural components of dry weight of the SSO samples

As illustrated in **Figure 4.1,** the percentage of glucose and xylose in the SSO samples, totally approximately 50%, is satisfactorily high with a low level of variation from the mean value. Therefore, SSO is a reliable feedstock for ethanol production. However, it should be noted that the blend of 20% wood waste in the SSO samples plays a significant role in increasing the carbohydrate content and reducing month to month fluctuations in the results because a relatively high amount of glucose and xylose is found in wood. It would be unadvisable to test organic waste on its own, because previous research (Claassen et al, 2000) found that the organic fraction of municipal solid waste (OFMSW) is not a perfect substrate for ethanol production if it is utilized alone, and it should be used as a co-substrate with another cellulosic feedstock. A blend of wood chips was added to the organic waste in this study because the co-substrating of the organic waste with construction and demolition wood waste not only enhances the potential fermentability of feedstock by increasing the amount of carbohydrate, but also decreases the month to month fluctuations in the results for characteristics analysis. Moreover, as it was explained in section 3.1.1, the addition of wood chips in the SSO has some other benefits in increasing the shelf life of the feedstock and making it more stable. It should also be mentioned that wood chips is a form of construction and demolition waste and it is free or even negatively costed feedstock. Therefore, it will not increase the cost of ethanol production; and it is beneficial for the overall waste management.

4.3 Theoretical Ethanol Production Potential of SSO

The theoretical ethanol production yield from the SSO can be calculated based on the average amount of each carbohydrate present in SSO. The actual yield of production will not be the same as the theoretical yield calculation, due to the limitations of substrate conversion efficiency and immaturity of the conversion technologies that were explained in chapter 2.

In the process of fermenting hexose and pentose sugars to ethanol, there is a myriad of chemical reactions, among which two are the most dominant. **Table 4.7** is a summary of the most significant reactions during the fermentation of biomass feedstock to ethanol after a proper pre-treatment step. The two most dominant reactions, as demonstrated by the very high conversion efficiencies, are shown in bold in **Table 4.7**. These results were observed in a study by NREL after a dilute acid pre-treatment, enzymatic saccharification, and co-fermentation of hexose and pentose sugars to ethanol (NREL, 2002).

Table 4.7 Reactions during the fermentation to ethanol (NREL, 2002)

Reaction	Reactant	Conversion Factor
Glucose → 2 Ethanol + 2 CO_2	**Glucose**	**0.95**
Glucose + Proper Nitrogen Source→ Cell Mass + H_2O + CO_2	Glucose	0.02
Glucose + $2H_2O$ → 2 Glycerol + O_2	Glucose	0.004
Glucose + $2CO_2$ → Succinic Acid + O_2	Glucose	0.006
Glucose → 3 Acetic Acid	Glucose	0.015
Glucose → 2 Lactic Acid	Glucose	0.002
3 Xylose → 5 Ethanol + 5 CO_2	**Xylose**	**0.85**
Xylose + Proper Nitrogen Source → Cell Mass + H_2O + CO_2	Xylose	0.019
3 Xylose + $5H_2O$→ 5Glycerol + $2.5O_2$	Xylose	0.003
Xylose + H_2O → Xylitol + 0.5 O_2	Xylose	0.046
3 Xylose + 5 CO_2 → 5 Succinic Acid + 2.5 O_2	Xylose	0.009
2 Xylose → 5 Acetic Acid	Xylose	0.014
3 Xylose → 5 Lactic Acid	Xylose	0.002

In **Table 4.7**, the conversion factor represents the likelihood of each reaction as compared to others during the fermentation process. The conversion factors for the fermentation of glucose and xylose to ethanol, in the main reactions are 0.95 and 0.85, respectively. This means that the most dominant reactions during the fermenting of glucose and xylose are for converting them into ethanol. Therefore, if a biological conversion process is properly designed, it can be expected that a high percentage of

glucose and xylose can be successfully fermented to ethanol. The theoretical amount of ethanol obtained from a complete conversion of SSO can be calculated based on the stoichiometry of the sugar fermentation equation. **Equations 4.1** and **4.2** express the conversion of glucose and xylose to ethanol, as follows:

$$C_6H_{12}O_6 \rightarrow 2\,C_2H_5OH + 2\,CO_2 \qquad (4.1)$$

Glucose Ethanol Carbon Dioxide

$$3C_5H_{10}O_5 \rightarrow 5\,C_2H_5OH + 5\,CO_2 \qquad (4.2)$$

Xylose Ethanol Carbon Dioxide

Based on the **Equation 4.1**, each mole of glucose produces two moles of ethanol and two moles of carbon dioxide. Therefore, the mass of ethanol produced per unit mass of glucose is given as:

$$\frac{1\text{ g Glucose}}{1} \times \frac{1\text{ mole Glucose}}{180\text{ g Glucose}} \times \frac{2\text{ mole Ethanol}}{1\text{ mole Glucose}} \times \frac{46\text{ g Ethanol}}{1\text{ mole Ethanol}} = 0.511\text{ g Ethanol} \qquad (4.3)$$

Based on the Equations 4.2, 3 moles of xylose produce 5 moles of ethanol and 5 moles of carbon dioxide. Therefore, the mass of ethanol produced per unit mass of glucose is given as:

$$\frac{1\text{ g Xylose}}{1}\times\frac{1\text{ mole Xylose}}{150\text{ g Xylose}}\times\frac{5\text{ mole Ethanol}}{3\text{ mole Xylose}}\times\frac{46\text{ g Ethanol}}{1\text{ mole Ethanol}}=0.511\text{g Ethanol} \quad (4.4)$$

According to **Table 4.7**, 95% conversion of glucose and 85% conversion of xylose to ethanol is possible (NREL, 2002). Therefore, each gram of glucose produces 0.95 × 0.511= 0.4854 ≈ 0.49 gram of ethanol and each gram of xylose produces 0.85 × 0.511= 0.434 ≈ 0.43 gram of ethanol under optimal conditions. Therefore, based on the mean values for glucose and xylose from the mass balance diagram in **Figure 4.1**, ethanol yield can be calculated for SSO using a 0.9 efficiency factor for saccharification commonly found in experiments done at NREL (NREL, 2002). **Table 4.8** and **Table 4.9** show the theoretical amount of ethanol produced from 1 tonne of SSO.

Table 4.8 Theoretical Ethanol yield from glucose

Dry SSO	1 tonne (1000 kg)
Glucose content	× 0.31
Saccharification efficiency	× 0.90 (NREL, 2002)
Ethanol stoichiometry yield	× 0.51
Glucose fermentation efficiency	× 0.95 (NREL, 2002)
Yield from glucose	135 kg ethanol = 171.3 L ethanol

Table 4.9 Theoretical Ethanol yield from xylose

Dry SSO	1 tonne (1000 kg)
Xylose content	× 0.19
Saccharification efficiency	× 0.90 (NREL, 2002)
Ethanol stoichiometry yield	× 0.51
Xylose fermentation efficiency	× 0.85 (NREL, 2002)
Yield from xylose	74 kg ethanol = 93.95 L ethanol

The total yield of ethanol production is the sum of the yields from glucose and xylose which is approximately 265 litres per tonne of dried SSO. Therefore, each dried tonne of SSO can be converted to 265 litres of ethanol. The City of Toronto collects approximately 100,000 tonnes of SSO per year. Based on **Table 4.1**, assuming 45% of the dry weight of SSO, 45,000 tonnes of dried SSO per year is available in the city of Toronto, from which 11,925,000 litres of ethanol can be produced. This is a considerable amount of ethanol that can be produced each year from an underutilized resource that currently is either combusted or sent to U.S for disposal.

The actual amount of ethanol production would be less than the theoretical yield, owing to some limitations in conversion and fermentation efficiencies. The actual amount of ethanol production is affected by the conversion efficiency factor in pre-treatment, saccharification, and fermentation. For instance, in the process of ethanol production by acid hydrolysis, Badger in his 2002 paper recommended on efficiency factor of 0.76 for saccharification, whereas in the above calculation, the factor of 0.9 was used for the

saccharification efficiency and release of glucose and xylose according to NREL (NREL, 2002).

4.4 Comparison of SSO with other Cellulosic Feedstocks

Figure 4.2 shows the comparison of the SSO composition with that of other cellulosic biomass resources, as reported by Wyman, (1994). The results for the compositional analysis of the SSO samples are comparable to other cellulosic feedstocks such as herbaceous energy crops, agricultural residue, and forest biomass. Total glucose, xylose, and other sugars are reasonably close to that of hardwood, agricultural residue, and herbaceous energy crops.

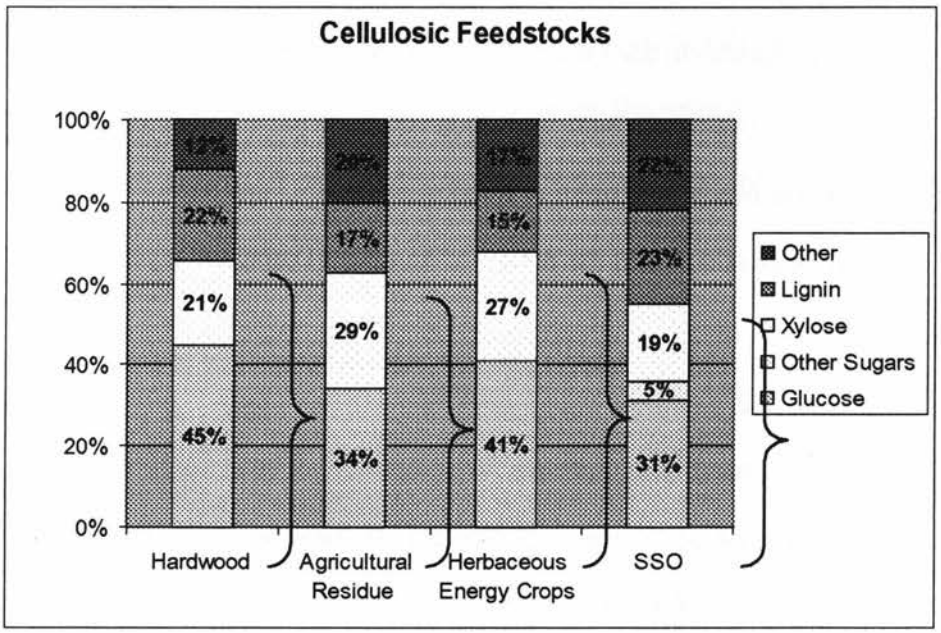


Figure 4.2 Comparison between the SSO samples and other Cellulosic feedstocks

As the distribution of the components is comparable to other cellulosic substrates, SSO has the potential to be utilized for commercial ethanol production. Interestingly, the carbohydrate content of the SSO sample is comparable to dedicated energy crops, for which the feedstock cost is about 33% of the total ethanol production costs (NREL, 2002). Since SSO is a waste and usually has a negative cost, therefore, utilizing SSO instead of dedicated energy crops can drastically reduce the costs of ethanol production.

Presently, SSO after curbside collection and processing by the ATS machine is mostly sent for combustion, composting, or disposal (Mike Crupi, Personal communication, 24 April 2009). However, results from this study indicate that the significant amount of polymeric saccharides in SSO invites other methods that may generate value-added compounds such as ethanol. It should be noted that, for such applications, the mobilization of the saccharides in SSO is a prerequisite. Moreover, the presence of lignin in the SSO samples is considerably high indicating the need for a proper pre-treatment step prior to biological conversion in order to fractionate the SSO and separate lignin. All these factors should be considered in the design of an ethanol production plant utilizing SSO as a feedstock.

Figure 4.3 illustrates the monthly fluctuations for compositional analysis of SSO over the period of study. It must be noted that a percentage of free sugars is lost during the analysis of samples because of washing with hot water and refluxing in 95% ethanol. Therefore, the amount of actual carbohydrates present in SSO is expected to be higher than that of the reported values.

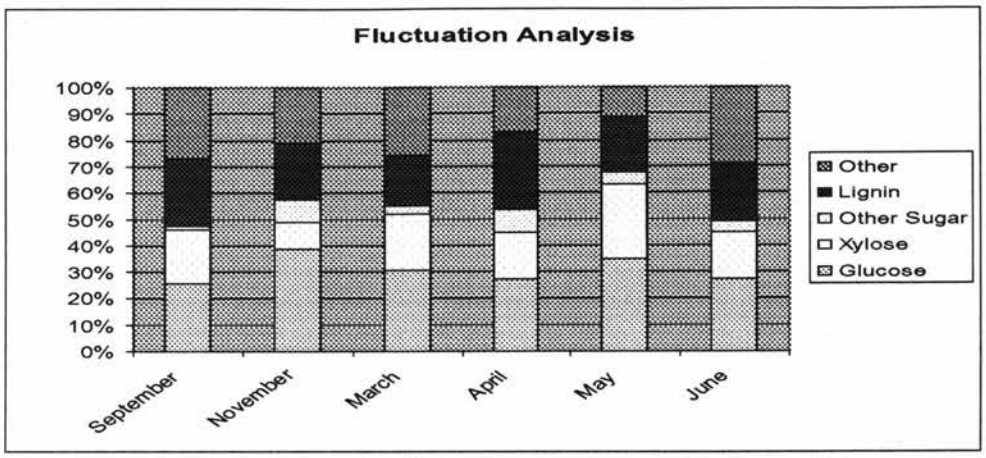


Figure 4.3 Fluctuation of the compositional components of SSO over the six-month of study

T- test for comparison of the means of each two consequent months of analysis

Table 4.10 shows the t-test results for the structural components of the SSO for the period of analysis. A t-test was conducted for each two consequent months in order to examine if the difference between the means is significant. The t-value for the comparison of each two months is listed under the second month in **Table 4.10**. For example, the t-value for the comparison of September and November is listed under the month of November.

Table 4.10 t- test for the monthly fluctuation of the SSO samples

Glucose	Sep 2008	Nov 2008	Mar 2009	Apr 2009	May 2009	June 2009
Mean	25.8	38.19	30.61	27.32	34.88	27.13
SD	2.9	2.8	1.01	3.7	5.04	3.1
t- value	-	**9.7**	**8.08**	**2.71**	**3.84**	**4.16**
Xylose	19.77	10.54	21.97	17.32	27.7	17.53
SD	3.06	1.3	0.15	3.03	4.03	2.3
t- value	-	**8.35**	**27.87**	**4.89**	**6.52**	**7**
Lignin	25.85	21.51	18.95	29.78	21.41	22.00
SD	3.7	2.1	1.64	0.78	2.3	0.56
t-value	-	**3.23**	**3.04**	**19.33**	**10.94**	**0.79**

With 95% confidence and the degree of freedom 18, the value of $t\alpha$ is 2.1. As can be seen in **Table 4.10**, the t-value in most of the tests is greater than t_α for glucose, xylose, and lignin. Therefore, the difference between the means for each two consequent months is significant, meaning that the means are statistically different from each other and the fluctuation in the results is obvious. However, this fluctuation is due to the heterogeneity of the SSO sample and the low standard deviation in each set of the measurement from month to month, which make the means of these months statistically distinct from each other. Although the t-test shows a significant difference between the means, it should be noted that they are in the certain range during the period of analysis.

To summarize, a number of points should be noted based on the compositional analysis of SSO samples and fluctuations in the results over the period of study:

• SSO has great potential to be utilized as a feedstock for biological ethanol production due to its high carbohydrate content. However, similar to other lignocellulosic biomass, the main problem in dealing with SSO as feedstock is the mobilization of monomeric sugars from the carbohydrate chains. This requires the design of an effective hydrolysis and saccharification units prior to the biological fermentation of SSO to ethanol.

- The high lignin content in SSO emphasizes a need for an effectively designed pre-treatment unit prior to biological conversion in order to remove or alter lignin and facilitate fermentation of carbohydrate.

- In general, about 30% of the final cost for cellulosic ethanol production belongs to the biomass feedstock. Dedicated energy crops are the most expensive cellulosic feedstocks that are currently used for cellulosic ethanol production. Therefore, utilizing SSO as a cellulosic feedstock can drastically reduce the total cost of producing ethanol. On the other hand, with the current debate about the food vs. fuel, it is important to look at the other renewable feedstock other than the human food.

- The ATS machine is effective in homogenizing the samples and reducing the fluctuation in results. It is also an effective physical pre-treatment for SSO samples due to milling, communition, heat treatment, and pressure effects. The combination of pressure, heat, and screwing renders the SSO to be a more homogenized and less odorous materials.

- The SSO after the pre-treatment by the ATS machine also has a potential for being utilized as a compost material. The effect of pressure and heat in the ATS machine significantly reduces the amount and dimension of the sharp foreign matter. However, the amount of some trace metals in the SSO may cause some restrictions in using the SSO as a compost. Also the stability and maturity of the

ATS processed SSO is not satisfactory for utilization as compost, requiring further treatment in the form of aeration to become an acceptable compost material.

- The idea of mixing the SSO with a blend of wood chips from the Construction and Demolition waste (C&D) is significantly beneficial because the mixer blend has a higher potential to be utilized as a feedstock for ethanol than the organic waste alone.

- The high amount of volatile organic compounds (VOC) in the SSO samples indicates sources of carbon other than carbohydrates; therefore, a design of a proper conversion process for the utilization of all volatile organic compounds is essential. The degradation of the remaining volatile organic carbons can be performed in a separate anaerobic digestion during the wastewater treatment process in ethanol plant. Anaerobic digestion will not only treat the effluent, but also produce biogas such as methane and carbon dioxide from volatile organic compounds which can be used to generate heat and power for the ethanol plant.

