PILOT-SCALE FERMENTATION OF OFFICE PAPER AND CHICKEN MANURE TO CARBOXYLIC ACIDS

A Thesis

by

ANDREW GARRET MOODY

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

May 2006

Major Subject: Chemical Engineering

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Approved by:

Chair of Committee,	Mark Holtzapple
Committee Members,	Charles Glover
	Cady Engler
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ABSTRACT

Pilot-scale Fermentation of Office Paper and Chicken Manure to Carboxylic Acids.

(May 2006)

Andrew Garret Moody, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Mark T. Holtzapple

This project focused on scaling up the laboratory fermentation of biomass to carboxylic acids. Four 1050-gallon tanks were used to simulate four-stage countercurrent fermentation. Most laboratory fermentations have been performed with 1-L fermentors. The purpose of the pilot plant was to show that the process is scalable.

The inocula were marine and terrestrial microorganisms. Office paper was used as an energy source, and chicken manure provided the necessary nutrients. The substrate was 80 wt% office paper and 20 wt% chicken manure. Calcium carbonate was used as a neutralizing agent and iodoform served as a methane inhibitor. The fermentor temperature was 40 ^{o}C and the pH was 6.0.

The highest total acid concentration obtained was 32.4 g/L, operating with a volatile solids loading rate (VSLR) of 1 g/(L liq ·d) and a liquid residence time (LRT) of 80 days. Typical laboratory VSLRs and LRTs are 3 to 10 g/(L liq ·d) and 10 to 30 days, respectively. Similar VSLRs and LRTs were not achieved at the pilot scale because the design was limited by the ability to effectively separate large amounts of solids and liquids.

The bulk of the effort was concentrated on overcoming temperature control and solids-handling issues. Design modifications included a redesigned temperature control system and a new material transfer method.

DEDICATION

I dedicate this thesis to my wonderful wife, Jessica. This work would not have been possible without her continuous love and support.

ACKNOWLEDGMENTS

I would like to express my appreciation to my advisor, Dr. Mark Holtzapple, for his guidance and support. His advice and encouragement was essential for the success of this project. I would also like to thank my committee members, Dr. Charles Glover and Dr. Cady Engler, for their continuous support. Much thanks also goes to Dr. Cesar Granda for his guidance and advice in overcoming process obstacles. I also express gratitude to Jim Lampley, Mark Carraba, and David Carraba for their generosity and support.

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CHAPTER I

INTRODUCTION

Economic and population growth has increased the global energy demand. In 2002, fossil fuels supplied 90% of the world's demand for energy, with crude oil accounting for 40% of this. According to many energy experts, global oil supplies can meet demands until oil production peaks somewhere between 2013 and 2020 (Selameh 2002). Because oil is exhaustible, methods to develop alternative energy will be mandatory.

To help alleviate demands on fossil fuel, Holtzapple *et al.* (1997) have developed the MixAlco process, which converts waste biomass into useful chemicals and fuels. Successful research has been conducted at Texas A&M University for the past 15 years to optimize the MixAlco process.

THE MIXALCO PROCESS

The MixAlco process can convert a variety of biomass sources including municipal solid waste, sewage sludge, agriculture residues, and energy crops. The basis of the process is fermentation, which uses acid-forming microorganisms found in ruminant animals, soil, compost, swamps, and marine environments to digest the biomass. A mixture of carboxylic acids is produced and can be chemically converted to mixed alcohol fuels using conventional chemical engineering methods. Figure I-1

This thesis follows the style of Biotechnology and Bioengineering.

shows a block diagram of the MixAlco process.

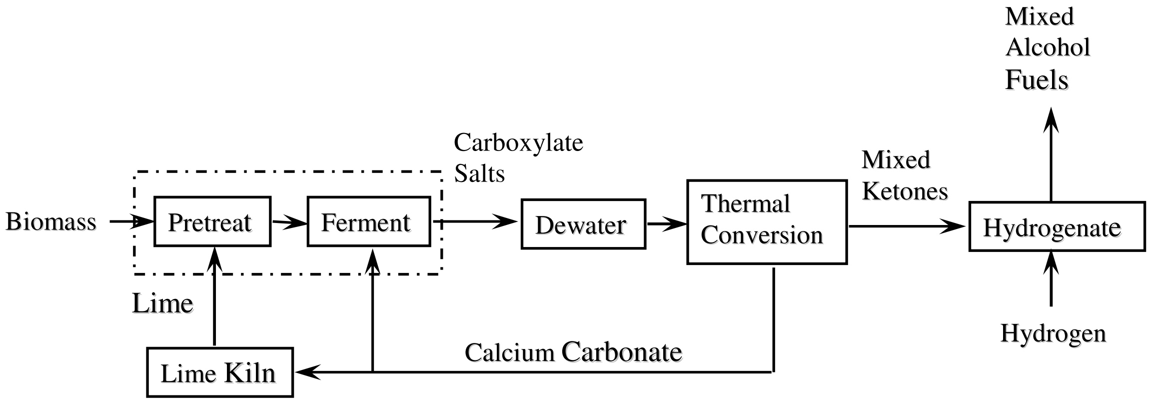


Figure I-1. The MixAlco process.

Biomass is first pretreated with lime (calcium hydroxide) to improve digestibility. Microorganisms then convert the biomass into carboxylic acids during the fermentation process. The microorganisms favor a near-neutral pH, thus calcium carbonate buffer is added to neutralize the acid products by forming carboxylate salts. The resulting pH is 5.8 to 6.2. The carboxylate salts are diluted in water and must be concentrated with a dewatering step. The concentrated carboxylate salts are then chemically converted to mixed ketones where calcium carbonate is a byproduct that can be recycled back to the fermentation. The calcium carbonate can also be sent to a kiln to make lime for pretreatment. The ketones are hydrogenated to form alcohols, which can be used as a source of fuel.

CARBOXYLIC ACID FERMENTATION

The bulk of the research on the MixAlco process has been conducted on the fermentation step because this step is a key part of the process. Carboxylate salt is the dominant end-product of the fermentation step; the protonated form of the minor product, carboxylic acid. The reaction of interest is the microbial conversion of biomass to carboxylic acids.

Inoculum

The conversion of biomass to carboxylic acids requires anaerobic microorganisms, which are found in a variety of environments. Anaerobic-acid forming microorganisms can be found in animal rumens, fresh or saline swamps, compost piles, soil, etc. Research suggests that marine microorganisms may improve biomass digestion because they have the ability to easily adapt to saline environments (Thanakoses, 2002).

Substrate

The fermentation process must also have the appropriate substrate (biomass) to achieve optimal results. The substrate must supply the proper energy and nutrient source for the microorganisms. The energy source is from a biomass that is high in carbohydrates such as paper, bagasse, and corn stover. These materials have a high lignocellulose content, which is composed mainly of hemicellulose, cellulose, and lignin. Microorganisms can digest hemicellulose and cellulose, but not lignin; therefore, biomass with low lignin content is preferred.

The appropriate nutrient source is also crucial for bacterial growth. Specifically, sufficient amounts of nitrogen, carbon, phosphorus, potassium, and sulfur are necessary for the microorganisms to thrive. High-nutrient biomass such as sewage sludge, chicken manure, and pig manure is important for successful fermentation (Chan, 2002). Additional nutrients may also be required. Caldwell and Bryant (1996) developed a medium high in the required nutrients that has been successfully used as a supplement in numerous studies. Urea has also been used as a nitrogen supplement in some instances (Ross, 1998).

Methane Inhibition

Carboxylic acids are only one of the products of the anaerobic fermentation of biomass; methane is also produced by methanogenesis. To prevent carboxylic acid loss to methane, a methanogen inhibitor is required. Ross (1998) found that iodoform could be used to completely inhibit methane production.

pH Effects

Another important condition to maintain is the pH. For carboxylic acid production, the pH should stay near neutrality. A pH above 6.2 encourages methanogenesis, and a pH below 5.8 is too acidic for acid forming microorganisms to survive (Thanakoses, 2002).

Oxygen Effects

Performing fermentations in the absence of oxygen is necessary because most acid-producing microorganisms are strict anaerobes. In laboratory studies, a reducing agent, such as cysteine, was added to the medium to eliminate oxygen. A nitrogen blanket was also used in laboratory fermentors to occupy headspace.

Biomass Pretreatment

Lignin is an inert aromatic polymer that surrounds cellulose and hemicellulose and reduces the susceptibility to microbial attack. Many methods have been developed to treat lignocellulose to improve biomass digestibility. Chemical pretreatment with lime, calcium hydroxide, is an inexpensive technique. Chang *et al.* (1999) studied lime pretreatment with a carbonated wash water and found that temperature and time impacted pretreatment severity; however, lime loading over 0.1 g $Ca(OH)_2$/g dry biomass and water loading had little effect. With short pretreatment times of 1 to 3 hours, high temperatures (85-135 ^{o}C) were required. With pretreatment times over 24 hours, temperatures as low as 65 ^{o}C were effective.

Thanakoses (2002) studied a different lime pretreatment method and found oxygen can be used as an oxidative agent to further degrade lignin. Biomass was pretreated by circulating water-saturated air through wet biomass loaded with various amounts of lime at ambient temperatures. High lime loadings (0.4 g $Ca(OH)_2$/g dry biomass) and long pretreatment times (16 weeks) were shown to improve biomass digestibility.

Thanakoses (2002) also found that physical pretreatment of partially digested biomass with sonication further improved biomass digestibility. Specifically, using a Fisher sonic dismembrator Model 300 at a power of 60 for 15 minutes was deemed optimal.

Countercurrent Continuous Fermentation

Biomass loses reactivity as it becomes digested; high carboxylic acid concentrations also inhibit biomass digestion (Loescher,1996). The best way to offset these inhibitions is to operate with a multi-stage countercurrent fermentation system. Solids and liquid are transferred in opposite directions. Fresh water is added to one end of the fermentation train (F4), and product is harvested at the other end (F1). At the same time, fresh biomass is added where the product is removed (F1), and indigestible biomass is removed where fresh water is added (F4) (see Figure I-2). Acid concentrations increase from left to right, and biomass digestion increases from right to left. This allows the highest acid concentrations to exist with the most reactive biomass (fresh biomass), and the lowest acid concentrations to exist with the least reactive biomass (most digested). This configuration allows maximum acid concentration and conversion.

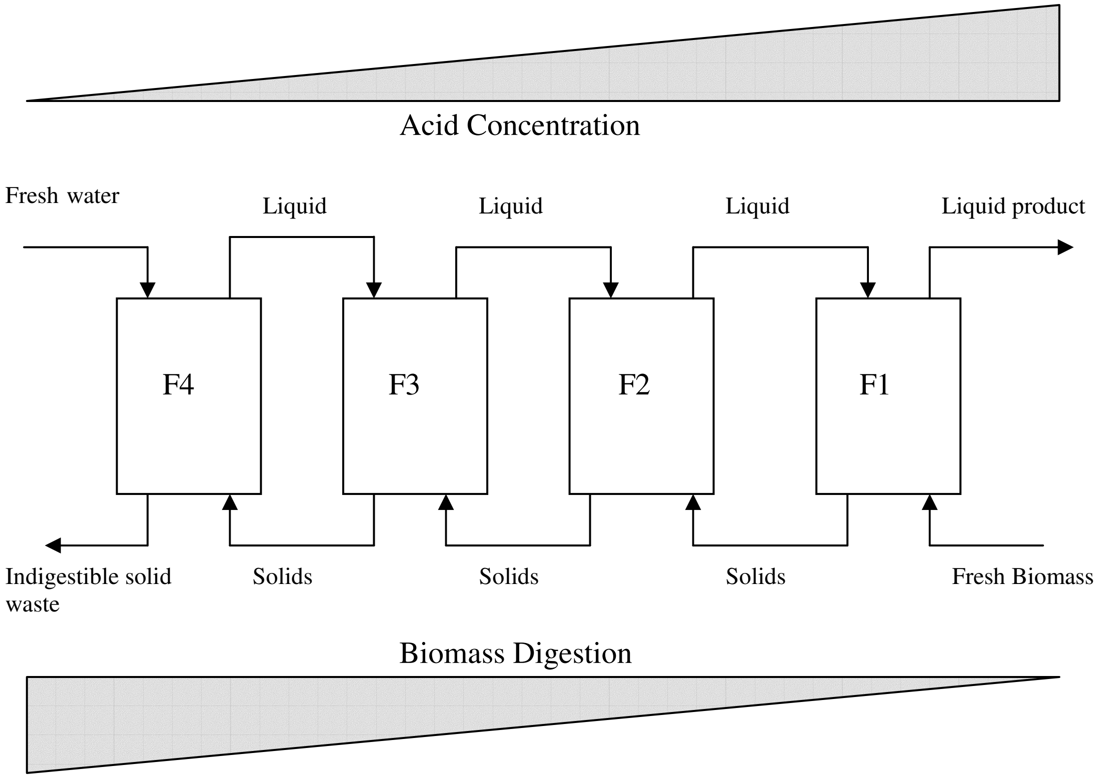


Figure I-2. Countercurrent fermentation.

USEFUL TERMS

The following definitions will be used in this research.

$$\text{yield} = \frac{\text{mass of carboxylic acids produced}}{\text{mass of VS fed}} \tag{I-1}$$

$$\text{conversion} = \frac{\text{mass of VS digested}}{\text{mass of VS fed}} \tag{I-2}$$

$$\text{total acid selectivity} = \frac{\text{mass of carboxylic acids produced}}{\text{mass of VS digested}} \tag{I-3}$$

$$\text{total acid productivity} = \frac{\text{mass of carboxylic adids produced}}{\text{TLV} \cdot \text{time}} \tag{I-4}$$

$$\text{volatile solids loading rate (VSLR)} = \frac{\text{VS fed/day}}{\text{TLV}} \tag{I-5}$$

$$\text{total liquid volume (TLV)} = \sum \overline{K_i} \cdot w + F_i \tag{I-6}$$

$$\text{liquid residence time (LRT)} = \frac{\text{TLV}}{\text{Q}} \tag{I-7}$$

where,

VS = volatile solids

$\overline{K_i}$ = average wet mass of solid cake in Fermentor *i* (g)

w = average liquid fraction of solid cake in Fermentor *i* (L liquid/g wet cake)

F_i = average volume of free liquid in Fermentor *i* (L)

Q = flowrate of liquid out of the fermentor train (L/d)

LITERATURE REVIEW

The fermentation of biomass to carboxylic acids has been intensively studied, thus a summary of the most important laboratory findings is warranted. The following is a synopsis of the most significant research done under Dr. Mark Holtzapple at Texas A&M University.

"Volatile Fatty Acid Fermentation of Biomass and Kinetic Modeling using the CPDM Method"

The following is a summary of the dissertation "Volatile Fatty Acid Fermentation of Biomass and Kinetic Modeling Using the CPDM Method" by Loescher in 1996. In this work, the Continuum Particle Distribution Modeling (CPDM) method for analyzing solid/fluid reaction systems, specifically biomass conversion, was developed. The CPDM method was proven effective by comparing the model to reaction data obtained from experiments conducted on the fermentation of biomass to volatile fatty acids (carboxylic acids). Reaction mechanisms have not been developed for biomass conversion to carboxylic acids because of the complexity. CPDM is a way to use experimental data to model and optimize these reactions for various reactor configurations, including batch, fed batch, continuous stirred tank reactors (CSTR), and plug flow reactors (PFR) systems. With this model it was determined that a continuous countercurrent CSTR cascade is the optimal reactor configuration for carboxylic acid fermentation.

Loescher also studied the effects of different conditions on carboxylic acid fermentation, including experiments on fermentations with differing artificially high carboxylic acid concentrations, varying substrate concentrations, increased nutrients, and varying inoculum amounts.

Concepts of CPDM

CPDM is based on the concept of a "continuum particle," which is defined by a cluster of particles that is always a representative sample of discrete particles. Despite the extent of the reaction, a "continuum particle" is always identical to the next. Loescher quantified a "continuum particle" as one gram of solids in the initial unreacted state. In the solid phase not one particle has the same conversion at any given time, thus it is necessary to describe the particle conversion as a conversion *distribution function*, $\hat{n}(x)$. The parameter x is defined as the solids conversion and ranges from 0 (unreacted) to 1 (totally reacted).

$$n_{x_1,x_2} = \text{number of particles/L with } x\text{'s between } x_1 \text{ and } x_2 = \int_{x_1}^{x_2} \hat{n}(x)dx \qquad \text{(I-8)}$$

As the solids are consumed, there is an unreactive mass that is left behind. This can be visualized as an inert solid phase skeleton that defines the particle regardless of the extent of the reaction. Based on this concept, the following relationship was developed, where n_o is the initial particle concentration (particle/L).

$$n_o = \int_0^1 \hat{n}(x)dx \qquad \text{(I-9)}$$

The solid/fluid interface reaction was defined as

$$\mathrm{R} \rightarrow \sum_{products} a_{oi}\ \underline{\mathrm{A}}_{\mathrm{i}} \tag{I-10}$$

where R is the solid phase reactant, $\underline{\mathrm{A}}$ represents a vector of all fluid-phase product species, *i*, and a_{oi} is the stoichiometric coefficient for species $\underline{\mathrm{A}}_i$.

The products of the solid/fluid interface reaction may react further in fluid-phase reactions, as described by

$$\sum_{reactants} a_{oi}\ \underline{\mathrm{A}}_{\mathrm{i}} \xrightarrow{r_k} \sum_{products} b_{ki}\ \underline{\mathrm{A}}_{\mathrm{i}} \tag{I-11}$$

where the rate expression for reaction *k* has the general form

$$r_k = f_k([\underline{\mathrm{A}}]) \tag{I-12}$$

Equilibrium conditions can be employed on reaction *k* to develop the following equation

$$\underline{\mathrm{A}}_{\mathrm{i}} = C_{\mathrm{i}}(\ [\underline{\mathrm{A}}],\ \hat{n}(x)\ ) \tag{I-13}$$

where C_i is the relationship between the particle conversion distribution function and the product concentration.

The overall reaction rate, *r*, was expressed as

$$r = \text{reaction rate (g/(L·h))} = \int_x \hat{r}(x,[\underline{A}])\hat{n}(x)dx \tag{I-14}$$

where $\hat{r}$ is the specific reaction rate (g/(particle·h), which depends on individual particle reactions and is a function of both particle conversion, *x*, and fluid-phase species concentrations, [$\underline{\mathrm{A}}$].

Batch experiments are conducted at varying reactant concentrations to collect information on each product species. To do this, the preceding principles needed minor modifications. In a batch reaction, all particles have the same conversion, *x*'. Thus,

$\hat{n}(x) = 0$ everywhere except x'. By using the Dirac delta function, δ, and Equation I-9, Equation I-14 was rewritten as

$$r = \int_0^1 \hat{r}(x,[\underline{A}])\hat{n}(x)dx = \int_0^1 \hat{r}(x,[\underline{A}])n_o\delta(x - x')dx = \hat{r}(x',[\underline{A}])n_o \tag{I-15}$$

Equation I-15 shows that the specific reaction rate can be directly observed in batch experiments and is proportional to the overall reaction rate. Thus, $\hat{r}$ (x,[$\underline{\mathrm{A}}$]) can be measured and correlated to data using the observed product species concentrations in [$\underline{\mathrm{A}}$] as a function of time and n_o. The function $g(\underline{\mathrm{A}}(t, n_o))$ can be defined to relate the product species concentration back to the solid-phase reactant concentrations through the reaction stoichiometry. $\underline{\mathrm{A}}(t, n_o)$ can be calculated from Equation I-13 or can be measured experimentally.

From Equation I-13 the following equation can be expressed

$$\hat{r}(x,[\underline{A}]) = \frac{1}{n_o} r = \frac{1}{n_o}\left[\frac{\partial g(\underline{A}(t,n_o))}{\partial t}\right]\Bigg|_{n_o} \tag{I-16}$$

The conversion, x, can be expressed as

$$x = \frac{g([\underline{A}])}{n_o s_o} \tag{I-17}$$

where s_o is the amount of reactant per "continuum particle" (one initial gram).

The data collected from batch experiments are in the form of $\{[\mathrm{A}], \mathrm{t}, \mathrm{n_o}\}_i$. Using Equations I-16 and I-17, a new data set can be made with the form $\{\hat{r}, x, [\underline{\mathrm{A}}]\}_i$. Then by data fit, the specific rate, $\hat{r}\,(x, [\underline{A}])$ can be obtained. From this function, other reactor configurations can be modeled.

One reactor configuration that Loescher simulated was a cascade CSTR. Figure I-3 illustrates a cascade CSTR configuration.

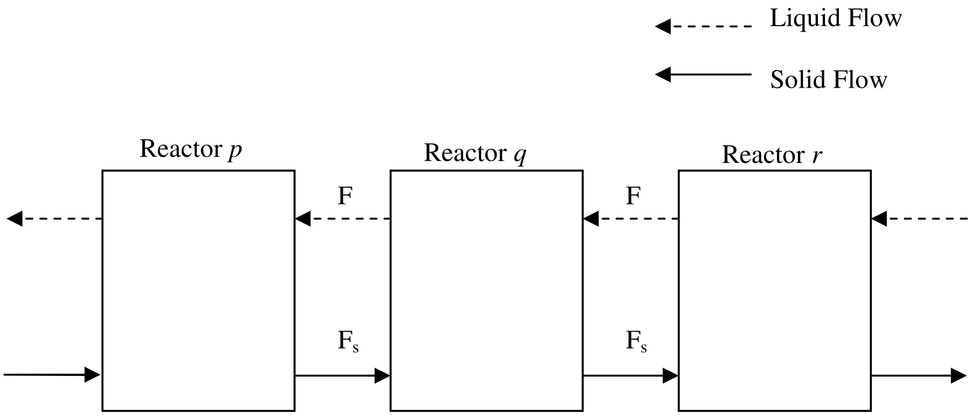


Figure I-3. Loescher: CSTR cascade.

The following equation was derived to simulate a cascade of CSTRs

$$[\underline{A}]^q i = [\underline{A}]^r i + \beta[\underline{A}]^p i + \tau_L\left[a_{oi}\int_0^1 \hat{r}(x,[\underline{A}]^q)\hat{n}(x)dx + \sum_{\text{reactants}}(b_{ki}-a_{ki})f_k([\underline{A}]^q)\right] \quad \text{(I-18)}$$

where

$$\beta = \frac{F_s}{F} \quad \text{(I-19)}$$

The following condition is also used

$$[\underline{A}]^q i = C_i([\underline{A}]^q, \hat{n}(x)) \quad \text{(I-20)}$$

Application of CPDM Modeling

Loescher studied a set of lignocellulose hydrolysis data for batch and CSTR reactors that had been obtained by South *et al*. (1993, 1995) and South (1994). This reaction model was based on a Langmuir adsorption model. Loescher showed a solid/fluid reaction rate ($\hat{r}$) based on the conversion of cellulose and fluid-phase reaction rates ($f_k([\underline{A}])$). Using these rate equations, Loescher calculated batch and CSTR data. He then developed a CPDM model which correlated well to the calculated data and showed that the reaction slowed as the conversion increased, and there was an inhibitor effect from high ethanol concentrations. This study served as the basis for assuming the empirical reaction model (Equation I-22) in countercurrent carboxylic acid fermentation.

Modeling Rumen Fermentation with CPDM

Loescher then conducted batch experiments on carboxylic acid fermentation to obtain data for a CPDM model. The batch fermentations were done in 1-L beakers and were intended to simulate carboxylic acid fermentation in bovine rumen. Temperature was controlled with an incubator, and the fermentor was kept well mixed with an orbital shaker plate. The fermentation conditions are summarized in Table I-1.

Table I-1. Loescher: Batch fermentation conditions.

Temperature	39 °C
pH	6.7
Energy Source	Mixed Winter Grass (MWG)
Nutrient Source	Caldwell and Bryant Medium
Neutralizing Agent	NaOH
Inoculum	Steer Rumen Fluid
Methane Inhibitor	1.0-mM 2-bromoethane-sulfonic acid, sodium salt (BES)

Experiments were performed at various substrate concentrations. The data were collected and fit to a polynomial equation of the form

$$[\mathrm{AcEq}](\mathrm{t}) = \mathrm{AcEq}(\mathrm{initial}) + \alpha t^{0.3} + \beta t^{0.4} + \gamma t^{0.5} \tag{I-21}$$

where AcEq stands for acetate equivalents. These data were then used to formulate an empirical reaction model with the assumed form

$$\hat{r} = \frac{a(1-x)^{b}}{1+cx^{d}+e(AcEq)^{f}} \tag{I-22}$$

The accuracy of the model was determined by an explained residual value shown by Equation I-23.

$$\text{Explained Residual (\%)} = 100\left[1-\frac{\sum_{data}(\hat{r}(data)-\hat{r}(x,[AcEq]))^{2}}{\sum_{data}\hat{r}(data)^{2}}\right] \tag{I-23}$$

Loescher's batch model had an explained residual value of 86.1%.

Next, Loescher conducted fermentation experiments with a single-stage CSTR. The fermentation conditions were the same as shown in Table I-1, but with various liquid residence times (LRTs), solids residence times (SRTs), feed particle concentrations, and reactor particle concentrations. The CPDM model was constructed and compared to the experimental results. The model predicted acid concentrations with an average error of 9.6 %.

Loescher then used the empirical rate equation to develop a model for a cocurrent and countercurrent CSTR cascade configuration. These data suggested that the SRT is the primary variable affecting biomass conversion, and LRT is the primary variable affecting product concentration. The simulation revealed the importance of multi-stage countercurrent reactor design. With constant liquid and solid residence times, the conversion and product concentration are dramatically improved with countercurrent operation.

Continuous Countercurrent Carboxylic Acid Fermentation

Loescher describes that high carboxylic acid concentrations impede biomass conversion, which is the basis for the use of countercurrent fermentation. Batch experiments were performed to analyze the effect of high carboxylic acid concentrations on biomass conversion. The same fermentor conditions shown in Table I-1 were used except the energy source was changed to rye grass. The results show that at low initial carboxylic acid concentrations (2.2 g/L) there was 80% conversion, and at high initial carboxylic acid concentrations (48.3 g/L) there was 32% conversion.

Similar batch fermentations were conducted with various pretreated (with $Ca(OH)_2$) and untreated substrate concentrations. Data showed untreated low substrate concentration (2.5 g/L) allowed high conversions (71%), and untreated high substrate concentrations (75 g/L) allowed low conversions (42%). However, with pretreated biomass, high conversions (> 80%) were achieved at substrate concentrations up to 25 g/L. This proves that high substrate concentrations lead to high carboxylic acid concentrations, which inhibit conversion. It was also concluded that pretreated biomass significantly increased conversion even with higher carboxylic acid concentrations.

The next experiment Loescher conducted was a countercurrent, anaerobic, four stage fermentation of pretreated rye grass and a bagasse alfalfa blend. Four 1-L reactors were used. The conditions were the same as shown in Table I-1, but rye grass and a bagasse alfalfa blend were used as a substrate, and changes were made with the inoculum at various times. Table I-2 shows the fermentation results.

Table I-2. Loescher: Countercurrent fermentation results.

Time(h)	0-355	355-628	628-1919	1919-3620	3620-4795	4795-5350	5350-7487	7487-8758	8758-1000
Substrate	RG	RG	RG	BA	BA	BA	BA	BA	BA
Substrate Conc. (g/L)	50	100	100	100	200	200	200	200	200
LRT (d)	8	8	12	12	12	12	12	12	12
SRT (d)	8	8	12	12	12	12	12	24	36
Inoculum	R	R	R	R	R	CM	CS	CS	CS
Conversion (%)	39	36	58	52	ND	42	18	36	30
Total Acid Conc. (g/L)	14	21	29	24	21	21	35	26	18

RG = Rye Grass
BA = Bagasse/Alfalfa
R = Rumen Fluid
CM = Compost Mix
CS = Compost/Swamp
ND = Not Determined

Loescher developed a CPDM model for the rye grass fermentation and achieved an explained residual value of 87.7%. The results of the CPDM model and Table I-2 show higher conversions and total acid concentrations were obtained with rye grass fermentation at constant substrate concentration, SRT, and LRT. In general, higher substrate concentrations, SRTs, and LRTs resulted in higher conversions and total acid concentrations.

Low pH

Further batch experiments were conducted to determine the effect of pH. The same fermentor conditions as shown in Table I-1 were used, except pretreated rye grass was used as an energy source and the pH was maintained at 5.8. The experimental data were used to develop a CPDM model. At low conversion (0 to 60%) and high carboxylic acid concentrations (>10 g/L), the reaction rate at a pH of 5.8 was 1% to 20% of the rate at a pH of 6.7. This suggests that the reaction was inhibited at a low pH. The inoculum used was taken from a rumen where the average pH is approximately 6.7, therefore the microorganisms may have been unable to adapt at a low pH.

Increased Nutrients

Batch experiments were conducted to show the effect of increased nutrients. The conditions were the same as shown in Table I-1 except that the energy source was pretreated rye grass, and various supplemental nutrients were added using the modified Caldwell and Bryant medium. CPDM was used to model the batch data. The experiment concluded that at high conversions (> 60%) and carboxylic acid concentrations up to 35 g/L, the reaction rate was increased by >20 fold with increased nutrients. At conversions below 60% the reaction rate was approximately the same. This implies that a lack of nutrients may contribute to the inhibition effects of high carboxylic acid concentrations.

Fermentation of Municipal Solid Waste and Sewage Sludge

Loescher conducted batch experiments to consider a combination of municipal solid waste (MSW) and municipal sewage sludge (SS) as a substrate. Twelve fermentations were run with various MSW and SS ratios. The fermentation conditions are shown in Table I-3.

Table I-3. Loescher: Conditions of MSW/SS fermentation.

Temperature	39 °C
pH	5.5-5.8
Carbohydrate Source	MSW
Nutrient Source	SS
Neutralizing Agent	$CaCO_3$
Inoculum	Steer Rumen Fluid
Methane Inhibitor	BES

The results of the experiment show acid concentrations were the highest (~21 g/L) with MSW/SS ratios between 60/40 and 80/20.

MSW had a low packing density, thus Loescher conducted another similar batch experiment using a mill to grind the solid waste to achieve higher substrate concentrations in the fermentors. The substrate ratio was 70/30 (MSW/SS) and used at a substrate concentration of 200 g/L. With a higher substrate concentration, a total acid concentration of approximately 25 g/L was achieved.

Loescher conducted other similar batch experiments and concluded that insufficient inoculum can lead to a lengthy lag phase in the fermentation. A long lag phase may also result from the microorganisms adapting to a lower pH. The effect of the addition of cellulose enzymes was also studied and shown to increase carboxylic acid production.

