5-2010

Anaerobic Fermentation of Hemicellulose Present in Green Liquor and Hot Water Extracts to Carboxylic Acids

Rakhi Reddy Baddam

ANAEROBIC FERMENTATION OF HEMICELLULOSE PRESENT IN GREEN LIQUOR AND HOT WATER EXTRACTS TO CARBOXYLIC ACIDS.

By

Rakhi Reddy Baddam

B.Tech. Jawaharlal Nehru Technological University, 2006

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Biological Engineering)

The Graduate School

The University of Maine

May, 2010

Advisory Committee:

G. Peter van Walsum, Associate Professor of Chemical Engineering, Advisor

Adriaan R.P. van Heiningen, Professor of Chemical Engineering

Paul J. Millard, Associate Professor of Biological Engineering

THESIS

ACCEPTANCE STATEMENT

On behalf of the Graduate Committee for Rakhi Reddy Baddam, I affirm that this manuscript is the final and accepted thesis. Signatures of all committee members are on file with the Graduate School at the University of Maine, 42 Stodder Hall, Orono Maine.

Committee Chair's Signature:

Date:

LIBRARY RIGHTS STATEMENT

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at The University of Maine, I agree that the Library shall make it freely available for inspection. I further agree that permission for "fair use" copying this thesis for scholarly purposes may be granted by the Librarian. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature:

Date:

ANAEROBIC FERMENTATION OF HEMICELLULOSE PRESENT IN GREEN LIQUOR AND HOT WATER EXTRACTS TO CARBOXYLIC ACIDS.

By Rakhi Reddy Baddam

Thesis Advisor: G. Peter vanWalsum

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
(in Biological Engineering)
May, 2010

Wood is composed of cellulose, hemicellulose and lignin. In the paper industry the cellulose fraction is the major resource used in paper production, whereas the hemicellulose and the lignin are usually burned for heat recovery. Recently, wood-derived hemicellulose extracts have received much attention for the production of valuable bioproduct fuels and chemicals. Mixed-culture microbial ecosystems are capable of converting biomass materials, such as wood extracts, into mixtures of carboxylic acids (C_1-C_7), which can in turn be purified and sold as products, or converted into other organic chemicals through chemical means. The relative concentrations of the acids produced in the fermentations vary depending upon the type of extracts used and also on the microbial communities employed, such as those growing at mesophilic or thermophilic temperatures or different buffered pH levels .

In this study, we were looking for the maximum production of carboxylic acids at varying temperatures using mesophilic and the thermophilic microbes growing on green liquor and hot water extracts. Steps had to be taken to restrict the growth of methanogenic cultures, thereby inhibiting the production of methane and enabling higher carboxylic acid accumulation. The inhibition of methane was done by adding iodoform at low

concentrations or using ammonium bicarbonate as a buffer. The buffering agents calcium carbonate and ammonium bicarbonate were tested as alternate means of maintaining neutral pH during acidogenic growth on pure sugars. Results from the pure sugar fermentations led to applying ammonium bicarbonate as the buffer of choice for wood extracts fermentations. During fermentations, samples were collected at specific time intervals and the pH, off-gas volume, off-gas composition and total sugar and carboxylic acid contents were measured Analytical methods used included GC for determining gas composition and GC and HPLC for determining acid and sugar concentrations..

Results indicated that mixed microbial cultures were capable of converting glucose and xylose sugars and hydrolyzing oligomeric hemicellulose without addition of supplemental enzymes. Conversion yields of organic acids to carbohydrate ranged from 50 to 80%, with lactic acid dominating in lower pH fermentations and acetic acid dominating in fermentations at closer to neutral pH. Methane production in all cases was detected at very low levels compared to CO_2 production rates.

With the benefits of autohydrolysis, high product yields and low operating costs due to the non-aseptic fermentation, conversion of aqueous wood extracts to carboxylic acids may be an economically attractive method of adding value to these extracts.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor Dr. Peter van Walsum for his continuous support and encouragement throughout my research work.

In addition, I would also like to take the opportunity to thank the members of my thesis committee, Dr. Adriaan van Heiningen and Dr. Paul Millard for accepting to spend their time and energy for the benefit of my thesis.

Special thanks to the fellow group members past and present for their valuable feedback on my work.

I would also like to thank the faculty, staff and students of the Department of Chemical and Biological Engineering, the Pulp and Paper Process Development Centre and the Forest Bioproducts Research Initiative (FBRI) for their support in various ways.

This work could not be completed without any funds. So I would like to thank National Science Foundation (EPSCoR Grant # 0554545) and by the Department of Energy (MIXALCO Grant # DE-FG36-08GO18165) for providing financial support to carry out this part of a research project.

I would like to thank my friends and colleagues for their continuous help and support during this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

The importance of using renewable resources to produce high end products that can replace non-renewable resources has become apparent in recent years. This is due to the use of non-renewable resources to satisfy our present energy needs, thereby leading to anticipated reduction of these resources over the next few decades. Use of fossil fuels also leads to a lot of environmental problems. To overcome these problems scientists and researchers are performing extensive research looking for other resources to replace the existing nonrenewable sources.

1.1. Background

There are various renewable resources that are available to replace our present energy sources, including food crops, wind energy, biomass and so on. The use of food crops has a high impact on the economy since the use of these for energy production has the tendency to increase food prices and reduce their availability as food for the community. An alternative and easily available resource is biomass. The most abundant available biomass is lignocellulose, and as such it is the best resource for the production of fuels and chemicals (Binder et al, 2008). Plant biomass, with abundant lignocellulose, was the source for our energy demands before the discovery of fossil fuels (Huber et al, 2006). There is increasing interest in moving our focus towards forest resources as a source of biomass for fuel. One means of doing this is to co-produce biofuels and bioproducts with existing wood-based industries, such as pulp and paper. Developing an integrated forest products bio-refinery alongside the pulp and paper industry will leverage

existing infrastructure for collection of raw material and processing of biomass. (vanHeiningen, 2006).

1.1.1. The Forest and its Products

The state of Maine, with almost 90% of its land covered by forest, is one of the major paper and forest product producing states in the United States. In addition to paper, there are other products derived from wood such as panel boards, building materials and so on. Wood, also known as lignocellulose, is comprised of cellulose, hemicellulose and lignin. Hemicellulose is the second most abundant component in wood, next to cellulose. The wood used in paper making is cooked at very high temperatures to remove hemicellulose and lignin from wood so as to retain the strength and quality of cellulose in the pulp. For hardwoods, the sugars collected as extracts from this processing are predominantly xylo-oligomeric sugars mixed with other sugars and acids (Tunc. M., 2008).

1.1.2. Lignocellulosic Material

Major sources for collecting wood extract sugars could be from kraft pulp mills or from pre-extraction of wood at panel board making industries. The total sugar content in these extracts varies depending the chemistry and temperature gradients used for pre-treating the wood prior to processing. A kraft pulp mill can use green liquor to extract some of the hemicellulose and lignin present in the woodchips while still retaining high pulp yield (van Heiningen, 2006). Thus the extract generated by using the green liquor is called green liquor extract. In contrast, at the panel board making industries, the wood could be treated with plain hot water, which results in the removal of greater amounts of sugars. Pre-extracting the wood used in panel board manufacturing has the benefit of

diminishing the damage that microbes can do to the wood panels and increases their useable life (Howell, 2009). The extracts thus generated by using hot water are referred to as hot water extracts.

1.1.3. Integrated Forest Product Research

Recently there has been much interest shown in these liquid extracts so as to completely use the sugars present in them to produce other useful bioproducts such as volatile organic acids, ethanol, and so on(Balaban 2003, van Heningen 2006). With the use of theses extracts there is a hope to diminish the use of food sources for energy production. The sugars present in wood extracts are dilute and when derived from hardwoods, have high content of acetic acid which may have high value in the market.

1.1.4. Mixalco^{TM} Process

Organic acids ranging from C2-C7 are the focus of this work. Any biodegradable material, especially lignocelluloses, can be converted in to volatile organic acids by the use of Mixalco^{TM} process, which makes use of a mixed culture of microbial organisms (Holtzapple et al, 1999). We are applying this method to determine the conversion of the hemicellulose extract rich in xylo-oligomeric sugars to carboxylic acids. Studies are being conducted comparing two buffers for the fermentation: $CaCO_3$ and NH_4HCO_3, in order to determine the best neutralizing agent for the fermentation. The goal is to keep the fermentation at a steady pH by converting acids in to carboxylate salts. The use of halogenated methane analogs (eg: bromoform, iodoform) has been shown to give good results in inhibiting methane production (Holtzapple et al, 1999). By inhibiting methane production, a higher concentration of carboxylic acids is produced by eliminating the potential hydrogen sink (Ross. 1998).

To best determine the conversion efficiency of the Mixalco^{TM} process directly on hemicellulose sugars, a standard sugar experiment was performed on a batch scale, with equal concentrations of monomeric glucose and xylose. Sugars alone offer no nutrient source, so the nutrient source was provided by adding corn steep liquor rich in nutrients to about 3% of the total sugar concentration. The batch experiments are performed anaerobically at two different temperatures: one at 37°C and the other at 55°C, to determine the best temperature for a high concentration of carboxylic acids.

1.2. Literature Review

1.2.1. Wood Composition

In general wood is defined as "a three-dimensional biopolymer composite composed of an interconnected network of cellulose, hemicelluloses and lignin with minor amounts of extractives and inorganics" (Rowell, 2005). An average elemental composition of any dry wood consists of about 50% carbon, 6% hydrogen, 44% oxygen, and inorganics in trace amounts. Wood types are differentiated in to hard woods and soft woods.

1.2.1.1. Cellulose:

Cellulose is the main constituent of wood comprising approximately 40-45% of the total wood. It is a homopolysaccharide composed of D-glucopyranose units, linked together by β-(1→4)- glycosidic bonds. The wood cellulose has an average degree of polymerization of at least 9000-10,000 and as high as 15,000 units. (Rowell, 2005;

Sjöström, 1993). The combination of all the cellulose and hemicelluloses in wood is known as Hollocellulose. (Rowell, 2005).

1.2.1.2. Hemicellulose:

Hemicelluloses, the second most abundant sugars next to cellulose, is a hetero polysaccharide made up of pentoses (D-Xylose, L-Arabinose) and hexoses(D-Glucose, D-Galactose and D-Mannose) with a few sugar acids. Most of the hemicelluloses have an average DP of 100-200. (Rowell, 2005). A characteristic difference in the structure and composition of hemicelluloses can be seen in the hardwood and softwoods. The principal hemicelluloses in softwood are galactoglucomannans (approx 20%) with a linear or possibly slightly branched chain build up of (1→4)- linked β-D-glucopyranose and β-D-mannopyranose and the other is arabinoglucoxylan (approx 5-10%) composed of a frame work containing (1→4)- linked β-D-xylopyranose units partially substituted by 4-O-methyl-α-D-glucoronioc acidgroups. The hardwood hemicelluloses consist of mainly glucuronoxylan (approx 15-30%) with O-acetyl-4-O-methylglucurono-β-D-xylan as a major component. Hardwood hemicellulose consists of small amounts of glucomannan (approx 2-5%) composed of β-D-glucopyranose and β-D-mannopyranoseunits linked by (1→4) bonds.(Sjöström, 1993).

1.2.1.3. Lignin:

Lignin is an amorphous, highly complex aromatic polymer of phenylpropanoid precursors considered to be an encrusting substance. The lignin content of hardwood is around 18-25% whereas for softwood the value is around 25-30%. All wood lignin consists mainly of guaiacyl, syringyl and *p*-hydroxyphenyl moieties. The methoxyl

content in soft wood is around 15-16% and for a hardwood is around 21%. (Rowell, 2005).

1.2.2. Hemicellulose Extraction

Hemicellulose extraction is the process of extracting some of the hemicellulose from wood chips or wood strands prior to processing the wood into pulp or the strands into oriented strand board. When done effectively, extraction can recover hemicellulose sugars while retaining, and in some cases even improving, the quality of the traditional wood product. The process of extracting hemicellulose is different for different products of interest. For example, the kraft pulping process is used in the paper making process, whereas hot water extraction would be performed on wood used for compound boards or panel boards.

1.2.3. Kraft Pulping (Green Liquor)

Kraft pulping is a widely used aqueous chemical pulping process, used in pulp and paper industries to obtain higher quantity and quality of pulp. The pulping process includes an intermediate stream (green liquor) that is an aqueous solution comprised of sodium hydroxide, sodium carbonate, sodium sulfide and sodium sulfate (Um. 2009). The quality of the pulp is dependent upon the concentration of the aqueous solution used in the pulping process. For extraction with green liquor, the higher the concentration the better the pulp yield and the lower the hemicellulose concentration. To maximize the total hemicellulose production while maintaining the pulp quality and quantity, a near neutral hemicellulose extraction process is being developed by using diluted green liquor as the extraction agent (Haibo Mao, 2009). Table 1.1 presents the basic composition of the green liquor in a kraft mill for the hemicellulose extraction process.

Table 1-1: Chemical composition of green liquor used in Kraft pulping extraction process.

Chemicals	Value as Na_2O	Molar concentration
Total titrated alkali(TTA)	3% on wood as Na_2O	
Sodium hydroxide (NaOH)	9.0 g/l as Na_2O	0.23M
Sodium sulfide (Na_2S)	29.1 g/l as Na_2O	0.12M
Sodium carbonate (Na_2CO_3)	70.0 g/l as Na_2O	0.66M
Sodium sulfate (Na_2SO_4)	0.8 g/l as Na_2O	0.002M
TTA	108.9 g/l as Na_2O	

In this process about 0.05% of Antharaquinone(AQ) (based on wood) may be added during the extraction to maintain the pulp yield (Sjöström, 1993). The extraction has been demonstrated in a 7 liter indirectly heated rocking digester which operated at 160°C for 110 Min with a liquid to wood ratio of 4 to 1 until the H-factor reached to 780 to 790 hrs.(H. Mao, 2008).

1.2.4. Hot Water Extraction Process

The hot water extraction process is simple aqueous extraction of hemicellulose from wood chips or strands by simply cooking them using hot, pressurized water. This process is also known as autohydrolysis (Lora, Jairo H. 1978) which uses naturally released wood acids to assist in catalyzing the extraction process. This helps to protect the wood panel boards or composite boards from termites or rotting of wood and increase their shelf life (Juan Jacobo Paredes, 2008; Dirol and Guyonnet, 1993; Troya and Navarette, 1994). Extraction of lignocellulosic material using water as a solvent is considered as a good option since the yield of sugars is high and the byproduct formation is low (Waleed Wafa Al-Dajani. 2009).

1.2.5. Microbial Fermentation

The use of green liquor and hot water extracts in bio-based product conversions is being investigated. The total sugar concentration in green liquor extract is typically lower when compared to hot water extracts (Um. 2009). The production of ethanol from hemicelluloses extracts is one conversion process that has been investigated (Walton, 2009) Walton reported that production of ethanol from green liquor extract is likely to be challenging to commercialize because of the low concentration of sugars and ethanol, the cost of hydrolyzing the hemicelluloses oligomers and the inhibitory agents present in the extract. Regardless of their source, fermentation of wood-derived sugars to ethanol is challenging because few microbes can convert all of the hemicellulose sugars to ethanol. Most organisms intended for this conversion have been genetically modified (GMOs), the use of which typically requires sterile operating conditions and expensive process equipment.

A recent approach to overcoming the problems of producing ethanol with GMOs in a sterile environment is to biologically convert these hemicelluloses to carboxylic acids such as acetic acid, propionic acid, butyric acid, and so on. These can then be chemically upgraded into mixed alcohols or hydrocarbons or sold at higher value as chemicals. The inocula for the mixed acid fermentation may be derived from any location where biomass is naturally decomposing in an anaerobic environment. Of particular interest are soils, and also samples from the ruminant fore stomach which has a thriving anaerobically grown cellulolytic microbial culture. The cultures collected from the shores of salt lakes have the capabilities growing at harsh conditions such as high salinity, high or low pH. Organisms can also be isolated from environments at mesophilic or thermophilic

conditions. These mixed cultures also have the ability of hydrolyzing the complex carbohydrates into individual sugar molecules, which eliminates the high costs associated with hydrolysis and further simplifies processing.

1.2.6. Mixalco^{TM} Process

The Mixalco^{TM} process is a patented technology developed by Mark T. Holtzapple at Texas A &M University. The features of the Mixalco^{TM} process were employed as early as 1914 in a patented process by Lefrance (Lefrance. L., 1918), to convert biomass to butyric acid, which was further converted to "Ketol" used as a motor fuel. In the MixAlco^{TM} process, biomass is used as the major feed stock and is converted in to carboxylic acids using a mixture of microbial cultures collected from terrestrial and marine sources. This process is performed in anaerobic conditions by limiting oxygen supply. Also known as the carboxylate platform, the MixAlco^{TM} process converts biomass into acetic acid and other carboxylic acids which are further upgraded to useful fuels and chemicals like aldehydes, keytones and alcohols (Holtzapple, 2009). The advantages of this process include using low value substrates, not using any sterile culture techniques and eliminating the need of any external enzymes. See figure 1.1 and 1.2. results from MixAlco^{TM} fermentations are listed in table 1.2.

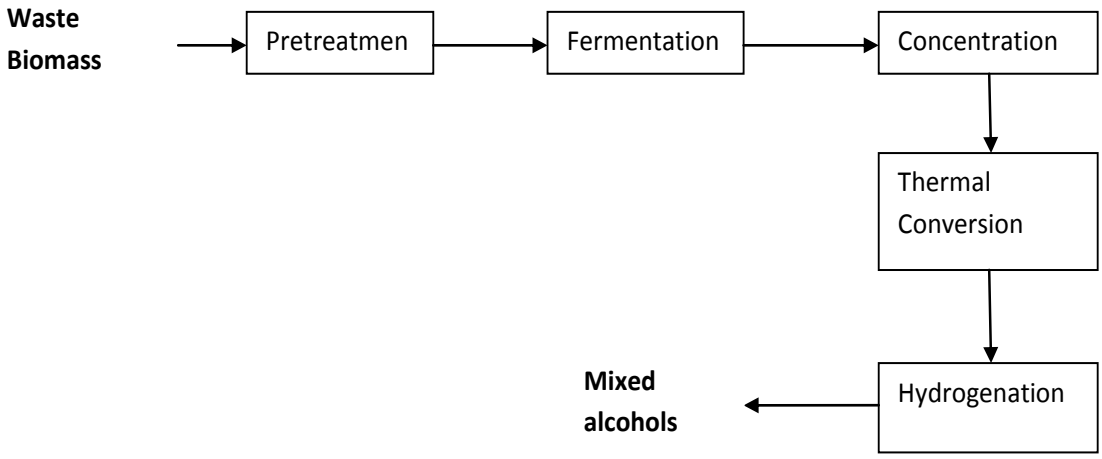


Figure 1-1: Mixalco^{TM} process(Holtzapple, 1997).

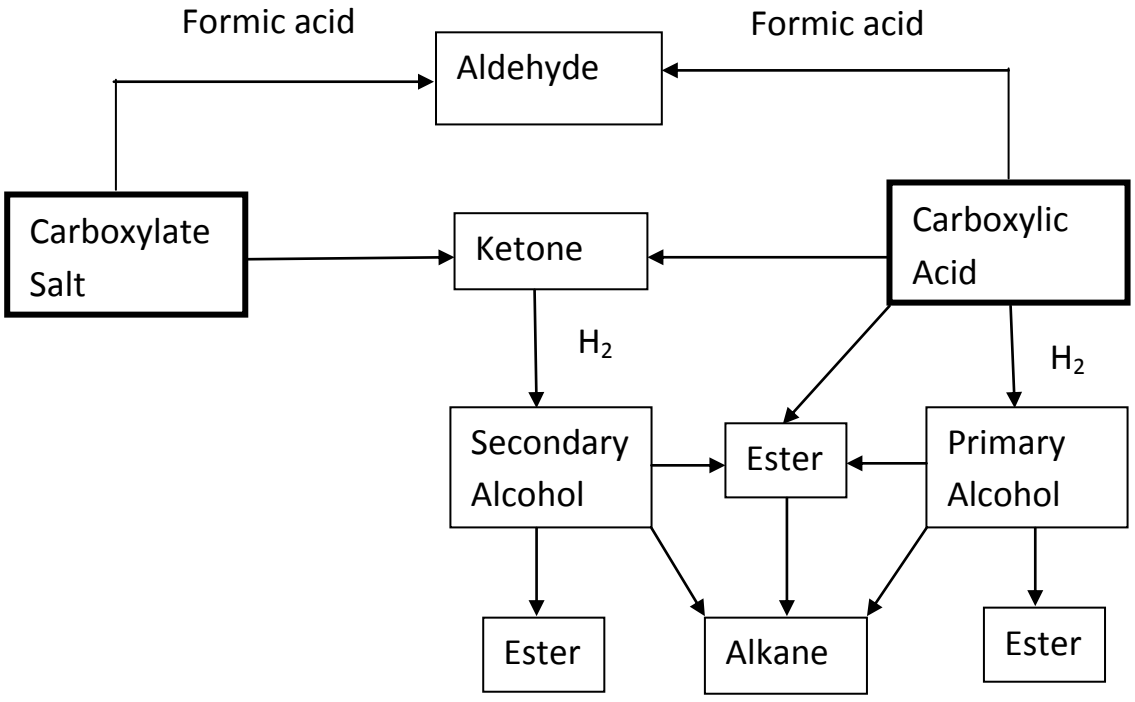


Figure 1-2: Carboxylate platform: overview of routes to chemicals and fuel products (Holtzapple, 1997).

Table 1-2: Discussions from previous papers on Mixalco^{TM} process on various biomass systems.

Serial number	Feed stock	Method adopted	Buffers used	Inoculum	temperature	Product concentration	Yield	references
1a	cattle manure(60% corn, 12% crude protein, 28% cotton seed hull)	counter current fermentation	calcium carbonate	rumen fluid	40°C	32.5	0.24	kyle ross (2001)
1b	80% municipal solid waste/ 20% sewage sludge	counter current fermentation	calcium carbonate	rumen fluid	40°C	26.5	0.34	kyle ross (2001)
2	80% municipal solid waste/ 20% sewage sludge	counter current fermentation	calcium carbonate	terrestrial inocula(rumen fluid, commertial and resedential compost piles and lake sediments), marine inocula(swamps along the coast of gulf of mexico)	55°C	20.5	0.41	Wen Ning Chan (2003)
3	80% sugarcane bagasse/ 20% chicken manure	counter current fermentation	calcium carbonate	terrestrial	40°C	18.7	0.338	piyarat Thanokeses, Nagat Abd Alla (2003)
				marine	40°C	16.2	0.359	
4	80% corn stover/ 20% pig manure	counter current fermentation	calcium carbonate	terrestrial inoculum (rumen fluid,compost and swamp lake sediments)	40°C	21.4	0.55	piyarat Thanokeses, Nagat Abd Alla (2003)
5a	80% paper/ 20% biosludge	counter current fermentation	calcium carbonate	terrestrial inoculum (rumen fluid,compost and swamp lake sediments)	40°C	19.5	0.42	Domke, Aiello-Mazzarri(2003)

Table 1-2 Continued.

Serial number	Feed stock	Method adopted	Buffers used	Inoculum	temperature	Product concentration	Yield	references
5b	80% paper/ 20% biosludge	counter current fermentation	calcium carbonate	terrestrial inoculum (rumen fluid,compost and swamp lake sediments)	40°C	16.8	0.46	Domke, Aiello-Mazzarri(2003)
6	80% municipal solid waste/ 20% sewage sludge	counter current fermentation	calcium carbonate	terrestrial inoculum (rumen fluid,compost and swamp lake sediments)	40°C	22.17	0.171	Aiello-Mazzarri, Coward-Kelly, Agbogbo (2005)
7	80% municipal solid waste/ 20% sewage sludge	counter current fermentation	calcium carbonate	terrestrial inoculum (rumen fluid,compost and swamp lake sediments)	40°C	26	0.18	Aiello-Mazzarri, Agbogbo (2005)
8	80% rice straw/ 20% chicken manure	counter current fermentation	calcium carbonate	Marine inoculum from previous cultures	40°C	25 - 40	0.16 - 0.29	Agbogbo (2006)
9a	80% rice straw/ 20% chicken manure (train A)	fixed-bed fermentation	calcium carbonate	Marine inoculum from previous cultures	40°C	F1 = 34.2 F2 - F4 = ~ 44	0.489 - 0.609	Agbogbo (2006)
9b	80% rice straw/ 20% chicken manure (train B)	fixed-bed fermentation	calcium carbonate	Marine inoculum from previous cultures	40°C	F1 = 30.5 F2 - F4 = ~ 48	0.563 - 0.669	Agbogbo (2006)
10a	100% Dairy manure	batch fermentation	calcium carbonate	Dairy manure and marine sediment cultures	40°C	8.5	0.2	Blackman and van Walsum (2009)

Table 1-2 Continued.

Serial number	Feed stock	Method adopted	Buffers used	Inoculum	temperature	Product concentration	Yield	references
10b	100% Dairy manure	batch fermentation	calcium carbonate	Dairy manure and marine sediment cultures	55°C	7.5	0.16	Blackman and van Walsum (2009)
11	80% ammonia-treated sugarcane bagasse/20% chicken manure	Four-stage counter current fermentation	Ammonium bicarbonate	Mixed culture of amrine microorganisms	55°C	27.6	0.104	Fu Z and Holtzapple (2010)
12	80% sugarcane bagasse /20% chicken manure	Four-stage counter current fermentation	calcium carbonate	Mixed culture of amrine microorganisms	55°C	15.5	0.18	Fu Z and Holtzapple (2010)
13	80% lime-treated sugarcane bagasse /20% chicken manure	Four-stage counter current fermentation	Ammonium bicarbonate	Mixed culture of amrine microorganisms using CBP method	55°C	31.3	0.14	Fu Z and Holtzapple (2010)

1.2.7. Consolidated Bioprocessing

Consolidated bioprocessing (CBP) involves the combination of four different biologically mediated transformations in to a single step transformation. As typically envisioned, biological conversion of biomass to liquid fuel could be accomplished by: 1) the production of sacchorarolytic enzymes (cellulase and hemicellulase); 2) hydrolysis of the lignocelluloses to monosugars (hexoses, pentoses); 3) fermentation of hexose sugars (glucose, mannose and galactose) and finally 4) fermentation of pentose sugars (xylose and arabinose). The motivation for consolidated bioprocessing is that it offers the potential for lower cost and higher yield efficiencies than processing a dedicated cellulose production (Lee Lynd, 2005). The properties required for a CBP process of both substrate utilization and specific product formation, such as ethanol, combined in a single microbe is currently not available (Lee Lynd, 2005). Developments can be pursued by two strategies: native cellulolytic strategy and recombinant cellulolytic strategy. Currently more importance is given to a native cellulolytic strategy involving engineering naturally occurring cellulolytic microorganisms to improve product related properties (yield and titer). In a mesophilic non cellulolytic enteric bacteria, the native cellulolytic strategy (CBP) was predicted to be economical, by the studies of the metabolic engineering of mixed acid fermentations under anaerobic conditions (Ingram et al., 1999). The term CBP, a synonym, was previously referred to as direct microbial conversion (DMC).

1.2.8. Anaerobic Digestion and Stages Adopted During Fermentation.

MixAlco^{TM} process employs an anaerobic digestion process to produce high yields of product of interest. The anaerobic digestion process is divided in to three stages

considering methane as the final product. These stages are hydrolysis, acid-formation and methanogenesis (Gerardi, 2003). The critical biochemical reactions within these stages are presented in the below figure 1.3.

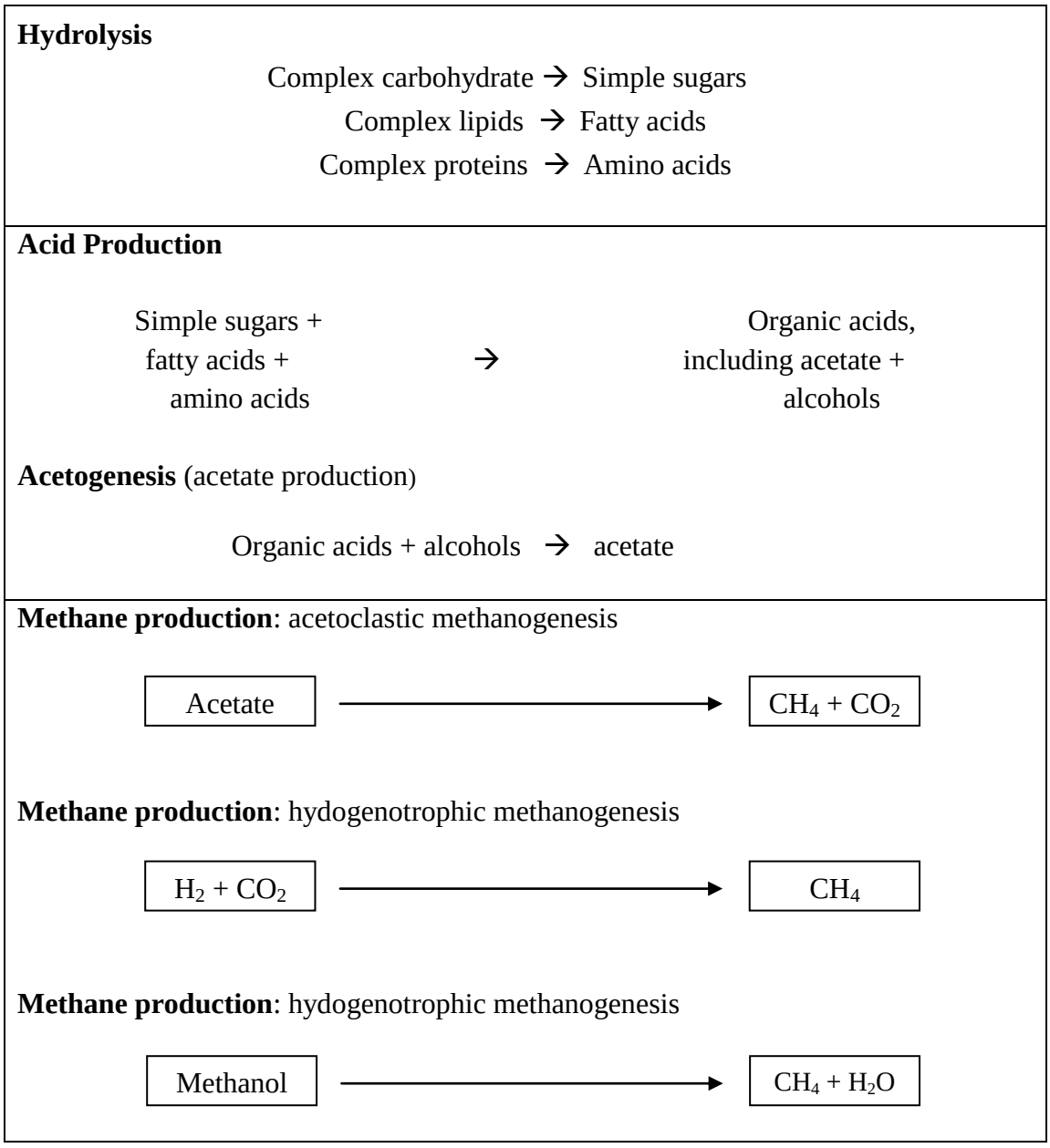


Figure 1-3: The critical biochemical reactions in the anaerobic digestion process with methane as the final product include hydrolysis, acid production, acetogenesis and methane production.

1.2.8.1. Stage 1:- Hydrolysis Stage

The complex insoluble compounds such as particulates and colloidal wastes especially of carbohydrates, fats and proteins are polymeric substances. These are large insoluble molecules consisting of many small molecules, soluble in solution, joined together by unique chemical bonds. Hydrolysis is the process of splitting (lysis) of a compound with water. These chemical bonds are broken down or simply hydrolyzed with the help of hydrolytic bacteria or facultative anaerobes and anaerobes (Gerardi, 2003).

1.2.8.2. Stage 2:- Acid–Forming Stage

The soluble compounds produced through hydrolysis or discharged as momomers to the digester follow the biodegradation steps through the process which is catalyzed by a large diversity of facultative anaerobes and anaerobes. This stage, called the acid-forming stage, results in the production of carbon dioxide, hydrogen gas, alcohols, organic acids and some organic nitrogen and organic sulfur compounds due to the degradation of amino acids and proteins. Of these, acetate is the most important (Gerardi, 2003).

Acetate, produced through the fermentation of organic compounds is also produced by the acetogenesis process. Acetogenesis is a step in the acid-forming stage that converts many of the acids and alcohols to acetate. The production of acetate is accomplished through the activity of acetogenic and acid-forming bacteria (Gerardi, 2003).

1.2.8.3. Stage 3:- Methanogenesis Stage

The methanogenesis stage is the final stage where methane is the final product in the process. Carbon dioxide and hydrogen gas are directly converted to acetate or

methane, acetate formed in the stage 2 is converted to methane using the methane-forming bacteria. Methane is also formed from some organic compounds other than acetate (Gerardi, 2003).

1.2.9. Lactate Fermentation

Lactate is a common product in many fermentative reactions. Lactate formation occurs when consuming sugars like glucose, fructose, galactose. mannose, saccharose, lactose, maltose and pentoses. Three biochemical reactions for lactate production from sugars (glucose) are represented below (Gerardi, 2003).

Glucose → 2 Lactate

Glucose → Lactate + Ethanol + CO_2

2 Glucose → 2 Lactate + 3 Acetate.

Lactic acid producing microorganisms can be divided in to two groups: bacteria and fungi. The fungal fermentative process by Rhizopus species is a simple fermentative process which converts the starch directly to lactic acid, although it requires a lot of aeration for this process. Whereas for a bacterial fermentation the process occurs in the anaerobic condition and most of the lactic acid producing bacteria belong to the lactobacillus species. It has also been shown that the temperature and pH plays a major role in the production of lactic acid (Y. J. Wee *et al.*, 2006). Ratin Datta in his review pointed out that most of the lactic acid produced in present days is in anaerobic fermentation process using calcium hydroxide as the buffering agent and trying to maintain the pH between 5 - 6. He has reported that a maximum of 90 wt% of lactic acid yield is being produced form a dextrose equivalent of carbohydrate as the source (R Datta, 2006).

1.2.10. Metabolic Pathways

Metabolic pathways in general defined as a series of chemical reactions taking place within a cell to produce a series of intermediate to final products. In this work, during mixed microbial anaerobic fermentation xylose is used as the major carbon source for the production of volatile fatty acids and alcohols (Temudo FM, 2008). Fermentation of xylose under both mesophilic and thermophilic conditions resulted in the production of a wide spectrum of products like acetate, propionate, butyrate, lactate, formate and some hydrogen (Zhao C, 2010). The figure 1.4 below represents the various likely metabolic pathways during the fermentation of xylose to specific products. The figure has been adapted from Zaho C, Tanaka.K, and Temudo F M. Tanaka K has reported that the xylose was catabolyzed by both pentose phosphate pathway/ glycolytic pathway and the phosphoketolase pathway. The switching to either of these pathways is dependent upon the concentration of xylose present and therefore can change the final concentration of lactate accumulation (Tanaka K., 2002; Temudo F M, 2008). The pentose phosphate pathway is the most common pathway but many bacteria also have the capacity to catalyze the phosphoketolase pathway (Temudo F M, 2008).

pentose phosphate pathway: 3 Xylose → 5 Pyruvate

phosphoketolase pathway: 3 Xylose → 3 Pyruvate + 3 Acetyl - P

Higher xylose concentration leads to adopting the pentose phosphate pathway and produces high amounts of lactate, while a lower xylose concentration adopts the phosphoketolase pathway and produces a lower amount of lactate when compared to acetate and ethanol.

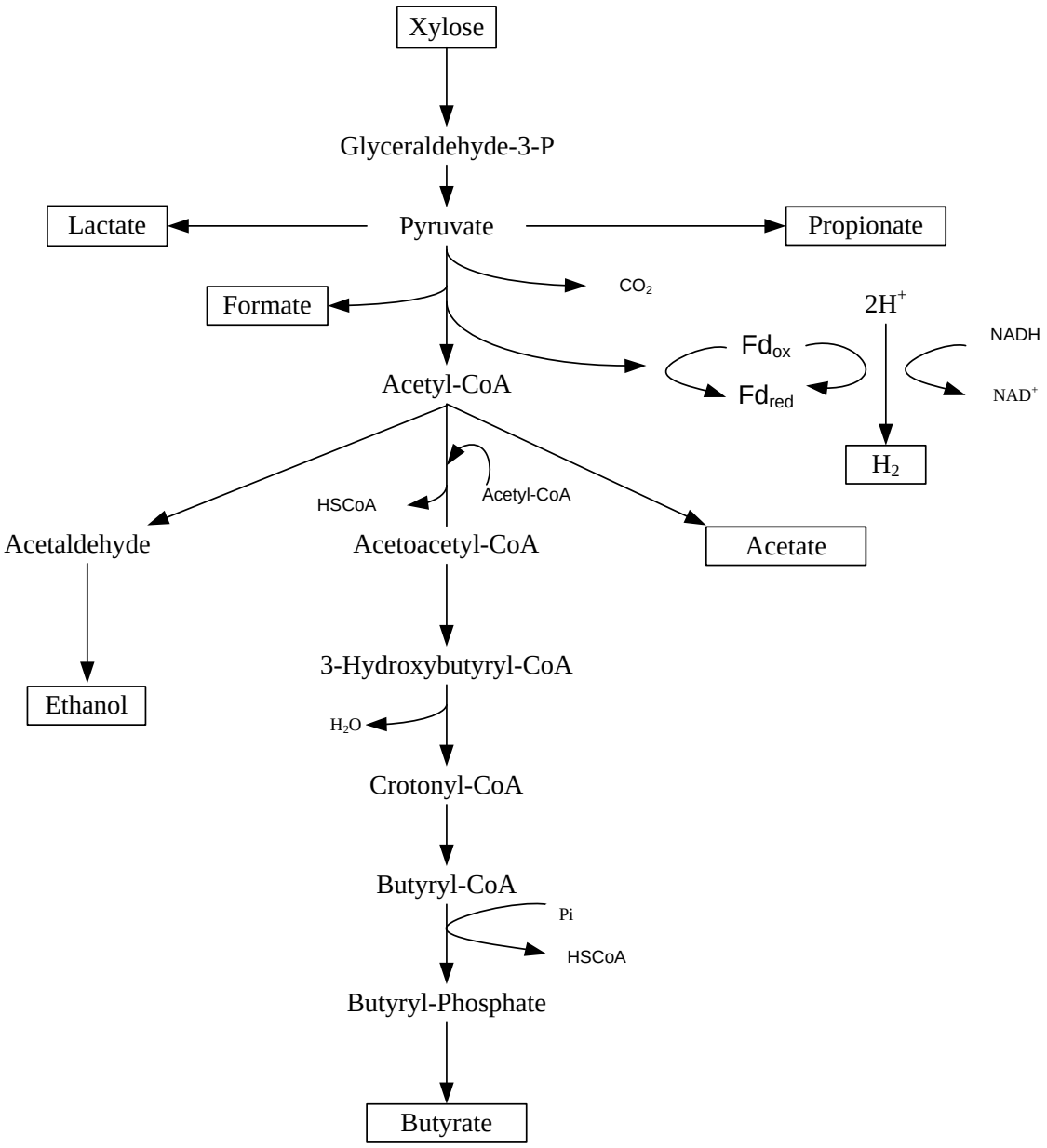


Figure 1-4: Metabolic pathway during anaerobic mixed microbial fermentation of Xylose to fuels and chemicals.

1.2.11. Modified MixAlco™ Process

People at the FBRI (forest bio-products research institute) are looking into ways to utilize the available renewable biomass sources in a highly integrated means for further production of bio based fuels and chemicals (van Heiningen, 2006). This concept leads to a modified kraft pulping process by integrating a hemicellulose extraction process prior to pulping for biofuel production. The steps that have been outlined for acid catalyzed hydrolysis and ethanol fermentation from extract (Walton, 2009) could be replaced with the Mixalco™ process, integrating its unit operations with the kraft pulp mill for the production of high value fuels and chemicals.

