CONTINUOUS FERMENTATION OF FOOD SCRAPS WITH CONSTANT pH CONTROL TO PRODUCE CARBOXYLIC ACIDS

A Thesis

by

STANLEY COLEMAN, JR.

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2007

Major Subject: Chemical Engineering

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Chair of Committee, Mark Holtzapple
Committee Members, Charles Glover
Robin Autenrieth
Head of Department, Michael Pishko

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ABSTRACT

Continuous Fermentation of Food Scraps with Constant pH Control

to Produce Carboxylic Acids. (December 2007)

Stanley Coleman, Jr., B.S., Prairie View A&M University

Chair of Advisory Committee: Dr. Mark Holtzapple

Global energy demands combined with environmental restrictions are fueling a move to alternative energy sources. Biofuels are formed from biomass; the MixAlco process is one such method. In this work, food scraps are explored as a potential feedstock to the MixAlco process. Batch fermentation with various temperatures, buffers, and pH control methods elucidated the behavior of food scraps during fermentation. The pH and reactor configuration were limiting factors when maximizing production. A fermentor was developed and tested with constant pH control. This resulted in elevated concentration (100 g/L) and selectivity (82%) of desired products.

The fermentation resulted in elevated concentrations, but low conversion of solids. The undigested material may serve as a nutrient source for fermenting lignocellulosic feedstocks. Combining various nutrient sources with lignocellulose, such as bagasse, resulted in additional production and further conversion. Multiple nutrient sources were tested resulting in total acid concentration ranging from 20.2 to 34.5 g/L.

To my Lord, the source of my strength
To my parents
To Juany, Lowell, Nella-Pea, and Jay-Dee
To Sarah, Ed, and the students in my after school mentoring program always remember that "*the man who wins is the man who thinks he can" – Walter D. Wintle*

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CHAPTER I
INTRODUCTION

1.1 Renewable Natural Resources

The year 2006 will be remembered for soaring energy costs with crude oil surpassing $70/barrel, and regular gasoline exceeding $3.00/gallon. International conflict, demand in developing countries, and extreme weather conditions contributed to the current explosive increase in energy costs. The root cause is the struggle to meet growing demand with limited natural resources. Non-renewable natural resources – such as petroleum, natural gas, and coal – cannot replenish themselves and must be used sparingly. In recent decades, the demand for energy has outpaced supply (Wells, 2005).

Because of the pressures on finite fossil resources, alternative energy sources must be developed. One such technology is the fermentation of biomass to biofuels. Biofuels directly substitute for fossil fuels in transportation and can be readily integrated into fuel supply systems (Council of the European Union, 2006). These fuels will have tremendous environmental and economical benefits (Greene, 2004). To increase utilization and profit margins, the biomass feedstock must be inexpensive, abundant, and highly fermentable. A material that fits these criteria is food scraps.

1.2 Food Scraps

Food scraps include uneaten food and wastes from residences, restaurants, and cafeterias. One benefit of using food scraps is availability. Food scraps are the single-largest component of the waste stream by weight in the United States (Environmental Protection Agency, 2003). Americans throw away more than 25% of the food prepared, about 96 billion pounds of food waste each year (EPA, 2003). In 2003, almost 12% of the total municipal solid waste (MSW) generated in American households was food scraps and less than 3% was recovered (EPA, 2003). This margin shows a great opportunity to recover and utilize food scraps in fermentation.

This thesis follows the style and format of Bioresource Technology.

Additional benefits of using food scraps are their high nutrient content and digestibility. Food scraps are composed mainly of nonstructural carbohydrates (sugar, starch, galactans, and pectins) which are readily digestible. Food scraps also contain a desirable ratio of carbon and nitrogen that support microbial growth. Carbon supplies energy and growth whereas nitrogen is used for protein and reproduction (Sherman, 1999). The availability, nutrient content, and digestibility of food scraps motivated this investigation for its use as a feedstock in the MixAlco process.

1.3 MixAlco Process

The MixAlco process generates mixed alcohols. In this process, biomass is pretreated to increase digestibility. The pretreated biomass is fermented by a mixed culture of microorganisms to produce carboxylic acids. To control pH, calcium carbonate can be added, thus forming carboxylic salts. The salts are concentrated then dried. This product can be thermally converted to ketones, which subsequently are hydrogenated to alcohols (Holtzapple *et al.*, 1999) (Figure 1-1).

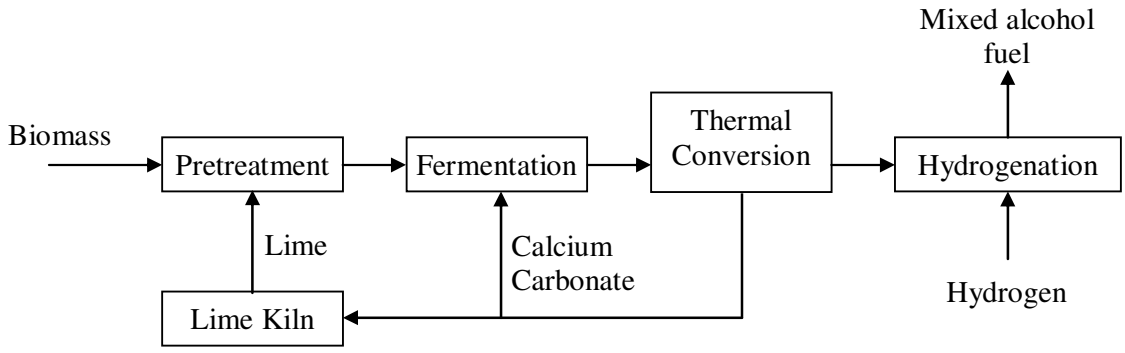

Figure 1-1. MixAlco process.

1.4 Current Food Scrap Disposal

Currently, food scraps are disposed in landfills, combusted in incinerators, or composted. In landfills, food scraps decompose under anaerobic conditions and produce methane, a greenhouse gas (GHG). Landfills are the largest human-related source of methane in the United States, accounting for 34% of all methane emissions (EPA, 2003). GHGs in the atmosphere will lead to major environmental changes and pose potentially significant risks to humans, social systems, and the natural world (EPA, 2002).

Incineration is commonly used to dispose of MSW, including food scraps. It reduces the volume of wastes and can produce electricity, but discharges pollutants. The major pollutants are dioxins, acid gases, nitrogen oxides, heavy metals, and particulates (The Parliamentary Office of Science and Technology, 2000). Adverse health effects – including respiratory, thyroid, heart disease, cancer and congenital abnormalities – have been associated with incinerator exposure (Allsopp, 2001).

Composting transforms organic waste into a valuable soil resource by bacteria, worms, or woodlice. Vineyard managers in California, who began using compost consisting mainly of food scraps, experienced increased soil microbial growth because of the increased presence of nutrients (Reed, 2002).

1.5 Buffer

Historically, the MixAlco process used $CaCO_3$ as the buffer. Calcium carbonate is inexpensive and can be converted into lime to pretreat biomass feedstock. Recent research shows that NH_4HCO_3 is an effective buffer. Ammonium bicarbonate produces higher acid concentrations at mesophilc conditions compared to $CaCO_3$ (Agbogbo, 2005). Additional advantages are inhibited methane production (Kayhanian, 1999; Parkin el al., 1980), effective pH control, and supplementation of nitrogen.

$CaCO_3$ and NH_4HCO_3 react with organic acids to form carboxylate salts, water, and carbon dioxide:

$$2CH_3(CH_2)_xCOOH + CaCO_3 \longrightarrow Ca(CH_3(CH_2)_xCOO)_2 + H_2O + CO_2 \qquad (1\text{-}1)$$

$$CH_3(CH_2)_xCOOH + NH_4HCO_3 \longrightarrow NH_4CH_3(CH_2)_xCOO + H_2O + CO_2 \qquad (1\text{-}2)$$

where x = 0, 1, 2, 3, 4, 5.

1.6 Objective

Food scraps are a potential feedstock in the MixAlco process. The environmental impacts associated with food scraps, digestibility, and the small margin recycled present an attractive opportunity. The research will accomplish the following:

- Explore food scrap fermentation through preliminary experiments. Parameters such as temperature, buffer, and pH control will be studied through batch fermentation.
- Use data from batch experiments to design a food scrap fermentor that will capitalize on the fermentation properties of food scraps.
- Find multiple uses for food scraps and the undigested residue.

CHAPTER II
MATERIALS AND METHODS

2.1 Substrates

The food scraps (FS) used in the following experiments were collected at a campus dining hall (Texas A&M University) and two local restaurants (Golden Corral and Taste of China). To ensure a representative sample, food scraps were collected for an entire week. This material was then chopped in a 9-cup General Electric food processor (model #106622F) to reduce particle size. The volume of FS eventually caused the 450-W geared-down motor to fail. The food processor was modified with a ½-hp motor with a direct drive-shaft. The FS were collected in a 114-L container and mixed to create a homogeneous mixture. The FS were packaged in 12 in × 15 in Zip Press polyethylene bags and then frozen.

Long-term continuous fermentation (72 days) of FS in the fermentor, designated the Pre-Digestion Unit (PDU), resulted in digested food scraps (DFS). This material consisted of two separate components: (1) partially digested oils and fats (OF), and (2) partially digested carbohydrates and vegetables (CV). After centrifuging the DFS, the oils and fats formed a layer above the liquids. The partially digested carbohydrates and vegetables were below the liquids.

Short-term batch fermentation (14 days) of FS in the PDU produced a DFS that consisted mostly of partially digested carbohydrates and vegetables and some partially digested oils and fats. The CV and OF were mixed and used in 1-L batch fermentations.

Pretreated bagasse was obtained from a 60-day air and lime laboratory-scale pile pretreatment (Jones, 2007). In this pretreatment method, a covered pile circulated water and air to decrease the lignin content and increased digestibility (Granda 2004).

Chicken manure (CM) was attained from the Poultry Science Department Pilot Plant at Texas A&M University.

2.2 Deoxygenated Water

Deoxygenated water was used to reduce the inhibitory effects of dissolved oxygen during anaerobic fermentation. Reducing agents such as sodium sulfide and L-cysteine hydrochloride hydrate 99% were added to increase the reducing potential. Details on the preparation of deoxygenated water can be found in Appendix C.

2.3 Inoculum

Marine inoculum was collected from coastal sites in Galveston, Texas (East Beach, 9th Street, 51st Street, and 8 Mile). Sediment was extracted from a 0.5-m hole and placed in a centrifuge bottle filled with deoxygenated water.

2.4 Dry Nutrients

Dry nutrients (Appendix F) were used in the preliminary batch experiments to determine if they are needed in the fermentation of food scraps. In previous experiments with lignocellulosic substrates, dry nutrients were used to sustain microbial growth.

2.5 Rotary Fermentors

A 1-L centrifuge bottle was used for the batch fermentation experiments. The bottle cap was modified to hold a rubber stopper. The rubber stopper served two purposes in the fermentor: (1) to form an airtight seal in the reactor, and (2) to serve as an accessible interface. A glass test tube that was cut and flared was inserted into the center of the stopper. The test tube was capped with a rubber septum and served as a sample port for gas measurements and analysis. The rubber stopper also allowed a stirrer to enter the reactor. The stirrer was designed to ensure that proper mixing occurred as the fermentor rotated (Figure 2-1). The fermentors were placed horizontally in an incubated roller apparatus.

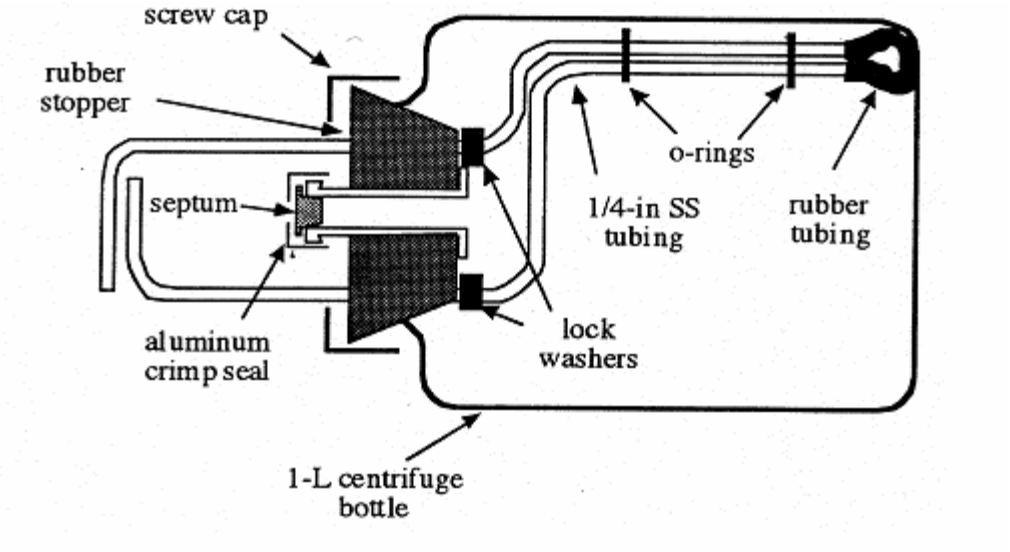

Figure 2-1. 1-L Centrifuge bottle bioreactor. (Ross, 1998)

2.6 Pre-Digestion Unit (PDU)

The PDU (Figure 2-2) consists of a 10-L B. Braun Biotech reaction chamber and four independent systems; a pH controller, gas displacement system, temperature controller, and a mixer.

pH Controller

The pH was maintained with an Omega panel-mounted pH controller. A pH electrode was fixed and sealed in the fermentation broth. Continuous pH data were fed to the controller. The controller was connected to a variable-flow peristaltic pump, which slowly dripped a 30% solution of NH_4HCO_3. This buffer solution converted carboxylic acids into ammonium salts, and maintained the pH within the optimal range (6.8 to 7.2).

Gas Displacement

The reactivity of food scraps produced large volumes of carbon dioxide as a by-product of fermentation. In previous experiments with 1-L centrifuge bottles, fermentors would burst or require constant CO_2 release. To address this problem, a gas displacement system was developed to prevent the negative effects of over pressurization. This system was designed to vent CO_2 at the top of the fermentor as it was produced. This gas traveled through 0.25-in PVC tubes into four 0.1-m-diameter transparent PVC pipes each 1.22 m tall. Each column was filled with a 20% NaCl solution that was displaced in a 60-L tank as CO_2 was produced. This setup allowed constant gas removal.

Temperature Controller

Mesophilic conditions were maintained with water circulation in a tubular heat exchanger within the fermentor. An Omega auto tune temperature controller maintained a 15-L container of DI water at 42^{o}C. Water was circulated using a 225-W pump. This temperature maintained 40^{o}C in the fermentor. Mesophilic conditions were used rather

than thermophilic, to minimize energy costs and provide a more stable fermentation: Both of these considerations are important factors in large-scale industrial application.

Mixer

Mixing speed is a major feature in the distribution and availability of microorganisms, substrates, and products. When optimized, it will improve microbial growth and reactor stability (Ganduri, 2004). To ensure proper mixing, a 115-W motor was used to drive a timing belt connected to a shaft in the fermentor. Three impellers were spaced 3 inches apart and extended through 4 L of the fluid in the fermentor. The speed was set at 50 rpm, and was effective in distributing reactor contents.

2.7 Analytical Methods

Gas produced in the 1-L fermentors was measured using a water displacement apparatus (Figure 2-3). A glass tube 1 m in height and 0.05 m in diameter had two flexible PVC tubes connected to the top: (1) to a vacuum pump and (2) to a syringe. The glass tube was placed in a container with a 20% solution of $CaCl_2$. Calcium chloride was used to inhibit microbial growth and resist CO_2 dissolving into the liquid. The vacuum pump raised the water to a measured height. The syringe was then inserted into the fermentor to vent the gas.

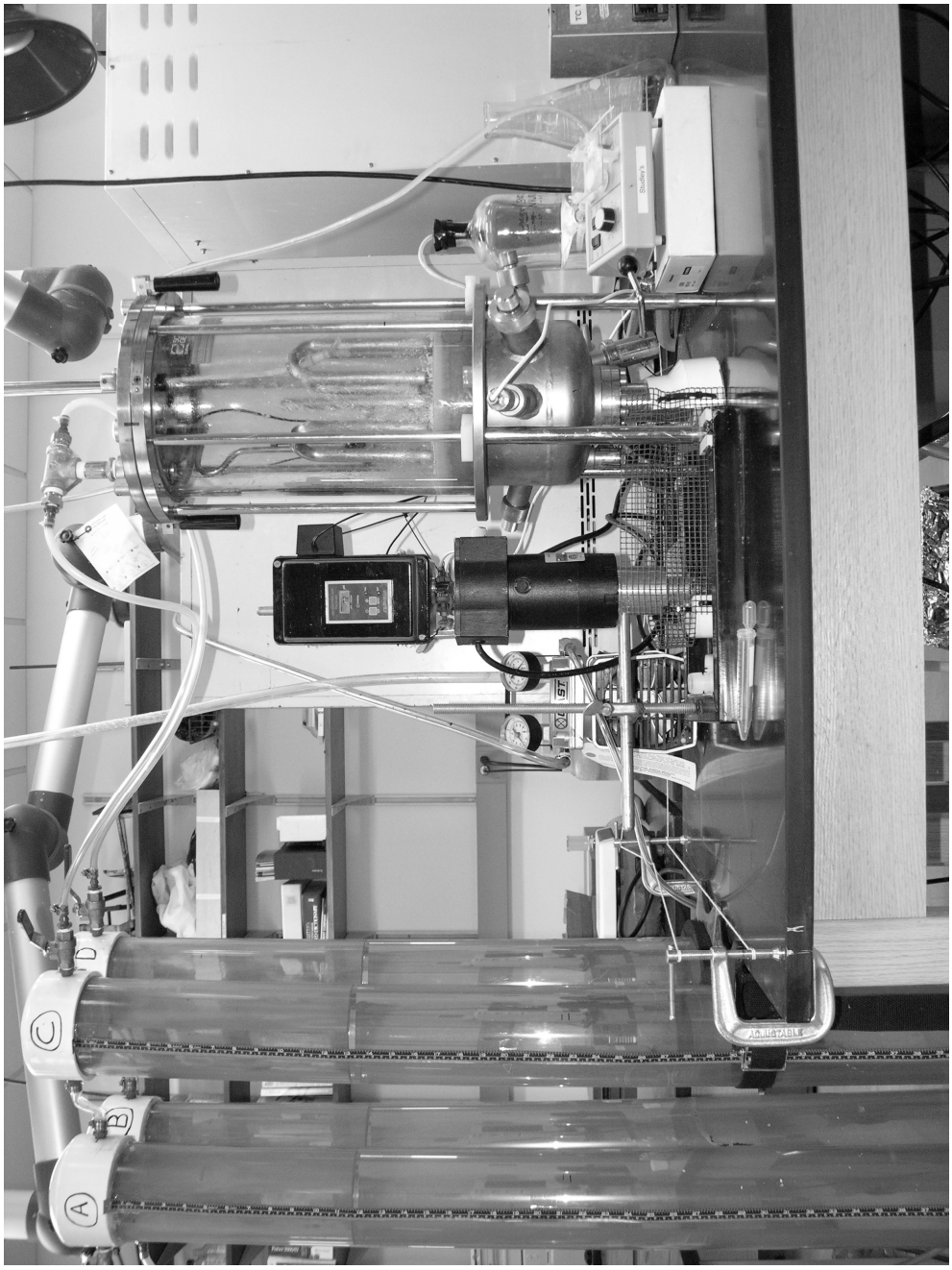

Figure 2-2. Pre-Digestion Unit (PDU).

An Agilent 6890 series gas chromatograph (GC) was used to determine acid concentration in liquid samples. Liquid samples were mixed with 1.162 g/L of 4-methly-n-valeric acid and 3-M phosphoric acid (Appendix F). Upon injection, the liquid was vaporized then carried by an inert gas (He) through a heated capillary column (J&W Scientific, model DB-FFAP). The sample traveled through the column and separated into pure components. Prior to exiting, it passed through a flame ionization detector, which recorded a peak at a characteristic retention time. These retention times and peak areas were used to find the concentration of products in the sample.

Gas samples taken from the fermentors were analyzed using a thermal conductivity detector (TCD) in the same Agilent 6890 series GC. Carbon dioxide and methane can be detected with a TCD. Abiotic CO_2 is produced from the reaction of carboxylic acids and the buffer. Based upon stoichiometry, 1 mole of abiotic CO_2 is produced for every 2 moles of acid. Biotic CO_2 is produced from the fermentation and was calculated by subtracting the abiotic CO_2 from the total CO_2.