Conclusion

1. Loescher developed the CPDM model to simulate the kinetics of complicated biomass conversion in various reactor configurations. The details of the model were created in the computer program *Mathmatica*.
2. CPDM showed that countercurrent fermentation could suppress the natural inhibitory effect of high carboxylic acid concentrations on microorganisms, thus achieving higher acid concentrations and higher conversions.
3. With only a few weeks of batch data, the CPDM method was able to predict acid concentrations and conversions at varying SRTs and LRTs. This enabled the optimization of countercurrent fermentation to be done in a very short amount of time.
4. Loescher determined that mixed winter grass, rye grass, bagasse, municipal solid waste, and sewage sludge were all acceptable substrates for carboxylic acid fermentation.
5. With batch experiments, he also concluded that pretreatment and supplemental nutrients improved acid production, whereas a lower pH tended to inhibit acid production.

“Production of Acetic Acid from Waste Biomass”

The following is a summary of Michael Ross’s 1998 dissertation, “Production of Acetic Acid from Waste Biomass.” Ross explored the feasibility of using feedlot manure and municipal solid waste with sewage sludge (MSW/SS) as substrates in the fermentation to produce carboxylic acids. The CPDM method developed by Loescher (1996) was also used to model the manure and MSW/SS fermentations. Research was conducted to determine the suitability of using iodoform as a methane inhibitor.

CPDM Modifications

In Loescher's derivation of the CPDM method he made the assumption that the inhibitory effects of carboxylic acid concentrations were proportional to an acetic acid equivalent (aceq) concentration. Ross found in MSS/SS fermentation the acid product profiles changed with varying conditions. This means that the ratio of moles of acid to moles of aceq, ϕ, was not always constant. To correct this, Ross modified Loescher's governing rate equation (Equation I-22) to reflect inhibition as a function of total molar acid concentration, instead of acetate equivalents. Equation I-24 shows the modified governing rate equation (A_e = aceq).

$$\hat{r} = \frac{a(1-x)^b}{1 + cx^d + e(\phi A_e)^f} \tag{I-24}$$

When applying the CPDM method, Ross found that the model could be fit to the data in two ways. First, with the data from each batch experiment, an empirical equation describing the acid concentration as a function of time could be determined with the form

$$[A] = \frac{a \cdot t}{1 + b \cdot t} \tag{I-25}$$

Differentiating Equating I-25 gave the rate as a function of time, and using this information one could construct a table of rates as a function of concentration and conversion. The overall rate equation parameters could then be obtained by minimizing the squared difference between the model predictions and the rates from the constructed table. This was the basis of Loescher's method and was designated as Model Fit I.

Alternatively, the overall rate equation (Equation I-24) could be directly fit to the batch experimental data. With respect to time, the overall rate equation is a first-order, non-linear differential equation and could be integrated numerically to give the acid concentration (and conversion) as a function of time. The parameters could then be determined by minimizing the squared difference between the experimental acid concentrations and the concentrations predicted from the integrated model. This was designated as Model Fit II.

In the laboratory, Ross determined that countercurrent fermentation was better modeled by simulating a series of fed-batch reactors, and the CSTR model should be used to design industrial-scale fermentors. This was because the fermentors used in the laboratory were run semi-continuously, rather than continuously. Ross designated this configuration of batch reactors, pseudo-continuous. The governing equations for this configuration are

$$\frac{dA_e}{dt} = \sum_i Ci \cdot \hat{r}(x_i, A_e) \tag{I-26}$$

$$\frac{dx_i}{dt} = \frac{\hat{r}(x_i, A_e)}{s_o} \tag{I-27}$$

The traditional "continuum particle" *distribution function* could not be used in this case because the feed was not continuous, thus the distribution function was not continuous making it difficult to manipulate. To curb this problem, the distribution function became a table of conversion intervals that contained the amount of particles in each interval. The amount of solids retained in each fermentor was determined by

$$M = \sum_{i} (c_i \cdot (1 - \bar{x}_i)) \qquad \text{(I-28)}$$

where,

M = the mass of particles at the end of the batch fermentation period (g)

c_i = the amount of continuum particles in the conversion interval *i* after the new conversion distribution table is calculated (g)

$\bar{x}_i$ = the median conversion in the conversion interval *i* (dimensionless)

To solve these equations it was necessary to iterate until a constant acid concentration was obtained in each fermentor at the end of the batch period.

Countercurrent Fermentation of Municipal Solid Waste and Sewage Sludge

Ross used three different fermentor designs in the countercurrent fermentation experiments. The first was designated the “Magic Fingers” (MF) design, which was composed of a stainless steel cylinder containing a rotating shaft. The shaft was equipped with metal pins that extended radially along the length of the shaft. When rotated, the pins meshed with stationary pins attached to the cylinder walls. The MF design was intended to improve mixing, but the design also made operation cumbersome.

The second design, termed the Centrifuge Bottle Fermentor I (CB I), included a 1-L centrifuge bottle that rested in a roller bottle apparatus to rotate the fermentor. The bottle was sealed with a rubber stopper equipped with exhaust tubes and a mixing bar to stir the contents as the fermentor rotated. This design improved operational procedures, but there were problems with keeping the fermentor sealed.

The third design was the Centrifuge Bottle Fermentor II (CB II). With the exception of the leak prone exhaust tubes, the design was similar to the CB I. Rather than using the exhaust tubes, a septum was used to seal the bottle, and a syringe was used to sample the gas at designated time intervals. Because this design was permanently sealed, care had to be taken to keep the fermentors from over pressurizing.

The substrate mixture was an 80/20 ratio of MSW/SS, also used by Loescher (1996). The inoculum was rumen fluid from a forage-fed steer. The acids produced were neutralized by $CaCO_{3.}$ All fermentations were done in anaerobic conditions, and the transfers were based on keeping a constant mass of wet cake in each fermentor. Data were not collected until the fermentors reached a pseudo-steady state. The conditions and results for some experiments are shown in Table I-4.

Table I-4. Ross: MSW/SS countercurrent fermentation conditions and results.

Fermentation	B	D	E	F	G	H
Temp (°C)	40	40	40	40	40	40
Number of Stages	4	4	4	4	4	4
LRT (d)	12.9	14.7	13.7	19.3	27.7	12
VSLR (g VS/L of liq·d)	7.9	6.8	6.9	3.3	3.4	3.4
F1 solid conc. (g VS/L of liq)	128	94	96	97	130	92
F2-4 solid conc. (g VS/L of liq)	128	125	133	134	130	126
VS/liquid feed ratio (g/g)	0.070	0.070	0.070	0.052	0.070	0.035
Fermentor design	MF	CB II	CB II	CB II	MF	CB II
Liq feed @ transfer (mL)	200	200	200	200	200	200
Solid feed @ transfer (dry g)	20	20	20	15	20	10
Transfer frequency (days)	1	2	2	3	2	2
Medium	2	3	3	3	2	3
Nutrients	1,3,4	3	3,4	3,4	1	5
Inhibitor Addition	No	No	No	No	No	No
pH	6-6.2	6-6.2	6-6.2	6-6.2	6-6.2	6-6.2
Tot. acid production (g acid/(L of liq·d)	2.28	1.17	1.39	1.13	0.94	0.82
	± 0.3	± 0.3	± 0.4	± 0.1	± 0.3	± 0.2
Tot. acid conc. (g acid/L of liq)	28.7	17.2	19.0	21.7	26.1	9.9
	± 4.3	± 3.8	± 5.4	± 2.8	± 8.2	± 2.5
Yield (g acid/g VS fed)	0.29	0.17	0.20	0.34	0.28	0.25
CH_4 prod. (g/(L of liq·d))	ND	0.082	0.101	0.077	ND	0.075

1. Modified Caldwell and Bryant medium
2. Deoxygenated water
3. 0.15 g urea to each fermentor at each transfer
4. 0.20 g dry nutrients to each fermentor at each transfer
5. 0.10 g urea to each fermentor at each transfer

As shown in Table I-4, the highest total acid concentrations were obtained at high LRTs, and the highest acid productions were achieved at high VSLRs.

Ross then conducted batch experiments to obtain modeling data for CPDM. Batch fermentations were executed at various MSW/SS ratios. Caldwell and Bryant medium was used along with the addition of urea. CPDM was performed for the CSTR cascade and pseudo-continuous configurations. The results of the CPDM simulation were compared to Fermentations B and F and are shown in Table I-5.

Table I-5. Ross: MSW/SS CPDM results.

Fermentation	Fermentor	Total Acid Concentration (g/L)				
			Model Fit II		Model Fit I	
		Experimental Results	Pseudo-continuous	CSTR-cascade	Pseudo-continuous	CSTR-cascade
B	1	28.7	26.1	25.1	23.8	23.2
	2	21.4	22.3	20.9	22.2	21.7
	3	14.2	16.3	15.0	19.5	18.7
	4	8.6	8.3	7.2	14.5	13.3
F	1	21.7	22.3	23.1	24.1	24.0
	2	13.8	15.6	15.3	21.9	22.2
	3	5.4	8.2	7.9	19.0	17.9
	4	1.1	3.5	2.8	11.3	9.3

Table I-5 shows the countercurrent model agreed well with the pseudo-continuous model, which indicates that the laboratory fermentations are a good comparison to a continuous cascade of CSTRs. The Model Fit II agreed better with the experimental results than did Model Fit I.

Countercurrent Fermentation of Feedlot Manure

Ross also conducted fermentation experiments with feedlot manure (60% corn, 12% crude protein, and 28% cottonseed hull), which were performed similar to that in the MSW/SS fermentation. The feedlot manure was first pretreated with lime. The conditions and results are shown in Table I-6.

Table I-6. Ross: Feedlot manure countercurrent fermentation conditions and results.

Fermentation	A	B	C	D	E
Temp (^{o}C)	40	40	40	40	40
Number of Stages	4	4	4	4	4
LRT (d)	7.9	8.3	13.1	13.0	12.3
VSLR (g VS/L of liq·d)	11.2	14.7	14.4	14.6	4.3
F1 solid conc. (g VS/L of liq)	123	123	178	178	123
F2-4 solid conc. (g VS/L of liq)	123	123	178	178	178
VS/liquid feed ratio (g/g)	0.070	0.091	0.122	0.122	0.046
Fermentor design	CB I	CB I	CB II	CB II	CB II
Liq feed @ transfer (mL)	200	200	100	100	200
Solid feed @ transfer (dry g)	20	30	20	20	15
Transfer frequency (days)	1	1	1	1	2
Medium	1	1	2	2	2
Iodoform (mg/ fermentor/L liq to Fermentor 4)	0	0	0	4	2
pH	5.7-6	5.7-6	5.7-6	5.7-6	5.7-6
Productivity (g acid/(L of liq·d)	2.68 ± 0.6	2.98 ± 0.7	2.48 ± 0.3	2.65 ± 0.3	1.03 ± 0.3
Tot. acid conc. (g acid/L of liq)	21.3 ± 4.6	24.8 ± 5.6	32.5 ± 4.1	34.5 ± 4.1	14.3 ± 3.9
Yield (g acid/g VS fed)	0.24	0.20	0.17	0.18	0.24
CH_4 prod. (g/(L of liq·d))	ND	ND	0.036	ND	ND

1. Modified Caldwell and Bryant medium
2. Deoxygenated water

High acid concentrations and productivities were achieved at high VSLRs, as shown in Table I-6.

Batch experiments were then performed to develop a CPDM model. Model Fit I and Model Fit II methods returned very similar parameters, thus only one modeled equation was used. The results were compared to Fermentations C and E, as shown in Table I-7.

Table I-7. Ross: Feedlot manure CPDM results.

Fermentation	Fermentor	Total Acid Concentration (g acid/L of liq) Experimental Results	Pseudo-Continuous	CSTR Cascade
C	1	32.4	32.0	32.5
	2	15.4	27.0	28.4
	3	7.5	19.8	21.8
	4	3.0	11.8	11.8
E	1	14.3	18.4	17.8
	2	6.2	9.0	7.5
	3	3.3	4.7	3.0
	4	1.7	2.5	1.1

Table I-7 shows the models predicted an accurate concentration for F1, but was not as accurate in predicting the other fermentor concentrations. The *Mathmatica* program developed by Loescher (1996) was also used to predict conversions and acid concentrations at various VSLRs and LRTs.

Methanogen Inhibitor Study

The effect of adding methanogen inhibitor (iodoform) was studied by comparing the results of both MSW/SS and feedlot manure fermentations before and after iodoform addition. The addition of 2 and 4-mg iodoform per L of liquid fed to each fermentor was tested. The 2 mg addition resulted in a greater than three fold decrease in methane production, and 4 mg of iodoform completely inhibited methane production; however, iodoform was shown to lower acetate selectivity.

Thermophilic Culture Study

Ross also conducted an experiment to evaluate the effects of a thermophilic fermentation. The fermentation was operated under the same conditions as in MSW/SS

Fermentation D except with a thermophilic culture of microorganisms and elevated operating temperatures. The thermophilic fermentation (60 ^{o}C) was more selective to acetate (79%), but the yield was reduced by half when compared to the mesophilic fermentation. However, addition of nutrients did increase the yield and productivity. Decreasing the temperature from 60 ^{o}C to 55 ^{o}C also increased the yield and productivity, but slightly decreased the selectivity.

Conclusion

1. Ross proved that MSW/SS and feedlot manure were feasible fermentation substrates.
2. Higher total acid concentrations (34.5 g/L) and productivities (2.98 g/L liq·d) were obtained with feedlot manure fermentation. However, higher acid yields (0.34 g acid/g VS fed) were obtained with MSW/SS fermentations.
3. CPDM was used to model countercurrent fermentation and matched experimental data with success. CPDM also showed the effect of various VSLRs and LRTs on the acid concentrations and conversion of feedlot manure and MSW/SS fermentations.
4. Thermophilic fermentation was found to increases acetic acid selectivity.
5. Experiments also suggested that 4 mg of iodoform per L of liquid fed to each fermentor could completely eliminate methane production.

“Fermentation of Industrial Biosludge, Paper Fines, Bagasse, and Chicken Manure to Carboxylate Salts”

The dissertation, “Fermentation of Industrial Biosludge, Paper Fines, Bagasse, and Chicken Manure to Carboxylate Salts” by Susan Domke in 1999 will be summarized in the following text. Domke’s objective was to determine the viability of fermenting various mixtures of biosludge, paper fines, bagasse, and chicken manure to produce carboxylic acids. The optimal energy to nutrient ratio was also studied and fermentations were modeled with CPDM.

Batch Studies

By conducting batch experiments, Domke determined that biosludge was a feasible nutrient source, but required the addition of the Caldwell and Bryant medium to obtain the best results. The addition of urea was also shown to improve acid production. Batch studies were performed to determine the optimal ratio of paper and biosludge. These data showed that paper to biosludge ratios of 80/20, 60/40, and 40/60 produced similar results in batch fermentors.

80/20 Paper to Biosludge Countercurrent Fermentation

Domke used two different styles of fermentors. The first fermentor (A) was an upright, glass, 1-L beaker previously described by Loescher (1996). This fermentor design did not allow proper mixing. The second fermentor (B) was the same design that Ross (1998) designated as CB II and was able to achieve better mixing.

The fermentors were operated countercurrently with four stages and a paper to biosludge ratio of 80/20. In this case, paper was the energy source and biosludge was the nutrient source. In Fermentation 4b, biosludge was substituted with chicken manure. The biosludge and chicken manure were pretreated with lime. The acids were neutralized with $CaCO_3$ and the inoculum was rumen fluid. All fermentations were done under anaerobic conditions. In Fermentation 4a, the effect of additional lime pretreatment between F3 and F4 was also studied. The condition and results of each experiment are shown in Table I-8.

Domke found that additional pretreatment between F3 and F4 was not beneficial, as shown in Table I-8. Chicken manure was determined to be a feasible nutrient source and gave similar results when compared to the fermentations performed with biosludge.

Table I-8. Domke: 80/20 Paper/biosludge countercurrent conditions and results.

Fermentation	1	2	3	4	4a	4b
Temp (^oC)	40	40	40	40	40	40
Addition condition change	No	No	No	No	1	2
LRT (d)	12.9	16	20	25	28	24
VSLR (g VS/L of liq·d)	11.6	11.8	3.9	2.0	2.0	2.1
F1 solid conc. (g VS/L of liq)	164	174	205	169	159	153
F2 solid conc. (g VS/L of liq)	232	220	212	237	243	213
F3 solid conc. (g VS/L of liq)	328	230	343	328	296	338
F4 solid conc. (g VS/L of liq)	417	247	518	372	312	355
VS/liquid feed ratio (g/g)	0.16	0.17	0.1	0.04	0.04	0.06
Fermentor design	A	A/B	B	B	B	B
Liq feed @ transfer (L)	0.2	0.2	0.2	0.15	0.15	0.15
Solid feed @ transfer (dry g)	40	40	20	10.5	10.5	10.5
Transfer frequency (days)	2	2	3	3	3	3
Medium	DW	DW	DW	DW	DW	DW
Urea (g/fermentor/L liq fed to F4)	1.0	1.0	0.5	0.7	0.7	0.7
Inhibitor	BES	b	b	i	i	i
Inhibitor addition (mg/fermentor/L fed to F4)	1.4	2.0	4.0	4.0	4.0	4.0
pH	5.7	5.6	5.9	5.8	6.1	5.7
Tot. acid conc. (g acid/L of liq)	19.6	17.8	16.8	20.2	18.2	20.7
wt% acetic acid	57	41	39	40	39	39
Conversion (g VS digested/g VS fed)	0.31	0.29	0.49	0.50	ND	0.58
Selectivity (g acid/g VS digested)	0.4	0.34	0.43	0.78	ND	0.48
Yield (g acid/g VS fed)	0.12	0.10	0.21	0.39	ND	0.28
Acid productivity (g acid/(L liq·d))	1.4	1.3	0.9	0.8	0.7	0.6
CH_4 productivity (g CH_4/(L liq·d))	ND	ND	0.01	0.0	0.002	0.0

1 = additional pretreatment of solids between F3 and F4
2 = used chicken manure instead of biosludge
DW = Deoxygenated water
b = Bromoform
i = Iodoform
ND = Not Determined

40/60 Paper to Biosludge Countercurrent Fermentation

Based on batch data, considering countercurrent fermentation with a 40/60 paper to biosludge ratio seemed necessary. The fermentation experiments were conducted similar to those with an 80/20 ratio, but additional nutrients were added and other inoculum sources were used in some cases (see Table I-9). The biosludge was pretreated with lime, and the inoculum was rumen fluid. Table I-9 shows the fermentation conditions and results of several experiments.

Table I-9. Domke: 40/60 Paper/biosludge countercurrent conditions and results.

Fermentation	6	7	7a	8	8a	9	11
Temp (^{o}C)	40	40	40	40	40	40	40
Addition changes made	No	1	2	1	2	1	1
LRT (d)	15	14	12	10.6	10.4	13	27
VSLR (g VS/L of liq·d)	7.5	7.5	7.5	3.4	3.4	5.4	12.4
F1 solid conc. (g VS/L of liq)	211	208	307	224	252	237	288
F2 solid conc. (g VS/L of liq)	315	315	235	346	341	296	142
F3 solid conc.(g VS/L of liq)	285	293	252	345	372	597	147
F4 solid conc. (g VS/L of liq)	281	370	299	367	347	295	203
VS/liquid feed ratio (g/g)	0.09	0.09	0.09	0.04	0.04	0.07	0.09
Fermentor design	B	B	B	B	B	B	B
Liq feed @ transfer (L)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Solid feed @ transfer (dry g)	25	12	12	12	12	19	23.5
Transfer frequency (days)	2	2	2	2	2	2	2
Medium	DW	DW	DW	DW	DW	DW	DW
Urea (g/fermentor/L liq fed to F4)	0.75	0.5	0.5	0.5	0.5	0.5	0.5
Inhibitor	b	i	i	i	i	i	i
Inhibitor addition (mg/fermentor/L fed to F4)	2.0	2.0	2.0	2.0	2.0	4.0	4.0
pH	6.3	6.2	6.1	6.6	6.4	5.9	6.0
Tot. acid conc. (g acid/L of liq)	18.7	20.4	20.4	16.4	17.7	17	29.9
wt% acetic acid	44	46	46	51	47	45	47
Conversion (g VS digested/g VS fed)	0.27	0.22	0.28	0.49	0.47	0.41	0.23
Selectivity (g acid/g VS digested)	0.67	0.82	0.52	0.73	0.85	0.61	0.37
Yield (g acid/g VS fed)	0.18	0.18	0.14	0.36	0.40	0.25	0.25
Acid productivity (g acid/(L liq·d))	1.3	1.3	1.1	1.2	1.3	1.4	3.1
CH_4 productivity (g CH_4/(L liq·d))	0.042	ND	ND	0.042	0.021	0.029	ND

1 = inoculum was from rumen fluid, soil, compost, and swamp
2 = additional nutrients were supplied from Caldwell &Bryant medium (1.0 g dry nutrient mix/fermentor/L to F4), and inoculum was from rumen fluid, soil, compost, and swamp
DW = Deoxygenated water
b = Bromoform
i = Iodoform
ND = Not Determined

Supplemental nutrients did not drastically improve fermentation results, as shown in Table I-9. The fermentation with a 40/60 ratio achieved similar results when compared to the fermentation with an 80/20 ratio.

80/20 Bagasse to Chicken Manure Countercurrent Fermentation

Domke also conducted experiments to determine the viability of using bagasse as an energy source and chicken manure as a nutrient source in countercurrent fermentation. She used an 80/20 ratio of bagasse to chicken manure with rumen fluid as the inoculum source. The chicken manure was pretreated with lime and calcium carbonate was used as a neutralizing agent. The fermentation conditions and results are shown in Table I-10.

Table I-10. Domke: 80/20 Bagasse/chicken manure countercurrent conditions and results.

Fermentation	12	13	14	14a
Temp (^oC)	40	40	40	40
Addition changes made	No	No	No	1
LRT (d)	16.5	10	10.5	10.5
VSLR (g VS/L of liq·d)	5.9	4.5	5.0	4.8
F1 solid conc. (g VS/L of liq)	228	359	228	222
F2 solid conc. (g VS/L of liq)	237	479	349	322
F3 solid conc.(g VS/L of liq)	294	518	414	406
F4 solid conc. (g VS/L of liq)	331	199	415	420
VS/liquid feed ratio (g/g)	0.04	0.04	0.04	0.04
Fermentor design	B	B	B	B
Liq feed @ transfer (L)	0.17	0.09	0.12	0.12
Solid feed @ transfer (dry g)	8.3	4.2	5.5	5.5
Transfer frequency (days)	1	1	1	1
Medium	C&B	C&B	C&B	C&B
Urea (g/fermentor/L liq fed to F4)	0.88	1.7	1.3	1.3
Inhibitor	i	i	i	i
Inhibitor addition (mg/fermentor/L fed to F4)	9.0	9.0	7.0	7.0
pH	6.4	6.7	6.6	6.8
Tot. acid conc. (g acid/L of liq)	10.8	11.8	10.1	10.3
wt% acetic acid	35	35	33	34
Conversion (g VS digested/g VS fed)	0.44	0.53	0.5	ND
Selectivity (g acid/g VS digested)	0.52	0.43	0.46	ND
Yield (g acid/g VS fed)	0.23	0.23	0.23	ND
Acid productivity (g acid/(L liq·d))	1.3	1.0	1.1	ND
CH_4 productivity (g CH_4/(L liq·d))	0.011	0.002	0.004	0.010

1 = additional pretreatment between F3 and F4
C&B = Modified Caldwell and Bryant medium
i = Iodoform
ND = Not Determined

In the bagasse/chicken manure experiments, the results showed that additional pretreatment between F3 and F4 was not beneficial, as can be seen in Table I-10. The bagasse/chicken manure fermentation also produced lower acid concentrations than the paper/biosludge fermentation.

Applying CPDM

Domke used CPDM to model the above countercurrent fermentations. Batch experiments were conducted at various substrate concentrations to obtain the data to model the paper/biosludge and bagasse/chicken manure systems. Domke simplified the method developed by Loescher (1996) and Ross (1998). Each batch experiment was fit to the equation

$$\text{aceq} = a + \frac{bt}{1+ct} \tag{I-29}$$

where aceq is the acetic acid equivalent concentration. The rate was then determined by the equation

$$r = \text{rate} = \frac{d(aceq)}{dt} = \frac{b}{(1+ct)^2} \tag{I-30}$$

The rate was converted into a specific rate, $\hat{r}_{calc}$, by dividing by the initial substrate present, S_o.

$$\hat{r}_{calc} = \frac{r}{S_o} \tag{I-31}$$

Then, using aceq from Equation I-29 and the conversion, x, calculated from experimental data, a least squares fit was conducted using Equations I-31 and I-32 to determine the parameters $e, f, g,$ and h.

$$\hat{r}_{pred} = \frac{e(1-x)^f}{1+g(\phi \cdot aceq)^h} \tag{I-32}$$

Equation I-32 was the governing rate equation that described the effect of conversion and product concentration. The numerator describes the conversion "penalty" function, and the denominator describes the inhibitory effect of the product concentration on the microorganisms as explained by South and Lynd (1994). Table I-11 shows the experimental data and the predicted results for some of Domke's countercurrent fermentations.

Table I-11. Domke: CPDM results.

Fermentor Condition	Fermentor	Experiment Tot. Acid Conc. (g/L)	CPDM Tot. Acid Conc. (g/L)
3	1	16.8	14.2
	2	14.7	10.4
	3	14.7	7.0
	4	9.8	3.6
	conversion	0.49	0.54
6	1	18.7	20.1
	2	13.3	15.2
	3	6.8	10.2
	4	3.7	5.6
	conversion	0.27	0.34
9	1	17.0	17.6
	2	12.5	12.2
	3	7.1	8.9
	4	3.4	4.6
	conversion	0.41	0.45
11	1	29.9	30.1
	2	23.1	28.1
	3	16.8	23.1
	4	10.5	13.2
	conversion	0.23	0.19
13	1	11.8	11.4
	2	4.6	9.1
	3	1.3	7.7
	4	0.6	4.9
	conversion	0.53	0.29

As shown in Table I-11, the model accurately predicted the experimental acid concentrations and conversions. Domke also used the rate models to predict acid concentrations and conversions at various VSLRs and LRTs using the CPDM program developed by Loescher (1996).

Conclusion

1. The paper/biosludge fermentation was found to be feasible. Various acid concentrations and conversions were obtained by varying the LRT and VSLR.
2. CPDM predicted acid concentrations in F1 within 10% in the paper/biosludge fermentations.
3. The CPDM model also predicted that high conversions and acid concentrations could be achieved with both paper/biosludge fermentations.
4. When biosludge was replaced with chicken manure, similar results were achieved.
5. An 80/20 ratio of paper to biosludge may be more beneficial than the 40/60 ratio, based on CPDM.
6. The level of methane was controlled well in the paper/biosludge fermentation with iodoform.
7. The bagasse/chicken manure fermentations were not as successful as the paper and biosludge.
8. With CPDM, F1 acid concentrations and conversions were predicted with less accuracy in the bagasse/chicken manure fermentations.

9. The CPDM method also predicted that the bagasse/chicken manure system could not reach an economical acid concentration of 22 g/L (Holtzapple *et al.*, 1999).
10. Bagasse/chicken manure fermentations also tended to produce higher order acids.
11. Large amounts of iodoform were required to suppress methane production in the fermentation of bagasse and chicken manure.
12. In general, supplemental nutrients were not necessary as long as urea was added.
13. Additional pretreatment between F3 and F4 was not beneficial.

“Conversion of Bagasse and Corn Stover to Mixed Carboxylic Acids Using a Mixed Culture of Mesophilic Microorganisms”

The following text summarizes the dissertation, “Conversion of Bagasse and Corn Stover to Mixed Carboxylic acids Using a Mixed Culture of Mesophilic Microorganisms” by Piyarat Thanakoses in 2002. Thanakoses describes the anaerobic, countercurrent fermentation of bagasse and corn stover under mesophilic conditions (40 ^{o}C). Nutrients were supplied by chicken and pig manure. Pretreatments with lime and sonication were also studied.

Batch Experiments

Batch studies were performed to achieve the optimal ratio of bagasse/chicken manure and corn stover/pig manure. Bagasse and corn stover were the energy sources and chicken and pig manure were the nutrient sources. Thanakoses found in both cases a 40/60 ratio of energy source to nutrient source provided the best results. However, she explained that in the United States, large amount of bagasse and corn stover are available making it more appropriate to have a higher energy to nutrient source ratio. For this reason, 80/20 ratios of bagasse/chicken manure and corn stover/pig manure were used throughout her work.

Batch experiments were also conducted to determine the effect of sonication on biomass digestion. Sonication was thought to break up inactive or dead microorganisms that may form around biomass. Research determined that sonication with a Fisher sonic dismembrator Model 300 at a power of 60 for 15 minutes was beneficial to biomass digestion.

Bagasse/Chicken Manure Fermentation

The countercurrent fermentor design was the same as the CB II used by Ross (1998). Bagasse and chicken manure were used with a ratio of 80/20, and the operating temperature was 40 ^{o}C. The inoculum used was terrestrial and included rumen fluid and compost material. The neutralizing agent was calcium carbonate. The medium was the modified Caldwell & Bryant medium. Iodoform was used as a methanogen inhibitor, and all fermentations were done under anaerobic conditions. Data were not collected until the system reached steady state. Table I-12 shows other experimental conditions and the results with various VSLRs and LRTs.

Table I-12. Thanakoses: 80/20 Bagasse/chicken manure countercurrent fermentation conditions and results.