- The high calorific value of the SSO samples indicates the potential of utilizing the SSO for combustion. The calorific value of a biomass sample is positively related to the lignin content of the biomass samples (Ayhan, 2002). The design of a combustion unit for the ethanol plant is highly beneficial to the overall economy of the plant because steam or electricity can be generated from the combustion of

the remaining lignin after fermentation. The resultant steam or electricity can be utilized in the plant.

Chapter 5 Biological Conversion of SSO to Ethanol

Based on the results of this study, a Process Flow Diagram (PFD) for the biological production of ethanol from SSO is being proposed in this chapter. The PFD is being proposed based on the currently available technologies and the characteristics of SSO as a feedstock. The results in Chapter 4 indicate the process configuration and support the design of the ethanol plant PFD. For example, the high carbohydrate contents of the SSO sample indicates the need for the design of the biological conversion unit for the fermentation of hexose and pentose sugars to ethanol.

5.1 General Pathway for Ethanol Production

The main compartments of the overall PFD of ethanol plants are pre-treatment, hydrolysis, fermentation, and ethanol recovery. There are also other operational units required for the process such as waste water treatment, combustion, and feedstock storage.

The general layout of the PFD for ethanol plants is outlined in **Figure 5.1,** in which the operational units are illustrated. Based on the available literature for design of ethanol plants, this study aims to propose the best currently existing conversion techniques and explain the design of each unit in detail. The interaction between the different operational units should also be considered in the design of ethanol plant. There are a number of commercial process simulator softwares for the rigorous mass and energy balance

calculation and design of ethanol plants, among which ASPEN Plus is the most common (Aspen Technologies Inc., USA).

The conversion economy for cellulosic ethanol production is a key obstacle to overcome. In order to achieve this goal, the research currently focuses on the alternatives that can reduce the production cost of cellulosic ethanol. The objective of this chapter is also to present updated technologies available for the reduction of cost in cellulosic ethanol production.

5.2 Steps for Biological Conversion of SSO to Ethanol

In general, the conversion process of cellulosic biomass such as SSO to ethanol includes four major steps:

1. Pre-treatment
2. Saccharification
3. Fermentation
4. Distillation

The main challenge in the conversion of cellulosic biomass to ethanol is the pre-treatment step. Unlike starch, cellulosic biomass demands a complicated process for the pre-treatment and liberation of its carbohydrates. Therefore, one of the main gaols is to design a viable and cost effective pre-treatment step in order to liberate the carbohydrates

from their bondage to lignin and make them more susceptible to the enzymatic hydrolysis and fermentation. The saccharification of hexose and pentose sugars to ethanol is also a challenge in terms of the cost. Distillation and separation of ethanol from the broth is the final process in the ethanol plant.

Figure 5.1 is a schematic diagram of the major steps for the proposed biological conversion of SSO to ethanol.

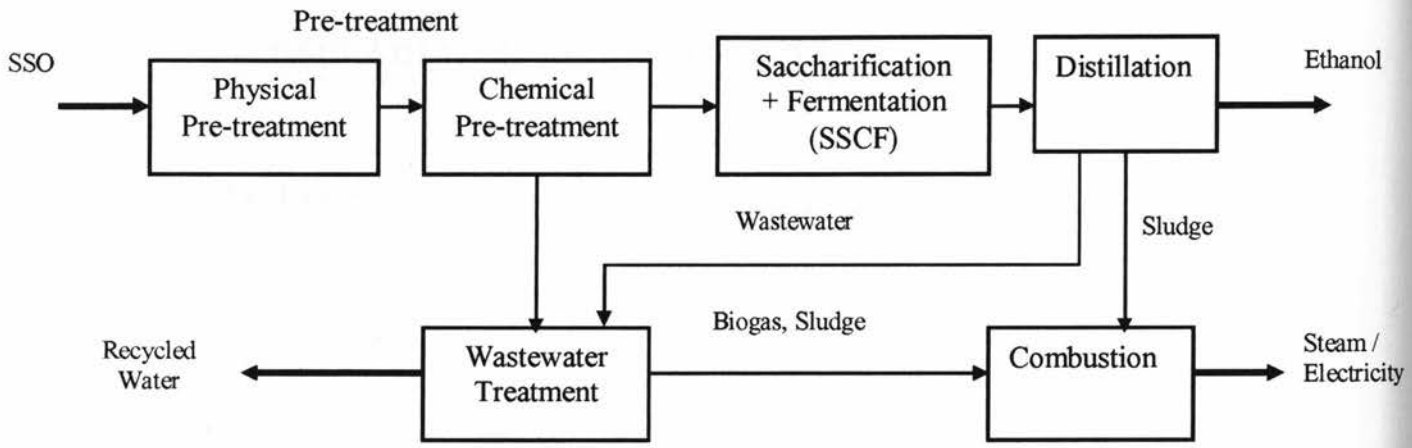


Figure 5.1 General pathways in the process of ethanol production

As illustrated in **Figure 5.1,** the PFD is comprised of pre-treatment, biological conversion, and distillation. The combustion and wastewater treatment units are also essential for a conversion plant as these units generate energy in the form of electricity and heat, and are beneficial to the overall economy of the ethanol plant. The biological conversion may have different configurations which will be explained later in this chapter.

5.2.1 Pre-treatment

As explained above, the main challenge for the conversion of SSO to ethanol is the pre-treatment step. Physical pre-treatment is an essential step in the pre-treatment of biomass material. The main task of physical pre-treatment is to reduce the size and increase the surface availability for the consequent hydrolysis. The types of physical pre-treatment were explained in Chapter 2. One of the promising techniques for the physical pre-treatment of biomass feedstock is the ATS machine. The ATS machine was introduced in Chapter 2 and will be further explained in the following section.

5.2.1.1 Physical Pre-treatment

The combination of high pressure and temperature in the ATS machine results in size reduction of the SSO feedstock and makes SSO a fibrous material. The treated product from the ATS machine is a homogenised and less odorous material that is more amenable to the subsequent chemical pre-treatment and processing.

One of the main advantages of the ATS machine is that it provides a less expensive process and demands less energy in comparison to the other physical pre-treatment. The friction caused by the plates inside the ATS machine elevates the temperature to 150 °C and produces pressure of 40 to 50 bars (Mike Crupi, Personal communication, 24 April 2009). Therefore, the process does not rely on the additional external energy source for heating. The process results in seven times densification of SSO within only a few

seconds of processing (Mike Crupi, personal communication, April 24, 2009). **Figure 5.2** illustrates the operation of the ATS machine.

Figure 5.2 Operation of ATS machine for pre-treatment of SSO

5.2.1.2 Chemical Pre-treatment

After the physical pre-treatment, SSO must undergo a proper chemical pre-treatment process in order to separate the carbohydrates from their bondage to lignin and alter the structure of the lignocellulosic materials. According to the results for carbohydrate and lignin, which indicate that SSO has a lignocellulosic structure with a relatively high amount of lignin and hemicellulose, there is a need for a proper chemical pre-treatment in order to make SSO more susceptible to the biological conversion. As discussed in Chapter 2, a myriad of chemical pre-treatment techniques have recently been developed and researched in order to make lignocellulosic biomass more digestible.

From the pre-treatment technologies that were described in Chapter 2, the method of dilute acid pre-treatment was selected as the best available technique based on the characteristics of SSO and the feasibility for commercialization. This is an economic method and the technology is more mature than the other techniques of pre-treatment (NREL, 2002). The method and the operational conditions are as follows.

Dilute/ Concentrated Acid Pre-treatment

The process of dilute acid pre-treatment should be designed after the ATS machine in order to solubilise hemicellulose and a small portion of cellulose. A small amount of lignin is also soluble in acid and this makes the cellulose more exposed to further hydrolysis. NREL proposed dilute acid pre-treatment in the design of ethanol plant for lignocellulosic biomass. Under the optimal condition, the efficiency factor for dilute acid pre-treatment could reach up to 90% (NREL, 2002).

The optimum conditions for dilute acid pre-treatment technique are summarized in **Table 5.1** (Sierra et al., 2008).

Table 5.1 Optimal Operational conditions for dilute acid pre-treatment (Sierra et al., 2008)

Acid Concentration	0.5% - 10%
Residence time	5-30 minutes
Temperature	140 °C-190° C
Pressure	4-13 atm

NREL used dilute acid pre-treatment by choosing sulphuric acid as the catalyst with a concentration of 1.1%, residence time of two minutes, temperature of 190 °C, pressure of 12.1 atm, and solid loading of 30% in the reactor. This pre-treatment is expected to have 90% efficiency in hydrolysing of hemicellulose and removing of lignin (NREL, 2002).

One of the problems associated with dilute acid pre-treatment technique is the degradation of sugars and the formation of inhibitory products during the process, resulting in lower ethanol yield and inhibition of the further fermentation process. Predominant inhibitory by-products formed during the dilute acid pre-treatment are furfural and hydroxyl methyl furfural (HMF). To remedy this situation, a detoxification unit must be designed after the dilute acid pre-treatment in order to remove these inhibitory products from the hydrolysate. NREL has suggested that the liquid and solid components after the pre-treatment to be flash cooled which causes the inhibitory products to evaporate and makes the remaining material more susceptible to fermentation.

After the pre-treatment by dilute acid and cooling of the materials, the liquid phase must be separated from the solid, so that the liquid phase may be detoxified to remove the furfural and other inhibitory components, naturalized by lime, and filtered out for the removal of gypsum. After filtering the liquid, the solid and liquid are then re-combined and sent together for further saccharification and co-fermentation (NREL, 2002).

5.2.2 Biological Conversion Process

After the proper pre-treatment, the material is ready for saccharification and fermentation to ethanol. The process of fermentation of lignocellulosic biomass such as SSO is essential in the overall process design. Design and control of the process of fermentation are complex tasks due to the complicated nature of the regimes and reactions during the fermentation. The complex behaviour of fermentation imposes great challenges on the process design and simulation (Cardona and Sanchez, 2007).

As shown in **Figure 5.3,** the biological conversion process may consist of one of the following processes:

- Separate Hydrolysis and Fermentation (SHF)
- Simultaneous Saccharification and Fermentation (SSF)
- Simultaneous Saccharification and Co-Fermentation (SSCF)
- Consolidated Bio-Processing (CBP)

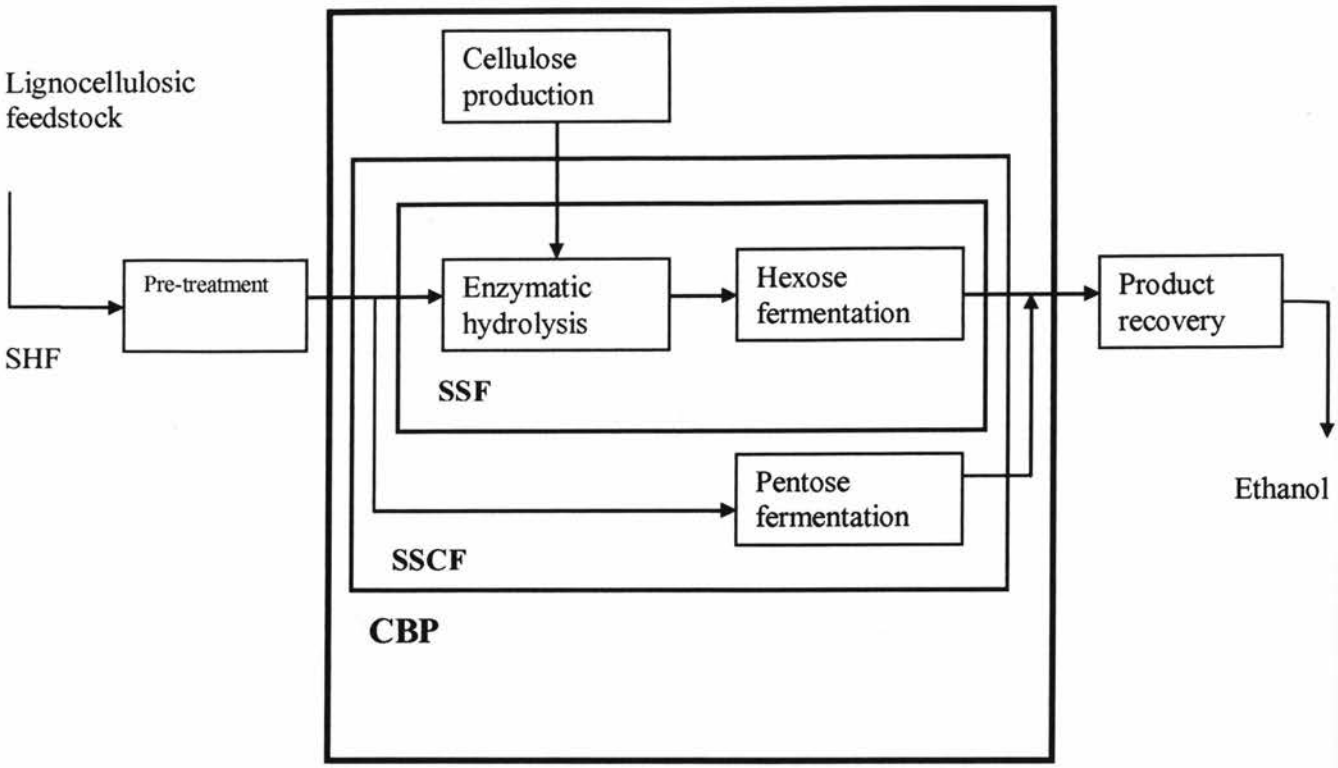


Figure 5.3 Different biological process configurations (Cardona and Sanchez, 2007)

As explained in Chapter 2, in SHF, the hydrolysis of carbohydrates is not performed in the same unit as the fermentation; whereas, in SSF, cellulose hydrolysis and hexose fermentation are conducted in the same unit and the fermentation of pentose sugars is performed separately. In SSCF, the saccharification, fermentation of hexose sugars and fermentation of pentose sugars are performed in one step. Finally, in CBP, the hydrolysis, saccharification, and fermentation of hexose and pentose sugars are all conducted in one step by employing one cellulolytic microorganism. Detailed explanations of all of these process configurations are in the following sections.

5.2.2.1 Separate Hydrolysis and Fermentation (SHF)

The hydrolysis of cellulose is performed by employing the cellulase enzyme. This is in fact a collection of enzymes including: a) endglucanase, which attacks along the cellulose fibres to reduce the degree of polymerization, b) exoglucanase, which attacks the ends of cellulose fibres in order to hydrolyse crystalline cellulose, and c) β-glucosidase, which hydrolyzes the resulting cellobiose to glucose. Several organisms are capable of producing these enzymes, including white rot fungi and bacteria in ruminant guts. The most industrialised organism for this purpose is Trichoderma reesei (NREL, 2002). The hydrolysed cellulose is then fermented by employing hexose consumer bacteria. The pentose resulting from the hydrolysis of hemicelluloses is fermented separately by utilizing pentose consumer bacteria.

5.2.2.2 Simultaneous Saccharification and Fermentation (SSF)

One of the significant advances in ethanol production pathway is the implementation of SSF, in which the enzymatic degradation of cellulose is combined with the fermentation of hexose sugars in one step. SSF has the potential to reach higher yields of ethanol in comparison with SHF (Wyman, 1996). SSF could significantly reduce the overall cost of the ethanol production by performing the cellulose hydrolysis and fermentation of hexose sugars simultaneously.

The implementation of SSF could significantly reduce the capital cost of the ethanol production because there is no need for a separate hydrolysis reactor. However, SSF demands a complex and difficult operation and control because of the oscillatory nature of the reactions and different optimal conditions required for hydrolysis and fermentation processes (Claassen et al., 2000). SSF is performed by utilizing the cellulase enzymes and the fermentation of hexose sugars by hexose fermenting bacteria such as Z. mobilis or modified yeast in a same unit; and the fermentation of pentose sugar is performed separately by employing a pentose fermenting bacteria.

5.2.2.3 Simultaneous Saccharification and Co-fermentation (SSCF)

SSCF includes the fermentation of pentose sugars in addition to the fermentation of hexose sugars in one step. Co-fermentation of hexose and pentose sugars is a prerequisite to obtain high ethanol yield and reduce the cost of ethanol production (Öhgren et al., 2006).

The process of SSCF implies a significant challenge to the overall production of ethanol because fermenting of hexoses and pentoses at the same time is a complex process. The choice of using SSCF in the PFD of ethanol production is highly influenced by the availability of a proper organism capable of utilizing pentose sugars and hexose sugars simultaneously (Mielenz, 2001). Recently, the pathway of cellulosic ethanol production has focused on the genetic manipulation of some organisms enabling them to ferment a variety of sugars at the same time. Examples of the genetically engineered bacteria are

Escherichia coli, Klebsiella, and Zymomonas mobilis; and the examples of genetically modified yeasts are Saccharomyces and Pichia species (Mielenz, 2001). Introducing the genes of pentose consumer organisms into some bacteria could enhance their ability for co-fermentation of hexose and pentose sugars for industrial applications (Wiedemann et al., 2008).

Recently, NREL has proposed a co-fermentation process capable of fermenting hexose and pentose sugars by employing the recombinant Zymomonas mobilis. Cellulose is first hydrolysed and saccharified by cellulase enzyme produced from Trichoderma reesei and the combination of hexose and pentose sugars from cellulose and hemicellulose are then fermented by recombinant Zymomonas mobilis (NREL, 2002).

5.2.2.4 Consolidated Bio-Processing (CBP)

CBP is a promising process configuration for ethanol production, in which only one microbial community is employed for cellulase production, hydrolysis, and fermentation of hexose and pentose sugars in one step. In fact, CBP is the desired culmination for the production of ethanol form lignocellulosic biomass. The most significant advantage of CBP is its potential to reduce the capital cost of ethanol by a factor of 8 (Lynd et al., 2005).