1.2.12. Nutrient Availability and Sources

For the optimal growth of an organism it needs nutrient sources. The availability of nutrients in the wood extracts are limited since the nutrients that are extracted during the cooking process are present in the wood only in low concentrations. For a non-aseptic biological process, the use of natural nutrient sources like chicken manure, horse or a cow manure, or sewage sludge, etc., is possible and provides a low cost nutrient source rich in macro and micronutrients. This is in contrast to aseptic fermentations which would likely find such biodiverse and poorly defined nutrient sources difficult to sterilize. The availability of nutrients can also be provided from the biproducts or waste products from a food processing plant. A good source of this type is corn steep liquor (CSL) from the corn wet milling industry. Its composition depends upon the type of processing selected, but in general this is very rich in nitrogen, vitamins, amino acids and certain growth simulators. In one study ,addition of nutrients as supplements to CSL did not show any

benefit on the growth of microbes and formation of ethanol as the product (Samuel Amartey, 1994). CSL consists of approximately 50% of dry matter.

1.2.13. Methane Inhibition

The microbial ecosystem samples from various sources such as the forestomach of ruminants or swampy places near salt water are composed of a complex anaerobic community of bacteria capable of growing under harsh conditions. These communities have varied types of organisms present in them, of which some are methanogens. During a MixAlco^{TM} anaerobic fermentation some amount of carbon and energy is lost in the form of methane. The only way to inhibit the production of methane is to inhibit the methanogens. There are various ways of doing this, which include using: halogenated methanes such as chloroform, bromoform, iodoform, carbon tetrachloride or methylene chloride (Bauchop, 1967; Chapula, 1980; Russell and Martin, 1985; Holtzapple, 1999); by using the Coenzyme M analogs like hydroxymethylglutaryl~SCoA (HMG-SCoA) reductase inhibitors (Miller and Wolin, 2001); or 2-Bromoethanesulfonic acid (2-BES) (Martin and Macy, 1985; Sauer and Teather, 1987). By inhibiting the methane production an increase in hydrogen production is observed resulting in higher carboxylic acid concentrations (Bauchop, 1967; Chapula, 1980, Martin and Macy, 1985). A high ammonium concentration can also inhibit the methane production in an anaerobic process of producing fuels and chemicals (Holtzapple, 2009).

1.3. Economics

During a kraft pulping process the soluble hemicellulose and lignin present in the spent liquor is combusted to produce steam and electricity and to regenerate the pulping

chemicals. The conversion of these low value hemicelluloses into value added bioproducts helps to improve the present economic opportunity (van Heiningen, 2006). The conversion of this to transportation fuels is the present interest.It is believed that MixAlco^{TM} processing technology may be more economical than production of ethanol through aseptic fermentation. As a comparison between the two technology approaches, recent studies by Holtzapple (2009) and NREL (2008) on conversion of lignocelluloses suggest that the Mixalco^{TM} process is more economical. The minimum ethanol selling price according to the NREL study for lignocellulose derived biochemically produced ethanol was recently estimated to be at $2.61/gal.(Humbird, Aden, 2008 NREL). Where as a municipal solid waste processing plant using hydrogen, generated from natural gas, can sell alcohols for $0.72/gal and gasoline for$1.14/gal. More comparable to the NREL scenario, a large plantation of energy crops could sell MixAlco^{TM} derived alcohols for $1.2/gal and gasoline for $1.85/gal. (Holtzapple, 2009).

CHAPTER 2. MATERIALS AND METHODS

2.1. Substrates

The liquid extracts that were used as raw material were collected from wood chip digesters located in the process development pilot plant in the Department of Chemical and Biological Engineering at the University of Maine. The digesters are designed to simulate pulping of wood chips in a continuous digester. Extracts used for this study were prepared by carrying out relatively mild condition extractions on hardwoods using either hot water or water mixed with green liquor, which is a highly buffered intermediate pulping stream in the kraft pulping process.

The green liquor and hot water extraction processes are carried out at varied levels of temperatures, chemical loadings, wood to liquor ratio and periods of reaction time. A typical H-factor for the green liquor extraction process was around 780 – 790 hrs and for hot water extraction process was about 350 – 380hrs.

The green liquor extract was a custom blended 1.44% green liquor used in extracting the hemicellulose extract from hard wood chips and hot water extracts were obtained by cooking aspen wood strands in water, so as to remove the hemicelluloses. The total sugar content in the green liquor extract was measured to be around 10.25g/L and the acetic acid content was around 11.8g/L where as the total sugar content in the hot water extract was measured to be around 27g/L and the acetic acid is about 9.6g/L.

2.2. Media and Nutrients

- Pure Sugar fermentation.

For the pure sugar fermentation process the sugars glucose and xylose were used. These were purchased off the shelf from Sigma Aldrich inc. About 10g/L of each were mixed together and used in the fermentation process for establishing a baseline of sugar conversion and determining optimum fermentation conditions for best product yields.

- Corn Steep Liquor

Various sources for obtaining the additional nutrients for the fermentation have been studied. One preferred in this work is corn steep liquor, which is a byproduct of corn wet-milling and a good source for organic nitrogen. About 3g/L for pure sugar fermentation and 5g/L for the wood extracts were used to enable good fermentation process. Corn steep liquor was purchased from the Sigma-Aldrich

2.3. Buffers

Calcium carbonate or ammonium bicarbonate were used as buffering agents. The buffer was added in its solid form so as to maintain an optimum pH between 6.5 and 7.3. Neutralizing the acids produced in the fermentation process maintains a neutral pH which results in better growth of the microorganisms. When using calcium carbonate, the excess buffer that is not consumed settles to the bottom of the fermentation vessel and is dissolved and consumed as needed. Ammonium bicarbonate dissolves completely upon addition and was added when the fermentation pH dropped below 5.8. For a green liquor extract the solution was already well buffered by the pulping salts and it did not require any buffer addition at the initiation of the experiment.

2.4. Inoculum

Inocula for this experiment were obtained from various sources, including secondary clarifier sludge from the waste water treatment facility at a kraft pulp mill, saline sediments from The Great Salt Lake in Utah and salt water sediment from the coast of Maine. To minimize the exposure to oxygen, these were collected in tightly sealed containers with the solids content having microbes covered with sea water.

2.5. Methanogen Inhibitor

Iodoform (CHI_3) was used as an inhibitor to prevent or diminish the production of methane. In several cases, methane production was low and no inhibitor was required. Excessive use of this chemical may harm the growth of the other microbes present in the mixed culture, and so should be used sparingly. The concentration required for effective methane inhibition is about 10 mg/L. As these compounds are sensitive to light and air, it is preferred that they be stored in a tinted glass and capped immediately after use. Prepare 20g Iodoform /L ethanol by dissolving 20 grams of Iodoform in 1 L of 99.8% pure ethanol. It is preferred to prepare these at the time of use in order to avoid prolonged storage which might affect its stability. When ammonium bicarbonate used as a buffer, the ammonia released also inhibited methanogenesis at neutral pH.

2.6. Batch Fermentation Process

Experiments were performed in batch mode to determine optimal productivity from the available sugar content of the extracts. All the batch experiments performed were carried out in duplicate or triplicate to determine reproducibility of results. The batch

fermentations were performed in 250 ml serum bottles with a narrow mouth (Wheaton science products # 223950). The mouth was sealed by using a rubber septum stopper (Wheaton # 224100-330) and an aluminum seal (Wheaton # 224187-01) which held the rubber stopper tightly to the mouth of the glass serum bottle. The rubber septa enabled collection and measurement the total gas produced during the fermentation by inserting a syringe needle connected to a gas accumulation and volume measurement device. All the materials were purchased from Fisher Scientific Inc.

To best determine the conversion efficiency of the Mixalco^{TM} process directly on hemicellulose sugars, a standard sugar experiment was performed on a batch scale, with equal concentrations of monomeric glucose and xylose. Sugars alone offer no nutrient source, so the nutrient source was provided by adding corn steep liquor rich in nutrients to about 3% of the total sugar concentration. The batch experiments were performed anaerobically at two different temperatures: one at 37°C and the other at 55°C, to determine the best temperature for a high concentration of carboxylic acids.

For the wood extract experiments, the bottles were filled with approximately 100ml of the wood extract in each bottle, which left sufficient head space for accumulation of produced gasses. These bottles were placed in a shaking incubator (Sartorius Certomat BS-1) with continuous shaking at about 200 rpm and constant temperature maintenance. Two different temperatures are investigated for the process: one at 37°C and the other at 55°C.

The gas and liquid samples were taken at regular intervals of time so as to determine the total acid productivity, carboxylic acid concentration, total yield, available sugar concentrations and to check for any methane production during the process.

2.7. Analytical Methods

2.7.1 Gas Samples

Prior to sampling, the bottles were removed from the shaking incubator and allowed to sit at room temperature for a period of time so that the temperature of the bottle could reach room temperature. The minimum required gas sample to run through a GC for gas analysis is about 2ml. The total gas samples collected were around 5- 10 ml depending on the fermentation process. As it was difficult to store the gas samples these were run through the GC as soon as they were collected from the bottle.

2.7.2 Gas Volume Measurement

The total gas volume in the serum vial was measured by connecting it to a gas volume measuring device and noting down the total liquid displacement in the device. This measurement device was assembled in the lab using a graduated glass burette, a rubber stopper and few connections. (see figure 2.1) It consisted of a 30% calcium chloride solution in a 2 liter beaker, which was used as a gas displacement fluid. The calcium chloride was added to limit the amount of carbon dioxide that dissolves in to the fluid. A graduated burette which has a capacity to measure 100ml of fluid was taken and the bottom kept open. It was arranged such that the bottom end was completely immersed in the fluid and no leaks were visible when the fluid was taken to the maximum level of the burette. The top of the burette was fitted with a rubber stopper with a small tube through the stopper. This tube was connected either to measure the gas volume in the serum bottle or to a vacuum pump, which was used to pull the liquid up into the burette before the measurement. The graduations on the tube were used to measure the total volume of the gas produced during the fermentation process.

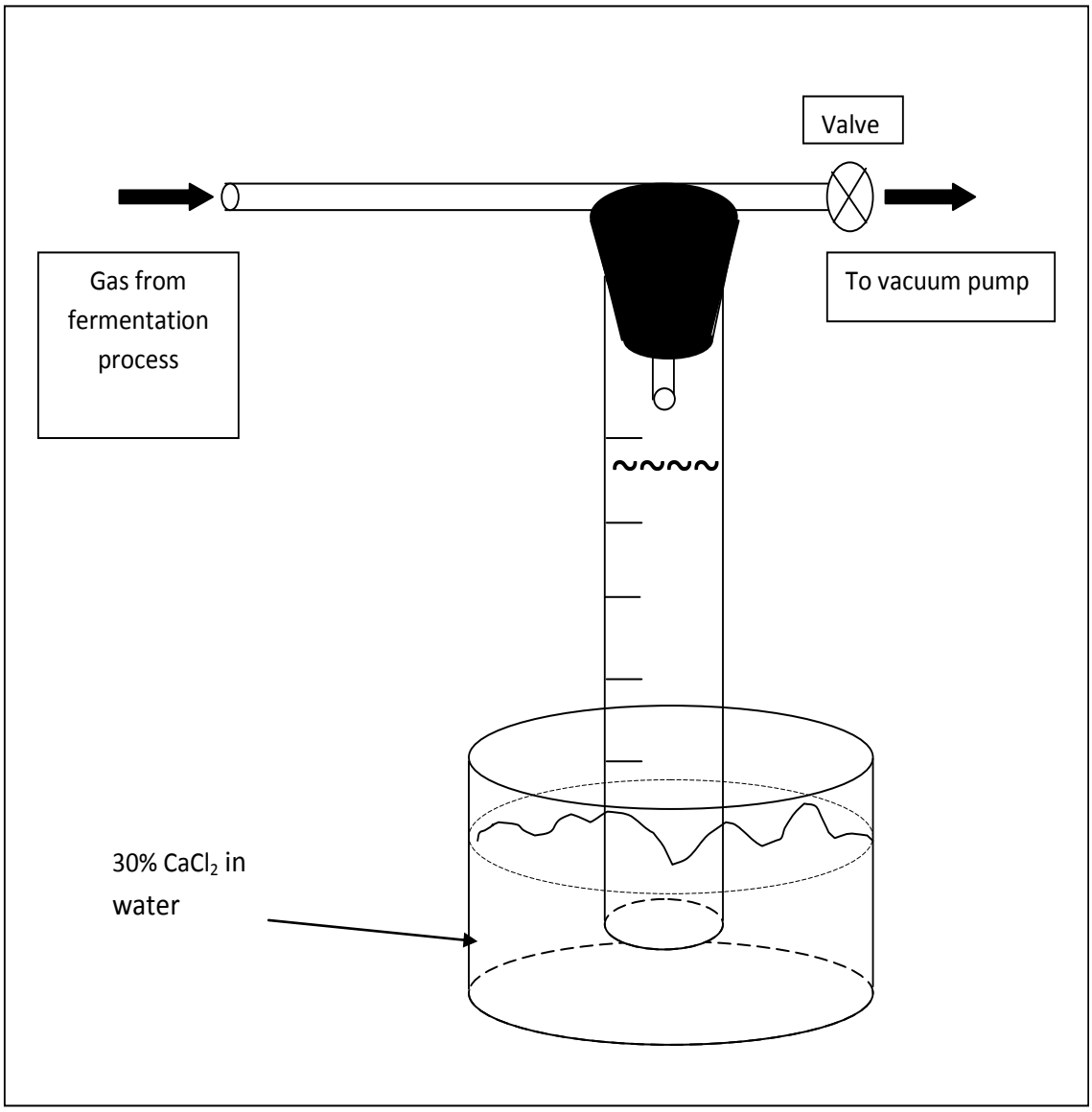


Figure 2-1: Gas volume measurement apparatus.

30% calcium chloride helps in reducing the possible water evaporation since the concentration level is considered to be at its minimum saturation level. The high salinity also has an inhibitory effect of microbial growth. It also helps in preventing any CO_2 adsorption since the chemical itself is made of a weak base and a strong acid and the pH of the solution is acidic (i.e., around pH 5.6). During sampling gas and before the start of the fermentation process for the day after sampling the pressure in the fermenter is made equal to the pressure in the head space in the measuring burette.

2.7.3 Gas Analysis

Methane and carbon dioxide concentrations in the produced gas were measured using a SRI multiple gas analyzer #2 with sulfur detector instrument, which has three columns: a 60 m MXT-1 capillary column, a Molecular Sieve 13X packed column and a HayeSep-D packed column (Restek U.S., Bellefonte. PA.), and are supplied by SRI Instruments. Detection is done using TCD, FID with methanizer and FPD detectors. The mobile phase used is helium gas. The minimum amount of sample needed to analyze was at least 2ml. typically a volume of 5ml -10ml was injected in to the GC for analysis. All the samples were injected manually using a 10ml syringe. A detailed sample running procedure is discussed in Appendix A.

2.7.4 Liquid Samples

Liquid samples were collected on a daily basis after the gas volume was measured. A total volume of approximately 1 ml was collected and was diluted with an appropriate volume of water so as to measure the products within the prescribed range of values. The samples were stored in a freezer in 1.5ml micro-centrifuge tubes. These collected samples were run through the HPLC and GC for determination of total acid production. The sample preparation methods are discussed in the appendices below.

2.7.5 HPLC Analysis for Sugars and Acids

The samples from the batch cultures were collected at regular intervals of time—typically on the order of once every 24 hours. The samples were analyzed to measure the total sugar concentrations in the reactor and also the total volatile solids present within the system. The measurement of the total sugars was analyzed on a Shimadzu Prominence HPLC system (Shimadzu scientific instruments, Columbus. MD.). which

was equipped with an Aminex HPX-87H column supplied by the Bio-Rad Laboratories. The eluent used for this column was 5mM H_2SO_4. The flow rate was maintained at 0.6mL/min through the column. The temperature of the column was set at 60°C. The liquid samples were mixed with fucose as an internal standard and run through the HPLC system. The data were measured using a refractive index detector set to 40°C. Detailed sample preparation and running procedures are discussed in Appendix B.

2.7.6 Gas Chromatography for Measuring Acids

The liquid samples collected from the batch cultures are also analyzed on a GC system. Acids in the sample were analyzed on a Shimadzu GC-2010 gas chromatograph with a capillary column "Stebilwax-DA" supplied by Restek Corporation. The analyzer was equipped with flame ionization detector. The sample of 10µl volume was injected in to the GC using an auto injector. These samples were mixed with 1.162gm/L of 4-methyl-n-valeric acid as an internal standard and acidified with 3M phosphoric acid (H_3PO_4) before injecting in to the GC. The column temperature was maintained at 50°C for 1 min and then increased from 50°C to 200°C at a rate of 20°C/min. and kept constant at 200°C for 1min. A detailed sample preparation and running procedures for Gas Chromatogram is described in detailed in Appendix C.

2.7.7 pH Analysis

The pH analysis is performed using the Orion pH probe, calibrated using the known pH standards of pH 7.0 and pH 10.01. This helped to provide accurate results.

2.7.8 Calculation of Abiotic CO_2

In general, according to the stoichiometric calculation for a mono-carboxylic acid such as acetic acid, one mole of hydroxide is required to neutralize one mole of protonated acid. The bases selected for these experiments were carbonate bases: $CaCO_3$ and NH_4HCO_3. When neutralizing acids these carbonate bases release CO_2, which in this study is referred to as abiotic CO_2.

For $CaCO_3$ the total amount of CO_2 released during neutralization is

$CaCO_3 + 2H^+ \rightarrow Ca^{2+} + H_2O + CO_2$ Equation-1

Thus two moles of acid require one mole of $CaCO_3$ to be neutralized into carboxylate salt, and results in the release of one mole of CO_2.

For NH_4HCO_3, the total amount of CO_2 released during neutralization is

$NH_4HCO_3 + H^+ \rightarrow NH_3 + H_2O + CO_2$ Equation-2

One mole of acid requires one mole of NH_4HCO_3 to neutralize in to carboxylate salt, and it releases one mole of CO_2 in to atmosphere.

The total biotic CO_2 produced during the fermentation process is the difference between the total CO_2 produced and the abiotic CO_2 generated by the neutralization process.

2.7.9 Dry Mass

The dry mass content in the liquid extract at the beginning and at the end of the experiment was measured by dispensing a small sample of about 10ml of the extracts in ceramic crucibles of 35ml volume and drying them in a convection oven at 105±5°C for an overnight period of time.

2.7.10 Ash

The ashing is done to the air dried extracts in a furnace at a temperature of 550°C for a period of 4 hrs. After combustion, the samples were massed to determine the ash content.

2.7.11 Measurement of Oligomeric Sugars

The hemicellulose present in hot water and green liquor are analyzed in two steps to determine the oligomeric and monomeric sugar concentrations. First, the raw sample is analyzed by direct injection in to the HPLC, which determines the sugar present in the unhydrolyzed extract. Second, the sample undergoes a one hour hydrolysis, carried out with 4% H_2SO_4 at 121°C in an autoclave. This hydrolyzed sample is then analyzed in the HPLC for total monomeric sugars. The yield in oligomeric sugars was calculated by the increase in total monosugar anhydride content resulting from the acid hydrolysis.

CHAPTER 3. RESULTS

3.1. Reproducibility of the Acidogenic Fermentation Process

The below graphs represents the best reproducibility of the fermented products when done in different bottles. These reproducible values represent the similarities between the four different bottles using the same buffer at the same temperature. The four replicate bottles represented included two bottles with iodoform addition, and two bottles without iodoform. It was found that the addition or exclusion of iodoform resulted in no observable differences between fermentations.

Example: Green liquor extract fermentation at 37°C

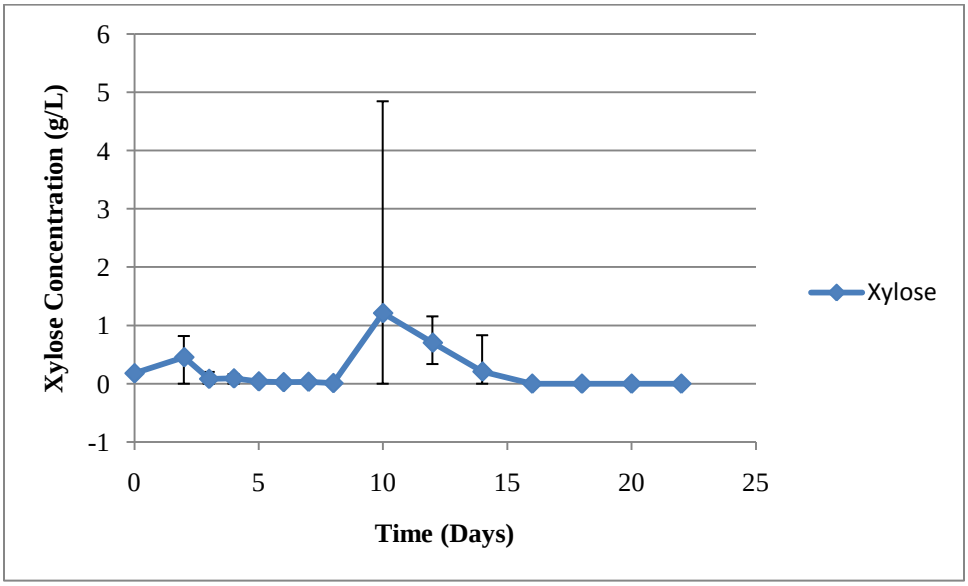


Figure 3-1: Auto hydrolysis of oligo-xylan to xylose sugar.

In the xylan auto hydrolysis step (fig 3.1) the total xylan conversion to xylose doesn't vary much during the fermentation process.

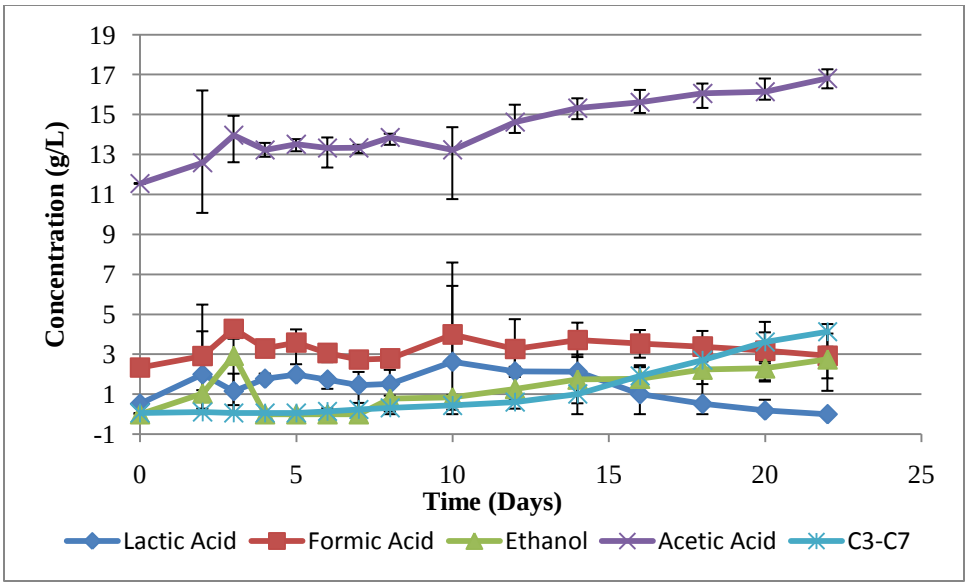


Figure 3-2: Total product concentration during acidogenic fermentation of GL extract.

The lactic acid production during the process in different bottles doesn't vary much as the final concentration of the lactic acid is converted to other products.

Formic acid production does not vary much and the final formic acid content is constant.

The total ethanol production increased during the process and the total difference in the total ethanol production is not more than 1.5g/l.

The acetic acid production during the process is quite good showing little difference in the acid production in each bottle. The total final acetic acid produced in each bottle varied by 0.5 – 1 g/l.

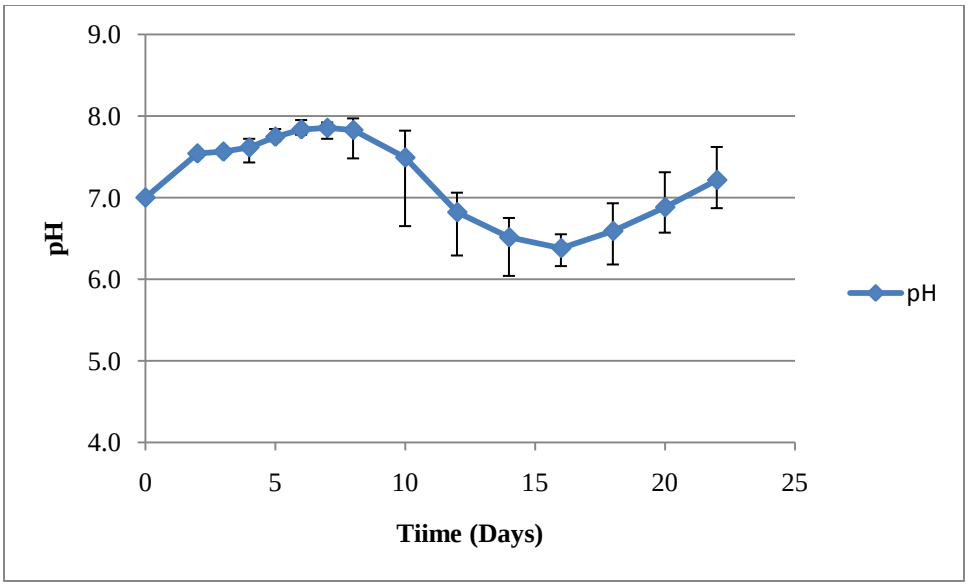


Figure 3-3: pH trend during the fermentation process.

The pH in the bottles during the fermentation seemed to be almost the same in each of the bottles.

3.2. Lactic Acid Production

We had observed from the results that lactic acid is being produced during the fermentation process and later being consumed to produce other acids. This process of product formation and consumption is characteristic of what is referred to as an intermediate product. Although lactic acid is not the target product for this fermentation process, it plays a major role as an intermediate compound during the process to give the final product of interest.

This has also been reported by other researchers who indicate that lactic acid is being produced as an earliest intermediate product during anaerobic fermentation process but is later diverted to produce small chain carboxylic acids.(. Kim *et al.,* 2003). These researchers had also observed that lactic acid is produced at lower pH during a thermophilic fermentation process.

3.3. Pure Sugar Fermentation

Before working with the hemicellulose extracts, a model experiment was conducted with a mixture of pure glucose and xylose to explore the optimum growth conditions for best product formation. The total amounts of sugars considered for this experiment were taken based on a rough calculation of the total sugars expected to be present in hardwood hemicellulose extracts. The studies were done with two different buffers at two different temperatures. The buffers used were calcium carbonate and ammonium bicarbonate. The temperatures at which the fermentations were performed were 55°C (thermophilic) and 37°C (mesophilic). The gas measurements were performed on a daily basis measuring the total methane and carbon dioxide produced and recording the volume of gas produced. Liquid samples were taken daily and run through the HPLC and GC. pH was measured each day after taking the samples.

Table 3-1 presents the experimental design for the comparison of the effect of the buffers on the microbial growth and product formation.

Table 3-1: Matrix table for buffer comparison on pure sugars.

Substrate: glucose 10g/L, xylose 20 g/L, corn steep liquor 3 g/L **Inoculum source: original (unadapted) marine inoculum**			
condition	**temperature**	**buffer**	**iodoform**
Fermentation	55°C	$CaCO_3$	No
	55°C	$CaCO_3$	Yes
	55°C	NH_4HCO_3	No
	55°C	NH_4HCO_3	Yes
	37°C	$CaCO_3$	No
	37°C	$CaCO_3$	Yes
	37°C	NH_4HCO_3	No
	37°C	NH_4HCO_3	Yes

All the experimental conditions were performed in duplicate. The iodoform added was dissolved in ethanol. The samples which did not have iodoform added were supplemented with ethanol so as to balance the total ethanol added to the different fermentation bottles. The addition of iodoform did not show any difference in the total product output as there was no methane produced in any of the bottles.

3.3.1. Glucose and Xylose Consumption During the Fermentation Process

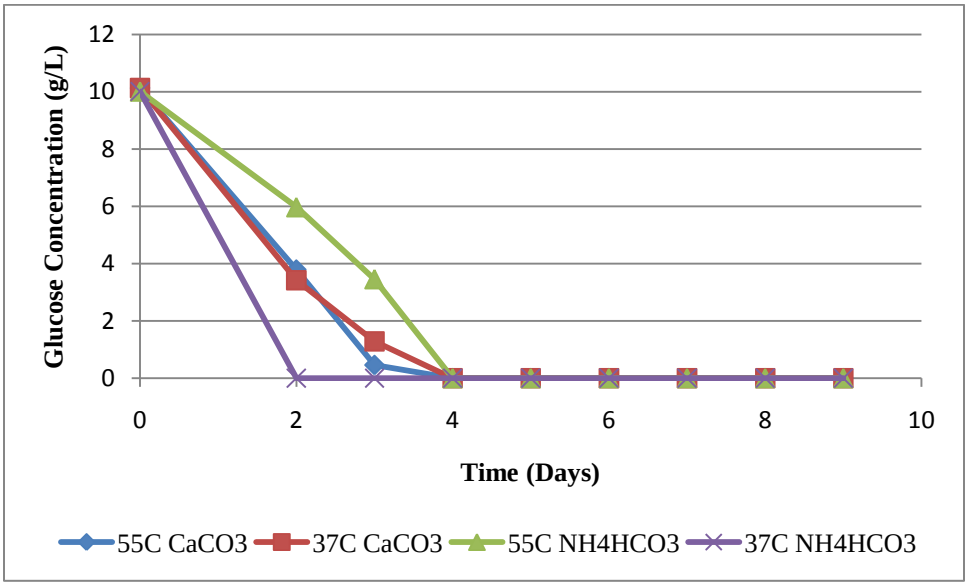


Figure 3-4: Glucose consumption for the four conditions.

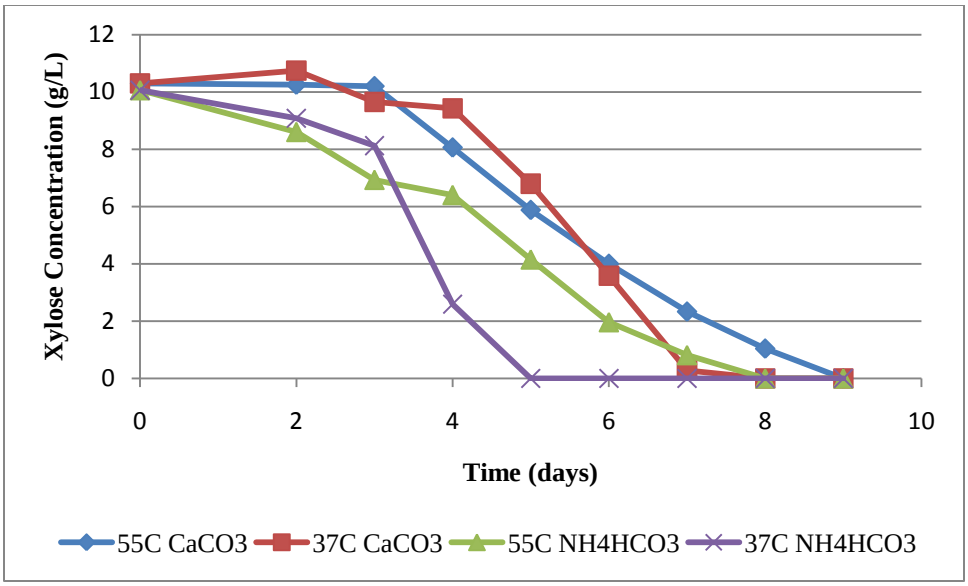


Figure 3-5: Xylose consumption at four different conditions.

The total glucose consumption was much faster than that of the xylose in all the conditions. The glucose consumption took place in a period of 2-4 days whereas the xylose was consumed for a minimum of 5 days to a maximum of 9 days.

3.3.2. Methane Production During the Fermentation Process

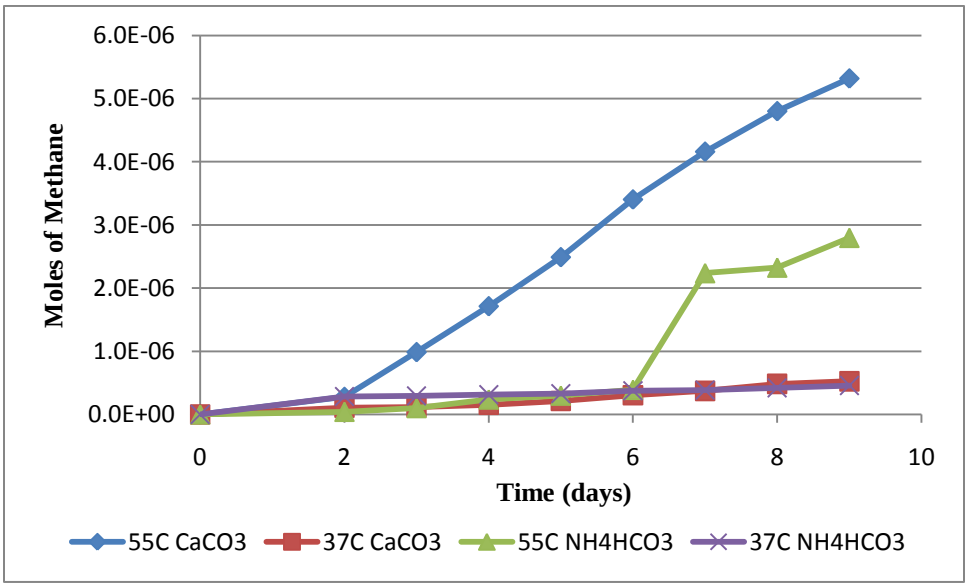


Figure 3-6: Total methane production at four fermentation conditions on pure sugars.

The total methane were measured on a daily basis. The methane was produced by converting acids to methane gas with the help of methanogen organisms. To restrict the methane production, iodoform was prepared ahead of time and was added when ever the methane production was observed to rise to levels above 0.001 moles. In all experiments, it was observed that methane production was very low and appeared to give little or no response to the addition of iodoform as a methane inhibitor.

3.3.3. $CaCO_3$ as a Buffer

3.3.3.1. Summary of Previous Findings

Prior to experiments on extracts, a preliminary experiment was performed with pure sugars. It was found that the total lactic acid content produced was the maximum possible: about 85% of the total sugar supplied as a substrate for the fermentation process. For a total sugar concentration of 20g/L about 16g/L of lactic acid was produced. This result was unexpected, as the literature typically reports accumulations of aliphatic acids that contain no alcohol groups, such as acetic, propionic, butyric and other straight chain acids up to a length of C_7. It was decided to repeat this experiment and also compare the effects of temperature and buffer type on the fermentation of pure sugars.

3.3.3.2. $CaCO_3$ Buffered Sugar Fermentation at 55°C

Fermentation of pure sugars was performed at 55°C to confirm the ability to consume both glucose and xylose, establish a baseline performance of the mixed microbes for total acid production and to explore the optimum fermentation conditions. It

was known from the literature that these mixed microbes had been used on a pretreated biomass but performance on a hemicellulose extract had not been demonstrated.

3.3.3.2.1. Sugar Consumption and Product Formation

The pure sugars glucose and xylose were fermented under anaerobic conditions. The results above show that the glucose was consumed within a period of 2- 4 days(figure 3.4), whereas the xylose was consumed more slowly over a period of 3-8 days in (figure 3.5). With the calcium carbonate as a buffer we can observe that the amount of lactic acid produced was much more than the anticipated acid yield (figure 3.7). The total lactic acid produced was between 140 to 180% of the initial sugars present, which was higher than the anticipated value and violates the mass balance. A potential explanation of this higher volume of lactic acid is discussed in appendix F showing that the volatile solids present in the inoculum might be a source for additional lactic acid production. The pH during the fermentation equilibrated to a value below pH 5.5, which appears to be a condition selective for lactic acid production (figure 3.7). Addition of excess calcium carbonate buffer did not raise the pH to a higher level. From this result it is hypothesized that the decrease in pH played a major role in the production of higher volumes of lactic acid than the aliphatic acids.

Figure 3.7 shows the accumulation of fermentation products at 55°C with calcium carbonate buffer. We can see that formic acid, ethanol and carboxylic acids are being produced in small quantities relative to lactic acid. The total formic acid produced was a bit greater than other acids.

The total ethanol production was measured to be in between 0.6g/L to 2g/L. On the 3rd day about 50µL of 20g/L iodoform dissolved in ethanol was added to inhibit

methanogens. After this addition an accumulation of ethanol was observed in the fermentation process. The iodoform addition resulted in an extra source of ethanol, contributing about 0.4g/L to the total ethanol accumulation.

The different non-lactic acids produced during this fermentation were observed to be between a minimum of 1g/L to a maximum of 2 g/L. which was is a low yield for the fermentation process. The pH appeared to play a major role in producing less carboxylic acid and thereby enabling increases in the lactic acid content during fermentation. Addition of any additional $CaCO_3$ buffer could not bring the pH to neutral condition. This appeared to be due to the weak buffering capacity of the $CaCO_3$ and possibly also the rate of fermentation exceeding the rate of buffer solubilization.

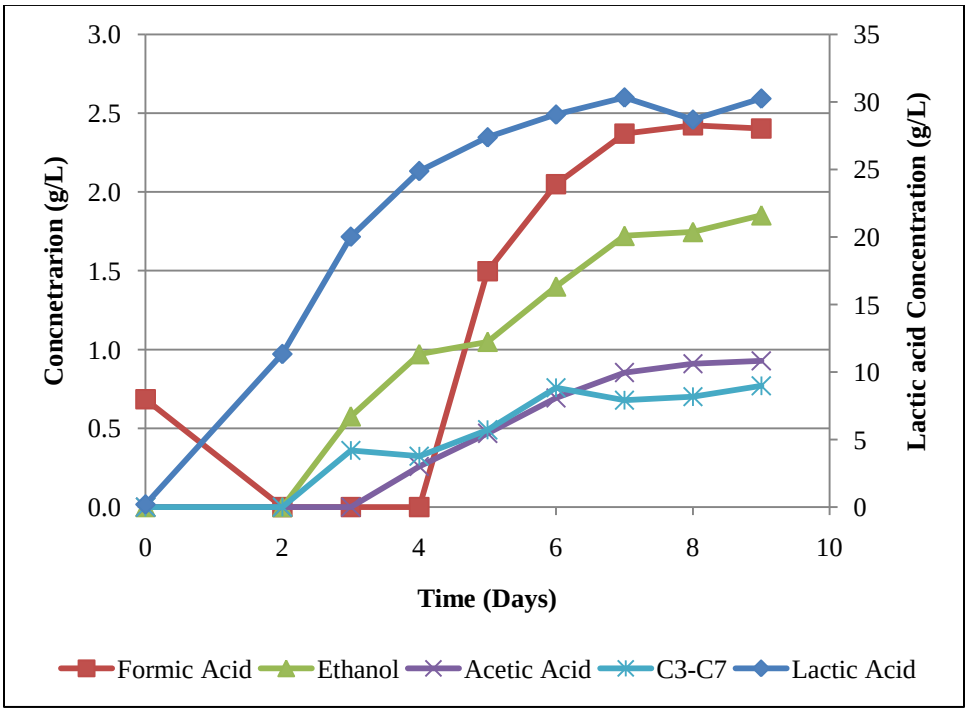


Figure 3-7: Production of ethanol, aliphatic acids and lactic acid at 55°C with calcium carbonate buffer on pure sugar fermentation.

3.3.3.2.2. CO_2 Production and pH Effects

The total carbon dioxide produced were measured on a daily basis. Figure 3.8 shows that the total CO_2 produced was between 0.012 to 0.015 moles.