Fermentation	A	C	D	F	I	J	L
Temp (^{o}C)	40	40	40	40	40	40	40
LRT (d)	11.7	13.5	9.7	13.1	12.1	20.5	20.0
VSLR (g VS/L of liq·d)	10.1	17.9	11.2	10.1	3.81	2.13	4.82
F1 solid conc. (g VS/L of liq)	119	124	98	124	127	131	159
F2-4 solid conc. (g VS/L of liq)	119	124	98	124	127	131	159
VS/liquid feed ratio (g/g)	0.093	0.131	0.081	0.091	0.039	0.039	0.083
Liq feed @ transfer (L)	0.1	0.15	0.15	0.08	0.10	0.10	0.15
Solid feed @ transfer (dry g)	11.7	25.0	15.6	9.3	5.0	5.00	15.0
Transfer frequency (days)	1	1	1	1	1	2	2
Medium	C&B	C&B	C&B	C&B	C&B	C&B	C&B
Urea (g/fermentor/L liq fed to F4)	1.00	0.67	0.67	1.25	1.0	1.0	0.67
Iodoform (mg/fermentor/L fed to F4)	16.0	10.7	5.3	20.0	24.0	24.0	10.7
pH	6.2	6.4	6.1	6.4	6.4	6.3	6.2
Tot. acid conc. (g acid/L of liq)	14.2	21.0	15.2	20.0	9.6	13.2	13.1
wt% acetic acid	35.7	39.6	35.6	40.1	35.4	35.1	38.5
Conversion (g VS digested/g VS fed)	0.43	0.413	0.385	0.438	0.538	0.600	0.484
Selectivity (g acid/g VS digested)	0.275	0.210	0.362	0.344	0.386	0.417	0.258
Yield (g acid/g VS fed)	0.118	0.087	0.139	0.151	0.208	0.250	0.125
Acid productivity (g acid/(L liq·d))	1.22	1.55	1.56	1.51	0.791	0.547	0.623
CH_4 productivity (g CH_4/(L liq·d))	0.0	0.0	0.0028	ND	0.0001	0.0001	0.0004

ND = Not determined
C&B = Caldwell & Bryant

Thanakoses found the productivity increased with high VSLRs, and the selectivity, yield, and conversion decreased, as seen in Table I-12.

Countercurrent fermentation studies were also conducted to analyze the effect of additional lime pretreatment between F3 and F4. Fermentations N and P used additional lime pretreatment and were performed under the same conditions as the above Fermentations D and J (see Table I-13). When comparing the results to Fermentations D and J, the acid concentration, productivity, selectivity, yield, and conversion were higher with additional lime pretreatment between F3 and F4.

Countercurrent experiments were also performed to study the effect of a combination of marine and terrestrial inocula sources at constant VSLRs and LRTs. The terrestrial inoculum was from rumen fluid and compost material and the marine inoculum was from sediment obtained from Galveston Bay. Fermentations AE and AF were identical to Fermentation I, and Fermentations AG and AH were identical to Fermentation J except for the inoculum source (see Table I-13). The acid productivity, selectivity, and yield were higher when compared to Fermentations I and J with solely terrestrial inoculum. In fermentations with 100% marine inoculum (AE and AH), the productivity, selectivity, and yield were slightly higher than the fermentations with 40% marine inoculum (AF and AG). At a higher VSLR and LRT, the fermentations with marine inoculum (AE, AF, AG, and AH) had higher conversions than fermentations with only terrestrial inoculum (I and J). CPDM was also used to confirm that marine inoculum increased acid concentrations and conversions.

Table I-13. Thanakoses: 80/20 Bagasse/chicken manure countercurrent fermentation conditions and results of additional experiments.

Fermentation	N	P	AE	AF	AG	AH
Temp (^{o}C)	40	40	40	40	40	40
Additional Condition	PT	PT	M	M/T	M	M/T
LRT (d)	9.7	20.5	12.1	12.1	20.5	20.5
VSLR (g VS/L of liq·d)	11.2	2.13	3.83	3.84	2.13	2.13
F1 solid conc. (g VS/L of liq)	100	130	128	128	127	128
F2-4 solid conc. (g VS/L of liq)	100	130	128	128	127	128
VS/liquid feed ratio (g/g)	0.081	0.039	0.039	0.039	0.039	0.039
Liq feed @ transfer (L)	0.15	0.10	0.1	0.1	0.1	0.1
Solid feed @ transfer (dry g)	15.6	5.00	5.0	5.0	5.0	5.0
Transfer frequency (days)	1	2	1	1	2	2
Medium	C&B	C&B	C&B	C&B	C&B	C&B
Urea (g/fermentor/L liq fed to F4)	0.67	1.0	1.0	1.0	1.0	1.0
Iodoform (mg/fermentor/L fed to F4)	5.3	25.0	24	24	24	24
pH	6.2	6.2	6.3	6.3	6.3	6.3
Tot. acid conc. (g acid/L of liq)	18.1	18.6	16.2	14.0	19.7	18.8
wt% acetic acid	39.6	33.4	45.5	44.9	45.1	44.9
Conversion (g VS digested/g VS fed)	0.410	0.630	0.538	0.556	0.760	0.695
Selectivity (g acid/g VS digested)	0.420	0.548	0.667	0.553	0.559	0.554
Yield (g acid/g VS fed)	0.172	0.345	0.359	0.308	0.425	0.385
Acid productivity (g acid/(L liq·d))	1.92	0.753	1.38	1.18	0.928	0.841
CH_4 productivity (g CH_4/(L liq·d))	0.0082	0.0002	0.0	0.0002	0.0002	0.0011

C&B = Caldwell & Bryant
PT = additional pretreatment between F3 and F4
M = 100% marine inoculum
M/T = 40% marine and 60% terrestrial inocula

Thanakoses also performed studies to analyze the effect of iodoform addition rate on acid and methane productions at constant VSLR and LRT. Data showed that methane production decreased with increasing inhibitor addition, but acid production was not affected.

Another experiment was conducted to evaluate the effect of pretreatment with air and lime on biomass before it is fermented. Studies were performed on pretreatment time and lime loading. She used a PVC pipe apparatus that contained various mixtures of lime and biomass with water saturated air circulated through it. The first study used a constant lime loading of 0.4 g/g dry biomass and pretreatment times of 2, 4, 8, 12, and

16 weeks. The second study used a constant pretreatment time of 4 weeks and lime loadings of 0.1, 0.15, 0.3, and 0.4 g/g dry biomass. Batch fermentations were used to develop a CPDM model to predict the effects. The results showed that at low VSLR (2 g/(L·d)) and low LRT (5 days), increased pretreatment time and lime loading did not affect acid concentration or conversion. However, at high VSLR (18 g/(L·d)) and high LRT (20.5 days), the acid concentration and conversion increased with pretreatment time and lime loading.

Corn Stover/Pig Manure Fermentation

For the fermentation of corn stover/pig manure the fermentor design was the same as in the bagasse/chicken manure fermentations. Corn stover and chicken manure were used with a ratio of 80/20, and the operating temperature was 40 °C. All fermentations were done under anaerobic conditions. The inoculum used was terrestrial and included rumen fluid and compost material. The neutralizing agent was calcium carbonate, and the medium was the modified Caldwell & Bryant medium. Iodoform was used as a methanogen inhibitor. Data were not collected until the system reached steady state. Table I-14 shows other experimental conditions and the results with various VSLRs and LRTs.

Table I-14. Thanakoses: 80/20 Corn stover/pig manure countercurrent fermentation conditions and results.

Fermentation	CP-A	CP-C	CP-F	CP-G	CP-H
Temp (^{o}C)	40	40	40	40	40
LRT (d)	11.2	11.0	20.54	15.9	24.9
VSLR (g VS/L of liq·d)	7.56	4.10	2.22	1.59	7.61
F1 solid conc. (g VS/L of liq)	98	95	133	97	141
F2-4 solid conc. (g VS/L of liq)	121	128	133	97	141
VS/liquid feed ratio (g/g)	0.079	0.050	0.020	0.039	0.157
Liq feed @ transfer (L)	0.2	0.2	0.10	0.2	0.1
Solid feed @ transfer (dry g)	20.0	10.0	5.0	5.0	20.0
Transfer frequency (days)	2	2	2	2	2
Medium	C&B	C&B	C&B	C&B	C&B
Urea (g/fermentor/L liq fed to F4)	0.50	0.50	1.0	0.50	1.0
Iodoform (mg/fermentor/L fed to F4)	12.0	12.0	24.0	12.0	24.0
pH	6.2	6.1	6.0	6.0	6.0
Tot. acid conc. (g acid/L of liq)	20.4	17.5	21.4	16.0	24.4
wt% acetic acid	42.2	42.2	37.6	39.8	40.9
Conversion (g VS digested/g VS fed)	0.468	0.613	0.765	0.770	0.460
Selectivity (g acid/g VS digested)	0.432	0.571	0.588	0.714	0.326
Yield (g acid/g VS fed)	0.203	0.350	0.450	0.550	0.150
Acid productivity (g acid/(L liq·d))	1.51	1.46	1.02	0.890	1.15
CH_4 productivity (g CH_4/(L liq·d))	0.0002	0.0042	0.0002	0.0008	0.0010

ND = Not determined
C&B = Caldwell & Bryant

As was shown in the experiments with bagasse/chicken manure, the productivity increased with VSLR, and the selectivity, yield, and conversion decreased (see Table I-14).

Thanakoses also conducted experiments with additional lime pretreatment between F3 and F4 on corn stover and pig manure and confirmed that it was beneficial. She also showed that pretreatment with sonication increased acid concentrations and conversions.

CPDM Application

Thanakoses conducted batch experiments for all substrates to develop a CPDM model as described by Loescher (1996) and Ross (1998). A comparison between some CPDM predictions and experimental data are depicted in Table I-15.

Table I-15. Thanakoses: CPCM results.

Fermentation	Exp. Acid Conc. (g/L)	Predicted Acid Conc. (g/L)	Error (%)	Experimental Conversion	Predicted Conversion	Error (%)
D	15.2	16.1	5.8	0.385	0.395	2.6
F	20.0	17.9	-10.6	0.438	0.373	-14.8
L	13.0	13.7	5.4	0.484	0.693	43.2
CP-A	20.4	18.7	-8.3	0.568	0.440	-6.0
CP-F	21.4	18.7	-12.6	0.765	0.770	0.7
CP-G	15.9	12.1	-23.9	0.770	0.886	15.1

In the fermentation of bagasse and chicken manure, the acid concentrations and conversions were predicted with an average error of 20.1% and 24.1%, respectively, as shown in Table I-15. For the corn stover and pig manure fermentations, the average errors in acid concentration and conversion were 13.4% and 11.66%, respectively.

Conclusion

1. Thanakoses determined that 80/20 ratios of bagasse/chicken manure and corn stover/pig manure were ideal.
2. With CPDM, Thanakoses found that bagasse/chicken manure fermentation was successful, even after Domke's (1999) predictions concluded that it was not. Thanakoses suggested the reason for this discrepancy was because Domke used rumen fluid as an inoculum source in her model development, whereas

Thanakoses used microorganisms from previous experiments that had already adapted to the environment. Therefore, Domke's predictions were less accurate.

3. Corn stover and pig manure were viable substrates.
4. Additional lime pretreatment between F3 and F4 was found beneficial unlike Domke's (1999) experiments. Thanakoses explained that poor mass balance closure in Domke's experiments was a contributing factor.
5. Fermentations with marine inoculum were more productive than with solely terrestrial inoculum.
6. Acid concentrations and conversions were increased with high lime loading rates and longer pretreatment times when VSLRs were high.
7. Sonication was another beneficial pretreatment procedure.

"Thermophilic Anaerobic Fermentation of Waste Biomass for Producing Acetic Acid"

The following is a summary of Wen Ning Chan's 2002 dissertation, "Thermophilic Anaerobic Fermentation of Waste Biomass for Producing Acetic Acid." Chen studied thermophilic fermentation (55 ^{o}C), as opposed to mesophilic fermentation (40 ^{o}C) because of the ability to increase acetic acid selectivity and reaction rate. Municipal solid waste/municipal sewage sludge (MSW/SS) and corn stover/pig manure (CS/PM) were used as substrates. Fermentations were also modeled with CPDM to analyze the effect of various VSLRs and LRTs.

Batch Studies

Chan conducted various batch experiments to gain information on thermophilic fermentation of MSW/SS and CS/PM. The optimal nutrient (dry Caldwell and Bryant medium) addition rate was found to be 0.67 g/(L liq·d). The optimal urea addition rate was determined to be 0.33 g/(L liq·d). Studies also showed that iodoform was a better methanogen inhibitor than 2-bromoethane-sulfonic acid (BES), but the addition of iodoform did not improve total acid concentration or selectivity. With batch experiments, it was also concluded that fermentation of corn stover/pig manure (CS/PM) produced higher total acid concentrations, but had lower acetic acid selectivity when compared to municipal solid waste/sewage sludge (MSW/SS) fermentation.

40/60 Corn Stover to Swine Manure Countercurrent Fermentation

Studies were conducted on thermophilic countercurrent fermentation of a 40/60 ratio of corn stover and pig manure, which was found to be optimal by Black (2000). The fermentor design was described by Ross (1998) and consisted of a sealed 1-L polypropylene centrifuge bottle that was rotated to achieve good mixing. The medium used was deoxygenated water with the addition of various nutrients (Caldwell and Bryant medium). The inoculum was terrestrial and was from rumen fluid, compost piles, and lake sediment. The acids were neutralized by the addition of calcium carbonate. The corn stover was pretreated with lime, and iodoform was used as the methanogen inhibitor. Data were not taken until steady state was reached. The conditions and results are shown in Table I-16.

Table I-16. Chan: 40/60 CS/PM countercurrent conditions and results.

Fermentation	CP1	CP2	CP3	CP4
Temp (^{o}C)	55	55	55	55
LRT (d)	13	12	14	15
VSLR (g VS/L of liq·d)	7.2	3.5	6.5	2.8
F1 solid conc. (g VS/L of liq)	231	321	328	344
F2 solid conc. (g VS/L of liq)	345	231	229	236
F3 solid conc.(g VS/L of liq)	364	269	254	258
F4 solid conc. (g VS/L of liq)	443	389	357	370
VS/liquid feed ratio (g/g)	0.08	0.04	0.08	0.04
Liq feed @ transfer (L)	0.2	0.16	0.14	0.2
Solid feed @ transfer (dry g)	20	8	15	10
Transfer frequency (days)	2	2	3	3
Nutrient (g/fermentor/L liq fed to F4)	1.0	1.25	1.4	1.0
Urea (g/fermentor/L liq fed to F4)	0.5	0.6	0.7	0.5
Iodoform (mg/fermentor/L fed to F4)	12	12	12	12
pH	6.0	6.0	6.0	6.0
Tot. acid conc. (g acid/L of liq)	18.3	17.0	25.1	15.2
wt% acetic acid	54.5	65.8	56.2	70.6
Conversion (g VS digested/g VS fed)	0.55	0.73	0.61	0.62
Selectivity (g acid/g VS digested)	0.35	0.51	0.44	0.56
Yield (g acid/g VS fed)	0.19	0.37	0.26	0.38
Acid productivity (g acid/(L liq·d))	1.5	1.3	1.8	0.98
CH_4 productivity (g CH_4/(L liq·d))	0.001	0.001	0.001	0.001

Chan evaluated the effects of differing VSLRs and concluded that with an increasing VSLR, acid productivity increases, and selectivity, conversion, and yield decrease, as shown in Table I-16.

80/20 Municipal Solid Waste to Sewage Sludge Countercurrent Fermentation

Chan used a MSW/SS ratio of 80/20 that was also used by Ross (1998). The MSW was pretreated with lime. The fermentation was done under the same conditions as described in the CS/SM fermentation with various LRTs and VSLRs. Numerous experimental conditions and results are represented in Table I-17.

Table I-17. Chan: 80/20 MSW/SS countercurrent conditions and results.

Fermentation	MS2	MS3	MS4	MS5	MS7	MS8	MS10
Temp (°C)	55	55	55	55	55	55	55
LRT (d)	21	23	19	19	29	25	21
VSLR (g VS/L of liq·d)	6.8	3.4	1.3	4.0	5.4	2.6	2.8
F1 solid conc. (g VS/L of liq)	173	172	225	202	188	171	194
F2 solid conc. (g VS/L of liq)	166	199	351	258	153	179	232
F3 solid conc.(g VS/L of liq)	197	286	389	355	159	227	310
F4 solid conc. (g VS/L of liq)	223	351	454	581	235	321	374
VS/liquid feed ratio (g/g)	0.11	0.06	0.02	0.06	0.1	0.06	0.06
Liq feed @ transfer (L)	1.1	1.14	0.98	0.96	1.2	0.97	1.1
Solid feed @ transfer (dry g)	20	15	5	10	25	10	12
Transfer frequency (days)	2	3	3	2	3	3	3
Nutrient (g/fermentor/L liq fed to F4)	1.4	1.0	1.25	1.4	1.25	1.25	1.25
Urea (g/fermentor/L liq fed to F4)	0.7	0.5	0.63	0.7	0.6	0.6	0.6
Iodoform (mg/fermentor/L fed to F4)	12	12	12	12	12	12	12
pH	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Tot. acid conc. (g acid/L of liq)	20.5	13.5	10.7	16.9	14.7	15.5	13.5
wt% acetic acid	68.2	71.1	86.4	73.2	64.3	80.2	79.9
Conversion (g VS digested/g VS fed)	0.28	0.38	0.69	0.44	0.30	0.45	0.44
Selectivity (g acid/g VS digested)	0.52	0.47	0.63	0.65	0.37	0.53	0.53
Yield (g acid/g VS fed)	0.15	0.18	0.41	0.29	0.11	0.24	0.23
Acid productivity (g acid/(L liq·d))	1.0	0.61	0.58	1.1	0.53	0.64	0.63
CH_4 productivity (g CH_4/(L liq·d))	0.001	0.001	0.001	0.001	0.001	0.001	0.001

With MSW/SS fermentation, it was also concluded that with an increasing VSLR, acid productivity increased, and selectivity, conversion, and yield decreased (see Table I-17).

80/20 Municipal Solid Waste to Sewage Sludge Countercurrent Fermentation with the Addition of Marine Inocula

Chan conducted countercurrent fermentation to determine the effect of marine inoculum. There were two fermentations performed, one using marine inoculum only (obtained from saltwater swamps), and one using marine and terrestrial inocula (obtained from rumen fluid, compost piles, and lake sediment). The conditions were the same as Fermentation MS3 with the exception of the source of the inoculum. Table I-18 compares the two experiments with MS3 (terrestrial only).

Table I-18. Chan: 80/20 MSW/SS countercurrent conditions and results with marine inoculum.

Fermentation	Marine Only Inoculum	Marine + Terrestrial Inocula	Terrestrial Only Inoculum
pH	6.0	6.0	6.0
Tot. acid conc. (g acid/L of liq)	13.5	12.8	13.5
wt% acetic acid	77.2	79.5	71.1
Conversion (g VS digested/g VS fed)	0.50	0.41	0.38
Selectivity (g acid/g VS digested)	0.39	0.44	0.47
Yield (g acid/g VS fed)	0.19	0.18	0.18
Acid productivity (g acid/(L liq·d))	0.65	0.62	0.61
CH_4 productivity (g CH_4/(L liq·d))	0.001	0.001	0.001

All three experiments produced very similar results, with the most noticeable difference being a slight variation in conversions, as shown in Table I-18.

CPDM

Chan conducted batch experiments to construct CPDM models of the countercurrent fermentations. The results of some the CPDM models are presented in Table 1-19.

Table I-19. Chan: CPCM results.

Fermentation	Exp. Acid Conc. (g/L)	Predicted Acid Conc. (g/L)	Error (%)	Experimental Conversion	Predicted Conversion	Error (%)
CP2	17.0	14.2	-16.5	0.73	0.80	9.6
CP4	15.2	15.0	-1.3	0.62	0.84	35.5
MS2	20.5	20.5	0.0	0.28	0.27	-3.6
MS5	16.9	14.5	10.8	0.44	0.36	-18.2
MS8	15.5	14.6	5.8	0.45	0.42	-6.8
MS10	13.5	13	3.7	0.44	0.41	-6.8

The model predicted acid concentrations better than conversions, as seen in Table I-19. Using the rate models she used the CPDM program developed by Loescher (1996) to develop a map of predicted conversions and product concentrations with various VSLRs and LRTs. The maps can be used to obtain the optimal operating parameters to achieve the desired acid concentration and conversion based on the substrate used. The CPD model of CS/PM fermentation showed that higher conversions and acid concentrations were obtained when compared to the MSW/SS fermentations. The map of MSW/SS fermentations with marine inoculum showed better results than with terrestrial inoculum only.

Conclusion

1. Thermophilic fermentation can achieve higher acetic acid selectivity than mesophilic fermentation.
2. For MSW/SS at high substrate concentrations, higher conversions and higher acid concentrations are attainable with thermophilic fermentation.
3. There was an inverse relationship between VSLR and selectivity with thermophilic fermentation, but with mesophilic fermentation, this relationship was not as evident.
4. Thermophilic fermentations tended to produce more methane than mesophilic systems.
5. Thermophilic CS/PM fermentations achieved higher acid concentrations and conversion than the thermophilic MSW/SS systems.

6. Fermentation results using marine inoculum versus terrestrial inoculum were improved, which could be due to the microorganism's ability to adapt better to saline conditions.
7. Iodoform was the best methanogen inhibitor.

Conclusion of Literature Review

Laboratory studies of the fermentation of biomass to produce carboxylic acids have been very successful. High acid concentrations and high conversions were obtained for a variety of substrates. The following is a list of general findings made in laboratory research.

1. The fermentation of biomass to carboxylic acids was optimal when there was a sufficient nutrient and energy source. The ratio varies depending on the substrates used.
2. In general, an 80/20 ratio of energy to nutrient source was ideal.
3. Municipal solid waste, municipal sewage sludge, bagasse, rye grass, swine and chicken manure, feedlot manure, corn stover, and paper were all feasible fermentation substrates.
4. If the substrates were rich enough in nutrients, additional nutrients (Caldwell and Bryant medium, and urea) were not necessary.
5. Countercurrent fermentation allowed higher acid concentrations and higher biomass conversions.

6. In general, high VSLRs allowed high acid concentrations but low conversions, and low VSLRs allowed high conversions but low acid concentrations.
7. High LRTs also allowed high acid concentrations.
8. Iodoform was determined to be the best methanogen inhibitor.
9. In general, the optimal pH was between 5.8 and 6.2.
10. Thermophilic fermentation conditions (55 ^{o}C) allowed for higher acetic acid selectivity.
11. Pretreatment with increased lime loading and pretreatment times increased biomass digestibility.
12. Additional pretreatment (lime and sonication) between F3 and F4 improved biomass digestibility.
13. CPDM was a powerful tool to determine optimal operating conditions for countercurrent fermentation with a variety of substrates in a short amount of time.

Table I-20 shows some of the highest acid concentrations and conversions obtained in the laboratory using countercurrent fermentation with various substrates. It is important to note that CPDM predicts that higher acid concentrations and conversions are possible at VSLRs and LRTs that were not practical to study on a laboratory scale. In general, higher acid concentrations were achieved at high VSLRs and/or high LRTs, and higher conversions were obtained with low VSLRs and high LRTs.

Table I-20. Summary of results of countercurrent fermentation in the laboratory.

Substrate	Ratio	Conversion (g VS digested/g VS fed)	Acid Conc. (g/L)	VSLR (g VS/L of liq·d)	LRT (d)
MSW/SS	80/20	ND	26.1	3.4	27.7
MSW/SS	80/20	0.69	10.7	1.3	19.0
Feedlot manure	100	ND	34.5	14.6	13.0
Paper/biosludge	80/20	0.50	20.7	2.1	24.0
Paper/biosludge	40/60	0.23	29.9	12.4	27.0
Paper/biosludge	40/60	0.49	16.4	3.4	10.6
Bagasse/chicken manure	80/20	0.41	21.0	17.9	13.5
Bagasse/chicken manure	80/20	0.76	19.7	2.13	20.5
Corn stover/pig manure	80/20	0.77	16.0	1.59	15.9
Corn stover/pig manure	80/20	0.46	24.4	7.61	24.9

INTRODUCTION TO PILOT-SCALE CARBOXYLIC ACID FERMENTATION

Laboratory studies have shown that carboxylic acid fermentation can be successfully performed. The next logical step before industrialization is to prove that fermentation can be performed on a larger scale. The research team of Dr. Mark Holtzapple has constructed a pilot plant on Texas A&M property next to the University Services Building.

The fermentors were designed and built in 1998 by Holtzapple and Praveen Vadlani, a post doctoral student for Dr. Holtzapple. The design consisted of four insulated, 1050-gallon fermentors. Material was mixed and moved by sludge pumps, and the temperature was controlled by a hot-water jacket. A more detailed description of the original fermentor design is described in Chapter III.

Vadlani operated the fermentors for several months in batch mode, but was never able to establish temperature control or operate continuously. The original design had flaws due to phase stratification behavior of the substrate; these problems are outlined in Chapter IV.

This research focused on achieving a working pilot plant-scale fermentation process. Many design improvements were necessary to accomplish countercurrent operation. This research describes the problems that were encountered and how they were solved.

CHAPTER II

MATERIALS AND METHODS

This chapter describes the fermentation substrates and how and where they were obtained. The fermentation conditions, design, and operation will also be discussed.

FERMENTATION INGREDIENTS

The substrates were office paper and chicken manure, and water was used as the fermentation medium. Calcium carbonate was used as the neutralizing agent. The inocula were from marine and terrestrial environments. Iodoform was used to inhibit methanogens.

Office Paper

The office paper was obtained from Document Destruction Inc., a document destruction company in Houston,. Document destruction companies typically shred proprietary company documents and then sell the paper to recycling companies. The document destruction company was contracted to ensure complete destruction of the documents; therefore, most companies would not relinquish the paper due to legal reasons. Document Destruction Inc. was the only company that agreed to sell the paper.

Eight tons of paper were delivered via truck to the pilot plant in the form of 1,200-lb bails. The paper was purchased for $0.12/ lb, and was stored in the loading dock area of the University Services Building next to the pilot plant. Eight tons were predicted to be sufficient to load the fermentors and to operate continuously for 6 months.

During the process of making paper it is pretreated, thus making further pretreatment unnecessary. Domke (1999) found that average office paper contained 0.14 g ash/g dry paper, thus 14% was indigestible.

Chicken Manure

Chicken manure was obtained from Feather Crest Farms, located east of Bryan on Hwy 21. Feather Crest Farms is an egg production facility that agreed to supply the manure free of charge. The manure produced gasses, which made it difficult to store in an enclosed container; thus, it was gathered and loaded into the fermentors immediately. Three tons of chicken manure were needed for the initial loading. It was shoveled into trash bags and transported via truck and trailer. During continuous operation, the manure was obtained every week and stored in 5-gallon buckets. It was important to leave room in the buckets for gas production; if the manure was stored for too long, the buckets would eventually explode. The average moisture content of the manure was approximately 80 wt%, and contained 0.41 g ash/g dry chicken manure (Domke, 1999).

Calcium Carbonate

Calcium carbonate was obtained from a farm supply store, Producers Cooperative in Bryan, Texas. For initial loading, 2,400 lb were needed. The calcium carbonate was purchased for $4.39 per 50-lb bag and transported via truck and trailer. During continuous operation, more bags were purchased as needed.

Inoculum

The main source of inoculum was marine microorganisms, which were used because of their ability to thrive in saline environments such as the carboxylate salt-rich fermentation broth. The marine microorganisms were collected in the Lagoon in Galveston, Texas. Directions: Take I-45 south to Galveston. I-45 will turn into Broadway in town. Go left on Seawall Blvd. and the Lagoon is on the right.

The microorganisms were collected with mud from under the water in the Lagoon. The mud was shoveled into 12 5-gallon buckets and topped off with sea water to prevent air space; the buckets were sealed with a lid. The marine microorganisms were added immediately to the fermentors to ensure their survival. Adding the inoculum was the last step in the loading of the fermentors.

Another source of inoculum was terrestrial microorganisms that were contained in the chicken manure. These microorganisms were introduced when the chicken manure was added to the fermentors.

Process Water

Water served as the basis of the fermentation medium. Several hundred gallons of water were added to each fermentor to homogenize the ingredients. De-chlorinated tap water was used to avoid killing the bacteria. The de-chlorination method is discussed later in this chapter. No reducing agent was added to eliminate oxygen, because it was assumed that the oxygen in the water would not have a significant effect on the anaerobic microorganisms.

Methane Inhibitor

Iodoform was used to inhibit methanogen growth and was purchased form Cole-Parmer Industries for $120 per 500 g. The iodoform is a powder and was dissolved in ethanol to make a solution 20 g/L. This was done to ensure the compound was evenly distributed in the fermentors. The iodoform solution addition rate was 200 to 400 mL/(d·fermentor). The iodoform was kept out of contact with direct sunlight, air, and excessive heat because of its susceptibility to degradation.

FERMENTATION CONDITIONS

The fermentors were loaded with a substrate consisting of 80 wt% office paper and 20 wt% chicken manure. This ratio was found to be optimum in the laboratory by Domke (1999). Each fermentor was loaded with approximately 1200 lb of paper and 300 lb of chicken manure (dry basis). Calcium carbonate was added on the basis of 0.4 lb $CaCO_3$/lb biomass (Holtzapple). Thus 600 lb of calcium carbonate was initially added to each fermentor. Fifteen gallons of marine-microorganism-rich mud was added to each fermentor to inoculate the system; terrestrial microorganisms were also introduced with the chicken manure. The ideal fermentation temperature was 40 ^{o}C (Holtzapple). The fermentors were kept as close to 40 ^{o}C as possible; however, changes in outside temperature sometimes made it difficult to maintain this temperature. Calcium carbonate was added to maintain an optimal pH of approximately 6; the microorganisms could not survive in acidic conditions (Holtzapple). Iodoform was added on the basis of 2 to 4 mg per L of reactor volume to inhibit methane production. It should also be noted that this is an anaerobic fermentation process and the fermentors were kept closed, but were not air tight. Gases produced by the fermentation purged air out of the system, but during material transfers, the medium was partially exposed to air.

FERMENTATION DESIGN

Fermentors

The fermentor design is illustrated in Figure II-1. The fermentation train consisted of four 1,050-gallon polyethylene cone bottom tanks made by K-Tanks in Houston, TX. Each fermentor was equipped with a 15-in screw-on lid on the top of the tank. The tanks were supported by a metal frame, stood approximately 10 ft tall, and they were accessed by a large rolling step ladder.

The fermentor temperature was regulated by a water jacket. A detailed version of the water jacket is shown in Figure II-2 and a simplified version can be seen in Figure II-1. The water jacket was constructed by wrapping several ½-inch hoses around the fermentor, which were connected by a manifold made of 3-in PVC pipe. Exiting the jacket, the water traveled through a flow indicator and then through an air trap (AT). The air trap collected air bubbles that accumulated in the system. Next, the water was pushed through a 30-gallon electric water heater by a ¼-hp March centrifugal pump

(CP). Before the heater, a pressure gauge (PI) was installed to monitor the pump performance. A temperature gauge (TI) was positioned after the heater to indicate the heater temperature. The hot water then flowed back into the water jacket. Just before entering the water jacket and just after exiting, temperature gauges were installed to monitor the inlet and outlet water temperatures. At the top of the fermentor, there was a vent valve installed to vent air out of the system upon addition of fresh water. Each water jacket circulation loop was also equipped with an expansion tank (ET) to absorb liquid expansion as the water was heated.