In CBP, there are five major biologically mediated phenomena accruing in one step. These phenomena include: a) production of cellulolytic enzyme, b) hydrolysis of

carbohydrates, c) fermentation of hexose sugars, d) fermentation of pentose sugars, and e) production of ethanol at a high rate. The feasibility of CBP at the industrial level depends on utilizing a suitable organism capable of doing all of these five tasks in one step. However, to date, there is no known organism capable of performing all of the combinations of these tasks in a single step (Cardona and Sanchez, 2007).

There are some cellulolytic organisms capable of direct hydrolysis and fermentation of cellulose to ethanol, among which the clostridia family is the most well known. For example, Clostridium thermocellum is able to grow on cellulose with 31% higher substrate conversion in CBP pathway than a system using Trichoderma reesei and Saccharomyces cerevisiae in SSCF. However, this bacterium cannot ferment pentose sugars (Cardona and Sanchez, 2007). Despite the fact that members of the Clostridia family are good candidates for the purpose of ethanol production, some of their characteristics cause drawbacks for industrial commercialization. For instance, low tolerance of clostridia to ethanol and other by-products, such as acetic acid and formic acid, causes a low concentration of ethanol production in the early stages of formation (Wyman, 1994).

Therefore, for the proposed PFD, CBP is not a feasible alternative at present. However, this feasibility depends upon the development and genetic modification of a microbial consortium which is capable of performing all the above five tasks in one step (Lynd et al., 2005). Research is currently being conducted (Leschine, 2007; Warnick et al., 2002;

Lynd et al., 2005) for the genetic modification of some organisms in order to increase their cellulolytic ability and ethanol tolerance.

Based on the above discussion on the different biological conversion strategies, the most feasible technology for the conversion of lignocellulosic biomass to ethanol is SSCF. Nevertheless, CBP is also a promising alternative and is considered as the culmination of the ethanol production pathway. However, in its present form, it is still not ready to be commercialized and suffers from some technological immaturities (Lynd et al., 2005).

Figure 5.4 is the proposed PFD for biological conversion of SSO to ethanol. As is shown in the figure, after the chemical pre-treatment and hydrolysis of hemicellulose, cellulose should also be hydrolysed via an enzymatic hydrolysis. The most dominant organism for the production of cellulase enzyme is Trichoderma reesei. The enzyme can also be produced in-situ in a separate reactor. The resultant saccharified liquid which also includes the saccharified hemicellulose and lignin must then be sent for the fermentation step. The co-fermentation of hexose and pentose sugars is performed in one step by employing the recombinant Zymomonas mobilis which is capable of utilizing both hexose and pentose sugars simultaneously. This is a SSCF conversion of SSO to ethanol and the reason for the design of a co-fermentation unit is the presence of xylose and other pentose sugars in the SSO samples, which was described in Chapter 4. Moreover, the combined fermentation of pentose and hexose sugars in SSCF is an economically viable solution in comparison with SHF and SSF because the process requires fewer reactors and operational units.

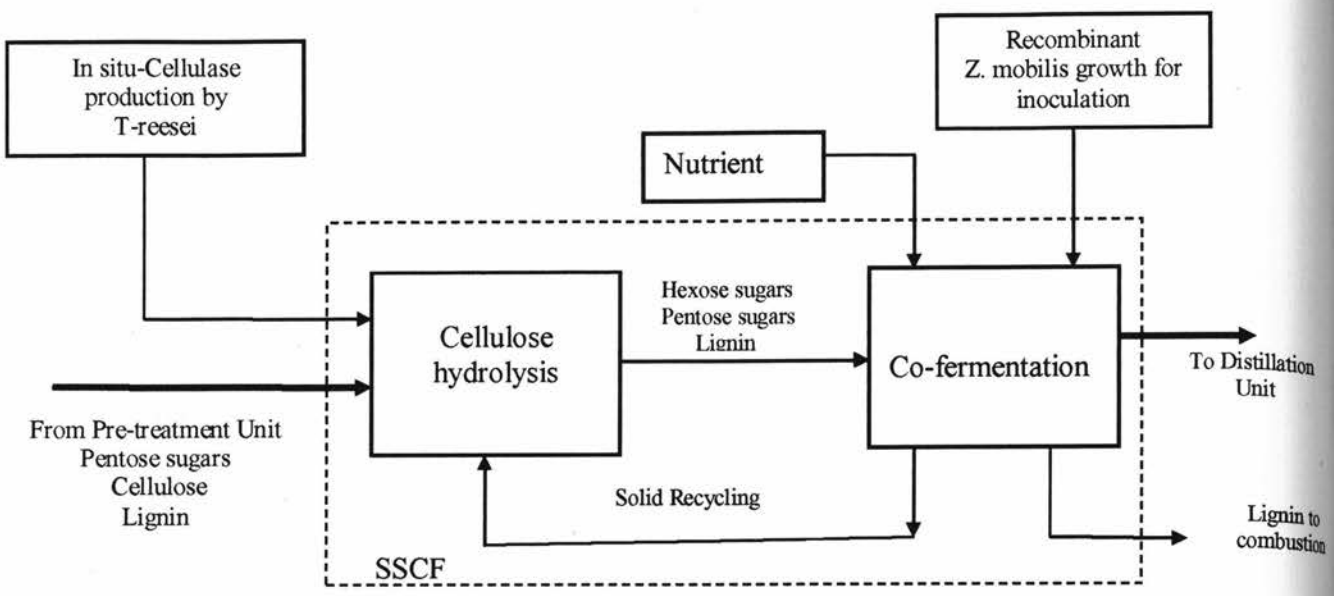


Figure 5.4 Proposed SSCF for the conversion of SSO to ethanol

5.2.3 Ethanol Recovery

After the fermentation terminates, ethanol must be separated from the fermentation broth. Ethanol should be recovered from the fermentation broth at the early stage of formation in order to minimize the inhibitory effect on the microbial growth rate (Cardona and Sanchez, 2007). Hence, all designed ethanol plants should have a separate ethanol recovery unit. There are many methods for removal of ethanol from the fermentation broth. Vacuumed separators, gas or steam strippers, membranes, and molecular sieves are all examples of removal technologies (Cardona and Sanchez, 2007).

A standard industrialized ethanol recovery unit is composed of a distillation and molecular sieve adsorption in order to recover ethanol from the raw fermentation beer and produce ethanol with 99.5% of purity. The process is composed of two distinct

columns. The first column removes the dissolved carbon dioxide and the second column concentrates the ethanol to a near azeotropic composition (NREL, 2002; Kwiatkowski et al., 2006).

Figure 5.5 is a simplified schematic diagram of a typical distillation unit in an ethanol plant. Distillation is a standard chemical separation concept, which is based on the difference in volatility (Wankat, 1988). According to this concept, in a mixture of ethanol and water at equilibrium, some of the ethanol is in the vapour phase above the liquid and some is in the liquid phase. Likewise, some of the water is in the vapour phase and some will be in the liquid phase. Due to higher volatility of ethanol than water, ethanol is more concentrated in the vapour phase. This phenomenon allows for the separation of ethanol from the water through subsequent vaporization, re-condensation and separation. However, after the ratio of ethanol to water exceeds 95.6 wt%, the difference in boiling point, which causes the separation, ceases. From this point, ethanol can no longer be separated from water. Hence, further separation is performed by employing a molecular sieve filter. Molecular sieves for drying ethanol are crystalline metal zeolites, which strongly adsorb water from the vapour/gas mixture, leading to the ethanol concentration of more than 99.6 %.

As illustrated in **Figure 5.5**, the first step is to separate ethanol from the fermentation beer by stripping in a beer column. The separated mixture of ethanol and water is then sent to the rectifier for further concentration and distillation. The solid stream from the beer column which is composed of lignin, cell mass, and other compounds is then sent for

combustion. Upon completion of the operation in the rectifier, the ethanol concentration usually reaches up to 91% (Kwiatkowski, 2006). As explained above, higher concentration of ethanol can be obtained upon the separation by molecular sieve in the next step. Hence, the vapour from the top of the rectifier is sent to the molecular sieve for further separation and then condensed into liquid ethanol. The liquid from the rectifier is further stripped in the stripper and re-rectified by being recycled into the rectifier. The process of ethanol distillation, which is illustrated in **Figure 5.5,** is a standard operational unit in chemical engineering with a high degree of certainty. However, in the overall energy demand of the PFD, distillation requires 12% (NREL, 2002) of the total energy consumption. This amount could be reduced depending upon further research and design of the innovative technologies for ethanol recovery. The proposed process configuration was based on the reviewed literature for the design of distillation units (NREL, 2002; Kwiatkowski, 2006; Cardona and Sanchez, 2007).

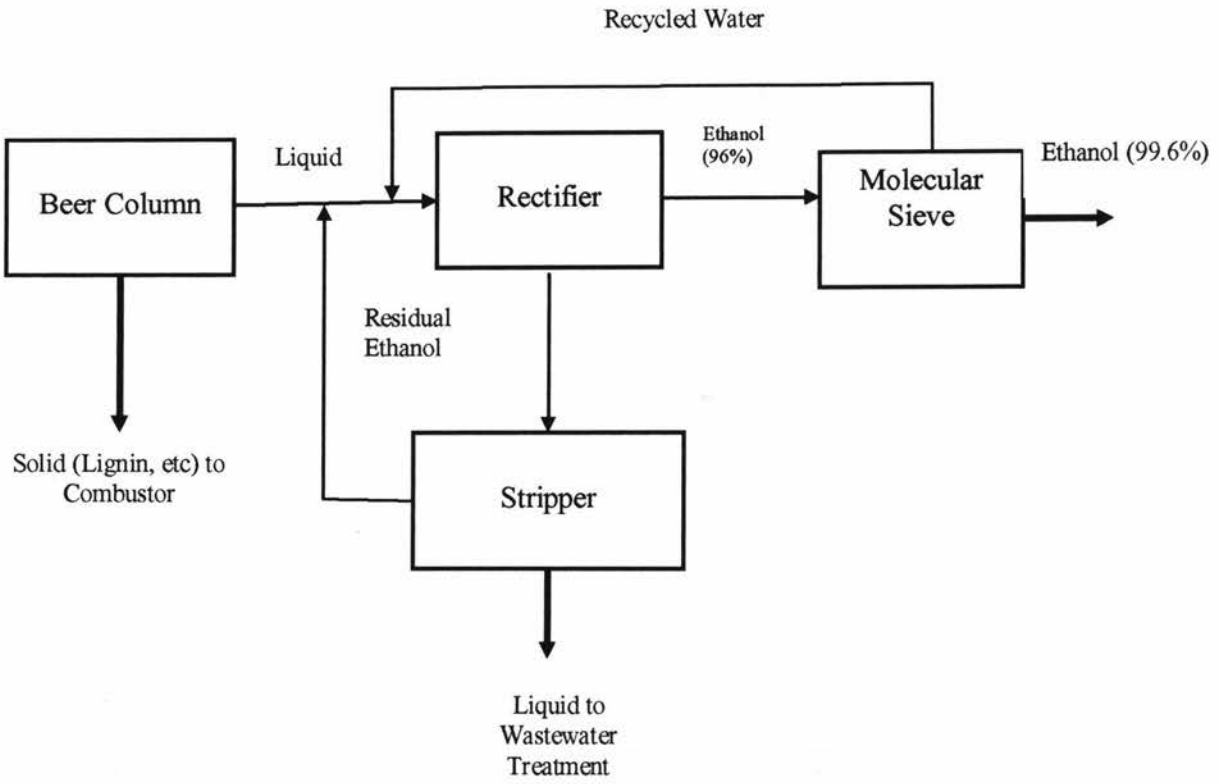


Figure 5.5 Simplified diagram for ethanol recovery unit (Kwiatkowski, 2006)

5.2.4 Wastewater Treatment

In addition to PFD for the conversion of lignocellulosic material, the plant should also be equipped with the proper infrastructure and facilities, including: waste management, and waste water treatment. The wastewater composition of the ethanol production process is highly dependant on the process configuration. However, some general treatment technologies are common for the waste water of all ethanol plants (Lynd, 1996).

Merrick Engineering Company in 1998 recommended an integration design for waste water treatment of ethanol plant, which is standard within the current ethanol industry in the range of 1 to 5 million gallons per day (NREL, 2002). This design is also used by NREL for cellulosic ethanol plant. The process is composed of screening, anaerobic and aerobic treatments, and filtration. The mass balance calculation can be performed by ASPEN Plus software package. However, as ASPEN Plus and other commercial simulators work in the steady state conditions, a design of an equalization basin is recommended by Merrick Engineering prior to the anaerobic digestion. The PFD for the waste water treatment of the ethanol plant is illustrated in **Figure 5.6**.

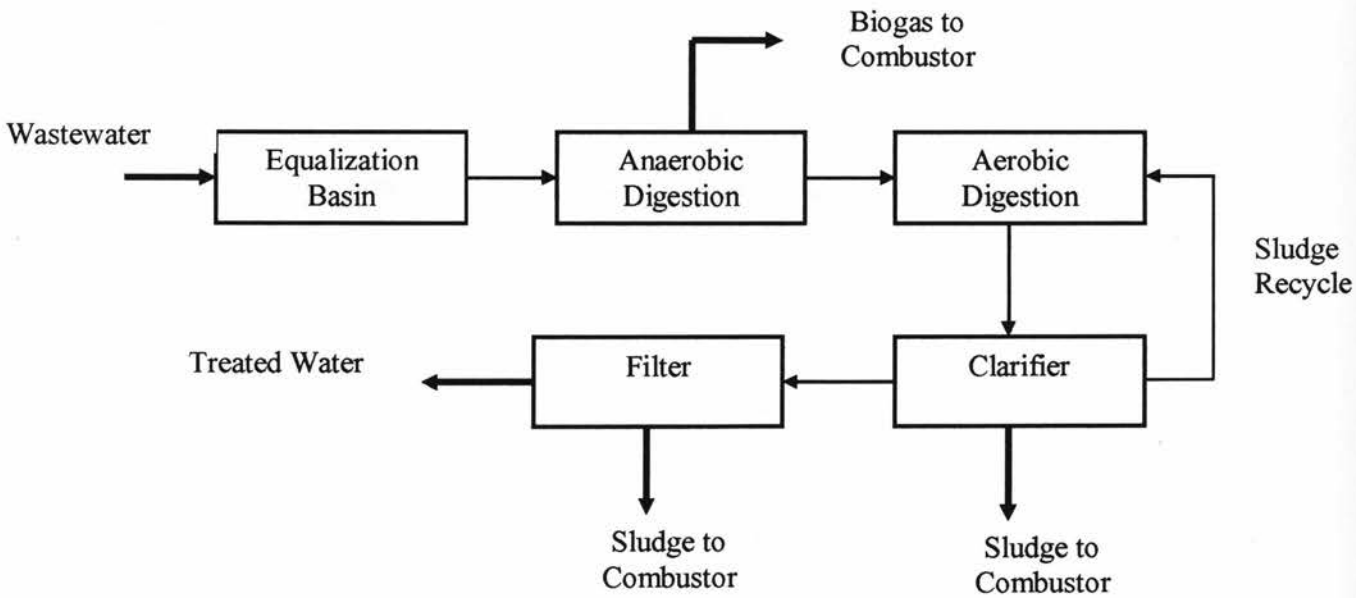


Figure 5.6 Wastewater treatment process of ethanol plant (NREL, 2002)

The outflow of the fermentor is first passed through a screen in order to remove the particulate matter. In the anaerobic digester, 90% of the organic compound is converted to methane and carbon dioxide in the ratio of 75% to 25%, and the majority of sulphate content is converted to hydrogen sulphide. The produced biogas is then sent to the combustion unit for the generation of steam and electricity. Liquid from the anaerobic digester is then pumped to the aeration basin for further removal of organic compounds. This system removes approximately 99.4% of the organic loading reported as chemical oxygen demand (COD) (g/l). After the aerobic treatment, materials are sent to the clarifier for separation. The treated wastewater after filtration by activated carbon filter is then recycled to the plant for utilizing, and the remaining sludge is pressed and dewatered by a belt conveyer filter press. The dewatered pressed solid is then sent for combustion (NREL, 2002).

The above process configuration for the wastewater treatment of an ethanol plant is not only beneficial for the pollution prevention from the plant, but also is a sustainable and cost-effective solution and an energetically viable design. The biogas generated from the anaerobic digester is 2.6% of the total fuel load supplied to the combustor. Moreover, combustion of lignin in the form of sludge after the anaerobic digester could produce a considerable amount of steam and electricity for the system (NREL, 2002).

Based on the above explanations for each compartment and the characteristic of SSO over the past few months, a PFD can be proposed in order to address the most feasible existing conversion method for commercialization. This PFD is mostly based on the

standard NREL design for the conversion of cellulosic biomass to ethanol with some modification and recommendation as well as with a comprehensive consideration to the characteristics of SSO as a feedstock. **Figure 5.7** is a schematic diagram for the conversion plant. As is shown in **Figure 5.7**, the main processes selected for the four steps of the biological conversion of SSO to ethanol are as follows:

1. Pre-treatment: the ATS machine is selected for the physical pre-treatment and the method of dilute acid is chosen as the chemical pre-treatment.

2. Saccharification: the cellulase enzyme from T-reesei is produced in situ for the saccharification of cellulose to glucose. Hemicellulose is also hydrolysed to xylose and other sugars during the dilute acid pre-treatment.

3. Fermentation: co-fermentation of glucose and xylose is conducted in one reactor by employing recombinant Z. mobilis bacterium.