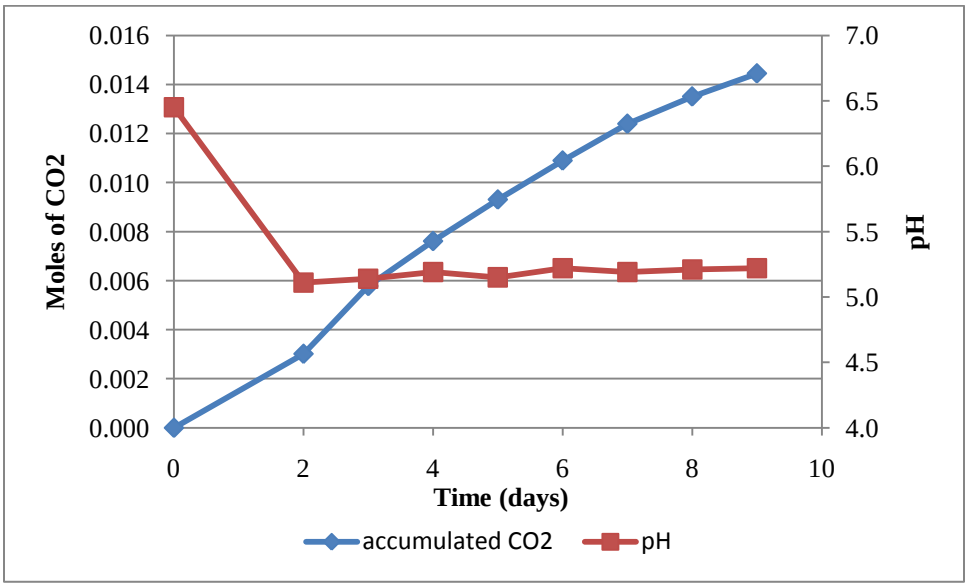


Figure 3-8: CO_2 production by sugar fermentations at 55°C, buffered with $CaCO_3$.

The total carbon dioxide produced was the accumulation of both the CO_2 produced during the neutralization called abiotic and the CO_2 produced during the fermentation process called biotic. The neutralization of the acids are performed by the buffer added at the initiation of the experiment. Approximately about 4 grams of $CaCO_3$ as a base is being added to each of the bottles. The $CaCO_3$ as a buffer cannot keep up the pH at the desired pH conditions. Calculated quantities of abiotic CO_2 are inconsistent as they exceeded the total amount of measured CO_2, resulting in a net biotic CO_2 having negative values, suggesting that CO_2 was consumed. This result is likely an outcome of the impossibly high lactic acid measurements made, which in turn increase the volume of abiotic CO_2 calculated. Thus the output data values made it difficult to approximate the total biotic CO_2 production.

3.3.3.2.3. Product Yield

The total acid production yield was almost double the theoretical product yield (Sara Walton, 2009), and as mentioned above, had a high concentration of lactic acid formation due to a low pH condition. The total product yield was close to 180%, which was 2.5 times more than the expected yield. This might be due to presence of unidentifiable organics added along with the inoculum.

3.3.3.3. $CaCO_3$ Buffered Fermentation at 37°C

The fermentation of pure sugars glucose and xylose was done prior to the extract fermentation to determine the optimum product yield and better baseline performance of mixed microbial system at mesophilic temperatures. These were performed in a temperature controlled shaking incubator set at 37°C. The experiment was helpful in determining the ability of the buffering by $CaCO_3$ at mesophilic (37°C) conditions.

3.3.3.3.1. Sugar Consumption and Product Formation

The fermentations were carried out on glucose and xylose under anaerobic conditions. The results from figure 3.4 show that the glucose was completely consumed in a 3- 4 day period and from figure 3.5, the xylose was completely consumed in a 7-8 day period. The use of calcium carbonate as a buffer has less capability of maintaining the pH neutral at the initial stages of the experiment, but later a steady increase in pH was observed (figure 3.10).

On the 3rd day about 50μL of 20g/L iodoform dissolved in ethanol was added to inhibit methanogens. After this addition an accumulation of ethanol was observed in the

fermentation process (figure 3.9). The iodoform addition resulted in an extra source of ethanol, contributing about 0.4g/L to the total ethanol accumulation. The amount of ethanol produced by fermentation was negligible, and any further additions were consumed during the fermentation process. There is no evidence of production of formic acid or ethanol at a later period.

From figure 3.9 it can be seen that the total carboxylic acid produced during the mixed microbial fermentation with calcium carbonate as a buffer at mesophilic condition was measured to be between 9.2g/L to 11.3g/L. The lactic acid produced at the beginning of the fermentation was consumed in the later phase of the fermentation process. It appears to have replaced glucose as a carbon source once the glucose was consumed. Figure 3.9 shows that the lactic acid formed by bioconversion of glucose by mixed microbes was later consumed and converted to aliphatic acids, evidenced in particular by an increased production of butyric acid.

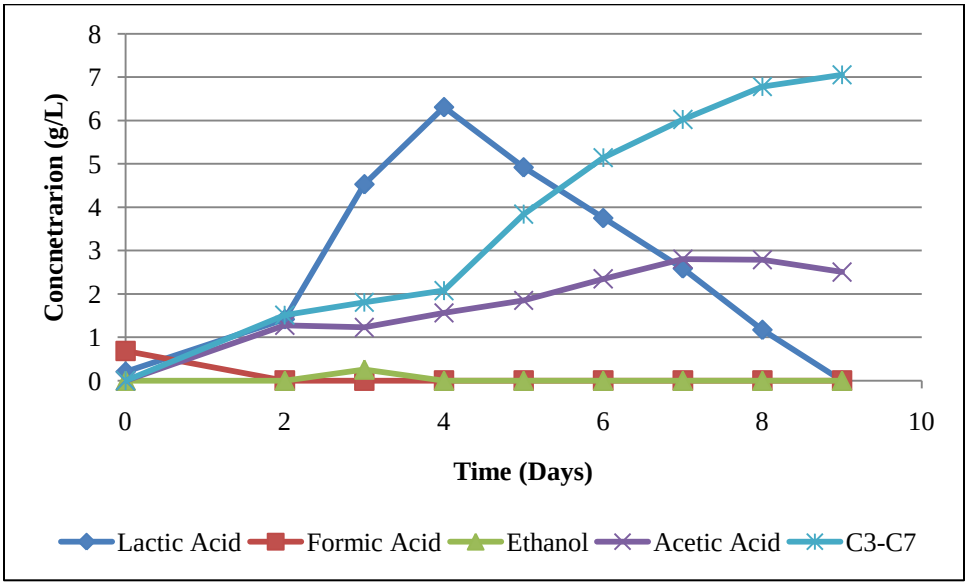


Figure 3-9: Production of ethanol, aliphatic acids and lactic acid at 37°C with calcium carbonate buffer on pure sugar fermentation.

The pH also played a major role in the production of carboxylic acids. From figure 3.10 we can observe that there was an increase in the pH from the 7th day of the fermentation, after the total sugar sources were completely depleted. It can be seen that there also appears to be a slight decrease in acetic acid with concomitant increase in C3 – C7 acids, which may have contributed to the increase in pH. Alternatively, it could have been simple reduction in fermentation rate which resulted in better buffering of the acids.

3.3.3.3.2. CO_2 Production and pH Effects

Methane and carbon dioxide are the 2 major gas constituents produced during the fermentation process. These were measured on a daily basis before liquid samples were prepared for further analysis.

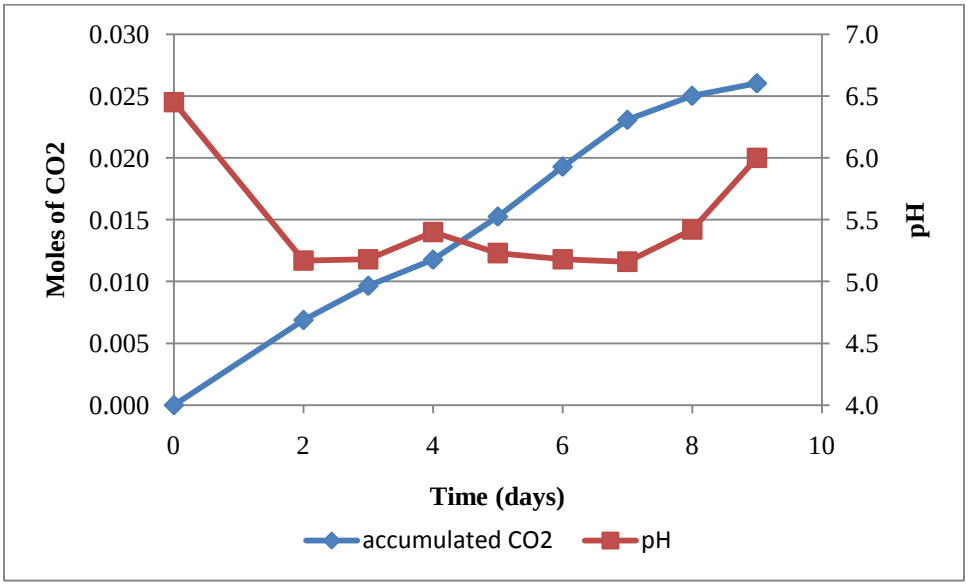


Figure 3-10: Gas production by sugar fermentations at 37°C, buffered with $CaCO_3$.

The results from figure 3.10 show that the total carbon dioxide produced was between 0.024 to 0.028 moles. This shows that as the fermentation progressed, CO_2 was released at a relatively steady rate from the fermentation broth. About 4 grams of calcium

carbonate was added to a volume of 100ml of media at the start of the experiment. The calcium carbonate could only maintain a low pH at the initial stage of the fermentation but later could reach up to the desired pH level of pH 6.0. CO_2 is released as soon as acids are produced and become neutralized by the buffer. It can be seen in figure 3-10 that as the CO_2 production slows down toward the end of the fermentation, the pH rises, suggesting that much of the CO_2 production during the fermentation was abiotic and tied to the low pH conditions requiring neutralization. The total biotic CO_2 produced was in the range of 0.017 to 0.021moles.

3.3.3.3.3. Product Yield

The final products produced were all aliphatic carboxylic acids and the yield ranged from 46% to 55% to the total substrate supplied during the fermentation process.

3.3.4. NH_4HCO_3 as a Buffer

In half of the sugar conversion experiments, ammonium bicarbonate was used as a buffering agent instead of calcium carbonate. Literature studies show that the ammonia present in ammonium bicarbonate acts as a methane inhibitor, inhibiting the methanogens and thus restricting the formation of methane gas (Kayhanian, 1999; Ryoh Nakakubo *et al*., 2007). Also, ammonium bicarbonate was much more soluble in water compared to calcium carbonate and could maintain a higher pH. Ammonium bicarbonate was therefore preferred as a good buffering source when compared to calcium carbonate.

3.3.4.1. NH_4HCO_3 Buffered Sugar Fermentation at 55°C

3.3.4.1.1. Sugar Consumption and Product Formation

The fermentations were carried on glucose and xylose under anaerobic conditions. These fermentations were performed in a temperature controlled shaking incubator at 55°C. Figure 3.4 shows that the glucose was completely consumed in a 3- 4 day period. but the xylose consumption continued for a period of 7-8 days (figure 3.5). The use of ammonium bicarbonate as a buffer helped to maintaining the pH at neutral, but later in the fermentation a slight increase in pH was observed (figure 3.12).

On the 3rd day about 50μL of 20g/L iodoform dissolved in ethanol was added to inhibit methanogens. The iodoform addition resulted in an extra source of ethanol, contributing about 0.4g/L to the total ethanol accumulation (figure 3-11). The amount of ethanol produced by fermentation was negligible, and any further additions were consumed during the fermentation process. There is no evidence of production of formic acid or ethanol at a later period.

Figure 3.11 shows that the initial lactic acid was produced during a period of using glucose as a sole carbon source, since when the glucose was depleted the organisms started converting the lactic acid to form other acids. The glucose consumption was complete by day 4, producing a maximum concentration of lactic acid. Afterwards, on day 5 the lactic acid concentration started decreasing with concurrent increase in C3-C7 acids, primarily butyric acid.

The formic acid also follows the same trend as the lactic acid in this case, and thereby increasing the carboxylic acid concentration. By the last day of the fermentation

the lactic acid and formic acid had apparently been converted to C2- C7 aliphatic carboxylic acids.

Ethanol was also formed during the fermentation process, which might be used as a carbon source at a later stage in the fermentation. The total ethanol produced was about 3g/L. which was too dilute to be commercially recoverable.

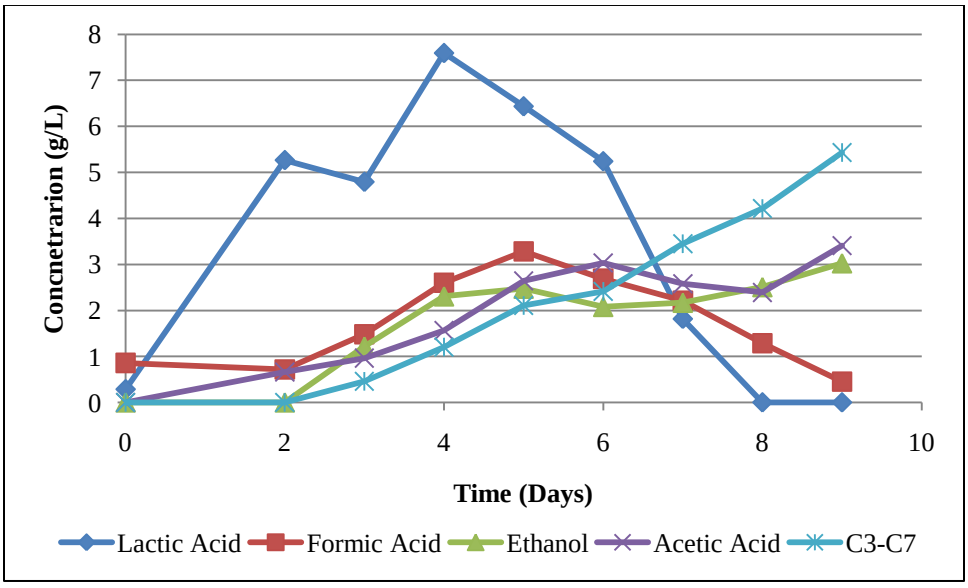


Figure 3-11: Production of ethanol, aliphatic acids and lactic acid at 55°C with NH_4HCO_3 buffer on pure sugar fermentation.

By the 10th day of the fermentation process the total carboxylic acid contenthad risen appreciably. The amount of acetic acid produced was almost equivalent to the other acids produced. The total concentrations of carboxylic acids were between 8.5g/L to 11.6g/L.

The pH of the fermentation process was maintained at near neutral. Whenever a decrease in pH was observed a further addition of ammonium bicarbonate helped in maintaining the neutral pH for better microbial growth and optimum product formation. Figure 3.11 shows the daily measured pH of the fermentation.

3.3.4.1.2. CO_2 Production and pH Effects

From figure 3.3 the total methane gas produced during this process was very little when compared to the total CO_2 produced.

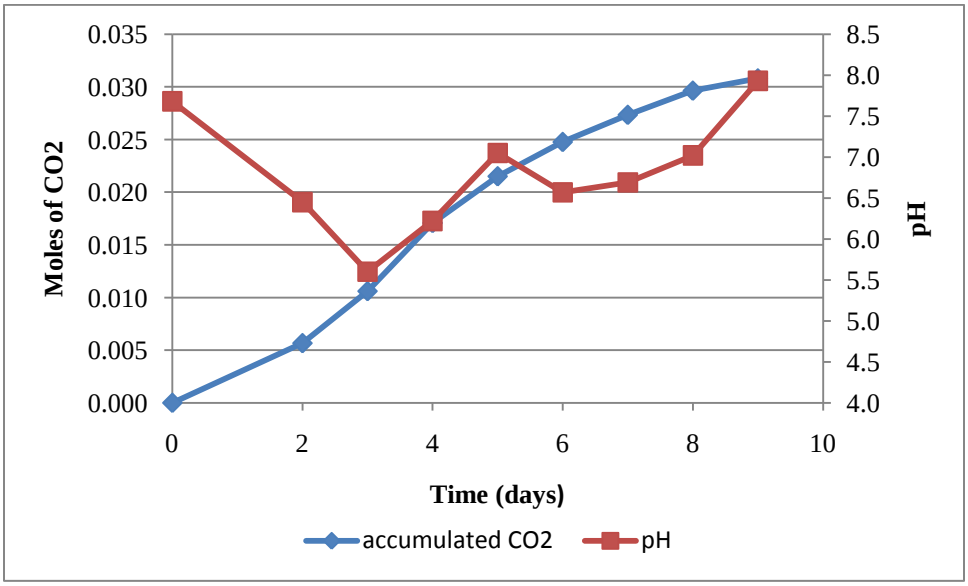


Figure 3-12: Gas production by sugar fermentations at 55°C, buffered with NH_4HCO_3.

Figure 3.12 shows the total CO_2 production during the fermentation process, measured to be around 0.024 – 0.03moles. This indicates that much product formation and neutralization with carbonate has been observed. Ammonium bicarbonate of about 0.5g was added on the day 3 and 0.5g was added on day 4 as to raise the pH to the required neutral pH of 6.0. Due to the addition of the ammonia buffer the pH was brought to the neutral. It can also be observed that on days 3 and 4 CO_2 was evolved at a faster rate than on subsequent days, suggesting large contributions from abiotic CO_2 after buffer addition.

The total biotic and abiotic carbon dioxide produced during the fermentation and neutralization process was calculated based on the acid concentration. The total biotic

CO_2 produced was in the ranges of 0.008 to 0.019 moles and the balance was abiotic produced from the neutralization of the total volume of acids present in the reactor.

3.3.4.1.3. Product Yield

The total products produced consisted primarily of carboxylic acids and the production was around 40% to 55% to the total substrate supplied during the fermentation process.

3.3.4.2. NH_4HCO_3 Buffered Sugar Fermentation at 37°C

3.3.4.2.1. Sugar Consumption and Product Formation

The fermentations carried out on pure sugars of glucose and xylose show that the glucose was completely consumed by the 2nd day of the fermentation (figure 3.4), which resulted in a sudden increase in the lactic acid concentration. Subsequent to completion of glucose consumption, the production of lactic acid was slowed down and then later consumed as a carbon source for the production of other acids (figure 3-13). The decrease in lactic acid concentration coincided with an increase in the total carboxylic acid concentration.

From figure 3.5 the xylose fermentation continued over a period of 5 days where the utilization was slow during the period that glucose was present but by the 3rd day the xylose consumption rate was increased dramatically. There was some sign of the formation of formic acid during the fermentation process, but this was ultimately consumed.

Figure 3.13 reveals that the total amount of ethanol production was drastically more when compared with the other buffers at varied temperatures. Of the four replicate samples, the maximum concentration of ethanol produced was 8.1g/L and the least was around 1.75g/L. The iodoform addition resulted in an extra source of ethanol, contributing about 0.4g/L to the total ethanol accumulation.

The changes in the concentration of ethanol during the fermentation process also have a big effect on the carboxylic acid concentration. The higher the ethanol concentration, the lower the total carboxylic acid concentration and vice versa, which is expected from the limited carbon available. It appears that after the lactic acid was consumed, the ethanol concentration started to slowly decline, indicating possible consumption of ethanol to yield more aliphatic acids. The major products produced in the carboxylic acids were acetic acid and butyric acids. Both of these constitute the maximum acid concentration in the products.

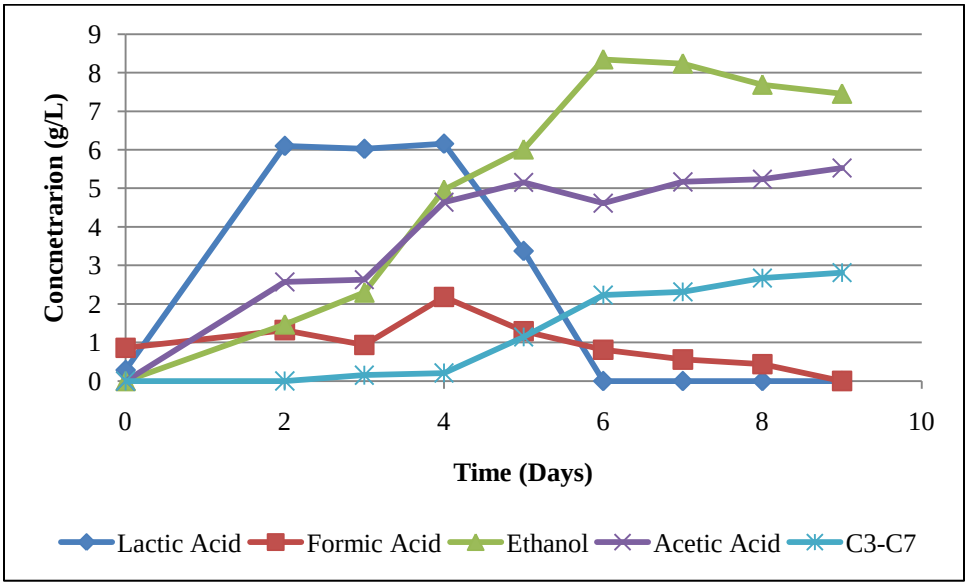


Figure 3-13: Production of ethanol, aliphatic acids and lactic acid at 37°C with NH_4HCO_3 buffer on pure sugar fermentation.

The changes in the pH were not much altered since ammonium bicarbonate helped in maintaining the optimum growth condition for the mixed microbial cultures. Any decrease in the pH was brought back to neutral by further adding ammonium bicarbonate to the broth. A steady increase in the pH paralleled an increase in the ethanol concentration, as can be observed from figure 3.13. The trend in the pH can be observed from figure 3.14.

3.3.4.2.2. CO_2 Production and pH Effects

On day 3 about 0.5 grams of ammonium bicarbonate was added to the fermentation bottle to bring back the pH to the desired level of pH6.0 for a good fermentation process. The increase in the addition of the buffer triggered an increase in the pH and also increased the total abiotic CO_2 production.

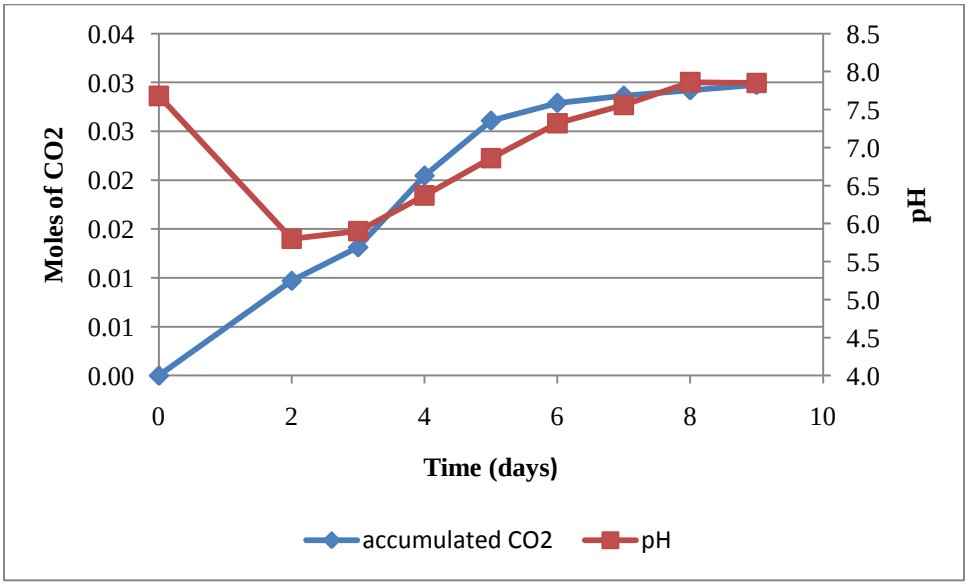


Figure 3-14: Gas production by sugar fermentations at 37°C, buffered with NH_4HCO_3.

The total carbon dioxide production was measured to be around 0.027 – 0.03 moles which shows more amounts of acids being formed during the fermentation process.

The total biotic CO_2 produced was in the ranges of 0.007 to 0.018 moles and the balance was abiotic produced from the total volume of substrate present in the reactor.

3.3.4.2.3. Product Yield

The total carboxylic acids produced during the fermentation process were around 40% to 68% to the total substrate supplied during the fermentation process. And the total product formation (carboxylic acids and ethanol) was around 77% to 93% of the total substrate supplied during the fermentation process.

A table summarizing results from the sugar fermentations is found below in the discussion section.

3.4. Hot Water Extract Fermentation

Hot water extract was collected from the pulp and paper processing unit of the University of Maine. The hot water extract was derived from aspen wood cooked at 160°C to attain an H-factor of 380hrs. This hardwood extract has xylose as a major constituent of the hemicellulose sugars. The table 4.5 represents the total sugars present in the unhydrolyzed and acid hydrolyzed hot water extract. It has very little nutrient content and adding an external nutrient source appeared to be necessary a good fermentative substrate for production of fuels and chemicals. The external nutrient source used in this experiment was corn steep liquor added at a concentration of 5g/L to the extract. The use of calcium carbonate as a buffer was insufficient for maintaining an optimum pH of above pH5.8 for microbial growth. Use of ammonium bicarbonate as a buffer generated higher production of non-lactic acids as it could maintain the optimum pH and could also contribute to inhibiting the methanogenisis. All the experimental conditions were performed in duplicate conditions. The iodoform added was dissolved in ethanol. The samples which did not have iodofrom added were supplemented with ethanol so that the total ethanol added to the fermentation bottles would be same. The addition of iodoform did not show any difference in the total product output as there was negligible methane produced in all of the bottles.

Compared to aseptic fermentations, the uses of mixed microbial cultures, in which each organism contributes a specific task or function to the overall process of breaking down biomass in an anaerobic environment, might help in producing useful fuels and chemicals with less capital cost. In addition, mixed cultures are not expected to require

preliminary hydrolysis of oligomeric-sugars since these cultures are capable of producing their own auto hydrolytic activity.

Table 3-2: Total sugar concentration in raw and acid-hydrolyzed hot water extract

Sample name	pH	Glucose	Xylose	Lactic Acid	Formic Acid	Acetic Acid
unhydrolyzed hot water	3.33	0.12	1.28	0.34	1.62	3.056
acid hydrolyzed hot water	1.00	1.54	25.29	0.77	0	9.57

Table 3-3: Matrix table for different experimental conditions on hot water extract for optimum product output.

Substrate: Hot water extract, corn steep liquor 5 g/L **Inoculum source: original (unadapted) marine inoculum**			
condition	**temperature**	**buffer**	**iodoform**
Fermentation	55°C	NH_4HCO_3	No
	55°C	NH_4HCO_3	Yes
	37°C	NH_4HCO_3	No
	37°C	NH_4HCO_3	Yes

3.4.1. Fermentative Autohydrolysis

Figure 3.15 presents the accumulation of xylose during a fermentation of hot water extract. As the fermentation progresses, xylo-oligomer sugars are broken down to simple mono sugars. While the released xylose is initially converted during the early stages of the fermentation, as fermentation slows down, the hydrolyzed xylose accumulates in the fermentation broth.

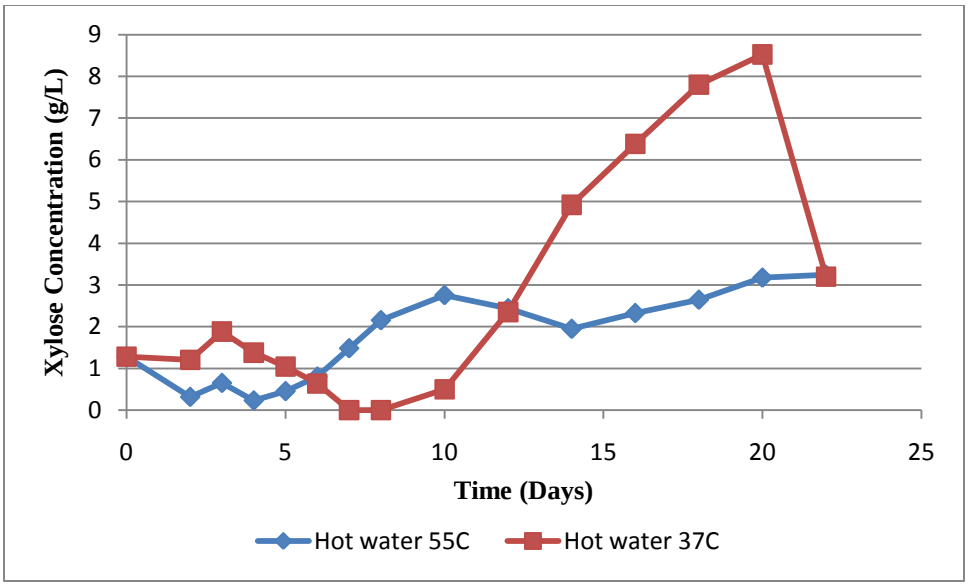


Figure 3-15: Accumulation of oligo-xylose to mono-xylose sugar at 55°C with NH_4HCO_3 buffer

3.4.2. Methane Production During Fermentation Process

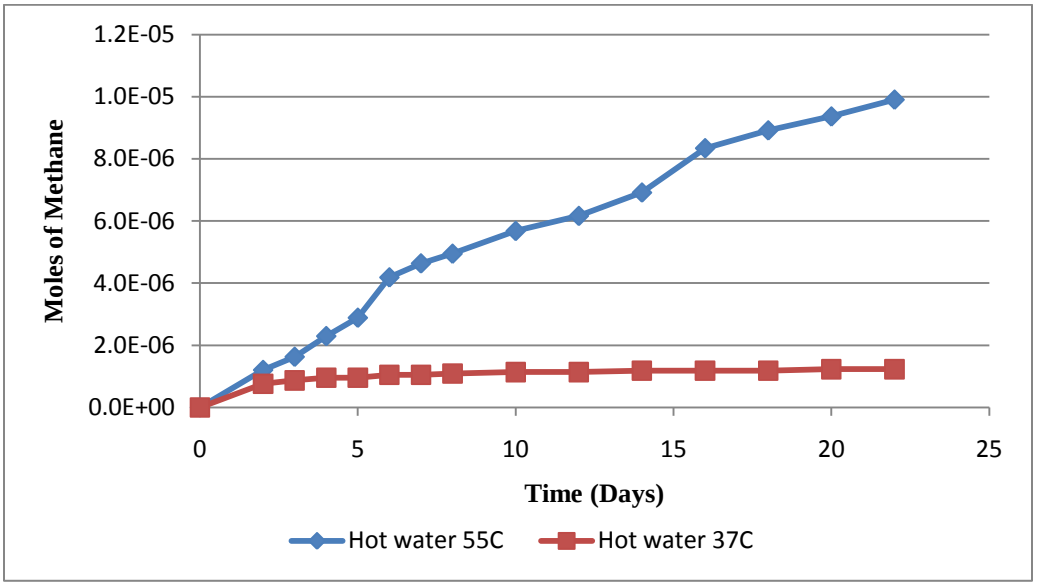


Figure 3-16: methane production during fermentation process at two different temperatures

The total methane produced during the fermentation process was negligible as the fermentation process proceeded much faster and also ammonium bicarbonate itself acts as a methane inhibitor. The figure 3.16 shows that the methane was produced but the

values are very low and can be neglected. On the other hand if we happened to see any increase in methane production during fermentation process, an addition of the methane analog iodoform was used to inhibit the methanogenesis process.

3.4.3. Hot Water Extract Fermentation at 55°C

These experiments were performed to determine the optimum operating conditions and the maximum product output at thermophilic condition using ammonium bicarbonate as a buffer.

3.4.3.1. Sugar Consumption and Product Formation

The unhydrolysed raw hot water extract has an initial acetic acid concentration of 3 g/L. After a prolonged period of fermentation for about 3 weeks the total acetic acid accumulated reached a maximum of about 9 to 13g/L. Of the total acetic acid concentration, 9.5 g/L of acid was attributed to acetate release from the acetylated oligo-xylose sugar complex (table 3.2). The balance was produced through conversion of carbohydrate to acetic acid. The complete acetic acid concentration is shown in the figure 3.17.

The production of lactic acid was also observed during the fermentation process. The total lactic acid produced was between 7-10g/L. The amount of lactic acid produced during this process was much higher than the other acids.

The amount of ethanol and formic acid formed are much smaller when compared to other acids formed during fermentation process. The accumulation of ethanol is due in part to the addition of methane inhibitor dissolved in ethanol. The iodoform addition

resulted in an extra source of ethanol, contributing about 0.8g/L to the total ethanol accumulation.

There wasn't much significant production of C3 – C7 carboxylic acids. The quantities of these are produced in minor levels and there was not much further increase even as the fermentation process was prolonged for a longer period of time.

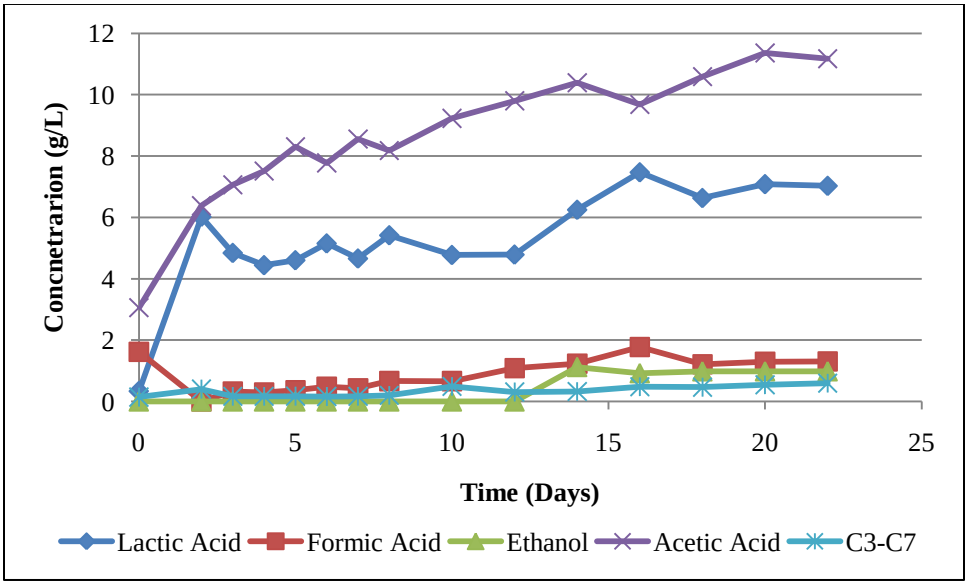


Figure 3-17: Production of ethanol, aliphatic acids and lactic acid at 55°C with NH_4HCO_3 buffer on hot water extract fermentation.

The pH of the fermentation process was continuously monitored on a daily basis. As the pH was noted to be decreasing, addition of ammonium bicarbonate was done to attain an optimum pH condition for a better microbial growth and product formation. The changes in the pH condition during the fermentation process are shown in figure 3.18.

3.4.3.2. CO_2 Production and pH Effects

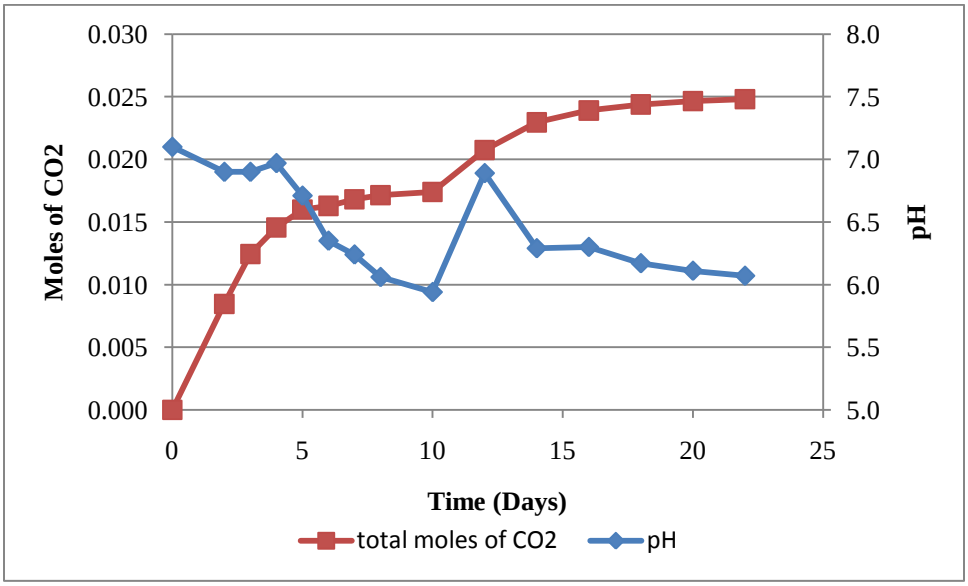


Figure 3-18: Gas production by fermentations of hot water extract at 55°C, buffered with NH_4HCO_3.

The total amount of CO_2 produced during the fermentation process included the biotic and the abiotic CO_2. The total carbon dioxide formed during the process was between 0.025 -0.027moles. Much of this might be abiotic CO_2 since the majority of the accumulated acetate is thought to be derived from hydrolysis of the xylan instead of fermention of sugars to acids.

The biotic CO_2 was the total gas derived from the metabolism of fermentation. The CO_2 produced from neutralizing the accumulated acids is referred to as abiotic CO_2. The total abiotic CO_2 was much greater than the biotic CO_2 since there was substantial accumulation of fermentation and hydrolysis derived acids. The total biotic CO_2 produced during the fermentation process was about 0.007 – 0.015 moles.

To maintain the optimum pH for the better microbial growth about 0.5 gms of NH_4HCO_3 was added on the day 10^{th} to the fermentations at both. It can be seen that this addition triggered a surge of CO_2 production as a result of rising pH.

3.4.3.3. Product Yield

The average product yield in fermenting the hot water extract at thermophilic conditions with ammonium bicarbonate as a buffer was about 71%, of which lactic acid was the predominant product of the fermentation process.

3.4.4. Hot Water Extract Fermentation at 37°C

Hot water extract was fermented using the mixed microbial cultures at mesophilic conditions with ammonium bicarbonate as a buffer. These experiments were performed to estimate the types of acids and the total product concentrations produced and to determine a baseline performance for pilot scale fermentation processes.

3.4.4.1. Sugar Consumption and Product Formation

The unhydrolysed raw hot water extract had an initial acetic acid concentration of 3 g/L. After a prolonged period of fermentation for about 3 weeks the total acetic acid accumulated reached to a maximum of about 10 to 16.5g/L of acid being produced. Of the total acetic acid produced, it is estimated that 9.5 g/L of acid was released from the acetylated oligo-xylose sugar complex. The rest derived from the conversion of hemicelluloses sugars to acetic acid. The total acid concentrations are shown in figure 3.19.

The production of lactic acid was also observed during the fermentation process. The total lactic acid produced was below 2g/L. The amount of lactic acid produced during this process was high at times and later it was further consumed and converted to other fermentation products.

The amount of ethanol and formic acid formed are a bit higher when compared to the results of the hot water extract fermentation at 55°C. The accumulation of ethanol might be due to the addition of methane inhibitor dissolved in ethanol. The total ethanol accumulation was recorded to be around 3- 4g/L and the formic acid concentration was recorded around 4.6- 7.2 g/L. The iodoform addition resulted in a contributing source of ethanol, contributing about 0.8g/L to the total ethanol accumulation.

There was a significant production of C3 – C7 carboxylic acids. The majority of these acids are produced at minor levels, but propionic and butyric acids contributed significantly to the total acid accumulation. The data values for the distribution of C3 – C7 acids are presented in appendix E.

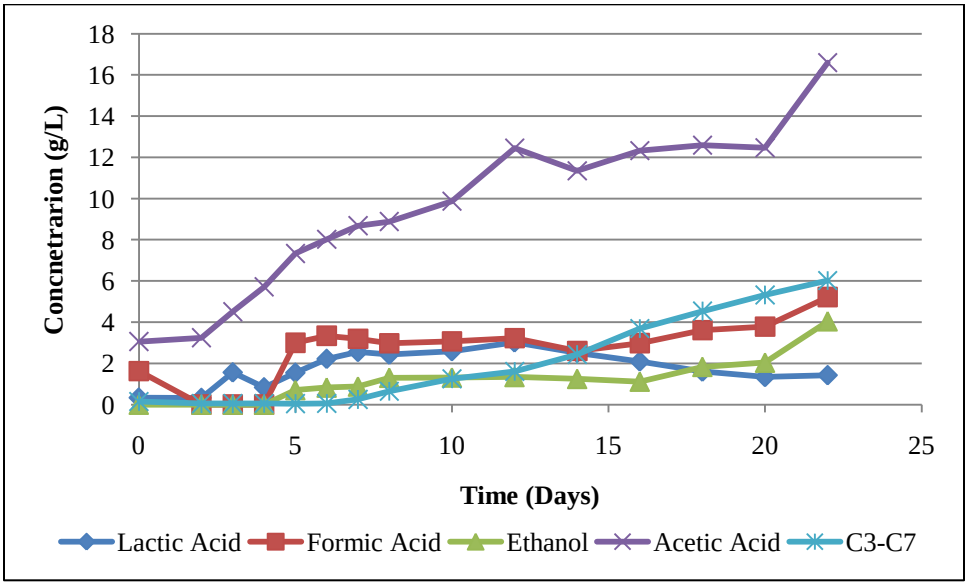


Figure 3-19: Production of ethanol, aliphatic acids and lactic acid at 37°C with NH_4HCO_3 buffer on hot water extract fermentation.