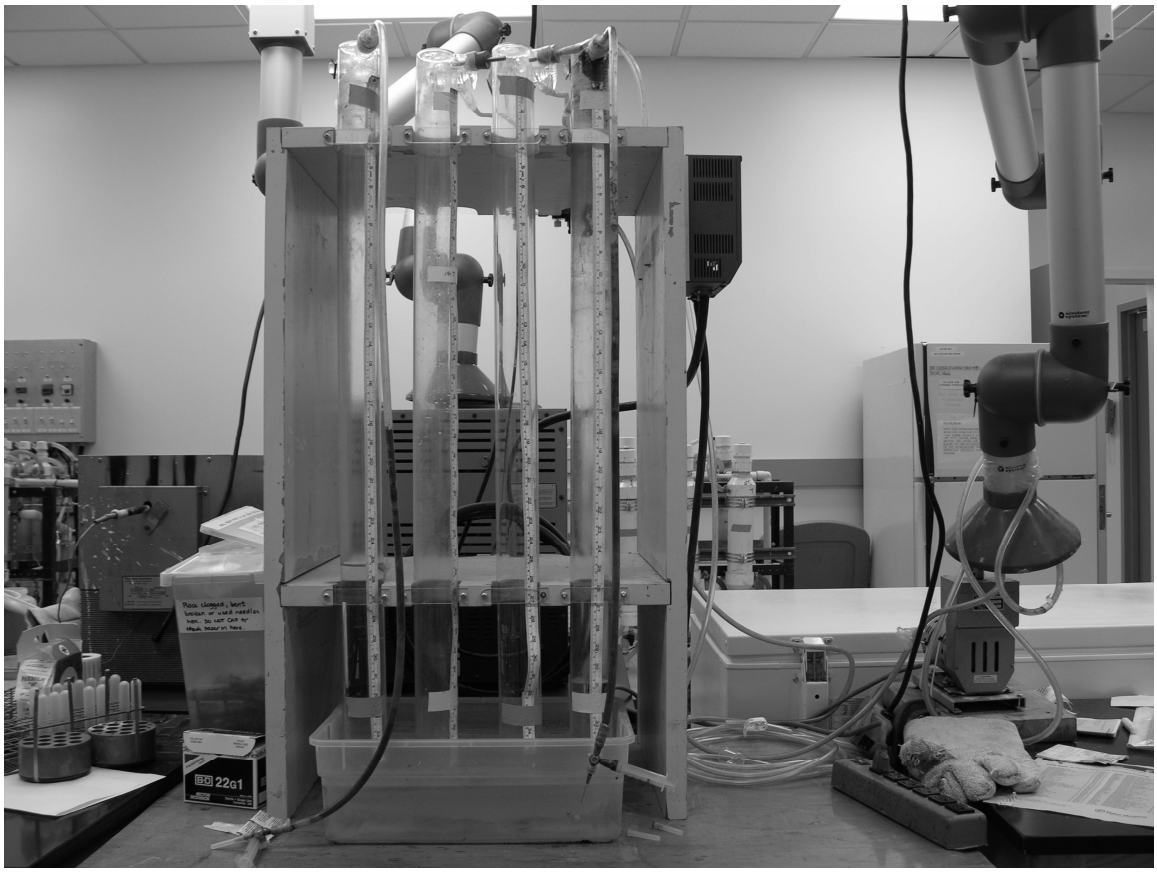

Figure 2-3. Water displacement instrument.

2.8 Fermentation Terms

At the end of the fermentation experiments, the data were used to calculate the following terms:

$$\text{Volatile Solids (VS)} = \text{Dry weight} - \text{Ash weight} \tag{2-1}$$

$$\text{Conversion } (x) = \frac{\text{VS digested}}{\text{VS fed}} \tag{2-2}$$

$$\text{Yield } (y) = \frac{\text{Total carboxylic acids produced}}{\text{VS fed}} \tag{2-3}$$

$$\text{Total acid productivity } (p) = \frac{\text{Total carboxylic acids produced}}{\text{Total liquid volume in fermentor} \times \text{time}} \tag{2-4}$$

$$\text{Total acid selectivity} = \frac{\text{Total carboxylic acids produced}}{\text{VS digested}} \tag{2-5}$$

$$\text{Liquid Residence Time (LRT)} = \frac{\text{Total liquid in fermentor}}{\text{Flow rate of liquid out of the fermentor}} \tag{2-6}$$

$$\text{Volatile Solids Loading Rate (VSLR)} = \frac{\text{VS feed to the system}}{\text{Total liquid in fermentor} \times \text{time}} \tag{2-7}$$

CHAPTER III
SUBSTRATE CHARACTERIZATION

The substrates used in the fermentation were characterized according to quantifiable aspects of the material. Properties such as moisture content, volatile solid content, sugar, protein, and fat were analyzed. This chapter will characterize substrates and present experimental results.

3.1 Moisture and Volatile Solid Content

Biomass contains volatile solids (VS) and ash (Figure 3-1). Anaerobic fermentation converts volatile solids to liquid and gaseous products, plus solid residues. The liquid products are carboxylic acids, extracellular proteins, and energy storage polysaccharides; the gaseous products are carbon dioxide and methane; and the solid residue contains ash and undigested VS (Agbogbo, 2006; Ross, 1998).

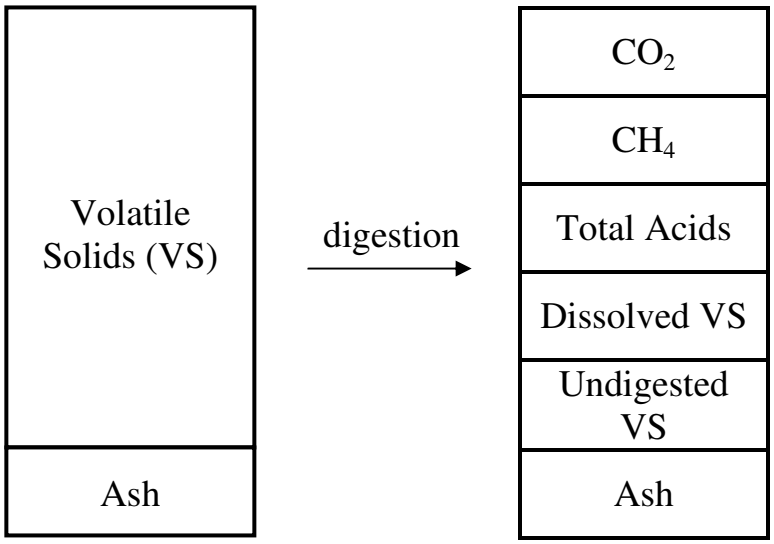

Figure 3-1. The digestion of biomass (Agbogbo, 2006).

The moisture content for food scraps (FS) was 0.648 g water/g raw FS, the ash content was 0.042 g ash/g dry FS, and the volatile solid (VS) content was 0.958 g VS/g dry FS.

Long-term continuous fermentation (72 days) of FS in the fermentor, designated the Pre-Digestion Unit (PDU), resulted in digested food scraps (DFS). This material consisted of two separate components: (1) partially digested oils and fats (OF), and (2) partially digested carbohydrates and vegetables (CV). After centrifuging the DFS, the oils and fats formed a layer above the liquids. The oils and fats had a moisture content of 0.610 g water/g raw OF, an ash content of 0.020 g ash/g dry OF, and a volatile solid content of 0.980 g VS/g dry OF.

The partially digested carbohydrates and vegetables, were below the liquids after centrifuging. The moisture content was 0.786 g water/g raw CV, the ash content was 0.041 g ash/g dry CV, and the volatile solid content was 0.959 g VS/g dry CV. On a wet basis, the DFS consisted of 0.6 g CV/g raw DFS and 0.4 g OF/g raw DFS.

Short-term batch fermentation (14 days) of FS in the PDU produced a DFS that consisted mostly of partially digested carbohydrates and vegetables and some partially digested oils and fats. The CV and OF were mixed and used in 1-L batch fermentations. The moisture content for the DFS was 0.657 g water/g raw DFS, the ash content was 0.574 g ash/g dry DFS, and the volatile solid (VS) content was 0.426 g VS/g dry DFS.

Pretreated bagasse was obtained from a 60-day air and lime laboratory-scale pile pretreatment (Jones, 2007). In this pretreatment method, a covered pile circulated lime and air to decrease the lignin content and increased digestibility (Granda 2004). The moisture content for the air and lime pretreated bagasse (after air drying for 2 days) was 0.062 g water/g raw bagasse, the ash content was 0.230 g ash/g dry bagasse, and the volatile solid content was 0.770 g VS/g dry bagasse.

Chicken manure (CM) was attained from the Poultry Science Department Pilot Plant at Texas A&M University. The moisture content for CM was 0.657 g water/g raw CM, the ash content was 0.426 g ash/g dry CM, and the volatile solid content was 0.574 g VS/g dry CM.

A summary of the moisture content, VS content, and ash content is shown in Table 3-1. The experimental procedures are in Appendix E.

Table 3-1. Results for moisture and volatile solid analysis

Substrate	**Moisture Content** (g water/g raw material)	**Volatile Solid Content** (g VS/g dry material)	**Ash Content** (g ash/g dry material)
Food Scraps	0.648	0.958	0.042
Continuous DFS	0.716	0.967	0.033
OF	0.610	0.980	0.020
CV	0.786	0.959	0.041
Batch DFS	0.657	0.426	0.574
Bagasse	0.062	0.770	0.230
CM	0.657	0.574	0.426

3.2 Sugar Analysis

Food Scraps contain carbohydrates, starches, and cellulose. These materials can be hydrolyzed to form monosaccharides and disaccharides. Monosaccharides and disaccharides – such as glucose, xylose, galactose, arabinose, and sucrose – are consumed by microorganisms and readily metabolize to carboxylic acids. Total sugars in the substrates were found by HPLC. The data are listed in Table 3-2.

Table 3-2. Sugars in various substrates (dry basis)

Substrate	Glucose (%)	Xylose (%)	Galactose (%)	Arabinose (%)	Sucrose (%)	Total (%)
FS	40.4	1.2	2.1	1.4	1.6	46.7
DFS Batch	5.8	1.6	1.8	1.5	0.0	10.7
DFS Continuous	5.1	0.7	0.0	0.0	0.0	5.8
OF	2.3	0.3	0.0	0.0	0.0	2.6
CV	8.6	1.5	0.0	1.3	0.0	11.4
CM	3.5	6.8	2.5	4.3	0.0	17.2

3.3 Crude Protein Analysis

Nitrogen is used for protein and reproduction. The total nitrogen in each substrate was found using a LECO FP 528 (LECO, 2003) located in Texas A&M University Department of Animal Science. This equipment vaporizes a solid sample and measures the gas by a thermal conductivity cell for nitrogen. Crude protein is calculated by a conversion factor. Nitrogen content, conversion factors, and crude protein content are displayed in Table 3-3.

Table 3-3. Nitrogen and protein content for various substrates (dry basis)

Substrate	**N_2 (%)**	**Conversion Factor**	**Crude Protein (%)**
FS	1.399	6.25	8.74
DFS Batch	1.039	6.25	6.49
DFS Continuous	3.220	6.25	20.13
OF	3.335	6.25	20.84
CV	3.143	6.25	19.64
CM	1.297	6.25	8.11

3.4 Fat Analysis

Fats do not dissolve in water, instead they congeal together in large masses which are less digestible. Fat content in the samples was obtained using a Soxhlet extractor. Petroleum ether was boiled and condensed in the apparatus for 4 hours. The ether was then evaporated leaving the fat in the flask. The total fat composition is shown in Table 3-4.

Table 3-4. Fat content for various substrates (dry basis)

Substrate	Fat (%)
FS	7.82
DFS Batch	6.81
DFS Continuous	15.01
OF	28.42
CV	6.16

CHAPTER IV
BATCH FERMENTATION

There are many factors to consider when using food scraps as a feedstock in the MixAlco process. To find the optimum operating parameters for food scraps, lab-scale batch fermentation was conducted in 1-L rotary fermentors. Carboxylic acid production with varying pH control, temperature, buffer, and inoculum source was investigated. These preliminary experiments illustrate the dynamics of batch food scrap fermentation and serve as a foundation for the development of the PDU. Detailed fermentation data are displayed in Appendix G.

4.1 Experiment 1

Comparing the digestibility of food scraps using various inoculum sources

Food scraps (100 dry g/L), 0.2 g dry nutrients (Appendix F), 0.2 g urea, and anaerobic water were placed in Fermentors C155, C255, N155, and N255. The pH was adjusted to 7 then 50 mL of inoculum was added to each reactor. Fermentors were accessed every other day to collect samples and adjust pH.

The first character in the reactor name identifies the buffer: C for calcium carbonate and N for ammonium bicarbonate. The second character represents experimental conditions:

(1) fresh inoculum from Galveston

(2) inoculum from previous bagasse and chicken manure fermentation (Fu, 2007)

The last two characters represent the temperature of the reactor: 55°C. Table 4-1 displays fermentor operating conditions for Experiment 1.

For all the batch fermentors discussed in this chapter, the following method applies: A 3-mL liquid sample was taken periodically. Additionally the pH of each reactor was measured and adjusted to the appropriate range (6.8 – 7.2) by adding dry buffer. After sampling, 120 μL of iodoform (20 g/L of iodoform dissolved in ethanol)

was added to inhibit methane production (Ross, 1998). When fermentors were open, a constant N_2 purge maintained anaerobic conditions.

Table 4-1. Data matrix for batch fermentation in Experiment 1

Reactor ID	**C155**	**C255**	**N155**	**N255**
Food Scrap - dry (g)	40	40	40	40
Buffer	$CaCO_3$	$CaCO_3$	NH_4HCO_3	NH_4HCO_3
Temperature (°C)	55	55	55	55
Inoculum Source	Galveston	***	Galveston	***
amount (mL)	50	50	50	50
Deoxygenated H_2O (mL)	276	276	276	276
Dry Nutrient (g)	0.2	0.2	0.2	0.2
Urea (g)	0.2	0.2	0.2	0.2
Iodoform (μL)	240	240	240	240

*** Inoculum from previous bagasse and chicken manure fermentation (Fu, 2007)

The rapid acid production displayed in Figures 4-1 and 4-2 showed that food scraps are a viable fermentable feedstock. It also showed that the inoculum harvested from new locations (East Beach, 9th Street, 51st Street and 8 Mile) contained a mixed culture of acid forming microorganisms. N155 had the highest acid concentration of 26 g/L as compared to 23 g/L in N255, and 17 g/L in C155 and C255. In each of the reactors, butyric acid was the major contributor to the total acid concentration (52 to 85%).

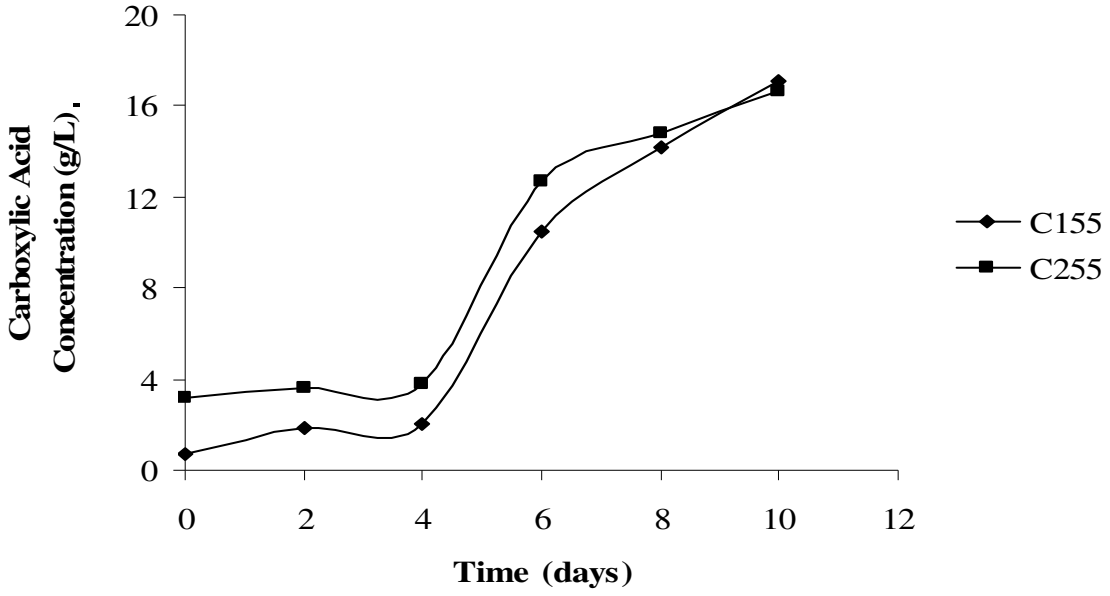

Figure 4-1. Total acid production with different inocula using $CaCO_3$ buffer.

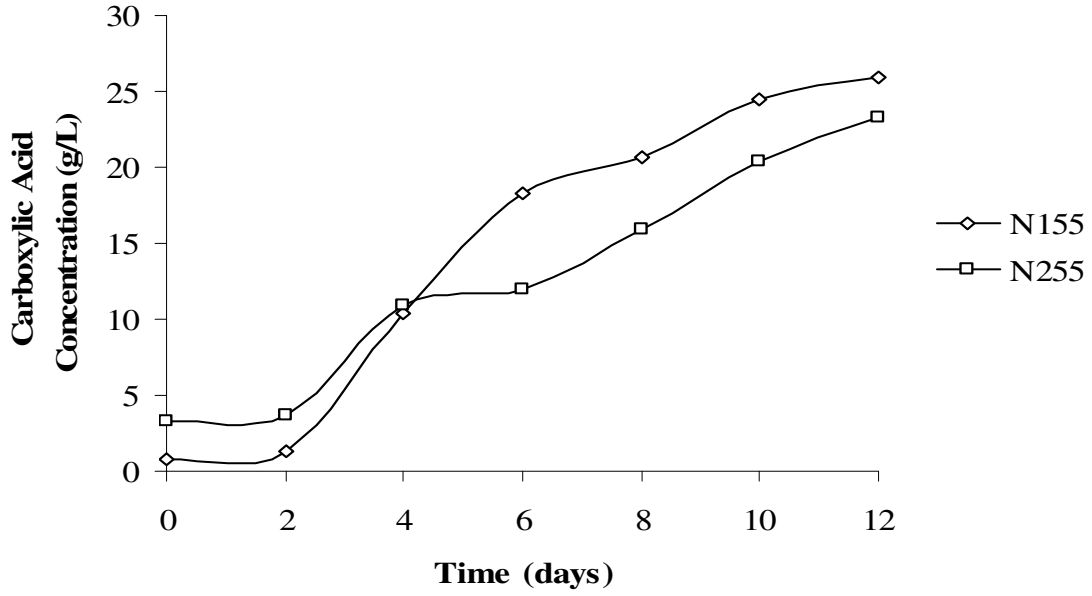

Figure 4-2. Total acid production with different inocula using NH_4HCO_3 buffer.

Proper pH control is vital to microorganism proliferation. In cases where the pH dropped below the optimal range, acid production was inhibited. The initial pH for all the reactors was 6.3. After two days, all of the reactors operated at a pH below 5.0. Ammonium bicarbonate was added to the reactors containing the ammonium buffer to raise the pH to 7. Controlling the pH for reactors containing the calcium buffer was difficult because calcium carbonate did not dissolve fully in the water. Lime was added to increase the pH to 7. Figures 4-3 and 4-4 contain the recorded pH for the fermentors.

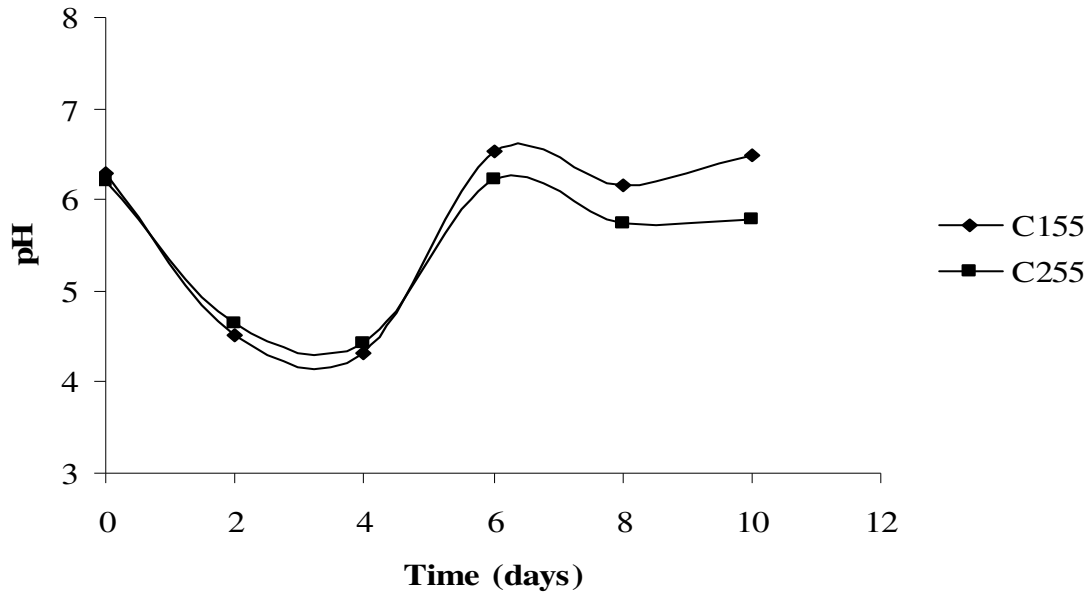

Figure 4-3. pH with different inocula using $CaCO_3$ buffer.