4. Ethanol distillation: a standard ethanol distillation unit is proposed for condensation and recovery of ethanol.

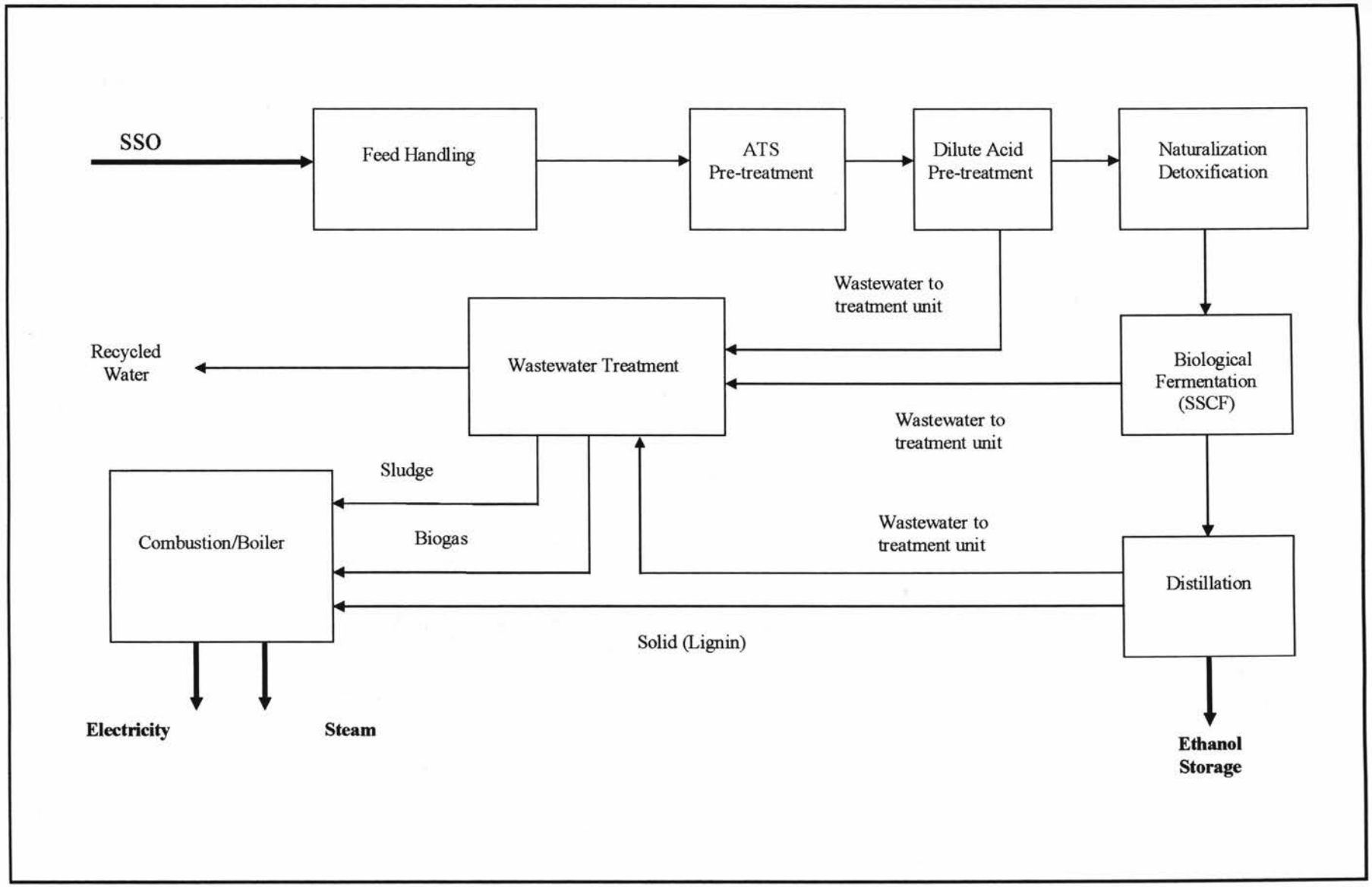


Figure 5.7 Simplified diagram for the biological conversion of SSO to ethanol

5.3 Cost of Ethanol

The overall cost of conversion of lignocellulosic biomass to ethanol depends on the contribution of all compartments of the process, among which feedstock cost and pre-treatment are the most dominants. Therefore, a viable and cost effective design for pre-treatment and an inexpensive resource of biomass feedstock like SSO could drastically reduce the overall cost of ethanol production.

The greatest contribution to the cost of ethanol is for feedstock, which is 31% of the final cost (NREL, 2002). Therefore, utilizing a feedstock like SSO, that has a negative price, could be a significant progress in the reduction of the overall cost for production of ethanol. The city of Toronto gives an incentive of approximately US$127 per tonne of SSO for utilization and diversion (Doug Beatty, personal communication, May 2007). The reason for such an incentive is because the City does not have a sustainable solution for the diversion of its organic wastes. After the costly collection and source separation, SSO is either sent to U.S for disposal, composted, or combusted, which are not economically and environmentally viable waste management solutions. This, in fact, invites the new technologies in utilizing SSO for the diversion to value- added energy products such as ethanol.

Based on the characteristics of the SSO, some conclusions can be obtained for the overall PFD in ethanol production. These conclusions are highlighted as follow:

- Introducing a negatively-costed feedstock such as SSO could drastically decrease the capital cost of ethanol. This fact along with the sufficiently high carbohydrate content in SSO could place greater emphases on the value of utilizing the SSO as a feedstock instead of dedicated energy crops or at least co-substrating of SSO with other cellulosic biomass.

- The high percentage of hexose and pentose sugars including glucose and xylose, in the SSO samples, which was reported in Chapter 4, indicates the need for the design of a SSCF unit which is capable of co-fermentation of both hexose and pentose sugars instead of other conversion techniques.

- Design of a unit exclusively for the combustion of solid residue is highly beneficial to the overall economy of the ethanol plant. The high percentage of lignin, which was consistently observed in the SSO samples for the six months of analysis, indicates the need for an effective pre-treatment process capable of lignin removal as well as a unit for lignin combustion and steam/electricity generation. The calorific value of the SSO samples, which was reported in Chapter 4, indicates the potential of energy generation by combustion of the post fermentation sludge.

- The anaerobic digester in the wastewater treatment plant produces biogas, which is 75% methane and 25% carbon dioxide. The design of the wastewater treatment unit is not only beneficial for pollution prevention and water recycling in the

plants, but also is advantageous for biogas generation because of the high percentage of volatile solids (80% of dry mass) present in the SSO samples. The high VOC content of the SSO samples indicates the presence of other carbon sources than the lignocellulosic carbohydrate, which have great potential for biochemical methane production.

- The detoxification unit must be designed to reduce the inhibitory compounds prior to fermentation. The recommendation for the detoxification unit is because of the heterogeneous and toxic nature of the SSO material in contrast with other biomass like corn stover. The presence of many unknown components in SSO material may result in the formation of certain inhibitory by-products during the acid hydrolysis pre-treatment. Therefore, thoroughly design of a detoxification unit prior to fermentation process is essential for the overall efficiency of the plant.

Chapter 6 Conclusions & Recommendations

6.1 Conclusions

This thesis confirmed that SSO is a potentially suitable feedstock for ethanol production through fractionation and bacterial fermentation. This research led to the development of a method utilizing waste biomass to produce ethanol. Therefore, the outcome of this research project benefits both the fields of waste management and the renewable energy sector in overcoming the current waste problems, energy crisis, and pollution prevention in the city of Toronto. From the observations and data that resulted from this study, the following conclusions may be summarized:

1. SSO has an excellent potential to be utilized as a feedstock for biological ethanol production due to its high carbohydrate content. Introducing a negatively-costed feedstock like SSO, which is amenable to ethanol production, can drastically decrease the final cost of ethanol. This fact along with the satisfactorily high carbohydrate contents in the SSO samples can place greater emphasis on the value of utilizing SSO as a feedstock instead of dedicated energy crops, or at least co-substrating of SSO with other cellulosic materials. However, similar to other lignocellulosic biomass, the main problem in dealing with SSO is separation of lignin and mobilization of monomeric sugars from the carbohydrate chains. This requires the design of an effective pre-treatment and saccharification unit prior to the biological fermentation of SSO to ethanol.

2. The high lignin content in SSO, which was reported in chapter 4, emphasizes the need for the design of an effective pre-treatment unit prior to biological conversion in order to remove or alter lignin, because it hinders the process of hydrolysis and fermentation of the carbohydrate.

3. The ATS machine is effective in homogenizing, reducing particle size, and increasing the shelf life of the samples. It is also an effective physical pre-treatment for SSO samples because it includes milling, size reducing, heating, and pulverizing. The combination of high temperature and high pressure causes the transformation of SSO to a more homogenous, stable, and fibrous product.

4. SSO after the pre-treatment by the ATS machine also has the potential to be utilized as compost. The effect of pressure and heat in the ATS machine significantly reduces the amount and dimensions of the sharp foreign matter. However, the amount of some heavy metals in SSO may cause some restrictions in using SSO as a category A compost. Also the stability of the SSO is lower than the acceptable level for compost according to the guidelines. Therefore, the outflow of the ATS machine should be further treated to enable it to become an acceptable compost material.

5. The idea of mixing SSO with a blend of wood chips from the construction and demolition waste (CD) is beneficial because the mixer blend has a higher potential to be utilized as a feedstock for ethanol than the organic waste alone.

The CD wood waste is also a negatively-costed waste and utilising it is beneficial to the overall waste management.

6. The high amount of volatile organic compounds (VOC) in the SSO samples indicates sources of carbon other than carbohydrates; therefore, a design of a proper conversion process for the utilization of all volatile organic compounds is essential. The degradation of the remaining volatile organic carbons can be performed in a separate anaerobic digestion during the wastewater treatment process in the ethanol plant. An anaerobic digester will not only treat the effluent, but also produce biogas such as methane and carbon dioxide from volatile organic compounds which can be used to generate heat and power for the ethanol plant.

7. The high calorific value of the SSO samples indicates the potential of utilizing SSO for combustion. The calorific value of a biomass sample is positively related to its lignin content (Demirbus, 2002). The design of a combustion unit for the ethanol plant is highly beneficial to the overall economy of the plant because steam or electricity can be generated from the combustion of the remaining lignin after fermentation. The resultant steam or electricity can be utilized in the plant.

8. Introducing the technology for converting SSO to ethanol, which was described in chapter 5, can lead to a better understanding of the design and cost estimation of the ethanol plant. This will provide more information for the decision makers in

municipalities and private sectors in order to find an environmentally sustainable solution for Toronto's waste problem.

In general, the main conclusion that can be drawn from this investigation is the amenability of SSO as a potentially reliable feedstock for ethanol production in the city of Toronto. Also, the proposed technology for the biological conversion that was presented in chapter 5 may provide a sustainable diversion of the organic waste to ethanol.

6.2 Recommendations

The research work presented has given a general understanding of the nature of SSO and the alternative waste management solutions in order to produce value-added energy products such as ethanol. This understanding could be enhanced by taking a detailed look at the design and feasibility assessment of the proposed technology for the conversion of SSO to ethanol. The following recommendations can be considered for the future work and directions.

1. A detailed analysis on the extractives and other unknown components in SSO should be conducted in order to have comprehensive information about the nature of SSO and provide more data in order to reduce the risk for the investment in such a heterogeneous feedstock.

2. Based on the observation of this study, more research is recommended for the design and optimization of an effective pre-treatment process in order to remove or alter lignin from SSO and make it more susceptible to the biological conversion to ethanol.

3. Design, implementation, and testing of a bench scale ethanol plant using technology proposed in chapter 5 are strongly recommended before the construction of a pilot plant.

4. A detailed study on the process of SSCF is recommended, which should include: the optimum enzyme loading and PH for femrnetaion, the conversion efficiency for the fermentation of hexose and pentose sugars to ethanol, and the optimum strain of microorganisms for enzyme production and microbial fermentation.

5. The proposed technology for the biological conversion of SSO to ethanol must be studied for design, simulation, and rigorous mass and energy balance calculations. There are some software packages available for the design and simulation of the ethanol plant among which Aspen Plus is one of the most popular.

6. More investigation on the consolidated bio-processing (CBP), for the conversion of SSO to ethanol in one step, is recommended for future works. CBP is considered as the ultimate goal of cellulosic ethanol production. The conversion of cellulosic feedstock like SSO to ethanol by CBP can not be achieved unless a

genetically modified organism is developed, which is capable of one-step conversion of cellulose to ethanol. A study on the genetic manipulation of a cellulolytic microorganism (e.g. clostridium phytofermentans) in order to enhance its cellulose degradation and ethanol production abilities is recommended.

7. Based on the results from chapter 4 for the compost characteristics of SSO, the heavy metals content of SSO is still too high to allow it to be considered as a category A compost. The development of fast and robust techniques for the removal of heavy metals from SSO is recommended for future work.

References

1. Aden, A., Ruth, M., Ibsen, K., Jechura, J., Neeves, K., Sheehan, J., et al. (2002). Lignocellulosic biomass to ethanol, process design and economics utilizing co-current dilute acid pre-hydrolysis and enzymatic hydrolysis for corn stover. National Renewable Energy Laboratory (NREL). Retrieved April 10, 2009, from:

   http://www.nrel.gov/docs/fy02osti/32438.pdf.

2. Baldasano, J. M., Soriano, C. (2000). Emission of greenhouse gases from anaerobic digestion processes, comparison with other MSW treatments. *Water Sci. Technol, 41(3), 275-82.*

3. BIOCAP Canada foundation (2002). Annual report for 2002/2003. Retrieved April 2, 2009, from:

   http://www.biocap.ca/images/pdfs/BIOCAP_Annual_2002-03.pdf

4. Biomass Energy Center (2008). Conversion technology. Retrieved September 2, 2008, from:

   http://www.biomassenergycentre.org.uk

5. Blades, R., (2006). Organic waste to energy, process feedstock. Retrieved February 12, 2009, from:

   http://www.wrighttech.ca/Organic%20Waste.htm

6. Boominathan, K., Reddy, C. A. (1992). CAMP-mediated differential regulation of lignin peroxidase and manganese-dependent peroxidase production in the white-rot basidiomycete Phanerochaete chrysosporium. *Proc. Natl. Acad. Sci, 89(12), 5586–90.*

7. Brenna, A. H., Hoagland, W., Schell, D. J. (1986). High temperature acid hydrolysis of biomass using an engineering-scale plug flow reactor: result of low solids testing. *Biotechnol. Bioeng. Symp. 17,53-70.*

8. Brown, R. M., Saxena, I. M., and Kudlicka, K. (1996). Cellulose biosynthesis in higher plant. *Trends in Plant Science, 1, 149–56.*

9. Browning, B. L. (1967). *Methods of wood chemistry*. New York, NY: Inter-science publishers.

10. Butts, R. (2005). City of Toronto waste diversion initiatives: zero waste to landfill by 2012. [PowerPoint slides, Presentation to Works Committee]. Retrieved March 10, 2009, from:

http://www.epa.gov/Region5/solidwaste/butts.pdf.

11. Canadian Bioenergy Association (2008). Biomass sources for bioenergy use in Canada. Fact sheet. Retrieved September 10, 2008, from:

http://www.canbio.ca/documents

12. Canadian Council of Minister of the Environment (CCME) (2005). Guidelines for Compost Quality. Retrieved August 2, 2008, from:

http://www.ccme.ca/assets/pdf/compostgdlns_1340_e.pdf

13. Cardona, C. A., Sanchez, O. J. (2007). Fuel ethanol production: Process design trends and integration opportunities. *Bioresource Technology, 98, 2415–57.*

14. Caulfield, D. E., Moore, W. E. (1974). Effect of varying crystallinity of cellulose on enzymatic hydrolysis. *Wood Sci, 6, 375–9.*

15. Chandler, J. A., Jewell, W. J., Gossett, J. M. (1980). Predicting methane fermentation biodegradability. *Biotechnology Bioengineering Symposium, 10 (10), 93-107.*

16. Chang, V. S., and Holtzapple, M. T. (2000). Fundamental factors affecting biomass enzymatic reactivity. *Applied Biochemistry and Biotechnology, 84(1), 5-37.*

17. Chang, V. S., Nagwani, M., Holtzapple, M. T. (1998). Lime pre-treatment of crop residues bagasse and wheat straw. *Applied Biochemistry and Biotechnology, 74, 135-59.*

18. Chang, V. S., Nagwani, M., Kim, C., and Holtzapple, M. T. (2000). Oxidative lime pre-treatment of high lignin biomass. *Applied Biochemistry and Biotechnology, 94, 1-28.*

19. City of Toronto. (2001).Waste diversion task force 2010 report. Toronto, Ontario. Retrieved November 20, 2006 from:

http://www.toronto.ca/taskforce2010/report.pdf

20. Claassen, P. A. M., Budde, A. M. W., and Lopez-Contreras, A. M. (2000). Acetone, Butanol and Ethanol production from domestic organic waste by solventogenic clostridia. *J. Mol .Microbial. Bioetechnol, 2(1), 39-44.*

21. Clark, T. A., Mackie, K. L. (1987). Steam explosion of the softwood Pinus radiate with sulphur dioxide addition. *Wood. Chem. Technol, 7, 373-403.*

22. De Baere, L. (1999). Anaerobic digestion of solid waste: state-of-the art. *Water Sci Technol,* 41(3), 283-90.

23. Demirbus A. (2002). Relationships between heating value and lignin, moisture, ash and extractive contents of biomass fuels. *Energy exploration & exploitation, 20(1), 105-11.*

24. Desvaux, M. (2005). Clostridium cellulolyticum: model organism of mesophilic cellulolytic clostridia. *FEMS Microbiology Reviews, 29, 741-764.*

25. Draude, K. M., Kurniawan, C. B., Duff, S. T. B. (2001). Effect of oxygen delignification on the rate and extent of enzymatic hydrolysis of lignocellulosic material. *Bioresour. Technol, 79, 113–20.*

26. Esteghlalian, A., Hashimoto, A. G., Fenske, J. J., Penner, M. H. (1997). Modeling and optimization of the dilute sulphuric acid pre-treatment of corn stover and poplarand switch grass. *Bioresour. Technol., 59, 129-36.*

27. Fan, L. T., Lee, Y. H., Beardmore, D. H. (1981). The influence of major structural features of cellulose on rate of enzymatic hydrolysis. *Biotechnol. Bioeng, 23, 419–24.*

28. Faye, M., Percy, B. (2007). Value added energy options from household organic waste. BSc Thesis. Ryerson University, Toronto, Ontario, Canada.