The pH of the fermentative system was maintained at near neutral. Whenever a decrease in pH was observed, addition of ammonium bicarbonate helped in maintaining the fermentation process at optimum microbial growth conditions at above pH 6.0. The changes in the pH condition during the fermentation process are shown in figure 3.20.

3.4.4.2. CO_2 Production and pH Effects

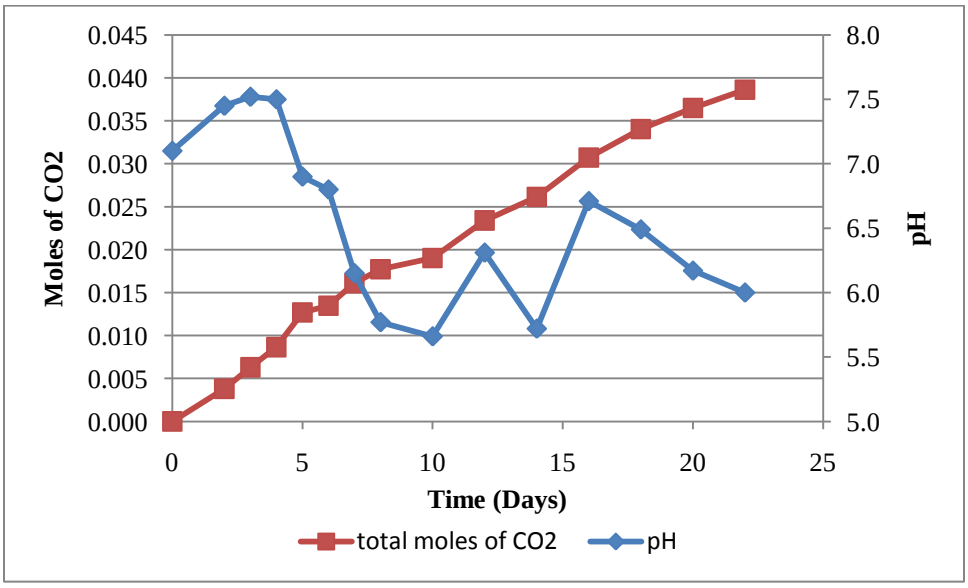


Figure 3-20: Gas production by fermentations of hot water extract at 37°C, buffered with NH_4HCO_3.

The total CO_2 produced during the fermentation process was the combination of biotic and abiotic CO_2. The total carbon dioxide formed during the process was between 0.036 - 0.039 moles. The total biotic CO_2 produced during the fermentation process was about 0.003 – 0.010 moles.

To maintain the optimum pH for the better microbial growth about 0.5 gms of NH_4HCO_3 was added on the day 10^{th} to the fermentations and about 0.5 gms of the same buffer was added on day 14^{th} during the fermentation process. In both cases, an increase in CO_2 release resulted after the addition of carbonate buffer.

3.4.4.3. Product Yield

The average product yield in fermenting the hot water extract at mesophilic conditions with ammonium bicarbonate as a buffer was about 84%, of which the fermentation derived acetic acid, propionic acid and butyric acid are the predominant product in the fermentation process. The data values are presented in appendix E.

3.5. Green Liquor Extract Fermentation

Green liquor extracts used in this experiments were collected from the pulp and paper processing unit at the University of Maine. These extracts are obtained by cooking the wood chips in green liquor composed of sodium salts and are cooked at about160°C for about 110min. The amount of hemicelluloses extracted in this process has xylose as the major constituent. Table 4.8 represents the total sugars present in the unhydrolyzed and acid hydrolyzed hot water extract. The nutrient availability in the extract is too low to support robust microbial growth. An external nutrient source was required to enhance microbial growth for producing useful fuels and chemicals. The external nutrient source used in this experiment was corn steep liquor added at a concentration of 5g/L. The use of calcium carbonate as a buffer did not help to maintain the optimum pH condition above pH 5.8, so use of ammonium bicarbonate as a buffer was preferred since it can maintain the optimum pH and also can inhibit methanogenisis. All the experimental conditions are performed in duplicate conditions. The iodoform added was dissolved in ethanol. The samples which did not have iodofrom added were injected with an equivalent amount of ethanol so as to balance the total ethanol added to the different fermentation bottles. The addition of iodoform didn't show any difference in the total product output as there was no methane produced in any of the bottles.

Green liquor extracts collected from the mill or research facility have very low concentrations of hemicelluloses sugars. The use of mixed microbial cultures might help to produce useful fuels and chemicals with lower capital cost than aseptic systems.

Table 3-4: Total sugar concentration in raw and acid-hydrolyzed green liquor extract

Sample name	pH	Glucose	Xylose	Lactic Acid	Formic Acid	Acetic Acid
unhydrolyzed green liquor	4.6	0	0.18	0.52	2.34	11.55
acid hydrolyzed green liquor	1.00	1.06	9.20	1.42	3.80	11.8

The uses of mixed microbial cultures have varied functions and that these are collected as a bulk sample with thousands of microbes, each of which contributes a specific task of function to the ensemble. The use of mixed microbial cultures was preferred so as to eliminate the need for preliminary hydrolysis and to include hydrolysis into the fermentation process where the microbes break down the oligomeric sugars to monomers and further continue the fermentation process.

Table 3-5: Matrix table for different experimental conditions on green liquor extract for optimum product output.

Substrate: Green liquor extract, corn steep liquor 5 g/L **Inoculum source: original (unadapted) marine inoculum**			
condition	**temperature**	**buffer**	**iodoform**
Fermentation	55°C	NH_4HCO_3	No
	55°C	NH_4HCO_3	Yes
	37°C	NH_4HCO_3	No
	37°C	NH_4HCO_3	Yes

3.5.1. Fermentative Autohydrolysis

The figure 3.21 below represents the gradual breakdown of oligomeric xylan to monomeric xylose where the auto enzyme hydrolyzed samples are consumed in the initial stages and later the total mono sugar concentration increases in time as the fermentation slows down.

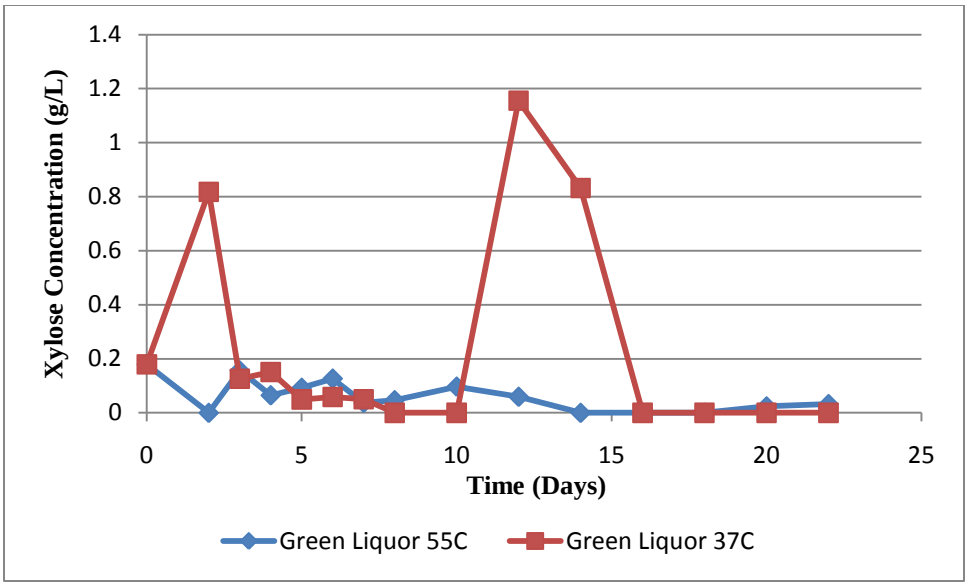


Figure 3-21: Conversion of oligo-xylose to mono-xylose sugar present in hot water extract at 55°C with NH_4HCO_3 buffer**.**

3.5.2. Methane Production During Fermentation Process

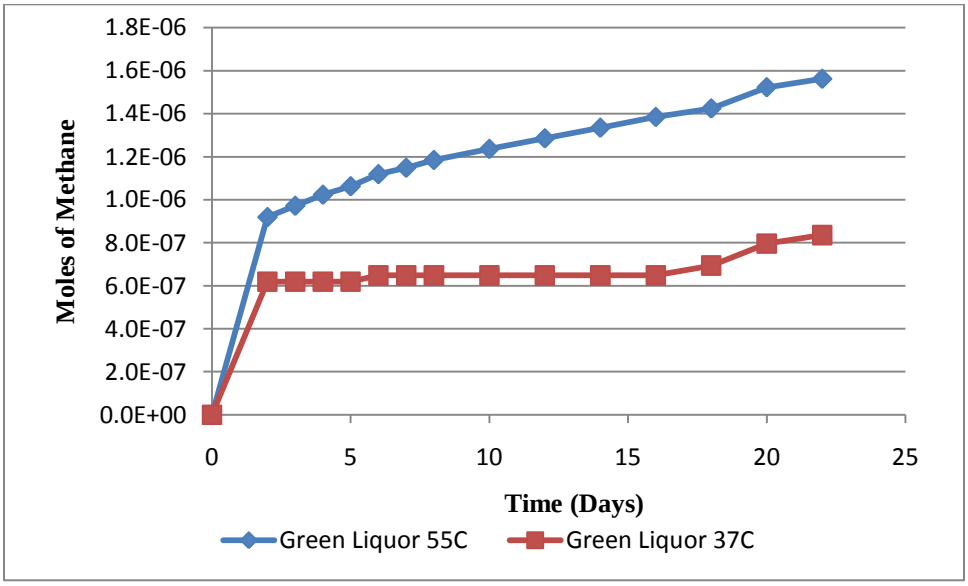


Figure 3-22: methane production during fermentation process at two different temperatures.

The total methane produced during the fermentation process was negligible as the fermentation process was continuously buffered by using ammonium bicarbonate which itself acts as a methane inhibitor. Figure 3.22 above shows that the methane was

produced, but the quantities are very low and can be neglected. On the other hand if any increase in methane production was observed during the fermentation process, an addition of the methane analog iodoform was used to inhibit the methanogenessis process.

3.5.3. Green Liquor Extract Fermentation at 55°C

These experiments were performed to determine the optimum operating conditions and the maximum product output at thermophilic condition using ammonium bicarbonate as a buffer.

3.5.3.1. Sugar Consumption and Product Formation

The unhydrolyzed raw green liquor extract has an initial acetic acid concentration of 11.4 g/L. After a prolonged period of fermentation for about 3 weeks the total acetic acid produced reaches to a maximum of about 12 to 15 g/L. Of the total acetic acid produced, 12 g/L of acid was released from the acetylated oligo-xylose sugar complex (Table 3-4). The rest resulted from the conversion of hemicelluloses to acetic acid. The total acid concentrations are shown in the figure 3.23.

There was no significant evidence of the production of other carboxylic acids ranging from C3 – C7. The quantities of these produced are in minor quantities and even if the experiment was prolonged further higher production may not have been achieved.

The ethanol at the initial extract was negligible, but its production was observed from day 8 and this might be due to the addition of methane inhibitor dissolved in ethanol. The iodoform addition resulted in an extra source of ethanol, contributing about 0.4g/L to the total ethanol accumulation.

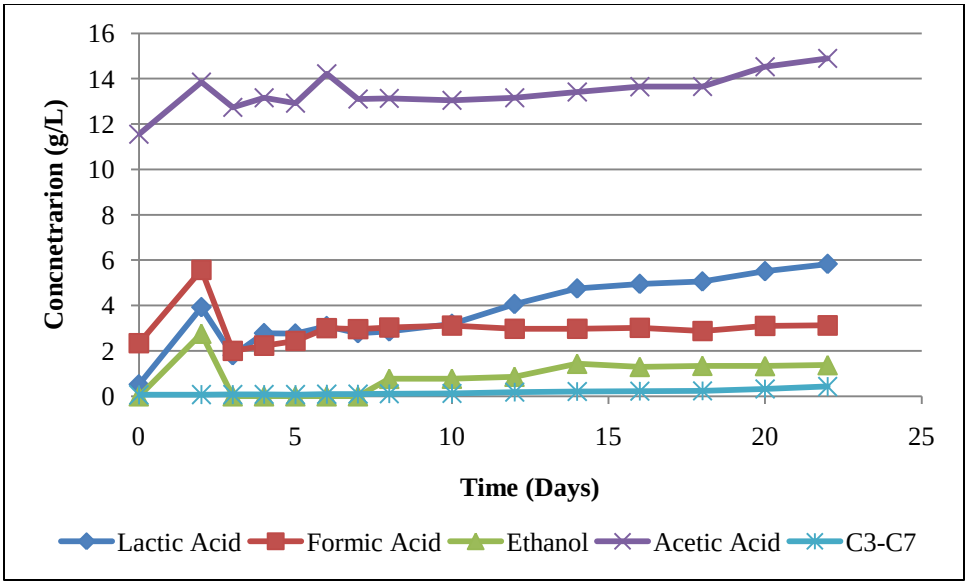


Figure 3-23: Production of ethanol, aliphatic acids and lactic acid at 55°C with NH_4HCO_3 buffer on green liquor extract fermentation.

The amount of formic acid at the outset was almost equal to the final concentration and throughout the fermentation process. There was no further gain or loss in the formic acid production.

Lactic acid production was large when compared with the other products. The total lactic acid produced in this experiments are about 2 – 6 g/L.

The pH of the fermentation process was continuously monitored on a daily basis. When the pH was noted to be decreasing, addition of ammonium carbonate was made to attain an optimum pH condition of pH above 6.0, which is beneficial for microbial growth and product formation. The changes in the pH condition during the fermentation process are shown in figure 3.24.

3.5.3.2. CO_2 Production and pH Effects

The total amount of CO_2 produced during the fermentation process included the release of biotic and abiotic CO_2. The total carbon dioxide formed during the process was 0.02 moles. The total CO_2 content was distributed between the biotic and the abiotic CO_2.

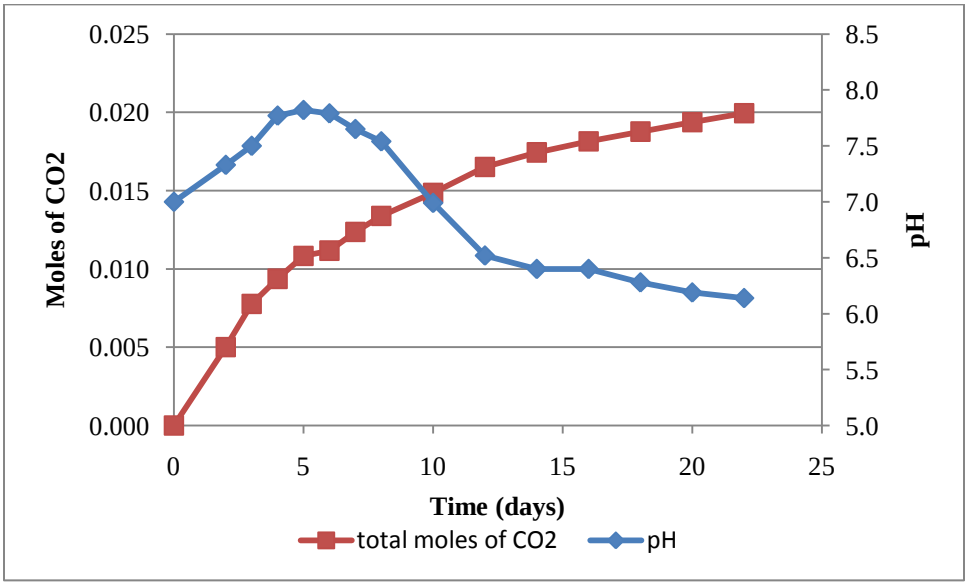


Figure 3-24: Gas production by green liquor extract fermentations at 55°C, buffered with NH_4HCO_3.

The total biotic CO_2 produced during the fermentation process was about 0.007 – 0.012 moles. The relationship between the pH and the CO_2 production can be seen clearly the more the CO_2 produced the decrease in the pH resulting in the neutralization of the acids formed during the fermentation process. There was no buffers added during the process as the pH at the initial increased and later decreased but still was above the expected pH range of 6.0.

3.5.3.3. Product Yield

The average product yield in fermenting the hot water extract at thermophilic conditions with ammonium bicarbonate as a buffer was about 49% of which lactic acid was the predominant product in the fermentation process.

3.5.4. Green Liquor Extract Fermentation at 37°C

Green liquor extract was fermented using mixed microbial cultures at mesophilic conditions with ammonium bicarbonate as a buffer. These experiments were performed to estimate the types of acids and the total product concentrations produced and to create a baseline performance for pilot scale fermentation processes.

3.5.4.1. Sugar Consumption and Product Formation

The unhydrolysed raw hot water extract had an initial acetic acid concentration of 11.4 g/L. After a prolonged period of fermentation for about 3 weeks the total acetic acid produced reached to a maximum of about 16.5g/L of acid. Of the total acetic acid produced, 12 g/L of acid was released from the acetylated oligo-xylose sugar complex. The rest resulted from the conversion of hemicellulose to acetic acid. The total acid concentrations are represented in the figure 3.25 below.

The production of lactic acid was also observed during the fermentation process. The highest lactic acid produced was below 2g/L. The amount of lactic acid produced during this process was high at times and later it was consumed within the system and converted through acid production.

The formic acid production was high at times in the initial period of the experiment but later was consumed for further product formation.

The amount of ethanol was a bit higher when compared to the results of the green liquor extract fermentation at 55°C. The accumulation of ethanol might be due in part to the addition of methane inhibitor dissolved in ethanol. The iodoform addition resulted in an extra source of ethanol, contributing about 0.4g/L to the total ethanol accumulation. The total ethanol accumulation was recorded to be around 3- 4g/L and the formic acid concentration was recorded at a maximum of around 4.6- 7.2 g/L before apparently being consumed.

There was a significant production of C3 – C7 carboxylic acids. The quantities of these are produced in minor levels and the maximum amount of the acids was contributed by propionic and butyric acids. The data values are presented in appendix E.

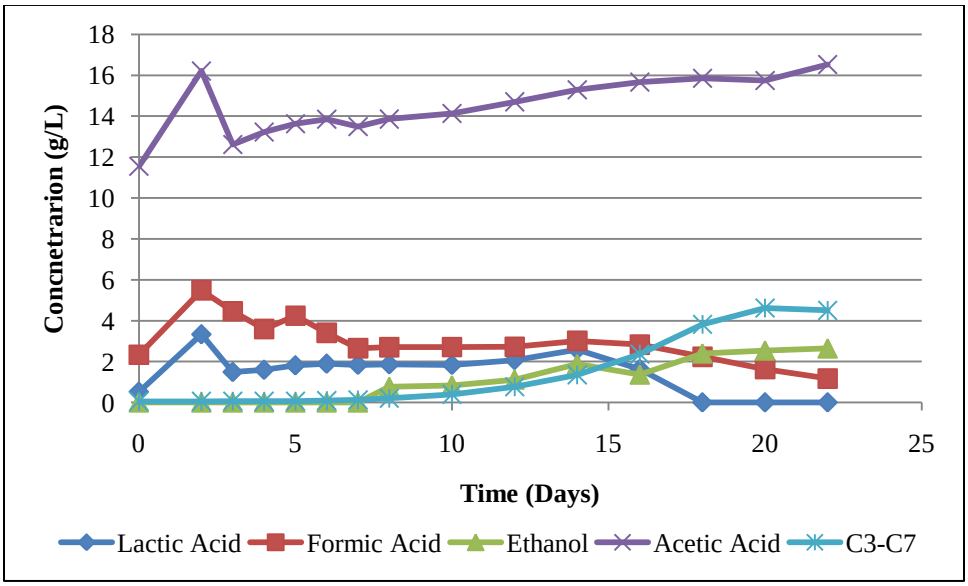


Figure 3-25: Production of ethanol, aliphatic acids and lactic acid at 37°C with NH_4HCO_3 buffer on green liquor extract fermentation.

The pH of the fermentation process was continuously monitored on a daily basis. If the pH was noted to be decreasing, addition of ammonium carbonate was made to attaining an optimum pH condition of pH above 6.0 which is considered beneficial for

microbial growth and product formation. The changes in the pH condition during the fermentation process are shown in figure 3.26.

3.5.4.2. CO_2 Production and pH Effects

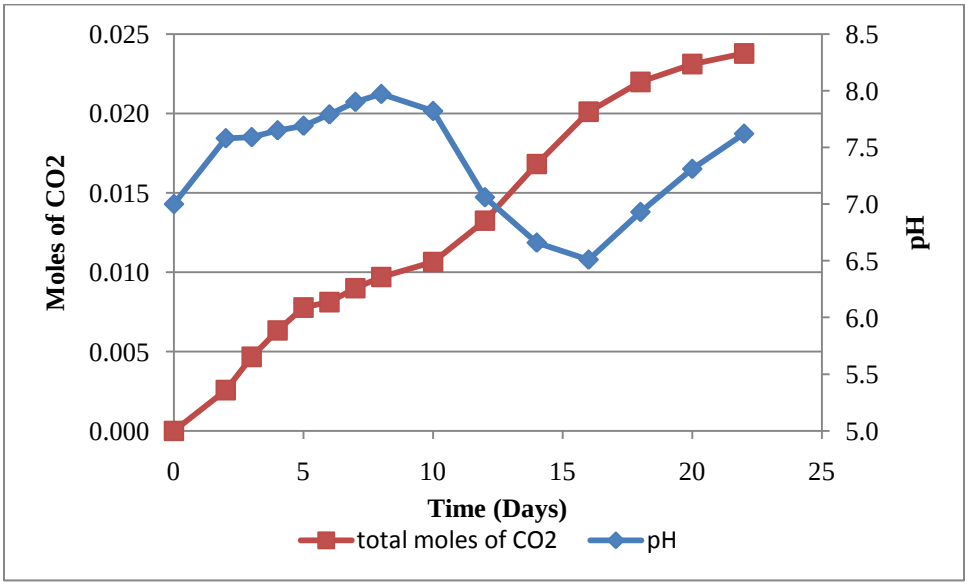


Figure 3-26: Gas production by green liquor extract fermentations at 37°C, buffered with NH_4HCO_3.

The total amount of CO_2 produced during the fermentation process comprised the biotic and the abiotic CO_2. The total carbon dioxide formed during the process was 0.022 to 0.024 moles. The total CO_2 content was distributed between the biotic and the abiotic CO_2. The total biotic CO_2 produced during the fermentation process was about 0.014 – 0.019 moles and the balance was the abiotic CO_2, resulting from the neutralization of the acids bound to the oligomeric sugar chains and the acids produced from fermentation.

The relationship between the pH and the CO_2 production can be seen clearly the more the CO_2 produced the decrease in the pH resulting in the neutralization of the acids formed during the fermentation process. There was no buffers added during the process as the pH at the initial increased and later decreased but still was above the expected pH

range of 6.0. Later the pH of the process was seemed to be increasing from day 16, and this might be due to more accumulation of the neutralized acids formed during the fermentation process.

3.5.4.3. Product Yield

The average product yield in fermenting the hot water extract at mesophilic conditions with ammonium bicarbonate as a buffer was about 64% of which total carboxylic acids are the predominant product in the fermentation process.

CHAPTER 4. DISCUSSION

Selected results from the above presentation of findings are summarized in tables 4-1 and 4-2, below. Table 4-1 shows results from the sugar fermentations, table 4-2 from the wood extract fermentations.

Consumption of Glucose and Xylose: Results from the sugar fermentations clearly show that glucose is a preferred substrate for the mixed cultures, and that xylose consumption is both slower and more lagged than glucose consumption. Interestingly, in some cultures the end of glucose consumption appears to trigger lactate consumption, and lactic acid that was produced from glucose fermentation is subsequently converted to other, and apparently more biostable, organic acids.

Choice of Buffer: In table 4.1 it shows that the pH in fermentations buffered with calcium carbonate was lower than those buffered with ammonium bicarbonate. Results in the literature on calcium buffered fermentations commonly report an operating pH of greater than 6.0, but general trends in product accumulation also appear to be related to the choice of buffer. Compared to their counterparts using ammonium bicarbonate buffer, the calcium carbonate fermentations consumed sugar more slowly, produced less acetic acid and produced more lactic acid. Other products such as formic acid, ethanol and C3-C7 acids showed mixed results between the buffers. The fermentation with calcium carbonate at 55^{o}C was anomalous in several regards—the lactic acid produced was very high, the other acids accumulations were very low, and it was the only one of the three fermentation conditions in which lactic and formic acids were not consumed in later

stages of the fermentation. If comparing only the buffer effects at 37^{o}C, in addition to the trends mentioned above, it can be seen that calcium carbonate buffered fermentation yielded higher mixed acid concentration, lower formic acid production, lower ethanol production and lower overall yield than the ammonia buffered fermentations. This revealed that ammonium bicarbonate is a good buffer when compared to that of calcium carbonate, which is in agreement with published results (Agbogbo, 2005).

Table 4-1: Summary of the pure sugar fermentation at thermophilic and mesophilic temperatures using calcium bicarbonate and ammonium bicarbonate as buffering agents

Characteristics	**Buffer**	**$CaCO_3$**	**$CaCO_3$**	**NH_4HCO_3**	**NH_4HCO_3**
	Temperature	**55°C**	**37°C**	**55°C**	**37°C**
steady state pH		5.2	5.2	6.5	6.8
maximum (final) acetic acid concentration [g/L]		0.93	2.8	3.4	5.52
maximum (final) C_3- C_7 acid concentration [g/L]		0.77	7.05	5.42	2.81
Maximum lactic acid concentration [g/L]		30.30	6.3	7.6	6.1
Final lactic acid concentration [g/L]		30.30	0	0	0
Maximum formic acid concentration [g/L]		2.42	0.68	3.28	2.18
Final formic acid concentration [g/L]		2.4	0	0.45	0
Maximum ethanol concentration [g/L]		1.85	0	0.03	8.34
glucose consumption time (days)		3	3	3	2
xylose consumption time (days)		8	7	7	4
Biotic CO_2 production (moles)		-0.004	0.02	0.019	0.018
Abiotic CO_2 production (moles)		0.019	0.006	0.012	0.011
CH_4 production (moles)		0	0	0	0
Total acid yield on sugars (%)		180	55	45	78

Methane Production: One proposed advantage of using ammonium bicarbonate as a buffer is that the ammonia will inhibit methanogens (Agbogbo, 2005). In the experiments comparing the two buffers on sugar fermentations, no significant difference

was seen between ammonia or calcium buffered cultures, nor did temperature or even addition of iodoform appear to have any influence on methane production. It is possible that the low pH of the calcium buffered fermentations inhibited methanogenesis as well as did the ammonia. The total methane produced in all cases was negligible, suggesting that the use of methane inhibitors was not necessary.

Table 4-2: Summary of the hot water and green liquor extract fermentations at thermophilic and mesophilic temperatures using ammonium bicarbonate as buffering agent.

		Hot water	**Hot water**	**Green Liquor**	**Green Liquor**
Characteristics	**Buffer**	**NH_4HCO_3**	**NH_4HCO_3**	**NH_4HCO_3**	**NH_4HCO_3**
	Temperature	**55°C**	**37°C**	**55°C**	**37°C**
steady state pH		6.23	6.03	6.23	7.14
initial acetic acid concentration [g/L]		3.03	3.05	11.5	11.5
maximum (final) acetic acid concentration [g/L]		11.2	16.6	14.9	16.5
maximum (final) C3- C7 acid concentration [g/L]		0.6	6.02	0.43	4.5
Maximum lactic acid concentration [g/L]		7.47	3.02	5.82	2.07
Final lactic acid concentration [g/L]		7.03	1.42	5.82	0
Maximum formic acid concentration [g/L]		1.78	5.21	3.11	4.45
Final formic acid concentration [g/L]		1.3	5.21	3.11	1.16
Maximum ethanol concentration [g/L]		0.98	4.04	1.37	2.64
Maximum xylose accumulation [g/L]		3.24	8.52	0.15	1.15
Final xylose accumulation [g/L]		3.24	3.2	0.03	0
Biotic CO_2 production (moles)		0.012	0.009	0.012	0.019
Abiotic CO_2 production (moles)		0.013	0.029	0.008	0.005
CH_4 production (moles)		0	0	0	0
Total acid yield on sugars (%)		81	100	87	48

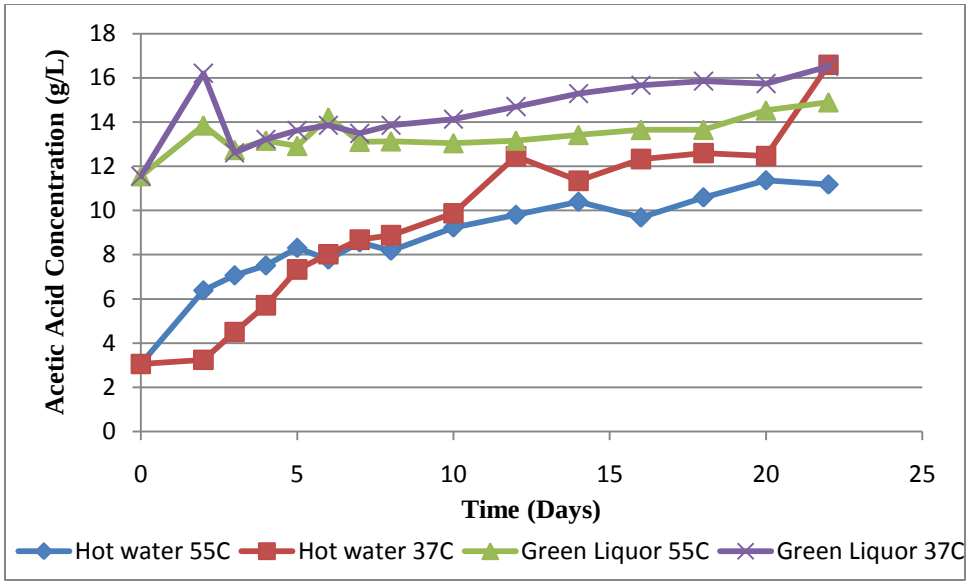


Figure 4-1: Acetic acid production at various fermentation conditions.

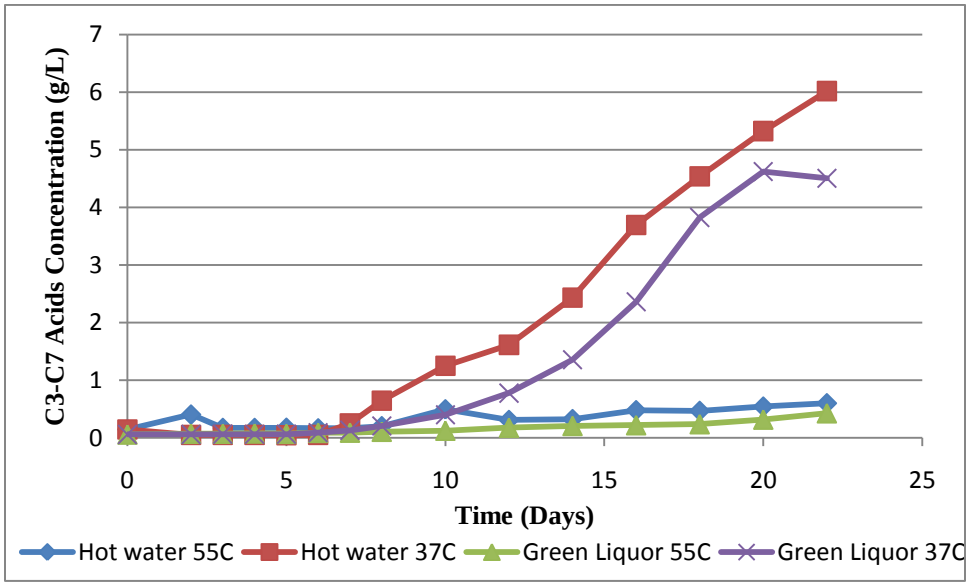


Figure 4-2: C_3-C_7 acids production at various fermentation conditions.

Temperature: Effects of temperature on product distribution can be best seen in the results from extract fermentations. It can be seen in table 4-2 and figure 4-1 and 4-2 that thermophillic cultures produce a higher ratio of acetic acid to C3-C7 acids. This tendency was noted on all of the wood extract fermentations and also the calcium

buffered sugar fermentations, and has been reported in other studies of mixed culture acidogenic digestion (Blackmann; vanWalsum, 2009). Acetic acid yields were higher for mesophilic fermentations, although total acid yield did not follow a temperature dependent pattern.

Consumption of Oligomeric Xylan: The total oligomeric xylan present in the extract was measured at the initiation of the experiment and after the fermentation process. The values are presented in the table 4.3 below.

Table 4-3: Oligomeric xylan present in the extract after fermentation

Fermentation process	**Initial**	**Final**	
Temperature		**55°C**	**37°C**
Hot Water Extract	23.999	11.524	5.832
Green Liquor Extract	9.024	1.166	0.144

Autohydrolysis: In the extract fermentations, at both temperatures conditions we can see that the mono sugars start accumulating, suggesting that the auto hydrolytic step of anaerobic digestion is taking place. Thus produced xylo monosugars are used in the fermentation to produce carboxylic acids, but as the acids accumulate, the fermentation slows down, and the free monosugars start to accumulate from the continuing hydrolysis.. The total accumulations of the xylose sugars are higher at mesophilic temperatures than at thermophilic temperatures. The autohydrolytic action of the mixed culture provides several advantages for extract processing. Autohydrolysis reduces the need for chemicals necessary for acid hydrolysis and subsequent neutralization, and also eliminates the generation of gypsum waste. This saves on costs for chemicals, steam, waste disposal and corrosion resistant process equipment. While enzymatic hydrolysis using purchased enzymes would offer these same advantages, hydrolytic enzymes are typically expensive

and thus the autohydrolytic mature of mixed cultures can generate substantial savings.

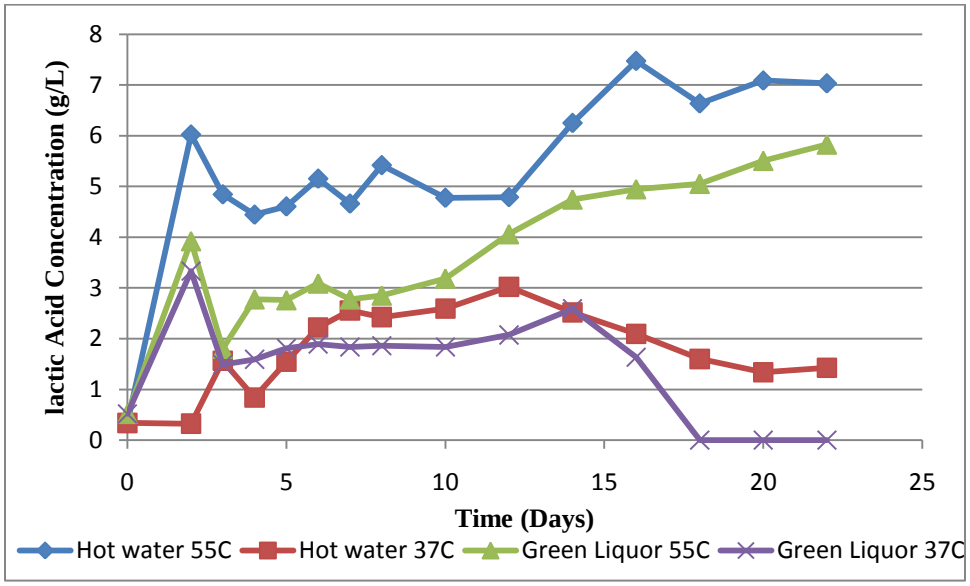


Figure 4-3: Lactic acid production at various fermentation conditions.

Production and Consumption of Lactic Acid: In several fermentations lactic acid was produced and then subsequently consumed. The literature on the MixAlco^{TM} process has not reported production of lactic acid, or the phenomenon of subsequent consumption. The Holtzapple research group typically reports pH values in their fermentations as being higher than pH 5.8, which according to results in this study would not result in an accumulation of lactic acid. Although speculative, it is also possible the fermentations reported by the Holtzapple research group could have been producing lactic acid that remained undetected. In this work, HPLC was used to quantify sugars, lactic and formic acids, but because the Holtzapple group used only the GC analytical method, they would not have detected lactic acid, and so it may have gone unnoticed. Production of lactic acid appears to be influenced by low pH and availability of glucose. In several fermentations, lactic acid was produced while glucose was available, but was then consumed once the glucose was exhausted. Mesophillic conditions appear to

encourage lactate consumption, while lower pH appears to discourage it. All the mesophillic fermentations consumed their lactic acid, while only thermophillic fermentations with pH 6.2 and above could do the same. If lactic acid were a desired product, it appears that running a mixed culture at thermophillic temperature and low pH would result in a high accumulation of this product, possibly enabling commercial production through this simple and robust process.

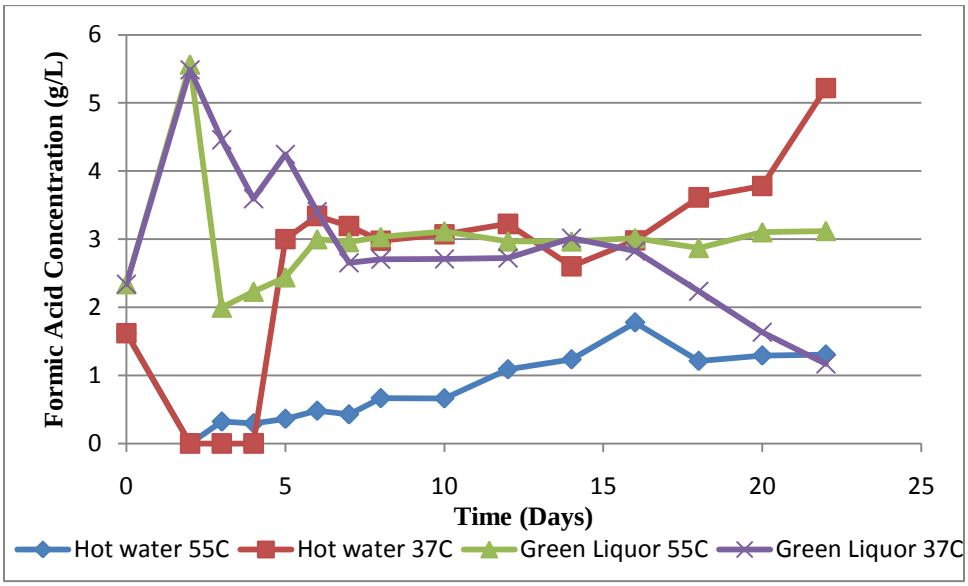


Figure 4-4: Formic acid production at various fermentation conditions.

Production and Consumption of Formic Acid: As with the lactic acid, formic acid was consumed in many of fermentations in which it was produced, and formic acid production and consumption have not been previously reported among the published studies on MixAlco™ fermentations. In general, higher pH and lower temperature favored the consumption of formic acid.

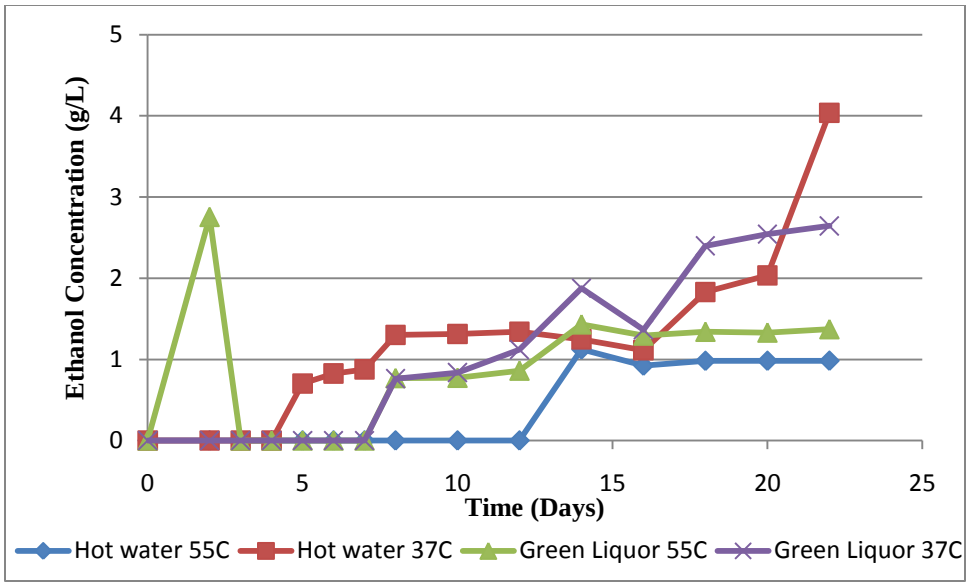


Figure 4-5: Ethanol production at various fermentation conditions.