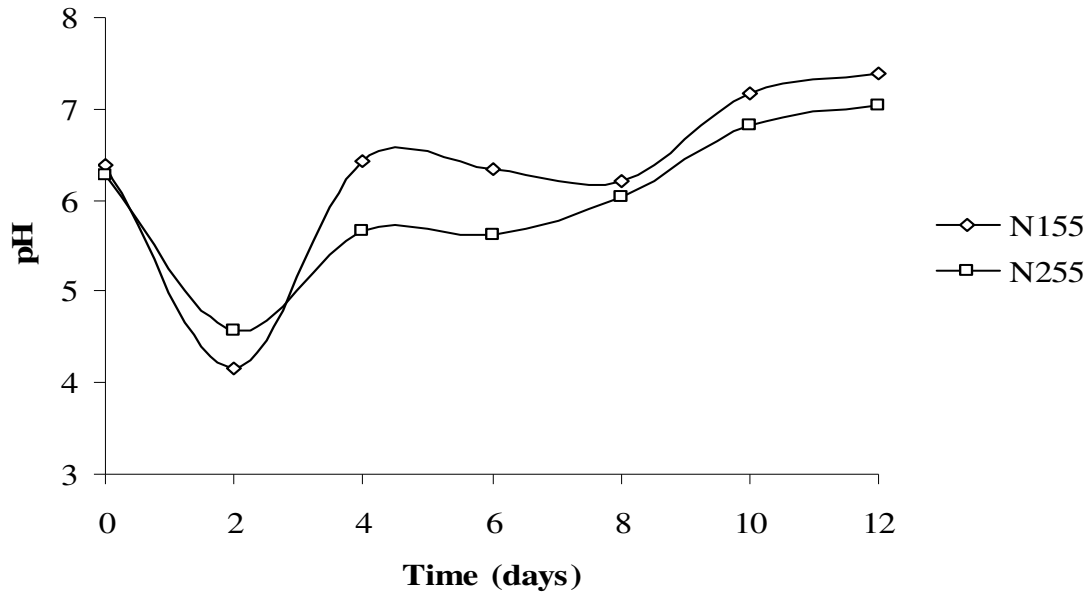

Figure 4-4. pH with different inocular using NH_4HCO_3 buffer.

4.2 Experiment 2

Food scraps as a nutrient source

In previous fermentations with a mixture of a lignocellulosic feedstock and a nutrient source (Agbogbo 2005, Thanakoses 2002, Ross 1998), dry nutrients and urea were added to initiate and sustain microorganism development. Experiment 2 will show that dry nutrients and urea are not necessary and that food scraps can serve as both a feedstock and nutrient source. This theory was tested at mesophilic and thermophilic conditions with each buffer type.

Food scraps (100 dry g/L) and anaerobic water were placed in Fermentors C340, C355, N340, and N355. The pH was adjusted to 7, and then 50 mL of inocula was added to each fermentor. Fermentors were accessed every other day to collect samples and adjust pH. Lime was added to increase the pH to 7 in fermentors using the $CaCO_3$ buffer.

The first character in the reactor name identifies the buffer: C for calcium carbonate and N for ammonium bicarbonate. The second character represents experimental conditions:

(3) No inorganic nutrients were added.

The last two numbers represent the temperature of the reactor: 55°C or 40°C. Table 4-2 shows fermentor conditions for Experiment 2.

Table 4-2. Data matrix for batch fermentation in Experiment 2

Reactor ID	**C340**	**C355**	**N340**	**N355**
Food Scrap - dry (g)	40	40	40	40
Buffer	$CaCO_3$	$CaCO_3$	NH_4HCO_3	NH_4HCO_3
Temperature (°C)	40	55	40	55
Inoculum Source	Galveston	Galveston	Galveston	Galveston
amount (mL)	50	50	50	50
Deoxygenated H_2O (mL)	276	276	276	276
Iodoform (µL)	240	240	240	240

The concentration data displayed in Figures 4-5 and 4-6 show that food scraps can produce carboxylic acids without the dry nutrient mixture. The use of food scraps instead of inorganic nutrients greatly reduces the cost for fermentation because it eliminates the use of expensive components.

N355 had the highest acid concentration of 20 g/L, as compared to 12 g/L in N340, 8 g/L in C340, and 2 g/L in C355. In each of the reactors, acetic acid was the major contributor to the total acid concentration (52 to 96%). Detailed data are contained in Appendix G.

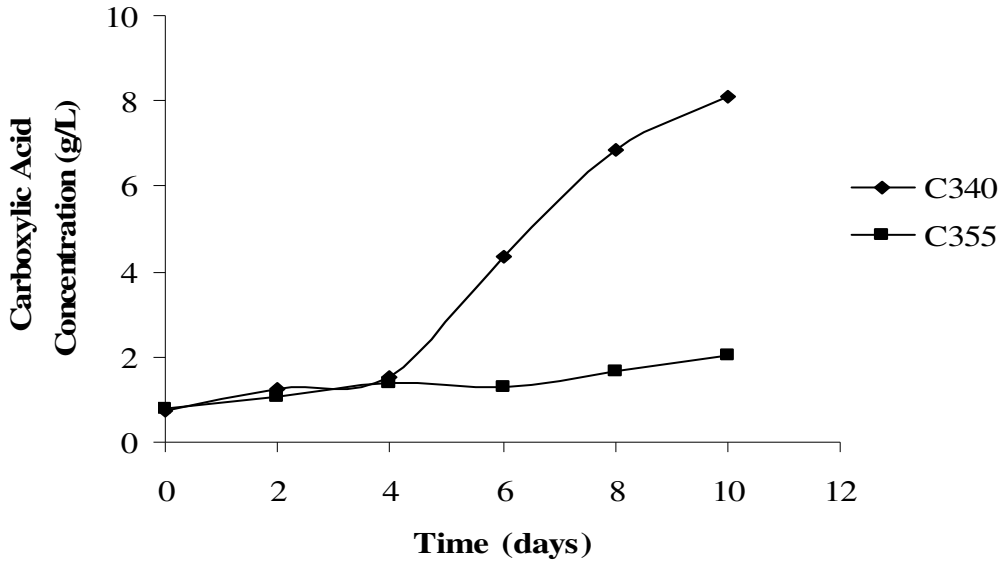

Figure 4-5. Total acid production at 40°C and 55°C using $CaCO_3$ buffer.

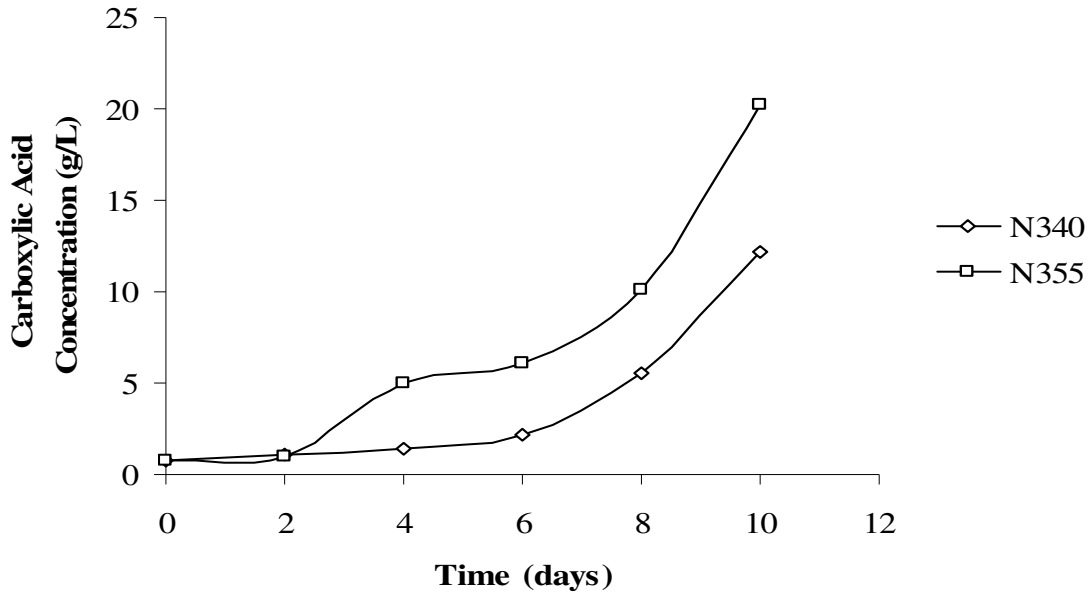

Figure 4-6. Total acid production at 40°C and 55°C using NH_4HCO_3 buffer.

Figures 4-7 and 4-8 show the pH of the fermentors at the given conditions. The average pH in C340 was 5.9, 5.2 in C355, 5.8 in N340, and 6.0 in N355. The low pH in the initial phase of the experiment caused low product concentrations.

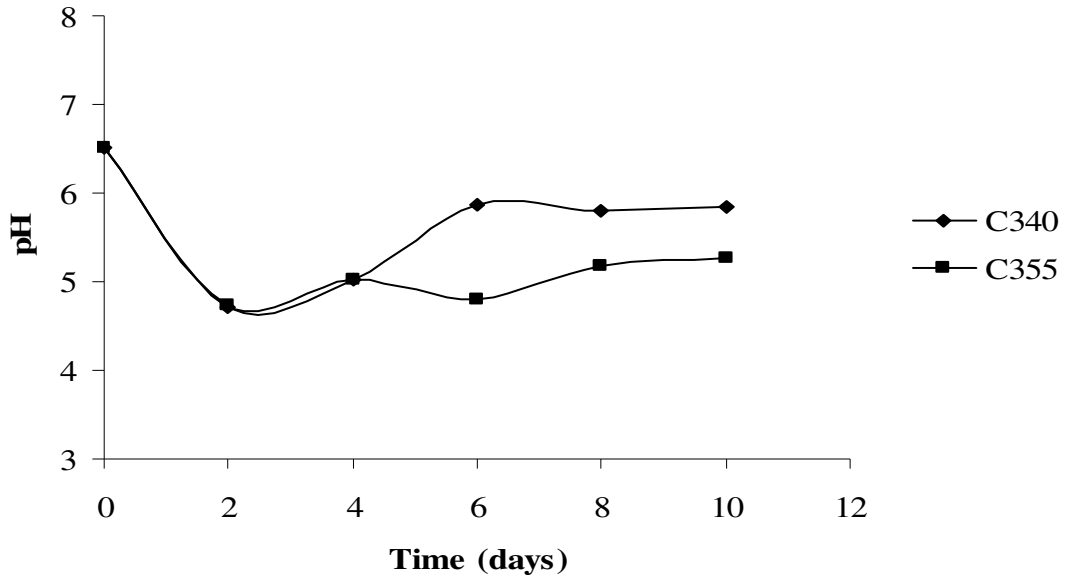

Figure 4-7. pH at 40°C and 55°C using $CaCO_3$ buffer and lime to adjust pH.

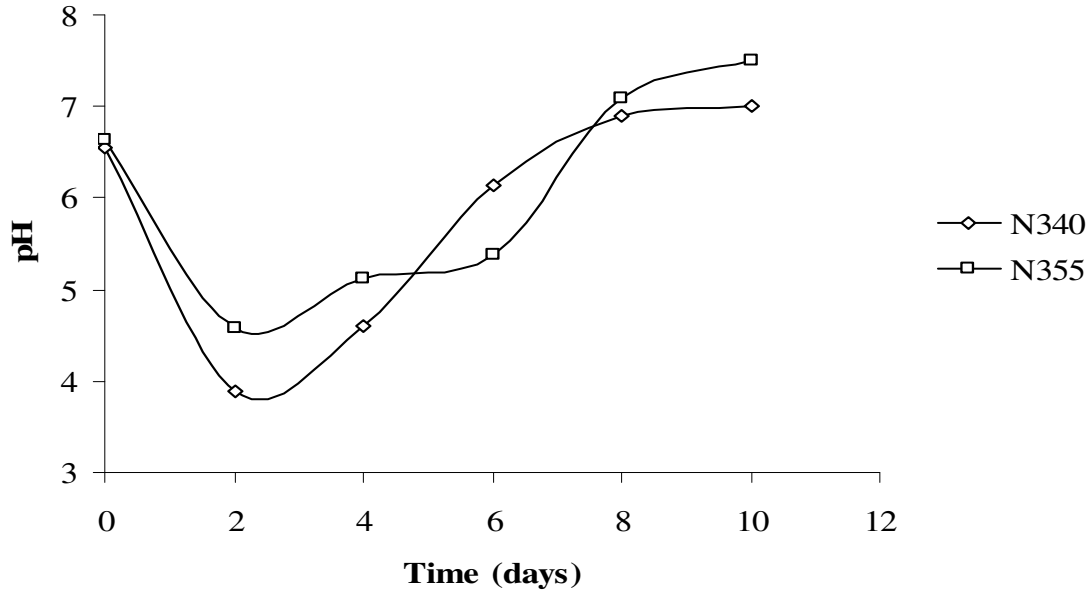

Figure 4-8. pH at 40°C and 55°C using NH_4HCO_3 buffer.

4.3 Experiment 3

pH Control

Fermentor pH was determined to be a major inhibitory factor in product formation by microorganisms (Agbogbo, 2005). This experiment showed the effectiveness of pH control in the conversion of food scraps. To control the pH, the fermentors were opened and adjusted every 2 to 4 hours during the initial stages.

Food scraps (100 dry g/L) and anaerobic water were placed in Fermentors C440, C455, N440, and N455. The pH was adjusted to 7 then 50 mL of inoculum was added to each reactor. C440 and C455 did not use lime to adjust pH; instead $CaCO_3$ was used despite its low solubility. N440 and N455 used NH_4HCO_3 as a buffer source. Table 4-3 displays fermentor conditions for Experiment 3.

Table 4-3. Data matrix for batch fermentation in Experiment 3

Reactor ID	**C440**	**C455**	**N440**	**N455**
Food Scrap - dry (g)	40	40	40	40
Buffer	$CaCO_3$	$CaCO_3$	NH_4HCO_3	NH_4HCO_3
Temperature (°C)	40	55	40	55
Inoculum Source	Galveston	Galveston	Galveston	Galveston
amount (mL)	50	50	50	50
Deoxygenated H_2O (mL)	276	276	276	276
Iodoform (μL)	240	240	240	240

The total acid concentrations and pH values in Experiment 3 are displayed in Figures 4-9 to 4-12. N440 had the highest acid concentration of 13.7 g/L, as compared to 13.3 g/L in C440, 8.6 g/L in N455, and 4.4 g/L in C355. Although pH was monitored frequently, there were instances where it was below 5.5 in the fermentors. The acid concentrations were lower than expected, but acetic acid was the major product (80 to 97%).

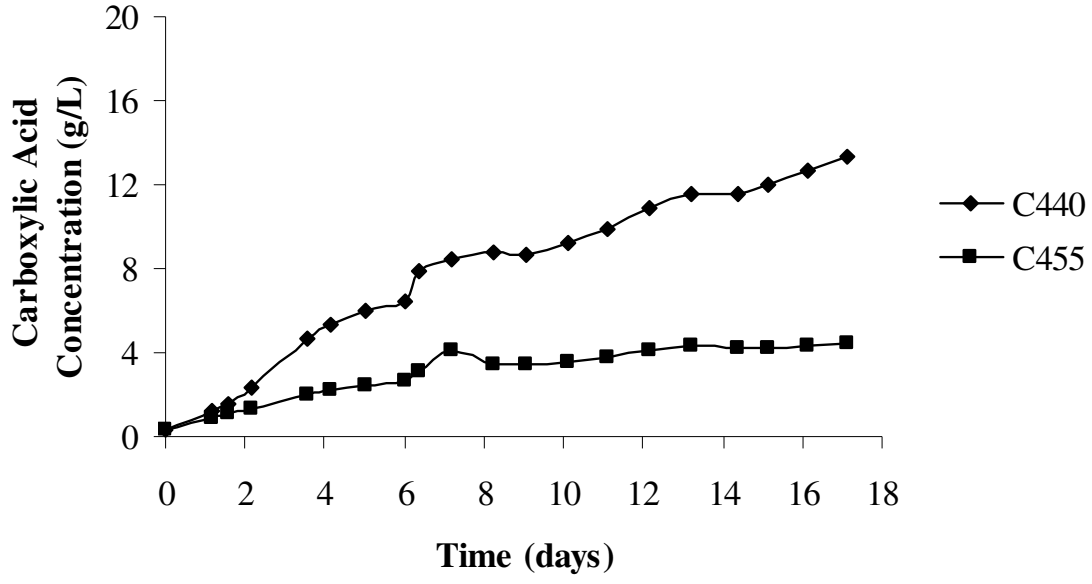

Figure 4-9. Total acid production at 40°C and 55°C using $CaCO_3$ buffer with constant pH control.

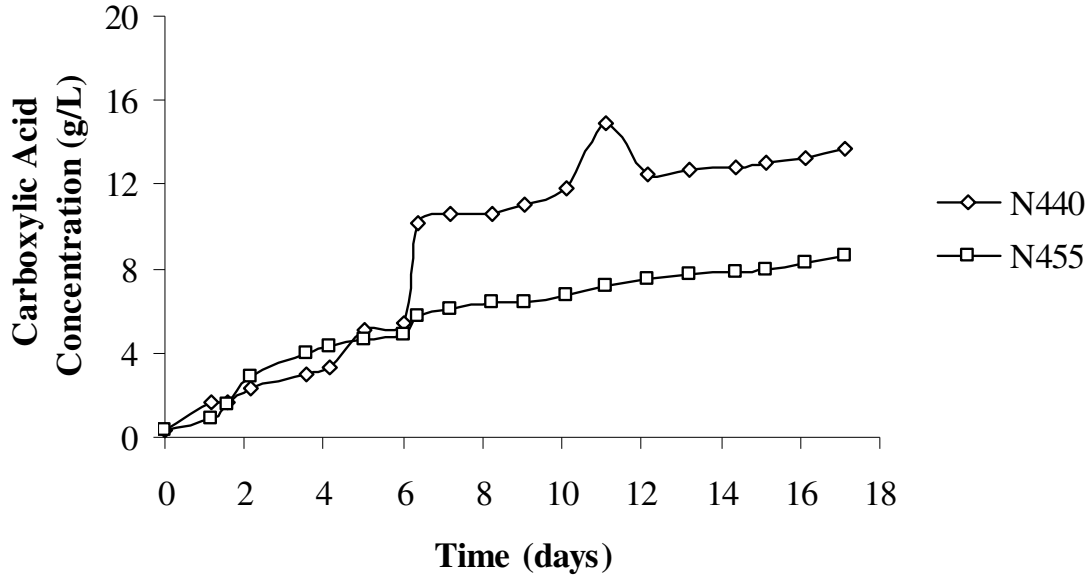

Figure 4-10. Total acid production at 40°C and 55°C using NH_4HCO_3 buffer with constant pH control.

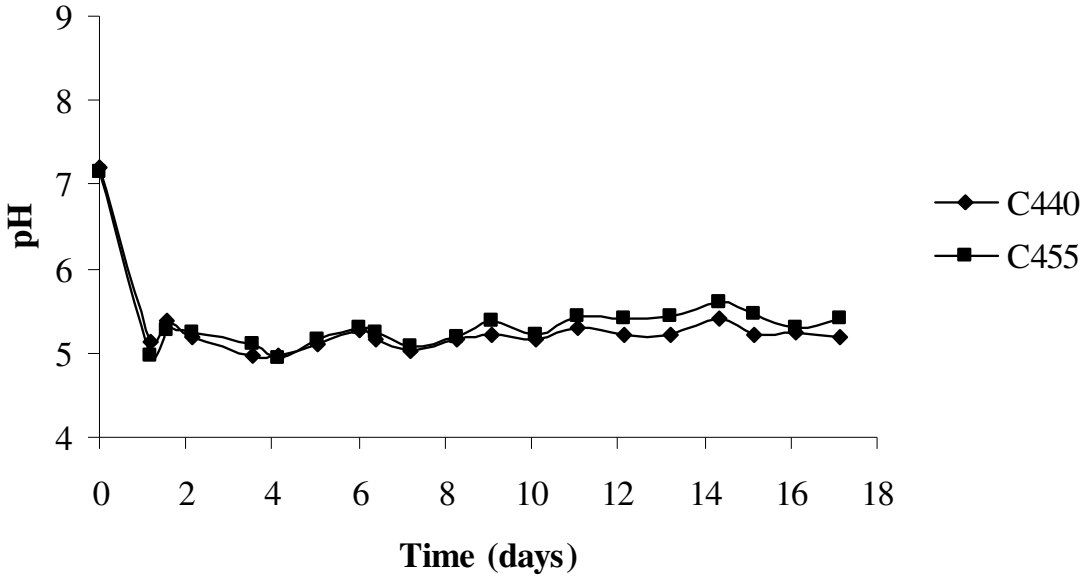

Figure 4-11. pH at 40°C and 55°C using $CaCO_3$ buffer.