29. Fengel, D., Wegener, G. (1989). *Wood chemistry, ultra structure and reactions.* Berlin, New York, U.S: W. de Gruyter.

30. Gaddy, J. L. (1992). Biological production of acetic acid from waste gases with Clostridium ljungdahlii. US Patent 5807722.

31. Government of Canada (2005). Five ethanol plants receive $46 million in Government of Canada funding. Retrieved July 6, 2005 from:

http://www.physorg.com/pdf4975.pdf

32. Grethlein, H. E. (1985). The effect of pore size distribution on the rate of enzymatic hydrolysis of cellulosic substrates. *Bio. Techno, 3, 155–60.*

33. Grethlein, H. E., Dill, T. (1993). The cost of ethanol production from lignocellulosic biomass- a comparison of selected alternative processes. Retrieved June 20, 2008 from:

http://www.osti.gov/bridge/servlets/purl/171246-xil24y/webviewable/

34. Grohmann, K., Mitchell, D. J., Himmel, M. E. (1989). The role of ester groups in resistance of plant cell wall polysaccharides to enzymatic hydrolysis. *Appl. Biochem. Biotechnol, 20, 45–61.*

35. Grohmann, K., Torget, R., Himmel, M. (1985). Optimization of dilute acid pre-treatment of biomass. *Biotechnol. Bioeng. Symp, 15, 59–80.*

36. Guffey, F. D., Wingerson, R. C. (2002). Fractionation of lignocellulosic biomass for fuel-grade ethanol production. Retrieved May 12, 2008 from:

http://www.osti.gov/bridge/servlets/purl/807155-kbdGYV/native/807155.pdf.

37. Hames, B., Ruiz, R., Scarlata, C., Sluiter, A., Sluiter, J., and Templeton, D. (2008). Preparation of Samples for Compositional Analysis Laboratory Analytical Procedure (LAP). Technical Report, NREL/TP-510-42620. Retrieved March 26, 2009, from:

http://www.nrel.gov/biomass/pdfs/42620.pdf

38. Haug, R.T. (1993). *The Practical Handbook of Compost Engineering (pp 307-30).* Boca Raton, Florida: Lewis Publishers.

39. Holtzapple, M. T., Lundeen, J. E., Sturgis, R., Lewis, J. E., and Dale, B. D. (1992). Pre-treatment of lignocellulosic municipal solid waste by ammonia fibre explosion (AFEX), *Applied Biochemistry and Biotechnology, 34, 5-21.*

40. Howard, R. L., Abotsi, E., Rensburg, J. E. L., Howard, S. (2003). Lignocellulose biotechnology: issues of bioconversion and enzyme production. *African Journal of Biotechnology, 2, 602-19.*

41. Ibrahim, M. (1998). Clean fractionation of biomass- steam explosion and extraction. MASc Thesis. Virginia Polytechnic Institute and State University. Retrieved December 12, 2008 from:

http://scholar.lib.vt.edu/theses/available/etd-2998-114756/unrestricted/e-front.pdf

42. Jansen, J. L. C., Spliid H., Hansen, T. L., Savard, A., Christensen, T. H. (2004). Assessment of sampling and chemical analysis of source-separated organic household waste, *Waste management, 24(6), 541-9.*

43. Kaar, W. E., Holtzapple, M. T. (2000). Using lime pre-treatment to facilitate the enzyme hydrolysis of corn stover. *Biomass and Bioenergy, 18(3), 189-99.*

44. Kim, Y., Mosier, N. S., Hendrickson, R., Ezeji, T., Blaschek, H., Dien, B., et al. (2007). Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. *Bio-resource Technology, 99, 5165–76.*

45. Kong, F., Engler, C. R., Soltes, E. J. (1992). Effect of cell-wall acetate, xylan backbone, and lignin on enzymatic hydrolysis of aspen wood. *Appl. Biochem. Biotechnol. 34/5, 23–35.*

46. Koullas, D. P., Christakopoulos, P. F., Kekos, D., Macris, B. J., Koukios, E. G. (1990). Effect of cellulose crystallinity on the enzymic hydrolysis of lignocellulosics by fusarium oxysporum cellulases. *Cellulose Chem. Technol., 24, 469–74.*

47. Kwiatkowski, J. R., McAloon, A. J., Taylor, F., and Johnston, D. B. (2006). Modeling the process and costs of fuel ethanol production by the corn dry-grind process. *Industrial crops and products, 23, 288-96.*

48. Leschine S. (2007). Biomass to biofuel technology: a novel bacterial catalyst for consolidated bioprocessing of biomass to ethanol. A report to the Institute for Massachusetts Biofuels Research (TIMBR). University of Massachusetts Amherst. Retrieved August 2009 from:

http://globalwarming.house.gov/tools/assets/files/0143.pdf

49. lignocellulosicc structure [Image]. Retrieved June 3, 2008, from:

http://www.ceres.net/AboutUs/AboutUs-Biofuels-Carbo.html

50. Lin, G. W. (2007). Investigation of a new effective bacterium for cellulosic degradation. MEng research project. Ryerson University. Toronto, Ontario, Canada.

51. Lynd, L. R. (1996). Overview and evaluation of fuel ethanol from cellulosic biomass: Technology, economics, the environment, and policy. *Annual Review of Energy and the Environment, 21, 403-65.*

52. Lynd, L. R., Cushman, J. H., Nichols, R. J., Wyman, C. E. (1991). Fuel ethanol from cellulosic biomass. *Science, 251(4999), 1318–23.*

53. Lynd, L. R., Willem, H. V. Z., McBride, J. E., and Laser, M. (2005). Consolidated bio-processing of cellulosic biomass: an update. *Current Opinion in Biotechnology, 16(5), 577-83.*

54. Mabee, W. E., Gregg, D. J., Arato, C., Berlin, A., Bura, R., Gilkes, N., et al. *(2006).* Updates on softwood-to-ethanol process development. *Applied Biochemistry and Biotechnology, 129, 55-70.*

55. Meta-Alvarez, J., Macé, S., and Liabrés, P. (2000). Anaerobic digestion of organic solid wastes. An overview of research achievements and prospective. *Bioresource Technology, 74(1), 3-16.*

56. Mielenz, J. R. (2001). Ethanol production from biomass: technology and commercialization status. *Current Opinion in Microbiology, 4(3), 324-29.*

57. Miller, M. M. (2001). Minerals Yearbook, Lime. Retrieved November 17, 2008, from:

http://minerals.usgs.gov/minerals/pubs.

58. Monosaccharids [Image]. Retrieved March , 26, 2008 from:

http://www.sciencecollege.co.uk/SC/biochemicals/cellulose.jpg

59. Mooney, C. A., Mansfield, S. D., Touhy, M. G., Saddler, J. N. (1998). The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods. *Bioresour. Technol., 64, 113–9.*

60. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., and Ladisch, M. (2005). Features of promising technologies for pre-treatment of lignocellulosic biomass. *Bioresource technology, 96, 673-86.*

61. Natural Resource Canada. (2008). Bioenergy systems publications. Retrieved February 2009 from:

http://canmetenergy-canmetenergie.nrcan-rncan.gc.ca/eng/bioenergy/publications/200843.html

62. Öhgren, K., Bengtsson, O., Marie, F., Grauslund, G., Galbe, M., Hahn-Hägerdal, B., et al. (2006). Simultaneous saccharification and co-fermentation of glucose and xylose in steam pre-treated corn stover at high fiber content with Saccharomyces cerevisiae. *Journal of Biotechnology, 126(4), 488-98.*

63. Owen, W. F., Stuckey, D. C., Healy, J. B., Young, L. Y. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research, 13(6), 485-92.*

64. Palmowski, L., and Muller, J. (1999). Influence of the size reduction of organic waste on their anaerobic digestion. *Water Science and Technology, 41(3), 155-62.*

65. Palonen, H. (2004). Role of lignin in the enzymatic conversion of lignocellulose. PhD thesis. Helsinki University of Technology. Espoo, Finland. Retrieved February 6, 2009, from:

http://www.vtt.fi/inf/pdf/publications/2004/P520.pdf

66. Pereira, A. N., Mobedshashi, M., and Ladish, M. R. (1988). Preparation of cellodextrins. *Meth. Enzymol, 160, 26–45.*

67. Puri, V. P. (1984). Effect of crystallinity and degree of polymerization of cellulose on enzymatic saccharification. *Biotechnol. Bioeng, 26, 1219–22.*

68. Ren, Z., Ward, T. E., Logan, B. E., and Regan, J. M. (2007). Characterization of the cellulolytic and hydrogen-producing activities of six mesophilic clostridium species. *Journal of Applied Microbiology, 103, 2258–66.*

69. Riggle, D. (1998). Acceptance Improves for large-scale anaerobic digestion. *Biocycle, 39(6), 51-5.*

70. Rivers, D. D., Emert, G. H. (1987). Lignocellulose Pre-treatment, A comparison of wet and dry ball attrition. *Biotechnology Letters, 9, 365-8.*

71. Ruffle, J. (2006). Pre-treatment and hydrolysis of recovered fibre for ethanol production. MASc Thesis. University of British Colombia. Retrieved March 14, 2009 from:

https://circle.ubc.ca/bitstream/2429/1369/1/ubc_2008_fall_ruffell_john.pdf

72. Sakai, S., Sawell, S. E., Chandler, A. J., Eighmy, T. T., Kosson, D. S., Vehlow, J., et al. (1996). World trends in municipal solid waste management. *Waste Management, 16, 5, 341-50.*

73. Sarkanen, K.V. (1980). Acid-catalyzed delignification of lignocellulosics. *Academic Press, 2, 129-44.*

74. Schwarz, W. H. (2001). The cellulosome and cellulose degradation by anaerobic bacteria. *Appl. Microbiol. Biotechnol, 56, 634–49.*

75. Shin, H. S., Youn J. H. (2005). Conversion of food waste into hydrogen by thermophilic acidogenesis. *Biodegradation, 16(1), 33-44.*

76. Sierra, R., Aaron, S., Cesar, G., Holtzapple, M. T. (2008). Producing fuels and chemicals from lignocellulosic biomass. Retrieved February 5, 2009 from:

http://www.aiche.org/uploadedFiles/SBE/MemberCenter/0808S10(2).PDF

77. Sinitsyn, A. P., Guakov, A. V., Vlasenko, E. Y. (1991). Effect of structural and physicochemical features of cellulosic substrates on the efficiency of enzymatic hydrolysis. *Appl. Biochem. Biotechnol, 30, 43-59.*

78. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter J., and Templeton, D. (2008). Determination of ash in biomass Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory (NREL). Retrieved June 28, 2008 from:

http://www.nrel.gov/biomass/pdfs/42622.pdf

79. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter J., Templeton, D., and Crocker, D. (2008). Determination of structural carbohydrates and lignin in

biomass. Laboratory Analytical Procedure (LAP). National Renewable Energy Laboratory (NREL). Retrieved March 2008 from:

http://www.nrel.gov/biomass/pdfs/42618.pdf

80. Sun, Y., and Cheng, J. (2002). Hydrolysis of lignocellulosic material for ethanol production, A Review. *Bioresource technology, 83, 1-11.*

81. Tampier, M., Smith, D., Bibeau, E., Beauchemin, P. A. (2004). Identifying environmentally preferable uses for biomass resources. Retrieved November 23, 2008, from:

http://www.for.gov.bc.ca/hts/bioenergy/Link_Rep/Envirochem_Bugwood.pdf

82. Technical Association of the Pulp and Paper Industry (TAPPI). (1988). T264 om-88. Preparation of wood for chemical analysis. *In Tappi Test Methods*. Atlanta, GA.

83. Thammasouk, K., Tandjo, D., and Penner, M. H. (1997). Influence of extractives on the analysis of herbaceous biomass. *J. Agric. Food Chem., 45 (2), 437–43.*

84. Thompson, D. N., Chen, H. C., Grethlein, H. C. (1992). Comparison of pre-treatment methods on the basis of available surface area. *Bioresource Technol, 39, 155-63.*

85. Torget, R., Werdene, P., Himmel, M., Grohmann K. (1990). Dilute acid pre-treatment of short rotation woody and herbaceous crops. *Appl. Biochem. Biotechnol, 24, 115-116.*

86. USEPA, (1996). Acid digestion of sediments, sludge and soils. method 3050B, Washington DC, U.S. Retrieved May 20, 2008, from:

http://www.epa.gov/waste/hazard/testmethods/index.htm

87. USEPA, (1993). Determination of total Kjeldahl nitrogen by semi-automated colorimetry, Washington DC, U.S. Retrieved May 20, 2009, from:

http://www.epa.gov/waste/hazard/testmethods/index.htm

88. Van Soet, P. J., Robertson, J. B., Lewis, B. A. (1991). Methods for determination of dietary fibre, neutral detergent fibre, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74, 10, 3583-97.*

89. Walsum, P.G., Shi, H. (2004). Carbonic acid enhancement of hydrolysis in aqueous pre-treatment of corn stover. *Bio-resource technology, 93, 217-26.*

90. Wankat, P.C. (1988). *Separations in Chemical Engineering: Equilibrium Staged Separations (pp 9-36).* Upper Saddle River, NJ: Prentice-Hall.

91. Warnick A., Methe A. B., and Leschine, B. S. (2002). Clostridium Phytofermentans sp. nov., a cellulolytic mesophile from forest soil. *International Journal of Systematic and Evolutionary Microbiology, 52, 1155-60.*

92. Weimer, P. J., Weston, W. M. (1985). Relationship between the fine structure of native cellulose and cellulose degradability by the cellulose complexes of Trichoderma reesie and Clostridium Thermocellum. *Biotechnol. Bioeng. 27, 1540–47.*

93. Wiedemann, B., Brat, D., Boles, E. (2008). Production of cellulosic ethanol with improved yeast strains. *Sugar Industry*, 133(10), 637-41.

94. Wright, J. D. (1983). High temperature acid hydrolysis of cellulose for alcohol fuel production. Retrieved August 11, 2008 from Energy Citation Database.

95. Wyman, C. E. (1994). Ethanol from lignocellulosic biomass- technologies, economics, and opportunities. *Bioresource Technology, 50, 1, 3-16.*

96. Wyman, C. E. (1999). Biomass ethanol: technical progress, opportunities and commercial challenges. *Annual Review of Energy and the Environment, 24, 189-226.*

97. Wyman, C. E., Goodman, B. J. (1993). Biotechnology for fuel, chemical, and materials from biomass. *Applied Biochemistry and Biotechnology, 39-40, 41-59*

98. Wyman, C.E. (1996). *Handbook on Bioethanol: Production and Utilization.* (pp 180-190 and 395-410).Washington, DC: Taylor and Francis.