Ethanol Production: As with lactic and formic acids, production of ethanol in mixed acid fermentations has not been reported by the Holtzapple research group, and again this could be a result of their GC method for measuring fermentation products, which does not detect lactic acid, formic acid or ethanol. Ethanol is an unexpected product, since it can be converted by mixed cultures to the more energetically advantageous acetate. While only minor consumption of ethanol was observed, it does seem likely that extended fermentation times could have resulted in reduced ethanol concentrations. In these experiments, mesophilic cultures tended to favor ethanol accumulation more than thermophilic cultures did.

Application: The above results reveal that for conversion of wood extracts to carboxylic acids, the fermentation of hot water extracts at mesophilic conditions using ammonium bicarbonate as the buffer is the preferred method, as the total amount of carboxylic acids formed is higher compared to the other extracts at different temperature conditions. There are also remaining amounts of ethanol, xylose, formic acid and lactic

acids present, which could potentially provide yet higher yields of aliphatic acids if the cultures could be more adapted to the growth conditions. The benefits of autohydrolysis and non asceptic operating conditions greatly reduce the capital and operating costs associated with fermentation conversion, and also result in a highly robust process that is likely to easily recover from process upset conditions.

Carbon Balance: The total carbon balance considered here is the average carbon balance of all the total products produced to the total carbon supplied in the substrate for the anaerobic fermentation process to be performed. The total carbons present in the carbon dioxide and methane are calculated for a 1L fermentation process system. The total carbons supplied through the inoculums and the total carbons from the microbial growth has not been considered in this balance sheet.

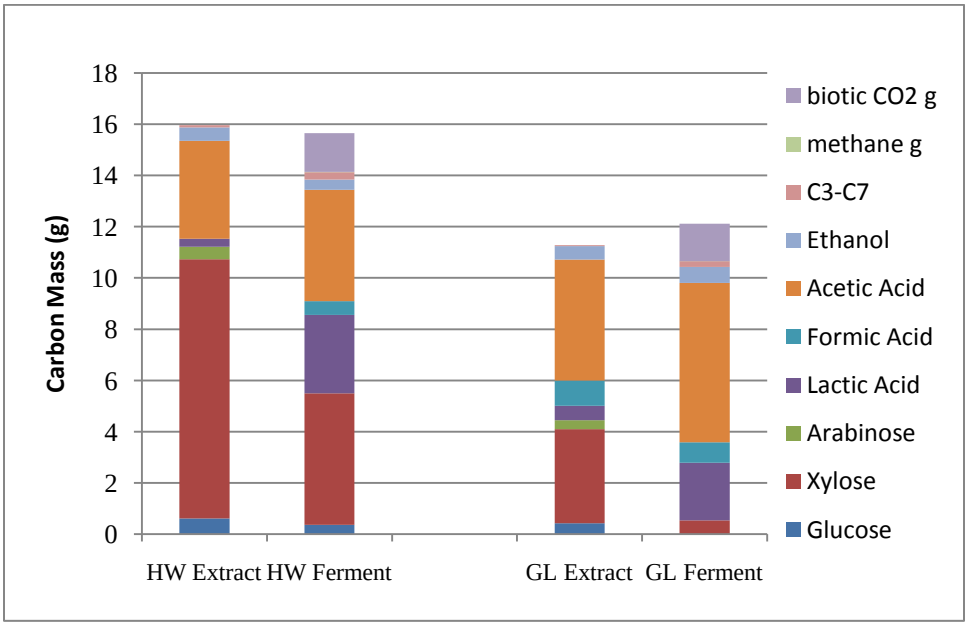


Figure 4-6: Total carbon input and total carbon output for fermentation of hot water and green liquor extracts at 55°C.

The bar graph above represents the total carbons input of each individual compound and the total product output of each individual compound is represented . this graph shows for the hot water and green liquor fermentation process performed at 55°C.

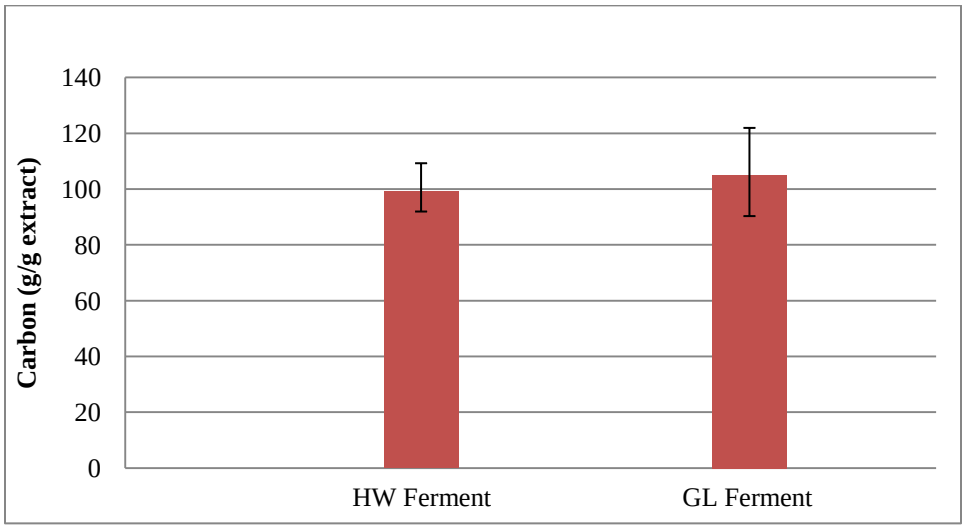


Figure 4-7: carbon balance for hot water and green liquor extracts fermented results at 55°C.

The bar graph (figure4.7) represents the percent carbon balance output with the respective extract carbon input. The values are quite close to 100% with a ±5% of difference. These values are calculated for fermentations performed at 55°C.

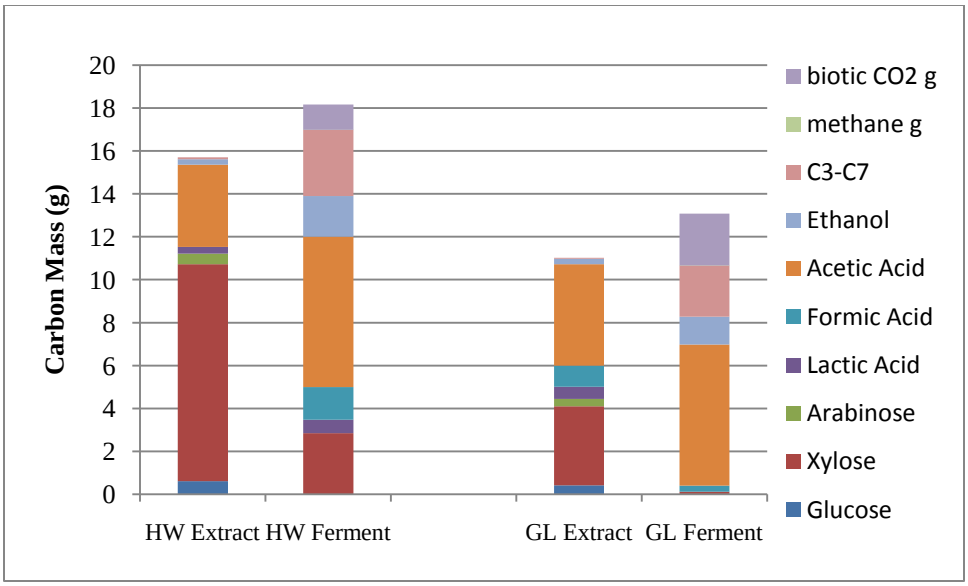


Figure 4-8: Total carbon input and total carbon output for fermentation of hot water and green liquor extracts at 37°C.

The bar graph above represents the total carbons input of each individual compound and the total product output of each individual compound is represented . this one shows for the hot water and green liquor fermentation process performed at 37°C.

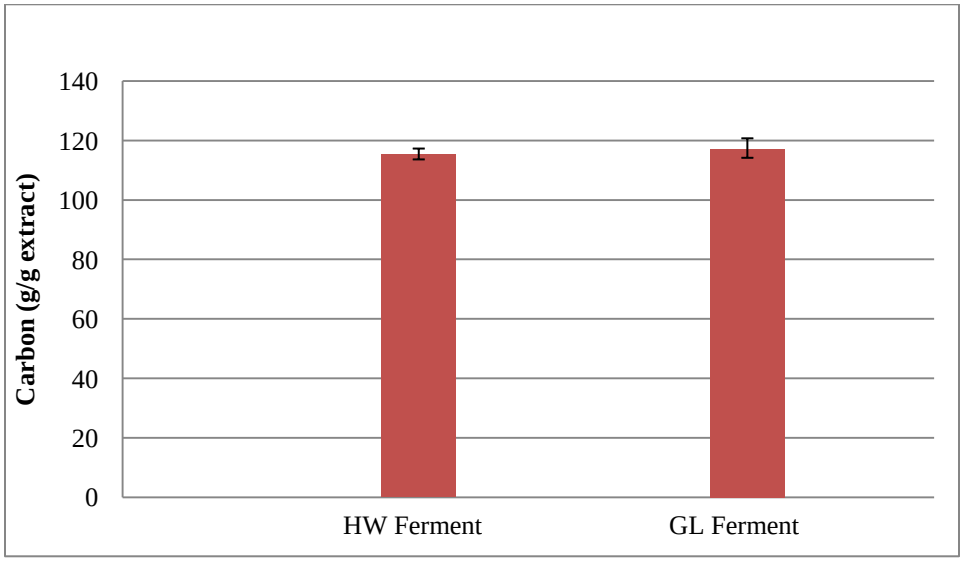


Figure 4-9: carbon balance for hot water and green liquor extracts fermented results at 37°C.

The bar graph (figure4.9) represents the percent carbon balance output with the respective extract carbon input. The values are relatively close to 100% with a ±16% of difference. These values are calculated for fermentations performed at 37°C.

There is some amount of the volatile solids added to the process during the time of addition of the inoculums to the fermentation bottles. The amounts of the volatile solids added are about 0.205g for 100ml of the sample. The total carbon content of this is unknown. Also the total carbon content for the microbial biomass grown during the fermentation is unknown as the bottles have other unknown compounds present that are combined with the cell mass during dry matter determination.

Future Work: For better understanding of the process of the experiment in terms of carbon consumed, a complete measurement of the total organic carbon present in the inoculum and the extract would be very helpful for closure of the mass balance.. Also the measurement of the total nitrogen present in the extracts and the nutrient source would helpful in determining the best guess of the C:N ratio resulting in the best progress of the microbial fermentation process.

CHAPTER 5. CONCLUSION

Results indicate that mixed microbial cultures derived from saline environments are capable of converting mixed sugars (glucose and xylose) to organic acids ($C_2 - C_7$). Variations in ambient pH and temperature conditions resulted in widely variable, but repeatable product profiles. Since these were derived from identical mixed culture innocula, this indicates strong dependence of product profile on culturing conditions. Optimal acid production was achieved at mesophillic temperature and neutral pH, resulting from the use of ammonium bicarbonate as buffer. These conditions provided the most rapid conversion and highest accumulation of organic acids. The calcium carbonate as a buffer agent could not keep up the pH neutral, which would have resulted in better production of carboxylic acid content, and therefore produced lactic acid as a major metabolic product.

The mixed microbial fermentation of hemicellulose sugars present in green liquor and hot water extracts were performed using ammonium bicarbonate as a buffer. It was found with both substrates that hydrolysis of the oligomeric sugars prior to fermentation was not necessary, as hydrolytic cultures in the mixed inoculum were able to break down the extracted xylo-oligomers into digestible xylose mono sugars. With hot water extract as the source of sugars, production of higher amounts of carboxylic acids was achieved at mesophilic conditions. At thermophilic condition the carboxylic acids from C_3- C_7 are produced in much lower quantities, and also lactic acid was produced during the process. Fermentations performed on the green liquor extract showed that the acetic acid production was very little as the acetic acid present in the extract might be inhibiting for

further acid production and thereby the total longer chain carboxylic acids, ranging from C_3 - C_7, were produced in greater quantities at mesophilic conditions. There was no evidence of formation of lactic acid at the mesophilic temperature. At thermophilic temperature the total lactic acid is a bit higher that the acetic acid produced from sugars.

From the overall results collected, it appears that hot water extracts will yield higher product concentrations than green liquor extracts; however both substrates are convertible into dilute streams of organic acids. Given the low cost of anaerobic digestion, the ease of autohydrolysis and the low cost of potential nutrient sources for the fermentation, acidogenic digestion may be an attractive technology for deriving value from wood extracts.

REFERENCES

Agbogbo FK, Holtzapple MT. (2006). Fermentation of Rice Straw/Chicken Manure to Carboxylic Acids Using a Mixed Culture of Marine Mesophilic Micoorganisms. *Applied Biochemistry and Biotechnology* 129-132:997-1014.

Agbogbo Frank K,. (2005). Fermentation of Rice Straw/Chicken Manure to Carboxylic Acids. Ph.D. Dissertation, Texas A&M University, College Station, Texas.

Agbogbo FK, Holtzapple MT. (2007). Fixed-Bed Fermentation of Rice Straw and Chicken Manure Using A Mixed Culture of Marine Mesophilic Microorganisms. *Bioresource Technology* 98(8): 1586-95.

Aiello-Mazzarri C, Agbogbo FK, Holtzapple MT. (2006). Conversion of Municipal Solid Waste to Carboxylic Acids Using A Mixed Culture of Mesophilic Microorganisms. *Bioresource Technoogyl* 97(1):47-56.

Aiello-Mazzarri C, Coward-Kelly G, Agbogbo FK, Holtzapple MT. (2005) Conversion of Municipal Solid Waste Into Carboxylic Acids by Anaerobic Countercurrent Fermentation: Effect of Using Intermediate Lime Treatment. *Applied Biochemistry and Biotechnology* 127(2):79-94.

Balaban. M, G. Uçar (2003) Estimation of Volatile Acids in Wood and Bark. *Holz als Roh- und Werkstoff* 61:465- 468.

Byung-Hwan Um, G. Peter van Walsum (2009) Acid Hydrolysis of Hemicellulose in Green LiquorPre-Pulping Extract of Mixed Northern Hardwoods *Applied Biochemistry and Biotechnology* 153:127–138.

Caitlin Howell, Juan Jacobo Paredes, Jody Jellison. (2009). Decay Resistance Properties of Hot Water Extracted Oriented Strandboard. *Wood and Fiber Science* 41(2): 201-208.

Cesar B. Granda, Mark T. Holtzapple, Luce G, Searcy K, Mamrosh DL. (2009) Carboxylate Platform: The Mixalco Process Part-2: Process Economics. *Applied Biochemistry and Biotechnology* 56(1-3): 107- 24.

Chan WN, Holtzapple MT. (2003) Conversion of Municipal Solid Wastes to Carboxylic Acids By Thermophilic Fermentation. *Applied Biochemistry and Biotechnology* 111(2):93-112.

Datta R, M Henry. (2006) Lactic Acid: Recent Advances in Products, Processes and Technologies - A Review. *Journal of Chemical Technology & Biotechnology* 81(7): 1119-1129.

Dirold and Guyonnetr(1993). The Improvement of Wood Durability by Rectification Process. *Int. Res. Group on Wood Preservation* Doc. No. IRG/WP93-40015.

Domke SB, Aiello-Mazzarri C, Holtzapple MT. (2004) Mixed Acid Fermentation of Paper Fines and Industrial Biosludge. *Bioresource Technology* 91(1):41-51.

Erin D. Blackman, G. Peter van Walsum. (2009). Production of Renewable Bioproducts and Reduction of Phosphate Pollution Through the Lime Pretreatment and Acidogenic Digestion of Dairy Manure. *Environmental Progress & Sustainable Energy* 28(1): DOI 10.1002/ep.

Fu Zhihong, Holtzapple MT. (2009) Fermentation of Sugarcane Bagasse and Chicken Manure to Calcium Carboxylates under Thermophilic Conditions. *Applied Biochemistry and Biotechnology* DOI: 10.1007/s12010-009-8748-z.

Fu Zhihong, Holtzapple MT. (2010) Consolidated Bioprocessing of Sugarcane Bagasse and Chicken Manure to Ammonium Carboxylates by A Mixed Culture of Marine Microorganisms. *Bioresource Technology* 101(8):2825-36.

Fu Zhihong, Holtzapple MT. (2010). Anaerobic Mixed-Culture Fermentation of Aqueous Ammonia-Treated Sugarcane Bagasse in Consolidated Bioprocessing. *Biotechnol Bioengineering*. 106(2): 216-227.

George W. Huber, Sara Iborra and Avelino Corma (2006). Synthesis of Transportation Fuels from Biomass: Chemistry, Catalysts, and Engineering. *Chemical Reviews*, Vol. 106, No. 9.

Humbird. D and A. Aden (2009) Biochemical Production of Ethanol from Corn Stover: 2008 State of Technology Model. Technical Report, *NREL/TP*-510-46214.

Ingram L. O, Aldrich H. C, Borges A. C. C, Causey T. B, Alfredo Martinez, Fernando Morales, Alif Saleh, S. A. Underwood, L. P. Yomano, S. W. York, Jesus Zaldivar, and Shengde Zhou. (1999). Enteric Bacterial Catalysts for Fuel Ethanol Production. *Biotechnolog. Progress* 15, 855-866.

Joseph B. Binder and Ronald T. Raines (2009). Simple Chemical Transformation of Lignocellulosic Biomass into Furans for Fuels and Chemicals. Journal of *American Chemical Society*. 131: 1979–1985.

Juan Jacobo Paredes, Heller and Stephen M. Shaler(2008). Oriented Strandrand Board (OSB) from Hot Water Extractedcted Wood. *Proceedings of Ecowood* 2008.

Kayhanian, M.(1999) Ammonia Inhibition in High-Solids Biogasification: An Overview and Practical Solutions, *Environmental Technology*, 20(4): 355 — 365.

M. Kim, C Y. Gomec, Y. Ahn, R. E. Speece. (2003) Hydrolysis and Acidogenesis of Particulate Organic Material in Mesophilic and Thermophilic Anaerobic Digestion. *Environmental technology*. 24(9): 1183-1190.

Lora, J.H., Wayman, M. (1978), Delignification of Hardwood by Autohydrolysis and Extraction, *Tappi* 61(6): 47–50.

Lefranc L. (1918). Fatty Acids. *British Patent*, 17,776.

Lynd Lee R, Willem H van Zyl, John E McBride, Mark Laser. (2005). Consolidated Bioprocessing of Cellulosic Biomass: an Update. *Current Opinion in Biotechnology* 16: 577-583.

Mao H, J. M. Genco, A. van Heiningen, H. Zou, J. Luo and H. Pendse (2008). Technical Economic Evualation of A Northern Hardwood Biorefinery Using the "Near-Neutral" Hemicellulose Pre-Extraction Process. *Pacific TAPPI/PIMA Winter Meeting*, Portland. Oregon.

Mao H, J. M. Genco, A. van Heiningen, H. Zou, J. Luo and H. Pendse (2009). Kraft Mill Biorefinary to Produce Acetic Acid and Ethanol: Technical Economic Analysis. Peer-Reviewed article, *BioResources* 5(2): 525-544.

Mark T. Holtzapple, Richard R. Davison, M. Kyle Ross, Salvador Aldrett-Lee, Murlidhar Nagwani, Chang-Ming Lee, Champion Lee, Seth Adelson, William Kaar, David Gaskin, Hiroshi Shirage, Nan-Sheng Chang, Vincent S. Chang, and Mitchell E. Loescher (1999) Biomass Conversion to Mixed Alcohol Fuels Using the MixAlco Process. *Applied Biochemistry and Biotechnology* 77–79: 609-631.

Mark T. Holtzapple, Cesar B. Granda. (2009) Carboxylate Platform: The Mixalco Process Part-1: Comparison of Three Biomass Conversion Platforms. *Applied Biochemistry and Biotechnol*ogy. 156(1-3): 95-106

Michael H. Gerardi. (2003). The Microbiology of Anaerobic Digesters. *John Wiley and Sons Inc*., Hoboken, New Jersey.

Roger M. Rowell. (2005). Hand Book of Wood Chemistry and Wood Composites, *Taylor and Francis Group*., New York.

Ross MK, Holtzapple MT. (2001) Laboratory Method for High-Solids Countercurrent Fermentations. *Applied Biochemistry and Biotechnology*. 94(2): 111-26.

Ross Michael Kyle(1998) Production of Acetic Acid from Waste Biomass. Ph.D. Dissertation, Texas A&M University, College Station, Texas.

Russels J. B, Martin S. A. (1984). Effects of Various Methane Inhibitors on the Fermentation of Amino Acids by Mixed Rumen Micro-organisms in Vitro. *Journal of Animal Science*. 59 (5): 1329-1338.

Ryoh Nakakubo, Henrik B. Møller, Anders M. Nielsen, and Juzo Matsuda. (2008) Ammonia Inhibition of Methanogenesis and Identification of Process Indicators during Anaerobic Digestion. *Environmental Engineering Science*, 28(10): 1487-1496.

Samuel Amartey, Thomas W. Jeffries. (1994). Comparision of Corn Steep Liquor with Other Nutrients in the Fermentation of D-Xylose by Pichia Stipitis CBS 6054. *Biotechnology Letters*. 16(2): 211-214.

Sauer. F. D, R. M. Teather. (1987). Changes in Oxidation Reduction Potentials and Volatile Fatty Acid Production by Rumen Bacteria When Methane Synthesis is Inhibited. *Journal of Dairy Science*. 70(9): 1835-1840.

Martin S. A, J. M. Macy. (1984). Effects of Monensin, Pyromellitic Diimide and 2-Bromoethanesulfonic Acid on Rumen Fermentation InVitro. *Journal of Animal Science*, 60(2): 544-550.

Sara Lynn Walton. (2009) Biological Conversion of Hemicellulose Extract into Value-Added Fuels and Chemicals. Ph.D. Dissertation, University of Maine, Orono, Maine.

Sjöström, Eero.(1993) Wood Chemistry Fundamentals and Applications; 2nd Edition, *Academy Press Inc*., San Diego, California.

Susan Burdick Domke (1999) Fermentation of Industrial Biosludge, Paper Fines, Bagasse and Chicken Manure to Carboxylate Salts. Ph.D. Dissertation, Texas A&M University, College Station, Texas.

Bauchop T. (1967). Inhibition of Rumen Methanogenesis by Methane Analogs. *Journal of Bacteriology*, 94(1): 171-175.

Tanaka K, Komiayama A, Sonomoto K, Ishizaki A, Hall S. J, Stanbury P. F. (2002). Two Different Pathways for D-xylose Metabolism and the Effect of Xylose Concentration on the Yield Coefficient of L-Lactate in Mixed-Acid Fermentation by the Lactic Acid Bacterium. *Applied Microbiology and Biotechnology* 60(1-2): 160-167.

Temudo M. F, Mato T. (2009). Xylose Anaerobic Conversion by Open-Mixed Cultures. *Applied Microbiology and Biotechnology* 82(2): 231-239.

Terry L. Miller, Meyer J. Wolin. (2001). Inhibition of Growth of Methane Producing Bacteria of the Ruminant Forestomach by Hydroxymethylglutaryl ~ SCoA Reductase Inhibitors. *Jouranl of Dairy Science*.84(6): 1445-1448.

Thanakoses P, Mostafa NA, Holtzapple MT. (2003) Conversion of Sugarcane Bagasse to Carboxylic Acids Using a Mixed Culture of Mesophilic Microorganisms. *Applied Biochemistry and Biotechnology*. 107(1-3): 523-46.

Thanakoses P, Black AS, Holtzapple MT. (2003) Fermentation of Corn Stover to Carboxylic Acids. *Biotechnology and Bioengineering*. 83(2): 191-200.

Troyam-Tand Navarettea(1994). Study of the Degradation of Rectified Wood through Ultrasonic and Gravimetric Techniques. *Int. Res. Group on Wood Preservation*. Doc. No. IRG/ WP94-40030.

Tunc M Sefik (2008) Hydrothermal Dissolution of Mixed Southern Hardwoods. *Holzforschung*. 62: 539-545.

Van Heningen, A. (2006) Converting A Kraft Pulp Mill into An Integrated Forest Products Biorefinery. *Pulp and Paper Canada* 107: 38-43.

Chapula W. (1980). Chemical Control of Rumen Microbial Metabolism. In: Rukebusch Y, Thivend P, editors. Digestive Physiology and Metabolism in Ruminants. London: *MTP Press*. P325-347.

Waleed Wafa Al-Dajani, Ulrike W. Tschirner. (2008). Pre-Extraction of Hemicelluloses and Subsequent Kraft Pulping Part I: Alkaline Extraction. *Tappi Journal*. 7(6): 3-8.

Waleed Wafa Al-Dajani, Ulrike W. Tschirner, Tryg Jensen. (2009). Pre-Extraction of Hemicelluloses And Subsequent Kraft Pulping Part Ii: Acid- And Autohydrolysis. *Tappi Jouranl*.8(9): 30-37.

Young-Jung Wee, Jin-Nam Kim And Hwa-Won Ryu. (2006) Biotechnological production of lactic acid and its recent applications. *Food Technol. Biotechnol*. 44(2): 163–172.

Zaho C, Karakashav D, Lu W, Wang H, Angelidaki I. (2010). Xylose Fermentation to Biofuels (Hydrogen and Ethanol) by Extreme Thermophilic (70 °C) Mixed Culture. *International Journal of Hydrogen Energy*.35(8): 3415-3422.

APPENDIX A

SRI'S MULTIPLE GAS ANALYZER #2 OPERATING PROCEDURE FOR GAS SAMPLE ANALYSIS

SRI's multiple gas analyzer #2 is equipped with 3 columns, 2 carrier gas flows and 4 detectors. These are constructed such that the change in the carrier gas flow is turned on to each column at different times during the run to separate the wide variety of peaks without any coelution.

Figure A-1: A typical SRI GC multiple gas analyzer with sulfur detector #2

Of the 3 columns, two are packed columns, one is a molecular sieve column that is helpful in separation of H_2, O_2, N_2, CH_4 and CO. One of the packed columns is a HayeSep-D column helpful in separation of compounds in the C_1 through C_6 range. The third column is a capillary column, MXT-60, which is connected in parallel to the HayeSep-D and is helpful in separating the hydrocarbons through C_{20}.

The carrier gas used was helium. At the start of the run, carrier gas flowing to the molecular sieve column is turned on while the carrier gas flow to the other two columns

is turned off. After a period of time during the run the molecular sieve carrier flow is turned off and the flow to the HayeSep-D and capillary columns is turned on.

Event time table for a ten port gas sampling valve for simultaneous injection of the same sample in to two different columns

Table A-1: Event time table for SRI GC multiple gas analyzer with sulfur detector

Event time	**Event**	**Event function**
0.000	Zero	Zero base line
0.000	B "ON"	Carrier #2 "OFF"
0.050	G "ON"	Valve in "INJECT"
8.50	A "ON"	Carrier #1 "OFF"
8.50	B "OFF"	Carrier #2 "ON"
13.000		End of run

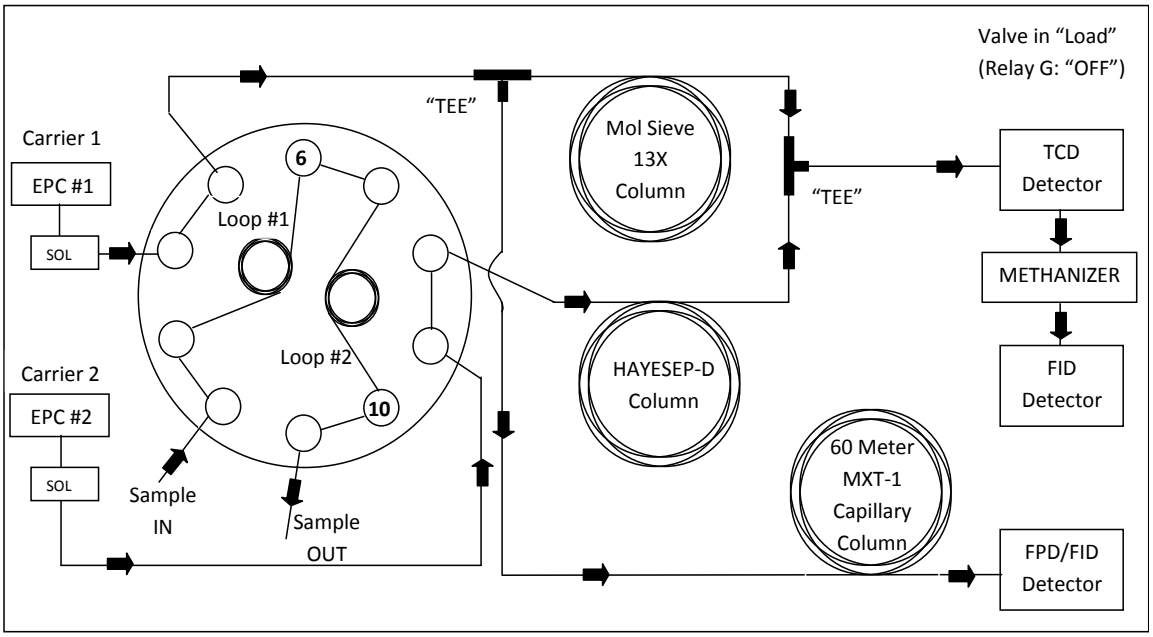


Figure A-2: Schematic diagram of the GC sampling system Load OFF

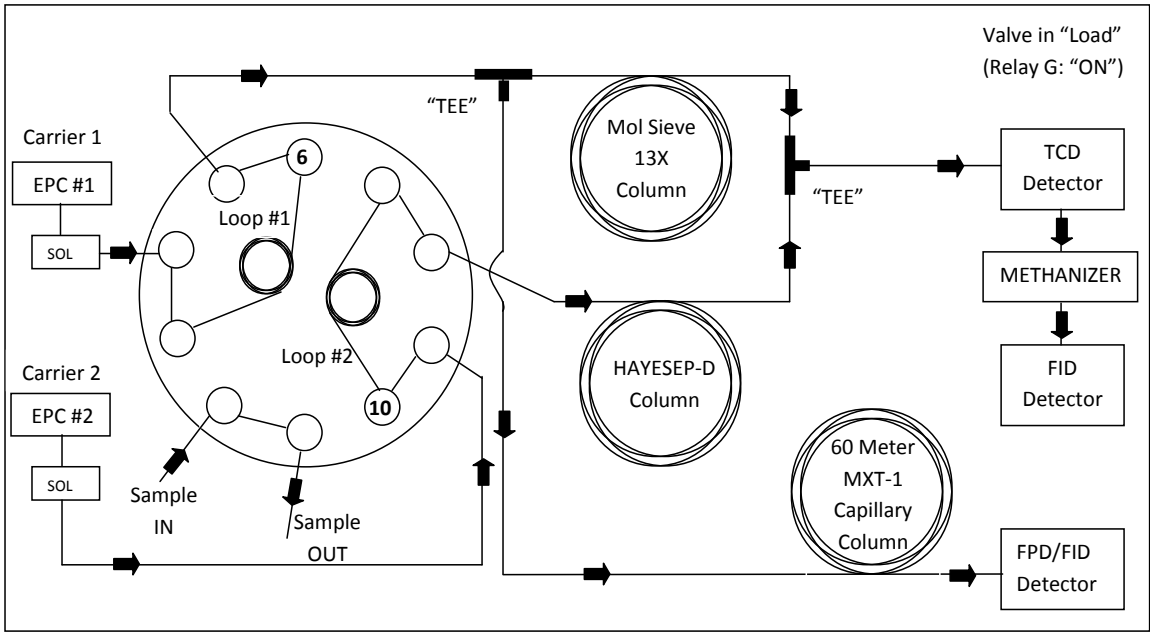


Figure A-3: Schematic diagram of the GC sampling system Load ON

The load positions of the sample in to the loop and later on to the columns with the carrier gas is shown in the above figure.

An event table is needed to change the load position during the runs. The detailed list of the events taking place during the run is shown on the above figure A.1.

Helium is used as the carrier gas in both the flow carriers A and B. H_2 is used as a combustion gas for the FID and the system is equipped with an internal air pump.

Run settings:

He flow pressure : 40 psig

H_2 flow pressure : 40 psig

Injection temperature : 60°C

Table A-2: Column temperature Program:

Initial Temp(°C)	Hold(Min)	Ramp(Min)	Final Temp(°C)
40.00	13.000	0.00	40.00

Table A-3: Detectors and their operating conditions:

Detectors		temperature	Gain	column	Carrier He	H_2	Air
FPD	PMT	150°C	High	60M MXT-1	21psi@ 20ml/min	32psi@ 39ml/min	3psi@150/ 20 ml/min
	FID	150°C	High	60M MXT-1	21psi@ 20ml/min	32psi@ 39ml/min	3psi@150/ 20 ml/min
FID (with Methanizer)		300°C	High	Molecular Sieve 13X/ HayeSep-D	20ml/min	20psi@ 25ml/min	5psi@ 250ml/min
TCD		150°C	Low	Molecular Sieve 13X/ HayeSep-D	20ml/min	-	-

Sample run:

1. Open the gas flow and power on the gas chromatogram. Check for the gas pressure controller. Wait for the injector, column and detector light to blink continuously to indicate it is ready for a sample.
2. Switch on the PMT switch on the front panel and the FID switch to low gain position.
3. Press the auto zero button to make sure the base line is constant
4. Using a 20ml syringe, withdraw 5-10 ml of sample from the fermentor and inject in to the ‘sample in’ port
5. Immediately start run either by pressing start run button on the front control panel on GC or by entering the ‘space bar’ on the computer.
6. The program is set to 13 minutes run a single sample
7. The relative compositions of the gases of interest from the sample are calculated using the area obtained from the GC with known standard composition in mole percent.

8. The standard from Scott gas mixtures purchase from Sigma Aldrich (#501697) is used.
9. The standard gas mixture is run at the beginning of the analysis for calibration and at the end of the analytical period to check consistency.

Shut down procedure.

1. Turn off the TCD and the PMT switches. Turn off the internal air pump. And power off the system.
2. Shut down the regulators on the cylinders.

Table A-4: Composition of the scott gas mixture is listed below

Component	**Gas concentration Moles (%)**
Carbon dioxide	5.00
Carbon monoxide	5.oo
Hydrogen	4.00
Methane	4.00
Nitrogen	5.00
Oxygen	5.00
Helium	Balance

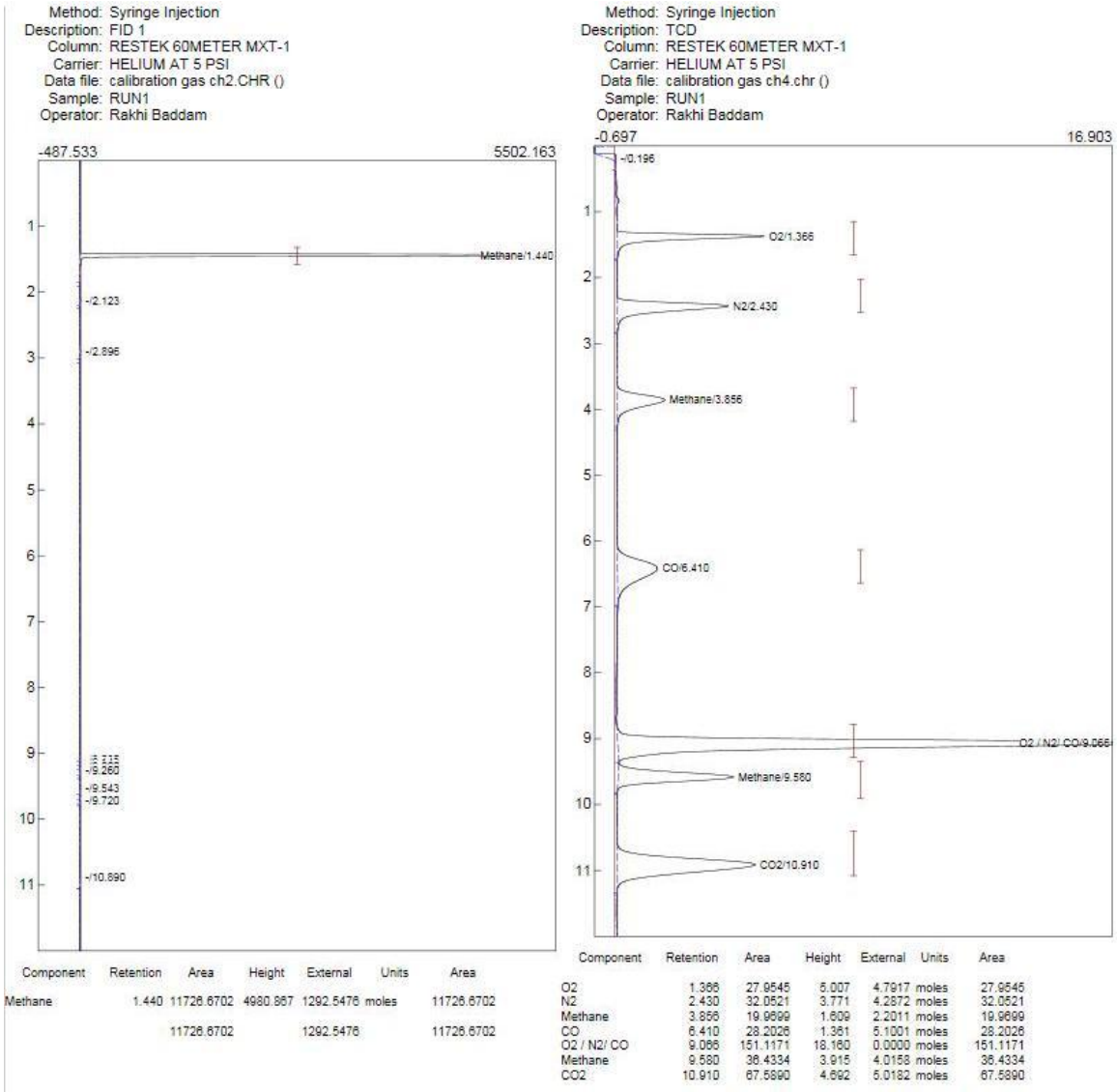


Figure A-4: The traces of gas measured using SRI GC- calibration standard gas run

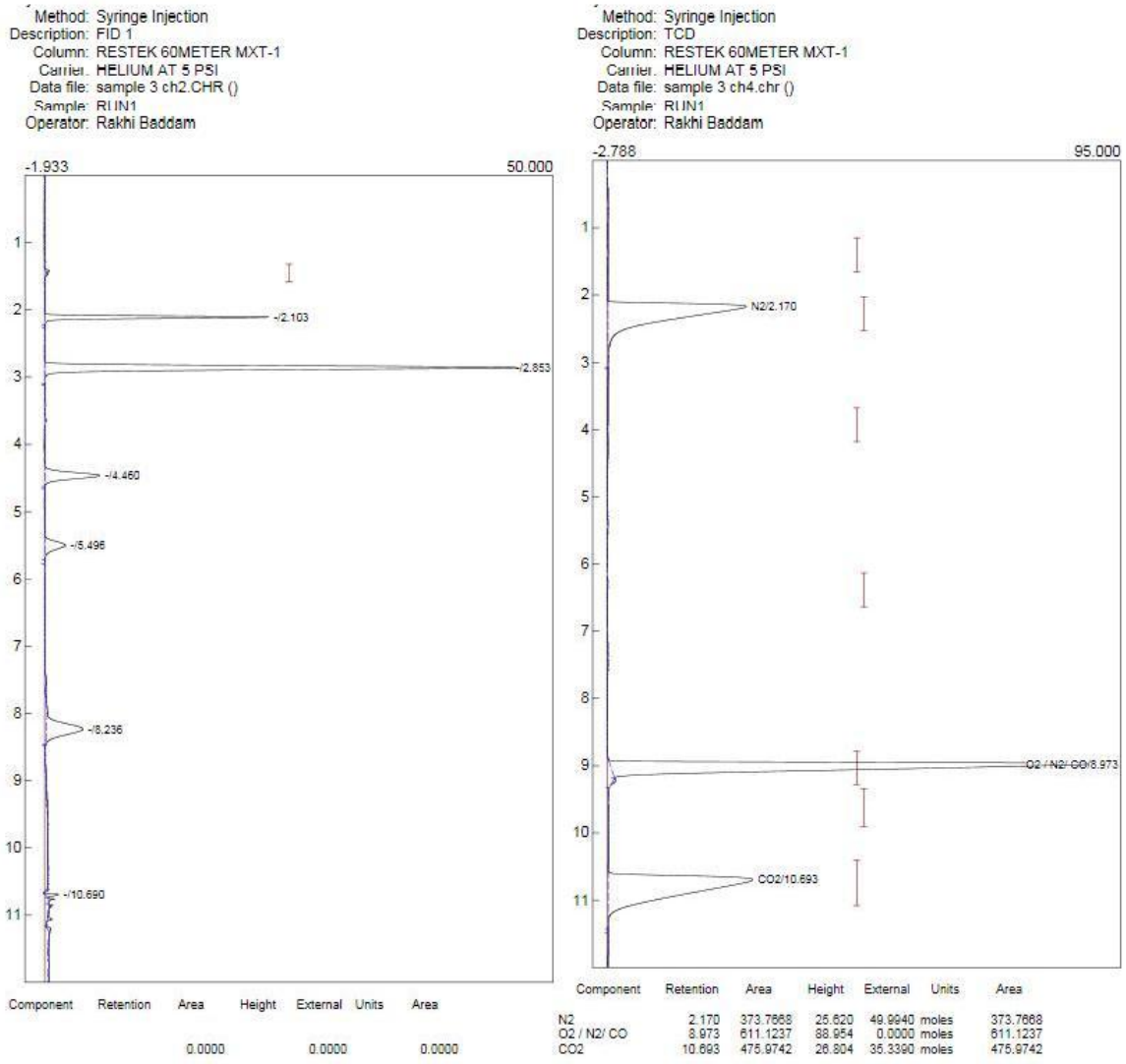


Figure A-5: The traces of gas measured using SRI GC- sample gas run

Methane is the only gas well detected using the FID. Other gases are also measured using the same detector. As methane is the point of interest its being reported in to this thesis.