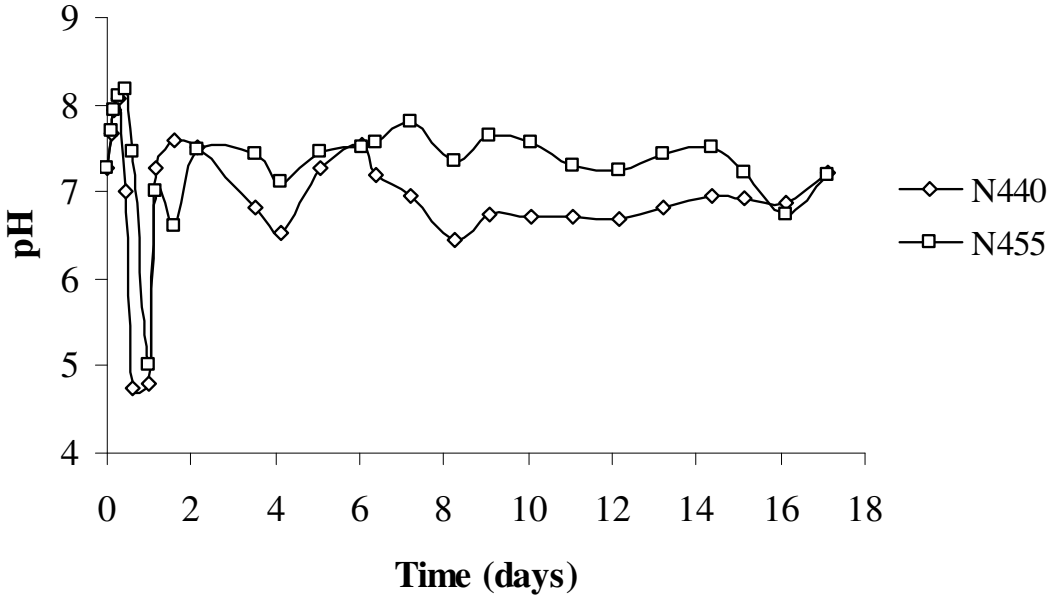

Figure 4-12. pH at 40°C and 55°C using NH_4HCO_3 buffer with constant pH control.

4.4 Experiment 4

Modified pH Control

The objective of this experiment was to convert acids to salts as they are formed by adding excess buffer. This method resulted in increased pH, and determined if acid production is greatly inhibited when pH is above or below the optimal range (6.8 – 7.2).

Food scraps (100 dry g/L) and anaerobic water were placed in Fermentors N540, N555, N640, and N655. The pH was adjusted to 7, and then 50 mL of inoculum was added to each reactor. All fermentors utilized ammonium bicarbonate as a buffer. The following experimental conditions were performed:

(5) addition of buffer hourly to adjust pH to 7

(6) addition of excess buffer

A 3-mL liquid sample was taken to monitor productivity and iodoform was added twice daily. This experiment did not test $CaCO_3$, as Experiment 3 determined that pH could not be maintained in the optimal range. Table 4-4 shows fermentor conditions for Experiment 4.

Table 4-4. Data matrix for batch fermentation in Experiment 4

Reactor ID	**N540**	**N555**	**N640**	**N655**
Food Scrap - dry (g)	40	40	40	40
Buffer	NH_4HCO_3	NH_4HCO_3	NH_4HCO_3	NH_4HCO_3
Temperature (°C)	40	55	40	55
Inoculum Source	Galveston	Galveston	Galveston	Galveston
amount (mL)	50	50	50	50
Deoxygenated H_2O (mL)	276	276	276	276
Iodoform (µL)	240	240	240	240

The total acid concentrations and pH values in Experiment 4 are displayed in Figures 4-13 to 4-16. N555 had the highest acid concentration of 30.0 g/L compared to 16.7 g/L in N540, 15.6 g/L in N640 and 15.3 g/L in N655. In each reactor, acetic acid was the major contributor to the total acid concentration (52 to 96%).

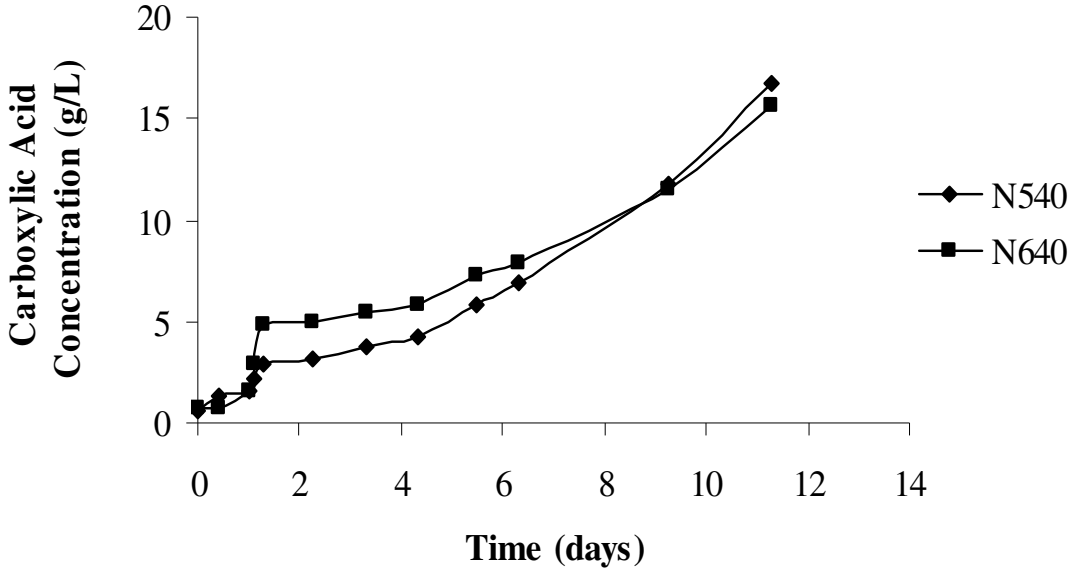

Figure 4-13. Total acid production at 40°C using hourly and stepwise buffer addition.

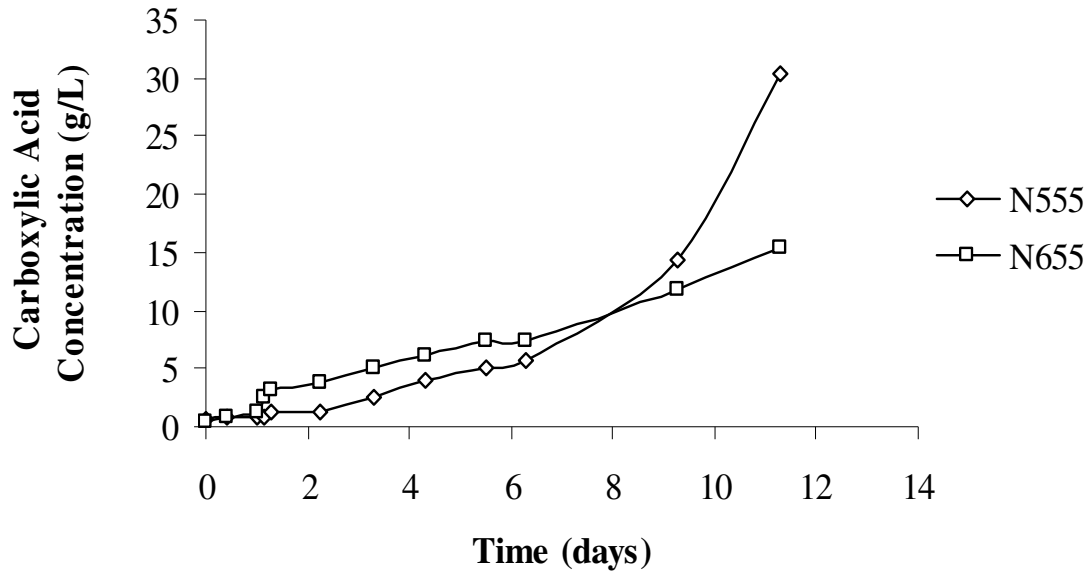

Figure 4-14. Total acid production at 55°C using hourly and stepwise buffer addition.

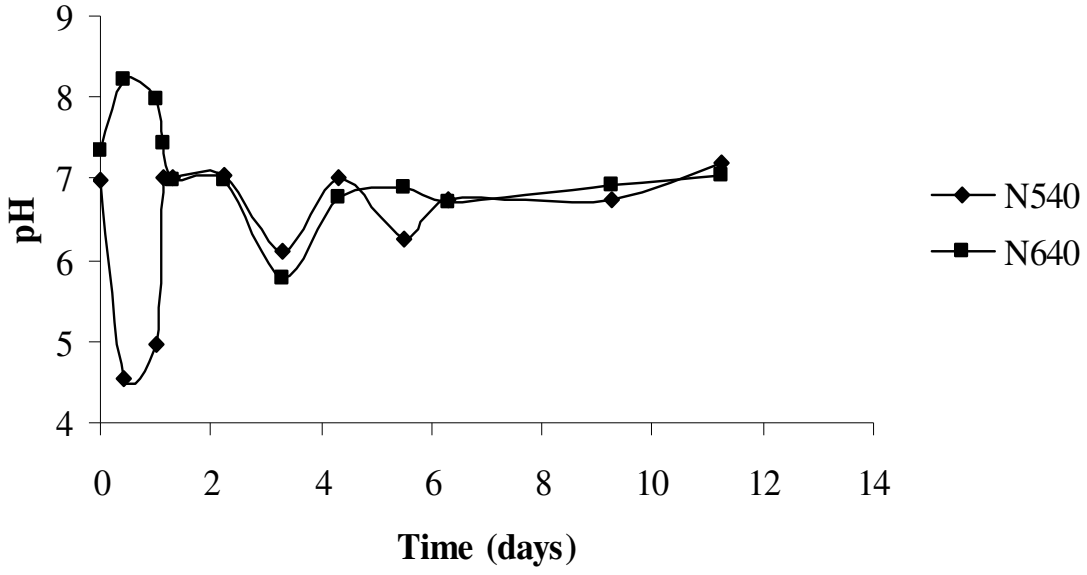

Figure 4-15. pH at 40°C using hourly and stepwise buffer addition.

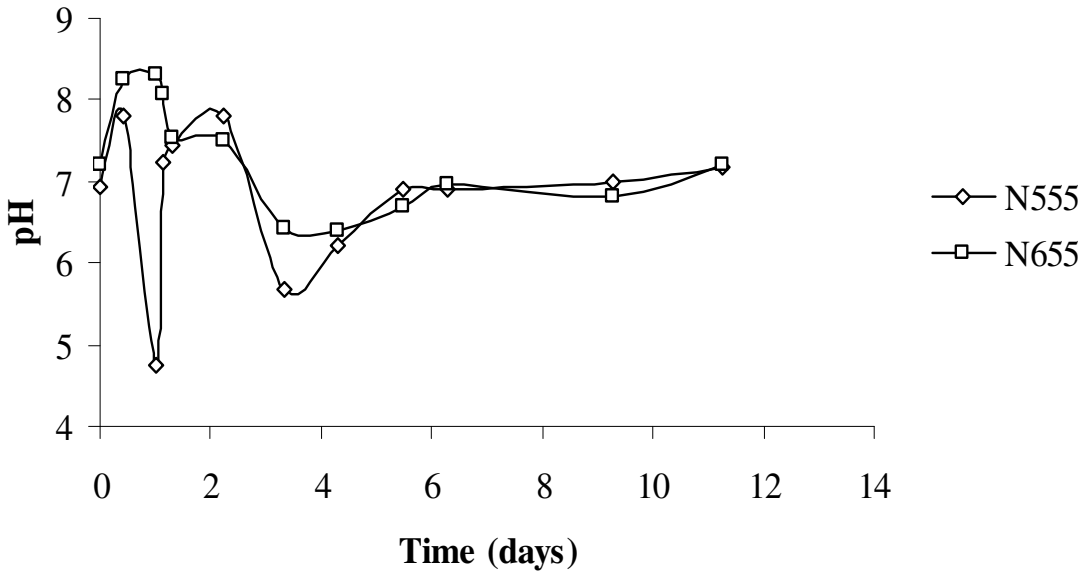

Figure 4-16. pH at 55°C using hourly and stepwise buffer addition.

4.5 Conclusion

The batch fermentations have proven that food scraps are a viable feedstock in the MixAlco process. Additionally, the following conclusions are made:

1) Food scraps are very reactive substrates and rapidly produce carboxylic acids thus lowering the pH. Constant pH control was necessary to maintain pH at neutrality.

2) Maintaining pH near neutrality for food scrap fermentation resulted in higher product concentrations of acetic acid. NH_4HCO_3 has proven to be an effective buffer and pH adjuster in batch fermentation.

3) Dry nutrients were not necessary for fermentation because food scraps contained the trace nutrients necessary for microorganism proliferation. This will have large economical impact on large-scale implementation.

4) Food scraps that are ground in a food processor to reduce particle size can be feed directly to fermentors.

CHAPTER V
CONTINUOUS FOOD SCRAP FERMENTATION

In 1-L fermentors with food scraps, pH could not be maintained at neutral, and reactors over-pressurized because of high CO_2 production. The result was minimal acid production. A new method was developed to maintain the pH in the optimal range (6.8 to 7.2) and to relieve gas pressure. Additionally, it was decided to scale-up from a 1-L centrifuge bottle to a 10-L fermentor. This design was named the Pre-Digestion Unit (PDU) (section 2.6).

5.1 Continuous Fermentation

Components of food scraps were readily digested in anaerobic fermentation as was observed in batch fermentation (Chapter IV). The PDU consisted of a continuous stirred tank reactor (CSTR) with constant pH control. The desired pH (6.8–7.2) was maintained by a 30% NH_4HCO_3 solution. The PDU was operated to convert mainly the most digestible portion of the food scraps. Although the acid concentration was high, the total conversion was low.

5.2 Fermentation Conditions

Food scraps (100 dry g/L) and anaerobic water (2.8 L) were added to the PDU. The initial pH was 4.8, so 13 g of dry NH_4HCO_3 was added to adjust the pH to 7. Inoculum (0.5 L) was added, and the fermentor was closed. The PDU initially operated in batch mode. After 9 days, the easily fermented carbohydrates were limited and acid concentration stabilized at 30 g/L. To provide a constant input of easily fermentable carbohydrates, the PDU was operated as a CSTR.

Fresh food scraps (40 dry g) were added and a slurry of partially digested food scraps (0.11 L) was removed daily. Additionally, a 6-mL sample was taken to be analyzed using gas chromatography. The PDU was purged with N_2 to maintain

anaerobic conditions. Detailed operation procedures for the PDU are in Appendices A and B.

Figure 5-1 shows that the acid concentration leveled to 100 g/L after 45 days. This steady-state concentration (±5 g/L average total acid concentration) was maintained for 27 days. The major products were acetic acid (82%), propionic acid (2%), and butyric acid (9%) as displayed in Figure 5-2. Steady-state data were used to determine yield, selectivity, and conversion (Table 5-1).

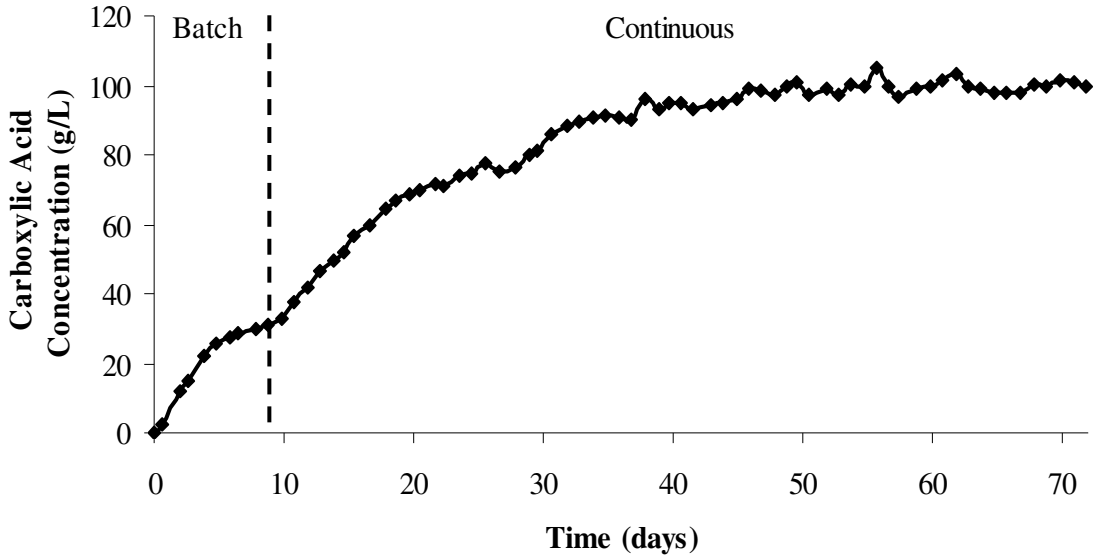

Figure 5-1. Total acid concentration from PDU at batch and continuous fermentation.

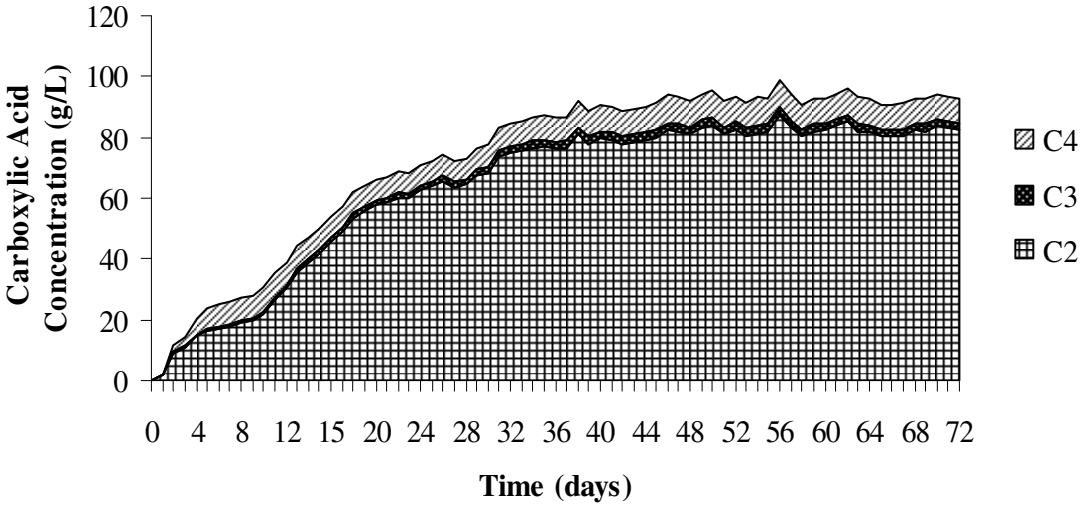

Figure 5-2. Acetic, propionic, and butyric acid produced in the PDU.

The pH was continuously adjusted to 7 by adding a 30% NH_4HCO_3 buffer solution. The average pH recorded during sampling was 6.8. Figure 5-3 shows pH data collected during fermentation. Acids were diluted by water contained in the buffer solution, and reactor volume was not held constant. The steady-state dosage of the buffer solution was 0.012 L buffer/(L reactor liquid·d).

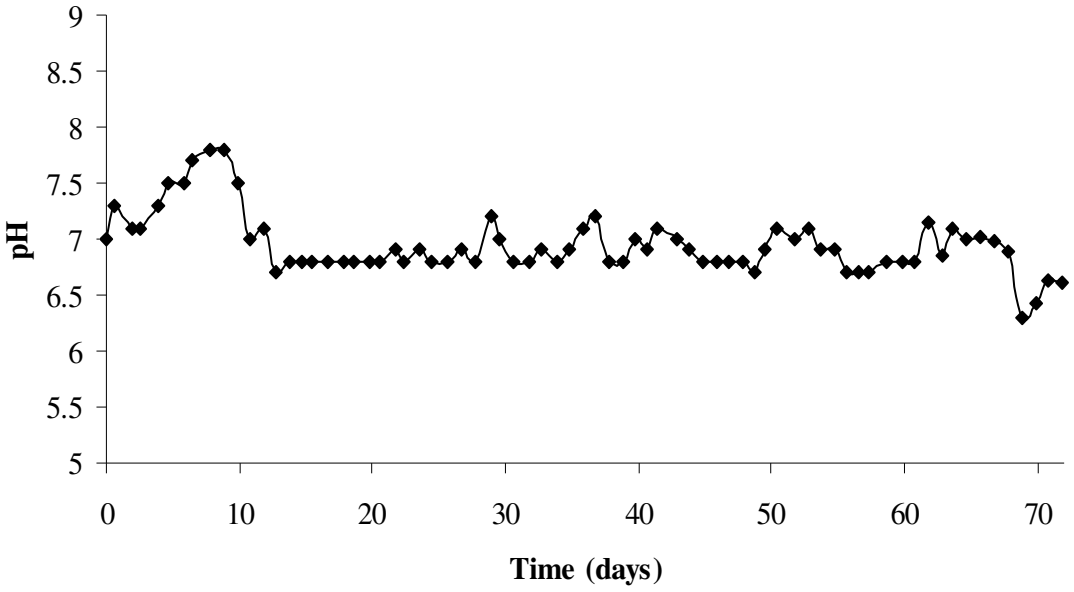

Figure 5-3. pH from PDU at batch and continuous fermentation.