Each fermentor had its own water jacket system with separate pumps (CP) and water heaters (WH) (see Figure II-2). There was also a line connecting all four water jacket systems with a fresh water source. The plumbing was done primarily in 1-in sch-40 PVC pipe. The water jackets and tanks were insulated with 4-in Styrofoam. The thermostat on the heaters could be varied between 100 ^{o}F and 150 ^{o}F (38 ^{o}C and 65 ^{o}C). The heater set point temperature was regulated to keep the fermentor temperature at 40 ^{o}C as the outside temperature changed. To monitor temperature, each fermentor was equipped with a temperature gauge with a 24-in thermocouple, as shown in Figure II-1.

To mix the contents of the fermentors and to transfer material in and out, a sludge pump was installed at the bottom of each fermentor. The sludge pumps were 1000 series progressive cavity pumps made by Moyno Pumps and were capable of forward and reverse operation. To mix the fermentors, the contents were circulated from the bottom of the fermentor to the top by the sludge pump. The piping was done in 3-in sch-40 PVC and included a main 3-in brass gate valve (V-1) at the top of the circulation line.

Due to the high viscosity of the fermentation medium, the piping was prone to clogging, thus making it necessary to install means to unclog the circulation line. To unclog the pipe, a 2-in PVC ball valve (V-3) was installed at the top of the fermentors in the 3-in circulation line. A 2-in flexible hose could be connected here and inserted in the top of the fermentor through the man-hole. The main gate valve (V-1) was shut and the pump was turned in reverse to suck thick material on the discharge side of the sludge pump or push material back through the suction-side of the sludge pump.

Gases are produced as a byproduct of the fermentation, so a pipe network was installed to vent the system. The vent pipes were positioned at the top of each fermentor and were connected to allow the gas to be gathered and sent through a gas scrubber. The scrubber was intended to absorb odorous gasses; however, it was not used because odor was never a problem. At the base of each fermentor vent there was a sample port installed where gas samples could be collected. The position of the vent network can be seen in Figure II-1.

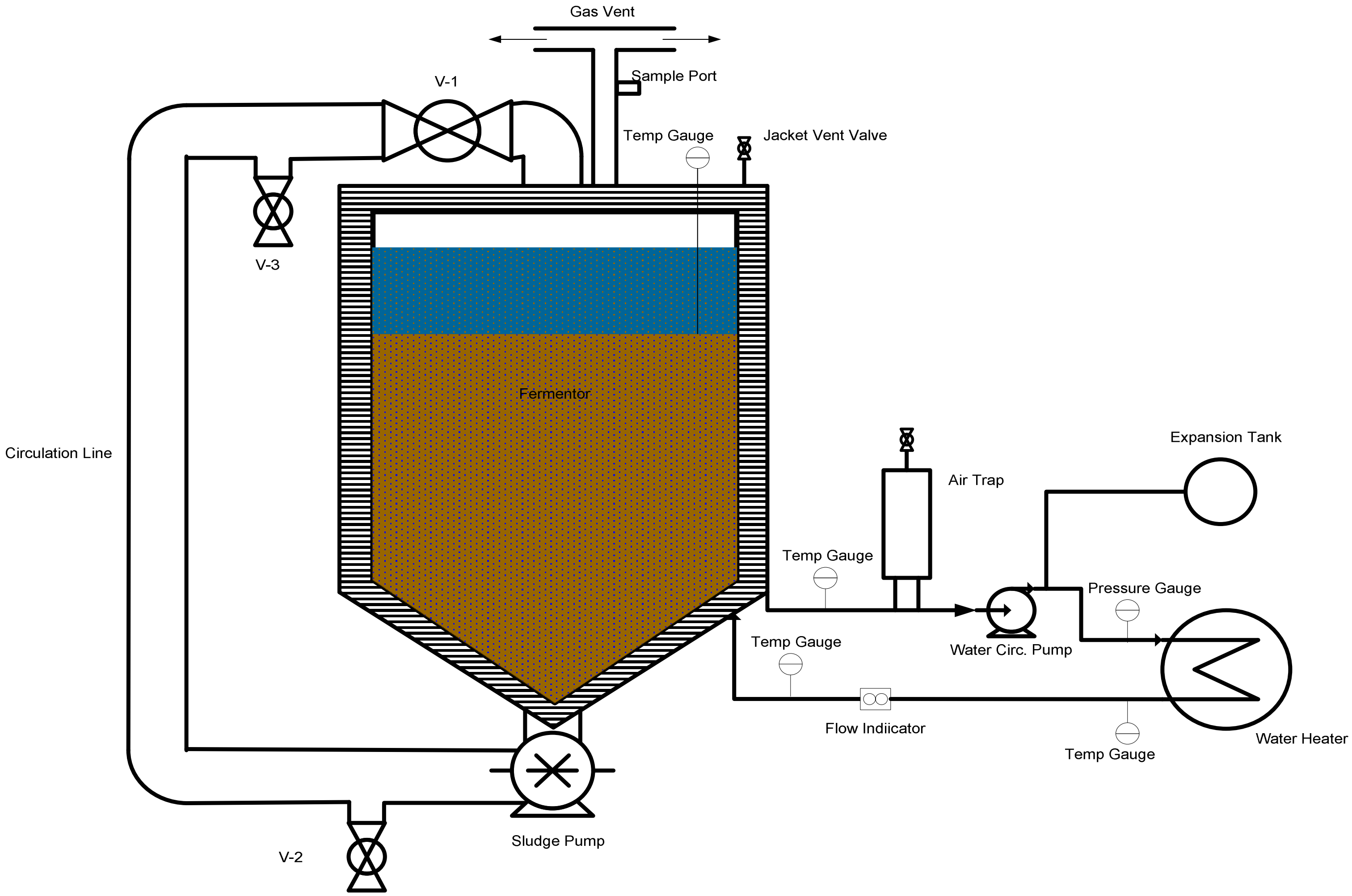


Figure II-1. Final fermentor design.

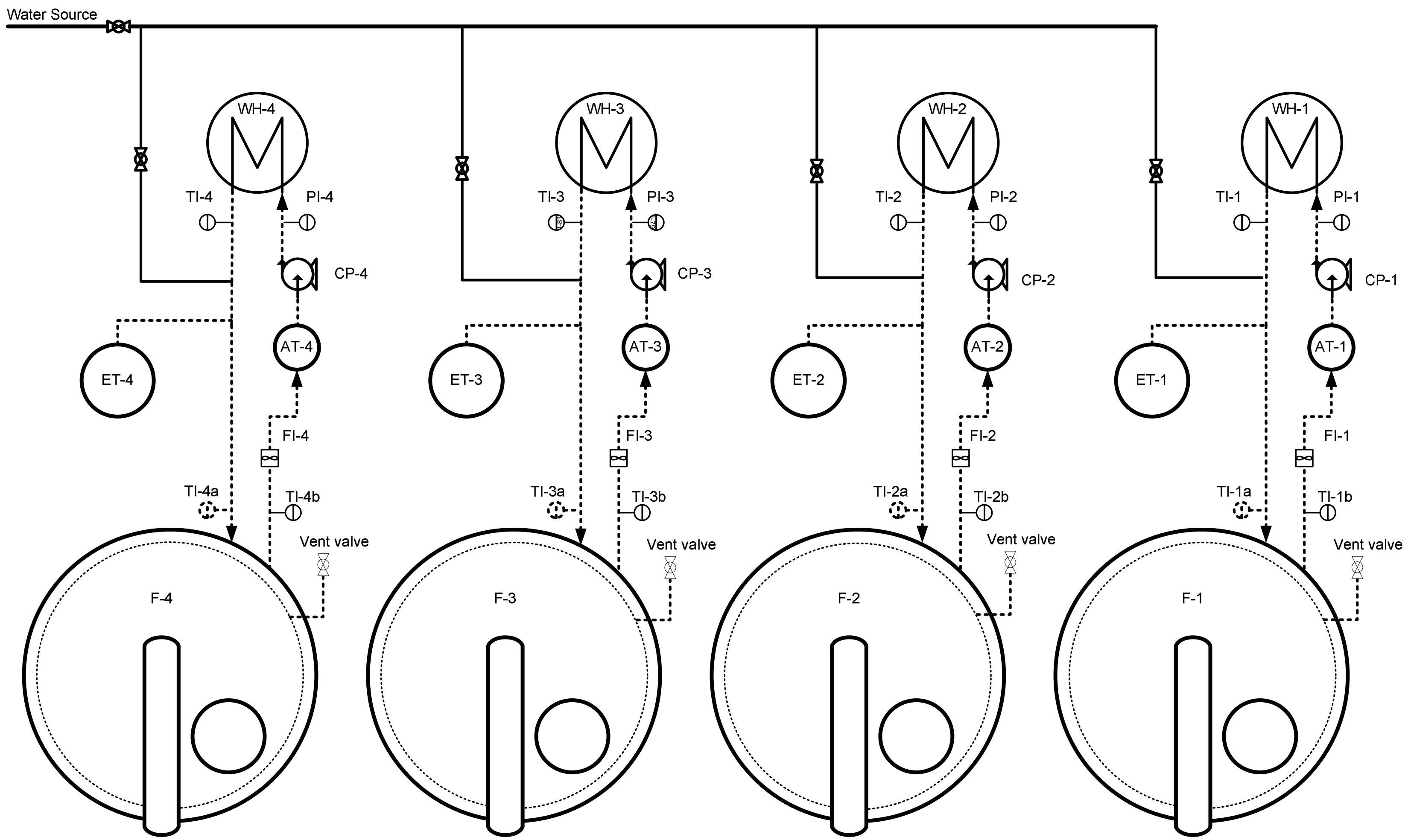


Figure II-2. Final water jacket design.

Solid/Liquid Separation

To operate countercurrently, undigested solids must be separated from the process liquid. The separation was done by filtration (see Figure II-3). A 2-in PVC ball valve (V-2) with a quick-disconnect fitting was installed at the bottom of the circulation line of each fermentor. This allowed a 2-in flexible hose to be easily connected to the circulation line. The other end of the hose was attached to a filter vessel.

The filter vessel is illustrated in Figure II-4. The vessel was 30 inches tall with a diameter of 18 inches and contained a removable strainer basket. The filter is common in the oilfield industry and was manufactured by Gooseneck Trailer, Inc. in Bryan. The inside of the vessel was coated with rust inhibitor to protect the metal from the corrosive fermentation medium. The fluid entered the top of the vessel and the solid-free liquid exited on the other side. The solids were collected in the strainer basket, which was removable and accessible through the lid on the top of the filter. The lid was secured by eight bolts and sealed with a rubber gasket. The filter contained a drain at the bottom, and a pressure gauge was installed to monitor the filter capacity. A vent valve was also installed to equalize pressure when filtration was complete.

The liquid exiting the filter was transferred to one of two places. When the liquid product was collected from the first fermentor, the liquid was transferred to the product tank. When liquid was to be transferred from one fermentor to another, it was moved to the measuring tank. Two-in flexible hoses were used to connect the filter to the tanks.

A detailed illustration of the product tank is shown in Figure II-5. The product tank was a 1,000-gallon polyethylene flat bottom tank made by K-Tank Industries. The product entered through the top of the tank and was drained from the bottom. A nitrogen blanket was used to prevent air from contacting the product. Nitrogen was supplied from a high-pressure cylinder obtained from Praxair in Bryan, TX. To ensure positive pressure on the product tank, a pressure reducing regulator was installed to supply nitrogen at about 1 psig. A back pressure regulator was also installed to relieve pressure if it increased to more than 1.5 psig.

The measuring tank was a 55-gallon cylindrical tank with a drain on the bottom of the curved side of the tank, as shown in Figure II-3. The tank rested on a stand and stood about 3 ft off of the ground. The tank was marked at 5-gallon intervals for measuring. Liquid was measured for the transfer and then pumped back into the appropriate fermentor.

The solids gathered in the filter were dumped into 5-gallon buckets and weighed. When enough solids were collected, the buckets were dumped manually back into the appropriate fermentor through the fermentor lids.

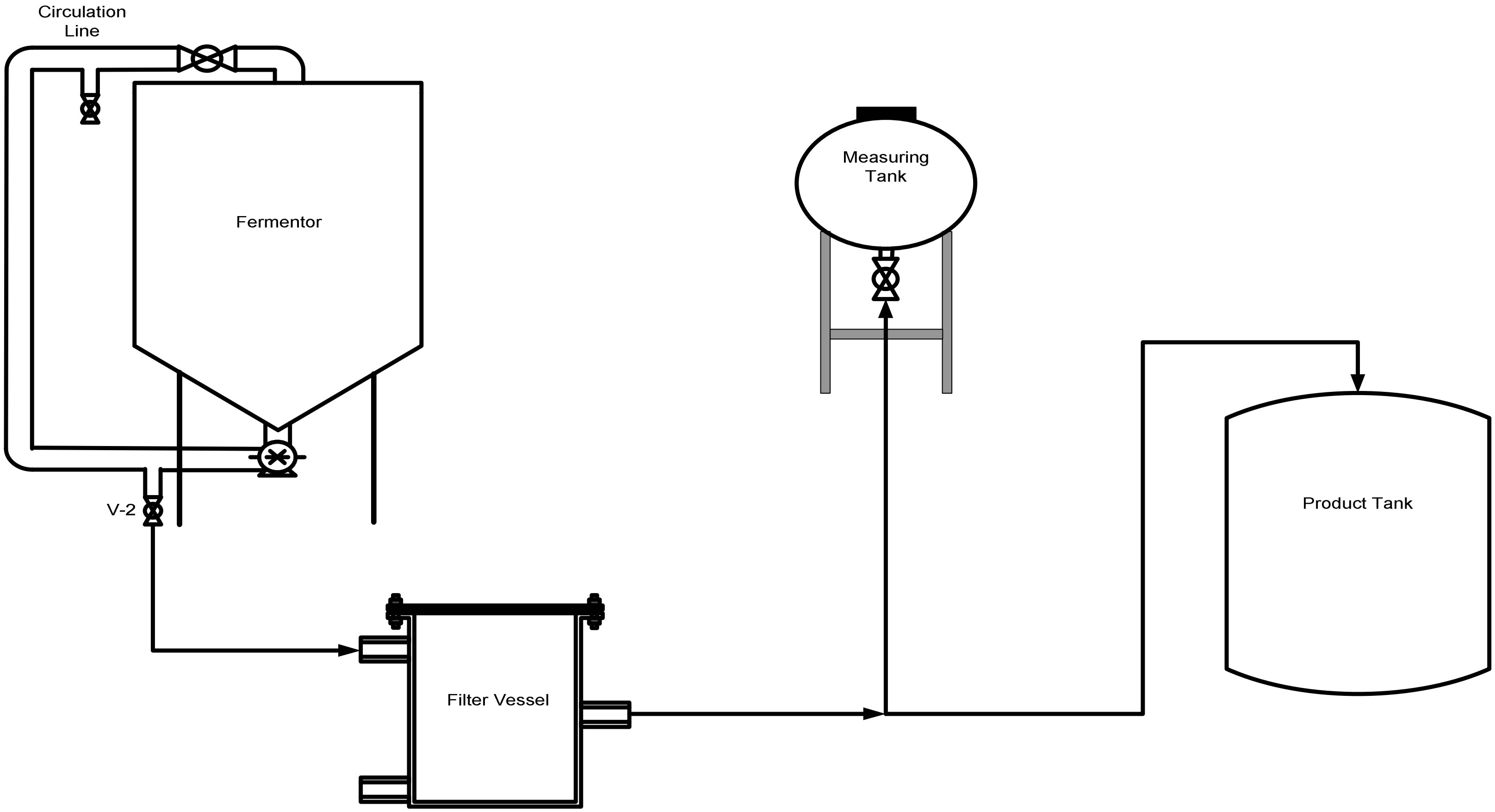


Figure II-3. Transfer method diagram.

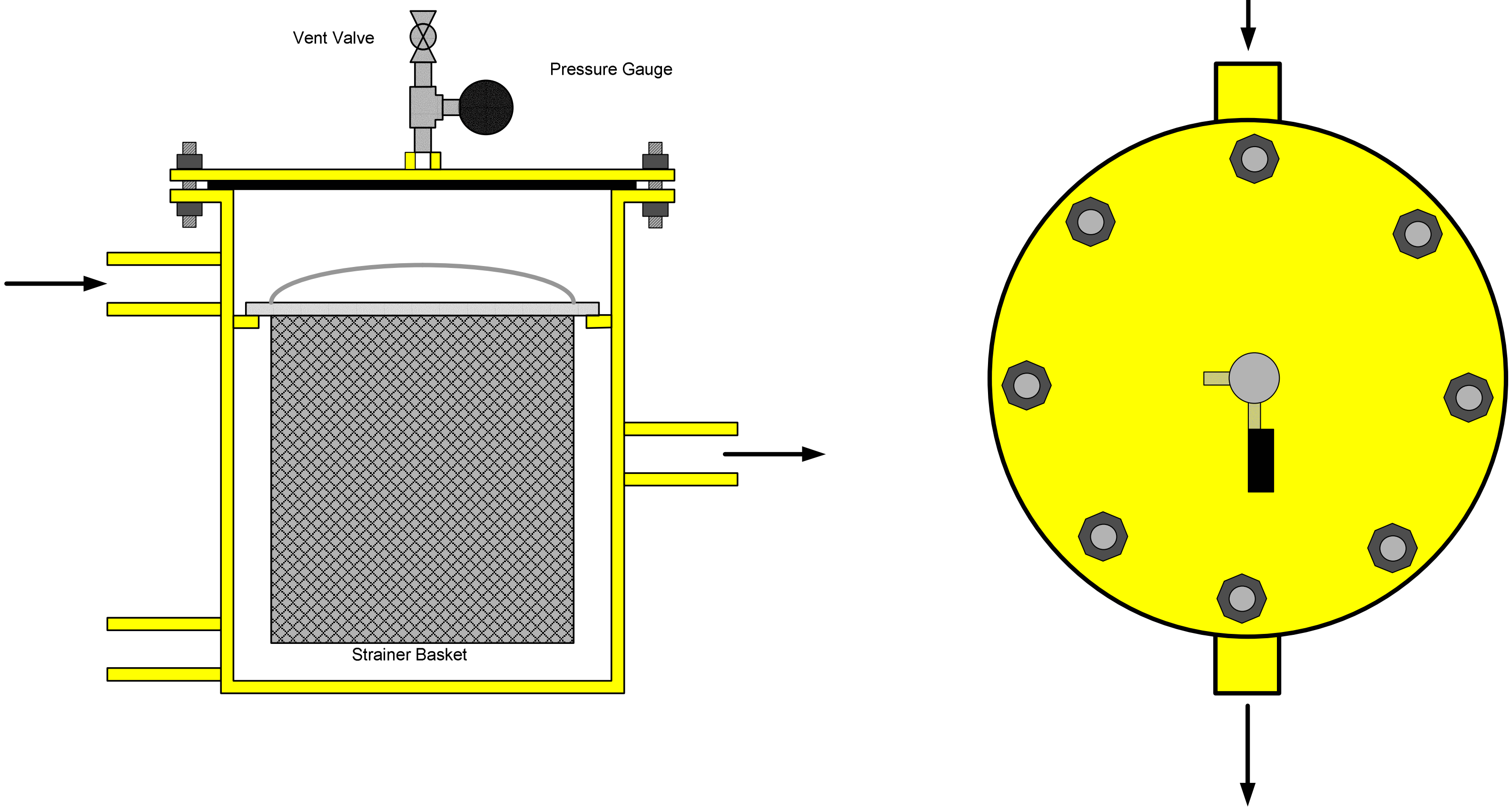


Figure II-4. Filter vessel design.

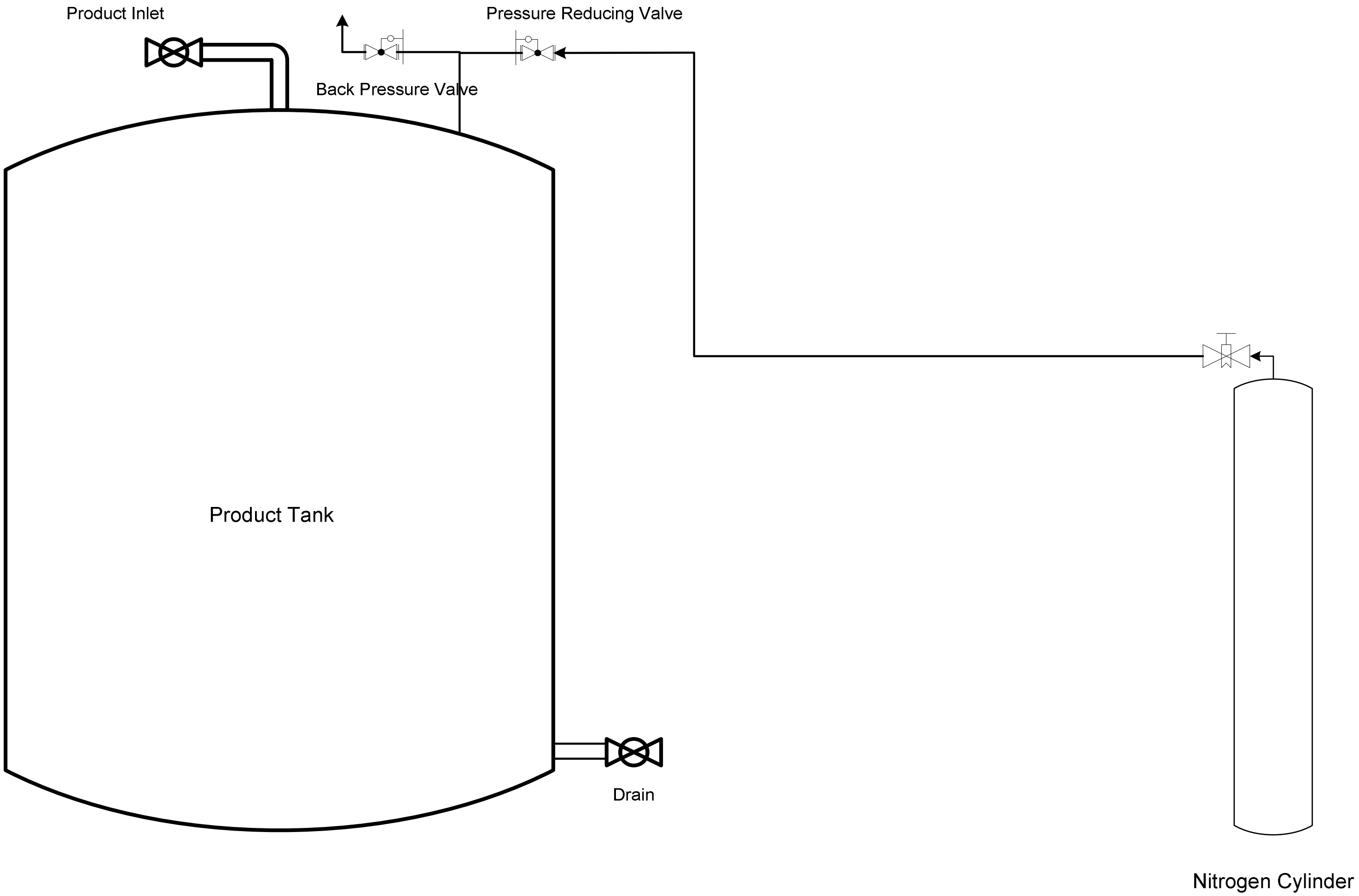


Figure II-5. Product tank design.

Water Treatment

It was necessary to remove chlorine from the tap water before it was used as process water; this was done with carbon adsorption beds. There were three vessels constructed out of 6-in PVC pipe that were approximately 3 ft tall. The vessels stood vertically and were connected with 1-in PVC pipe and ball valves to allow operation in series and in parallel (see Figure II-6). After the chlorine was removed in the carbon beds, the de-chlorinated water was stored in the water storage tank, which was the same style as the product tank. An 1/8-hp centrifugal pump was installed in the drain line to pump water to the fermentors. The piping was done in 1-in PVC. An overview of the entire equipment layout is shown in Figure II-7.

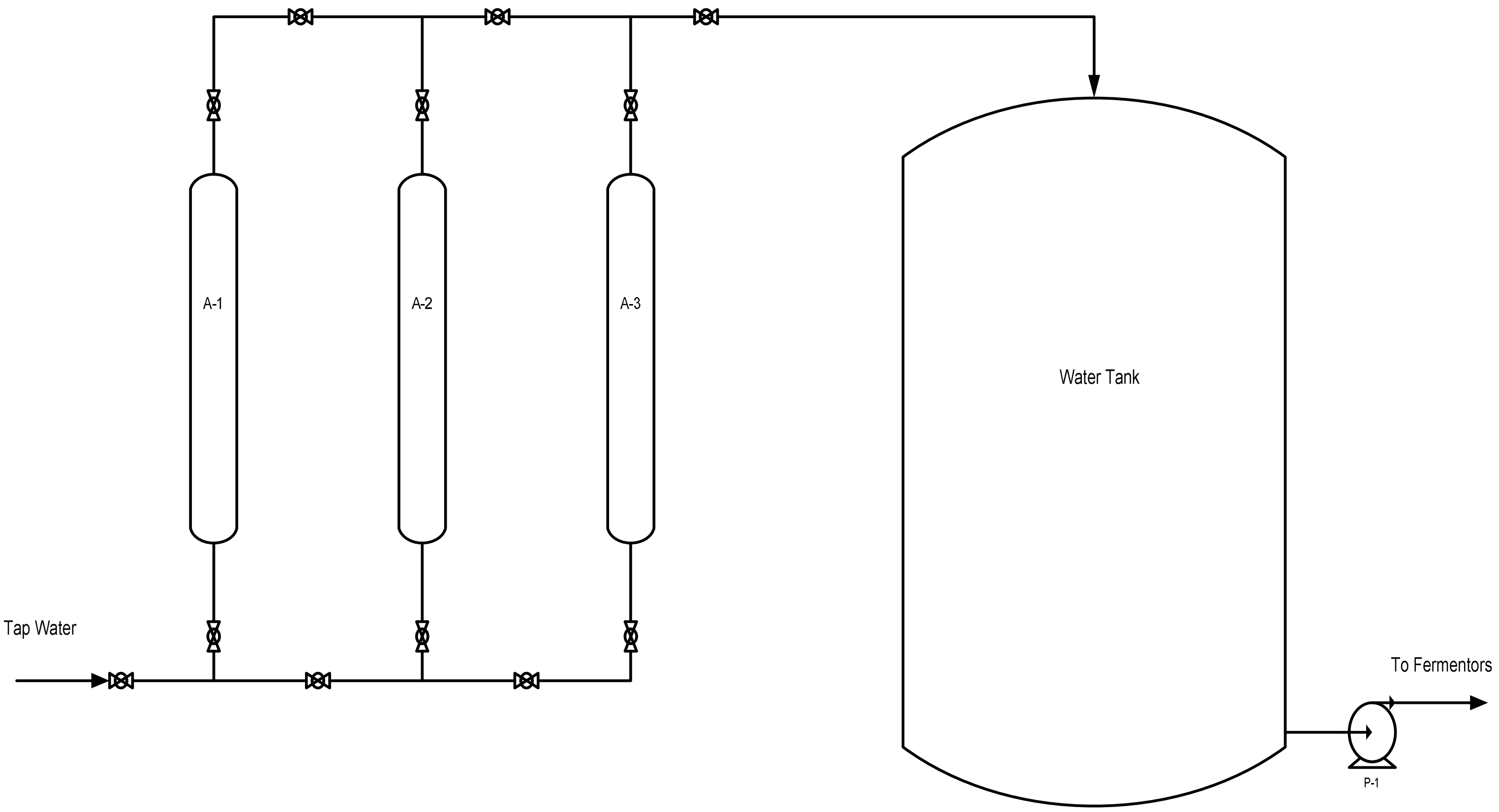


Figure II-6. Water treatment design.

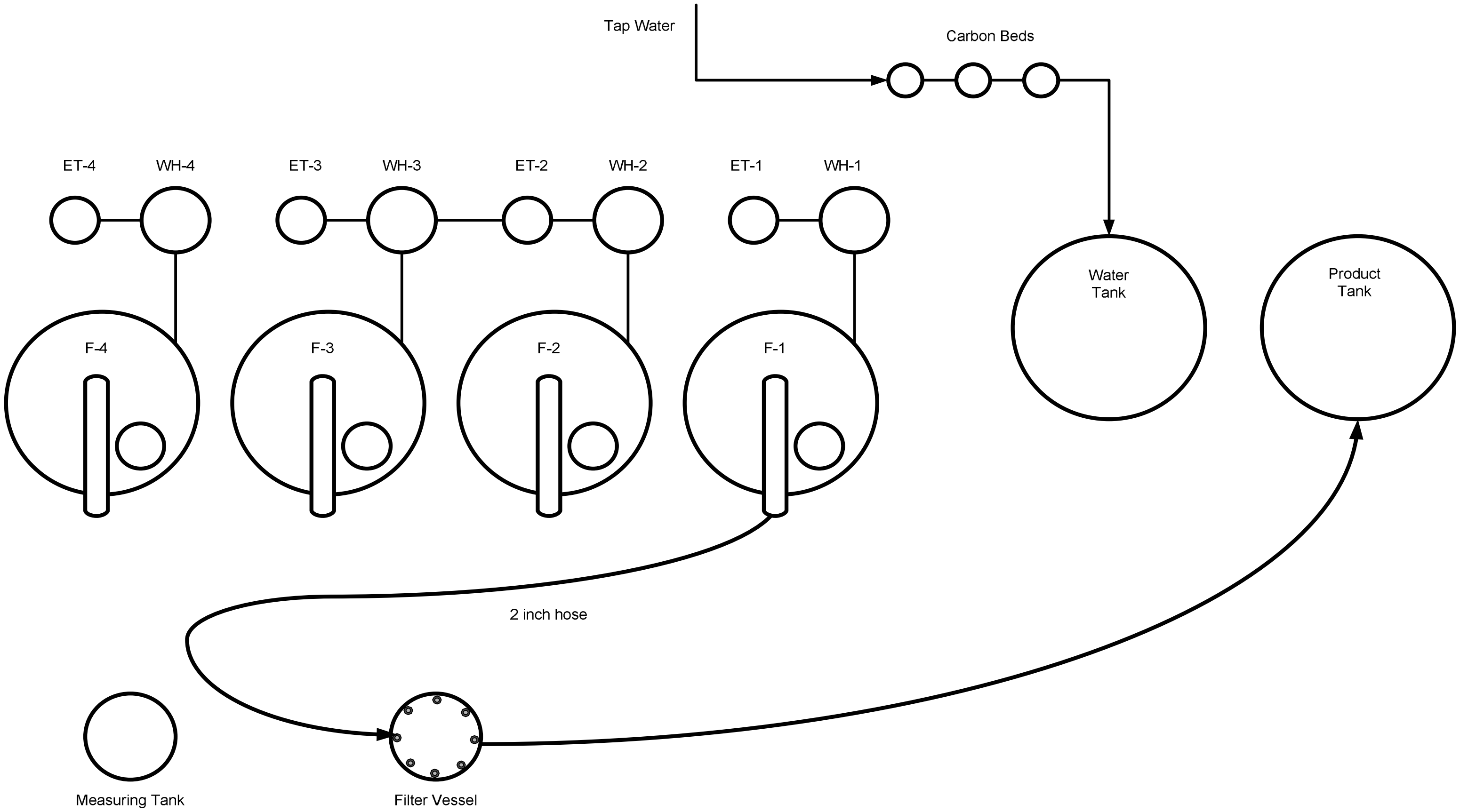


Figure II-7. Overall plant layout.