Gases like CO_2 and N_2 are best measured using the TCD. As the process is proceeded anaerobically no presence of O_2 can be observed. Trace amounts of methane and CO are not detected by using TCD.

APPENDIX B

HPLC ANALYSIS FOR SUGARS AND ACIDS

The HPLC system used was a Shimadzu Prominence HPLC system This system is used to measure some of the acids and the sugars during the fermentation process. The system was equipped with six different instruments to generate the data of our interest.
A typical HPLC system used in this work is listed in below figure B.1

Figure B-1: Shimadzu Prominence HPLC system

- The first and foremost one is the SCL 10Avp (system controller) that controls the other instruments inline and transfers the data produced by the detectors to the computer.
- LC 10AT vp is an isocratic pump that moves the mobile phase through the column and maintains a constant flow rate throughout the process. The flow rate is maintained at 0.6ml/min
- SIL 20AC is the auto sampler used for injecting the sample in to the column. This is equipped with a refrigeration system so the temperature is maintained at 4°C all the time.
- CTO 10Avp is the oven which holds the column and guard column. The temperature maintained in this is 60°C.
- The type of the column used is an Aminex HPX-87H column by BIO-RAD. The column is equipped with a guard column protecting the column from entering impurities.

- The mobile phase used is 5mM sulfuric acid (H_2SO_4). The mobile phase is degassed using Helium gas to remove any air bubbles present in it
- SPD 10 AV (wavelength at 280nm) and RID10A (cell temperature at 40°C) are the UV-visible and refractive index detectors respectively.

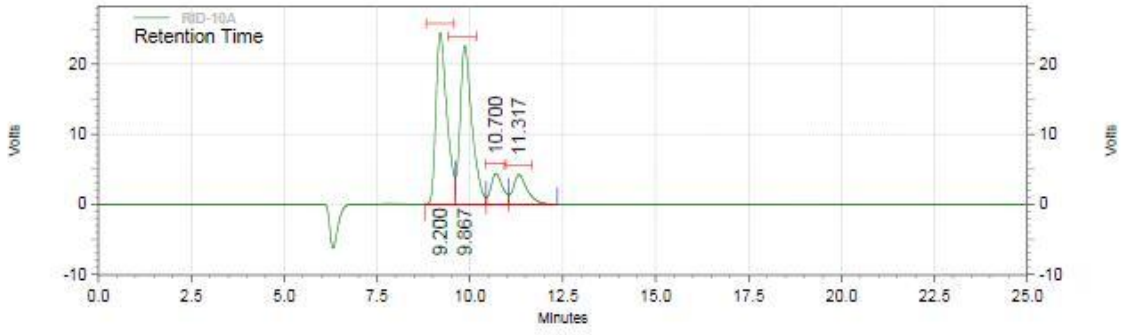
Sample preparation:

1. If the samples are stored in a fridge, then thaw and mix the sample well using a vortex shaker.
2. Pipette 1 ml of sample in to 5 ml of water making the total dilution factor of 6 in to a sample preparation vial. Make sure to follow the correct pipette procedures for good results.
3. Mix equal volumes of sample and internal standard in to a separate sample vial. The internal standard used is Fucose (1g/L).
4. Mix the contents well and place it in a micro centrifuge tube.
5. Cap the tube and centrifuge at 15,000 rpm (Eppendorf easy spin plus) for 10 minutes. This allows for settling heavy particles in the liquid to the bottom of the tube.
6. Pipette the supernatant liquid in to a glass GC vial and cap immediately. The GC vials and caps are ordered from fisher scientific company with catalog numbers 03-395C and 03-396A respectively.
7. This sample is now ready to run on the HPLC or else it can be stored in the freezer to run the sample at a later time. For use at a later period remove the samples from the freezer, thaw and mix well before using.
8. Samples are placed on the sampling tray and the run is started. Make sure the system is running in a good condition and that the entire general operating procedures had been followed.
9. Calibration is being done by using a known standard containing sugars and acids at different concentrations. The outputs from unknown samples are compared with the known concentrations of the standard mixture.
10. The different samples analyzed through the HPLC are Glucose, XMG (Xylose, Mannose and Galactose), Formic acid, Lactic acid, Acetic acid and Ethanol.

11. The standard carboxylic mixtures prepared in the lab is as follows:

Glucose	:	5g/L	Lactic acid	:	5g/L
Xylose	:	5g/L	Formic acid	:	5g/L
Fucose	:	2g/L	Acetic acid	:	5g/L
(Internal standard)			Ethanol	:	5g/L

Data File: C:\Rakhi's Data Files\sept 15, 2009\5gpl sugar standard.dat
Method: C:\EZStart\Projects\Default\Method\Martin-test25min.met
Acquired: 9/15/2009 9:22:49 AM
Printed: 4/19/2010 4:17:59 PM



RID-10A Results

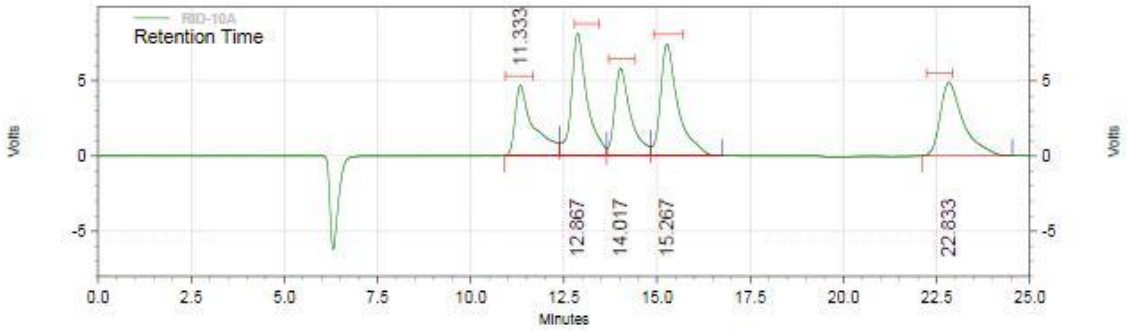
Retention Time	Area	Area %	Height	Height %
9.200	501909	41.38	24484	43.95
9.867	504791	41.62	22625	40.61
10.700	97234	8.02	4383	7.87
11.317	109006	8.99	4221	7.58

Figure B-2: The traces of sugars and acids measured using HPLC- calibration standard run for sugars.

12. Retention time for the compounds measured using the HPLC system(fig b.2 and b.3)

Compound	**Retention Time (Min)**
Glucose	9.20
Xylose, Mannose, Galactose (XMG)	9.867
Arabinose	10.70
Fucose (internal standard)	11.32
Formic Acid	12.86
Lactic Acid	14.02
Acetic Acid	15.27
Ethanol	22.83

Data File: C:\Rakhi's Data Files\sept 18,2009\5gpl acid standard.dat
Method: C:\EZStart\Projects\Default\Method\Martin-test25min.met
Acquired: 9/18/2009 2:49:37 PM
Printed: 4/19/2010 4:16:43 PM

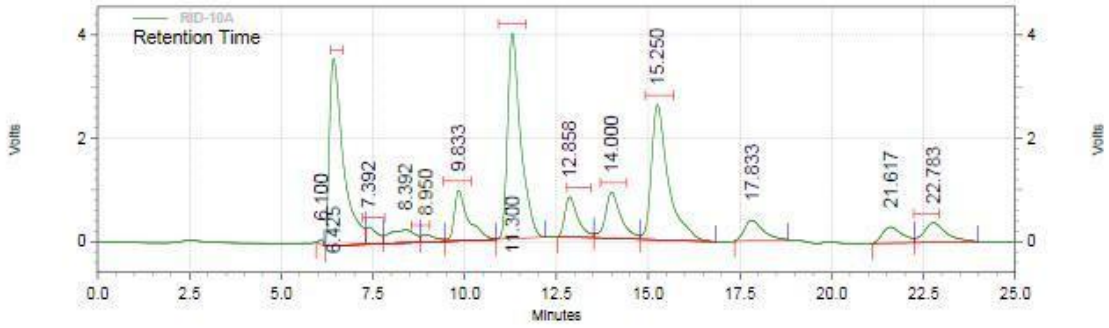


RID-10A Results

Retention Time	Area	Area %	Height	Height %
11.333	164534	15.88	4682	15.12
12.867	239866	23.14	8154	26.33
14.017	176459	17.03	5800	18.73
15.267	244806	23.62	7446	24.05
22.833	210766	20.34	4882	15.77

Figure B-3: The traces of sugars and acids measured using HPLC- calibration standard run for acids

Data File: C:\Rakhi's Data Files\Oct 25, 2009\Hot Water 37 sample 4 14.dat
Method: C:\EZStart\Projects\Default\Method\Martin-test50min.met
Acquired: 10/25/2009 4:00:28 PM
Printed: 4/26/2010 10:15:06 AM



RID-10A Results

Retention Time	Area	Area %	Height	Height %
6.100	753	0.18	100	0.68
6.425	94994	22.95	3611	24.47
7.392	7048	1.70	330	2.24
8.392	12157	2.94	257	1.74
8.950	3811	0.92	144	0.98
9.833	25936	6.27	974	6.60
11.300	97778	23.62	3972	26.92
12.858	19127	4.62	774	5.24
14.000	26398	6.38	894	6.06
15.250	84139	20.33	2619	17.75
17.833	13814	3.34	395	2.68
21.617	11846	2.86	309	2.09
22.783	16135	3.90	378	2.56

Figure B-4: The traces of sugars and acids measured using HPLC- sample run

All the sugars and acids as shown in the standards are being well measured for the sample injected . we can also see some unknown compounds also as well

APPENDIX C

GAS CHROMATOGRAM ANALYSIS FOR VOLATILE ACIDS IN LIQUID SAMPLES

Shimadzu GC 2010 is used to analyze the liquid samples for determining the volatile acids produced during the fermentation process. This instrument is equipped with an auto sampler. The injection port is a split with a temperature controller. This can accommodate only one column with a single FID detector. The column used in this entire process is Stabilwax-DA (supplied by Restek corporation. USA). The typical system looks like the one in below figure c.1

Figure C-1: Shimadzu GC 2010

Run Settings:

H_2 flow	:	40 ml/min
Air flow	:	400 ml/min
He flow	:	49.1 ml/min
Injection temperature	:	60°C

Table C-1: Column temperature Program

Rate(°C)	Temperature(°C)	Hold(min)	Remarks
-	50	0.5	Initial temperature
20	200	0.0	Ramp up temperature
50	230	2.5	Column cleanup

Detector : FID
Detector temperature : 250°C

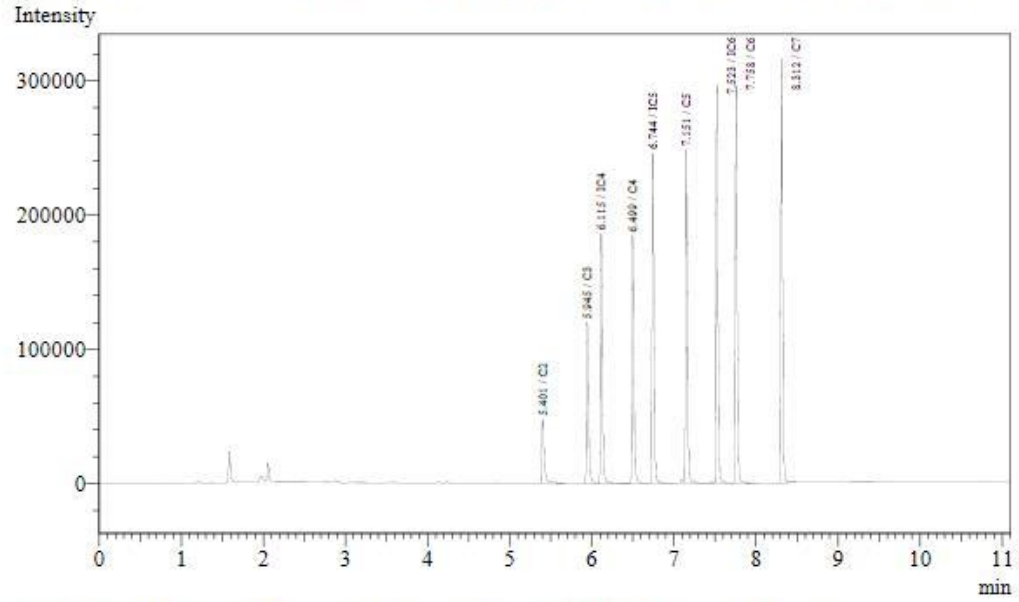
Sample run:

1. If the samples are stored in a fridge, then thaw and mix the sample well using a vortex shaker.
2. Pipette 1 ml of sample in to 5 ml of water making the total dilution factor of 6 in to a sample preparation vial. Make sure to follow the correct pipette procedures for good results.
3. Take 1 ml of the above sample in to a separate sample preparation vial. To it add 1 ml of 10mM 4methyl-n-valeric acid(1.162g/L) and 1 ml of 3M Phosphoric acid (H_3PO_4).
   - 4methyl-n-valeric acid serves as the internal standard.
   - Phosphoric acid is used as an acidifying agent, so as to acidify the volatile acids in the sample and enable vaporization as soon as they are injected in to the injection port.
4. Mix the contents well and transfer it in to a micro centrifuge tube.
5. Cap the tube and centrifuge at 15,000 rpm in Eppendorf easy spin plus for 10 minutes. This allows heavy particles in the liquid to settle at the bottom of the tube.
6. Pipette the supernatant liquid in to a glass GC vial and cap immediately. The GC vials and caps are ordered from fisher scientific company with catalog numbers 03-395C and 03-396A respectively.

7. This sample is now ready to run on the GC, or else it can be stored in the freezer to run the sample at a later time. For use at a later period remove the samples from the freezer, thaw and mix well before using.
8. Before starting the GC make sure all the gas cylinders are turned on and have sufficient amount of gas for the run to be completed. The GC is equipped with an auto sampler and so make sure everything is working properly. Samples are placed on the sampling tray and then the run is started.
9. Calibration is being done by using a standard carboxylic acid mixture (Matreya LLC Inc., #1075) run through the GC. The output is compared with the known concentrations of the standard mixture. Later the samples are run through the GC.
10. The standard carboxylic acid Mixture from Matreya LLC Inc (#1075) is as follows:

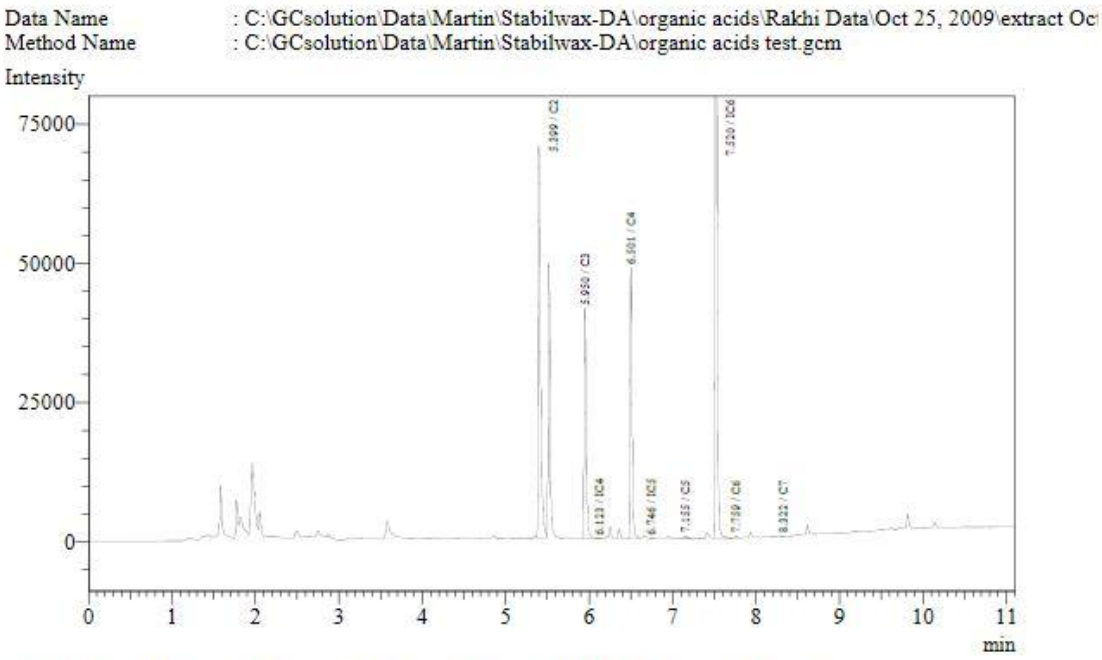
Acetic Acid :	0.601g/L	Valeric acid :	1.021g/L
Propionic Acid:	0.741g/L	Isocaproic acid:	1.162g/L
Isobutyric acid:	0.881g/L		(Internal standard)
Butyric acid :	0.881g/L	Caproic acid :	1.162g/l
Isovaleric acid :	1.021g/L	Heptanoic aicd:	1.302g/L

Data Name : C:\GCsolution\Data\Martin\Stabilwax-DA\organic acids\Rakhi Data\Oct 19, 2009\extract Oc
Method Name : C:\GCsolution\Data\Martin\Stabilwax-DA\organic acids\Rakhi Data\Oct 19, 2009\extract Oc



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	5.401	101055	48051	0.601	gpl	S	1	C2
2	5.945	211688	119952	0.741	gpl		2	C3
3	6.115	308799	186284	0.881	gpl	SV	3	IC4
4	6.499	306118	184403	0.881	gpl	SV	4	C4
5	6.744	391406	246055	1.021	gpl	SV	5	IC5
6	7.151	392701	248644	1.021	gpl	SV	6	C5
7	7.523	460254	296927	0.000	gpl	SV	7	IC6
8	7.758	458585	295921	1.162	gpl	SV	8	C6
9	8.312	460067	316073	1.302	gpl		9	C7
Total		3090673	1942310					

Figure C-2: The traces of volatile carboxylic acids measured using Shimadzu GC-calibration standard run



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	5.399	142747	70138	1.784	gpl		1	C2
2	5.950	72582	41346	0.530	gpl		2	C3
3	6.123	205	77	0.001	gpl		3	IC4
4	6.501	80169	48679	0.465	gpl		4	C4
5	6.746	172	72	0.001	gpl		5	IC5
6	7.155	683	420	0.003	gpl		6	C5
7	7.520	216618	139683	0.000	gpl		7	IC6
8	7.759	506	305	0.002	gpl		8	C6
9	8.322	153	80	0.001	gpl		9	C7
Total		513835	300800					

Figure C-3: The traces of volatile carboxylic acids measured using Shimadzu GC-volatile liquid sample run

The solubility of IC_4, IC_5, C_6 and C_7 are too small to be detected on the GC and the others like C_3 and C_4 are detected and measured well. C_2 is also detected using the GC but is being interfered with by the presence of the xylose.

APPENDIX D

MEASUREMENT OF VOLATILE SOLIDS CONTENT

1. Record the weight of an empty crucible.
2. Pipette about 5 ml of the extract in to the crucible and record its weight.
3. Place the crucible in an oven set at 105°C± 5°C for a day.
4. Record the dry weight of the crucible after it has been cooled down to room temperature in a vacuum desiccator.
5. Now move the crucibles in to a muffle furnace with temperatures set to 550°C. the ashing process is done for about 4 hours
6. Record the ash weight of the crucible after it has been cooled down to room temperature in vacuum desiccators.

The total volatile solid (VS) content is calculated by:

$$Volatile\ Solids = S6 - S4$$

APPENDIX E

SUGAR AND ACIDS DATA SHEETS

Total sugars consumption and acids production concentrations during fermentation process. The tables below present raw data from replicate experiments at the different conditions investigated.

Source	Buffer	Temperature	Growth type	Tables
Glucose and Xylose	$CaCO_3$	55°C	Control	E-1 to E-4
	$CaCO_3$	55°C	Methne Inhibition	E-5 to E-8
	$CaCO_3$	37°C	Control	E-9 to E-12
	$CaCO_3$	37°C	Methne Inhibition	E-13 to E-16
	NH_4HCO_3	55°C	Control	E-17 to E-20
	NH_4HCO_3	55°C	Methne Inhibition	E-21 to E-24
	NH_4HCO_3	37°C	Control	E-25 to E-28
	NH_4HCO_3	37°C	Methne Inhibition	E-29 to E-32

Source	Buffer	Temperature	Growth type	Tables
Hot Water Extract	NH_4HCO_3	55°C	Control	E-33 to E-36
	NH_4HCO_3	55°C	Methne Inhibition	E-37 to E-40
	NH_4HCO_3	37°C	Control	E-41 to E-44
	NH_4HCO_3	37°C	Methne Inhibition	E-45 to E-48

Source	Buffer	Temperature	Growth type	Tables
Green Liquor Extract	NH_4HCO_3	55°C	Control	E-49 to E-52
	NH_4HCO_3	55°C	Methne Inhibition	E-53 to E-56
	NH_4HCO_3	37°C	Control	E-57 to E-60
	NH_4HCO_3	37°C	Methne Inhibition	E-61 to E-64

Table E-1: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #1).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	6.45	10.123	10.294	0.000	0.206	0.685	0.000	0.000	0.000	0.086	-0.086
2	5.11	0.000	11.022	0.000	27.932	0.000	0.000	0.000	0.003	0.015	-0.013
3	5.12	0.000	9.793	0.990	16.064	0.000	0.454	0.000	0.006	0.009	-0.003
4	5.25	0.000	8.109	1.147	18.721	0.000	1.398	0.000	0.008	0.011	-0.002
5	5.22	0.000	6.956	1.019	20.609	0.000	2.860	0.000	0.009	0.012	-0.003
6	5.33	0.000	6.202	1.305	25.128	1.314	2.093	0.000	0.010	0.016	-0.005
7	5.22	0.000	5.627	0.000	29.182	1.657	1.365	0.000	0.011	0.018	-0.006
8	5.26	0.000	3.124	1.353	27.362	1.608	2.341	0.000	0.012	0.017	-0.005
9	5.22	0.000	1.733	1.474	28.635	1.885	1.957	0.000	0.013	0.018	-0.005

Table E-2: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #2).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	6.45	10.123	10.294	0.000	0.206	0.685	0.000	0.000	0.000	0.086	-0.086
2	5.11	3.787	10.250	0.000	11.342	0.000	0.000	0.000	0.003	0.006	-0.003
3	5.14	0.458	10.198	0.576	20.023	0.000	0.361	0.000	0.006	0.011	-0.005
4	5.19	0.000	8.058	0.971	24.885	0.000	0.583	0.000	0.008	0.014	-0.006
5	5.15	0.000	5.878	1.049	27.383	1.497	0.959	0.000	0.009	0.017	-0.007
6	5.22	0.000	3.999	1.400	29.066	2.050	1.450	0.000	0.011	0.018	-0.007
7	5.19	0.000	2.330	1.721	30.331	2.370	1.533	0.000	0.012	0.019	-0.007
8	5.21	0.000	1.039	1.746	28.688	2.423	1.612	0.000	0.014	0.018	-0.005
9	5.22	0.000	0.000	1.852	30.250	2.403	1.698	0.000	0.014	0.019	-0.004

Table E-3: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #1).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.454	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	0.443	0.000	0.000	0.000	0.073	0.000	0.248	0.634
5	0.429	0.000	0.000	0.225	0.230	0.340	0.530	1.106
6	0.528	0.119	0.087	0.080	0.084	0.105	0.277	0.814
7	0.588	0.197	0.014	0.051	0.048	0.039	0.150	0.279
8	0.573	0.119	0.046	0.104	0.176	0.216	0.342	0.765
9	0.603	0.043	0.052	0.061	0.093	0.160	0.244	0.700

Table E-4: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #2).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.361
4	0.259	0.000	0.000	0.000	0.000	0.000	0.000	0.324
5	0.468	0.036	0.000	0.020	0.018	0.020	0.000	0.397
6	0.693	0.083	0.053	0.137	0.008	0.031	0.078	0.368
7	0.854	0.105	0.000	0.324	0.031	0.003	0.058	0.157
8	0.911	0.025	0.002	0.317	0.025	0.014	0.059	0.260
9	0.928	0.023	0.002	0.377	0.010	0.017	0.061	0.280

Table E-5: Substrates usage and product formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #1).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	6.45	10.123	10.294	0.000	0.206	0.685	0.000	0.000	0.000	0.086	-0.086
2	5.05	4.505	10.880	0.000	12.804	0.000	0.118	0.000	0.004	0.007	-0.003
3	5.16	0.000	9.187	0.545	19.904	0.000	0.388	0.000	0.008	0.011	-0.003
4	5.17	0.000	5.542	0.831	26.446	0.000	0.565	0.000	0.010	0.015	-0.004
5	5.12	0.000	1.994	0.988	32.532	1.162	1.007	0.000	0.013	0.019	-0.006
6	5.32	0.000	0.162	1.132	35.933	1.556	5.487	0.000	0.014	0.024	-0.009
7	5.37	0.000	0.116	1.099	36.491	1.354	0.934	0.000	0.015	0.021	-0.006
8	5.43	0.000	0.104	1.039	33.265	1.530	1.093	0.000	0.015	0.019	-0.005
9	5.43	0.000	0.104	1.027	35.369	1.472	1.131	0.000	0.015	0.020	-0.005

Table E-6: Substrates usage and product formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #2).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	6.45	10.123	10.294	0.000	0.206	0.685	0.000	0.000	0.000	0.086	-0.086
2	5.05	6.449	10.823	0.000	5.713	0.000	0.536	0.000	0.005	0.004	0.002
3	5.09	0.124	4.936	1.082	28.781	0.000	0.261	0.000	0.009	0.016	-0.007
4	5.22	0.000	0.000	1.439	36.877	1.332	0.660	0.000	0.013	0.022	-0.009
5	5.29	0.000	0.000	1.368	36.542	1.488	0.883	0.000	0.014	0.022	-0.008
6	5.53	0.000	0.000	1.473	41.302	1.576	5.443	0.000	0.014	0.027	-0.012
7	5.57	0.000	0.000	1.438	37.466	1.515	0.834	0.000	0.014	0.022	-0.007
8	5.57	0.000	0.000	1.266	34.795	1.490	0.856	0.000	0.014	0.020	-0.006
9	5.45	0.000	0.000	1.340	36.704	1.604	0.952	0.000	0.014	0.021	-0.007

Table E-7: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #1).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.118	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.388	0.000	0.000	0.000	0.000
4	0.000	0.000	0.000	0.338	0.000	0.000	0.000	0.228
5	0.205	0.032	0.000	0.507	0.010	0.022	0.000	0.231
6	0.316	0.024	0.021	4.960	0.101	0.000	0.036	0.029
7	0.368	0.022	0.000	0.457	0.002	0.002	0.017	0.067
8	0.384	0.020	0.000	0.457	0.026	0.011	0.032	0.164
9	0.428	0.016	0.002	0.458	0.017	0.012	0.032	0.165

Table E-8: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #2).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.536	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.261	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	0.487	0.000	0.000	0.037	0.000	0.000	0.000	0.135
5	0.595	0.021	0.000	0.061	0.019	0.000	0.027	0.159
6	0.685	0.021	0.019	4.580	0.081	0.000	0.036	0.020
7	0.676	0.025	0.000	0.067	0.003	0.000	0.013	0.050
8	0.616	0.021	0.001	0.072	0.010	0.005	0.025	0.106
9	0.700	0.015	0.084	0.072	0.014	0.006	0.027	0.117

Table E-9: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #1).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	6.45	10.123	10.294	0.000	0.206	0.685	0.000	0.000	0.000	0.086	-0.086
2	5.07	2.961	10.559	0.000	1.062	0.000	3.011	0.000	0.006	0.003	0.003
3	5.15	0.000	8.732	0.866	0.985	0.000	4.934	0.000	0.010	0.004	0.006
4	5.3	0.000	7.140	0.000	0.242	0.000	5.655	0.000	0.013	0.004	0.009
5	5.13	0.000	5.044	0.000	0.000	0.000	8.458	0.000	0.016	0.005	0.011
6	5.06	0.000	1.513	0.000	0.000	0.000	10.374	0.000	0.021	0.007	0.014
7	5.16	0.000	0.000	0.000	0.000	0.000	11.152	0.000	0.023	0.007	0.016
8	5.24	0.000	0.000	0.000	0.000	0.000	12.234	0.000	0.024	0.008	0.016
9	5.36	0.000	0.000	0.000	0.000	0.000	11.264	0.000	0.024	0.007	0.017

Table E-10: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #2).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	6.45	10.123	10.294	0.000	0.206	0.685	0.000	0.000	0.000	0.086	-0.086
2	5.15	2.655	10.356	0.000	1.244	0.000	3.732	0.000	0.007	0.003	0.004
3	5.14	0.000	8.515	0.941	0.667	0.000	7.028	0.000	0.013	0.005	0.008
4	5.25	0.000	7.081	0.000	0.000	0.000	7.541	0.000	0.017	0.005	0.012
5	5.15	0.000	4.332	0.000	0.000	0.000	9.587	0.000	0.021	0.006	0.014
6	5.13	0.000	0.529	0.000	0.000	0.000	10.783	0.000	0.025	0.007	0.018
7	5.27	0.000	0.000	0.000	0.000	0.000	10.924	0.000	0.027	0.007	0.020
8	5.29	0.000	0.000	0.000	0.000	0.000	11.061	0.000	0.028	0.007	0.021
9	5.4	0.000	0.000	0.000	0.000	0.000	11.174	0.000	0.028	0.007	0.021

Table E-11: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #1).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1.415	0.000	0.000	1.596	0.000	0.000	0.000	0.000
3	2.009	0.000	0.000	2.925	0.000	0.000	0.000	0.000
4	2.624	0.000	0.000	2.944	0.000	0.000	0.028	0.059
5	3.382	0.027	0.025	4.767	0.167	0.005	0.037	0.048
6	4.405	0.033	0.004	5.806	0.058	0.000	0.033	0.035
7	4.940	0.025	0.000	6.103	0.024	0.003	0.033	0.024
8	4.852	0.019	0.022	6.938	0.020	0.016	0.104	0.264
9	4.965	0.033	0.040	6.120	0.016	0.007	0.051	0.031

Table E-12: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #2).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1.440	0.000	0.000	2.292	0.000	0.000	0.000	0.000
3	2.612	0.000	0.000	4.416	0.000	0.000	0.000	0.000
4	3.191	0.051	0.000	4.231	0.000	0.000	0.000	0.067
5	3.852	0.026	0.023	5.455	0.145	0.000	0.046	0.041
6	3.928	0.021	0.004	6.689	0.068	0.000	0.037	0.036
7	4.235	0.020	0.000	6.564	0.043	0.000	0.042	0.019
8	4.206	0.026	0.043	6.533	0.010	0.006	0.083	0.155
9	4.436	0.035	0.077	6.492	0.017	0.006	0.073	0.038

Table E-13: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #1).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	6.45	10.123	10.294	0.000	0.206	0.685	0.000	0.000	0.000	0.086	-0.086
2	5.17	3.419	10.747	0.000	1.416	0.000	2.782	0.000	0.007	0.003	0.004
3	5.18	1.284	9.647	0.257	4.530	0.000	3.033	0.000	0.010	0.004	0.005
4	5.4	0.000	9.423	0.000	6.309	0.000	3.637	0.000	0.012	0.006	0.006
5	5.23	0.000	6.795	0.000	4.920	0.000	5.688	0.000	0.015	0.006	0.009
6	5.18	0.000	3.586	0.000	3.750	0.000	7.490	0.000	0.019	0.007	0.013
7	5.16	0.000	0.284	0.000	2.588	0.000	8.823	0.000	0.023	0.007	0.016
8	5.42	0.000	0.000	0.000	1.172	0.000	9.568	0.000	0.025	0.006	0.019
9	6	0.000	0.000	0.000	0.000	0.000	9.559	0.000	0.026	0.006	0.020

Table E-14: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #2).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	6.45	10.123	10.294	0.000	0.206	0.685	0.000	0.000	0.000	0.086	-0.086
2	5.2	3.058	10.325	0.000	1.024	0.000	3.262	0.000	0.007	0.003	0.004
3	5.2	1.083	9.557	0.827	4.784	0.000	3.088	0.000	0.010	0.005	0.005
4	5.37	0.000	9.437	0.000	6.965	0.000	3.157	0.000	0.012	0.006	0.006
5	5.29	0.000	7.539	0.000	6.610	0.000	5.114	0.000	0.015	0.007	0.008
6	5.25	0.000	4.383	1.429	5.810	0.000	6.824	0.000	0.018	0.007	0.011
7	5.23	0.000	1.137	0.000	3.692	0.000	8.036	0.000	0.022	0.007	0.016
8	5.36	0.000	0.000	0.000	1.395	0.000	9.009	0.000	0.025	0.006	0.019
9	5.9	0.000	0.000	0.000	0.000	0.000	9.185	0.000	0.026	0.005	0.021

Table E-15: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #1).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1.272	0.000	0.000	1.510	0.000	0.000	0.000	0.000
3	1.226	0.000	0.000	1.807	0.000	0.000	0.000	0.000
4	1.561	0.023	0.029	1.987	0.000	0.000	0.000	0.036
5	1.850	0.028	0.028	3.584	0.120	0.000	0.044	0.035
6	2.347	0.028	0.003	4.954	0.099	0.000	0.033	0.027
7	2.800	0.021	0.000	5.861	0.088	0.000	0.036	0.016
8	2.788	0.025	0.017	6.568	0.003	0.004	0.057	0.106
9	2.504	0.049	0.092	6.805	0.011	0.005	0.060	0.033

Table E-16: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using $CaCO_3$ as a buffer (replicate #2).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1.355	0.000	0.000	1.907	0.000	0.000	0.000	0.000
3	1.263	0.000	0.000	1.825	0.000	0.000	0.000	0.000
4	1.542	0.015	0.006	1.568	0.000	0.000	0.000	0.026
5	1.884	0.019	0.022	3.045	0.085	0.000	0.033	0.027
6	2.082	0.021	0.002	4.580	0.081	0.000	0.036	0.020
7	2.150	0.024	0.002	5.751	0.051	0.000	0.042	0.015
8	1.846	0.020	0.002	6.983	0.003	0.000	0.077	0.077
9	1.900	0.034	0.005	7.094	0.008	0.007	0.115	0.022

Table E-17: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #1).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.68	10.010	10.059	0.000	0.286	0.859	0.000	0.000	0.000	0.002	-0.002
2	6.53	6.185	8.750	0.000	5.682	0.493	0.432	0.000	0.005	0.008	-0.003
3	5.4	2.933	7.208	0.932	8.139	1.235	1.038	0.000	0.009	0.013	-0.004
4	6.59	0.000	6.029	0.933	12.086	1.945	2.555	0.000	0.015	0.021	-0.005
5	6.82	0.000	4.856	1.652	6.122	1.981	4.440	0.000	0.020	0.016	0.004
6	6.6	0.000	2.638	1.704	0.000	2.032	6.878	0.000	0.024	0.012	0.012
7	6.38	0.000	0.777	1.339	0.000	1.384	7.189	0.000	0.027	0.011	0.016
8	6.32	0.000	0.048	1.263	0.000	1.193	7.993	0.000	0.029	0.012	0.017
9	6.91	0.000	0.000	0.000	0.000	0.956	8.122	0.000	0.030	0.012	0.018

Table E-18: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #2).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.68	10.010	10.059	0.000	0.286	0.859	0.000	0.000	0.000	0.002	-0.002
2	6.45	5.971	8.602	0.000	5.268	0.720	0.660	0.000	0.006	0.008	-0.003
3	5.6	3.457	6.930	1.216	4.795	1.483	1.426	0.000	0.011	0.010	0.014
4	6.22	0.000	6.401	2.307	7.596	2.601	2.771	0.000	0.017	0.017	-0.041
5	7.05	0.000	4.151	2.474	6.437	3.286	4.753	0.000	0.022	0.020	0.001
6	6.57	0.000	1.962	2.078	5.243	2.688	5.448	0.000	0.025	0.018	0.006
7	6.69	0.000	0.812	2.168	1.816	2.212	6.033	0.000	0.027	0.014	0.013
8	7.02	0.000	0.000	2.508	0.000	1.286	6.608	0.000	0.030	0.011	0.019
9	7.93	0.000	0.000	3.027	0.000	0.454	8.837	0.000	0.031	0.012	0.019

Table E-19: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #1).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.432	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	1.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	1.905	0.042	0.003	0.476	0.005	0.000	0.000	0.123
5	1.512	0.101	0.021	2.649	0.010	0.000	0.022	0.124
6	1.598	0.175	0.023	4.926	0.017	0.021	0.017	0.102
7	1.843	0.305	0.005	4.963	0.007	0.019	0.012	0.036
8	2.046	0.438	0.006	5.352	0.012	0.033	0.022	0.084
9	2.295	0.554	0.006	5.118	0.013	0.028	0.022	0.085

Table E-20: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #2).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.660	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.964	0.000	0.000	0.462	0.000	0.000	0.000	0.000
4	1.566	0.017	0.000	1.096	0.000	0.000	0.000	0.092
5	2.645	0.056	0.028	1.897	0.008	0.012	0.017	0.091
6	3.033	0.084	0.024	2.194	0.019	0.006	0.013	0.075
7	2.583	0.100	0.006	3.290	0.008	0.007	0.011	0.030
8	2.391	0.236	0.004	3.881	0.009	0.015	0.016	0.057
9	3.408	1.346	0.005	3.972	0.012	0.016	0.019	0.060