Table 5-1. Fermentation results in the PDU

FERMENTATION IN PDU	
Total volatile solids fed (g/d)	40
Total liquid volume (L)	7.7
Temperature (°C)	40
Slurry output (L/d)	0.111
Frequency of transfers	daily
Average pH	6.8
Total acid productivity (g/(L·d))	1.39
Maximum acid concentration (g/L)	104
Steady-state acid concentration (g/L)	99.6
VS digested (g/d)	21.6
LRT (d)	69.4
VSLR (g VS/(L·d))	5.05
Yield (g total acids/g VS fed)	0.275
Selectivity (g total acids/g VS digested)	0.496
Conversion (g VS digested/g VS fed)	0.555

5.3 Conclusions

The experiment conducted in the PDU showed that food scraps have great potential as a feedstock in the MixAlco process. It also showed that when the pH is controlled, elevated acid production is obtained. The following conclusions are made:

1) The concept and design behind the PDU was effective and allowed high acid yields and effective pH control.

2) At a VSLR of 5.05 g VS/(L·d) and LRT of 69.4 days, 100 g/L of carboxylic acids was produced at steady state. The VSLR introduced large volumes of easily digestible material for microorganisms.

3) The NH_4HCO_3 buffer delivery solution and pH controller maintained pH in the desired range and allowed increased acid production.

4) The amount of acids produced required addition of buffer solution at a rate of 0.012 L buffer/(L reactor liquid·d). A significant amount of water was introduced using the 30% NH_4HCO_3 solution.

5) Homogeneous mixing was not achieved at 50 rpm with the current stirrer design. The agitation rate must allow homogeneous mixing without disrupting acid-forming microorganisms.

CHAPTER VI
BATCH FERMENTATION OF BAGASSE AND FOOD SCRAPS

Currently, the MixAlco process combines pretreated lignocellulosic material (e.g., bagasse) and a nutrient source (e.g., chicken manure) to form carboxylic acids via fermentation. Chicken manure has proven to be a productive and available nutrient source, it has toxic contaminants that prevent its use in MixAlco products that may enter the food or feed markets. Batch fermentation in 1-L rotary fermentors was conducted using various forms of food scraps as an alternative nutrient source.

6.1 Disadvantages of Chicken Manure

In previous laboratory studies, chicken manure (CM) has been the primary nutrient source in the fermentation to carboxylic acids. It has disadvantages in introduction to the large-scale production in the MixAlco process. To supply the necessary amount of CM for the MixAlco process, it must be collected from a confined animal feeding operation (CAFO). The poultry industry efficiently cultivates chickens by using antibiotics to maintain health and development. In large quantities, products such as arsenic, heavy metals, and dioxins found in antibiotics pose a potential threat to human health.

Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) and p-arsanilic acid are the most extensively used arsenic feed additives in the poultry industry. They are used to control coccidial intestinal parasites in poultry thereby improving feed efficiency (Momplaisir, 2001). Ingestion of arsenic by humans can contribute to cancers of skin, bladder, and lung. Additional health risks include gastrointestinal, neurological, dermal, hematological, cardiovascular, peripheral vascular, and immune system effects (Bates et al., 1992; Tsula et al., 1995).

In addition to arsenic, other heavy metals such as lead, mercury, cadmium, manganese, aluminum, chromium, copper, and zinc are used to prevent disease and increase feed efficiency (Jackson et al., 2003). Metals are notable for their wide

dispersion, and tendency to accumulate in tissue becoming toxic even at relatively minor levels of exposure (Hu, 2002). The toxicity of metals most commonly affects the brain and kidneys.

Dioxins are chlorinated aromatic compounds that can accumulate in fatty tissue and may increase the risk of tumors and other undesirable health effects. Chicken feed may become contaminated by ball clay, which naturally contains dioxins, when used as a desiccant to enhance flowability during processing (Hardin, 2001).

Arsenic, heavy metals, and dioxins limit the market for downstream products, including carboxylic salts, esters, aldehydes, amides, and ketones. Carboxylic salts used as animal feeds have the potential of containing trace amounts of these contaminants that may harm animals. Esters, which are used as flavor additives for human food, may also become contaminated from the use of manure as a nutrient source.

6.2 Alternatives

Various forms of food scraps can be used as an alternative to using chicken manure as a nutrient source in the MixAlco process. Fresh food scraps (FS) have proven to be a viable feedstock. In addition to fresh food scraps, continuous and batch fermentation conducted in the Pre-Digestion Unit (PDU) produced other potential nutrient sources.

Continuous fermentation in the PDU resulted in partially digested food scraps. This material was composed of oils and fats (OF), and carbohydrates and vegetables (CV). The materials can be separated because OF floated to the top whereas CV settled to the bottom of the fermentor. Centrifuging expedites this process, but in large-scale operation a settling vessel can achieve similar separation.

Digested food scraps from batch fermentation will have fewer readily fermentable carbohydrates, thus reducing the necessity of continuous pH control. The following experiments compare the feasibility of utilizing the various forms of food scraps as a nutrient source.

6.3 Fermentation Conditions

Experiments were conducted in six 1-L rotary fermentors to illustrate the effectiveness of various forms of food scraps as a nutrient source. An 80:20 ratio of lignocellulosic material to nutrient source was used in each fermentor. Therefore air-and-lime pretreated bagasse (32 g dry) was placed in six 1-L rotary fermentors. Various forms of nutrient source (8 g dry) were also placed in the fermentors to initiate growth and supplement nutrients. The various nutrient sources are described below:

Fermentor 1 (F1):

F1 contained fresh food scraps (FS) as a nutrient source. It has been shown that FS are a readily fermentable substrate (Chapter IV). Controlling the pH was difficult because of the large volume of fermentable carbohydrates. The goal was to utilize FS as a nutrient source to allow better pH control.

Fermentor 2 (F2):

Digested food scraps (DFS) obtained from short-term (14-d) batch fermentation in the PDU was used to determine if pH is more stable with reduced amounts of fermentable carbohydrates.

Fermentor 3 (F3):

F3 contained digested food scraps (DFS) obtained from continuous fermentation in the PDU at steady state. This material consisted of 40:60 mixture of OF and CV on a wet basis. The steady-state acid concentration in the PDU was 100 g/L. Products from the continuous fermentation have produced high acid yields, but low conversion. This product will show the usefulness of the solids from the steady-state discharge.

Fermentor 4 (F4):

Partially digested OF obtained from continuous fermentation in the PDU at steady state were separated to see the effectiveness as a nutrient source. Fats and oils have inhibitory effects on fermentation (Angelidaki and Ahiring, 1992; Hanaki et al., 1981).

Fermentor 5 (F5):

Partially digested CV were separated from the DFS obtained from continuous fermentation in the PDU at steady state. This material contained readily fermentable carbohydrates and was not inhibited by OF.

Fermentor 6 (F6):

Chicken manure was used as a control to compare the effectiveness of the various forms of food scraps.

Liquids in the fermentor (350 mL) consisted of deoxygenated water and moisture from the associated substrates. The pH was adjusted to 7 then 50 mL of marine inoculum was added to the fermentors. Each fermentor utilized NH_4HCO_3 buffer and operated at mesophilic conditions (40°C). A 3-mL liquid sample was taken periodically. Additionally, the pH of each reactor was measured and adjusted to the appropriate range (6.8 – 7.2) by adding of dry buffer. When fermentors were opened, a constant N_2 purge maintained anaerobic conditions.

6.4 Experimental Results

Figure 6-1 shows fermentation data for the batch fermentations with pretreated bagasse and various nutrient sources. F6 was the control and gave a base-line for fermentation results. It had a total acid concentration of 34.4 g/L after 40 days. F5 had a slightly higher total acid concentration of 34.5 g/L. F1 resulted in a total acid concentration of 31.6 g/L, 30.6 g/L in F3, 24.7 g/L in F4, and 20.2 g/L in F2. Detailed acid concentration data are presented in Appendix G.

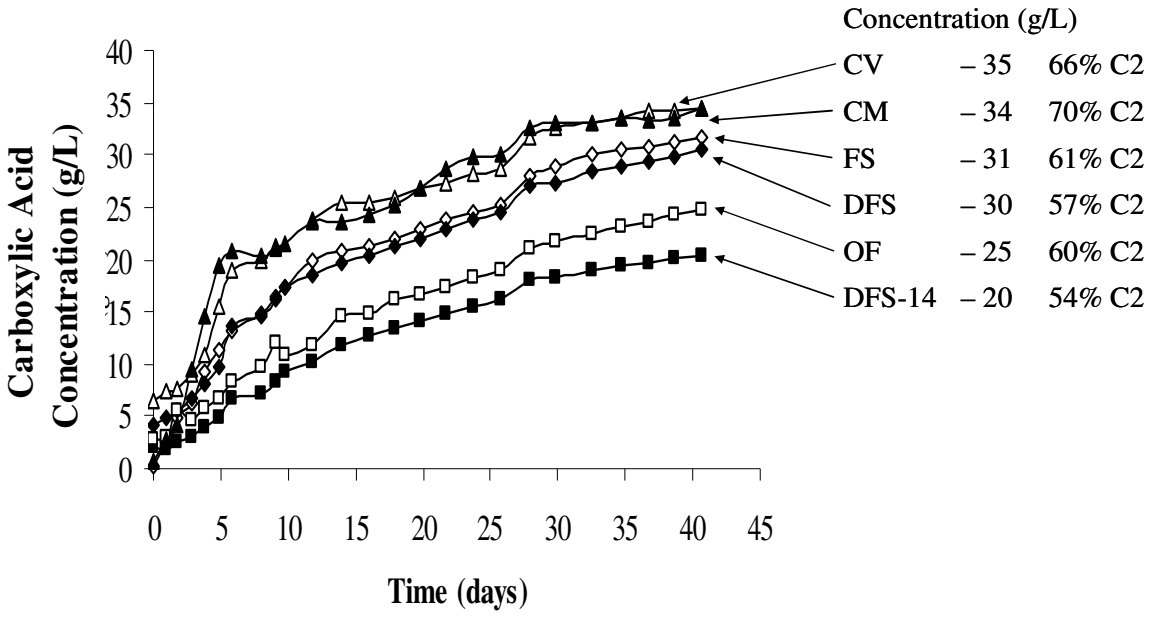

Figure 6-1. Total acid production for fermentors containing pretreated bagasse and various nutrient sources.

Maintaining neutral pH in the fermentors was one of the goals in this experiment. Initially, pH was adjusted daily and remained in the desired range (6.8 – 7.2). After 14 days, the pH stabilized and NH_4HCO_3 was added every 4 to 6 days for the duration of the experiment. The pH in the fermentors was graphed in Figures 6-2 to 6-4.

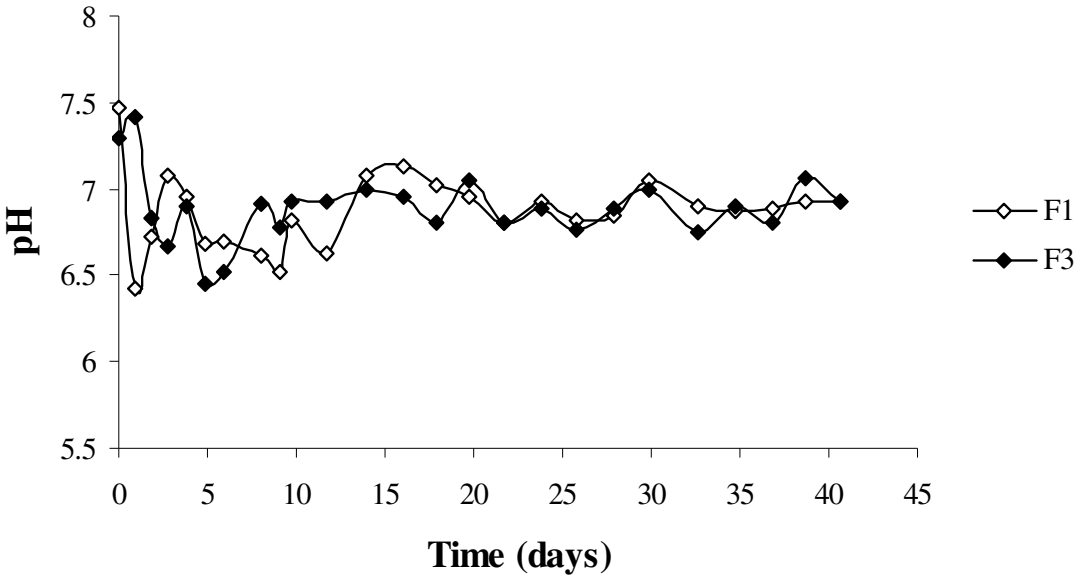

Figure 6-2. pH in fermentor containing fresh food scraps (F1) and digested food scraps obtained from continuous fermentation (F3).

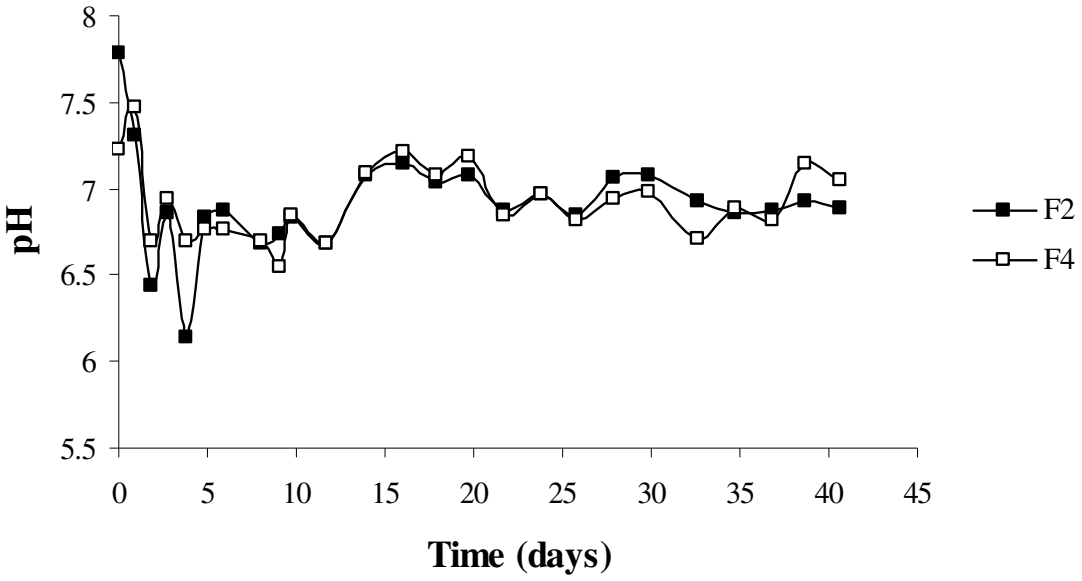

Figure 6-3. pH in fermentors containing digested food scraps from batch fermentation (F2), and partially digested oils and fats (F4).

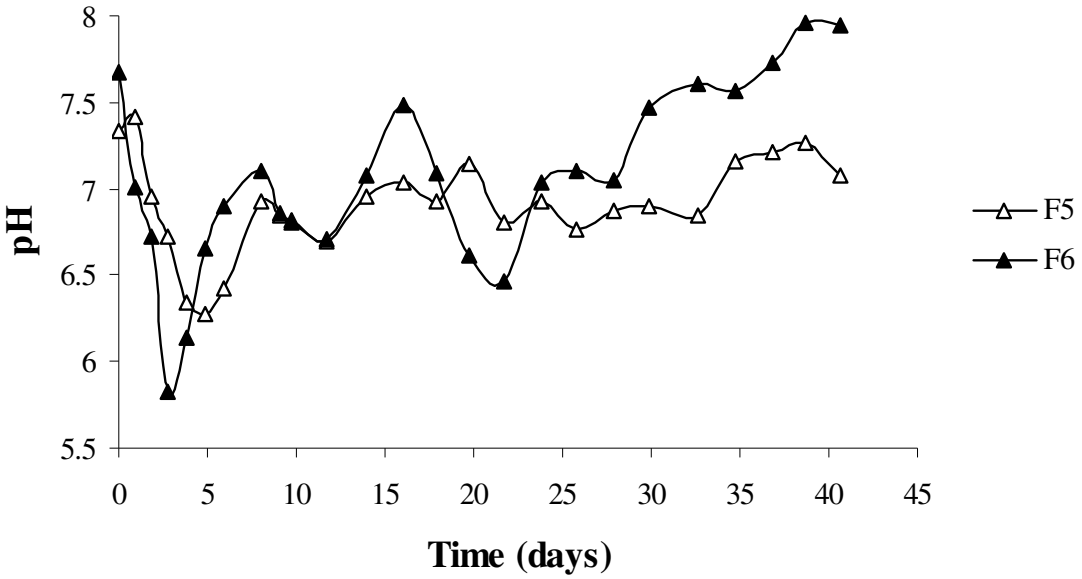

Figure 6-4. pH in fermentors containing partially digested carbohydrates and vegetables (F5), and chicken manure (F6).

6.5 Conclusions

The experiment tested air-and-lime pretreated bagasse with various nutrient sources mixed at 80% and 20% respectively. The following conclusions are made:

1) Fresh food scraps maintained pH in the desired range (6.8 to 7.2) when used as a nutrient source without continuous pH control. In previous experiments (Chapter IV), the pH dropped below 5.5 because of the abundance of fermentable carbohydrates. Acid production decreased, but a more stable reaction occurred compared to FS fermented alone. Fresh food scraps used as a nutrient source required less frequent addition of NH_4HCO_3.

2) DFS from 14-day batch fermentation resulted in the lowest acid concentration because the essential nutrients and the easily fermentable sugars were digested in the PDU.

3) DFS from continuous fermentation in the PDU had high acid yields because it retained the necessary components for use as a nutrient source. It was slowed by the OF, but did not require separation of CV and OF, which would have had associated energy demands.

4) Oils and fats slowed fermentation and resulted in lower acid yields when mixed with carbohydrates and vegetables.

5) Carbohydrates and vegetables had a similar fermentation profile as chicken manure. When separated from the continuous fermentation output, this product may be substituted for chicken manure.

CHAPTER VII
CONCLUSIONS

This work explored food scraps (FS) as a feedstock and a nutrient source in the MixAlco process. Preliminary experiments showed that FS are a very reactive substrate. FS rapidly produce carboxylic acids thus lowering the pH. Constant pH control was necessary to maintain a neutral pH. NH_4HCO_3 proved to be an effective buffer and pH controller.

A Pre-Digestion Unit (PDU) was developed to maintain neutral pH during FS fermentation. The design and concept behind the PDU allowed high acid concentration (100 g/L at steady state) and effective pH control (average 6.8). The PDU was operated as a continuous stirred tank reactor with a 5.05 g/(L·d) volatile solid loading rate (VSLR) and a 69.4-day liquid residence time. The conversion was 0.55 g VS digested/g VS fed and resulted in a considerable volume of undigested volatile solids.

Batch fermentation of various forms of partially digested FS from the PDU and pretreated bagasse was conducted to produce mixed carboxylic acids from the undigested volatile solids. The acid concentrations obtained ranged from 20.2 to 34.5 g/L. Partially digested FS can be used as a nutrient source and further digested to increase conversion obtained from the PDU.

The results obtained from this work lead to the idea of a PDU in series with a countercurrent fermentor. The PDU will be fed FS and operate with constant pH control. After achieving steady state, the output stream will be a mixture of high-concentration acids and low conversion-digested food scraps. The acids will be collected and the solids will advance to further fermentation. A pretreated lignocellulosic material such as bagasse will be mixed with the partially digested FS at an 80:20 ratio. The material will be fed in a countercurrent fermentor resulting in high solids conversion and carboxylic acid outputs.

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APPENDIX A
PDU OPERATION

PROCEDURE FOR PDU START-UP

1. Fill fermentor with 10 L of water and allow temperature to stabilize to 40°C. Check that mixing apparatus is functioning appropriately.
2. Seal the fermentor and check that all the components have an airtight seal.
3. Raise the 20% sodium chloride solution into the CO_2 recovery columns using the vacuum pump, then open valves.
4. Record the liquid level and allow it to stand for 20 minutes. If the level is the same, then there is a good seal. If not, there is a seal failure. Some typical leakage points are pH probe entry, buffer solution lid, CO_2 column connection, or gas sampling port.
5. Calibrate pH meter and buffer addition system using a separate handheld meter.
6. Drain out all fermentor contents and allow it to dry.
7. Add 100 g/L of food scraps to the fermentor. Then add deoxygenated water. Cover the fermentor lid with plastic wrap and flush with N_2.
8. Using a separate handheld pH meter with 0.01 accuracy, record fermentor pH. The value should match value displayed on the panel pH controller.
9. Add dry NH_4HCO_3 step-wise to the PDU to increase pH to 7.0. Allow ample time between additions to allow full dissolving and mixing.
10. Remove plastic wrap, flush the PDU with N_2 then mix in 0.5 L of marine inocula. Take a 6-mL sample, which will be the initial time.
11. Seal the PDU then open valves on CO_2 recovery columns.

PROCEDURE FOR PDU SHUT-DOWN

The fermentation broth will be used for further experimentation, and care must be taken to ensure that all products are collected and separated.