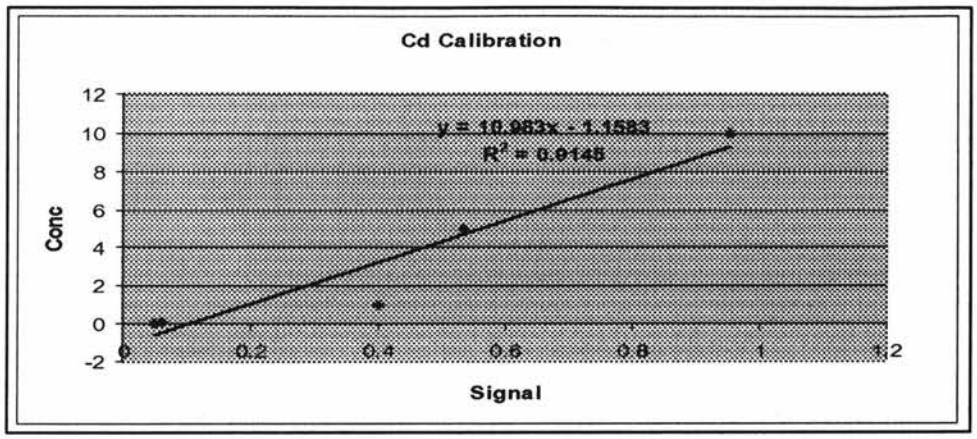
99. Yoon, H. H. (1998). Pre-treatment of lignocellulosic biomass by auto hydrolysis and aqueous ammonia percolation. *Korean J. Chem. Eng, 15(6), 631–36.*

100. Zhang, M., Eddy, C., Deanda, K., Finkelstein, M., Picataggio, S. (1995). Metabolic engineering of a pentose metabolism pathway in ethanologenic Zymomonas mobilis. *Science, 267, 240−43.*

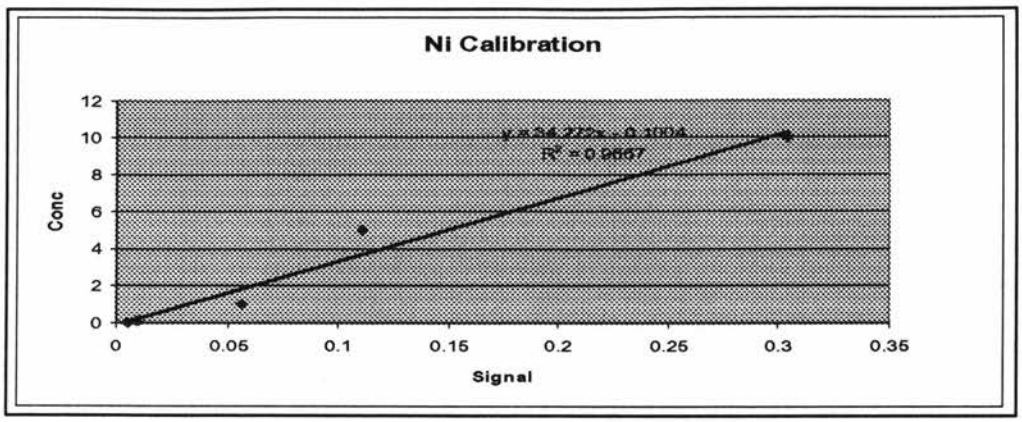
101. Zhu, L., O'Dwyer, J. P., Chang, V. S., Granda, C. B., and Holtzapple, M. T. (2007). Structural features affecting biomass enzymatic digestibility. *Bioresource Technology, 99, 3817–28.*

Appendix A

Heavy Metals



*** The concentration of Cd is less than 2 ppm for the period of study**



*** The concentration of Ni is less than 2 ppm for the months of Sep 08, Nov 08, Mar 09, A**

Heavy Metals

May-09

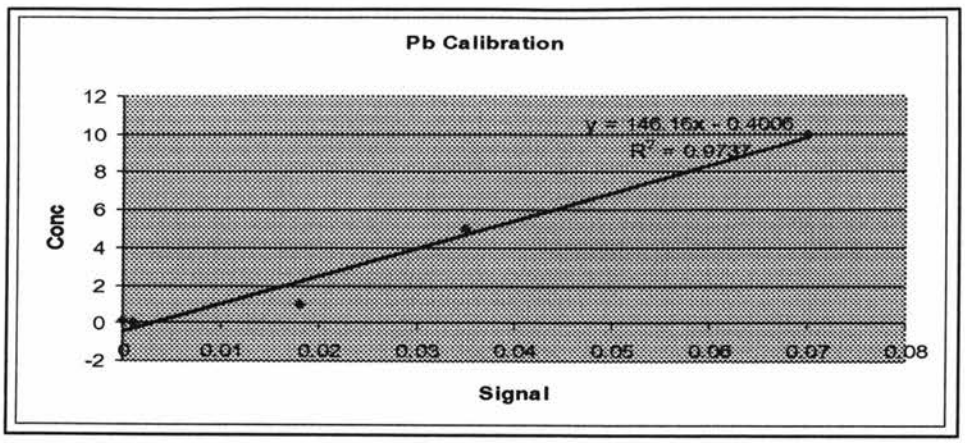
Ni
Less than 2

Jun-09

Ni
3.68
3.67
3.66
3.66
3.65

Summary Statistics	
Mean	3.664
Standard Error	0.005099
Median	3.66
Standard Deviation	0.011402
Sample Variance	0.00013
Range	0.03
Minimum	3.65
Maximum	3.68
Sum	18.32
Count	5
Largest(1)	3.68
Smallest(1)	3.65
Confidence Level(95.0	0.014157

Heavy Metals



*** The concentration of Pb is less than 2 ppm for the months of Sep 08, and Nov 08.**

Heavy Metals

Mar-09

Pb
62.25
62.25
62.25
63.2
62.25

Summary Statistics	
Mean	62.4408
Standard Error	0.189801
Median	62.252
Standard Deviation	0.424407
Sample Variance	0.180121
Range	0.95
Minimum	62.25
Maximum	63.2
Sum	312.204
Count	5
Largest(1)	63.2
Smallest(1)	62.25
Confidence Level(95.0%)	0.526971

Apr-09

Pb
3.78
3.69
3.78
3.78
3.7

Summary Statistics	
Mean	3.7508
Standard Error	0.022835
Median	3.788
Standard Deviation	0.051061
Sample Variance	0.002607
Range	0.098
Minimum	3.69
Maximum	3.788
Sum	18.754
Count	5
Largest(1)	3.788
Smallest(1)	3.69
Confidence Level(95.0%)	0.0634

Heavy Metals

May-09

Pb
237.64
252.3
245.65
243.6
235.4

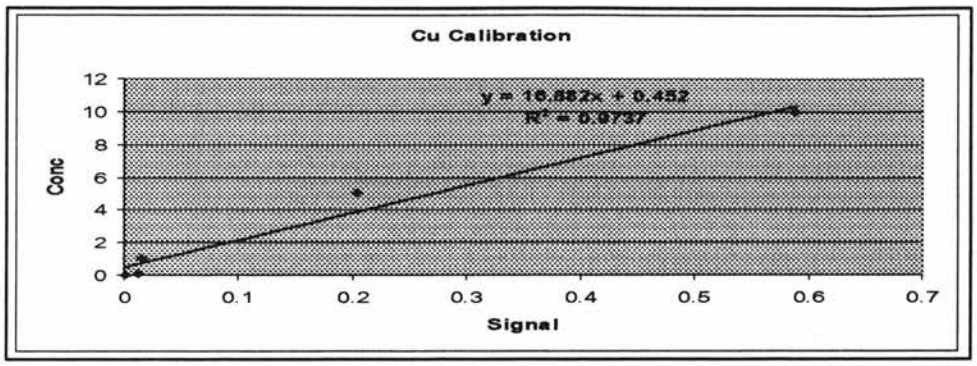
Summary Statistics	
Mean	242.9188
Standard Error	3.002385
Median	243.6
Standard Deviation	6.713536
Sample Variance	45.07157
Range	16.9
Minimum	235.4
Maximum	252.3
Sum	1214.594
Count	5
Largest(1)	252.3
Smallest(1)	235.4
Confidence Level(95.0%)	8.335956

Jun-09

Pb
18.06
15.69
16.87
15.69
16.87

Summary Statistics	
Mean	16.64092
Standard Error	0.444097
Median	16.8783
Standard Deviation	0.993032
Sample Variance	0.986112
Range	2.3738
Minimum	15.6914
Maximum	18.0652
Sum	83.2046
Count	5
Largest(1)	18.0652
Smallest(1)	15.6914
Confidence Level(95.0%)	1.233012

Heavy Metals



Sep-08

Cu
85.71
87.4
87.5
85.3
84.7

Summary Statistics	
Mean	86.12436
Standard Error	0.56599
Median	85.7168
Standard Deviation	1.265593
Sample Variance	1.601725
Range	2.8
Minimum	84.7
Maximum	87.5
Sum	430.6218
Count	5
Largest(1)	87.5
Smallest(1)	84.7
Confidence Level(95.0%)	1.571441

Nov-08

Cu
72.21
68.83
68.87
71.9
72.5

Summary Statistics	
Mean	70.8632
Standard Error	0.826391
Median	71.9
Standard Deviation	1.847865
Sample Variance	3.414606
Range	3.6652
Minimum	68.8348
Maximum	72.5
Sum	354.316
Count	5
Largest(1)	72.5
Smallest(1)	68.8348
Confidence Level(95.0%)	2.294428

Heavy Metals

Mar-09

Cu
154.93
170.12
171.8
178.56
178.54

Summary Statistics	
Mean	170.7935
Standard Error	4.321232
Median	171.8
Standard Deviation	9.662569
Sample Variance	93.36525
Range	23.6348
Minimum	154.933
Maximum	178.5678
Sum	853.9676
Count	5
Largest(1)	178.5678
Smallest(1)	154.933
Confidence Level(95.0%)	11.99766

Apr-09

Cu
100.91
97.53
99.22
87.4
98.7

Summary Statistics	
Mean	96.75444
Standard Error	2.399685
Median	98.7
Standard Deviation	5.365859
Sample Variance	28.79244
Range	13.5056
Minimum	87.405
Maximum	100.9106
Sum	483.7722
Count	5
Largest(1)	100.9106
Smallest(1)	87.405
Confidence Level(95.0%)	6.662593

Heavy Metals

May-09

Cu
303.49
297.87
295.05
296.5
297.8

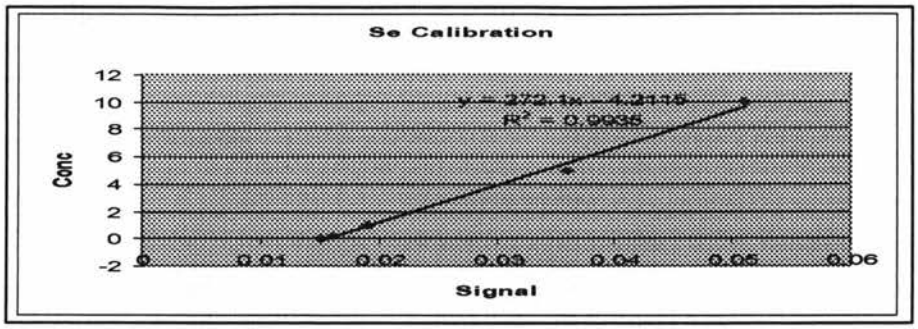
Summary Statistics	
Mean	298.1436
Standard Error	1.433102
Median	297.8
Standard Deviation	3.204514
Sample Variance	10.26891
Range	8.441
Minimum	295.0536
Maximum	303.4946
Sum	1490.718
Count	5
Largest(1)	303.4946
Smallest(1)	295.0536
Confidence Level(95.0%	3.97893

Jun-09

Cu
114.33
114.33
116.03
122.84
112.63

Summary Statistics	
Mean	116.036
Standard Error	1.785807
Median	114.3333
Standard Deviation	3.993185
Sample Variance	15.94553
Range	10.2162
Minimum	112.6306
Maximum	122.8468
Sum	580.18
Count	5
Largest(1)	122.8468
Smallest(1)	112.6306
Confidence Level(95.0%	4.958195

Heavy Metals



Sep-08

Se
Less than 2

Nov-08

Se
41.2
41.42
ND
ND
ND

Summary Statistics	
Mean	41.31
Standard Error	0.11
Median	41.31
Standard Deviation	0.155563
Sample Variance	0.0242
Range	0.22
Minimum	41.2
Maximum	41.42
Sum	82.62
Count	2
Largest(1)	41.42
Smallest(1)	41.2
Confidence Level(95.0%)	1.397683

Heavy Metals

Mar-09

Se
41.42
41.34
41.42
41.42
41.23

Summary Statistics	
Mean	41.366
Standard Error	0.037363
Median	41.42
Standard Deviation	0.083546
Sample Variance	0.00698
Range	0.19
Minimum	41.23
Maximum	41.42
Sum	206.83
Count	5
Largest(1)	41.42
Smallest(1)	41.23
Confidence Level(95.0%)	0.103737

Apr-09

Se
41.42
41.42
41.9
41.42
42.01

Summary Statistics	
Mean	41.632
Standard Error	0.130782
Median	41.42
Standard Deviation	0.292438
Sample Variance	0.08552
Range	0.58
Minimum	41.42
Maximum	42
Sum	208.16
Count	5
Largest(1)	42
Smallest(1)	41.42
Confidence Level(95.0%)	0.36311

Heavy Metals

May-09

Se
41.42
68.63
41.43
68.54
42.31

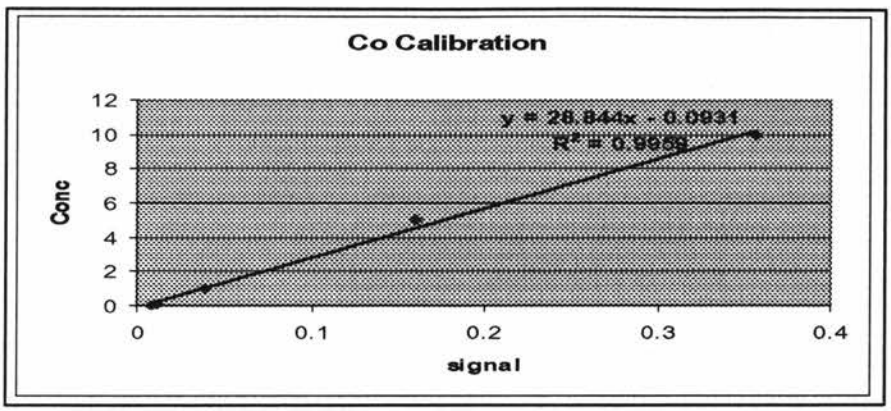
Summary Statistics	
Mean	52.466
Standard Error	6.582553
Median	42.31
Standard Deviation	14.71904
Sample Variance	216.65
Range	27.21
Minimum	41.42
Maximum	68.63
Sum	262.33
Count	5
Largest(1)	68.63
Smallest(1)	41.42
Confidence Level(95.(	18.2761

Jun-09

Se
50.18
50.18
77.6
91.31
91.31

Summary Statistics	
Mean	72.1216
Standard Error	9.299253
Median	77.606
Standard Deviation	20.79376
Sample Variance	432.3805
Range	41.133
Minimum	50.184
Maximum	91.317
Sum	360.608
Count	5
Largest(1)	91.317
Smallest(1)	50.184
Confidence Level(95.(	25.81886

Heavy Metals



*** The Concentration of Co is less than 2 ppm for the months of Sep 08, and Nov 08.**

Mar-09

Co
22.85
19.98
17.09
22.85
21.2

Summary Statistics	
Mean	20.798
Standard Error	1.074114728
Median	21.2
Standard Deviation	2.401793546
Sample Variance	5.76861224
Range	5.7688
Minimum	17.0908
Maximum	22.8596
Sum	103.99
Count	5
Largest(1)	22.8596
Smallest(1)	17.0908
Confidence Level(95.0%)	2.982220578

Apr-09

Co
2.66
2.66
2.72
2.67
2.41

Summary Statistics	
Mean	2.62752
Standard Error	0.055263065
Median	2.6688
Standard Deviation	0.123571971
Sample Variance	0.015270032
Range	0.31
Minimum	2.41
Maximum	2.72
Sum	13.1376
Count	5
Largest(1)	2.72
Smallest(1)	2.41
Confidence Level(95.0%)	0.153434867

Heavy Metals

May-09

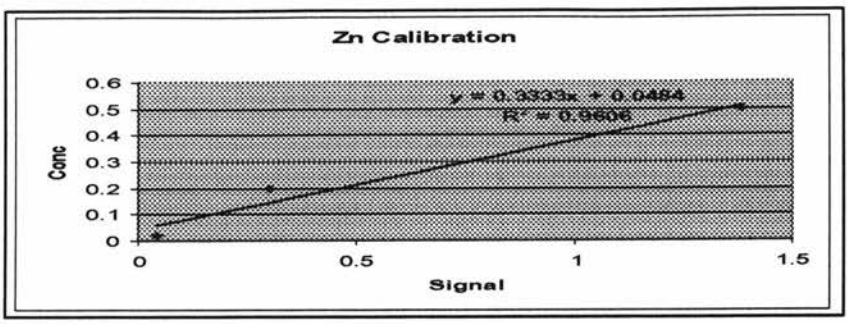
Co
2.66
2.67
2.66
2.67
2.69

Summary Statistics	
Mean	2.67
Standard Error	0.005477
Median	2.67
Standard Deviation	0.012247
Sample Variance	0.00015
Range	0.03
Minimum	2.66
Maximum	2.69
Sum	13.35
Count	5
Largest(1)	2.69
Smallest(1)	2.66
Confidence Level(95.0%	0.015207

Jun-09

Co
Less than 2

Heavy Metals



Sep-08

Zn
408.14
622.8
586.8
659.8
598.6

Summary Statistics	
Mean	575.228
Standard Error	43.59706
Median	598.6
Standard Deviation	97.486
Sample Variance	9503.52
Range	251.66
Minimum	408.14
Maximum	659.8
Sum	2876.14
Count	5
Largest(1)	659.8
Smallest(1)	408.14

Nov-08

Zn
21.15
21.15
17.18
21.23
20.91

Summary Statistics	
Mean	20.32833
Standard Error	0.787237
Median	21.1574
Standard Deviation	1.760315
Sample Variance	3.098708
Range	4.04313
Minimum	17.18687
Maximum	21.23
Sum	101.6417
Count	5
Largest(1)	21.23
Smallest(1)	17.18687
Confidence Level(95.0%)	2.18572

Heavy Metals

Mar-09

Zn
7.82
6.52
9.15
8.05
7.68

Summary Statistics	
Mean	7.849648
Standard Error	0.420697
Median	7.8254
Standard Deviation	0.940707
Sample Variance	0.88493
Range	2.63307
Minimum	6.52553
Maximum	9.1586
Sum	39.24824
Count	5
Largest(1)	9.1586
Smallest(1)	6.52553
Confidence Level(95.0%)	1.168042

Apr-09

Zn
1.25
1.85
1.75
2.22
2.1

Summary Statistics	
Mean	1.840804
Standard Error	0.167414
Median	1.85933
Standard Deviation	0.374349
Sample Variance	0.140137
Range	0.96657
Minimum	1.25939
Maximum	2.22596
Sum	9.20402
Count	5
Largest(1)	2.22596
Smallest(1)	1.25939
Confidence Level(95.0%)	0.464816