OPERATIONAL PROCEDURE

Batch Operation

When the fermentors were loaded, the process was operated in batch mode to let the microorganisms adapt to their new environment and to establish an initial acid concentration. In batch mode, the primary concern was to maintain the proper fermentation conditions and to monitor acid concentrations.

It was important to maintain a fermentor temperature of 40 ^{o}C, which was controlled by adjusting the water heater thermostats. The changing outside temperature was the primary cause of temperature fluctuations.

It was also important to control the pH between 5.8 and 6.2. The pH was tested every week with pH test strips or by a digital pH monitor. If the pH dropped, more calcium carbonate was added to neutralize acid production.

Methanogen inhibition was also critical to successful fermentation. Biomass conversions would greatly decrease without a proper methanogen inhibitor. It was necessary to add 200 to 400 mL of the 20-g iodoform/L ethanol solution to each fermentor every day (Holtzapple). To mix the inhibitor, the sludge pumps were used to circulate the fermentor contents. The biomass was pumped from the bottom of the fermentors to the top. Because there was no mechanism to evenly distribute the biomass as it entered the top of the fermentor, it was prone to channeling through the middle of the fermentor. This made it difficult to thoroughly mix the inhibitor, thus it was sometimes necessary to manually assist by mixing with a shovel. The mixing process usually lasted between 1 and 2 hours per day.

It was necessary to take samples of the gas product to analyze the methane concentration. Approximately every week, gas samples were taken from the vent system in each fermentor and brought to the laboratory to be analyzed with the gas chromatograph. Ideally the methane concentration would be 0 wt%, but it was difficult to maintain a level lower than 5 wt%. If the methane concentration elevated above 5 wt%, it was necessary to take action to bring it back down. It was common procedure to add a double dose of inhibitor for a few days and to extend the mixing time to ensure an even distribution.

To monitor acid production, liquid samples were taken every 2 days while in batch mode. After the fermentors were well mixed, samples were taken and stored in a freezer until they could be analyzed with the gas chromatograph. A description of the procedure for analyzing data is described in Appendix I.

Countercurrent Operation

Countercurrent operation was a semi-continuous mode of operation. Every 3 days, solids and liquids were separated and transferred in opposite directions in the fermentation train to simulate solid and liquid countercurrent flow. At one end, the product was harvested and fresh biomass was added. Undigested biomass was discarded and fresh water was added at the other end. See Figure II-8 for a simplified illustration of the countercurrent operation.

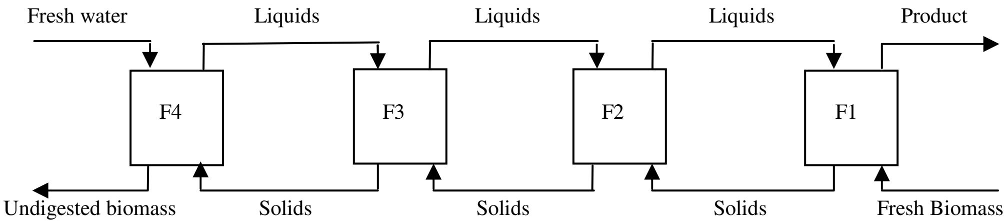


Figure II-8. Countercurrent flow diagram.

After the fermentors were well mixed, the material transfer started with Fermentor 1 (F1). The material was pumped with the sludge pump into the filter vessel to separate the solid biomass from the liquid product. The liquid exited the filter and was moved to the product storage tank (see Figure II-3 and II-7). When the filter reached maximum capacity, the procedure was stopped and the solids were gathered from the filter and set aside. This process was repeated until enough solids and liquid were obtained. When filtration was complete, fresh biomass was added to F1. The same procedure was performed with F2, but the liquid was moved to a measuring tank instead of the product tank, and there was no fresh biomass added. An accurate way to measure the volume in the fermentors did not exist, thus the liquid was held in the measuring tank until enough was collected. The liquid was then pumped into F1 and the solids from F1 were added to F2. The solids from F2 were set aside for F3. Following the same procedure, liquid was moved from F3 to F2 and from F4 to F3. Solids were transferred from F2 to F3 and from F3 to F4. Solids taken from F4 were discarded as waste, and fresh water was added to F4. The amounts transferred varied as a better understanding of the process was achieved, but the same transfer procedure was used throughout. A more detailed description of the countercurrent operational procedure is included in Appendix A.

During the countercurrent operation, maintaining the fermentor temperature at 40 ^{o}C, the pH around 6, and the methane concentration below 5 wt% was still important. Liquid samples were taken on transfer days (every 3 days) instead of every 2 days.

Solids loading and liquid residence time will be discussed later. More changes were made, as more was learned about the behavior of the system, and is described in detail in Chapter V.

CHAPTER III

INITIAL DESIGN AND IDEAS

The initial design of the pilot-scale fermentors was based on observations at the laboratory scale. In the laboratory the fermentation medium appeared to separate into two strata. The solids settled to the bottom and the liquid formed a solid-free liquid layer above the solids. The principle behind the pilot plant fermentor design was based on the assumption that the solids settled and a liquid level could be established above the solids. The reality was the medium did not immediately, if at all, separate into two strata. The fermentor contents did not separate into a solid-free liquid phase, which made the principles of the initial design obsolete. This chapter will describe the initial design ideas and the way the process was intended to work. The next chapter will explain design flaws and how they were corrected. Refer to Figures III-1 and III-2 for a detailed illustration of the original fermentor design.

TEMPERATURE CONTROL

Temperature was an important variable to manage. There were initially two means of controlling the fermentor temperature. The first was the water jacket system described in Chapter II, where water was heated by electric water heaters and circulated through tubes that wrapped the fermentors. At first, there was only one 30-gallon water heater and one main circulation pump for all four water jacket systems. Before the hot water entered each water jacket, there were two smaller auxiliary centrifugal pumps in series. It turned out that one heater and one main pump were not sufficient (see Figure III-2). The details of this issue will be discussed in Chapter IV.

The second method of controlling the temperature was circulating the process liquid through two heat exchangers and then evenly distributing the warm fluid through the fermentor. Figures III-1 and III-2 show the circulation loop in detail. The heat exchanger circulation loop for each fermentor contained an internal filter, a circulation pump, two heat exchangers (HEX), a temperature control valve (TC-1), a flow meter, and a distributor. Hot water from an electric 30-gallon water heater was used to supply heat to the heat exchangers. Originally, there was only one heater and one utility water circulation pump for all four fermentors.

Figure III-2 shows the original design of both the water jacket system and the hot water system for the heat exchangers. A circulation pump (CP-1) pushed water through the water heater (WH-1) to supply heat to all four heat exchangers (HEX). Before the circulation pump (CP-1), there was an air trap (AT-1) to collect air that accumulated in the system. Also connected to the pipe network, was an expansion tank (ET-1) used to absorb expansion from the heated water. The water jacket system was set up similar to the heat exchanger system. The water jacket system contained a circulation pump (CP-2), a water heater (WH-2), an air trap (AT-2), an expansion tank (ET-2), and two additional smaller auxiliary pumps (AP) before each fermentor.

In the heat exchanger circulation loop, the internal filter was intended to suspend in the liquid layer above the solids (see Figure III-1). It was made of perforated 2-in PVC pipe wrapped with a plastic mesh. The filter was designed to collect the process

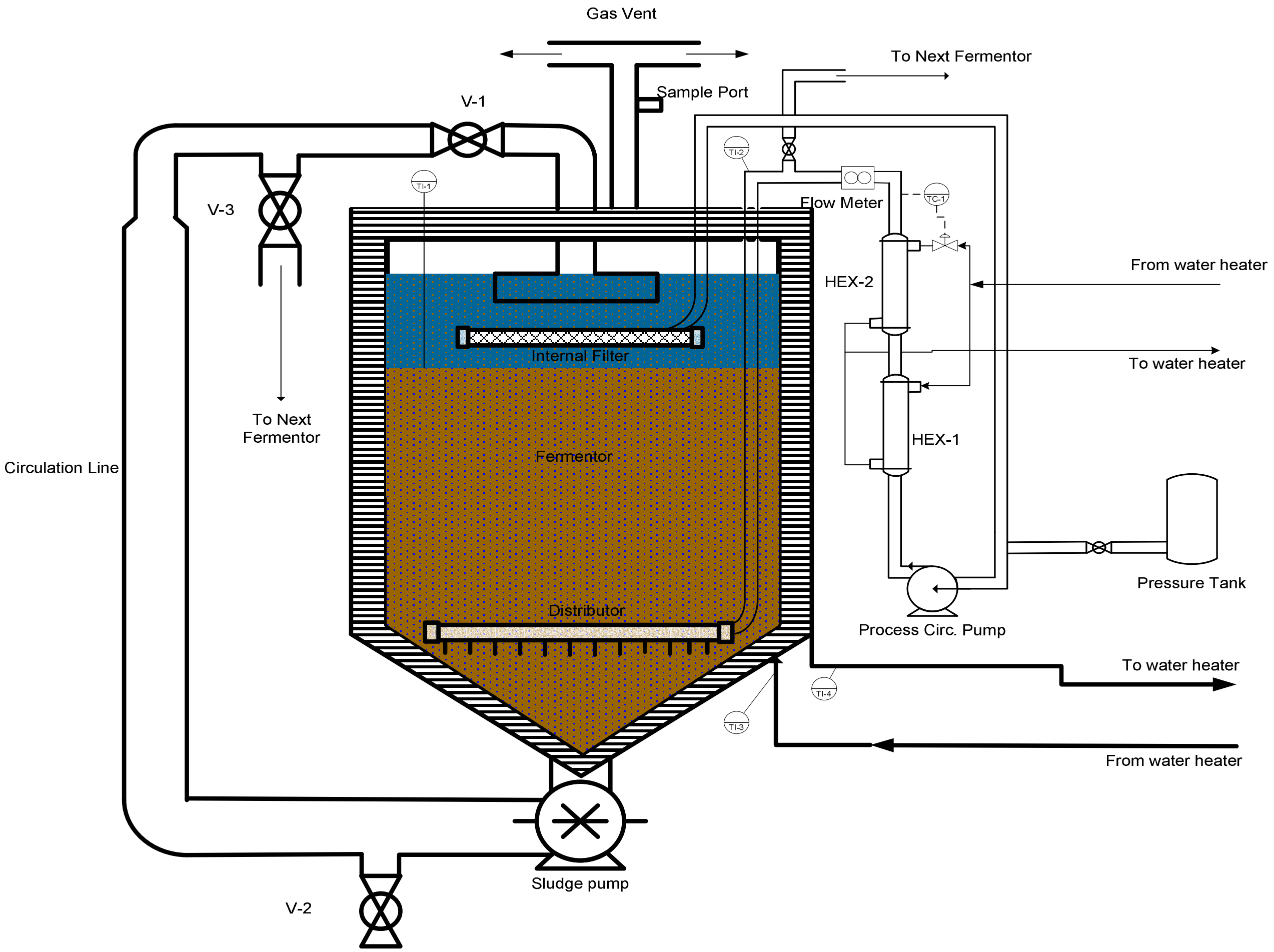


Figure III-1. Original fermentor design.

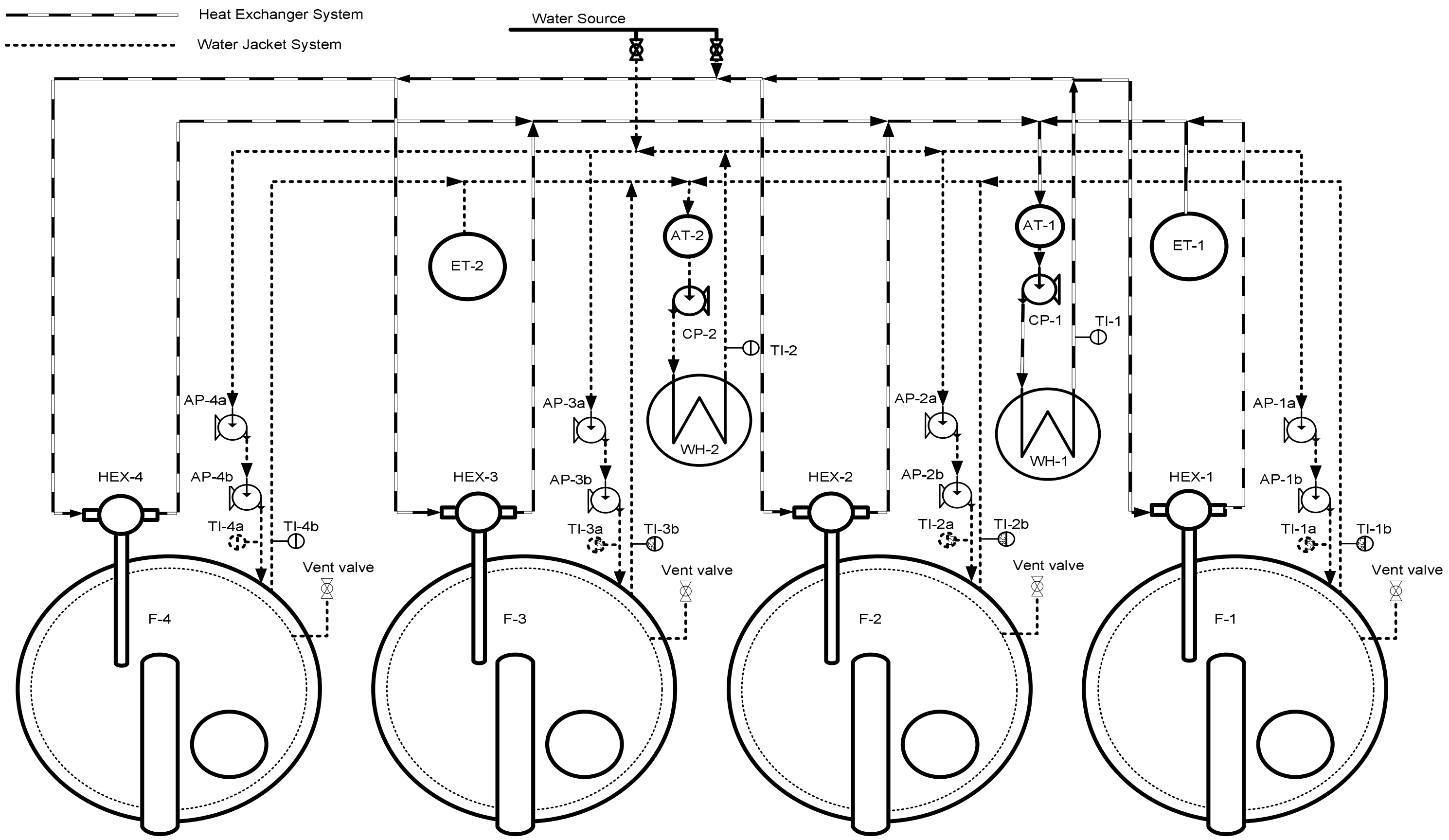


Figure III-2. Original heating system design.

liquid while rejecting the solids. To prevent leaks, the filter was piped through the top of the fermentor tank with 1-in PVC pipe. The liquid was sucked through the filter by a March ¼-hp centrifugal pump. The process circulation pump was intended to operate with positive suction head, but instead was forced to suck the liquid up and out of the fermentors, which made it very difficult to keep the pump primed. The pump then pushed the liquid through two stainless steel concentric tube heat exchangers (HEX). The process liquid was passed through the inner tube and hot utility water was passed through the outer tube. The utility water for all four fermentors was heated by one 30-gallon water heater. The temperature was maintained with a temperature control valve (TC-1) that would sustain a temperature exiting the exchangers by regulating the hot water flow to the second exchanger.

Next, the process fluid passed through a flow meter before entering the top of the fermentor. The warm fluid was then pushed to the bottom of the fermentor to the distributor. The distributor consisted of a large and small ring of 1-in PVC pipe joined together to form an inner and outer ring. On the bottom of the pipe rings there were 1/8-in check valves positioned approximately every 3 inches. The check valves allow only outward flow to keep solids from entering and plugging up the distributor. The heat exchange circulation loop was intended to take liquid from the top of the fermentor, heat it, and return it to the bottom to create an evenly heated and well mixed fermentation medium.

Solids caking on the internal filter and restricting flow was anticipated. To solve this problem, there was a pressure tank installed to back flush the internal filter. Fluid was able to be pumped into the pressure vessel against a diaphragm and then could be shut in. When necessary, the pressurized fluid could be surged into the system to flush the filter.

MIXING

A main circulation loop was used to do the bulk of the mixing. The settled solids were predicted to form a heavy sludge that would be pumped from the bottom of the fermentor to the top through 3-in PVC pipe with a sludge pump, as described in Chapter II. The initial design, however, used 2-in PVC pipe at the top of the loop because it reduced the cost of valves and fittings. Another difference was that in the initial design, the 2-in pipe entered the top of the fermentor and was connected to a tee that would be submerged in the process fluid (see Figure III-1).

SOLID/LIQUID SEPARATION

The most important aspect of the design was the ability to separate solids and liquids for countercurrent operation. Solids would be transferred in one direction and liquids in the other direction. The initial design was again based on the prediction that the solids settled. Each fermentor was equipped with a heat exchange circulation loop as described above. At the top of each loop, before the pipe re-entered the fermentors, there was a connecting line to the adjacent fermentor (see Figure III-1). This allowed valves to be switched and flow to be directed toward the next fermentor. The amount of liquid transferred could be monitored by reading the flow meter and timing the transfer.

Transferring solids was done much like transferring liquids. The main circulation loop was connected to the adjacent fermentor similar to the heat exchange loop but in the opposite direction. Valves could be turned to divert sludge flow to the next fermentor. The liquid content in the sludge was assumed to be insignificant. Instead of measuring the mass or volume of solids transferred, the design intended to maintain a constant solids level by measuring the height of the solids settled below the liquid. As long as this level was maintained, approximately the same amount of solids would be transferred to each fermentor.

When the pilot plant was built in 1998, the fermentors were operated for only a short time. They were never operated continuously, only in batch mode. Temperature control was never established and solid and liquid transfers were never accomplished. It was only until now that it was discovered that there must be major changes to the initial design. The design was constructed on a principle that turned out to be false, thus the plan was to adapt the existing fermentor design to the new conditions.

CHAPTER IV

DESIGN MODIFICATIONS AND SUBSTRATE STRATIFICATION BEHAVIOR

This chapter describes a timeline of how the pilot-scale fermentors have evolved from the initial design to how they are operated today. This will include explanations of design problems and how they were solved. The stratification behavior of the fermentation substrate will also be discussed because of its operational impact.

CLEAN-UP AND MAINTENANCE

After initial construction in 1998 the fermentors were only operated for a short time, and were not used since. Biomass remained in the unused fermentors. A vacuum truck was used to suck out the leftover sludge. During the clean up, the heat exchanger circulation loop was found to be plugged in several places and all of the check valves in the distributor were clogged. The pipe network was cleaned out and the integrity was tested. Many leaks were repaired and hot pipes were re-insulated. Because the equipment was idle for several years, the pumps and water heaters were tested, and the necessary repairs were made.

INITIAL SYSTEM IMPROVEMENTS

Heat Exchanger Loop

Before operation could begin, some improvements to the existing system were necessary. Refer to Figure IV-1 for a modified fermentor design. Due to the observed plugging of the circulation loop, a finer screen was installed on the internal filter. It was anticipated that a finer screen would not be sufficient to stop solid particles from clogging the check valves in the distributor, so two strainers were installed on the discharge side of the circulation pump to clean the process fluid before it reached the distributor. The ideal position for the strainers was before the pump, but there would not be adequate pressure to push the liquid through the strainers. Two Banjo T Strainers were installed in parallel with valves positioned so as to service one strainer while the other was on line. A differential pressure gauge (DP) was used to determine when the strainers needed to be cleaned.

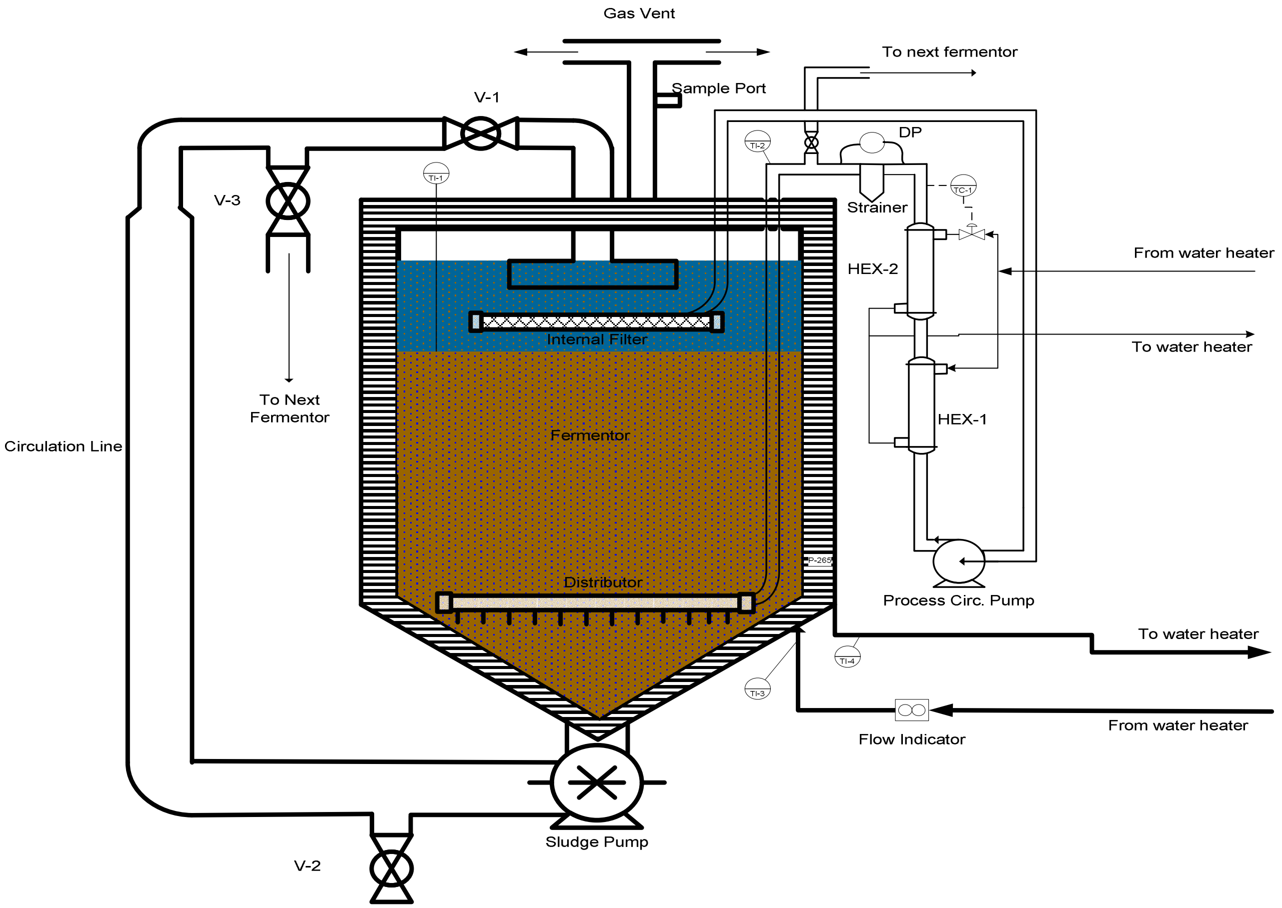


Figure IV-1. Modified fermentor design.

Heating Systems

The heating systems for the heat exchangers and the water jackets also required some modifications. Initially there was only one heater and one main circulation pump to supply hot water to all four water jackets, and only one heater and one main circulation pump to supply heat to all the heat exchangers. For the initial heating system design refer to Figure III-2 in Chapter III.

The same problems were encountered in both the water jacket heating system and the heat exchanger heating system. Because there was not a way to confirm water flow in either system, flow indicators and pressure gauges were installed first. The fermentors were then filled with water to test the temperature control, and the design was determined to be inadequate. It was concluded that one water heater (WH) per system could not supply enough heat to all four fermentors. In addition, one main circulation pump (CP) in both systems could not move enough water to supply the heating system of each fermentor. The pumps pushed water through the path of least resistance which was to the two closest fermentors (F-2 and F-3). There was very little water delivered to the farthest two fermentors (F-1 and F-4).

Thus, two more heaters and circulation pumps were installed, as well as two expansion tanks and two air traps. There were now two heaters for the heat exchanger system and two for the water jacket system. With the additional heaters, the system could obtain the desired temperature of 40 ^{o}C. The modified heating system design is shown in Figure IV-2. The system included four water heaters (WH), four expansion tanks (ET), four circulation pumps (CP), four air traps (AT), eight auxiliary pumps (AP),

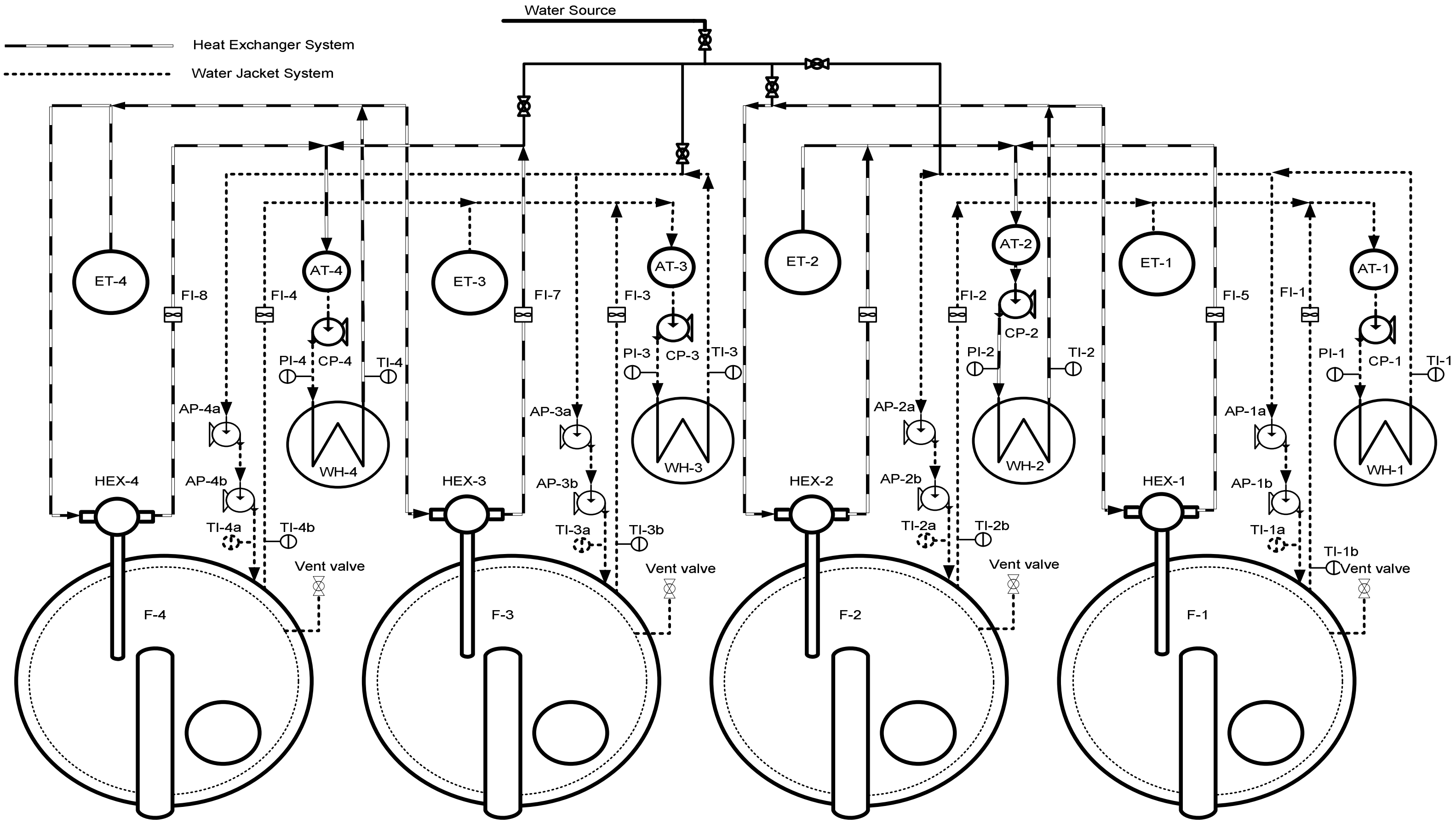


Figure IV-2. Modified heating system design.

eight flow indicators (FI), and numerous pressure gauges (PI), and temperature gauges (TI).