1. Collect five 1-L centrifuge bottles, remove lids, and then label each bottle. Record the weight of each bottle. This value will be used in volatile solid (VS) analysis.
2. Close valves on CO_2 recovery columns, then open the reactor.
3. Place fermentor drain tube in a 5-L flask, open discharge valve, and empty out all reactor contents.
4. Pour fermentation broth into the 1-L centrifuge bottles and seal.
5. Turn off master power supply.
6. Disconnect pH probe and CO_2 recovery lines, then remove reactor lid. Place aside to be cleaned.
7. Collect any additional solids that did not drain out of the PDU and place into the 1-L centrifuge bottles.
8. Centrifuge bottles for 25 minutes at 4000 rpm and 25°C. Set brake at 5 on the centrifuge.
9. After centrifuging, the bottles will have three distinct layers. Oils and fats (OF) will be on top, the liquid acid solution below and carbohydrates and vegetables on the bottom. Separate each component and then record the weight of each. These values will be used in VS analysis.
10. Thoroughly clean all components of the PDU, then reassemble.
11. Turn on master power and check that each system is functioning properly.
12. Next fill PDU with 10 L of DI water and allow it to mix for 10 minutes. Then drain water into a bucket and discard.
13. Repeat Step 12 four times to ensure that PDU is clean and uncontaminated.

APPENDIX B
SAMPLING AND MATERIAL HANDLING

Date: _____ Time: _____

Status Check:

Buffer sol level Measure Δz of buffer solution _____

Buffer Pump ☐ Mixer ☐ Water Temp (42°C) ☐

pH _____

CO_2 Recovery Measure Δz of $NaCl_2$ solution _____

Procedure:

1. Unscrew six screws with screwdriver
2. Label orange cap tube with name, date and time
3. Open valve for N_2 purge
4. Lift and slide over the reactor lid
5. Use a pipet to extract 6 mL of fermentation broth
6. Use graduated cylinder, funnel, and spoon to extract 111 mL of fermentation broth
7. Empty in the appropriate centrifuge bottle
8. Add fresh food scraps in the reactor (40 g dry)
9. Replace reactor top and tighten screws firmly
10. Purge for one additional minute
11. Close reactor opening then turn off gas valves
12. Place samples in the freezer
13. Rinse off equipment
14. Wipe off counter top

Notes:

_____

_____

_____

APPENDIX C
DEOXYGENATED WATER PREPARATION

Deoxygenated water containing cysteine hydrochloride and sodium sulfide was used in all fermentation experiments.

1. Pour 5 L of distilled water into a large glass container (6-L total volume).
2. Boil distilled water under a nitrogen purge for 5 min.
3. Seal lid with plastic wrap and cool the boiled water to room temperature.
4. Add 0.275 g/L cysteine hydrochloride and 0.275 g/L sodium sulfide.
5. Stir the solution and pour into storage bottles with a nitrogen purge. Be sure to fill the bottles completely and close the lid tightly.

APPENDIX D
CARBOXYLIC ACIDS ANALYSIS

For carboxylic acids analysis, at least 3 mL of liquid should be withdrawn from the fermentor and placed in a 15-mL conical bottom centrifuge tube. If not used immediately, the samples must be stored at -15°C. At the moment of the analysis, if the sample has been stored in the freezer, thaw and vortex the sample before beginning the procedure.

GC LIQUID SAMPLE PREPARATION

1. Centrifuge the liquid sample for 15 min at 3500 rpm.
2. Pipette 1 mL of the broth into a 15-mL round-bottom ultracentrifuge tube.
3. Add to the same tube, 1 mL of 10-mM of internal standard 4-methyl-valeric acid (1.162 g/L internal standard, ISTD).
4. Add to the same tube, 1 mL of 3-M phosphoric acid to acidify the sample and allow the carboxylic acids to be released in the GC injection port.
5. Cap the tube and vortex.
6. Centrifuge the mixture at 15,000 rpm in the IEC B-20A centrifuge (Industrial Equipment Co., Needham Hts., MA). Due to the poor refrigeration system in the centrifuge, simply accelerate the centrifuge to 15,000 rpm and immediately turn to zero rpm. (Be sure that the temperature is lower than 25°C before using).
7. Pipette1 mL of the centrifuge mixture into a glass GC vial and cap. The sample in the vial is ready to be analyzed. If the sample will not be analyzed immediately, it can be stored in the freezer. If frozen, care should be taken to thaw and vortex the sample before the GC analysis.

GC OPERATION

1. Before starting the GC, check the gas supply cylinders (compressed hydrogen, zero grade helium, and compressed zero-grade air from Praxair, Bryan, TX) to insure at least 100 psig pressure in each. If there is not enough gas, switch cylinders and place an order for new ones.
2. Establish gas flow by setting the regulators in 40 psig for hydrogen, 60 psig for helium, and 50 psig for air.
3. Check the solvent and waste bottles on the injection tower. Fill the solvent bottles with methanol, and be sure the waste bottles are empty.
4. Make sure the column head pressure gauge on the GC indicates the proper pressure (15 psig). Low head pressure usually indicates a worn-out septum. Replace the septum before starting the GC.
5. Up to 100 samples can be loaded in the auto sampler plate. Place the samples in the auto sample racks, not leaving empty spaces between samples. Place volatile acid standard mix (Matreya, Inc. #1075) solutions every 50 samples for calibration.
6. Check the setting conditions in the method:
    a. Oven temperature = 50°C
    b. Ramp = 20°C/min
    c. Inlet temperature = 230°C
    d. Detector temperature = 250°C
    e. H_2 flow = 40 mL/min
    f. He flow = 180 mL/min
    g. Air flow = 400 mL/min
7. Start the GC on the computer by selecting the method with the setting conditions above mentioned. Set and load the sequence of samples to run. Once the conditions are reached and the green start signal is on the screen, start the run sequence. Details about operation, setting sequence and calibration are in Agilent 6890 instrument manual.

8. Periodically check back to ensure that the equipment is working properly. Be sure to indicate the number of samples and any maintenance performed (changes of septum, gas cylinders, liner, etc.) in the GC logbook.
9. When finished running the sequence, turn the GC on standby and close air and hydrogen cylinder valves.

APPENDIX E
VOLATILE SOLIDS ANALYSIS

PROCEDURE FOR PRODUCT LIQUID

When approximately 900 mL of product liquid have been collected, take the collection bottle out of the freezer and leave the bottle to be thawed overnight. Sometimes, there is a small amount of solid particles in the collected product liquid that were inadvertently washed into the liquid collection bottle. To ensure an accurate measure, this amount of solids also needs to be analyzed for VS, so Steps 10–16 are needed.

1. Record the weight of the full collection (without cap).
2. Centrifuge the liquid collection bottle to separate any solids that might be in the liquid. Use the centrifuge for 20 min at 3500 rpm. When finished, decant all the supernatant liquid into a large clean empty container, being careful not to lose any solids from the bottles.
3. Record the weight of an empty 500-mL Erlenmeyer flask.
4. Add approximately 3 g $Ca(OH)_2$ to the empty container and record weight.
5. Add approximately 100 g of supernatant liquid to the container and record the weight. Mix well. Throw away the rest of the liquid.
6. Record the label and weight of a clean, dry, 150-mL crucible (Crucible A).
7. Pour, while mixing, approximately 70 g of the lime and liquid product mix into Crucible A. Record weight of the Crucible A + liquid mix.
8. Dry the crucible at 105°C for two days in the drying oven. Place the crucible in a vacuum dessicator and allow it to cool to room temperature before weighing. Record the weight of the crucible.
9. Ash the crucible at 575°C for at least 2 h. Remove the crucible from the ashing oven and place it in a vacuum dessicator and allow it to cool to room temperature. Record the ash weight of the crucible.
10. Record the weight of the collection bottle after pouring off all the liquid.

11. Record the label and weight of a clean, dry, 150-mL crucible (Crucible B).
12. Add approximately 3 g of $Ca(OH)_2$ to Crucible B and record weight.
13. Mix the remaining content of the liquid collection bottle, and pour carefully approximately 70 g into Crucible B. Mix well the lime and solids, and record the weight of the crucible.
14. Dry the crucible at 105°C as in Step 8.
15. Ash the crucible at 550°C as in Step 9.
16. Wash, dry and record the weight of the empty liquid collection bottle (without cap)

The amount of VS in the supernatant liquid is calculated as

$$\mathrm{VS}_{\mathrm{dissolved}}\ (\mathrm{g\ VS}) = \frac{(\mathrm{W8 - W9})}{\left(\frac{\mathrm{W7 - W6}}{\mathrm{W5 - W3}}\right)\times\left(\frac{\mathrm{W5 - W4}}{\mathrm{W1 - W10}}\right)}$$

$$\mathrm{VS}_{\mathrm{dissolved}}\ (\mathrm{g\ VS/(g \cdot d)}) = \frac{\dfrac{(\mathrm{W8 - W9})}{\left(\frac{\mathrm{W7 - W6}}{\mathrm{W5 - W3}}\right)\times\left(\frac{\mathrm{W5 - W4}}{\mathrm{W1 - W10}}\right)}}{\mathrm{collected\ time\ period}}$$

The amount of VS in the solid residue present in the liquid is calculated as

$$\mathrm{VS}_{\mathrm{solid\ residue}}\ (\mathrm{g\ VS}) = \frac{(\mathrm{W14 - W15})}{\left(\frac{\mathrm{W13 - W15}}{\mathrm{W10 - W16}}\right)}$$

In all the formulas, W*i* is the weight recorded in the i^{th} step.

PROCEDURE FOR SOLID RESIDUE

1. Record the weight of the full collection bottle (without cap).
2. Empty the solids into a clean empty container, and mix very well. Be careful not to lose any solids from the bottle.
3. Record the label and weight of a clean, dry, 150-mL crucible.
4. Remove a representative sample of approximately 10 g of solid product into the crucible, and record the weight of the crucible
5. Dry the crucible at 105°C for 2 days in the drying oven. Place the crucible in a vacuum dessicator and allow cooling to room temperature before weighing. Record the dry weight of the crucible.
6. Ash the crucible at 550°C for at least 2 h. Remove quickly the crucible from the ashing oven and place it in a vacuum dessicator and allow cooling to room temperature. Record the ash weight of the crucible.
7. Record the weight of the empty liquid collection bottle (without cap).

The amount of VS in the solid is calculated as

$$VS_{solid} = \frac{(W5 - W6)}{\left(\frac{W4 - W3}{W1 - W7}\right)}$$

The amount of VS in one gram of collected solid is calculated as

$$VS_{g\ solid}\ (\text{g VS/gsolids}) = \frac{(W5 - W6)}{(W4 - W3)}$$

Again, in all the formulas, W*i* represents the weight recorded in the i^{th} step.

APPENDIX F
DRY NUTRIENT MIXTURE

The formulation for the dry nutrients mix used in all fermentation experiments was recommended by Ross (1998). The components of the dry nutrients mixture are given in Table E-1.

Table E-1: Dry nutrients mixture

Component	Amount (g/100 g of mixture)
K_2HPO_4	16.3
KH_2PO_4	16.3
NH_2SO_4	16.3
NaCl	32.6
$MgSO_4$ $7H_2O$	6.8
$CaCl_2$ $2H_2O$	4.4
HEPES (N-2-Hydrocyethyl piperazine-N'-2 ethanesulfonate)	0.86
Hemin	0.71
Nicotinamide	0.71
p-Aminobenzoic acid	0.71
Ca-panyothenate	0.71
Folic acid	0.35
Pyrixodal	0.35
Riboflavin	0.35
Thiamin	0.35
Cyanocobalamin	0.14
Biotin	0.14
EDTA	0.35
$FeSO_4$ $7H_2O$	0.14
$MnCl_2$	0.14
H_3BO_3	0.021
$CoCl_2$	0.014
$ZnSO_4$ $7H_2O$	0.007
$NaMoO_4$ $7H_2O$	0.0021
$NiCl_2$	0.0014
$CuCl_2$	0.0007

APPENDIX G

TABLES 1 – 6

Table 1A: Fermentation data for C155

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	% C2	pH
0	0.6999	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6999	100.00	6.28
2	1.7372	0.0000	0.0000	0.0000	0.0000	0.1001	0.0000	0.0000	1.8374	94.55	4.52
4	1.9236	0.0000	0.0000	0.0000	0.0000	0.0714	0.0000	0.0000	1.9950	96.42	4.32
6	0.6071	0.0000	0.0000	9.7394	0.0731	0.0639	0.0000	0.0000	10.4835	5.79	6.53
8	1.7576	0.0808	0.0000	12.2013	0.1017	0.0000	0.0000	0.0000	14.1415	12.43	6.15
10	2.2008	0.1334	0.0000	14.6124	0.1357	0.0000	0.0000	0.0000	17.0822	12.88	6.49
										Average:	5.72

Table 1B: Fermentation data for C255

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	% C2	pH
0	2.4785	0.0000	0.0000	0.6492	0.0000	0.0000	0.0446	0.0000	3.1724	78.13	6.21
2	2.9023	0.0000	0.0000	0.6044	0.0000	0.1072	0.0000	0.0000	3.6138	80.31	4.65
4	3.1119	0.0000	0.0000	0.5995	0.0000	0.1061	0.0000	0.0000	3.8175	81.52	4.43
6	0.9448	0.0000	0.0000	11.6721	0.0000	0.0668	0.0000	0.0000	12.6837	7.45	6.23
8	2.2435	0.0000	0.0000	12.5177	0.0000	0.0000	0.0000	0.0000	14.7612	15.20	5.74
10	3.0761	0.0000	0.0000	13.6021	0.0000	0.0000	0.0000	0.0000	16.6782	18.44	5.79
										Average:	5.51

Table 1C: Fermentation data for N155

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	% C2	pH
0	0.7280	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7280	100.00	6.38
2	1.2203	0.0000	0.0000	0.0000	0.0000	0.1041	0.0000	0.0000	1.3243	92.14	4.16
4	0.7431	0.0000	0.0000	9.5398	0.0000	0.1125	0.0000	0.0000	10.3954	7.15	6.43
6	5.9962	0.1418	0.0904	11.9133	0.1185	0.0000	0.0000	0.0000	18.2601	32.84	6.35
8	8.0111	0.2117	0.1021	12.1749	0.1290	0.0000	0.0000	0.0000	20.6289	38.83	6.20
10	10.4879	0.2707	0.1239	13.3838	0.1735	0.0000	0.0000	0.0841	24.5239	42.77	7.18
12	11.6568	0.5227	0.0000	13.5606	0.2153	0.0000	0.0000	0.0000	25.9554	44.91	7.38
										Average:	6.30

Table 1D: Fermentation data for N255

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	% C2	pH
0	2.5550	0.0000	0.0000	0.6561	0.0000	0.0000	0.0452	0.0000	3.2563	78.46	6.27
2	3.0553	0.0000	0.0000	0.5934	0.0000	0.0985	0.0000	0.0000	3.7471	81.54	4.57
4	1.1885	0.0000	0.0000	9.6296	0.0000	0.1042	0.0458	0.0000	10.9681	10.84	5.67
6	2.2729	0.0000	0.0000	9.7560	0.0000	0.0000	0.0000	0.0000	12.0289	18.90	5.63
8	4.0419	0.0953	0.0000	11.6773	0.0526	0.0000	0.0000	0.0000	15.8672	25.47	6.04
10	6.9634	0.1447	0.0000	13.1740	0.0896	0.0000	0.0000	0.0000	20.3716	34.18	6.82
12	9.2236	0.2023	0.0000	13.7576	0.1194	0.0000	0.0000	0.0000	23.3028	39.58	7.04
										Average:	6.01

Table 2A: Fermentation data for C340

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	% C2	pH
0	0.7586	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7586	100.00	6.52
2	1.1645	0.0000	0.0000	0.0000	0.0000	0.0775	0.0000	0.0000	1.2420	93.76	4.70
4	1.5230	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.5230	100.00	5.03
6	2.9784	0.0855	0.0000	1.3094	0.0000	0.0000	0.0000	0.0000	4.3733	68.10	5.87
8	4.5026	0.1133	0.0000	2.1108	0.0623	0.0000	0.0000	0.0622	6.8512	65.72	5.80
10	5.2257	0.1262	0.0000	2.7021	0.0590	0.0000	0.0000	0.0000	8.1129	64.41	5.85
										Average:	5.63

Table 2B: Fermentation data for C355

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	% C2	pH
0	0.7843	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7843	100.00	6.51
2	0.9636	0.0000	0.0000	0.0000	0.0000	0.0810	0.0000	0.0000	1.0447	92.24	4.73
4	1.3775	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.3775	100.00	5.02
6	1.3185	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.3185	100.00	4.81
8	1.5989	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0503	1.6491	96.95	5.18
10	1.9452	0.0000	0.0000	0.0743	0.0000	0.0000	0.0000	0.0000	2.0195	96.32	5.26
										Average:	5.25

Table 2C: Fermentation data for N340

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	% C2	pH
0	0.7919	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7919	100.00	6.55
2	1.0607	0.0000	0.0000	0.0000	0.0000	0.0720	0.0000	0.0000	1.1327	93.64	3.88
4	1.4105	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.4105	100.00	4.60
6	2.1554	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2.1554	100.00	6.14
8	4.3476	0.1195	0.0000	1.0518	0.0000	0.0000	0.0000	0.0000	5.5189	78.78	6.90
10	6.9612	0.2793	0.0875	4.5215	0.2241	0.0000	0.0000	0.0863	12.1598	57.25	7.01
										Average:	5.85

Table 2D: Fermentation data for N355

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	% C2	pH
0	0.7538	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7538	100.00	6.63
2	0.8596	0.0000	0.0000	0.0000	0.0000	0.0757	0.0000	0.0000	0.9353	91.90	4.58
4	0.6756	0.0000	0.0000	4.2393	0.0000	0.0852	0.0000	0.0000	5.0000	13.51	5.13
6	1.8098	0.0000	0.0000	4.2678	0.0000	0.0000	0.0000	0.0000	6.0776	29.78	5.38
8	4.6884	0.0737	0.0000	5.2998	0.0000	0.0000	0.0000	0.0000	10.0619	46.60	7.09
10	10.5601	0.7258	0.2685	7.9634	0.6583	0.0000	0.0000	0.0600	20.2362	52.18	7.51
										Average:	6.05

Table 3A: Fermentation data for C440

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	%C2	pH
0.00	0.3542	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3542	100.0	7.20
1.17	1.1670	0.0000	0.0000	0.0000	0.0000	0.0930	0.0000	0.0000	1.2600	92.6	5.12
1.58	1.5393	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.5393	100.0	5.38
2.17	2.3151	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2.3151	100.0	5.19
3.54	4.7187	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	4.7187	100.0	4.97
4.13	5.3668	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	5.3668	100.0	4.96
5.04	6.0195	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	6.0195	100.0	5.11
6.04	6.4504	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	6.4504	100.0	5.28
6.38	7.9097	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	7.9097	100.0	5.17
7.21	8.4404	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	8.4404	100.0	5.02
8.25	8.7287	0.0000	0.0000	0.0000	0.0000	0.0875	0.0000	0.0000	8.8162	99.0	5.16
9.08	8.6980	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	8.6980	100.0	5.22
10.08	9.2340	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	9.2340	100.0	5.15
11.08	9.8544	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	9.8544	100.0	5.29
12.17	10.9140	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	10.9140	100.0	5.21
13.21	11.5255	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	11.5255	100.0	5.21
14.35	11.6045	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	11.6045	100.0	5.41
15.15	12.0255	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	12.0255	100.0	5.22
16.13	12.7179	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	12.7179	100.0	5.23
17.13	13.2229	0.0000	0.0000	0.0629	0.0000	0.0000	0.0000	0.0000	13.2858	99.5	5.20

Table 3B: Fermentation data for C455

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	%C2	pH
0.00	0.3271	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3271	100.0	7.16
1.17	0.7880	0.0000	0.0000	0.0000	0.0000	0.0878	0.0000	0.0000	0.8757	90.0	4.96
1.58	1.0970	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0970	100.0	5.27
2.17	1.3510	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.3510	100.0	5.24
3.54	1.9944	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.9944	100.0	5.10
4.13	2.2081	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2.2081	100.0	4.93
5.04	2.4155	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2.4155	100.0	5.16
6.04	2.6308	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2.6308	100.0	5.31
6.38	3.0693	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	3.0693	100.0	5.23
7.21	4.1096	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	4.1096	100.0	5.07
8.25	3.3342	0.0000	0.0000	0.0656	0.0000	0.0000	0.0000	0.0000	3.3998	98.1	5.20
9.08	3.3805	0.0000	0.0000	0.0709	0.0000	0.0000	0.0000	0.0000	3.4514	97.9	5.38
10.08	3.5130	0.0000	0.0000	0.0853	0.0000	0.0000	0.0000	0.0000	3.5983	97.6	5.22
11.08	3.6671	0.0000	0.0000	0.0966	0.0000	0.0000	0.0000	0.0000	3.7637	97.4	5.45
12.17	3.9547	0.0000	0.0000	0.1121	0.0000	0.0000	0.0000	0.0000	4.0668	97.2	5.40
13.21	4.2015	0.0000	0.0000	0.1254	0.0000	0.0000	0.0000	0.0000	4.3268	97.1	5.45
14.35	4.0807	0.0000	0.0000	0.1201	0.0000	0.0000	0.0000	0.0000	4.2008	97.1	5.59
15.15	4.1121	0.0000	0.0000	0.1185	0.0000	0.0000	0.0000	0.0000	4.2306	97.2	5.47
16.13	4.2138	0.0000	0.0000	0.1203	0.0000	0.0000	0.0000	0.0000	4.3341	97.2	5.30
17.13	4.3310	0.0000	0.0000	0.1224	0.0000	0.0000	0.0000	0.0000	4.4534	97.3	5.40