Heavy Metals

May-09

Zn
27.82
25.15
25.15
26.5
27.1

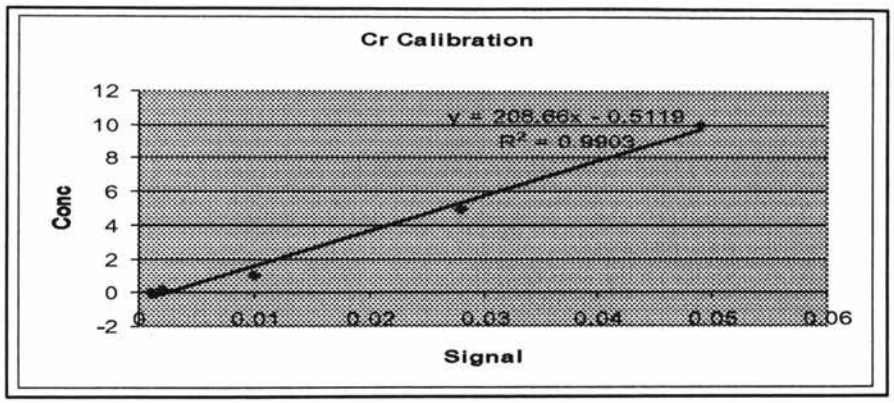
Summary Statistics	
Mean	26.34748
Standard Error	0.529262
Median	26.5
Standard Deviation	1.183467
Sample Variance	1.400593
Range	2.6664
Minimum	25.157
Maximum	27.8234
Sum	131.7374
Count	5
Largest(1)	27.8234
Smallest(1)	25.157
Confidence Level(95.0%)	1.469468

Jun-09

Zn
13.56
18.71
16.46
16.14
15.57

Summary statistics	
Mean	16.09389
Standard Error	0.826055
Median	16.14006
Standard Deviation	1.847116
Sample Variance	3.411836
Range	5.14488
Minimum	13.56762
Maximum	18.7125
Sum	80.46944
Count	5
Largest(1)	18.7125
Smallest(1)	13.56762
Confidence Level(95.0%)	2.293497

Heavy Metals



Sep-08

Cr
Less than 2

Nov-08

Cr
Less than 2

Mar-09

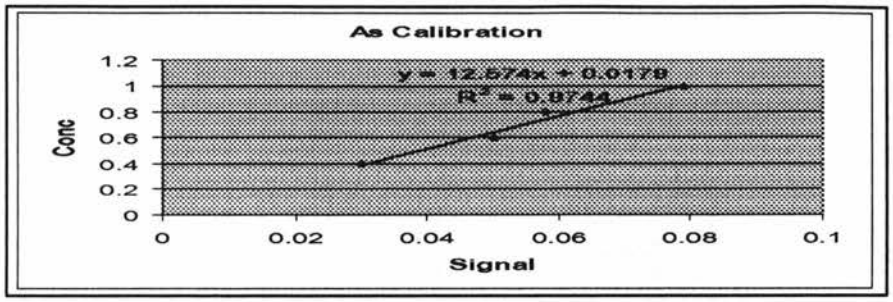
Cr
32.27
32.27
33.4
32.27
32.76

Summary Statistics	
Mean	32.5964
Standard Error	0.221852
Median	32.274
Standard Deviation	0.496075
Sample Variance	0.246091
Range	1.126
Minimum	32.274
Maximum	33.4
Sum	162.982
Count	5
Largest(1)	33.4
Smallest(1)	32.274
Confidence Level(95.0%	0.615959

Apr-09

Cr
Less than 2

Heavy Metals



Sep-08

As
29.4
24.2
29.4
25.8
33.1

Summary Statistics	
Mean	28.38
Standard Error	1.557048
Median	29.4
Standard Deviation	3.481666
Sample Variance	12.122
Range	8.9
Minimum	24.2
Maximum	33.1
Sum	141.9
Count	5
Largest(1)	33.1
Smallest(1)	24.2
Confidence Level(95.0%)	4.32306

Nov-08

As
34.5
30.7
24.4
39.5
29.4

Summary Statistics	
Mean	31.7
Standard Error	2.53239
Median	30.7
Standard Deviation	5.662597
Sample Variance	32.065
Range	15.1
Minimum	24.4
Maximum	39.5
Sum	158.5
Count	5
Largest(1)	39.5
Smallest(1)	24.4
Confidence Level(95.0%)	7.031042

Heavy Metals

Mar-09

As
34.2
30.71
32.1
34.48
31.23

Summary Statistics	
Mean	32.54452
Standard Error	0.767668
Median	32.1
Standard Deviation	1.716558
Sample Variance	2.946571
Range	3.7722
Minimum	30.7102
Maximum	34.4824
Sum	162.7226
Count	5
Largest(1)	34.4824
Smallest(1)	30.7102
Confidence Level(95.0%)	2.131388

Apr-09

As
21.9
23.16
21.9
24.42
21.78

Summary Statistics	
Mean	22.63716
Standard Error	0.513143
Median	21.9084
Standard Deviation	1.147422
Sample Variance	1.316576
Range	2.6432
Minimum	21.78
Maximum	24.4232
Sum	113.1858
Count	5
Largest(1)	24.4232
Smallest(1)	21.78
Confidence Level(95.0%)	1.424712

Heavy Metals

May-09

As
26.93
26.78
26.93
26.5
28.4

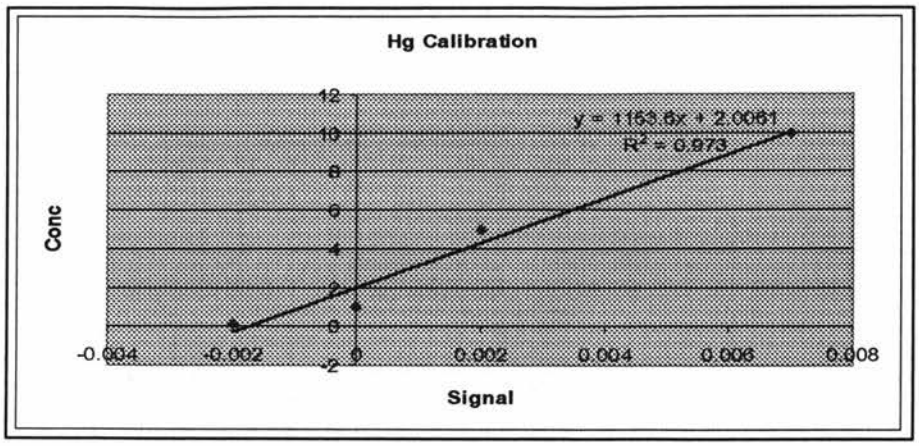
Summary Statistics	
Mean	27.1112
Standard Error	0.331983
Median	26.938
Standard Deviation	0.742338
Sample Variance	0.551065
Range	1.9
Minimum	26.5
Maximum	28.4
Sum	135.556
Count	5
Largest(1)	28.4
Smallest(1)	26.5
Confidence Level(95.0%	0.921734

Jun-09

As
35.4
30.71
39.51
33.25
36.99

Summary Statistics	
Mean	35.16888
Standard Error	1.515212
Median	35.4
Standard Deviation	3.388117
Sample Variance	11.47934
Range	8.8018
Minimum	30.7102
Maximum	39.512
Sum	175.8444
Count	5
Largest(1)	39.512
Smallest(1)	30.7102
Confidence Level(95.0%	4.206903

Heavy Metals



*** The concentration of Hg is less than 2 ppm for the months of Sep 08, Nov 08**

Heavy Metals

May-09

Hg
Less than 2

Jun-09

Hg
18.71
16.46
16.14
15.57
16.5

Summary Statistics	
Mean	16.68036
Standard Error	0.534344
Median	16.46986
Standard Deviation	1.194829
Sample Variance	1.427617
Range	3.1331
Minimum	15.5794
Maximum	18.7125
Sum	83.40182
Count	5
Largest(1)	18.7125
Smallest(1)	15.5794
Confidence Level(95.	1.483577

Appendix B

Lignin

Sep-08

Lignin
26.03%
26.87%
26.70%
25.70%
20.65%
24.30%
34.20%
21.20%
27.01%
25.90%

Sammary Statistics	
Mean	0.25856
Standard Error	0.011736
Median	0.25965
Standard Deviation	0.037111
Sample Variance	0.001377
Range	0.1355
Minimum	0.2065
Maximum	0.342
Sum	2.5856
Count	10
Largest(1)	0.342
Smallest(1)	0.2065
Confidence Level(95.0%)	0.026548

Nov-08

Lignin
22.12%
22.90%
20.98%
19.76%
21.01%
17.87%
19.76%
24.02%
21.80%
24.96%

Summary Statistics	
Mean	0.21518
Standard Error	0.006708
Median	0.21405
Standard Deviation	0.021212
Sample Variance	0.00045
Range	0.0709
Minimum	0.1787
Maximum	0.2496
Sum	2.1518
Count	10
Largest(1)	0.2496
Smallest(1)	0.1787
Confidence Level(95.0%)	0.015174

Lignin

Mar-09

Lignin
18.51%
18.51%
22.22%
18.51%
18.51%
18.99%
16.12%
21.01%
19.01%
18.19%

Summary Statistics	
Mean	0.18958
Standard Error	0.00519636
Median	0.1851
Standard Deviation	0.01643234
Sample Variance	0.00027002
Range	0.061
Minimum	0.1612
Maximum	0.2222
Sum	1.8958
Count	10
Largest(1)	0.2222
Smallest(1)	0.1612
Confidence Level(95.0%)	0.01175499

Apr-09

Lignin
29.62%
30.03%
29.62%
30.33%
29.62%
29.62%
28.01%
31.01%
29.89%
30.09%

Summary Statistics	
Mean	0.29784
Standard Error	0.0024086
Median	0.29755
Standard Deviation	0.00761668
Sample Variance	5.8014E-05
Range	0.03
Minimum	0.2801
Maximum	0.3101
Sum	2.9784
Count	10
Largest(1)	0.3101
Smallest(1)	0.2801
Confidence Level(95.0%)	0.00544864

Lignin

May-09

Lignin
18.51%
22.22%
22.22%
25.92%
22.22%
18.51%
18.70%
22.32%
21.38%
22.12%

Summary Statistics	
Mean	0.21412
Standard Error	0.007288
Median	0.2217
Standard Deviation	0.023047
Sample Variance	0.000531
Range	0.0741
Minimum	0.1851
Maximum	0.2592
Sum	2.1412
Count	10
Largest(1)	0.2592
Smallest(1)	0.1851
Confidence Level(95.0%)	0.016487

Jun-09

Lignin
22.02%
22.12%
22.01%
22.65%
22.10%
21.90%
20.56%
22.03%
22.10%
22.54%

Summary Statistics	
Mean	0.22003
Standard Error	0.001774
Median	0.22065
Standard Deviation	0.005611
Sample Variance	3.15E-05
Range	0.0209
Minimum	0.2056
Maximum	0.2265
Sum	2.2003
Count	10
Largest(1)	0.2265
Smallest(1)	0.2056
Confidence Level(95.0%)	0.004014

Appendix C

Moisture and VOC

Sep-08

Moisture
52.25%
49.03%
51.55%
52.99%
48.95%
52.84%
50.84%
51.79%
56.30%
53.27%

Summary Statistics	
Mean	0.51981
Standard Error	0.006796
Median	0.5202
Standard Deviation	0.02149
Sample Variance	0.000462
Range	0.0735
Minimum	0.4895
Maximum	0.563
Sum	5.1981
Count	10
Largest(1)	0.563
Smallest(1)	0.4895
Confidence Level(95.0%)	0.015373

Nov-08

Moisture
53.09%
53.90%
52.52%
52.80%
52.05%
51.83%
51.56%
54.32%
51.12%
52.21%

Summary Statistics	
Mean	0.5254
Standard Error	0.0032
Median	0.52365
Standard Deviation	0.010121
Sample Variance	0.000102
Range	0.032
Minimum	0.5112
Maximum	0.5432
Sum	5.254
Count	10
Largest(1)	0.5432
Smallest(1)	0.5112
Confidence Level(95.0%)	0.00724

Moisture and VOC

Sep-08

VOC
86.20%
82.29%
81.38%
82.87%
79.26%
79.20%
81.20%
82.30%
86.10%
82.70%

Summary Statistics	
Mean	0.8235
Standard Error	0.007529
Median	0.82295
Standard Deviation	0.023809
Sample Variance	0.000567
Range	0.07
Minimum	0.792
Maximum	0.862
Sum	8.235
Count	10
Largest(1)	0.862
Smallest(1)	0.792
Confidence Level(95.0%)	0.017032

Nov-08

VOC
91.39%
89.20%
90.39%
90.39%
89.79%
89.83%
89.72%
89.71%
90.61%
90.40%

Summary Statistics	
Mean	0.90143
Standard Error	0.001955
Median	0.9011
Standard Deviation	0.006182
Sample Variance	3.82E-05
Range	0.0219
Minimum	0.892
Maximum	0.9139
Sum	9.0143
Count	10
Largest(1)	0.9139
Smallest(1)	0.892
Confidence Level(95.0%)	0.004422

Moisture and VOC

Mar-09

Moisture
64.86%
56.41%
59.27%
61.77%
63.17%
60.62%
59.35%
63.96%
60.71%
59.17%

Summary Statistics	
Mean	0.60929
Standard Error	0.008115
Median	0.60665
Standard Deviation	0.025663
Sample Variance	0.000659
Range	0.0845
Minimum	0.5641
Maximum	0.6486
Sum	6.0929
Count	10
Largest(1)	0.6486
Smallest(1)	0.5641
Confidence Level(95.0%)	0.018358

Apr-09

Miosture
57.65%
58.08%
61.38%
56.70%
57.60%
58.10%
57.05%
56.30%
56.10%
55.10%

Summary Statistics	
Mean	0.57406
Standard Error	0.005343
Median	0.57325
Standard Deviation	0.016898
Sample Variance	0.000286
Range	0.0628
Minimum	0.551
Maximum	0.6138
Sum	5.7406
Count	10
Largest(1)	0.6138
Smallest(1)	0.551
Confidence Level(95.0%)	0.012088

Moisture and VOC

Mar-09

VOC
67.40%
63.40%
65.40%
65.20%
68.90%
65.01%
60.80%
63.40%
66.50%
65.40%

Summary Statistic	
Mean	0.65141
Standard Error	0.00716
Median	0.653
Standard Deviation	0.022642
Sample Variance	0.000513
Range	0.081
Minimum	0.608
Maximum	0.689
Sum	6.5141
Count	10
Largest(1)	0.689
Smallest(1)	0.608
Confidence Level(95.0%)	0.016197

Apr-09

VOC
93.46%
90.36%
92.00%
92.10%
90.70%
93.70%
93.45%
92.10%
90.32%
91.20%

Summary Statistics	
Mean	0.91939
Standard Error	0.004076
Median	0.9205
Standard Deviation	0.012888
Sample Variance	0.000166
Range	0.0338
Minimum	0.9032
Maximum	0.937
Sum	9.1939
Count	10
Largest(1)	0.937
Smallest(1)	0.9032
Confidence Level(95.0%)	0.009219

Moisture and VOC

May-09

Moisture
56.99%
56.87%
57.87%
52.39%
55.97%
56.02%
55.70%
56.01%
55.89%
55.40%

Summary Statistics	
Mean	0.55911
Standard Error	0.004552
Median	0.5599
Standard Deviation	0.014395
Sample Variance	0.000207
Range	0.0548
Minimum	0.5239
Maximum	0.5787
Sum	5.5911
Count	10
Largest(1)	0.5787
Smallest(1)	0.5239
Confidence Level(95.0%)	0.010298

Jun-09

Miosture
56.58%
56.58%
56.82%
54.81%
56.59%
56.70%
55.40%
54.30%
56.60%
56.10%

Summary Statistics	
Mean	0.56048
Standard Error	0.002828
Median	0.5658
Standard Deviation	0.008942
Sample Variance	8E-05
Range	0.0252
Minimum	0.543
Maximum	0.5682
Sum	5.6048
Count	10
Largest(1)	0.5682
Smallest(1)	0.543
Confidence Level(95.0%)	0.006397

Moisture and VOC

May-09

VOC
97.12%
96.46%
96.14%
96.06%
93.57%
97.10%
97.60%
95.40%
90.43%
97.11%

Summary Statitics	
Mean	0.95699
Standard Error	0.006902
Median	0.963
Standard Deviation	0.021826
Sample Variance	0.000476
Range	0.0717
Minimum	0.9043
Maximum	0.976
Sum	9.5699
Count	10
Largest(1)	0.976
Smallest(1)	0.9043
Confidence Level(95.0%)	0.015614

Jun-09

VOC
91.90%
90.16%
90.92%
92.54%
93.10%
92.10%
93.20%
90.20%
90.89%
91.80%

Summary Statistics	
Mean	0.91681
Standard Error	0.003497
Median	0.9185
Standard Deviation	0.011059
Sample Variance	0.000122
Range	0.0304
Minimum	0.9016
Maximum	0.932
Sum	9.1681
Count	10
Largest(1)	0.932
Smallest(1)	0.9016
Confidence Level(95.0%)	0.007911

Appendix D

Sep-08

Glucose
28.70%
29.93%
26.49%
25.68%
21.82%
29.63%
25.34%
21.41%
25.79%
23.60%

Summary Statistics	
Mean	0.25839
Standard Error	0.009474
Median	0.25735
Standard Deviation	0.029958
Sample Variance	0.000898
Range	0.0852
Minimum	0.2141
Maximum	0.2993
Sum	2.5839
Count	10
Largest(1)	0.2993
Smallest(1)	0.2141
Confidence Level(95.0%)	0.021431

Nov-08

Glucose
32.90%
38.83%
35.22%
37.50%
35.40%
39.34%
41.16%
39.87%
41.81%
39.94%

Summary Statistics	
Mean	0.38197
Standard Error	0.009105
Median	0.39085
Standard Deviation	0.028792
Sample Variance	0.000829
Range	0.0891
Minimum	0.329
Maximum	0.4181
Sum	3.8197
Count	10
Largest(1)	0.4181
Smallest(1)	0.329
Confidence Level(95.0%)	0.020596