FERMENTOR LOADING

The next step was loading the fermentors with biomass. Office paper was shredded and baled by the document destruction company. Ideally the paper would have the consistency of confetti, but when the paper was delivered, it was not as shredded as the sample that was previously observed. A large amount of the paper was in long shreds as opposed to tiny particles. Despite the unfavorable paper consistency, it was loaded anyway, and took 2 weeks to load all four fermentors by hand. The ingredients were added dry and then the fermentors were filled with water. A problem arose as the water was added. The strands of paper became heavy and clingy when water was absorbed, and formed a thick interwoven mat in the fermentors. The internal filter was only supported by one pipe attached to the top of the fermentor (see Figure IV-1). The wet paper latched onto the filter and put enough weight on it to cave in the top of the first fermentor. Special care had to be taken to keep the heavy paper from damaging the fermentor tanks and the internal piping. This was the first indication of problems to come with the stratification behavior of the paper sludge.

SUBSTRATE STRATIFICATION BEHAVIOR

The first several weeks of operation were performed in batch mode to allow the microbial culture adapt and grow, and to build the acid concentration as the biomass was digested. The consistency of the substrate changed as it became more digested, but at no time did the solids totally settle. At any given time, the fermentors had multiple phases that differed in solid density. The consistency of the fermentors was broken into three stages, which evolved over several months of digestion (see Figure IV-3). Before all three stages could develop, fresh biomass was added during the continuous mode; however, the fourth fermentor saw the most digested biomass over time. Thus, it was predicted that if the fermentors were left in batch mode, the substrate would behave in all the fermentors as it did in the fourth fermentor. This idea will aid in the understanding of the phase stratification in the fermentors.

The first stage lasted approximately one month after the initial loading, from the end of April to the end of May, 2005. During the first stage, the paper would clump together and suspend in the water and gradually form a paste that was homogenous throughout the fermentor. The paper was thick and difficult to pump and mixing was very difficult in this stage. The biomass would often plug the suction-side of the sludge pump. To put this in perspective, a shovel was only able to penetrate the medium a few feet. Only liquid that channeled through the solids could be circulated.

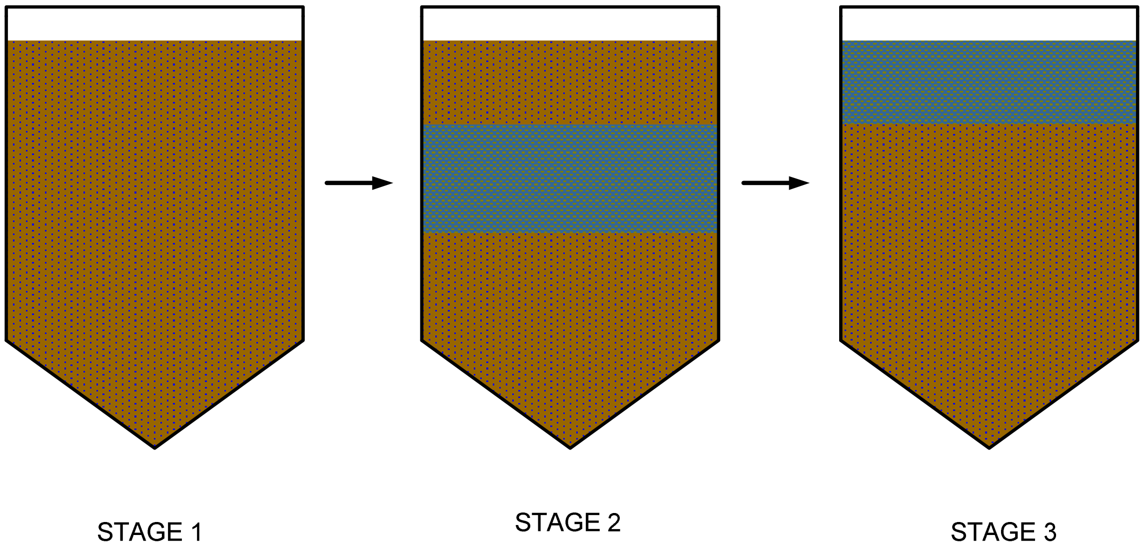


Figure IV-3. Substrate stratification behavior with time.

The second stage lasted over one month, from the end of May to the beginning of July, 2005. This stage lasted much longer in the first fermentor because fresh biomass was continuously added during countercurrent operation. In this stage, more of the paper was digested by the microorganisms, and as the paper broke down, it would settle to the bottom. There were three layers, where the most undigested paper floated and the most digested paper settled. In between the settled and floating layers was a slurry of suspended particles. The settled and more digested biomass was easier to pump, but the movable fluid still channeled through the fermentors. Sufficient mixing was difficult to achieve without the aid of a shovel.

In the third stage, the microorganisms had broken down the paper into small particles that were unable to clump together and float on the surface. In this stage, there was a settled solid phase and a suspended slurry phase. The biomass was much easier to pump and the mixing was improved. Eventually, the second, third, and fourth fermentors developed into what resembles this third stage.

If no fresh biomass was added and the substrate was never disturbed by mixing, the solids would continue to settle. Due to the slow nature of the digestion and regular mixing, the biomass never settled completely to the bottom. Regardless of how much time passed, there was never a solid-free liquid level established.

After continuous operation was established, each fermentor would adopt a more consistent substrate stratification behavior. The first fermentor was the most congested because the biomass was the least digested and the fourth fermentor was the most fluid because the biomass was the most digested. Figure IV-4 illustrates how the medium would typically behave when the fermentors were operated continuously.

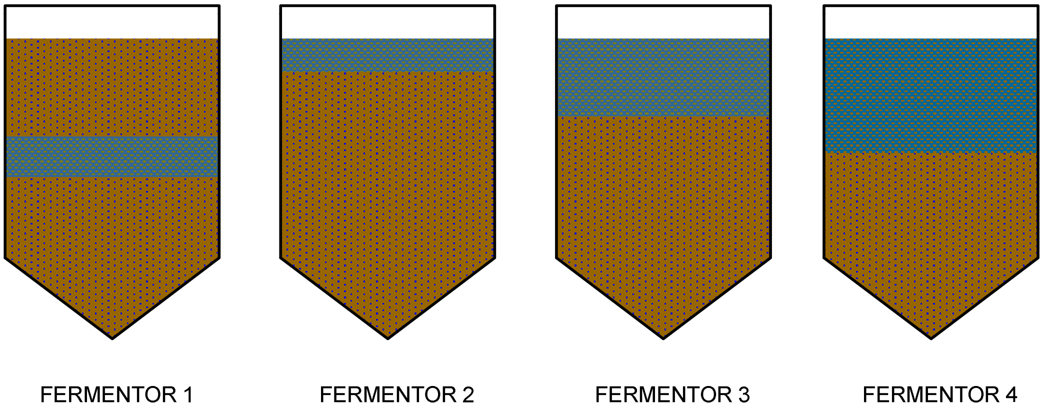


Figure IV-4. Substrate stratification in each fermentor.

EFFECTS OF SUBSTRATE STRATIFICATION

The original design anticipated being able to circulate process fluid to control temperature and transfer liquid, which required a clean liquid layer to form. When the fermentors were loaded, the fresh paper by itself filtered the water, enabling circulation. However, as soon as the biomass started to break down, this was no longer possible. The finer screen on the internal filter would reject the particles down to 1-mm, but smaller particles would pass. The amount of solids suspended in the liquid was vastly underestimated; smaller particles would instantly fill the T strainers. If the strainers were bypassed, the process circulation pump only lasted a few minutes before seizing. The fluid was observed as it was pumped through the flow meters. It was obvious the solid content was excessively high, making it impossible for the current system to handle these conditions.

For a new circulation loop design to be feasible, several issues had to be considered. A much larger strainer would be required for the filter system, and a new pump would be necessary to handle the abrasive material. If the internal filter clogged too often, continuous back flushing of the system would be required. Priming the circulation pump was a difficult process because the pump had to lift fluid out of the top of the fermentor, which would add to the complications after every back flush. Thus, the decision was made to abandon the idea of a heat exchanger circulation loop. Without the circulation loop, the original method of transferring liquid was also not possible, thus major changes had to be made.

SUFFICIENT TEMPERATURE CONTROL

Because the substrate stratification behavior eliminated the use of the heat exchanger circulation loop, the main priority became re-establishing temperature control. Maintaining the desired fermentor temperature was necessary to ensure the health of the microorganisms. The heat exchanger circulation loop was now obsolete; however the water jacket system was still operable. In the spring, the outside temperature was warm, so the system required minimal heat to maintain the fermentors at 40 ^{o}C. Fortunately, the two heaters supplied sufficient heat to the water jackets to sustain the desired temperature, but this system was not ideal. Response to disturbances from outside temperature changes was slow. This system was only able to raise the fermentor temperature approximately 1 ^{o}C every 24 hours. Despite the slow response time, adequate temperature control was sustained for the duration of the summer.

However, when the outside temperature decreased in the fall, the two heaters were not sufficient. Two new heaters and the additional necessary equipment were installed in December, so each fermentor now had one heater. The two original heaters that supplied the heat exchangers were still available, but were over 20 years old and in poor condition, thus were not used. The final water jacket heating system is illustrated in Figure IV-5. Even with four heaters, maintaining the desired temperature was still difficult. The time delay in the temperature response made it difficult to counter major outside temperature declines. Another disadvantage was the heater set points had to be changed manually, which further delayed the response. This system was still not optimal, but was used nevertheless. Improvements to this system should be considered.

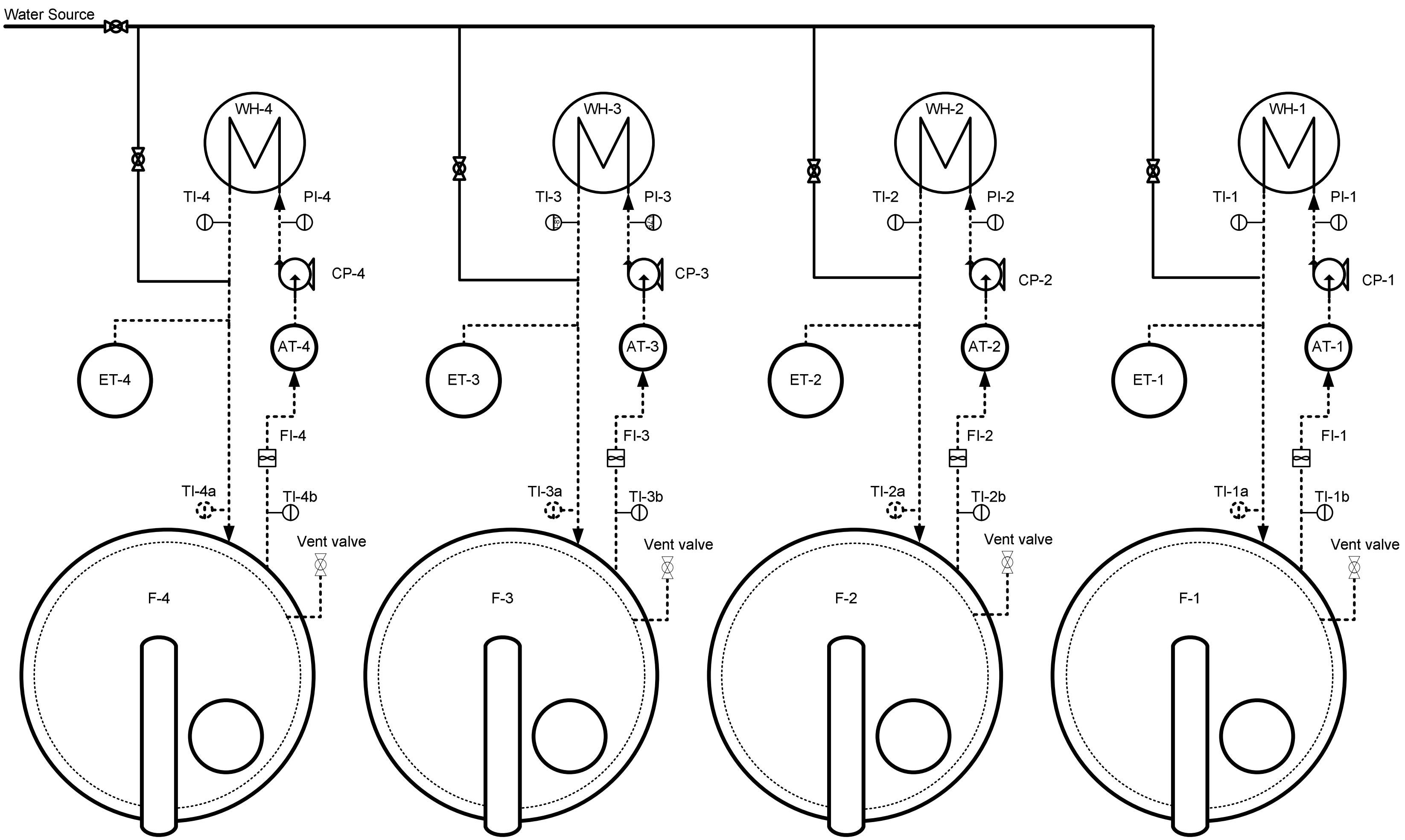


Figure IV-5. Final water jacket design.

NEW TRANSFER METHOD

A new solid and liquid transfer method was developed due to the inadequacies of the original design. After observing how the substrate behaved, separating the solids and liquid by means other than gravity was necessary. The solution was to perform the separation by filtration by using pressure supplied by the sludge pumps as the driving force.

A large vessel commonly used in the oilfield industry was chosen as the separation device. The filter vessel and operational procedure are described in more detail in Chapter II. The medium was pushed by the sludge pumps through the filter. Solids collected in the strainer basket and liquid exited from the other side of the vessel. The resulting liquid contained approximately 5 to 10% solids by volume. The collected solids were still saturated with liquid with a moisture content of about 75 wt%.

When the filter vessel reached maximum capacity, the process was stopped. The vessel was opened by loosening eight bolts that secured the lid. The strainer basket was removed and the solids were transferred into buckets by hand. The liquid was measured in a holding tank or in the product tank. This process was repeated until enough liquid and solids were obtained for the particular fermentor. The solids were then transferred to the appropriate fermentor manually. This process worked well, but was very time consuming and physically intensive. Depending on the amount transferred, the process sometimes took as long as 8h. Despite the labor demand, this method was adopted because it worked well and was a very inexpensive solution.

MIXING ISSUES

Sufficient mixing of the fermentors was important to keep the inhibitor evenly distributed and to blend in the fresh and transferred biomass. Mixing was performed by circulating the reactor contents with the sludge pump, as described previously. The consistency of the fermentor contents caused several mixing problems. The inability to achieve proper mixing resulted in higher methane production and product concentration gradients, which affected product conversion. Mixing was very important and was one of the main limiting factors in the design.

Fresh Paper

Because the paper was not finely shredded, the fresh biomass in the first fermentor clumped together and formed an impenetrable, floating layer. It was common for the partially digested biomass to be pumped on top of the thick layer of paper, but unable to pass through. When the majority was pumped to the top, the suction-side of the sludge pump would clog, making it necessary to turn the pump in reverse to transfer the movable fluid back to the bottom. Often, the only way to achieve sufficient mixing was to pump the movable fluid back and forth to the top and bottom. The conclusion was made that improved mixing could be achieved with more finely shredded paper.

Dr. Holtzapple donated his residential chipper/shredder for use at the pilot plant. Before every biomass addition, the paper was shredded into a confetti-like material. The finely shredded paper aided in homogenizing the reactor contents and improved mixing, as well as helped to prevent clogging.

Circulation Loop Clogging

The circulation loops clogged often during mixing. As described previously, the original design used 2-in PVC pipe at the top of the circulation loop. The turns in the pipe, especially the exiting tee, were very prone to clogging. Figure IV-6 shows the original and modified circulation loops. The red circles show areas that were susceptible to clogging. On two separate occasions, the circulation loop became plugged without notice. Pressure increased and ruptured the PVC pipe. In addition to causing broken pipes and a mess, it also placed unwanted strain on the sludge pumps. The design was changed by replacing the 2-in pipe with 3-in pipe. The exiting tee was also removed. Installing a 3-in tee would improve mixing, however the inside of the loaded fermentors were inaccessible for installation (see Figure IV-6 for the modification). The increased pipe size prevented the circulation loop from clogging again.

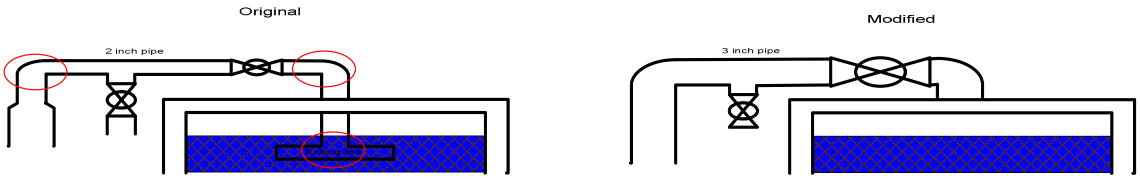


Figure IV-6. Circulation loop modifications.

Channeling

Another challenge was preventing the circulated biomass from channeling. After the exiting tee was removed from the circulation loop, the biomass entered the top center of the fermentor. Naturally, the biomass would migrate through the core of the fermentor back to the bottom where it was pumped around again. This channeling effect greatly affected proper mixing. The substrate that resided along the walls of the fermentors stayed in place, allowing only the middle to be well mixed. The immediate solution was to manually aid mixing with a shovel. This was very time consuming and did not help in the area opposite of the manhole; this area was too far out of reach.

Another issue that increased channeling and impeded mixing was the piping left in the fermentors from the heat exchanger circulation loop. The internal filter and distributor were left in the fermentors because it was not feasible to remove them. As discussed earlier, the piping inside the fermentors was too heavy and caused the top of the tanks to cave in. When the discovery was made that the internal piping was not going to be used, it was cut from the tops of the fermentors. An attempt was made to try to pull the pipe to the surface and disassemble it, but it was too heavy. To remove the piping, it would be necessary to almost completely empty the fermentors. The contents would have to be transferred into a holding tank and then pumped back into the fermentor with an auxiliary sludge pump. There was not a large enough holding tank nor an auxiliary pump available, so the piping was left in the fermentors.

The abandoned pipes were believed to exacerbate the mixing problems because the piping obstructed flow and created an obstacle for the medium to flow around. This also contributed to poor mixing and efforts should be made to remove the piping in the future.

Proper mixing was very critical to managing methane production. Without proper mixing, the inhibitor could not be distributed evenly, and methane production would increase. Poor mixing also affected the microorganism's ability to consume the biomass. The freshest biomass was the most susceptible to microbial attack. If the fresh biomass was not evenly distributed, the microorganisms had to migrate towards the preferred biomass. Improper carbohydrate-to-nutrient ratios could also result. Therefore, proper mixing was essential and further design improvements should be considered.

PUMP FAILURE

There were also challenges with the sludge pump operations. The pumps worked well, but their performance declined gradually over time. The pumps would first lose their ability to create suction when operated in reverse, which was crucial during the transfer procedure. The pump consisted of a rotor and stator. The rotor was made of chrome-plated steel and was machined into a cork screw shape. The rotor was housed in a special rubber sleeve called the stator which channeled the fluid as it built pressure.

The pump on the second fermentor failed first, and when dismantled, significant damage was exposed on the rotor. The necessary parts were replaced, but not until the pump on the third fermentor broke down, was the cause of the failure discovered. Small metal pieces were found in the pump housing and some wrapped around the rotor. The metal was remains of paper clips and staples from the shredded paper. The metal would collect in the pump and eventually wear grooves in the metal rotor that would let fluid slip by. Eventually all four pumps were equipped with a new rotor and stator.

Special care was taken to remove any noticeable metal pieces during solid and liquid transfer procedures. Also special attention was given when fresh paper was added to ensure no metal entered the fermentors. A more proactive pump maintenance program should be considered because the parts are very expensive to replace and require a great deal of work to install. The parts are also uncommon and take several days to ship.

PRODUCT STORAGE

Another design issue was the lack of means to store the carboxylic acid product. Limiting oxygen exposure was preferred because over-exposure contributed to product degradation (Holtzapple). A 1000-gallon polyethylene tank was available for use at the pilot plant. To limit oxygen contact, the best solution was to use a nitrogen blanket in the tank. However, the tank was not air tight and not made to operate under pressure. The least expensive solution was to weld the polyethylene tank to be air tight. To prevent tank failure, the nitrogen blanket was used at very low pressures. The product tank is described in more detail in Chapter II.

SUMMARY OF DESIGN FLAWS AND SOLUTIONS

Table IV-1 summarizes the original design flaws and how the problems where solved. It also includes problems that were not anticipated and how they were solved.

Table IV-1. Summary of process problems.

SUBJECT	ANTICIPATED ISSUE	SOLUTION TO ANTICIPATED ISSUE	ACTUAL ISSUE	SOLUTION TO ACTUAL ISSUE
Temperature Control	Heat process fluid	Circulate liquid through heat exchangers	Equipment could not handle solids	Abandon heat exchanger idea
	Create thermal barrier	Water jacket supplied by water heater	Not enough heat	Use one water heater per fermentor
Solids and Liquid Transfer Method	Clean liquid layer on top of solids	Pump liquid layer with circulation pump	Equipment could not handle solids	Use sludge pump to push fluid through filter to separate solids and liquids
	Solids would settle to the bottom	Pump settled solids with sludge pump	Too much liquid in sludge	
Measuring Transferred Liquid	Needed to measure amount of liquid transferred	Use flow meter	Equipment could not handle solids	Use measuring tank after filtration
Measuring Transferred Solids	Needed to measure amount of solids transferred	Measure settled solids height under liquid	Solids did not settle	Weigh solids after filtration
Mixing	Fermentor contents needed to be mixed	Mix by circulating fluid with sludge pump	Pipes in circulation loop clogged	Replace with larger diameter pipe
	Distribute inhibitor evenly and blend fresh biomass	Mix by circulating fluid with sludge pump	Poor mixing	Assist mixing manually
Fresh Biomass	Needed fresh paper source	Obtain finely shredded paper	Finely shredded paper not available, constipated fermentors	Shred paper to finer consistency with chipper/shredder before adding
Pump Failure	Not anticipated	Not anticipated	Metal from paper damaged pumps	Repair pumps and sort fresh paper
Product Storage	Not anticipated	Not anticipated	Needed storage tank for product with limited oxygen exposure	Used polyethylene tanks with nitrogen blanket

CHAPTER V

RESULTS AND DISCUSSION

When the project began, many questions existed concerning how operational procedures would be performed. A lot was unknown about the substrate stratification behavior upon scaling up the process. The objective was to prove that laboratory results could be reproduced on a larger scale. As more was learned, the bulk of the effort was concentrated on overcoming process limitations based on material handling issues. The results were not ideal, but very important lessons were learned about the process on a more industrial scale.

The basic data that were collected included liquid product samples, gas samples, fermentor temperature, and pH. Later in the project, solids samples were taken to analyze fermentor solids ratios. Overall material balances were not calculated because it was not feasible to monitor all mass entering and exiting the system. This also prevented overall biomass conversion and acid selectivity from being evaluated. Steady state was never achieved, thus it was not meaningful to calculate acid yield and productivity.

As the project developed, the parameters were modified to adapt to process limitations. The process can be broken down into seven stages. Over the course of the project there were two batch mode intervals and five different countercurrent modes of operation. The batch modes were designated as Batch Mode I and II, whereas the countercurrent modes were designated as Countercurrent Mode I, II, III, IV, and V. This chapter will discuss the different operational modes in chronological order, as well as the effect of fermentor solids ratios and the overall results.

BATCH MODE I

Operation Conditions

The fermentor loading process was started on April 5, 2005 and was completed on April 15, 2005. The fermentors were operated in batch to allow the bacteria culture to establish and adapt to the new environment. As the system was idle, the acid concentrations were also allowed to build. At this point, it was discovered that the heat exchanger circulation loop was inadequate. This also eliminated the use of the original solid and liquid transfer method that was necessary for continuous operation. Batch Mode I (BM1) was intended to last for approximately 2 weeks, but was prolonged while a new transfer method was investigated. Batch operation continued for approximately 2 months and ended on June 16, 2005.

Results

Figure V-1 shows the total carboxylic acid concentrations in each fermentor. The first samples were collected on April 19, 2005. The fermentors were loaded starting with Fermentor 4 (F4) and ending with Fermentor 1 (F1). F4 had the highest initial total acid concentration because it was loaded first, and F1 had the lowest initial concentration because it was loaded last. The acid concentrations increased as expected.

The highest acid concentration during batch operation was in F4 at 29.5 g/L. Table V-1 shows the average acid compositions from BM1.

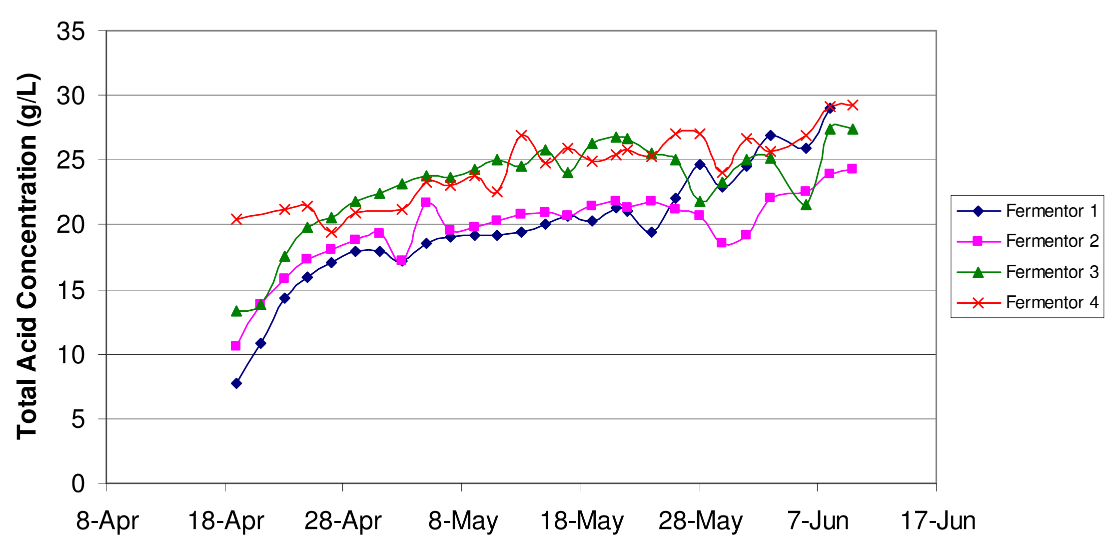


Figure V-1. Batch Mode I: Total acid concentrations in each fermentor.

Table V-1. Batch Mode I: Acid compositions (wt %).

Acetic Acid	33.0
Propionic Acid	11.9
Butyric Acid	30.3
Valeric Acid	3.1
Caproic Acid	17.8
Other Higher Acids	3.9

Gas samples were taken periodically in syringes and then transported to the laboratory to be analyzed on the gas chromatograph (GC). Successfully running samples from all four fermentors proved to be very difficult. Injecting the samples into the GC was a cumbersome process; often the samples would not register on the machine. Also, gases tended to leak out of the syringes making data collection more difficult. Even with two samples per fermentor, obtaining results for all four fermentors was challenging. Two or three attempts were often made to obtain data for all four fermentors. Thus, successful gas sample data were only collected every couple of weeks. Table V-2 shows methane concentrations during BM1.

Table V-2. Batch Mode I: Methane concentrations (wt%).

Date	F1	F2	F3	F4
27-Apr	2.1	1.7	1.5	1.3
10-May	5.2	6.4	2.1	3.1
24-May	2.1	9.3	6.1	8.7
8-Jun	2.8	10.2	5.1	3.0

The methane concentrations typically stayed below 10 wt%. If the methane concentration increased over 5 wt%, a double dose of inhibitor was added and special care was taken to thoroughly mix the fermentors. The pH was recorded periodically and consistently stayed between 5.75 and 6.

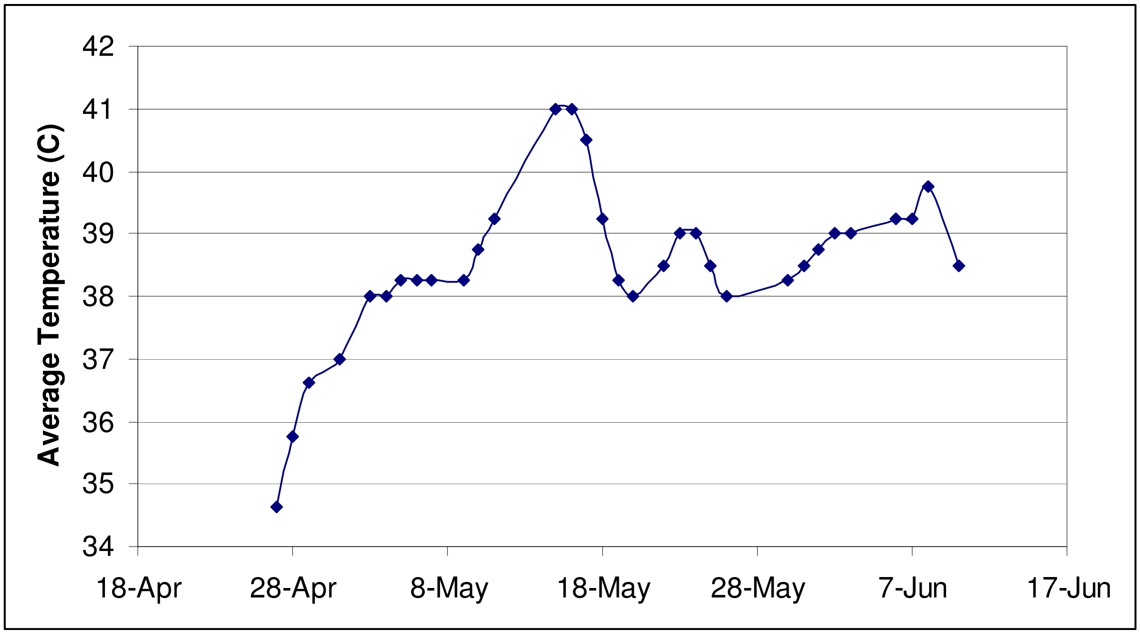


Figure V-2. Batch Mode I: Average fermentor temperature.

Figure V-2 displays the average temperature over all four fermentors. After start-up, the temperature was reasonably maintained closed to 40 ^{o}C throughout BM1.