Table 3C: Fermentation data for N440

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	%C2	pH
0.00	0.3534	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3534	100.0	7.28
1.17	1.5960	0.0000	0.0000	0.0000	0.0000	0.0880	0.0000	0.0000	1.6840	94.8	7.28
1.58	1.6756	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.6756	100.0	7.60
2.17	2.2835	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2.2835	100.0	7.50
3.54	2.9823	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2.9823	100.0	6.83
4.13	3.2700	0.0000	0.0000	0.0818	0.0000	0.0000	0.0000	0.0000	3.3519	97.6	6.52
5.04	4.8352	0.0972	0.0000	0.1559	0.0000	0.0000	0.0000	0.0000	5.0883	95.0	7.27
6.04	4.7262	0.1109	0.0000	0.1935	0.0000	0.0000	0.0000	0.3310	5.3616	88.1	7.54
6.38	8.4085	0.2481	0.0000	1.4684	0.0000	0.0000	0.0000	0.0000	10.1250	83.0	7.20
7.21	8.9196	0.2451	0.0000	1.4396	0.0000	0.0000	0.0000	0.0000	10.6043	84.1	6.94
8.25	8.9318	0.2468	0.0000	1.4378	0.0000	0.0000	0.0000	0.0000	10.6164	84.1	6.45
9.08	9.2738	0.2577	0.0000	1.5113	0.0000	0.0000	0.0000	0.0000	11.0429	84.0	6.75
10.08	9.8785	0.2600	0.0000	1.6094	0.0591	0.0000	0.0528	0.0000	11.8598	83.3	6.71
11.08	12.5255	0.3401	0.0000	1.9687	0.0671	0.0000	0.0560	0.0000	14.9574	83.7	6.71
12.17	10.4112	0.2638	0.0000	1.7066	0.0637	0.0000	0.0589	0.0000	12.5043	83.3	6.68
13.21	10.5594	0.2665	0.0000	1.7549	0.0783	0.0000	0.0600	0.0000	12.7192	83.0	6.83
14.35	10.6154	0.2666	0.0000	1.8184	0.0908	0.0000	0.0612	0.0000	12.8524	82.6	6.96
15.15	10.7975	0.2699	0.0000	1.8710	0.0909	0.0000	0.0631	0.0000	13.0924	82.5	6.93
16.13	10.7814	0.2720	0.0000	2.0551	0.0738	0.0000	0.0649	0.0000	13.2472	81.4	6.86
17.13	11.0357	0.2771	0.0000	2.2275	0.0721	0.0000	0.0701	0.0000	13.6824	80.7	7.22

Table 3D: Fermentation data for N455

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	%C2	pH
0.00	0.3742	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3742	100.0	7.28
1.17	0.8391	0.0000	0.0000	0.0000	0.0000	0.0919	0.0000	0.0000	0.9311	90.1	7.00
1.58	1.4775	0.0000	0.0000	0.0000	0.0000	0.0529	0.0000	0.0000	1.5304	96.5	6.61
2.17	2.8192	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2.8192	100.0	7.49
3.54	4.0105	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	4.0105	100.0	7.43
4.13	4.2656	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	4.2656	100.0	7.10
5.04	4.6197	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	4.6197	100.0	7.45
6.04	4.8598	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	4.8598	100.0	7.50
6.38	5.6195	0.0000	0.0000	0.0735	0.0000	0.0000	0.0000	0.0000	5.6930	98.7	7.57
7.21	5.9175	0.0000	0.0000	0.1126	0.0000	0.0000	0.0000	0.0000	6.0301	98.1	7.79
8.25	6.2371	0.0000	0.0000	0.1564	0.0000	0.0000	0.0000	0.0000	6.3935	97.6	7.35
9.08	6.2296	0.0000	0.0000	0.1663	0.0000	0.0000	0.0000	0.0000	6.3959	97.4	7.65
10.08	6.5830	0.0000	0.0000	0.1971	0.0000	0.0000	0.0000	0.0000	6.7801	97.1	7.56
11.08	6.9081	0.0000	0.0000	0.2261	0.0000	0.0000	0.0000	0.0000	7.1342	96.8	7.31
12.17	7.2154	0.0000	0.0000	0.2542	0.0000	0.0000	0.0000	0.0000	7.4696	96.6	7.24
13.21	7.4101	0.0000	0.0000	0.2806	0.0000	0.0000	0.0000	0.0000	7.6907	96.4	7.44
14.35	7.5139	0.0000	0.0000	0.3043	0.0000	0.0000	0.0000	0.0000	7.8182	96.1	7.52
15.15	7.6105	0.0000	0.0000	0.3201	0.0000	0.0000	0.0000	0.0000	7.9306	96.0	7.22
16.13	7.8572	0.0797	0.0000	0.3634	0.0000	0.0000	0.0000	0.0000	8.3003	94.7	6.75
17.13	8.1559	0.0852	0.0000	0.4034	0.0000	0.0000	0.0000	0.0000	8.6445	94.3	7.19

Table 4A: Fermentation data for N540

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	%C2	pH
0.00	0.5718	0.0856	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6574	87.0	6.98
0.42	1.0432	0.0944	0.0000	0.0000	0.0000	0.1679	0.0000	0.0000	1.3055	79.9	4.53
1.00	1.4718	0.0881	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.5599	94.4	4.97
1.13	2.1211	0.0897	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2.2108	95.9	7.01
1.29	2.7006	0.0990	0.0000	0.0000	0.0000	0.1255	0.0000	0.0000	2.9251	923	7
2.25	3.0150	0.0969	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	3.1118	969	7.04
3.31	3.6586	0.1146	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	3.7733	97.0	6.1
4.31	4.0806	0.1221	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	4.2027	97.1	7.02
5.50	5.5804	0.1332	0.0000	0.1018	0.0000	0.0000	0.0000	0.0000	5.8154	96.0	6.27
6.29	6.5932	0.1452	0.0000	0.1719	0.0000	0.0000	0.0000	0.0000	6.9102	95.4	6.73
9.27	7.7243	0.1954	0.0000	3.7502	0.0605	0.0000	0.0000	0.0000	11.7305	65.8	6.75
11.27	8.3731	0.2882	0.0000	7.9194	0.1684	0.0000	0.0000	0.0000	16.7491	50.0	7.19

Table 4C: Fermentation data for N555

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	%C2	pH
0.00	0.5873	0.0801	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6674	88.0	6.92
0.42	0.7148	0.0840	0.0000	0.0000	0.0000	0.1446	0.0000	0.0000	0.9434	75.8	7.81
1.00	0.6748	0.0000	0.0000	0.0000	0.0000	0.1226	0.0000	0.0000	0.7975	84.6	4.75
1.13	0.7511	0.0745	0.0000	0.0000	0.0000	0.1220	0.0000	0.0000	0.9475	79.3	7.24
1.29	0.9708	0.0907	0.0000	0.0000	0.0000	0.2166	0.0000	0.0000	1.2781	76.0	7.43
2.25	1.0680	0.0772	0.0000	0.0000	0.0000	0.1204	0.0000	0.0000	1.2656	84.4	7.8
3.31	2.5631	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	2.5631	100.0	5.69
4.31	3.9853	0.0000	0.0000	0.0000	0.0000	0.0600	0.0000	0.0000	4.0453	98.5	6.23
5.50	5.0524	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	5.0524	100.0	6.89
6.29	5.6110	0.0000	0.0000	0.0915	0.0000	0.0000	0.0000	0.0000	5.7024	98.4	6.9
9.27	9.4969	0.1478	0.0000	4.6197	0.0000	0.0000	0.0000	0.0000	14.2645	66.6	6.99
11.27	15.0945	1.0867	0.3426	12.7128	1.0721	0.0000	0.0000	0.0000	30.3088	49.8	7.17

Table 4B: Fermentation data for N640

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	%C2	pH
0.00	0.5791	0.0814	0.0000	0.0000	0.0000	0.0961	0.0000	0.0000	0.7565	76.5	7.33
0.42	0.5291	0.0000	0.0000	0.0000	0.0000	0.2209	0.0000	0.0000	0.7500	70.6	8.21
1.00	1.5310	0.0000	0.0000	0.0000	0.0000	0.0800	0.0000	0.0000	1.6110	95.0	7.97
1.13	2.8160	0.0000	0.0000	0.0000	0.0000	0.1368	0.0000	0.0000	2.9528	95.4	7.44
1.29	4.7275	0.0000	0.0000	0.0000	0.0000	0.1031	0.0000	0.0000	4.8306	97.9	6.97
2.25	4.9161	0.0000	0.0000	0.0000	0.0000	0.1136	0.0000	0.0000	5.0297	97.7	6.99
3.31	5.4828	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	5.4828	100.0	5.77
4.31	5.8598	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	5.8598	100.0	6.77
5.50	6.8991	0.0000	0.0000	0.4138	0.0000	0.0000	0.0000	0.0000	7.3129	94.3	6.9
6.29	7.3615	0.0000	0.0000	0.5088	0.0000	0.0000	0.0000	0.0000	7.8703	93.5	6.71
9.27	7.6363	0.2126	0.0000	3.6759	0.0000	0.0000	0.0000	0.0000	11.5247	66.3	6.91
11.27	8.3367	0.4178	0.0662	6.5136	0.1978	0.0000	0.0515	0.0000	15.5836	53.5	7.05

Table 4D: Fermentation data for N655

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	TOTAL (g/L)	%C2	pH
0.00	0.5023	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5023	100.0	7.2
0.42	0.6257	0.0807	0.0000	0.0000	0.0000	0.1259	0.0000	0.0000	0.8323	75.2	8.26
1.00	1.0267	0.0815	0.0000	0.0000	0.0000	0.1770	0.0000	0.0000	1.2851	79.9	8.31
1.13	2.2838	0.0000	0.0000	0.0000	0.0000	0.1558	0.0000	0.0000	2.4396	93.6	8.06
1.29	2.9173	0.0000	0.0000	0.0000	0.0000	0.2035	0.0000	0.0000	3.1208	93.5	7.52
2.25	3.7610	0.0000	0.0000	0.0000	0.0000	0.1301	0.0000	0.0000	3.8911	96.7	7.51
3.31	5.0727	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	5.0727	100.0	6.43
4.31	6.0775	0.0000	0.0000	0.0000	0.0000	0.0745	0.0000	0.0000	6.1520	98.8	6.39
5.50	7.4092	0.0000	0.0000	0.0000	0.0000	0.0596	0.0000	0.0000	7.4688	99.2	6.69
6.29	7.3573	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	7.3573	100.0	6.96
9.27	9.2720	0.1325	0.0000	2.4401	0.0000	0.0000	0.0000	0.0000	11.8446	78.3	6.81
11.27	10.2426	0.1890	0.0000	4.8841	0.0000	0.0000	0.0000	0.0000	15.3157	66.9	7.2

Table 5: Fermentation Data for PDU

Time (day)	C2 (g/L)	C3 (g/L)	IC4 (g/L)	C4 (g/L)	IC5 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)	% C2
0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00
0.65	2.2075	0.0000	0.0000	0.1281	0.0000	0.0000	0.0000	0.0000	2.3356	94.51
1.94	9.1545	0.1375	0.2366	2.1416	0.4698	0.0000	0.0000	0.0000	12.1400	75.41
2.56	11.1605	0.1582	0.2744	2.9809	0.5533	0.0000	0.0000	0.0000	15.1272	73.78
3.88	14.7356	0.3084	0.4539	5.3585	1.0063	0.0000	0.0000	0.0000	21.8626	67.40
4.72	16.3946	0.4819	0.5314	6.7997	1.1951	0.0000	0.0000	0.0000	25.4027	64.54
5.81	17.3258	0.6919	0.5717	7.4541	1.2869	0.0000	0.0000	0.0000	27.3304	63.39
6.50	17.8833	0.7875	0.5889	7.5522	1.3195	0.0000	0.0000	0.4600	28.5914	62.55
7.81	18.8807	0.8884	0.6183	7.7387	1.3954	0.0000	0.0000	0.5053	30.0268	62.88
8.81	19.4708	0.9243	0.6348	7.8475	1.4239	0.0000	0.0000	0.5614	30.8628	63.09
9.89	21.5743	0.9760	0.6766	7.7920	1.5274	0.0000	0.0000	0.0000	32.5463	66.29
10.83	26.3019	1.0500	0.7056	7.9259	1.5888	0.0000	0.0000	0.0000	37.5722	70.00
11.83	30.4595	1.0983	0.7075	7.5927	1.6241	0.0000	0.0000	0.5537	42.0357	72.46
12.76	35.3419	1.1770	0.7273	7.5094	1.6368	0.0000	0.0000	0.0468	46.4391	76.10
13.79	38.7793	1.2258	0.7286	7.2655	1.6403	0.0000	0.0000	0.0586	49.6981	78.03
14.64	41.4647	1.2488	0.7184	6.9777	1.6069	0.0000	0.0000	0.0729	52.0895	79.60
15.46	45.8650	1.3354	0.7416	6.9848	1.6484	0.0000	0.0000	0.0839	56.6591	80.95
16.68	49.0476	1.4023	0.7508	6.7671	1.6663	0.0000	0.0491	0.0965	59.7797	82.05
17.87	53.4352	1.5138	0.7853	6.9395	1.7491	0.0000	0.0651	0.1226	64.6105	82.70
18.63	55.8883	1.5657	0.8164	6.7960	1.7785	0.0000	0.0000	0.1500	66.9949	83.42
19.74	57.6700	1.5532	0.8076	6.5904	1.7383	0.0000	0.0000	0.1671	68.5266	84.16
20.51	58.4105	1.6417	0.8249	6.8564	1.8245	0.0000	0.0000	0.1860	69.7439	83.75
21.72	60.1764	1.6848	0.8464	6.7620	1.8792	0.0000	0.0824	0.2174	71.6486	83.99
22.31	59.9762	1.6824	0.8342	6.5986	1.8470	0.0000	0.0838	0.2446	71.2668	84.16
23.60	62.5640	1.7945	0.8838	6.5795	1.8724	0.0000	0.0930	0.2687	74.0558	84.48
24.43	63.7870	1.8378	0.0000	6.8095	1.9285	0.0000	0.0000	0.3038	74.6665	85.43
25.60	65.6492	1.8984	0.9047	6.8648	1.9868	0.0000	0.0000	0.3511	77.6551	84.54
26.64	63.3772	1.8920	0.8696	6.7117	1.9208	0.0000	0.1038	0.3882	75.2633	84.21
27.79	64.4844	1.9528	0.8837	6.8471	1.9511	0.0000	0.1132	0.4176	76.6499	84.13
28.91	67.3457	2.0373	0.9166	7.1748	2.0221	0.0000	0.1173	0.4890	80.1028	84.07
29.54	68.3740	2.0508	0.9183	7.2724	2.0178	0.0000	0.1141	0.5732	81.3205	84.08

30.64	73.3623	2.1688	0.0000	7.6596	2.1019	0.0000	0.0000	0.6707	85.9633	85.34
31.83	74.7507	2.2713	1.0093	7.6509	2.1425	0.0000	0.0000	0.7449	88.5697	84.40
32.69	75.6004	2.2189	0.9736	7.6750	2.1163	0.0000	0.0000	0.8504	89.4346	84.53
33.85	76.6498	2.3043	1.0171	7.8622	2.1554	0.0000	0.0000	0.9325	90.9213	84.30
34.78	77.0129	2.3270	1.0201	7.8882	2.1744	0.0000	0.0000	0.9886	91.4112	84.25
35.88	76.3327	2.2197	0.9814	7.7395	2.1367	0.0000	0.0000	1.0965	90.5065	84.34
36.75	76.5230	2.2553	0.0000	7.9979	2.1603	0.0000	0.0000	1.1889	90.1255	84.91
37.82	80.9174	2.3723	1.0260	8.4309	2.2733	0.0000	0.0000	1.3152	96.3351	84.00
38.88	77.9581	2.3315	0.9964	8.3263	2.1925	0.0000	0.0000	1.3612	93.1661	83.68
39.69	79.5678	2.3695	1.0157	8.5130	2.2436	0.0000	0.0000	1.4558	95.1654	83.61
40.60	79.2174	2.3772	1.0036	8.5311	2.2148	0.0000	0.0000	1.6127	94.9568	83.42
41.47	78.0494	2.3123	0.9857	8.3369	2.1635	0.0000	0.0000	1.5667	93.4144	83.55
42.85	78.6857	2.3679	0.9905	8.3650	2.1984	0.0000	0.0000	1.5954	94.2029	83.53
43.87	79.2698	2.4798	1.0563	8.5690	2.2106	0.0000	0.0000	1.6009	95.1863	83.28
44.87	79.9827	2.5621	1.0886	8.7634	2.3151	0.0000	0.0000	1.6275	96.3393	83.02
45.88	82.1758	2.5635	1.0838	9.1121	2.3868	0.0000	0.0000	1.7270	99.0491	82.96
46.78	82.0769	2.6122	1.1165	8.7619	2.3526	0.0000	0.0000	1.8692	98.7893	83.08
47.81	80.9070	2.4577	1.0498	8.5552	2.2947	0.0000	0.0000	1.9606	97.2250	83.22
48.76	83.0906	2.5049	1.0649	8.7284	2.3268	0.0000	0.0000	2.0970	99.8126	83.25
49.51	84.0715	2.5219	1.0631	8.7740	2.3017	0.0000	0.0000	2.1773	100.9095	83.31
50.42	80.8164	2.4952	1.0420	8.5768	2.2495	0.0000	0.0000	2.2206	97.4004	82.97
51.78	82.4563	2.4438	1.0253	8.5095	2.2372	0.0000	0.0000	2.2852	98.9573	83.33
52.78	80.6046	2.3925	1.0085	8.4422	2.2250	0.0000	0.0000	2.3497	97.0225	83.08
53.72	81.2172	2.7145	1.1371	9.5981	2.5085	0.0000	0.2556	2.6950	100.1260	81.11
54.78	81.8298	2.5063	1.0648	8.6055	2.2707	0.0000	0.0000	3.2788	99.5560	82.19
55.65	87.5264	2.5618	0.0000	8.9162	2.3597	0.0000	0.0000	3.6116	104.9757	83.38
56.58	82.9581	2.4126	1.0367	8.6607	2.2734	0.0000	0.0000	2.6127	99.9542	83.00
57.36	80.2799	2.3036	0.9926	8.2841	2.1868	0.0000	0.0000	2.6126	96.6597	83.05
58.69	81.8802	2.3270	1.0154	8.3805	2.2368	0.0000	0.0000	3.5197	99.3597	82.41
59.83	82.2048	2.3608	1.0172	8.4727	2.2435	0.0000	0.0000	3.4681	99.7671	82.40
60.79	83.5669	2.3508	1.0290	8.4814	2.2833	0.0000	0.0000	3.6612	101.3726	82.44
61.79	85.2246	2.3790	1.0421	8.5303	2.3137	0.0000	0.0000	3.8097	103.2994	82.50
62.79	82.0849	2.4008	1.0442	8.7848	2.3651	0.0000	0.0000	3.0603	99.7401	82.30
63.63	81.7177	2.3192	1.0122	8.4558	2.2617	0.0000	0.0000	3.0441	98.8108	82.70

64.71	80.3746	2.3049	1.0034	8.2770	2.2776	0.0000	0.0000	3.7830	98.0204	82.00
65.68	80.3925	2.3019	1.0057	8.3256	2.2785	0.0000	0.0000	3.7409	98.0450	82.00
66.82	80.4104	2.2988	1.0080	8.3741	2.2795	0.0000	0.0000	3.6988	98.0697	81.99
67.81	82.1834	2.2874	1.0008	8.3517	2.2440	0.0000	0.0000	3.9453	100.0127	82.17
68.79	82.0254	2.2565	0.9854	8.2311	2.1980	0.0000	0.0000	3.9754	99.6718	82.30
69.88	83.6251	2.2965	0.9969	8.4193	2.2494	0.0000	0.0000	3.9886	101.5757	82.33
70.85	83.1241	2.2504	0.9744	8.2852	2.1874	0.0000	0.0000	4.0720	100.8935	82.39
71.83	82.3583	2.1760	0.9445	8.0501	2.1292	0.0000	0.0000	4.1213	99.7794	82.54

Table 6A: Fermentation data for Bagasse and Food Scraps (F1)