Sep-08

Xylose
19.08%
20.02%
11.54%
21.78%
20.93%
21.02%
21.17%
21.55%
21.67%
19.02%

Summary Statistics	
Mean	0.19778
Standard Error	0.009691
Median	0.20975
Standard Deviation	0.030645
Sample Variance	0.000939
Range	0.1024
Minimum	0.1154
Maximum	0.2178
Sum	1.9778
Count	10
Largest(1)	0.2178
Smallest(1)	0.1154
Confidence Level(95.0%)	0.021922

Nov-08

Xylose
12.40%
10.50%
8.60%
9.90%
11.40%
9.90%
8.60%
12.10%
11.10%
10.90%

Summary Statistics	
Mean	0.1054
Standard Error	0.00414
Median	0.107
Standard Deviation	0.013091
Sample Variance	0.000171
Range	0.038
Minimum	0.086
Maximum	0.124
Sum	1.054
Count	10
Largest(1)	0.124
Smallest(1)	0.086
Confidence Level(95.0%)	0.009365

Sep-08

Mannose
1.60%
1.32%
1.65%
Less than 3

Summary Statistics	
Mean	0.015233
Standard Error	0.001027
Median	0.016
Standard Deviation	0.001779
Sample Variance	3.16E-06
Range	0.0033
Minimum	0.0132
Maximum	0.0165
Sum	0.0457
Count	3
Largest(1)	0.0165
Smallest(1)	0.0132
Confidence Level(95.0%)	0.004418

Nov-08

Mannose
7.99%
10.15%
6.63%
9.50%
9.09%
8.10%
6.67%
7.80%
6.87%
7.10%

Summary Statistics	
Mean	0.0799
Standard Error	0.003922
Median	0.07895
Standard Deviation	0.012402
Sample Variance	0.000154
Range	0.0352
Minimum	0.0663
Maximum	0.1015
Sum	0.799
Count	10
Largest(1)	0.1015
Smallest(1)	0.0663
Confidence Level(95.0%)	0.008872

Mar-09

Glucose
31.58%
28.72%
29.43%
31%
31.82%
29.80%
31.35%
30.54%
31.32%
30.54%

Summary Statistics	
Mean	0.3061
Standard Error	0.003204
Median	0.3077
Standard Deviation	0.010132
Sample Variance	0.000103
Range	0.031
Minimum	0.2872
Maximum	0.3182
Sum	3.061
Count	10
Largest(1)	0.3182
Smallest(1)	0.2872
Confidence Level(95.0%)	0.007248

Apr-09

Glucose
24.26%
27.47%
22.64%
22.64%
26.70%
35.29%
28.60%
28.31%
28.37%
28.96%

Summary Statistics	
Mean	0.27324
Standard Error	0.011715
Median	0.2789
Standard Deviation	0.037045
Sample Variance	0.001372
Range	0.1265
Minimum	0.2264
Maximum	0.3529
Sum	2.7324
Count	10
Largest(1)	0.3529
Smallest(1)	0.2264
Confidence Level(95.0%)	0.0265

Mar-09

Xylose
21.70%
22.35%
21.96%
21.96%
21.96%
21.96%
21.96%
21.96%
21.96%
21.96%

Summary Statistics	
Mean	0.21973
Standard Error	0.000492
Median	0.2196
Standard Deviation	0.001556
Sample Variance	2.42E-06
Range	0.0065
Minimum	0.217
Maximum	0.2235
Sum	2.1973
Count	10
Largest(1)	0.2235
Smallest(1)	0.217
Confidence Level(95.0%)	0.001113

Apr-09

Xylose
14.74%
17.37%
21.31%
16.90%
18.01%
21.84%
19.14%
17.80%
13.09%
13.09%

Summary Statistics	
Mean	0.17329
Standard Error	0.009603
Median	0.17585
Standard Deviation	0.030366
Sample Variance	0.000922
Range	0.0875
Minimum	0.1309
Maximum	0.2184
Sum	1.7329
Count	10
Largest(1)	0.2184
Smallest(1)	0.1309
Confidence Level(95.0%)	0.021723

Mar-09

Mannose
2.70%
2.80%
2.60%
2.50%
3.80%
2.64%
3.65%
3.55%
2.52%
3.55%

Summary Statistics	
Mean	0.03031
Standard Error	0.001686
Median	0.0275
Standard Deviation	0.005331
Sample Variance	2.84E-05
Range	0.013
Minimum	0.025
Maximum	0.038
Sum	0.3031
Count	10
Largest(1)	0.038
Smallest(1)	0.025
Confidence Level(95.0%	0.003814

Apr-09

Mannose
8.05%
10.30%
11.40%
11.03%
11.97%
4.80%
8.10%
9.94%
11.45%
9.64%

Summary Statistics	
Mean	0.09668
Standard Error	0.006878
Median	0.1012
Standard Deviation	0.021751
Sample Variance	0.000473
Range	0.0717
Minimum	0.048
Maximum	0.1197
Sum	0.9668
Count	10
Largest(1)	0.1197
Smallest(1)	0.048
Confidence Level(95.0%	0.015559

May-09

Glucose
38.66%
39.74%
31.70%
30.77%
38.66%
38.30%
40.67%
26.30%
34.67%
29.34%

Summary Statistics	
Mean	0.34881
Standard Error	0.015943
Median	0.36485
Standard Deviation	0.050418
Sample Variance	0.002542
Range	0.1437
Minimum	0.263
Maximum	0.4067
Sum	3.4881
Count	10
Largest(1)	0.4067
Smallest(1)	0.263
Confidence Level(95.0%)	0.036067

Jun-09

Glucose
30.49%
24.11%
24.55%
24.11%
29.33%
25.88%
29.74%
22.66%
30.62%
29.81%

Summary Statistics	
Mean	0.2713
Standard Error	0.00993
Median	0.27605
Standard Deviation	0.031401
Sample Variance	0.000986
Range	0.0796
Minimum	0.2266
Maximum	0.3062
Sum	2.713
Count	10
Largest(1)	0.3062
Smallest(1)	0.2266
Confidence Level(95.0%)	0.022463

May-09

Xylose
26.86%
27.15%
29.72%
31.87%
31.51%
33.33%
29.21%
22.94%
21.95%
23.22%

Summary Statistics	
Mean	0.27776
Standard Error	0.012767
Median	0.2818
Standard Deviation	0.040374
Sample Variance	0.00163
Range	0.1138
Minimum	0.2195
Maximum	0.3333
Sum	2.7776
Count	10
Largest(1)	0.3333
Smallest(1)	0.2195
Confidence Level(95.0%)	0.028882

Jun-09

Xylose
21.31%
15.50%
14.60%
16.93%
16.93%
21.57%
15.80%
16.98%
17.60%
18.30%

Summary Statistics	
Mean	0.17552
Standard Error	0.007297
Median	0.16955
Standard Deviation	0.023076
Sample Variance	0.000532
Range	0.0697
Minimum	0.146
Maximum	0.2157
Sum	1.7552
Count	10
Largest(1)	0.2157
Smallest(1)	0.146
Confidence Level(95.0%)	0.016507

May-09

Mannose
5.06%
4.35%
4.23%

Summary Statistics	
Mean	0.045467
Standard Error	0.00259
Median	0.0435
Standard Deviation	0.004486
Sample Variance	2.01E-05
Range	0.0083
Minimum	0.0423
Maximum	0.0506
Sum	0.1364
Count	3
Largest(1)	0.0506
Smallest(1)	0.0423
Confidence Level(95.0%)	0.011144

Jun-09

Mannose
2.89%
2.06%
3.96%
6.08%
3.37%
6.11%
3.45%
2.98%
3.34%
2.34%

Summary Statistics	
Mean	0.03658
Standard Error	0.004414
Median	0.03355
Standard Deviation	0.013959
Sample Variance	0.000195
Range	0.0405
Minimum	0.0206
Maximum	0.0611
Sum	0.3658
Count	10
Largest(1)	0.0611
Smallest(1)	0.0206
Confidence Level(95.0%)	0.009986

May-09

Galactose
1.76%
3.23%
1.53%
1.22%
1.54%
3.20%
1.78%
1.98%
1.23%
1.96%

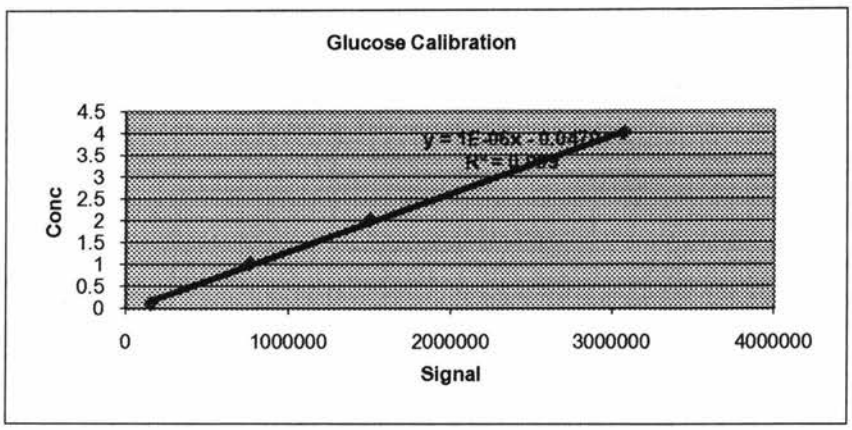
Summary Statistics	
Mean	0.01943
Standard Error	0.002276
Median	0.0177
Standard Deviation	0.007197
Sample Variance	5.18E-05
Range	0.0201
Minimum	0.0122
Maximum	0.0323
Sum	0.1943
Count	10
Largest(1)	0.0323
Smallest(1)	0.0122
Confidence Level(95.0%)	0.005149

Jun-09

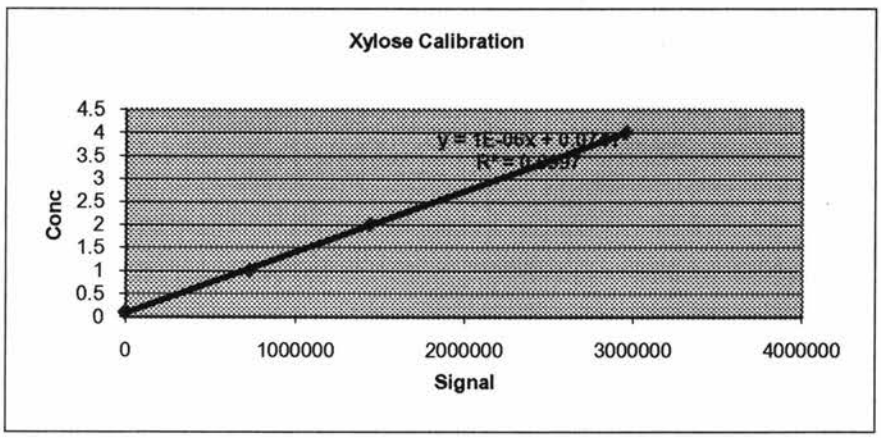
Galactose
Less than 3

Calibration Curves

STD NO	Conc (g/l)	Peack
1	4	3073760
2	2	1501697
3	1	761664.6
4	0.1	155353.4

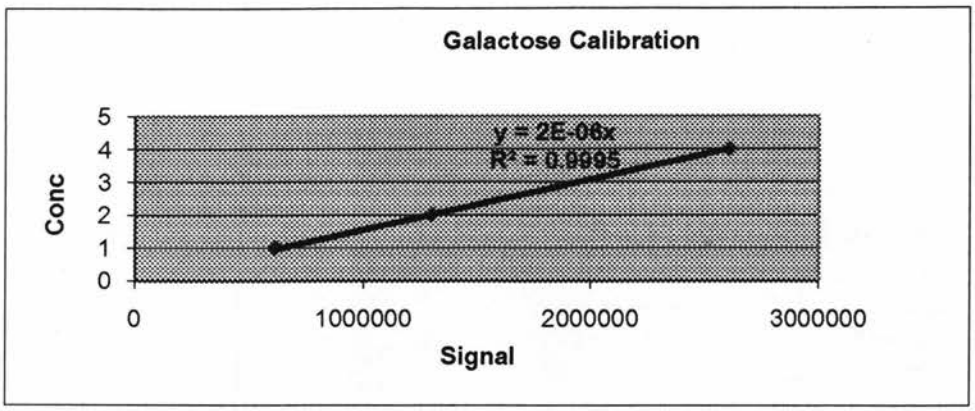


STD NO	Conc (g/l)	Peack
1	4	2957912
2	2	1441113
3	1	728847
4	0.1	2040.36

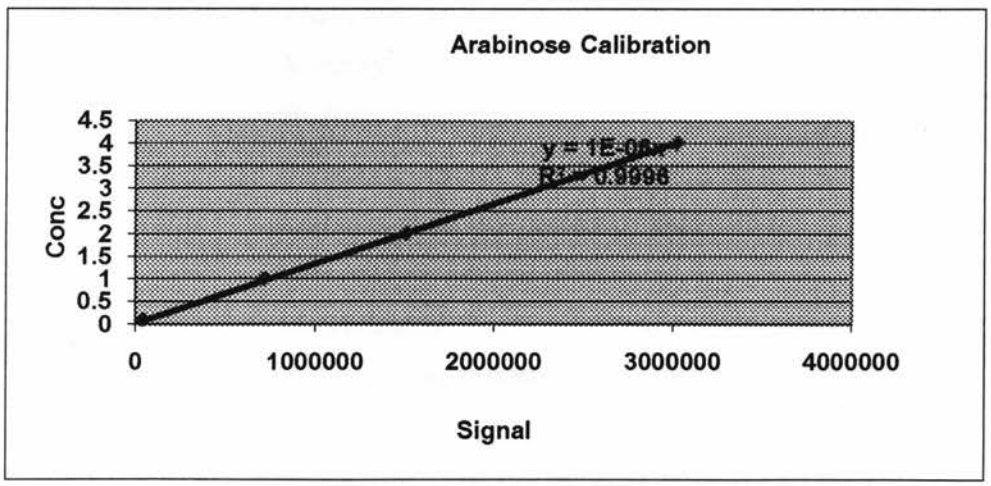


Calibration Curves

STD NO	Conc (g/l)	Peack
1	4	2610473
2	2	1303325
3	1	618985.3
4	0.1	78158.8

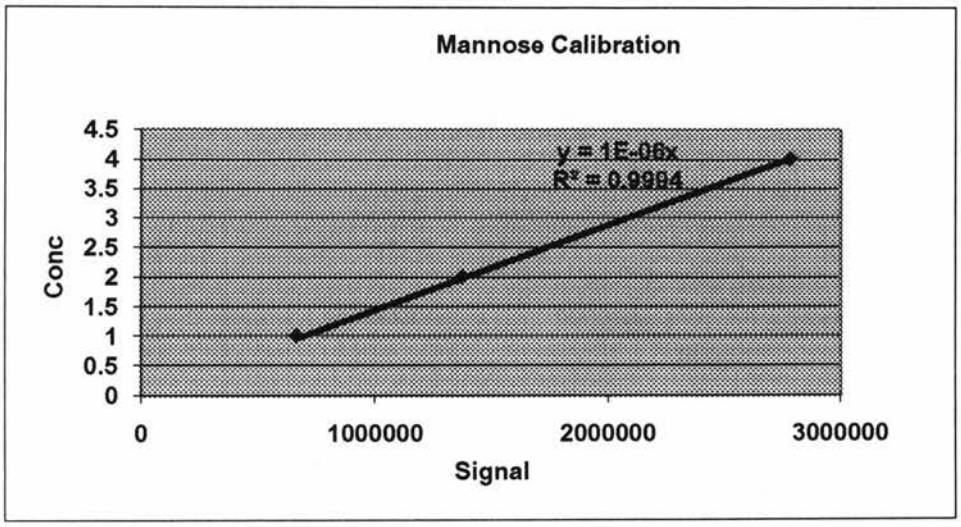


STD NO	Conc (g/l)	Peack
1	4	3023437
2	2	1514969
3	1	723206
4	0.1	45716.2



Calibration Curves

STD NO	Conc (g/l)	Peack
1	4	2785808
2	2	1374447
3	1	662269.2



Glossary

AFEX	Ammonia fibre explosion
AIS	Acid insoluble lignin
As	Arsenic
ASTM	American Society for Testing and Materials
ATS	Aufberestungs technology and system thermal screw machine
CBP	Consolidated bio-processing
CCME	Canadian Council of the Minister of the Environment
Cd	Cadmium
CD	Construction and demolition
Co	Cobalt
COD	Chemical oxygen demand
Cr	Chromium
Cu	Copper
DMF	Direct microbial fermentation
DP	Degree of polymerization
EPA	U.S. Environmental Protection Agency
FLAA	Flame ionization atomic absorption
Hg	Mercury
HMF	Hydroxy methylfurfural
HPLC	High performance liquid chromatography
L%	Lignin content as a percent of total solids
LAP	Laboratory analytical procedure
Mo	Molybdenum
MSW	Municipal solid waste
Mt	Mega tonne
Ni	Nickel
NREL	National Renewable Energy Laboratory
OCE	Ontario Center of Excellence

OFMSW	Organic fraction of municipal solid waste
Pb	Lead
PFD	Process flow diagram
RI	Refractive Index
Se	Selenium
SHF	Separate hydrolysis and fermentation
SSCF	Simultaneous saccharification and co-fermentation
SSF	Simultaneous saccharification and fermentation
SSO	Source separated organic waste
STEX	Steam explosion
TAPPI	Technical Association of the Pulp and Paper Industry
TKN	Total Kjeldahl nitrogen
TS	Total solids
UV	Ultraviolet
VOC	Volatile organic compounds
VS%	Volatile solids as percent of total solids
Zn	Zinc