COUNTERCURRENT MODE I

Operation Conditions

As soon as a new transfer procedure was developed and tested, continuous operation began. Countercurrent Mode I (CM1) lasted approximately 6 weeks, from June 16 to August 1, 2005. Solid and liquid transfers were performed once every 3 days. The VSLR and LRT were determined based on experiments performed on the laboratory scale. Depending on the system, a typical VSLR in the laboratory was between 3 and 10 g /(L liquid · d). A typical LRT was between 10 and 30 days. Operating the pilot plant with similar VSLRs and LRTs was impractical because of the amount of material that needed handling. These parameters were significantly reduced for operation to be feasible. Appendix F describes how the VSLR and LRT were determined for the pilot plant. The parameters actually used for CM1 were a VSLR of 1 g /(L liquid · d) and a LRT of 80 days. This was equivalent to a biomass loading rate of 80 lbs every 3 days (27 lbs/day) and a liquid transfer rate of 120 gallons every 3 days (40 gal/day).

The VSLR and LRT decided upon were based on what was thought to be reasonable, considering the equipment available and the amount of labor required. If operation went smoothly, these parameters were to be changed to better simulate laboratory procedures.

The procedure for CM1 was performed as follows: every 3 days, 120 gallons of fresh water was added to F4, 120 gallons of liquid was transferred from F4 to F3, 120 gallons was transferred from F3 to F2, 120 gallons was transferred from F2 to F1, and then 120 gallons was harvested from F1 as product. In the other direction, 80 lb of fresh biomass was added to F1, 80 lb of digested biomass was transferred from F1 to F2, 80 lb was transferred from F2 to F3, 80 lb from was transferred F3 to F4, and 16 lb was removed from F4 as waste. Only 16 lb of biomass was removed from F4 because the assumption was made that 80% of the biomass was digested by the time it reached F4 (Holtzapple).

Results

Figure V-3 shows the total acid concentration over time for all four fermentors. The acid profiles behaved as predicted. The acid concentration increased in F1 and reached a maximum on July 20 with a value of 32.4 g/L. The acid concentrations for the other three fermentors decreased as expected, with F4 having the lowest acid concentration of 10 g/L on July 29, 2005.

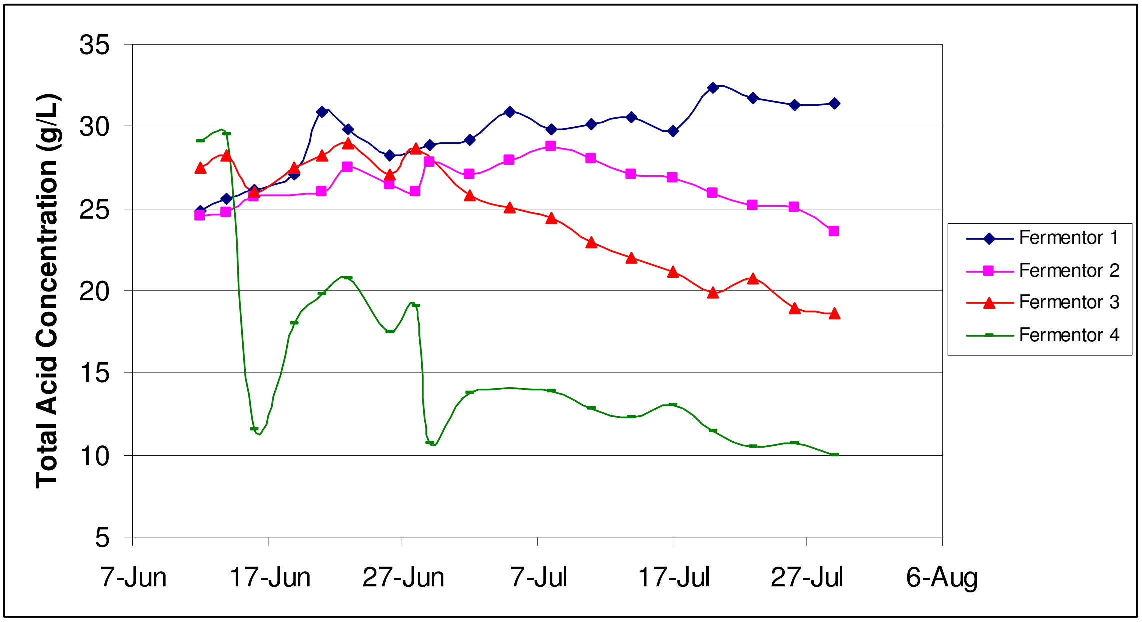


Figure V-3. Countercurrent Mode I: Total acid concentration in each fermentor.

Table V-3. Countercurrent Mode I: Methane concentrations (wt%).

Date	F1	F2	F3	F4
22-Jun	5.9	5.1	8.1	10.1
12-Jul	2.9	8.2	3.9	7.6
26-Jul	4.7	3.1	3.4	6.5

The methane concentrations are shown in Table V-3. Lower methane concentrations were preferred, but this was difficult to obtain without improved mixing. The pH consistently stayed between 5.75 and 6.5.

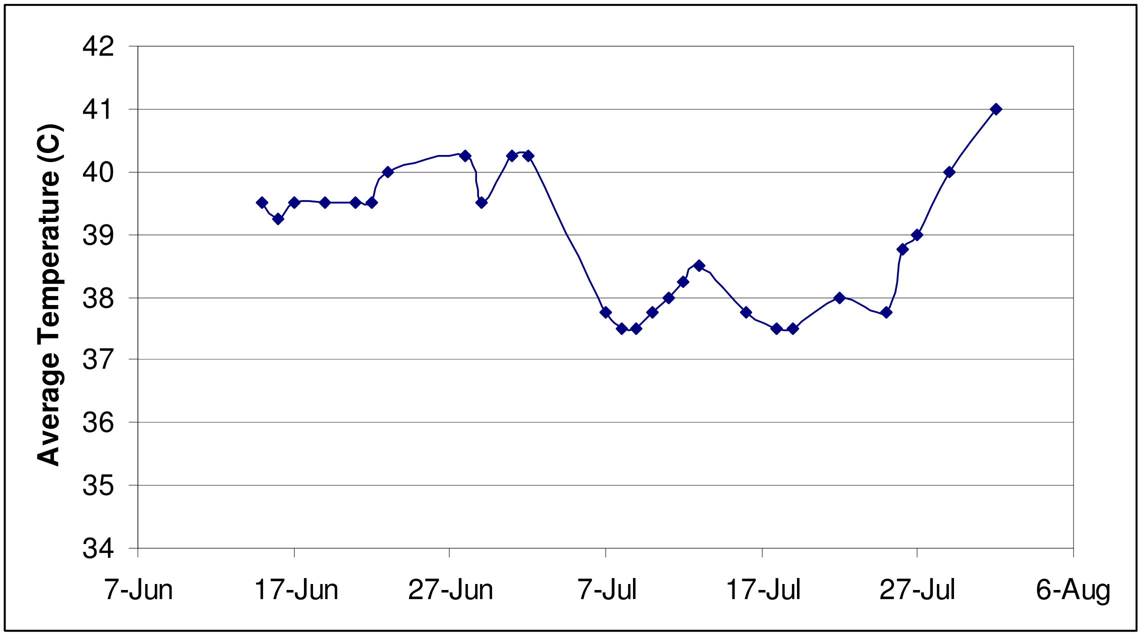
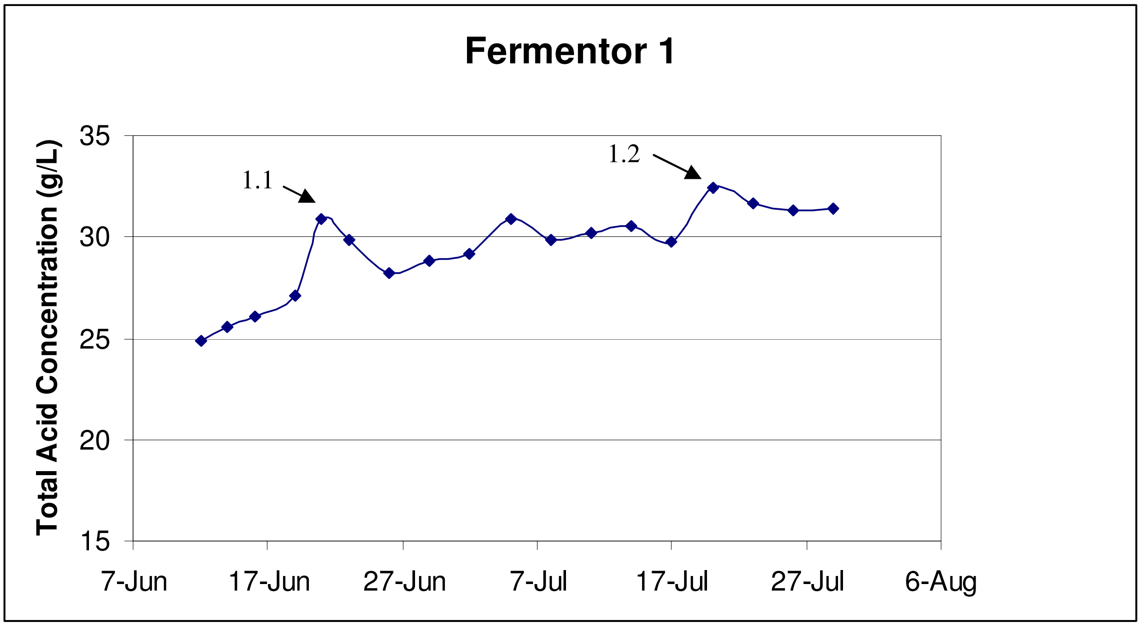


Figure V-4. Countercurrent Mode I: Average fermentor temperature.

The fermentor average temperature was not a major problem in CM1, as shown in Figure V-4. It was controlled close to the desired temperature of 40 °C.



FigureV-5. Countercurrent Mode I: Fermentor 1 acid concentration.

Figure V-5 shows the F1 total acid concentration. The acid concentration in F1 is the most important because this is where product is harvested. There are two main points on the acid profile that are worth discussing. At Point 1.1 (June 30, 2005), the system went for 13 days without material being transferred because of modifications being made to the transfer procedure. The concentration increased to over 30 g/L, but as soon as material was transferred again, the immediate effect was a decline in acid concentration. This decline is observed because the liquid transferred into F1 was lower in acid concentration and had a diluting effect. Acids production continued, and reached a maximum concentration of 32.4 g/L at Point 1.2 (July 20, 2005). At this time period, there were very noticeable changes in the fermentation substrate stratification behavior. The substrate had gradually become very thick and dense, and started to congest the fermentor. Being able to continue with the countercurrent operation depended on the sludge pump's ability to move the medium to achieve solid and liquid separation. The microorganisms could not break down the biomass fast enough to prevent the inlet of the sludge pump from clogging. However, the higher solids content resulted in the highest acid concentration. Obtaining higher acid concentrations were limited by the ability to achieve solid and liquid separation.

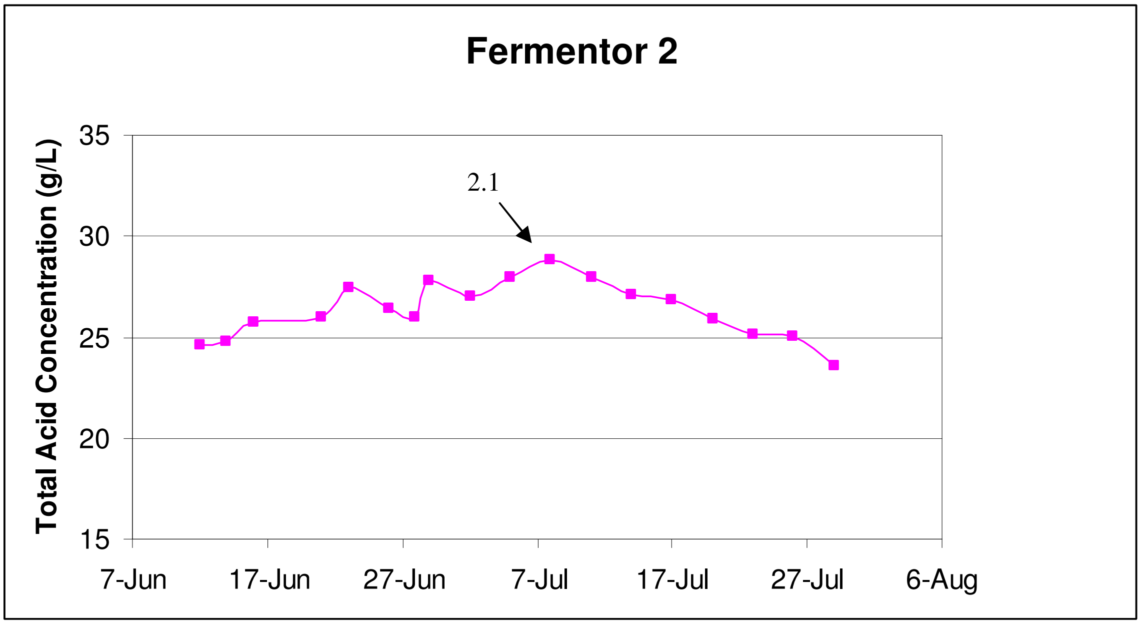


Figure V-6. Countercurrent Mode I: Fermentor 2 acid concentration.

Figure V-6 shows the acid concentration profile in F2. The acid concentration continued to rise in F2 until Point 2.1 (July 8, 2005). After this point, the acid concentration declined with time. The concentration increased until the lower concentrated liquid from F3 started to dilute the acid concentration in F2. Microorganisms could not produce enough acid to overcome this dilution factor. Another contributing factor was that the microorganisms were fed more partially digested biomass, as opposed to fresh biomass. This trend was expected, due to the nature of the countercurrent fermentation process.

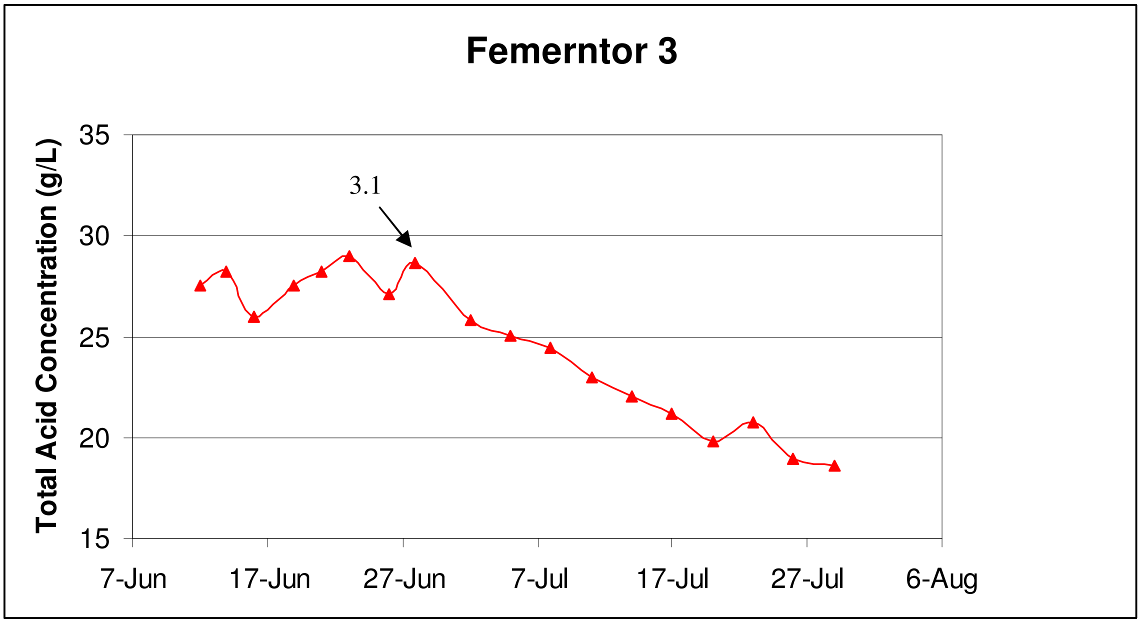


Figure V-7. Countercurrent Mode I: Fermentor 3 acid concentration.

The same trend occurred in F3 as shown in Figure V-7. The acid concentration continued to build until Point 3.1 (June 28, 2005), and then declined. However, the decline in concentration occurred sooner in F3 because the liquid transferred form F4 contained even lower acid concentrations.

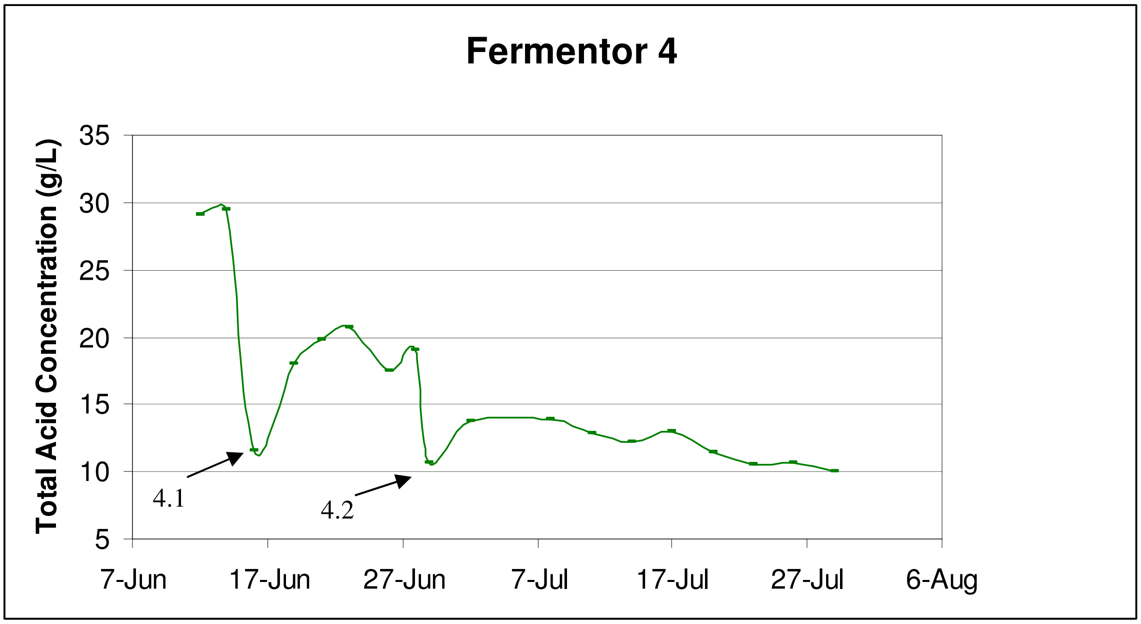


Figure V-8. Countercurrent Mode I: Fermentor 4 acid concentration.

The acid concentration profile in F4 is shown in Figure V-8. The sharp decline in acid concentration at Point 4.1 (June 16, 2005) occurred immediately after fresh water was added to the fermentor. Because of complications with the procedure, the next transfer did not occur until June 26, 2005. During this time, the acid concentration started to increase again. The decline at Point 4.2 (June 29, 2005) was a result of the addition of fresh water on June 26, 2005. As continuous operation continued, the acid concentration steadily declined.

The fermentation process was operated successfully until the end of CMI. The overall acid production was increasing and methane production was maintained below 10 wt%. Solids handling issues forced operational changes to be made, but the results observed between June 21 and Aug 1, 2005, are promising. This was a time period of only 42 days. However, if the process was not limited by the material handling issues, a reasonable assumption could be made that the results would improve upon reaching steady state. Table V-4 shows the results of CM1.

Table V-4. Countercurrent Mode I: Results

VSLR	g /(L liquid · d)	1
LRT	days	80
Avg. Total Acid Product Conc.	g/L	29.6
Acetic Acid	wt%	39.6
Propionic Acid	wt%	8.9
Butyric Acid	wt%	26.8
Valeric Acid	wt%	3.4
Caproic Acid	wt%	18.1
Other Higher Acids	wt%	3.2

The average acid concentration is high (29.6 g/L) because of a long LRT. The average acetic acid composition was 39.6 wt%. To maintain or even improve these results, a steady state would have to be reached, but unfortunately changes were required to continue operation.

COUNTERCURRENT MODE II

Operation Conditions

In F1, the substrate mixture had become excessively thick, making it extremely difficult to pump any material out of F1. The solids loading rate was determined to be too high and needed to be reduced to maintain countercurrent operation. The addition of fresh biomass to F1 was stopped to let it digest to a manageable level. The transfer procedure was intended to continue the same as CM1, except that no fresh biomass was added to F1, which delayed steady state. This was the basis of Countercurrent Mode II

(CM2). This mode only lasted 11 days, from August 1 to August 11, 2005. Because of solids handling issues and equipment failures, a consistent solids and liquid transfer rate was not established. Several setbacks occurred during this time.

Because F1 was so congested, it was not possible to add 120 gallons for the transfer. The solids and liquid separation process started by pumping out of F1 into the filter vessel, but the suction-side of the pump clogged almost immediately. The pump was switched to reverse operation and fluid was sucked from the top of the tank to unclog the pump. The separation process was then continued until the pump clogged again. This process would be repeated several times until it was impossible to extract any more fluid from the fermentor. During these 11 days, it was never possible to remove the desired volume of liquid from F1. To maintain the liquid level, the same amount of liquid that was gathered from F1 would be transferred to the remaining three fermentors.

It was also observed that the solid content in F2, F3, and F4 was gradually decreasing as the biomass became more digested. Instead of adding fresh biomass to F1, 20 lb of fresh biomass was added to the other three fermentors after each transfer.

Results

Figure V-9 shows the acid concentrations in each fermentor. CM2 is represented between the two dotted lines. During the transfer on August 7, 2005 the sludge pump on F2 broke down. Solids or liquid could not be removed from F2, but the liquid product had already been taken from F1. F1 needed liquid to replace what was lost as product, thus F2 was bypassed and liquid from F3 was transferred to F1. Because the acid concentration was much lower in F3, the transferred liquid diluted the product in F1. This is shown by Point 1 (August 11, 2005) in Figure V-9. The failure of the second sludge pump (Pump 2) ended CM2. The highest total acid concentration in CM2 was 31.3 g/L. The average acid compositions are shown in Table V- 5.

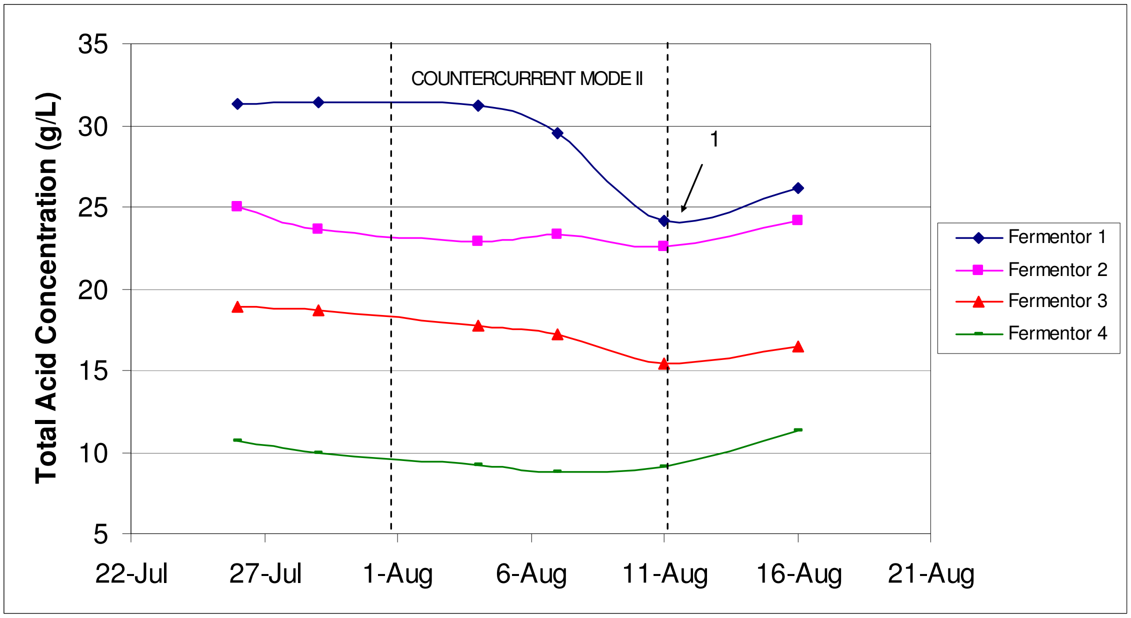


Figure V-9. Countercurrent Mode II: Total acid concentration in each fermentor.

Table V-5. Countercurrent Mode II: Acid compositions (wt%).

Acetic Acid	45.5
Propionic Acid	8.8
Butyric Acid	23.7
Valeric Acid	3.2
Caproic Acid	15.6
Other Higher Acids	3.3

Table V-6. Countercurrent Mode II: Methane concentrations (wt%).

Date	F1	F2	F3	F4
5-Aug	6.1	4.2	5.8	8.2

The methane concentrations are reported in Table V-6. The pH remained between 5.8 and 6.

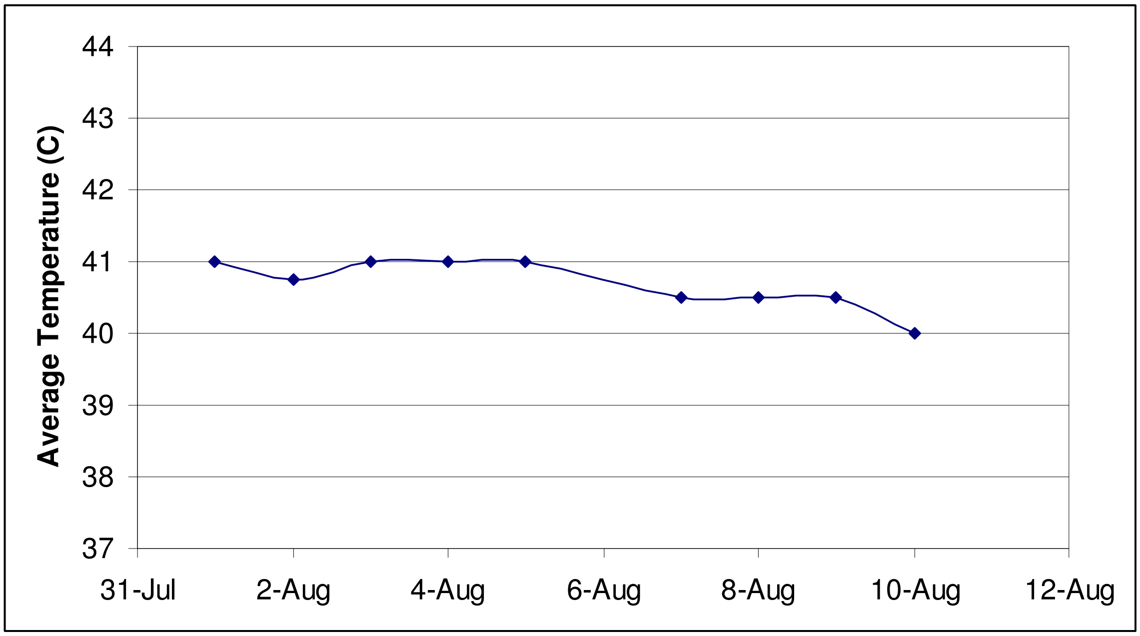


Figure V-10. Countercurrent Mode II: Average fermentor temperature.

In CM2, the temperature was successfully maintained close to the desired set point of 40 °C, as shown in Figure V-10.

Conclusions were not drawn from CM2 because the duration of this mode was short-lived. The results were not analyzed because of the small amount of data collected. The primary concern became repairing Pump 2.

BATCH MODE II

Operation Conditions

Continuous operation was delayed because of the failure of Pump 2. Continuous operation with only three fermentors was possible; however the decision was made to wait until all four fermentors were working. During this time, the process was left in batch mode. Batch Mode II (BM2) lasted approximately one month, from August 11 to September 8, 2005. The parts necessary to fix the pump were ordered, but due to complications with the order, it took nearly 4 weeks for their arrival.

Results

Figure V-11 shows the acid concentration profiles in each fermentor. While the fermentors were idle, the acid concentrations increased in all four fermentors. The acid concentration in F1 was recovering from being diluted during CM2. F2, F3, and F4 increased because the acid had time to become more concentrated when the fermentors were not countercurrently operated. An increase in acid concentration was expected. The highest total acid concentration in BM2 was 30.3 g/L. The average acid compositions are shown in Table V-7.

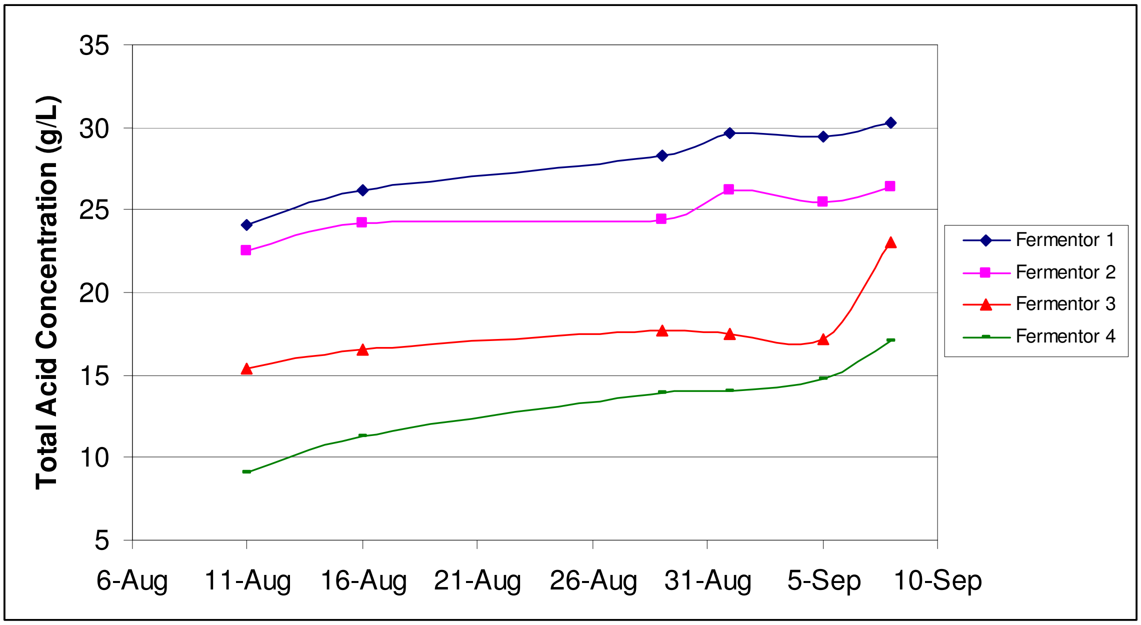


Figure V-11. Batch Mode II: Total acid concentration in each fermentor.

Table V-7. Batch Mode II: Acid compositions (wt%).