Table E-21: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #1).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.68	10.010	10.059	0.000	0.286	0.859	0.000	0.000	0.000	0.002	-0.002
2	6.05	2.750	6.920	1.392	1.379	0.395	3.368	0.000	0.008	0.007	0.001
3	5.4	0.589	5.331	1.872	3.506	0.798	3.553	0.000	0.012	0.011	0.002
4	6.33	0.000	2.038	4.349	0.000	2.739	6.112	0.000	0.018	0.014	0.004
5	7.16	0.000	0.073	5.191	0.000	3.707	7.999	0.000	0.021	0.018	0.003
6	7.5	0.000	0.000	4.712	0.000	3.563	8.180	0.000	0.022	0.018	0.004
7	7.83	0.000	0.000	4.773	0.000	3.156	8.450	0.000	0.023	0.018	0.006
8	8.18	0.000	0.000	4.605	0.000	2.077	6.649	0.000	0.024	0.014	0.010
9	8.23	0.000	0.000	4.265	0.000	0.539	11.648	0.000	0.025	0.017	0.008

Table E-22: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #2).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.68	10.010	10.059	0.000	0.286	0.859	0.000	0.000	0.000	0.002	-0.002
2	6.17	1.948	9.669	1.400	6.194	0.588	1.274	0.000	0.008	0.005	0.003
3	5.55	0.000	8.357	2.025	9.268	0.933	1.542	0.000	0.012	0.007	0.004
4	5.85	0.000	4.679	5.662	11.887	3.336	3.443	0.000	0.017	0.013	0.005
5	7.45	0.000	3.132	0.000	12.586	4.607	4.693	0.000	0.021	0.015	0.006
6	7.21	0.000	1.179	0.000	11.743	4.108	5.637	0.000	0.024	0.015	0.009
7	7.5	0.000	0.133	3.083	10.027	2.296	6.854	0.000	0.026	0.013	0.014
8	7.82	0.000	0.000	2.185	7.690	0.363	10.141	0.000	0.028	0.012	0.016
9	7.97	0.000	0.000	1.900	6.220	0.000	11.514	0.000	0.029	0.012	0.017

Table E-23: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #1).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1.750	0.067	0.122	1.428	0.000	0.000	0.000	0.000
3	1.985	0.000	0.000	1.569	0.000	0.000	0.000	0.000
4	2.654	0.043	0.032	3.305	0.000	0.000	0.000	0.078
5	3.414	0.054	0.029	4.389	0.000	0.015	0.019	0.080
6	3.805	0.059	0.005	4.239	0.004	0.005	0.011	0.053
7	4.342	0.100	0.008	3.950	0.010	0.006	0.011	0.023
8	6.083	0.021	0.000	0.469	0.007	0.006	0.015	0.048
9	6.868	0.770	0.015	3.890	0.030	0.011	0.016	0.047

Table E-24: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #2).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1.274	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	1.459	0.000	0.000	0.083	0.000	0.000	0.000	0.000
4	3.366	0.000	0.000	0.024	0.000	0.000	0.000	0.053
5	4.529	0.023	0.021	0.046	0.000	0.000	0.015	0.059
6	5.449	0.035	0.018	0.075	0.000	0.000	0.012	0.049
7	6.670	0.040	0.002	0.106	0.005	0.000	0.010	0.022
8	8.971	0.056	0.013	0.186	0.038	0.044	0.178	0.655
9	11.020	0.077	0.007	0.336	0.015	0.005	0.014	0.040

Table E-25: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #1).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.68	10.010	10.059	0.000	0.286	0.859	0.000	0.000	0.000	0.002	-0.002
2	5.9	0.000	9.036	1.539	5.769	1.240	2.691	0.000	0.008	0.013	-0.005
3	5.84	0.000	4.537	4.406	2.278	0.000	5.921	0.000	0.015	0.012	0.003
4	6.5	0.000	0.000	6.259	0.000	0.000	10.244	0.000	0.022	0.016	0.007
5	6.76	0.000	0.000	6.138	0.000	0.000	12.292	0.000	0.025	0.019	0.006
6	7.02	0.000	0.000	5.956	0.000	0.000	13.394	0.000	0.026	0.020	0.006
7	7.16	0.000	0.000	5.681	0.000	0.000	13.830	0.000	0.026	0.021	0.006
8	7.36	0.000	0.000	3.786	0.000	0.000	13.747	0.000	0.027	0.020	0.007
9	7.41	0.000	0.000	5.095	0.000	0.000	13.968	0.000	0.027	0.020	0.007

Table E-26: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #2).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.68	10.010	10.059	0.000	0.286	0.859	0.000	0.000	0.000	0.002	-0.002
2	5.7	0.000	6.563	2.222	0.000	1.485	4.836	0.000	0.010	0.010	0.052
3	5.47	0.000	2.386	3.299	0.000	0.696	7.083	0.000	0.017	0.011	0.006
4	6.6	0.000	0.027	4.205	0.000	0.000	9.882	0.000	0.023	0.014	0.009
5	6.96	0.000	0.000	3.274	0.000	0.000	13.780	0.000	0.025	0.019	0.006
6	7.16	0.000	0.000	0.923	0.000	0.000	13.962	0.000	0.026	0.019	0.007
7	7.22	0.000	0.000	2.441	0.000	0.000	13.684	0.000	0.026	0.019	0.007
8	7.32	0.000	0.000	2.203	0.000	0.000	13.495	0.000	0.027	0.018	0.008
9	7.35	0.000	0.000	1.751	0.000	0.000	13.701	0.000	0.027	0.019	0.008

Table E-27: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #1).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	2.691	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	4.952	0.273	0.000	0.695	0.000	0.000	0.000	0.000
4	8.687	0.335	0.025	1.157	0.016	0.000	0.000	0.025
5	10.056	0.565	0.023	1.574	0.032	0.000	0.013	0.030
6	10.921	0.678	0.088	1.611	0.053	0.007	0.010	0.025
7	11.310	0.796	0.057	1.571	0.066	0.000	0.011	0.018
8	11.128	0.842	0.065	1.547	0.076	0.008	0.018	0.064
9	11.418	0.813	0.059	1.541	0.083	0.010	0.019	0.026

Table E-28: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #2).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	2.264	0.000	0.000	2.572	0.000	0.000	0.000	0.000
3	3.338	0.044	0.000	3.701	0.000	0.000	0.000	0.000
4	5.873	0.076	0.008	3.902	0.000	0.000	0.000	0.023
5	7.431	0.399	0.022	5.844	0.032	0.000	0.018	0.034
6	8.387	0.383	0.029	5.057	0.052	0.006	0.014	0.034
7	8.564	0.391	0.031	4.606	0.057	0.007	0.014	0.014
8	8.442	0.424	0.033	4.460	0.057	0.013	0.018	0.048
9	8.680	0.454	0.035	4.417	0.058	0.011	0.018	0.028

Table E-29: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #1).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.68	10.010	10.059	0.000	0.286	0.859	0.000	0.000	0.000	0.002	-0.002
2	5.82	0.000	9.120	1.416	5.822	1.242	2.117	0.000	0.010	0.013	-0.003
3	6.01	0.000	8.242	2.126	5.735	1.110	2.660	0.000	0.013	0.013	0.000
4	6.47	0.000	0.515	7.268	4.680	2.254	4.880	0.000	0.021	0.017	0.004
5	7.02	0.000	0.000	8.828	0.000	1.463	5.805	0.000	0.024	0.011	0.013
6	7.57	0.000	0.000	10.064	0.000	1.057	6.733	0.000	0.025	0.011	0.014
7	7.77	0.000	0.000	9.243	0.000	1.079	6.787	0.000	0.026	0.012	0.014
8	8	0.000	0.000	8.335	0.000	0.782	6.947	0.000	0.026	0.011	0.015
9	7.98	0.000	0.000	8.115	0.000	0.000	7.946	0.000	0.027	0.011	0.016

Table E-30: Substrates usage and Product formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #2).

Days	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.68	10.010	10.059	0.000	0.286	0.859	0.000	0.000	0.000	0.002	-0.002
2	5.8	0.000	9.077	1.470	6.098	1.320	2.569	0.000	0.010	0.014	-0.004
3	5.9	0.000	8.124	2.295	6.022	0.935	2.777	0.000	0.013	0.013	0.014
4	6.37	0.000	2.591	4.961	6.155	2.180	4.845	0.000	0.020	0.019	0.002
5	6.86	0.000	0.000	6.009	3.374	1.293	6.292	0.000	0.026	0.016	0.010
6	7.319	0.000	0.000	8.344	0.000	0.808	6.839	0.000	0.028	0.011	0.017
7	7.56	0.000	0.000	8.237	0.000	0.558	7.481	0.000	0.029	0.012	0.017
8	7.86	0.000	0.000	7.688	0.000	0.437	7.904	0.000	0.029	0.012	0.017
9	7.85	0.000	0.000	7.454	0.000	0.000	8.341	0.000	0.030	0.011	0.018

Table E-31: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #1).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	2.004	0.000	0.000	0.113	0.000	0.000	0.000	0.000
3	2.482	0.031	0.000	0.147	0.000	0.000	0.000	0.000
4	4.227	0.016	0.005	0.608	0.000	0.000	0.000	0.023
5	3.393	0.043	0.008	2.317	0.004	0.004	0.012	0.023
6	3.903	0.438	0.007	2.345	0.006	0.000	0.008	0.026
7	4.059	0.442	0.009	2.243	0.007	0.003	0.009	0.016
8	4.113	0.461	0.017	2.266	0.026	0.005	0.015	0.043
9	5.108	0.478	0.030	2.228	0.052	0.008	0.015	0.027

Table E-32: Total carboxylic acid formation during the mixed microbial fermentation of pure sugars using NH_4HCO_3 as a buffer (replicate #2).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	2.569	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	2.627	0.000	0.000	0.150	0.000	0.000	0.000	0.000
4	4.637	0.013	0.006	0.170	0.000	0.000	0.000	0.019
5	5.150	0.061	0.007	1.036	0.004	0.004	0.009	0.020
6	4.610	0.094	0.011	2.077	0.006	0.003	0.012	0.027
7	5.166	0.224	0.013	2.040	0.010	0.004	0.009	0.014
8	5.234	0.465	0.017	2.121	0.015	0.008	0.013	0.032
9	5.529	0.502	0.027	2.199	0.037	0.007	0.015	0.025

Table E-33: Substrates usage and Product formation during the mixed microbial fermentation of hot water extract (replicate #1).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.10	0.115	1.284	0.000	0.339	1.619	3.204	0.000	0.000	0.009	-0.009
2	6.92	0.000	0.598	0.000	4.103	0.000	8.237	0.000	0.009	0.017	-0.008
3	6.79	0.000	0.000	0.000	4.903	0.560	8.170	0.000	0.014	0.019	-0.006
4	6.61	0.000	0.293	0.000	5.363	0.770	9.320	0.000	0.016	0.022	-0.006
5	6.24	0.000	0.929	0.000	5.413	0.784	9.868	0.000	0.018	0.023	-0.005
6	6.04	0.000	1.674	0.000	5.860	0.716	10.014	0.000	0.018	0.023	-0.005
7	6.00	0.251	2.449	0.000	7.329	1.146	9.839	0.000	0.018	0.025	-0.006
8	5.92	0.000	4.715	0.000	6.029	1.633	11.623	0.000	0.019	0.027	-0.008
10	5.85	0.000	4.722	0.000	5.914	0.999	10.253	0.000	0.019	0.023	-0.004
12	6.09	0.000	3.374	0.737	5.837	1.511	11.335	0.000	0.022	0.026	-0.003
14	6.72	0.000	3.316	1.297	6.946	1.943	12.273	0.000	0.024	0.029	-0.004
16	6.36	0.000	3.219	1.322	7.735	2.159	12.863	0.000	0.026	0.030	-0.005
18	6.04	0.000	3.349	1.355	7.966	2.309	12.882	0.000	0.027	0.031	-0.004
20	5.94	0.081	3.871	1.440	8.257	2.504	13.401	0.000	0.027	0.032	-0.005
22	5.96	0.000	4.273	1.472	8.625	2.561	13.620	0.000	0.027	0.032	-0.005

Table E-34: Substrates usage and Product formation during the mixed microbial fermentation of hot water extract (replicate #2).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.10	0.115	1.284	0.000	0.339	1.619	3.204	0.000	0.000	0.009	-0.009
2	6.90	0.000	0.318	0.000	6.022	0.000	6.785	0.000	0.008	0.018	-0.009
3	6.90	0.000	0.653	0.000	4.844	0.321	7.235	0.000	0.012	0.018	-0.005
4	6.97	0.319	0.234	0.000	4.445	0.296	7.682	0.000	0.015	0.018	-0.003
5	6.71	0.357	0.457	0.000	4.606	0.362	8.479	0.000	0.016	0.019	-0.003
6	6.35	0.000	0.804	0.000	5.154	0.481	7.941	0.000	0.016	0.019	-0.003
7	6.24	0.000	1.485	0.000	4.662	0.427	8.718	0.000	0.017	0.019	-0.002
8	6.06	0.000	2.156	0.000	5.419	0.665	8.381	0.000	0.017	0.020	-0.003
10	5.94	0.000	2.758	0.000	4.777	0.663	9.723	0.000	0.017	0.021	-0.003
12	6.89	0.000	2.441	0.000	4.790	1.088	10.109	0.000	0.021	0.022	-0.001
14	6.29	0.000	1.949	1.123	6.251	1.234	10.714	0.000	0.023	0.025	-0.002
16	6.30	0.000	2.327	0.922	7.472	1.776	10.159	0.000	0.024	0.026	-0.002
18	6.17	0.000	2.646	0.982	6.634	1.212	11.056	0.000	0.024	0.025	0.000
20	6.11	0.000	3.176	0.982	7.086	1.291	11.901	0.000	0.025	0.026	-0.002
22	6.07	0.000	3.243	0.983	7.030	1.304	11.771	0.000	0.025	0.026	-0.001

Table E-35: Total carboxylic acid formation during the mixed microbial fermentation of hot water extract (replicate #1).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	3.060	0.016	0.001	0.009	0.012	0.017	0.018	0.071
2	6.399	0.157	0.048	0.117	0.085	0.246	0.261	0.925
3	7.317	0.053	0.035	0.041	0.055	0.066	0.117	0.487
4	8.373	0.063	0.042	0.049	0.064	0.071	0.137	0.522
5	9.043	0.065	0.037	0.053	0.064	0.048	0.115	0.442
6	9.188	0.078	0.041	0.053	0.062	0.053	0.123	0.415
7	8.849	0.115	0.048	0.069	0.078	0.065	0.141	0.474
8	10.612	0.098	0.054	0.062	0.081	0.070	0.148	0.497
10	9.677	0.059	0.013	0.023	0.031	0.024	0.076	0.351
12	10.557	0.083	0.041	0.049	0.063	0.053	0.113	0.375
14	11.578	0.167	0.012	0.024	0.041	0.029	0.085	0.336
16	11.871	0.480	0.019	0.023	0.032	0.030	0.080	0.329
18	11.946	0.599	0.037	0.043	0.067	0.048	0.069	0.073
20	12.302	0.618	0.054	0.068	0.073	0.068	0.093	0.125
22	12.731	0.547	0.035	0.042	0.050	0.045	0.069	0.101

Table E-36: Total carboxylic acid formation during the mixed microbial fermentation of hot water extract (replicate #2).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	3.060	0.016	0.001	0.009	0.012	0.017	0.018	0.071
2	6.382	0.124	0.003	0.014	0.011	0.029	0.035	0.188
3	7.064	0.019	0.003	0.008	0.007	0.003	0.019	0.111
4	7.512	0.025	0.001	0.008	0.007	0.003	0.020	0.106
5	8.305	0.031	0.004	0.012	0.008	0.002	0.020	0.098
6	7.777	0.029	0.002	0.009	0.007	0.003	0.020	0.093
7	8.552	0.034	0.002	0.010	0.007	0.003	0.020	0.091
8	8.179	0.072	0.003	0.008	0.007	0.002	0.019	0.090
10	9.228	0.231	0.009	0.032	0.014	0.016	0.056	0.138
12	9.800	0.171	0.005	0.017	0.010	0.003	0.021	0.081
14	10.390	0.202	0.004	0.010	0.008	0.005	0.021	0.073
16	9.680	0.348	0.004	0.008	0.008	0.005	0.024	0.084
18	10.589	0.428	0.003	0.005	0.008	0.002	0.017	0.005
20	11.359	0.503	0.002	0.003	0.006	0.003	0.016	0.008
22	11.171	0.554	0.003	0.003	0.008	0.002	0.019	0.010

Table E-37: Substrates usage and Product formation during the mixed microbial fermentation of hot water extract (replicate #1).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.10	0.115	1.284	0.000	0.339	1.619	3.204	0.000	0.000	0.009	-0.009
2	6.79	0.000	0.000	0.000	4.962	0.000	6.651	0.000	0.009	0.016	-0.007
3	6.77	0.000	0.316	0.000	5.343	0.540	7.876	0.000	0.014	0.020	-0.006
4	6.65	0.000	0.326	0.000	5.390	0.546	8.704	0.000	0.016	0.021	-0.005
5	6.37	0.000	0.507	0.000	5.317	0.487	8.963	0.000	0.017	0.021	-0.004
6	6.14	0.000	0.685	0.000	5.366	0.466	9.107	0.000	0.018	0.021	-0.003
7	6.02	0.000	1.126	0.000	5.487	0.507	9.188	0.000	0.018	0.021	-0.003
8	6.00	0.000	0.965	0.000	5.499	0.546	9.535	0.000	0.018	0.021	-0.003
10	5.88	0.000	1.551	0.000	5.499	0.649	9.749	0.000	0.019	0.022	-0.003
12	6.87	0.000	2.265	0.545	5.257	0.966	9.868	0.000	0.022	0.022	0.000
14	6.12	0.000	1.851	0.955	7.368	1.593	10.259	0.000	0.024	0.026	-0.001
16	5.98	0.000	2.906	0.891	7.707	1.551	9.838	0.000	0.025	0.025	0.000
18	5.85	0.000	3.536	0.959	7.474	1.585	9.566	0.000	0.025	0.024	0.001
20	5.85	0.000	3.419	0.800	7.548	1.735	9.597	0.000	0.026	0.024	0.001
22	5.86	0.000	3.838	0.899	7.803	1.861	9.939	0.000	0.026	0.025	0.001

Table E-38: Substrates usage and Product formation during the mixed microbial fermentation of hot water extract (replicate #2).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.10	0.115	1.284	0.000	0.339	1.619	3.204	0.000	0.000	0.009	-0.009
2	6.88	0.000	0.729	0.000	4.815	0.000	6.572	0.000	0.009	0.016	-0.007
3	6.82	0.000	0.000	0.000	5.139	0.476	7.777	0.000	0.014	0.019	-0.006
4	6.82	0.000	0.000	0.000	5.168	0.550	8.627	0.000	0.016	0.021	-0.005
5	6.59	0.000	0.244	0.000	5.068	0.549	9.047	0.000	0.018	0.021	-0.003
6	6.35	0.000	0.638	0.000	5.072	0.493	9.424	0.000	0.018	0.021	-0.003
7	6.26	0.000	1.268	0.000	5.230	0.421	9.118	0.000	0.018	0.021	-0.002
8	6.21	0.000	1.961	0.000	5.320	0.432	9.500	0.000	0.019	0.021	-0.002
10	5.99	0.000	2.797	0.000	5.429	0.675	9.810	0.000	0.019	0.022	-0.003
12	7.06	0.000	2.079	0.000	5.802	0.648	10.063	0.000	0.022	0.022	0.000
14	6.48	0.000	0.952	0.768	7.969	0.683	9.228	0.000	0.025	0.023	0.002
16	6.14	0.000	0.876	0.745	8.765	0.982	9.426	0.000	0.026	0.024	0.001
18	6.04	0.000	0.929	0.764	8.512	0.985	9.011	0.000	0.026	0.023	0.003
20	5.97	0.000	1.294	0.738	9.678	1.088	9.509	0.000	0.027	0.025	0.001
22	5.94	0.000	1.373	0.745	9.980	1.082	9.299	0.000	0.027	0.025	0.002

Table E-39: Total carboxylic acid formation during the mixed microbial fermentation of hot water extract (replicate #1).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	3.060	0.016	0.001	0.009	0.012	0.017	0.018	0.071
2	6.506	0.016	0.001	0.009	0.012	0.017	0.018	0.071
3	7.771	0.025	0.002	0.012	0.007	0.001	0.013	0.047
4	8.603	0.023	0.002	0.011	0.006	0.002	0.015	0.041
5	8.852	0.040	0.002	0.012	0.006	0.003	0.014	0.035
6	8.971	0.065	0.004	0.009	0.007	0.003	0.015	0.032
7	9.044	0.078	0.002	0.009	0.008	0.002	0.014	0.030
8	9.381	0.092	0.003	0.005	0.005	0.002	0.014	0.034
10	9.466	0.185	0.004	0.012	0.016	0.006	0.022	0.038
12	9.669	0.137	0.003	0.008	0.008	0.004	0.014	0.025
14	9.206	0.983	0.003	0.037	0.004	0.004	0.014	0.009
16	9.635	0.128	0.002	0.007	0.011	0.003	0.017	0.036
18	9.258	0.198	0.003	0.059	0.014	0.003	0.029	0.003
20	9.378	0.137	0.002	0.048	0.003	0.003	0.022	0.003
22	9.480	0.289	0.009	0.088	0.017	0.007	0.038	0.010

Table E-40: Total carboxylic acid formation during the mixed microbial fermentation of hot water extract (replicate #2).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	3.060	0.016	0.001	0.009	0.012	0.017	0.018	0.071
2	6.479	0.021	0.000	0.006	0.012	0.005	0.015	0.033
3	7.706	0.022	0.002	0.009	0.005	0.001	0.012	0.020
4	8.548	0.022	0.002	0.011	0.007	0.004	0.014	0.020
5	8.962	0.034	0.002	0.012	0.007	0.003	0.012	0.015
6	9.247	0.080	0.004	0.018	0.009	0.006	0.025	0.035
7	8.979	0.089	0.002	0.008	0.007	0.001	0.014	0.017
8	9.367	0.088	0.003	0.011	0.005	0.001	0.013	0.013
10	9.666	0.095	0.002	0.012	0.006	0.001	0.015	0.012
12	9.848	0.171	0.003	0.007	0.009	0.002	0.013	0.011
14	9.013	0.161	0.003	0.005	0.009	0.004	0.016	0.017
16	9.191	0.179	0.003	0.005	0.007	0.003	0.017	0.021
18	8.758	0.214	0.002	0.004	0.008	0.002	0.020	0.003
20	9.293	0.186	0.002	0.005	0.006	0.002	0.013	0.002
22	9.059	0.198	0.002	0.011	0.005	0.002	0.016	0.005

Table E-41: Substrates usage and Product formation during the mixed microbial fermentation of hot water extract (replicate #1).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.10	0.115	1.284	0.000	0.339	1.619	3.204	0.000	0.000	0.009	-0.009
2	7.45	0.254	1.205	0.000	0.326	0.000	3.294	0.000	0.004	0.006	-0.002
3	7.52	0.000	1.883	0.000	1.565	0.000	4.555	0.000	0.006	0.009	-0.003
4	7.50	0.000	1.378	0.000	0.839	0.000	5.767	0.000	0.009	0.010	-0.002
5	6.90	0.000	1.043	0.704	1.547	3.000	7.378	0.000	0.013	0.020	-0.007
6	6.80	0.000	0.639	0.827	2.220	3.340	8.075	0.000	0.013	0.022	-0.008
7	6.15	0.000	0.000	0.878	2.554	3.194	8.933	0.000	0.016	0.023	-0.007
8	5.77	0.000	0.000	1.300	2.426	2.977	9.525	0.000	0.018	0.023	-0.005
10	5.66	0.000	0.498	1.315	2.592	3.072	11.122	0.000	0.019	0.025	-0.006
12	6.31	0.000	2.352	1.340	3.021	3.224	14.060	0.000	0.023	0.030	-0.007
14	5.72	0.000	4.922	1.248	2.516	2.600	13.776	0.000	0.026	0.027	-0.001
16	6.71	0.000	6.382	1.114	2.094	2.982	16.017	0.000	0.031	0.030	0.000
18	6.49	0.000	7.798	1.830	1.601	3.613	17.127	0.000	0.034	0.032	0.002
20	6.17	0.000	8.524	2.036	1.340	3.781	17.786	0.000	0.036	0.032	0.004
22	6.00	0.000	3.200	4.037	1.428	5.219	22.610	0.000	0.039	0.041	-0.003

Table E-42: Substrates usage and Product formation during the mixed microbial fermentation of hot water extract (replicate #2).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.10	0.115	1.284	0.000	0.339	1.619	3.204	0.000	0.000	0.009	-0.009
2	7.49	0.694	2.014	0.000	0.923	0.000	3.666	0.000	0.004	0.007	-0.003
3	7.42	0.000	1.885	0.000	0.531	0.275	4.312	0.000	0.006	0.008	-0.002
4	7.13	0.000	0.959	0.000	0.635	3.112	6.712	0.000	0.010	0.018	-0.008
5	6.87	0.000	0.674	0.960	1.257	4.169	7.839	0.000	0.013	0.023	-0.009
6	6.45	0.143	0.168	1.204	1.807	4.658	8.435	0.000	0.014	0.025	-0.011
7	6.28	0.000	0.059	1.331	2.082	4.677	8.702	0.000	0.016	0.025	-0.010
8	5.98	0.000	0.000	1.751	2.199	4.506	9.055	0.000	0.017	0.025	-0.009
10	5.76	0.000	0.000	1.750	2.168	4.264	9.596	0.000	0.017	0.025	-0.008
12	6.06	0.000	0.249	1.639	2.482	5.170	11.852	0.000	0.021	0.030	-0.009
14	5.91	0.000	1.080	1.608	1.154	3.963	14.374	0.000	0.024	0.030	-0.006
16	6.81	0.000	2.102	1.720	0.645	4.633	17.871	0.000	0.028	0.035	-0.007
18	6.47	0.000	4.161	2.823	0.459	4.919	18.050	0.000	0.032	0.035	-0.003
20	6.21	0.000	5.707	2.351	4.982	8.493	16.409	0.000	0.034	0.043	-0.008
22	6.11	0.000	5.817	2.984	1.336	4.092	16.295	0.000	0.036	0.030	0.006

Table E-43: Total carboxylic acid formation during the mixed microbial fermentation of hot water extract (replicate #1).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	3.060	0.016	0.001	0.009	0.012	0.017	0.018	0.071
2	3.245	0.018	0.000	0.007	0.006	0.001	0.012	0.005
3	4.507	0.014	0.001	0.011	0.005	0.002	0.011	0.004
4	5.716	0.015	0.001	0.012	0.004	0.002	0.011	0.006
5	7.333	0.009	0.001	0.010	0.005	0.001	0.012	0.006
6	8.024	0.012	0.001	0.008	0.005	0.001	0.013	0.009
7	8.682	0.214	0.002	0.011	0.005	0.002	0.012	0.006
8	8.883	0.597	0.003	0.009	0.005	0.005	0.012	0.011
10	9.872	1.211	0.002	0.010	0.006	0.001	0.012	0.007
12	12.447	1.568	0.005	0.010	0.006	0.002	0.012	0.009
14	11.345	2.106	0.004	0.291	0.006	0.003	0.014	0.007
16	12.325	2.609	0.006	1.040	0.005	0.006	0.015	0.011
18	12.591	2.862	0.007	1.635	0.005	0.011	0.012	0.003
20	12.463	3.127	0.007	2.149	0.005	0.016	0.014	0.005
22	16.593	3.179	0.007	2.787	0.005	0.021	0.014	0.005

Table E-44: Total carboxylic acid formation during the mixed microbial fermentation of hot water extract (replicate #2).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	3.060	0.016	0.001	0.009	0.012	0.017	0.018	0.071
2	3.619	0.017	0.000	0.008	0.005	0.002	0.012	0.003
3	4.266	0.014	0.002	0.011	0.005	0.002	0.010	0.003
4	6.656	0.016	0.002	0.012	0.006	0.002	0.011	0.007
5	7.784	0.015	0.002	0.013	0.006	0.001	0.012	0.006
6	8.385	0.015	0.002	0.010	0.005	0.002	0.011	0.005
7	8.635	0.034	0.001	0.010	0.004	0.001	0.011	0.006
8	8.861	0.160	0.002	0.007	0.005	0.001	0.011	0.007
10	9.182	0.375	0.002	0.008	0.009	0.002	0.012	0.008
12	11.121	0.660	0.005	0.038	0.006	0.002	0.012	0.008
14	12.271	1.493	0.004	0.568	0.007	0.004	0.015	0.011
16	14.449	1.905	0.006	1.474	0.007	0.006	0.017	0.010
18	13.743	1.916	0.004	2.356	0.006	0.007	0.012	0.006
20	10.938	2.113	0.005	3.311	0.006	0.013	0.013	0.011
22	10.171	1.990	0.007	4.080	0.006	0.016	0.012	0.012

Table E-45: Substrates usage and Product formation during the mixed microbial fermentation of hot water extract (replicate #1).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.10	0.115	1.284	0.000	0.339	1.619	3.204	0.000	0.000	0.009	-0.009
2	7.43	0.740	1.581	3.371	0.667	0.000	3.019	0.000	0.004	0.006	-0.002
3	7.43	0.000	1.927	0.000	0.399	0.277	4.068	0.000	0.007	0.008	-0.001
4	7.33	0.000	1.404	0.000	0.540	2.046	6.813	0.000	0.009	0.016	-0.007
5	6.93	0.000	1.283	0.000	0.699	2.199	8.440	0.000	0.013	0.019	-0.006
6	6.67	0.000	1.103	0.678	0.814	2.479	8.964	0.000	0.013	0.020	-0.007
7	6.30	0.000	0.666	0.661	0.970	2.622	9.864	0.000	0.015	0.022	-0.006
8	5.89	0.000	0.525	1.154	1.293	2.166	10.036	0.000	0.017	0.021	-0.004
10	5.72	0.000	0.785	1.096	1.385	2.301	10.249	0.000	0.018	0.022	-0.004
12	6.33	0.000	1.301	1.167	1.634	2.458	11.450	0.000	0.022	0.024	-0.002
14	5.77	0.000	2.170	1.005	2.757	3.356	11.321	0.000	0.024	0.026	-0.002
16	7.09	0.000	2.619	1.000	3.096	3.298	12.261	0.000	0.028	0.027	0.001
18	6.82	0.000	2.470	1.810	2.829	3.842	16.269	0.000	0.033	0.033	0.000
20	6.78	0.000	4.089	1.379	3.230	4.258	17.824	0.000	0.037	0.036	0.001
22	6.75	0.000	2.813	2.443	3.381	4.660	19.077	0.000	0.040	0.038	0.002

Table E-46: Substrates usage and Product formation during the mixed microbial fermentation of hot water extract (replicate #2).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.10	0.115	1.284	0.000	0.339	1.619	3.204	0.000	0.000	0.009	-0.009
2	7.43	0.764	1.714	3.297	0.287	0.000	3.074	0.000	0.004	0.005	-0.002
3	7.45	0.423	1.859	0.000	0.000	0.411	4.902	0.000	0.006	0.009	-0.002
4	7.15	0.000	1.218	0.000	0.469	3.048	6.709	0.000	0.010	0.018	-0.008
5	6.63	0.000	0.698	1.072	0.918	4.417	8.254	0.000	0.014	0.023	-0.009
6	6.67	0.000	0.458	1.072	1.506	4.491	8.468	0.000	0.015	0.024	-0.010
7	6.46	0.000	0.000	1.049	1.917	4.616	9.011	0.000	0.016	0.026	-0.009
8	6.08	0.138	0.116	1.681	2.198	4.614	9.306	0.000	0.017	0.026	-0.009
10	5.84	0.121	0.215	1.649	2.353	4.505	9.817	0.000	0.018	0.026	-0.009
12	6.09	0.000	1.030	1.569	2.524	4.436	11.839	0.000	0.022	0.029	-0.007
14	5.83	0.000	1.763	1.462	2.720	4.456	12.350	0.000	0.024	0.030	-0.006
16	6.83	0.000	1.482	1.353	4.122	5.892	13.479	0.000	0.028	0.035	-0.007
18	6.60	0.000	1.356	2.432	3.419	5.587	15.672	0.000	0.031	0.036	-0.005
20	6.31	0.000	1.602	3.015	3.669	6.561	17.978	0.000	0.034	0.041	-0.007
22	6.13	0.000	1.818	3.721	3.777	7.236	19.758	0.000	0.035	0.044	-0.009

Table E-47: Total carboxylic acid formation during the mixed microbial fermentation of hot water extract (replicate #1).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	3.060	0.016	0.001	0.009	0.012	0.017	0.018	0.071
2	2.948	0.016	0.000	0.007	0.001	0.028	0.011	0.007
3	4.019	0.014	0.000	0.011	0.006	0.002	0.011	0.005
4	6.761	0.017	0.002	0.009	0.007	0.002	0.011	0.005
5	8.393	0.011	0.002	0.012	0.003	0.002	0.011	0.005
6	8.919	0.010	0.001	0.009	0.004	0.001	0.011	0.007
7	9.722	0.090	0.002	0.027	0.004	0.002	0.011	0.007
8	9.717	0.262	0.002	0.030	0.006	0.002	0.011	0.006
10	9.703	0.486	0.004	0.029	0.005	0.002	0.012	0.007
12	10.626	0.765	0.004	0.026	0.006	0.002	0.013	0.008
14	10.268	0.983	0.003	0.037	0.004	0.004	0.014	0.009
16	10.875	1.190	0.003	0.158	0.005	0.003	0.015	0.011
18	13.522	1.805	0.003	0.917	0.004	0.004	0.013	0.003
20	13.878	2.315	0.006	1.592	0.005	0.010	0.013	0.006
22	14.426	2.557	0.005	2.058	0.003	0.009	0.013	0.007

Table E-48: Total carboxylic acid formation during the mixed microbial fermentation of hot water extract (replicate #2).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	3.060	0.016	0.001	0.009	0.012	0.017	0.018	0.071
2	2.981	0.020	0.000	0.006	0.006	0.035	0.012	0.012
3	4.855	0.013	0.001	0.013	0.005	0.002	0.010	0.004
4	6.663	0.012	0.003	0.009	0.005	0.002	0.011	0.005
5	8.211	0.010	0.001	0.009	0.005	0.002	0.011	0.005
6	8.419	0.012	0.002	0.008	0.005	0.003	0.011	0.007
7	8.946	0.032	0.002	0.008	0.007	0.001	0.011	0.005
8	9.193	0.078	0.001	0.007	0.007	0.001	0.013	0.006
10	9.522	0.256	0.003	0.011	0.005	0.002	0.012	0.006
12	11.230	0.570	0.003	0.009	0.006	0.003	0.012	0.006
14	11.727	0.577	0.002	0.015	0.004	0.004	0.013	0.009
16	12.167	0.830	0.005	0.445	0.005	0.004	0.014	0.009
18	13.641	1.112	0.003	0.893	0.004	0.005	0.011	0.003
20	15.217	1.417	0.005	1.306	0.007	0.007	0.013	0.006
22	16.517	1.535	0.005	1.671	0.005	0.007	0.013	0.006

Table E-49: Substrates usage and Product formation during the mixed microbial fermentation of gereen liquor extracts (replicate #1).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.00	0.000	0.179	0.000	0.521	2.338	11.607	0.000	0.000	0.025	-0.025
2	7.27	0.000	0.158	0.000	1.558	3.202	13.359	0.000	0.005	0.031	-0.025
3	7.59	0.000	0.164	0.000	2.367	1.806	11.303	0.000	0.008	0.025	-0.017
4	7.75	0.000	0.000	0.000	2.345	1.592	11.687	0.000	0.010	0.025	-0.015
5	7.67	0.000	0.127	0.000	2.241	1.535	11.424	0.000	0.012	0.024	-0.012
6	7.51	0.000	0.106	0.000	2.221	1.463	11.644	0.000	0.012	0.024	-0.012
7	7.30	0.000	0.316	0.000	1.921	1.197	10.543	0.000	0.014	0.021	-0.007
8	7.01	0.000	0.520	0.000	1.856	1.017	10.138	0.000	0.015	0.020	-0.005
10	6.50	0.000	0.582	0.000	1.800	0.880	9.327	0.000	0.016	0.018	-0.002
12	6.41	0.000	0.808	0.000	3.337	0.744	17.972	0.000	0.017	0.032	-0.015
14	6.23	0.000	0.063	1.516	4.069	1.301	20.624	0.000	0.018	0.037	-0.020
16	6.17	0.000	0.000	1.469	3.903	0.910	20.319	0.000	0.018	0.036	-0.017
18	6.09	0.000	0.000	0.665	1.806	0.458	9.965	0.000	0.019	0.017	0.002
20	6.09	0.000	0.000	1.424	3.818	1.112	20.356	0.000	0.019	0.035	-0.016
22	6.12	0.000	0.000	1.511	3.911	1.058	21.520	0.000	0.020	0.036	-0.017

Table E-50: Substrates usage and Product formation during the mixed microbial fermentation of gereen liquor extracts (replicate #2).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.00	0.000	0.179	0.000	0.521	2.338	11.607	0.000	0.000	0.025	-0.025
2	7.27	0.000	0.358	0.000	1.356	0.000	11.123	0.000	0.005	0.020	-0.014
3	7.49	0.000	0.202	0.000	1.408	1.236	12.358	0.000	0.008	0.024	-0.016
4	7.68	0.000	0.000	0.000	2.369	1.775	12.616	0.000	0.010	0.027	-0.017
5	7.68	0.000	0.130	0.000	2.060	1.514	11.950	0.000	0.012	0.024	-0.013
6	7.56	0.000	0.104	0.000	2.095	1.573	11.939	0.000	0.012	0.024	-0.012
7	6.63	0.000	0.265	0.000	0.843	1.147	11.824	0.000	0.015	0.022	-0.007
8	6.46	0.000	0.079	0.481	1.518	0.710	10.382	0.000	0.016	0.019	-0.003
10	6.40	0.000	0.023	0.820	2.994	1.035	19.495	0.000	0.017	0.035	-0.017
12	6.19	0.000	0.376	0.517	0.773	0.429	11.769	0.000	0.019	0.019	-0.001
14	6.09	0.000	0.000	0.805	1.711	0.438	11.170	0.000	0.020	0.019	0.001
16	6.11	0.000	0.000	0.801	1.727	0.493	11.037	0.000	0.020	0.019	0.002
18	6.04	0.000	0.000	0.741	1.710	0.505	11.167	0.000	0.021	0.019	0.002
20	6.11	0.000	0.000	0.778	1.724	0.443	11.867	0.000	0.021	0.019	0.002
22	6.25	0.000	0.000	0.890	0.909	0.487	14.094	0.000	0.022	0.022	0.000

Table E-51: Total carboxylic acid formation during the mixed microbial fermentation of gereen liquor extracts (replicate #1).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	11.549	0.024	0.001	0.009	0.003	0.000	0.015	0.007
2	11.059	0.016	0.001	0.009	0.008	0.001	0.015	0.014
3	12.282	0.034	0.000	0.013	0.007	0.000	0.013	0.008
4	12.534	0.038	0.002	0.013	0.006	0.001	0.013	0.007
5	11.852	0.048	0.005	0.014	0.007	0.001	0.015	0.009
6	11.836	0.059	0.003	0.012	0.005	0.001	0.015	0.009
7	11.704	0.081	0.003	0.012	0.005	0.000	0.013	0.006
8	10.235	0.099	0.003	0.011	0.006	0.005	0.014	0.010
10	19.184	0.260	0.003	0.015	0.007	0.001	0.016	0.008
12	11.255	0.380	0.004	0.079	0.008	0.003	0.027	0.011
14	10.490	0.477	0.006	0.126	0.012	0.007	0.037	0.014
16	10.333	0.464	0.008	0.160	0.009	0.009	0.045	0.011
18	10.447	0.477	0.005	0.170	0.010	0.008	0.049	0.001
20	10.910	0.658	0.006	0.215	0.009	0.011	0.055	0.004
22	12.997	0.799	0.005	0.215	0.009	0.012	0.053	0.004