Time days	C2 g/L	C3 g/L	IC4 g/L	C4 g/L	IC5 g/L	C5 g/L	C6 g/L	C7 g/L	Total g/L	%C2	pH
0.00	0.2353	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0854	0.3207	73.37	7.47
0.89	1.6971	0.0000	0.0000	0.5618	0.0000	0.0000	0.0000	0.2250	2.4840	68.32	6.43
1.82	2.5492	0.2106	0.0000	1.7647	0.0000	0.0000	0.0000	0.2275	4.7519	53.64	6.72
2.81	3.0790	1.0128	0.0000	2.2524	0.0000	0.0000	0.0000	0.0000	6.3443	48.53	7.08
3.85	5.6360	1.4201	0.0000	2.3028	0.0000	0.0000	0.0000	0.0000	9.3589	60.22	6.95
4.85	7.3300	1.6956	0.0000	2.3420	0.0000	0.0000	0.0000	0.0000	11.3676	64.48	6.68
5.88	8.7391	1.9505	0.0000	2.4237	0.0000	0.0498	0.0000	0.0000	13.1632	66.39	6.69
8.04	9.8840	2.2531	0.0575	2.5806	0.0000	0.0659	0.0000	0.0000	14.8410	66.60	6.61
9.03	11.0443	2.4450	0.0676	2.7559	0.0000	0.0792	0.0000	0.0000	16.3921	67.38	6.52
9.76	11.5846	2.5697	0.0799	2.8715	0.0610	0.0923	0.0000	0.0000	17.2590	67.12	6.82
11.75	13.3455	2.9550	0.1011	3.1590	0.0860	0.1217	0.0596	0.0000	19.8280	67.31	6.63
14.00	13.9357	3.0957	0.1140	3.3262	0.1021	0.1445	0.0766	0.0000	20.7948	67.02	7.08
16.01	14.2413	3.1262	0.1240	3.3833	0.1119	0.1662	0.0923	0.0000	21.2453	67.03	7.13
17.84	14.6972	3.1722	0.1392	3.5125	0.1233	0.1938	0.1133	0.0000	21.9516	66.95	7.02
19.76	15.2286	3.2315	0.1624	3.7444	0.1344	0.2409	0.1536	0.0000	22.8958	66.51	6.96
21.72	15.5884	3.2509	0.2001	4.0847	0.1496	0.3202	0.2315	0.0000	23.8254	65.43	6.8
23.78	15.6822	3.1864	0.2418	4.3853	0.1636	0.4206	0.3380	0.0000	24.4180	64.22	6.93
25.81	15.8516	3.1483	0.2759	4.7604	0.1764	0.5281	0.4817	0.0000	25.2225	62.85	6.82
27.87	17.5705	3.2763	0.3227	5.2949	0.1933	0.6738	0.6552	0.0000	27.9867	62.78	6.84
29.85	17.9419	3.2510	0.3471	5.5392	0.2052	0.7494	0.7708	0.0000	28.8045	62.29	7.05
32.60	18.5886	3.2636	0.3739	5.8898	0.2202	0.8438	0.9167	0.0000	30.0965	61.76	6.9
34.67	18.8448	3.2404	0.3867	5.9704	0.2281	0.8883	0.9865	0.0000	30.5453	61.69	6.87
36.78	18.7928	3.2626	0.4060	6.1038	0.2374	0.9279	1.0449	0.0000	30.7755	61.06	6.88
38.67	19.1084	3.3079	0.4191	6.2064	0.2456	0.9496	1.0835	0.0000	31.3205	61.01	6.92
40.72	19.2317	3.3205	0.4303	6.2448	0.2532	0.9722	1.1217	0.0000	31.5743	60.91	6.92

Average pH
6.87

Table 6B: Fermentation data for Bagasse and Digested food scraps obtained from short-term batch fermentation (F2)

Time days	C2 g/L	C3 g/L	IC4 g/L	C4 g/L	IC5 g/L	C5 g/L	C6 g/L	C7 g/L	Total g/L	%C2	pH
0.00	1.1263	0.1302	0.0000	0.5193	0.0682	0.0000	0.0000	0.1740	2.0180	55.81	7.78
0.89	1.1568	0.1056	0.0000	0.4985	0.0644	0.0000	0.0000	0.0000	1.8253	63.37	7.31
1.82	1.8646	0.0980	0.0000	0.5005	0.0613	0.0000	0.0000	0.0000	2.5244	73.86	6.44
2.81	2.1364	0.2584	0.0000	0.5305	0.0000	0.0000	0.0000	0.0000	2.9253	73.03	6.86
3.85	2.6353	0.7442	0.0000	0.5441	0.0000	0.0000	0.0000	0.0000	3.9237	67.16	6.14
4.85	3.2784	1.0674	0.0000	0.5680	0.0000	0.0000	0.0000	0.0000	4.9138	66.72	6.83
5.88	4.2399	1.5683	0.0000	0.7052	0.0000	0.0870	0.0000	0.0000	6.6004	64.24	6.87
8.04	4.1583	1.7696	0.0000	0.8426	0.0000	0.2635	0.1012	0.0000	7.1352	58.28	6.68
9.03	4.8290	2.0922	0.0000	0.9376	0.0000	0.3477	0.1347	0.0000	8.3412	57.89	6.73
9.76	5.2685	2.3242	0.0000	0.9928	0.0000	0.4019	0.1541	0.0000	9.1415	57.63	6.83
11.75	5.7098	2.5243	0.0576	1.1568	0.0000	0.5141	0.1985	0.0000	10.1611	56.19	6.68
14.00	6.5694	2.8522	0.0673	1.4584	0.0000	0.6850	0.2670	0.0000	11.8992	55.21	7.07
16.01	6.9784	2.8655	0.0767	1.6156	0.0000	0.8267	0.3272	0.0000	12.6902	54.99	7.14
17.84	7.3087	2.8267	0.0913	1.7602	0.0000	0.9581	0.3862	0.0000	13.3313	54.82	7.03
19.76	7.6235	2.8051	0.1078	2.0195	0.0000	1.1371	0.4811	0.0000	14.1740	53.78	7.07
21.72	7.8886	2.7827	0.1285	2.2458	0.0000	1.2878	0.5742	0.0000	14.9074	52.92	6.87
23.78	8.2812	2.7876	0.1489	2.3162	0.0518	1.3666	0.6330	0.0000	15.5853	53.13	6.97
25.81	8.5307	2.7871	0.1687	2.4705	0.0547	1.4728	0.7223	0.0000	16.2068	52.64	6.84
27.87	9.7262	2.9416	0.1921	2.6388	0.0589	1.6092	0.8218	0.0464	18.0350	53.93	7.06
29.85	9.7862	2.9094	0.2033	2.7391	0.0607	1.6938	0.9060	0.0510	18.3494	53.33	7.07
32.60	10.0275	2.9010	0.2179	2.8730	0.0638	1.7911	1.0004	0.0551	18.9297	52.97	6.92
34.67	10.3312	2.9285	0.2280	2.9853	0.0674	1.8477	1.0537	0.0579	19.4999	52.98	6.86
36.78	10.4594	2.9141	0.2376	2.9517	0.0717	1.8638	1.0816	0.0632	19.6431	53.25	6.87
38.67	10.7166	2.9290	0.2465	3.0164	0.0741	1.8925	1.1106	0.0651	20.0509	53.45	6.92
40.72	10.8997	2.8858	0.2545	2.9922	0.0763	1.9245	1.1425	0.0636	20.2390	53.85	6.88

Average pH
6.91

Table 6C: Fermentation data for Bagasse and digested food scraps obtained from continuous fermentation (F3)

Time days	C2 g/L	C3 g/L	IC4 g/L	C4 g/L	IC5 g/L	C5 g/L	C6 g/L	C7 g/L	Total g/L	%C2	pH
0.00	3.6267	0.0984	0.0000	0.3744	0.0000	0.0000	0.0000	0.1458	4.2454	85.43	7.29
0.89	4.1236	0.1079	0.0000	0.4285	0.1270	0.0000	0.0000	0.1467	4.9337	83.58	7.41
1.82	4.6328	0.0984	0.0572	0.4747	0.1333	0.0000	0.0000	0.1387	5.5351	83.70	6.83
2.81	5.3405	0.4413	0.0000	0.5942	0.0975	0.0000	0.0000	0.1960	6.6695	80.07	6.67
3.85	5.9879	1.1174	0.0640	0.6976	0.0799	0.0000	0.0000	0.1752	8.1220	73.72	6.9
4.85	6.9399	1.4045	0.0663	1.1162	0.0672	0.0000	0.0000	0.1370	9.7312	71.32	6.45
5.88	8.7528	2.2500	0.0851	2.3437	0.0700	0.0652	0.0000	0.0822	13.6490	64.13	6.52
8.04	8.7102	2.5770	0.1021	3.0413	0.0755	0.0000	0.1206	0.0000	14.6267	59.55	6.91
9.03	8.8145	2.5852	0.1105	3.6609	0.0839	0.4610	0.3946	0.0000	16.1105	54.71	6.78
9.76	9.0803	2.6236	0.1161	3.9939	0.0898	0.7536	0.7197	0.0000	17.3770	52.25	6.93
11.75	9.6403	2.8382	0.1240	3.9507	0.0991	0.9535	0.9556	0.0000	18.5614	51.94	6.93
14.00	10.2376	3.0091	0.1361	4.0300	0.1074	1.0990	1.1067	0.0000	19.7259	51.90	6.99
16.01	10.4908	3.0539	0.1485	4.1237	0.1111	1.2526	1.2620	0.0000	20.4425	51.32	6.96
17.84	10.8169	3.0856	0.1603	4.2509	0.1180	1.3949	1.4067	0.0000	21.2332	50.94	6.81
19.76	11.0416	3.1011	0.1729	4.4534	0.1251	1.5397	1.5677	0.0000	22.0015	50.19	7.05
21.72	11.3346	3.1045	0.1892	4.6140	0.1344	1.6615	1.7074	0.0438	22.7895	49.74	6.8
23.78	11.7867	3.1368	0.2150	4.7527	0.1473	1.7848	1.8597	0.0497	23.7327	49.66	6.88
25.81	12.0737	3.1507	0.2300	4.9830	0.1585	1.8983	2.0044	0.0554	24.5540	49.17	6.77
27.87	13.8659	3.3609	0.2562	5.1433	0.1716	2.0581	2.2034	0.0653	27.1247	51.12	6.89
29.85	13.9413	3.3065	0.2678	5.1592	0.1816	2.0979	2.2612	0.0692	27.2847	51.10	6.99
32.60	14.5534	3.3754	0.2850	5.2706	0.1953	2.1968	2.3889	0.0753	28.3406	51.35	6.75
34.67	15.0741	3.4493	0.2946	5.1341	0.2018	2.2236	2.4286	0.0819	28.8880	52.18	6.9
36.78	15.9471	3.5013	0.3051	4.6452	0.2092	2.2362	2.4465	0.0796	29.3701	54.30	6.8
38.67	16.5626	3.5373	0.3120	4.4086	0.2156	2.2458	2.4583	0.0814	29.8216	55.54	7.06
40.72	17.3239	3.6107	0.3212	4.2571	0.2234	2.2849	2.5023	0.0866	30.6102	56.60	6.93

Average pH
6.89

Table 6D: Fermentation data for Bagasse and Partially digested oils and fats (F4)

Time days	C2 g/L	C3 g/L	IC4 g/L	C4 g/L	IC5 g/L	C5 g/L	C6 g/L	C7 g/L	Total g/L	%C2	pH
0.00	2.3049	0.0000	0.0000	0.2509	0.0783	0.0000	0.0000	0.1081	2.7423	84.05	7.23
0.89	2.5249	0.0000	0.0000	0.2721	0.0876	0.0000	0.0000	0.1034	2.9880	84.50	7.47
1.82	4.6713	0.0997	0.0574	0.4781	0.1337	0.0000	0.0000	0.1451	5.5853	83.64	6.7
2.81	3.5741	0.4389	0.0000	0.3768	0.0567	0.0000	0.0000	0.1157	4.5622	78.34	6.94
3.85	4.1263	1.1140	0.0000	0.4377	0.0000	0.0000	0.0000	0.1233	5.8013	71.13	6.7
4.85	4.8320	1.3989	0.0000	0.4863	0.0000	0.0000	0.0000	0.0934	6.8106	70.95	6.77
5.88	5.6978	1.7841	0.0000	0.6645	0.0000	0.0589	0.0000	0.0760	8.2813	68.80	6.77
8.04	6.1649	2.0580	0.0545	1.0298	0.0000	0.2473	0.0911	0.0000	9.6457	63.91	6.69
9.03	7.2701	2.3197	0.0683	1.6145	0.0000	0.5025	0.2116	0.0000	11.9866	60.65	6.55
9.76	6.7733	2.2014	0.0620	1.3843	0.0000	0.4006	0.1594	0.0000	10.9810	61.68	6.84
11.75	6.8945	2.1984	0.0705	1.7017	0.0000	0.6008	0.2857	0.0000	11.7516	58.67	6.68
14.00	8.4900	2.6611	0.0927	2.1739	0.0000	0.8288	0.4212	0.0000	14.6678	57.88	7.09
16.01	8.5363	2.5091	0.1018	2.2310	0.0524	0.9180	0.4911	0.0000	14.8397	57.52	7.21
17.84	9.2775	2.5790	0.1194	2.4497	0.0611	1.0656	0.5943	0.0000	16.1467	57.46	7.08
19.76	9.4230	2.5183	0.1342	2.6886	0.0688	1.1791	0.7105	0.0000	16.7225	56.35	7.18
21.72	9.7717	2.4696	0.1539	2.8038	0.0764	1.2822	0.8199	0.0000	17.3775	56.23	6.85
23.78	10.3288	2.4689	0.1748	2.8500	0.0861	1.3501	0.9053	0.0438	18.2077	56.73	6.97
25.81	10.7456	2.4708	0.1936	2.9266	0.0953	1.4188	0.9887	0.0483	18.8877	56.89	6.82
27.87	12.3410	2.6404	0.2182	3.1204	0.1042	1.5329	1.1064	0.0573	21.1208	58.43	6.94
29.85	12.6039	2.6304	0.2308	3.2818	0.1108	1.6022	1.1962	0.0619	21.7180	58.03	6.98
32.60	12.9442	2.6397	0.2446	3.4273	0.1181	1.6817	1.3025	0.0650	22.4232	57.73	6.71
34.67	13.5820	2.6854	0.2529	3.3269	0.1227	1.7030	1.3381	0.0674	23.0784	58.85	6.89
36.78	14.0101	2.7526	0.2655	3.3037	0.1289	1.7385	1.3786	0.0727	23.6505	59.24	6.82
38.67	14.6077	2.8120	0.2738	3.2894	0.1338	1.7792	1.4196	0.0745	24.3901	59.89	7.15
40.72	14.9264	2.8313	0.2805	3.2052	0.1377	1.7995	1.4456	0.0746	24.7009	60.43	7.05

Average pH
6.92

Table 6E: Fermentation data for Bagasse and Partially digested carbohydrates and vegetables (F5)

Time days	C2 g/L	C3 g/L	IC4 g/L	C4 g/L	IC5 g/L	C5 g/L	C6 g/L	C7 g/L	Total g/L	%C2	pH
0.00	5.2679	0.1419	0.0635	0.5369	0.1509	0.0000	0.0000	0.2527	6.4137	82.13	7.34
0.89	6.1270	0.1528	0.0817	0.6683	0.1908	0.0000	0.0000	0.2085	7.4292	82.47	7.42
1.82	6.1992	0.1460	0.0832	0.7183	0.1867	0.0000	0.0000	0.2039	7.5373	82.25	6.95
2.81	7.0278	0.4898	0.0877	0.9188	0.1578	0.0000	0.0000	0.2747	8.9565	78.47	6.72
3.85	7.9238	1.4647	0.0925	1.1167	0.1306	0.0000	0.0000	0.2474	10.9757	72.19	6.34
4.85	10.3713	2.1408	0.0948	2.5028	0.1062	0.0000	0.0000	0.1873	15.4032	67.33	6.28
5.88	11.6023	2.6568	0.1102	4.1838	0.1134	0.0777	0.0000	0.1018	18.8460	61.56	6.43
8.04	11.4002	2.8454	0.1307	5.0121	0.1257	0.1596	0.1071	0.0000	19.7808	57.63	6.93
9.03	11.7878	2.9099	0.1395	5.5377	0.1421	0.3283	0.3333	0.0000	21.1785	55.66	6.84
11.75	12.8204	3.0853	0.1617	5.8298	0.1601	0.8198	1.0487	0.0000	23.9257	53.58	6.7
14.00	13.5675	3.2438	0.1836	5.9677	0.1746	0.9758	1.2689	0.0000	25.3818	53.45	6.96
16.01	13.8355	3.1875	0.1934	5.7521	0.1818	1.0370	1.3497	0.0000	25.5371	54.18	7.04
17.84	14.2926	3.1560	0.2028	5.6375	0.1898	1.0759	1.4010	0.0000	25.9555	55.07	6.92
19.76	14.8965	3.1761	0.2128	5.7135	0.1997	1.0955	1.4239	0.0000	26.7180	55.75	7.14
21.72	15.5598	3.1810	0.2238	5.5584	0.2106	1.1098	1.4435	0.0000	27.2869	57.02	6.81
23.78	16.4906	3.2005	0.2336	5.3897	0.2194	1.1287	1.4634	0.0000	28.1258	58.63	6.92
25.81	17.0300	3.1685	0.2391	5.3175	0.2258	1.1292	1.4694	0.0000	28.5794	59.59	6.77
27.87	19.8923	3.3472	0.2618	5.1761	0.2426	1.1985	1.5471	0.0000	31.6656	62.82	6.87
29.85	20.3784	3.3509	0.2707	5.5609	0.2567	1.1978	1.5526	0.0000	32.5681	62.57	6.9
32.60	20.9041	3.3111	0.2789	5.5441	0.2670	1.1976	1.5471	0.0000	33.0498	63.25	6.85
34.67	21.3440	3.3089	0.2895	5.5483	0.2756	1.1992	1.5577	0.0000	33.5233	63.67	7.16
36.78	22.0054	3.3499	0.3013	5.4245	0.2868	1.2192	1.5759	0.0000	34.1630	64.41	7.21
38.67	22.3087	3.3212	0.3115	5.2933	0.2938	1.2106	1.5614	0.0000	34.3005	65.04	7.27
40.72	22.7739	3.2885	0.3406	5.0924	0.3015	1.2064	1.5556	0.0000	34.5589	65.90	7.08

Average pH
6.91

Table 6F: Fermentation data for Bagasse and Chicken Manure (F6)

Time days	C2 g/L	C3 g/L	IC4 g/L	C4 g/L	IC5 g/L	C5 g/L	C6 g/L	C7 g/L	Total g/L	%C2	pH
0.00	0.5119	0.1505	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6624	77.28	7.68
0.89	2.2606	0.3924	0.0000	0.1043	0.0000	0.0000	0.0000	0.0000	2.7573	81.99	7.01
1.82	3.2754	0.6714	0.0000	0.1506	0.0000	0.0000	0.0000	0.0000	4.0974	79.94	6.72
2.81	6.6961	1.4681	0.0000	1.4221	0.0000	0.0000	0.0000	0.0000	9.5862	69.85	5.83
3.85	9.5575	1.8644	0.0000	2.9789	0.0000	0.0594	0.0000	0.0000	14.4602	66.10	6.14
4.85	12.6888	2.2405	0.0000	4.0965	0.0000	0.1304	0.1784	0.0000	19.3346	65.63	6.65
5.88	13.0929	2.3373	0.0653	4.4894	0.0565	0.2147	0.4987	0.0000	20.7549	63.08	6.9
8.04	12.5196	2.3315	0.0751	4.6824	0.0762	0.2305	0.4988	0.0000	20.4141	61.33	7.11
9.03	12.7209	2.4566	0.0877	4.8423	0.0966	0.2436	0.5027	0.0000	20.9503	60.72	6.86
9.76	13.0463	2.6276	0.0981	4.9477	0.1129	0.2569	0.5086	0.0000	21.5981	60.41	6.8
11.75	14.2147	3.2672	0.1312	5.0777	0.1691	0.2871	0.5070	0.0000	23.6540	60.09	6.71
14.00	14.0830	3.2940	0.1636	5.0886	0.2302	0.2980	0.5066	0.0000	23.6640	59.51	7.08
16.01	14.4699	3.3131	0.2011	5.1051	0.2718	0.3062	0.5110	0.0000	24.1782	59.85	7.49
17.84	15.4690	3.3424	0.2291	5.1045	0.2863	0.3115	0.5086	0.0495	25.3009	61.14	7.09
19.76	16.8054	3.3572	0.2550	5.1359	0.2743	0.3140	0.5086	0.0606	26.7110	62.92	6.62
21.72	18.6738	3.4340	0.2871	5.1155	0.2711	0.3177	0.5042	0.0000	28.6034	65.29	6.47
23.78	19.7056	3.5009	0.3306	5.1544	0.2821	0.3252	0.5084	0.0449	29.8522	66.01	7.03
25.81	19.9530	3.4052	0.3594	5.1354	0.3043	0.3232	0.5067	0.0000	29.9872	66.54	7.1
27.87	22.5234	3.5210	0.4078	5.0370	0.3364	0.3257	0.5237	0.0000	32.6749	68.93	7.05
29.85	22.8773	3.5185	0.4305	5.0702	0.3577	0.3280	0.5229	0.0000	33.1051	69.11	7.47
32.60	22.9281	3.4302	0.4603	4.9934	0.3901	0.3341	0.5190	0.0573	33.1125	69.24	7.61
34.67	23.2747	3.4524	0.4840	5.0566	0.4164	0.3403	0.5236	0.0649	33.6129	69.24	7.56
36.78	23.5826	3.4808	0.5100	4.8325	0.4394	0.0000	0.5314	0.0000	33.3767	70.66	7.73
38.67	23.7223	3.4978	0.5296	4.8855	0.4593	0.0000	0.5318	0.0000	33.6262	70.55	7.96
40.72	24.0164	3.4919	0.5512	4.8436	0.4841	0.3500	0.5345	0.0959	34.3677	69.88	7.95

Average pH
7.06

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