Table E-52: Total carboxylic acid formation during the mixed microbial fermentation of gereen liquor extracts (replicate #2).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	11.549	0.024	0.001	0.009	0.003	0.000	0.015	0.007
2	11.787	0.023	0.001	0.009	0.011	0.001	0.015	0.008
3	10.939	0.037	0.003	0.015	0.009	0.001	0.013	0.003
4	12.574	0.045	0.002	0.014	0.007	0.002	0.013	0.005
5	11.676	0.066	0.003	0.013	0.006	0.002	0.015	0.007
6	12.195	0.079	0.003	0.013	0.005	0.001	0.015	0.009
7	11.636	0.092	0.003	0.011	0.006	0.001	0.014	0.008
8	11.332	0.102	0.004	0.013	0.006	0.001	0.015	0.007
10	11.434	0.192	0.004	0.022	0.011	0.001	0.021	0.014
12	11.632	0.151	0.004	0.014	0.008	0.002	0.016	0.009
14	12.274	0.128	0.005	0.016	0.007	0.002	0.018	0.010
16	13.149	0.152	0.004	0.011	0.008	0.002	0.019	0.009
18	12.174	0.172	0.007	0.066	0.009	0.001	0.030	0.001
20	12.395	0.192	0.004	0.123	0.006	0.003	0.042	0.001
22	13.206	0.221	0.008	0.159	0.007	0.004	0.052	0.003

Table E-53: Substrates usage and Product formation during the mixed microbial fermentation of gereen liquor extracts (replicate #1).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.00	0.000	0.179	0.000	0.521	2.338	11.607	0.000	0.000	0.025	-0.025
2	7.33	0.059	0.000	2.756	3.923	5.567	13.919	0.000	0.005	0.039	-0.034
3	7.50	0.000	0.156	0.000	1.804	1.994	12.808	0.000	0.008	0.027	-0.019
4	7.77	0.000	0.065	0.000	2.778	2.228	13.234	0.000	0.009	0.029	-0.020
5	7.82	0.000	0.093	0.000	2.760	2.439	12.989	0.000	0.011	0.029	-0.018
6	7.79	0.000	0.127	0.000	3.089	2.999	14.305	0.000	0.011	0.032	-0.021
7	7.65	0.000	0.037	0.000	2.776	2.957	13.198	0.000	0.012	0.030	-0.017
8	7.54	0.000	0.047	0.770	2.852	3.031	13.234	0.000	0.013	0.030	-0.016
10	6.99	0.000	0.097	0.773	3.188	3.115	13.171	0.000	0.015	0.030	-0.015
12	6.52	0.000	0.059	0.861	4.061	2.967	13.335	0.000	0.017	0.030	-0.014
14	6.40	0.000	0.000	1.431	4.745	2.967	13.623	0.000	0.017	0.031	-0.013
16	6.40	0.000	0.000	1.293	4.948	3.015	13.866	0.000	0.018	0.031	-0.013
18	6.28	0.000	0.000	1.340	5.054	2.871	13.889	0.000	0.019	0.031	-0.012
20	6.19	0.000	0.024	1.330	5.507	3.102	14.845	0.000	0.019	0.033	-0.013
22	6.14	0.000	0.032	1.372	5.828	3.120	15.322	0.000	0.020	0.033	-0.013

Table E-54: Substrates usage and Product formation during the mixed microbial fermentation of gereen liquor extracts (replicate #2).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.00	0.000	0.179	0.000	0.521	2.338	11.607	0.000	0.000	0.025	-0.025
2	7.33	0.059	0.000	2.756	3.923	5.567	13.919	0.000	0.005	0.039	-0.034
3	7.50	0.000	0.156	0.000	1.804	1.994	12.808	0.000	0.008	0.027	-0.019
4	7.77	0.000	0.065	0.000	2.778	2.228	13.234	0.000	0.009	0.029	-0.020
5	7.82	0.000	0.093	0.000	2.760	2.439	12.989	0.000	0.011	0.029	-0.018
6	7.79	0.000	0.127	0.000	3.089	2.999	14.305	0.000	0.011	0.032	-0.021
7	7.65	0.000	0.037	0.000	2.776	2.957	13.198	0.000	0.012	0.030	-0.017
8	7.54	0.000	0.047	0.770	2.852	3.031	13.234	0.000	0.013	0.030	-0.016
10	6.99	0.000	0.097	0.773	3.188	3.115	13.171	0.000	0.015	0.030	-0.015
12	6.52	0.000	0.059	0.861	4.061	2.967	13.335	0.000	0.017	0.030	-0.014
14	6.40	0.000	0.000	1.431	4.745	2.967	13.623	0.000	0.017	0.031	-0.013
16	6.40	0.000	0.000	1.293	4.948	3.015	13.866	0.000	0.018	0.031	-0.013
18	6.28	0.000	0.000	1.340	5.054	2.871	13.889	0.000	0.019	0.031	-0.012
20	6.19	0.000	0.024	1.330	5.507	3.102	14.845	0.000	0.019	0.033	-0.013
22	6.14	0.000	0.032	1.372	5.828	3.120	15.322	0.000	0.020	0.033	-0.013

Table E-55: Total carboxylic acid formation during the mixed microbial fermentation of gereen liquor extracts (replicate #1).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	11.549	0.024	0.001	0.009	0.003	0.000	0.015	0.007
2	11.787	0.023	0.001	0.009	0.011	0.001	0.015	0.008
3	10.939	0.037	0.003	0.015	0.009	0.001	0.013	0.003
4	12.574	0.045	0.002	0.014	0.007	0.002	0.013	0.005
5	11.676	0.066	0.003	0.013	0.006	0.002	0.015	0.007
6	12.195	0.079	0.003	0.013	0.005	0.001	0.015	0.009
7	11.636	0.092	0.003	0.011	0.006	0.001	0.014	0.008
8	11.332	0.102	0.004	0.013	0.006	0.001	0.015	0.007
10	11.434	0.192	0.004	0.022	0.011	0.001	0.021	0.014
12	11.632	0.151	0.004	0.014	0.008	0.002	0.016	0.009
14	12.274	0.128	0.005	0.016	0.007	0.002	0.018	0.010
16	13.149	0.152	0.004	0.011	0.008	0.002	0.019	0.009
18	12.174	0.172	0.007	0.066	0.009	0.001	0.030	0.001
20	12.395	0.192	0.004	0.123	0.006	0.003	0.042	0.001
22	13.206	0.221	0.008	0.159	0.007	0.004	0.052	0.003

Table E-56: Total carboxylic acid formation during the mixed microbial fermentation of gereen liquor extracts (replicate #2).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	11.549	0.024	0.001	0.009	0.003	0.000	0.015	0.007
2	13.848	0.017	0.001	0.009	0.003	0.002	0.019	0.019
3	12.736	0.031	0.002	0.014	0.006	0.000	0.014	0.004
4	13.158	0.031	0.004	0.013	0.007	0.001	0.013	0.007
5	12.919	0.032	0.002	0.011	0.003	0.001	0.014	0.006
6	14.218	0.041	0.003	0.015	0.006	0.001	0.014	0.007
7	13.107	0.051	0.002	0.012	0.006	0.000	0.013	0.006
8	13.128	0.065	0.002	0.009	0.006	0.000	0.014	0.009
10	13.047	0.083	0.003	0.010	0.006	0.001	0.015	0.007
12	13.159	0.130	0.004	0.011	0.008	0.001	0.015	0.007
14	13.416	0.157	0.003	0.011	0.008	0.002	0.017	0.010
16	13.647	0.167	0.003	0.011	0.008	0.002	0.017	0.010
18	13.652	0.195	0.004	0.010	0.008	0.002	0.016	0.001
20	14.528	0.230	0.004	0.048	0.009	0.003	0.022	0.002
22	14.897	0.257	0.005	0.115	0.009	0.003	0.034	0.002

Table E-57: Substrates usage and Product formation during the mixed microbial fermentation of gereen liquor extracts (replicate #1).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.00	0.000	0.179	0.000	0.521	2.338	11.607	0.000	0.000	0.025	-0.025
2	7.51	0.000	0.439	0.000	2.014	1.627	10.542	0.000	0.003	0.023	-0.020
3	7.57	0.000	0.000	3.670	2.028	4.613	14.143	0.000	0.005	0.035	-0.030
4	7.66	0.000	0.000	0.000	1.840	3.281	12.945	0.000	0.007	0.030	-0.023
5	7.72	0.000	0.049	0.000	2.116	3.849	13.204	0.000	0.008	0.031	-0.023
6	7.83	0.000	0.045	0.000	1.856	2.918	12.420	0.000	0.009	0.028	-0.019
7	7.87	0.000	0.040	0.000	2.113	3.122	13.206	0.000	0.010	0.029	-0.020
8	7.95	0.000	0.040	0.780	2.218	2.616	13.748	0.000	0.010	0.029	-0.018
10	7.81	0.000	0.000	0.770	2.211	2.528	14.023	0.000	0.011	0.029	-0.017
12	7.00	0.000	0.870	0.952	2.573	2.373	14.531	0.000	0.014	0.029	-0.015
14	6.75	0.000	0.000	1.064	2.935	2.859	15.560	0.000	0.018	0.032	-0.014
16	6.16	0.000	0.000	0.952	2.353	2.937	16.490	0.000	0.020	0.032	-0.012
18	6.18	0.000	0.000	1.507	2.073	3.081	17.684	0.000	0.021	0.033	-0.012
20	6.73	0.000	0.000	1.697	0.722	3.271	20.350	0.000	0.023	0.035	-0.012
22	7.11	0.000	0.000	1.794	0.000	2.878	20.823	0.000	0.024	0.033	-0.010

Table E-58: Substrates usage and Product formation during the mixed microbial fermentation of gereen liquor extracts (replicate #2).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.00	0.000	0.179	0.000	0.521	2.338	11.607	0.000	0.000	0.025	-0.025
2	7.58	0.000	0.818	0.000	3.332	5.485	16.262	0.000	0.003	0.042	-0.040
3	7.59	0.000	0.126	0.000	1.488	4.460	12.673	0.000	0.005	0.032	-0.027
4	7.65	0.000	0.151	0.000	1.594	3.594	13.281	0.000	0.006	0.031	-0.024
5	7.69	0.000	0.050	0.000	1.812	4.244	13.690	0.000	0.008	0.033	-0.025
6	7.79	0.000	0.058	0.000	1.894	3.405	13.941	0.000	0.008	0.031	-0.023
7	7.90	0.000	0.051	0.000	1.839	2.653	13.616	0.000	0.009	0.029	-0.020
8	7.97	0.000	0.000	0.763	1.861	2.704	14.063	0.000	0.010	0.029	-0.019
10	7.82	0.000	0.000	0.838	1.836	2.710	14.528	0.000	0.011	0.029	-0.019
12	7.06	0.000	1.156	1.121	2.074	2.724	15.475	0.000	0.013	0.031	-0.017
14	6.66	0.000	0.832	1.878	2.590	3.014	16.638	0.000	0.017	0.033	-0.016
16	6.51	0.000	0.000	1.366	1.635	2.828	18.023	0.000	0.020	0.033	-0.013
18	6.93	0.000	0.000	2.399	0.000	2.234	19.672	0.000	0.022	0.032	-0.010
20	7.31	0.000	0.000	2.543	0.000	1.634	20.362	0.000	0.023	0.031	-0.008
22	7.62	0.000	0.000	2.644	0.000	1.169	21.025	0.000	0.024	0.030	-0.007

Table E-59: Total carboxylic acid formation during the mixed microbial fermentation of gereen liquor extracts (replicate #1).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	11.549	0.024	0.001	0.009	0.003	0.000	0.015	0.007
2	10.264	0.021	0.001	0.008	0.008	0.220	0.014	0.006
3	14.087	0.019	0.000	0.015	0.005	0.002	0.011	0.004
4	12.885	0.019	0.002	0.015	0.005	0.001	0.013	0.005
5	13.161	0.010	0.001	0.009	0.005	0.002	0.011	0.005
6	12.346	0.019	0.001	0.028	0.006	0.001	0.013	0.006
7	13.068	0.030	0.005	0.074	0.008	0.000	0.013	0.006
8	13.483	0.063	0.005	0.166	0.008	0.001	0.015	0.007
10	13.670	0.108	0.010	0.203	0.009	0.001	0.015	0.007
12	14.077	0.138	0.013	0.254	0.011	0.015	0.015	0.008
14	14.766	0.327	0.011	0.423	0.006	0.001	0.017	0.008
16	15.078	0.731	0.014	0.633	0.007	0.002	0.017	0.009
18	15.330	1.154	0.018	1.151	0.003	0.011	0.016	0.002
20	16.081	1.826	0.030	2.378	0.014	0.002	0.018	0.002
22	16.311	1.677	0.037	2.762	0.014	0.004	0.016	0.002

Table E-60: Total carboxylic acid formation during the mixed microbial fermentation of gereen liquor extracts (replicate #2).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	11.549	0.024	0.001	0.009	0.003	0.000	0.015	0.007
2	16.204	0.024	0.001	0.009	0.003	0.000	0.015	0.007
3	12.611	0.022	0.002	0.016	0.006	0.001	0.012	0.004
4	13.221	0.018	0.003	0.013	0.006	0.001	0.013	0.006
5	13.626	0.023	0.002	0.012	0.006	0.001	0.014	0.006
6	13.852	0.047	0.002	0.014	0.005	0.002	0.014	0.005
7	13.486	0.066	0.001	0.041	0.003	0.000	0.014	0.005
8	13.858	0.092	0.004	0.079	0.008	0.001	0.015	0.006
10	14.127	0.156	0.011	0.202	0.010	0.001	0.015	0.006
12	14.700	0.219	0.016	0.504	0.010	0.002	0.016	0.007
14	15.284	0.294	0.027	0.994	0.010	0.002	0.017	0.008
16	15.662	0.881	0.029	1.414	0.007	0.003	0.018	0.009
18	15.848	1.135	0.035	2.622	0.009	0.003	0.017	0.001
20	15.742	1.280	0.042	3.254	0.019	0.006	0.017	0.003
22	16.523	1.192	0.045	3.227	0.018	0.005	0.015	0.001

Table E-61: Substrates usage and Product formation during the mixed microbial fermentation of gereen liquor extracts (replicate #1).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.00	0.000	0.179	0.000	0.521	2.338	11.607	0.000	0.000	0.025	-0.025
2	7.58	0.000	0.000	4.150	1.410	3.687	13.842	0.000	0.003	0.032	-0.030
3	7.56	0.000	0.203	3.487	0.650	3.776	14.287	0.000	0.005	0.032	-0.027
4	7.72	0.000	0.163	0.000	1.629	3.229	13.296	0.000	0.006	0.030	-0.024
5	7.84	0.000	0.000	0.000	1.957	3.751	13.538	0.000	0.007	0.032	-0.024
6	7.95	0.000	0.000	0.000	1.899	3.314	13.353	0.000	0.008	0.030	-0.022
7	7.92	0.000	0.037	0.000	1.889	2.647	13.479	0.000	0.009	0.028	-0.020
8	7.48	0.000	0.000	0.927	1.980	3.058	14.176	0.000	0.010	0.030	-0.020
10	6.65	0.000	4.845	0.966	6.423	7.591	10.990	0.000	0.014	0.038	-0.025
12	6.29	0.000	0.338	1.866	3.178	4.755	15.768	0.000	0.016	0.036	-0.020
14	6.04	0.000	0.000	1.843	2.980	4.582	15.959	0.000	0.018	0.036	-0.018
16	6.55	0.000	0.000	2.453	0.000	4.160	17.228	0.000	0.019	0.033	-0.013
18	6.80	0.000	0.000	2.639	0.000	4.049	18.862	0.000	0.021	0.034	-0.014
20	6.92	0.000	0.000	2.462	0.000	3.702	18.573	0.000	0.022	0.033	-0.011
22	7.26	0.000	0.000	3.280	0.000	3.600	21.260	0.000	0.023	0.036	-0.013

Table E-62: Substrates usage and Product formation during the mixed microbial fermentation of gereen liquor extracts (replicate #2).

Day	pH	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Total Carboxylic Acids	Total Methane (moles)	Total CO2 (moles)	Moles of Abiotic CO_2	Moles Biotic CO_2
0	7.00	0.000	0.179	0.000	0.521	2.338	11.607	0.000	0.000	0.025	-0.025
2	7.49	0.000	0.563	0.000	1.204	0.862	10.133	0.000	0.003	0.020	-0.017
3	7.53	0.000	0.000	4.540	0.455	4.228	14.995	0.000	0.005	0.034	-0.029
4	7.43	0.000	0.047	0.000	2.031	3.048	13.651	0.000	0.007	0.031	-0.023
5	7.73	0.000	0.057	0.000	2.041	2.500	13.854	0.000	0.009	0.029	-0.021
6	7.77	0.000	0.000	0.000	1.269	2.603	14.131	0.000	0.009	0.029	-0.020
7	7.72	0.000	0.000	0.000	0.000	2.517	13.975	0.000	0.010	0.027	-0.017
8	7.91	0.000	0.000	0.657	0.000	2.775	14.657	0.000	0.011	0.028	-0.017
10	7.68	0.000	0.000	0.827	0.000	3.135	15.135	0.000	0.012	0.029	-0.017
12	6.93	0.000	0.449	1.125	0.732	3.200	15.120	0.000	0.014	0.030	-0.015
14	6.60	0.000	0.000	2.148	0.000	4.382	17.162	0.000	0.017	0.034	-0.016
16	6.30	0.000	0.000	2.327	0.000	4.214	18.400	0.000	0.020	0.035	-0.014
18	6.45	0.000	0.000	2.376	0.000	4.166	18.788	0.000	0.022	0.035	-0.013
20	6.57	0.000	0.000	2.476	0.000	4.097	19.724	0.000	0.023	0.035	-0.012
22	6.87	0.000	0.000	3.255	0.000	4.036	20.638	0.000	0.024	0.036	-0.012

Table E-63: Total carboxylic acid formation during the mixed microbial fermentation of gereen liquor extracts (replicate #1).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	11.549	0.024	0.001	0.009	0.003	0.000	0.015	0.007
2	13.788	0.022	0.001	0.008	0.006	0.001	0.013	0.004
3	14.230	0.019	0.001	0.015	0.006	0.002	0.012	0.003
4	13.236	0.017	0.003	0.014	0.006	0.001	0.013	0.006
5	13.479	0.017	0.004	0.014	0.005	0.001	0.013	0.006
6	13.268	0.027	0.005	0.021	0.009	0.002	0.014	0.006
7	13.361	0.050	0.006	0.033	0.008	0.001	0.014	0.006
8	14.039	0.068	0.006	0.030	0.009	0.000	0.015	0.007
10	10.766	0.098	0.012	0.081	0.011	0.001	0.016	0.006
12	15.491	0.109	0.007	0.129	0.009	0.001	0.016	0.006
14	15.413	0.332	0.007	0.174	0.006	0.002	0.017	0.008
16	15.479	0.701	0.010	0.996	0.005	0.011	0.017	0.009
18	16.485	1.007	0.010	1.335	0.007	0.001	0.016	0.000
20	15.928	0.990	0.014	1.617	0.008	0.002	0.015	0.001
22	17.137	1.447	0.021	2.611	0.011	0.008	0.019	0.005

Table E-64: Total carboxylic acid formation during the mixed microbial fermentation of gereen liquor extracts (replicate #2).

Day	C2	C3	IC4	C4	IC5	C5	C6	C7
0	11.549	0.024	0.001	0.009	0.003	0.000	0.015	0.007
2	10.076	0.021	0.002	0.008	0.006	0.000	0.016	0.004
3	14.937	0.023	0.003	0.013	0.006	0.001	0.011	0.002
4	13.580	0.032	0.002	0.012	0.006	0.001	0.013	0.006
5	13.768	0.045	0.003	0.012	0.006	0.001	0.013	0.006
6	13.841	0.101	0.006	0.157	0.004	0.002	0.014	0.006
7	13.412	0.115	0.005	0.417	0.006	0.001	0.014	0.005
8	14.015	0.153	0.004	0.448	0.007	0.006	0.015	0.008
10	14.366	0.218	0.010	0.492	0.011	0.014	0.016	0.007
12	14.189	0.272	0.012	0.613	0.009	0.002	0.016	0.007
14	15.811	0.353	0.009	0.953	0.009	0.003	0.017	0.007
16	16.232	0.929	0.010	1.194	0.005	0.002	0.018	0.009
18	16.551	0.899	0.009	1.307	0.004	0.002	0.015	0.000
20	16.802	1.059	0.017	1.821	0.007	0.002	0.014	0.002
22	17.263	1.148	0.022	2.175	0.010	0.003	0.016	0.002

Total mono-sugars and acetic acid present in the hot water and green liquor extract before and after fermentation using acid-hydrolysis step on both control and methane inhibition steps and total biotic and abiotic CO_2 formed during fermentation.

Table E-65: Total sugars, acids and total abiotic and biotic CO_2 formed and yields of the fermentation process.

Sample Name	Glucose	Xylose	Ethanol	Lactic Acid	Formic Acid	Acetic Acid	C3-C7	Moles of Abiotic CO_2	Moles of Biotic CO_2	Yield acids/ sugars
acid hydrolyzed hot water extract	1.539	25.284	0.000	0.771	0.000	9.569	0.030	0.000	0.000	0.000
Hot Water at 55°C control 1	1.025	13.445	1.160	9.215	3.429	12.634	0.889	0.020	0.007	1.257
Hot Water at 55°C control 2	0.930	12.808	0.760	7.655	2.060	10.851	0.600	0.013	0.012	0.812
Hot Water at 55°C inhibitor 1	0.000	11.154	0.838	7.517	1.509	10.807	0.459	0.011	0.014	0.625
Hot Water at 55°C inhibitor 2	0.806	11.125	0.734	10.007	1.257	9.921	0.239	0.012	0.015	0.732
Hot Water at 37°C control 1	0.000	7.116	3.623	1.567	5.852	17.503	6.017	0.029	0.009	1.007
Hot Water at 37°C control 2	0.000	7.831	4.151	0.520	6.105	15.549	6.124	0.026	0.010	0.910
Hot Water at 37°C inhibitor 1	0.000	7.146	2.533	4.680	6.308	16.867	4.651	0.031	0.009	1.086
Hot Water at 37°C inhibitor 2	0.000	10.183	3.183	3.860	7.769	17.631	3.241	0.032	0.003	1.275
acid hydrolyzed green liquor extract	1.060	9.203	0.000	1.420	3.791	11.800	0.033	0.000	0.000	0.000
Green Liquor at 55°C control 1	1.011	1.905	1.291	4.635	2.154	20.801	0.483	0.012	0.007	1.533
Green Liquor at 55°C control 2	0.484	0.800	0.649	1.869	0.520	12.997	1.097	-0.005	0.027	-0.064
Green Liquor at 55°C inhibitor 1	0.598	1.175	0.885	4.705	2.347	13.070	0.454	0.003	0.017	0.424
Green Liquor at 55°C inhibitor 2	0.000	1.345	1.213	5.625	3.041	15.547	0.425	0.008	0.012	0.866
Green Liquor at 37°C control 1	0.000	0.419	1.473	0.000	3.012	16.857	4.512	0.009	0.015	0.720
Green Liquor at 37°C control 2	0.000	0.323	2.504	0.000	1.047	16.433	4.503	0.005	0.019	0.480
Green Liquor at 37°C inhibitor 1	0.000	0.775	2.577	0.000	3.666	17.703	4.124	0.011	0.011	0.859
Green Liquor at 37°C inhibitor 2	0.000	0.439	1.714	0.000	4.079	17.521	3.375	0.011	0.013	0.780

APPENDIX F

VOLATILE SOLIDS CALCULATION

Table F-1: Total volatile solids present at the initial and at the end of the fermentation for a given sample (averaged results).

	wet mass	moisture mass	dry mass	ash mass	VS	% cahnge in VS during fermentation
HW	5.156	4.930	0.226	0.004	0.223	
GL	5.132	4.961	0.171	0.061	0.110	
inoculum	10.195	2.874	7.321	7.116	0.205	
HW 55°C	5.745	5.455	0.290	0.056	0.234	-0.904
HW 37°C	5.744	5.528	0.216	0.051	0.165	-30.080
GL 55°C	5.864	5.520	0.343	0.079	0.264	111.306
GL 37°C	5.677	5.483	0.194	0.085	0.109	-9.622

Volatilc solids addition.

Assumptions on difference in product formation on pure sugar fermentation at thermophilic temperatures using calcium carbonate as the buffer. We have seen that the total lactic acid produced during the fermentation is abnormally higher than the theoretical limits suggested by a carbon mass balance or laboratory results on conversion of wood extracts to lactic acid, demonstrated by Sara Walton growing a pure culture of Bacillus Coagulans (Walton, 2009). This might be due to the addition of some additional organics contained within the inocula collected from the swamps along the shores having decomposing organics. The total volatile solids added with the inoculums was calculated to be around 0.2 grams, and this might contribute to the higher product formation. The table of the volatile solids calculation can be found in appendix D.

APPENDIX G

CARBON BALANCE

Table G-1: The total carbon balance in to the and out of the system during the fermentation process.

Sample	Glucose	Xylose	Arabinose	Lactic Acid	Formic Acid	Acetic Acid	Ethanol	C3	IC4	C4	IC5	C5	C6	C7	Methane (g)	biotic CO_2 (g)	total carbons
Feed solutions																	
acid hydrolyzed hot water extract 55°C	0.616	10.114	0.486	0.308	0.000	3.827	0.522	0.008	0.001	0.005	0.007	0.010	0.011	0.046	0	0.000	15.960
acid hydrolyzed green liquor extract 55°C	0.424	3.681	0.339	0.568	0.989	4.720	0.522	0.012	0.000	0.005	0.002	0.000	0.009	0.004	0	0.000	11.275
acid hydrolyzed hot water extract 37°C	0.616	10.114	0.486	0.308	0.000	3.827	0.261	0.008	0.001	0.005	0.007	0.010	0.011	0.046	0	0.000	15.700
acid hydrolyzed green liquor extract 37°C	0.424	3.681	0.339	0.568	0.989	4.720	0.261	0.012	0.000	0.005	0.002	0.000	0.009	0.004	0	0.000	11.014
Products																	
Hot Water at 55°C control 1	0.410	5.378	0.000	3.686	0.895	5.054	0.605	0.266	0.019	0.023	0.030	0.026	0.043	0.066	0.011	0.928	17.438
Hot Water at 55°C control 2	0.372	5.123	0.000	3.062	0.537	4.340	0.397	0.270	0.002	0.002	0.005	0.001	0.012	0.006	0.001	1.513	15.643
Hot Water at 55°C inhibitor 1	0.000	4.461	0.000	3.007	0.394	4.323	0.437	0.141	0.005	0.048	0.010	0.004	0.024	0.006	0.001	1.813	14.674
Hot Water at 55°C inhibitor 2	0.322	4.450	0.000	4.003	0.328	3.968	0.383	0.096	0.001	0.006	0.003	0.001	0.010	0.003	0.000	1.900	15.476
Hot Water at 37°C control 1	0.000	2.846	0.000	0.627	1.527	7.001	1.890	1.546	0.004	1.520	0.003	0.012	0.009	0.003	0.000	1.165	18.153
Hot Water at 37°C control 2	0.000	3.132	0.000	0.208	1.593	6.220	2.166	0.968	0.004	2.225	0.004	0.009	0.008	0.008	0.000	1.301	17.846
Hot Water at 37°C inhibitor 1	0.000	2.858	0.000	1.872	1.646	6.747	1.322	1.244	0.003	1.122	0.002	0.005	0.008	0.004	0.000	1.109	17.941
Hot Water at 37°C inhibitor 2	0.000	4.073	0.000	1.544	2.027	7.052	1.661	0.747	0.003	0.912	0.003	0.004	0.008	0.004	0.000	0.383	18.419
Green Liquor at 55°C control 1	0.405	0.762	0.000	1.854	0.562	8.320	0.674	0.220	0.001	0.003	0.003	0.001	0.008	0.001	0.000	0.932	13.747
Green Liquor at 55°C control 2	0.194	0.320	0.000	0.747	0.136	4.495	0.339	0.389	0.002	0.117	0.005	0.007	0.033	0.003	0.000	3.396	10.183
Green Liquor at 55°C inhibitor 1	0.239	0.470	0.000	1.882	0.612	5.228	0.462	0.108	0.004	0.086	0.004	0.002	0.032	0.002	0.000	2.185	11.318
Green Liquor at 55°C inhibitor 2	0.000	0.538	0.000	2.250	0.793	6.219	0.633	0.125	0.003	0.063	0.005	0.002	0.021	0.002	0.000	1.462	12.115
Green Liquor at 37°C control 1	0.000	0.168	0.000	0.000	0.786	6.743	0.769	0.816	0.020	1.506	0.008	0.002	0.010	0.001	0.000	1.841	12.670
Green Liquor at 37°C control 2	0.000	0.129	0.000	0.000	0.273	6.573	1.306	0.580	0.025	1.760	0.011	0.003	0.009	0.001	0.000	2.410	13.079
Green Liquor at 37°C inhibitor 1	0.000	0.310	0.000	0.000	0.956	7.081	1.344	0.704	0.011	1.424	0.007	0.005	0.012	0.003	0.000	1.443	13.302
Green Liquor at 37°C inhibitor 2	0.000	0.176	0.000	0.000	1.064	7.008	0.894	0.559	0.012	1.186	0.006	0.002	0.010	0.001	0.000	1.662	12.579

APPENDIX H

HPLC ANALYSIS FOR CARBOXYLIC ACIDS

The HPLC system used was the Shimadzu prominence HPLC system. This system is used to measure various compounds by using a specific column for the compounds. The entire system was equipped in a manner similar to the one explained in Appendix B, although the pump used in this was a low pressure gradient solvent mixing pump, different from that used in the sugar measuring HPLC system. The column used was specific in its ability to separate a wide variety of carboxylic acids.

- The first and foremost component of the HPLC is the CBM 20A (system controller) that controls the other instruments inline and transfers the data produced by the detectors to the computer.
- LC 20AD uses a parallel-type double plunger solvent delivery method. The pump has a low pressure gradient elution specification with 4 different solvents. The low pressure gradient mixing pump moves the mobile phase through the column and maintains a constant flow rate throughout the process. The flow rate is maintained at 1ml/min. This system is connected to an in line DGU $20A_5$ degasser that helps in removing any gas from the solvents.
- SIL 20A is the auto sampler used for injecting the sample in to the column. About 15µL of sample is injected in each run.
- CTO 20A is the oven which holds the column and guard cartridge. The temperature maintained in this is 37°C.
- The type of the column used is an Acclaim ® Organic Acid (OA) column (# 062902) by Dionex Corporation, Sunnyvale, CA. The column is equipped with a guard cartridge (# 062925) protecting the column from entering impurities.
- The mobile phase used is 2.5mM Methanesulfonic acid (CH_3SO_3H) and Acetonitrile (C_2H_3N). These are mixed in the ratios of 55/45% during the process of the run. The mobile phase is degassed in the degasser to remove any air bubbles present in it

- SPD 20 AV (wavelength at 210nm) and RID10A (cell temperature at 40°C) are the UV-visible and refractive index detectors, respectively.
- Time programming for running a gradient mixture is as follows.

  The pump is set at low pressure gradient mixing with a flow rate of 1ml/Min.

  This pump has capability of mixing four different gradients, named solvent A through solvent D.
- Acetonitrile is considered as solvent A and methnesulfonic acid as solvent B.

  During a standby mode the pump is set to run on 100% solvent B

Table H-1: Time program for multi gradient pumping during a sample run.

Time (Min)	Module	Action	Value (%conc.)
1.50	Pumps	Pump B	100
15.50	Pumps	Pump B	55
21.00	Pumps	Pump B	55
24.75	Pumps	Pump B	100
25.10	Controller		

The sample preparation method is similar to that explained in appendix C. The standards carboxylic acid mixture used is the same purchased from Matreya LLC (#1075).

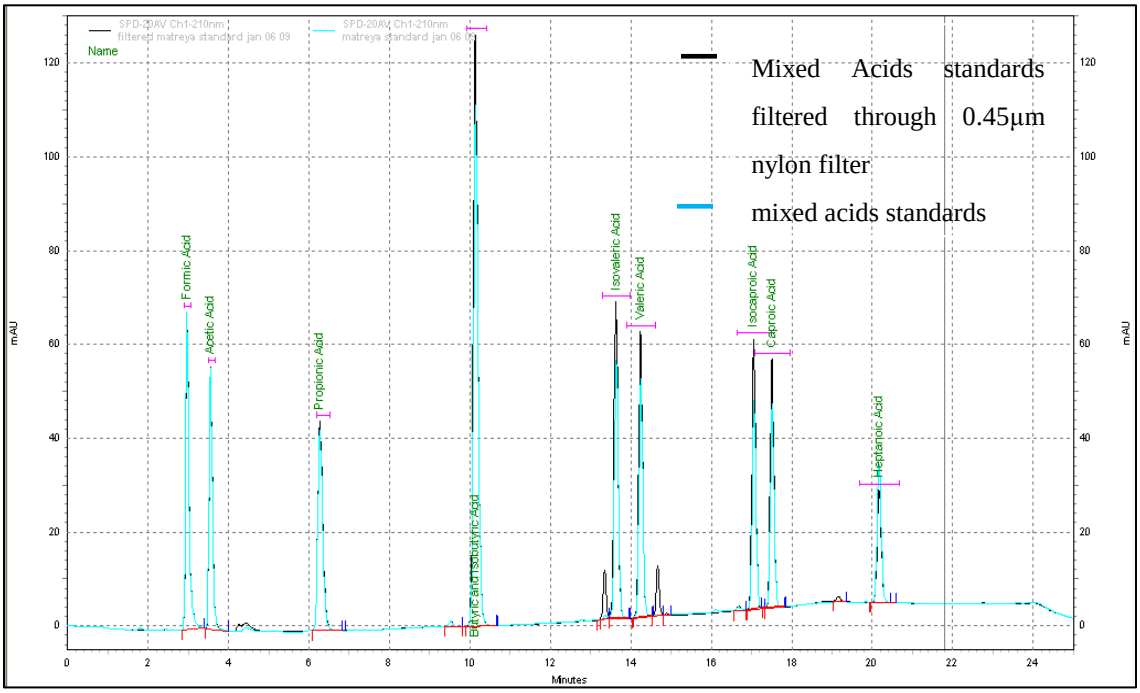


Figure H-1: Matreya standards at 37°C.through acclaim OA column.

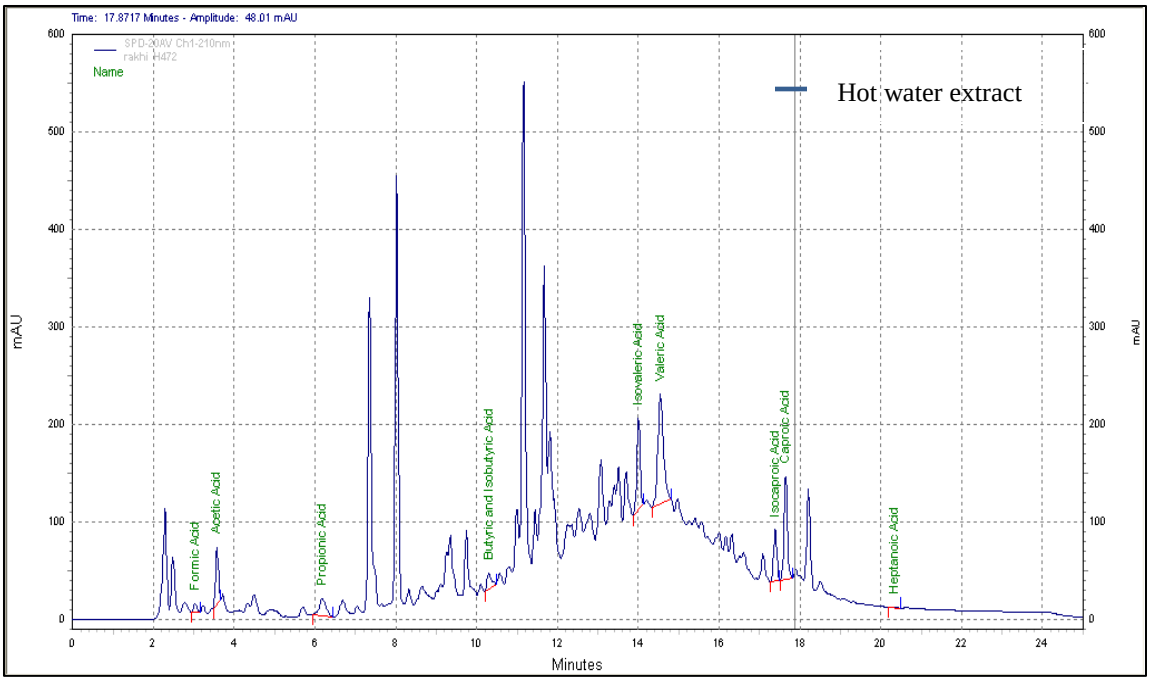


Figure H-2: Hot water extract sample through Acclaim OA column.

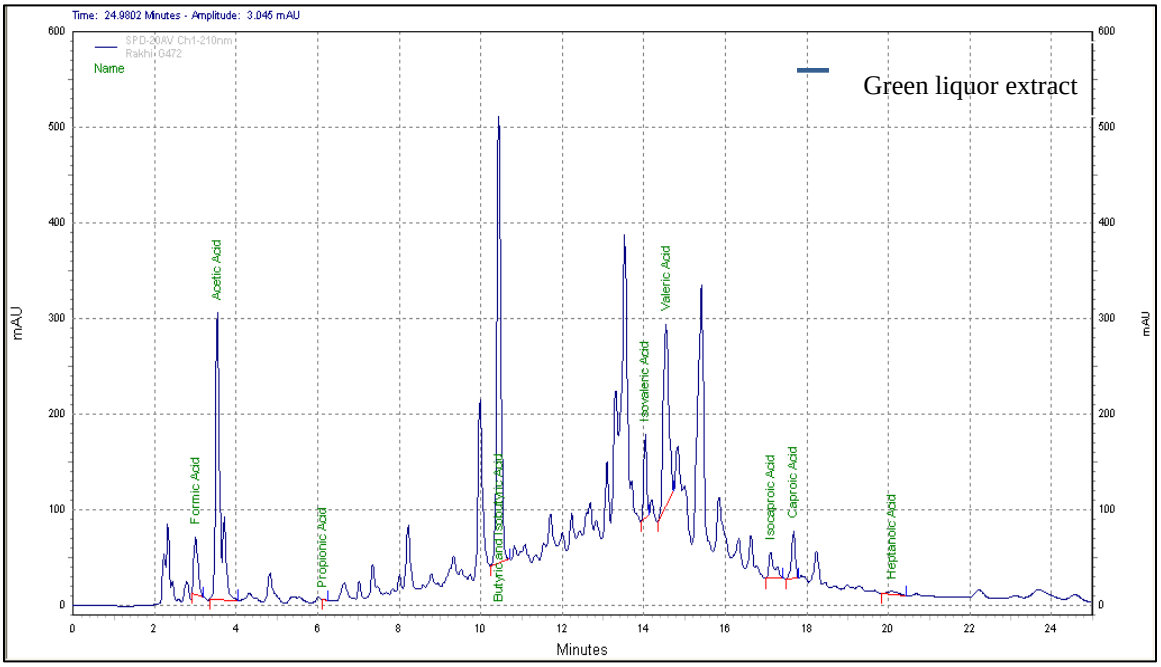


Figure H-3: Green liquor extract sample through Acclaim OA column.

The above results show that from fig F.1, use of nylon filters added few peaks to the standard samples. From figures F.2 and F.3 it is shown that it is very hard to associate specific peaks with the peaks of the standard, as there are a lot of noisy peaks in the sample which interfere with the peaks of interest. The use of Phosphoric acid as an acidifying agent also added a few other peaks to the sample making it much more difficult to separate the peaks. This is especially observed with $C_4 – C_7$ acid peaks.

BIOGRAPHY OF THE AUTHOR

Rakhi Reddy Baddam was born on August 29th, 1983. He grew up in a small village during his child hood. At the age of 4 years his family moved to Hyderabad, India to provide a good education for their children. He completed his Secondary School Certificate (SSC) in 2000 with distinction. With an interest in becoming an engineer, he opted to take Mathematics as a major in his Intermediate college level and passed out with distinction in the year 2002. He received his Bachelor of Technology in Biotechnology in 2006 from Jawaharlal Nehru Technological University, Hyderabad, India. He joined the Department of Chemical and Biological Engineering at the University of Maine as a graduate student to pursue his masters degree in Biological engineering.

Rakhi Baddam is a candidate for the Master of Science degree in Biological Engineering from The University of Maine in May 2010.