Acetic Acid	45.5
Propionic Acid	8.5
Butyric Acid	23.6
Valeric Acid	3.1
Caproic Acid	15.8
Other Higher Acids	3.4

In BM2, the methane concentration was not observed to rise above 10 wt%, as shown in Table V-8. The pH in each fermentor stayed consistently close to 6, and an average temperature of 40 ^{o}C was closely maintained, as shown in Figure V-12.

Table V-8. Batch Mode II: Methane concentrations (wt%).

Date	F1	F2	F3	F4
17-Aug	5.6	8.7	4.1	8.8
6-Sep	4.1	7.7	3.8	9.4

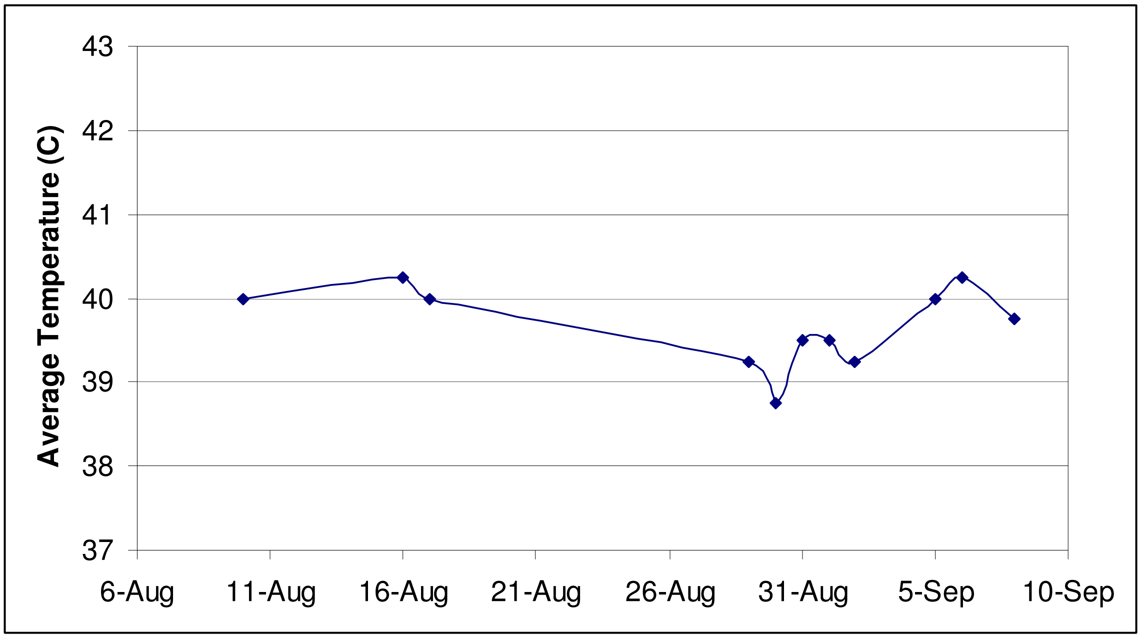


Figure V-12. Batch Mode II: Average fermentor temperature.

COUNTERCURRENT MODE III

Operation Conditions

Continuous operation started again as soon as Pump 2 was repaired. After existing in batch mode for several weeks, F1 became more fluid. The normal transfer procedure was continued, but operated at a lower VSLR and a longer LRT. It was determined in the beginning of CM2 that the solids loading rate was too high. The

VSLR now used was 0.25 g/(L liquid · d) and the LRT used was 318 days. The new VSLR was ¼ of the VSLR used in CM1, and the LRT was 4 times longer. These parameters were significantly different than what has been used on the laboratory scale, but changes were necessary to continue operation. If the parameters were unchanged, F1 would soon become constipated and continuous operation would have to be stopped again. During Countercurrent Mode III (CM3), 20 lb of fresh biomass was added and 30 gallons of product was harvested every 3 days. If operation went smoothly, plans were made to increase these rates.

Another observation made was that the solids content in the last three fermentors was continually decreasing. This became apparent due to the gradual decrease in the amount of solids obtained in the filtration process from F2, F3, and F4. In CM1, the same amount of biomass was transferred to each fermentor except for out of F4. If the same amount of mass is transferred to each fermentor and there is mass lost due to consumption, then naturally the solid content would decrease. This seemed to hold true for all fermentors, except F1. To correct this issue and maintain the fresh biomass feed rate, less mass should be transferred to each fermentor in the direction of F1 to F4, to account for biomass consumption. The biomass digestion rate in each fermentor was unknown, so it would have to be assumed. The amount of solids transferred to each fermentor was reduced by 25 wt% to account for mass that was consumed. The amount transferred from F1 to F2 was 16 lb, from F2 to F3 was 12 lb, from F3 to F4 was 8 lb and out of F4 was 4 lb. This was an attempt to keep the solids content in the last three fermentors from getting too low.

Results

Figure V-13 shows the total acid concentration profiles for each fermentor. When operation switched from batch to continuous mode, the immediate effect was a decrease in acid concentration in all fermentors. This was again from a dilution effect from the transfer of lower concentrated liquid from the adjacent fermentor.

On September 24, 2005 bad weather was expected from Hurricane Rita. For safety reasons, the transfer scheduled on that day was cancelled. Effects on the data were not observed, except for in F4. Because there was no transfer for 5 days, the acid concentration increased in F4, as shown by Point 4 (September 26, 2005). When normal operation continued, the acid concentration came back down. The highest acid concentration in F1 was 29.6 g/L, shown by Point 1 (October 5, 2005).

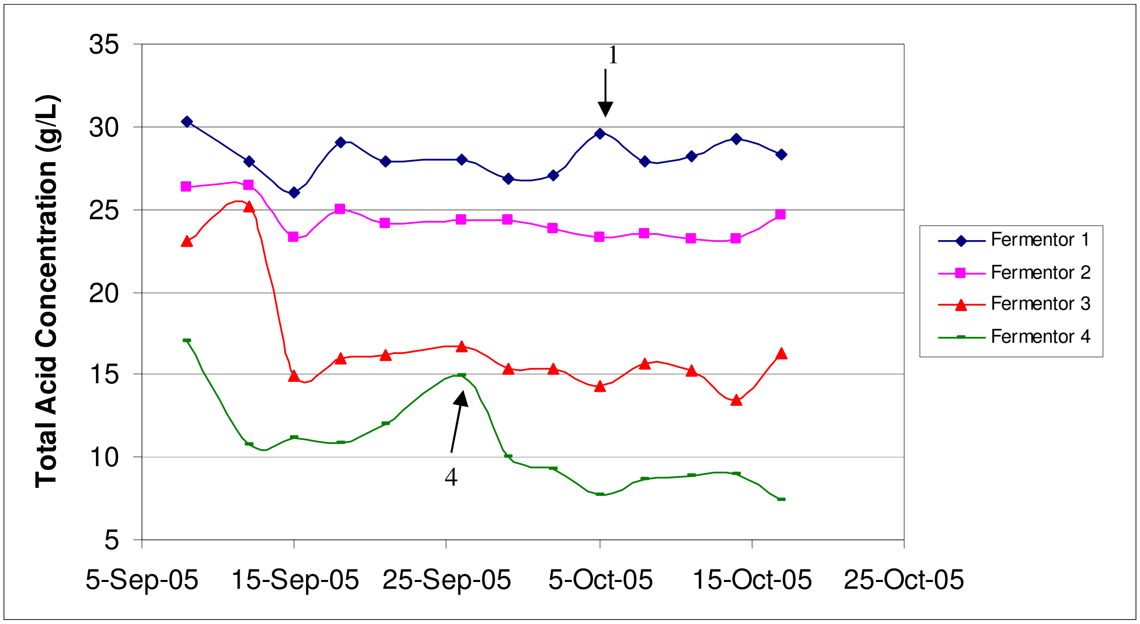


Figure V-13. Countercurrent Mode III: Total acid concentration in each fermentor.

Table V-9. Countercurrent Mode III: Methane concentrations (wt%).

Date	F1	F2	F3	F4
16-Sep	2.8	2.0	4.8	7.2
28-Sep	4.3	8.1	3.5	9.7
12-Oct	7.1	11.9	7.4	14.1

The methane production became more of a problem at the end CM3, as Table V-9 shows. Additional inhibitor was added for the last couple of weeks to bring the methane concentrations back down. Extensive mixing was also employed to ensure that the inhibitor was evenly distributed. The pH was successfully maintained between 6 and 6.5 in all fermentors.

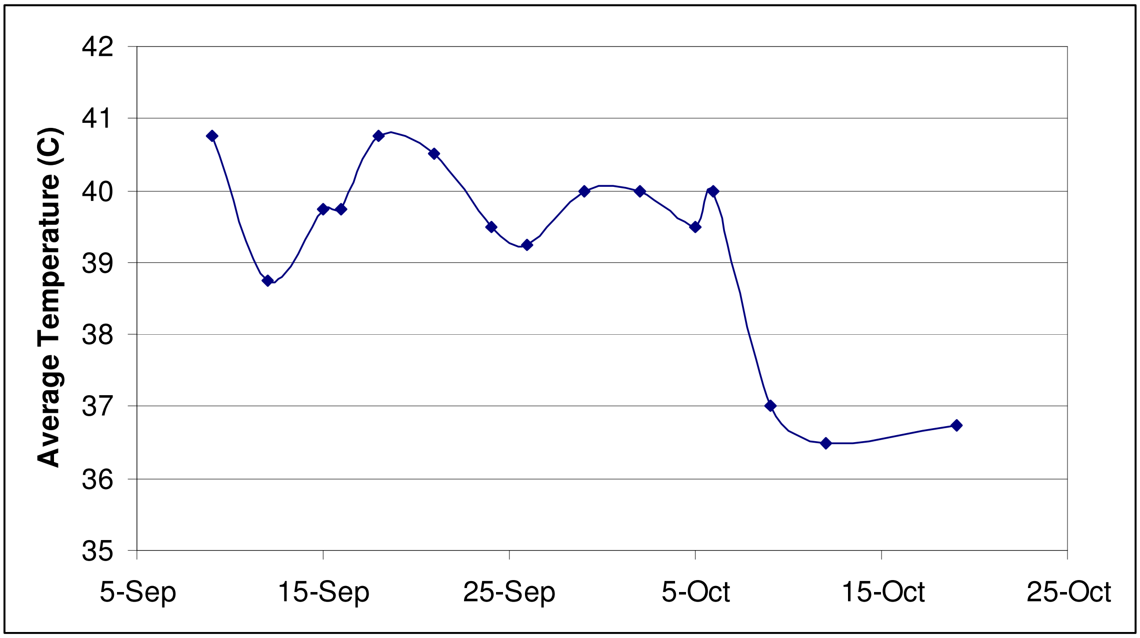


Figure V-14. Countercurrent Mode III: Average fermentor temperature.

Figure V-14 shows the average fermentor temperature during CM3. Temperature was controlled around 40 ^{o}C until October 6, 2005. A cold front decreased the outside temperature, and the water heaters could not supply enough heat to counter the temperature decline. The heater set points were increased, but several days were required for the fermentor temperatures to respond.

Although the fermentor temperatures declined, little effect on the acid concentrations was observed at this time. The concentration profiles remained steady during the cold period, as shown in Figure V-13.

Even with a lower VSLR, F1 still became congested. Complications persisted when trying to remove material from F1, but because the amount of material that needed to be removed was much less than before, the transfers were always possible. The consistency of the fresh paper was determined to be a major factor in causing congestion in F1. If the paper was shredded into a finer consistency, the substrate stratification behavior in F1 was thought to improve.

The reduced solids and liquid loading rates decreased process performance significantly. The reduced VSLR had the most impact on the data. Decreased liquid rates correlated to a longer LRT, which should help increase total acid concentration. However, the microorganisms were exposed to less fresh biomass, which limited productivity. The average total acid concentration in F1 was 28 g/L with an average acetic acid composition of 46.2 wt%. The acetic acid composition of the product was about 6 mol% higher than in CMI. Although not obvious, the lower temperature may have played a minor role in the lower acid production. Increased methane production may have also been a contributing factor. Steady state was not achieved. The results of Countercurrent Mode III are shown in Table V-10.

Table V-10. Countercurrent Mode III: Results.

VSLR	g /(L liquid · d)	0.25
LRT	days	318
Avg. Total Acid Product Conc.	g/L	28.0
Acetic Acid	wt%	46.2
Propionic Acid	wt%	9.7
Butyric Acid	wt%	21.8
Valeric Acid	wt%	3.3
Caproic Acid	wt%	15.4
Other Higher Acids	wt%	3.7

COUNTERCURRENT MODE IV

Operation Conditions

Countercurrent Mode IV (CM4) started on October 17, 2005 and ended on December 7, 2005. It lasted approximately 7.5 weeks. The main focus during this operational mode was to increase the solids content in the last three fermentors and to maintain the maximum solid content in all four fermentors. The solid content was steadily decreasing in the last three fermentors. Acid production was thought to improve if each fermentor contained the maximum solids content. The maximum solids content was the maximum amount of solids that could be contained in the fermentor without jeopardizing the ability to make solid and liquid transfers.

A relationship between acid production and the solids content in each fermentor appeared to exist based on observations from CMI. Data were collected to determine a solids ratio in each fermentor during CM4 and during the next operational mode, CM5.

The effects of the solids ratio in these two modes of operation will be discussed later, in the *Effects of Solids Ratio* section of this chapter. The method used to determine the solids ratio is explained in Appendix H.

The countercurrent continuous operation was still performed using the same conditions as CM3. The VSLR was 0.25 g/(L liquid · d), and the LRT was 318 days. The difference was that periodically fresh solids were added to the last three fermentors to build the solids content. Between October 17 and November 1, 2005, 700 lb of fresh biomass was distributed between F2, F3, and F4. The fresh biomass caused operational problems so additions were stopped until CM5. Smaller amount should have been added over longer periods of time to prevent the fermentors from becoming congested.

Another concern was trying to prevent F1 from becoming too congested. The plan was to further shred the office paper to a finer consistency. This was believed to improve the sludge pumps ability to handle the substrate. During CM4, the office paper was shredded again with Dr. Holtzapple's residential chipper shredder. Before each biomass addition, the paper was shredded into a fluffy confetti-like consistency.

Results

Figure V-15 shows the total acid concentration in each fermentor. The concentration profiles in CM4 are more irregular because of changes in operational conditions, including fermentor temperature decreases and changes in solids ratios. The effects of the solids ratio will be discussed later, as mentioned above.

On November 7, 2005 the sludge pump on F3 (Pump 3) broke down. From November 7 to December 1, 2005, F3 was bypassed until the pump could be repaired. During this time, the acid concentration increased, as shown in Figure V-15, because there was no dilution effect from liquid transfers.

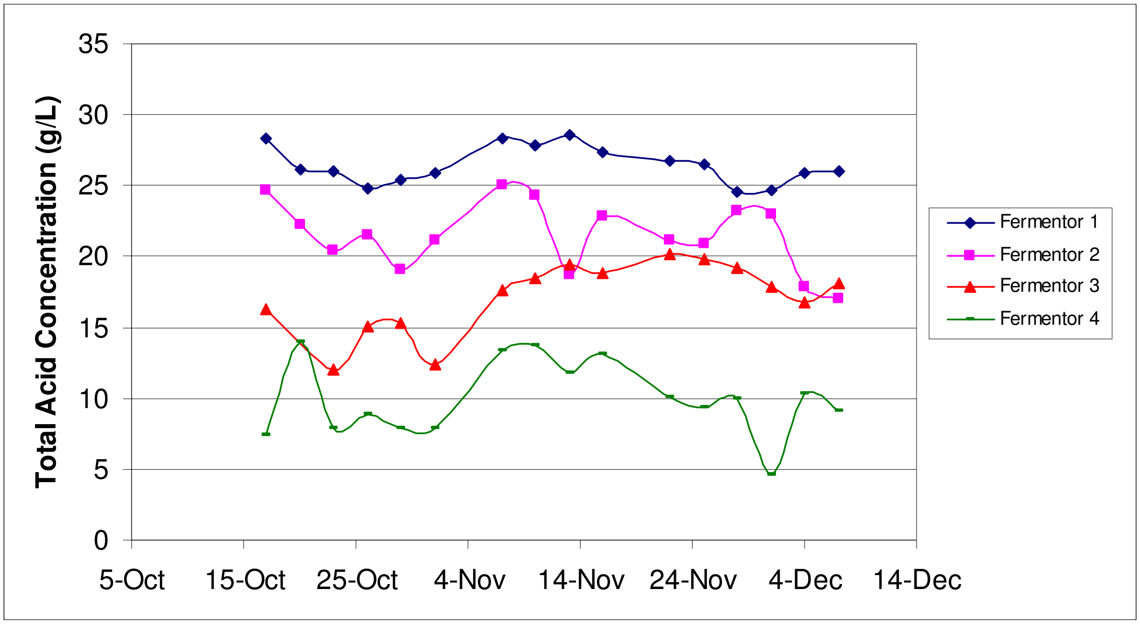


Figure V-15. Countercurrent Mode IV: Total acid concentration in each fermentor.

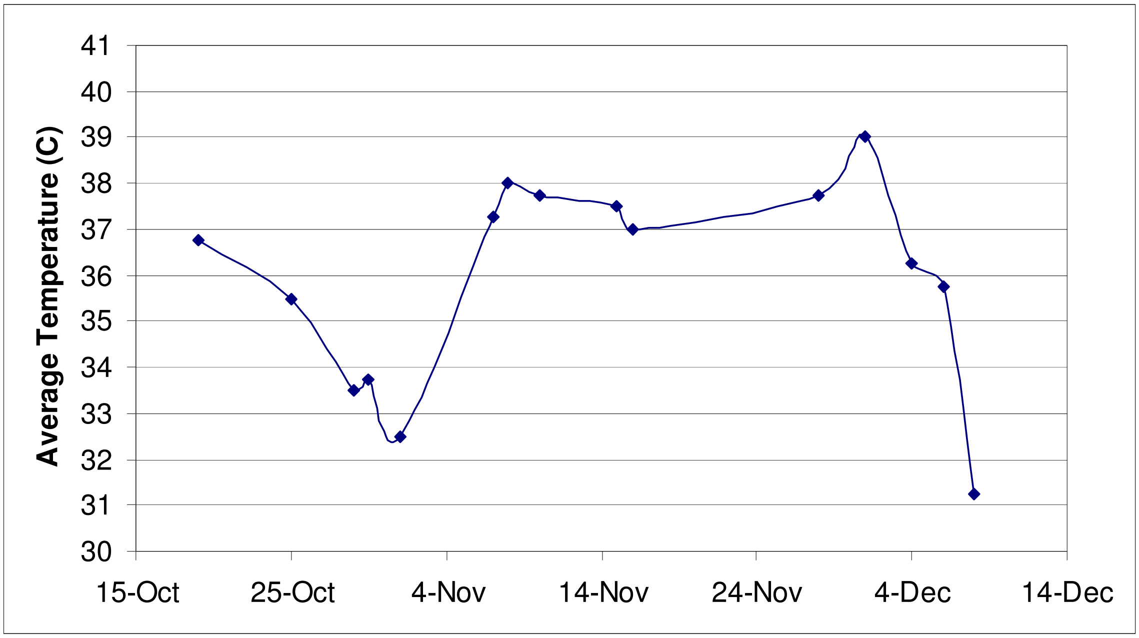


Figure V-16. Countercurrent Mode IV: Average fermentor temperature.

Temperature control became more of an issue in CM4, as shown in FigureV-16. Outside temperature declines caused the average fermentor temperature to drop. Changes to the temperature control system were now considered to better manage the fermentor temperatures.

The acid concentrations shown in Figure V-15 roughly followed the temperature trend in Figure V-16. In general the acid concentrations decreased with the temperature in late October, then increased through November with warmer temperatures, and decreased again in early December as it cooled.

Table V-11. Countercurrent Mode IV: Methane concentrations (wt%).

Date	F1	F2	F3	F4
19-Oct	7.12	9.9	6.2	23.5
25-Oct	8.5	8.7	7.6	11.9
1-Nov	7.5	6.7	6.4	5.9
16-Nov	9.5	14.3	8.7	19.2

The methane concentrations in each fermentor are shown in Table V-11. Methane concentrations were slightly elevated in CM4. This is mainly due to the inability to achieve good mixing. Increasing solids ratios in the fermentors seemed to affect mixing. Methane concentrations were especially high in F4. In mid October, there was a deceased bird found in the process water tank. The decaying bird encouraged methanogenesis and contaminated the water that was added to F4. The bird was removed, and the contaminated water was replaced with fresh water. This helped bring methane levels back down. Methane levels in F4 increased again in mid November, but were believed to be contributed to poor mixing.

Controlling the pH was still not a problem in CM4. The pH consistently remained between 6 and 6.5.

The results of CM4 are shown in Table 5-9. The original VSLR was 0.25 g/(L liquid · d), but the fresh biomass added to the other fermentors needed to be accounted for. The new VSLR was 0.92 g/(L liquid · d). The increased solid content in F2, F3, and F4 helped acid production in those fermentors, but did not carry through to F1. The ideal way to increase the solids content would be to add more biomass to F1, and then distribute more partially digested biomass out of F1 to the other three fermentors. This was not possible because the fresh biomass congested F1 preventing the ability to transfer material at all. Thus, the additional biomass did not directly affect the product concentration.

Therefore, there was a general decrease in performance in this mode of operation. The average total acid concentration in F1 was 26.3 g/L and the average acetic acid composition was 48.4 wt%, as shown in Table V-12. The inability to maintain the desired fermentor temperature played a major role in the decrease in acid production. The slight increase in methane production could have also influenced the product concentration. The fermentor solids ratio also contributed and will be discussed shortly.

Table V-12. Countercurrent Mode IV: Results.

VSLR	g /(L liquid · d)	0.92
LRT	days	318
Avg. Total Acid Product Conc.	g/L	26.3
Acetic Acid	wt%	48.4
Propionic Acid	wt%	11.8
Butyric Acid	wt%	18.5
Valeric Acid	wt%	3.3
Caproic Acid	wt%	13.6
Other Higher Acids	wt%	4.3

Although acid production decreased slightly, some success was reached from an operational stand point. Shredding the paper before it was added to F1 significantly improved the fermentor consistency. The biomass was now easier to mix and was less apt to clogging the sludge pump. The shredding procedure helped the overall process from an operational perspective, but the solids ratio was still high enough to affect transfer procedures.

During CM4 the solids ratio was determined to be optimal between 85 and 120 dry g/(L of slurry) (0.7-1.0 dry lb/(gal of slurry)). F1 consistently stayed above this level. Shredding the paper helped, but the solid content in F1 still needed to be reduced to ease operations.

COUNTERCURRENT MODE V

Operation Conditions

The purpose of countercurrent operation was re-evaluated in Countercurrent Mode V (CM5). The objective is to offset natural inhibitions to maximize biomass conversion and achieve a higher concentrated product. To take advantage of this operational method, the VSLR must be high enough to make an impact. In the previous two modes of operation, the normal VSLR was 0.25 g /(L liquid · d), which is equivalent to 6.7 pounds of dry biomass per day. If biomass consumption was taken into account, then the amount transferred between each fermentor decreases from F1 to F4. In each fermentor there was initially 1,500 lb of total dry biomass. This means that less than 1% of the mass of total solids in each fermentor was transferred per day. If the VSLR is compared to the total amount of solids in the system, it could be assumed that the solids transfers were insignificant and had very little effect on biomass digestion.

Based on this idea, adding fresh biomass to each fermentor to maximize the solids ratio and only transferring liquid through the fermentor train was logical. This would maximize the acid concentration in each fermentor, thus maximizing the product concentration. The disadvantage was that biomass would not be removed from the system, thus indigestible biomass would accumulate.

During CM5, only liquids were transferred and more effort was concentrated on maintaining a high and consistent solids ratio in each fermentor. Based on the calculated solids ratio, fresh biomass would be added to each fermentor as needed to try to obtain a solids ratio between 85 and 120 dry g/(L of slurry) (0.7-1.0 dry lb/(gal of slurry)). During this time period, 400 lb of fresh biomass was distributed between all four fermentors. The LRT remained the same at 318 days. This mode lasted approximately 5 weeks, from December 7, 2005 to January 12, 2006.

As shown in CM4, temperature control was not able to be maintained; the water heaters could not supply sufficient heat. Plans were made to install additional water heaters, and the installation was completed on December 21, 2005.

Results

The total acid concentration profiles in each fermentor are shown in Figure V-17, and the average fermentor temperature is represented in Figure V-18. Temperature control was obviously a problem. The low temperatures limited acid production in the beginning of CM5. When the new heaters were installed on December 21, 2005 and the temperature started to increase, so did the acid concentrations in F2, F3, and F4. However, acid concentrations in F1 did not increase. From December 16 to December 31, 2005 no fresh biomass was added to F1 in order to reduce the solids ratio to improve operations. This directly affected acid production. On the other hand, biomass was added to the other 3 fermentors which also contributed to the increase in acid production in F2, F3, and F4.

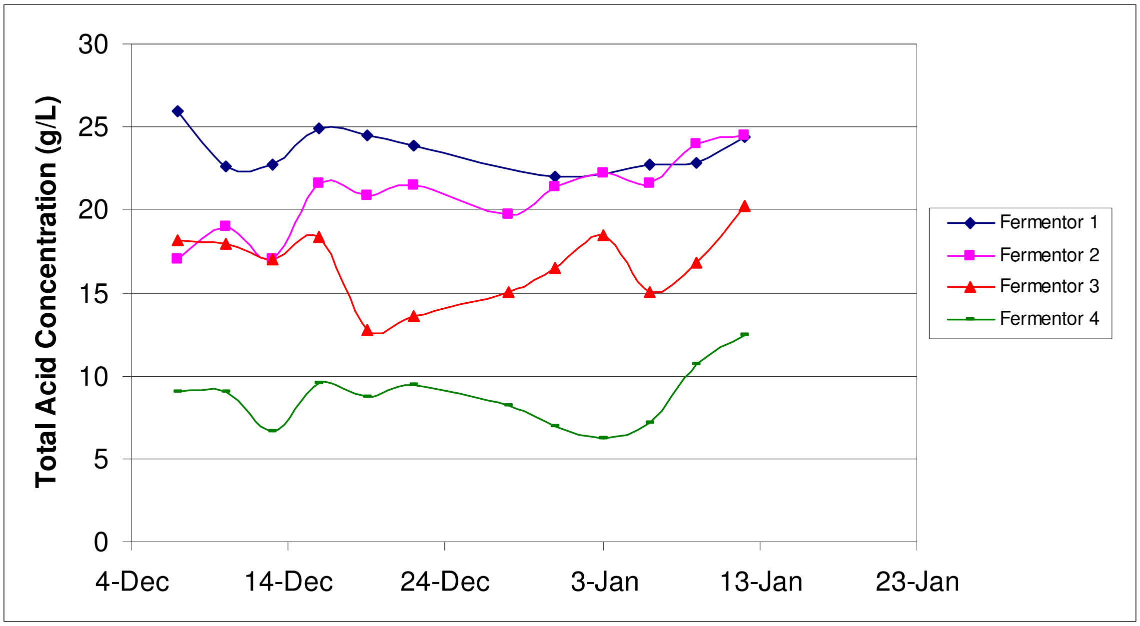


Figure V-17. Countercurrent Mode V: Total Acid concentration in each fermentor.

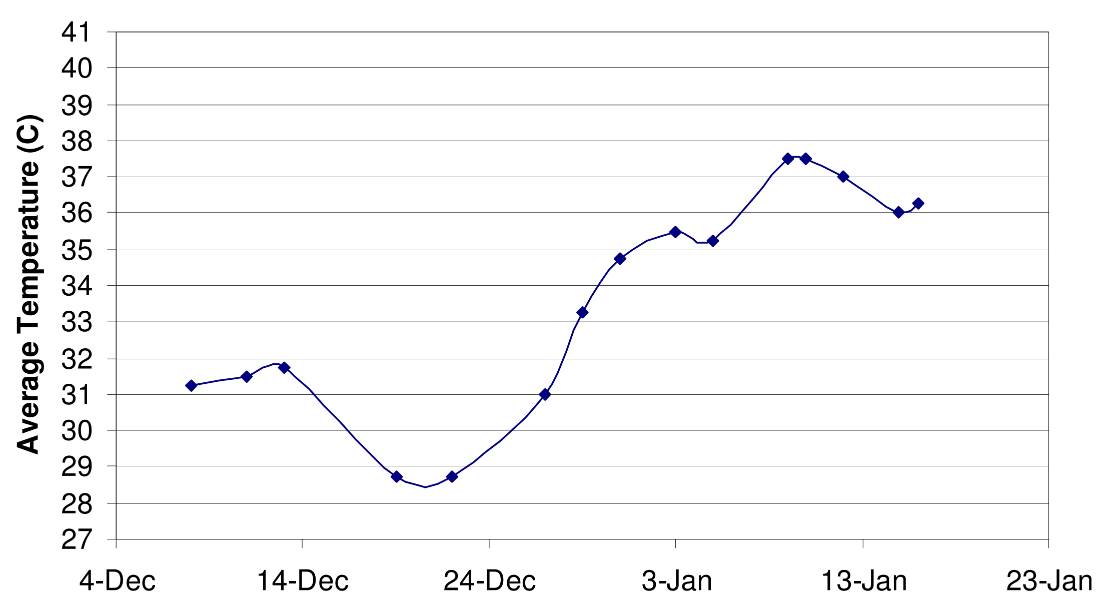


Figure V-18. Countercurrent Mode V: Average fermentor temperature.

There were no gas samples taken in CM5 because of problems with the gas chromatograph. The pH was still maintained between 6 and 6.5.

The traditional countercurrent method was not used in this mode. Biomass was added to all fermentors as opposed to only F1. Steady state was not achieved because of the operational changes. To determine the VSLR, the biomass feed rate must be known. To determine a feed rate the total biomass added was divided by the duration in days. The results of CM5 are shown in Table V-13.

Table V-13. Countercurrent Mode V: Results.

VSLR	g /(L liquid · d)	0.43
LRT	days	318
Avg. Total Acid Product Conc.	g/L	23.4
Acetic Acid	wt%	49.5
Propionic Acid	wt%	10.6
Butyric Acid	wt%	13.6
Valeric Acid	wt%	3.9
Caproic Acid	wt%	15.7
Other Higher Acids	wt%	6.7

The average total acid concentration in F1 was 23.4 g/L and the average acetic acid composition was 49.5 wt%, as shown in Table V-13. There were many factors that could have affected acid production. Temperature was obviously an issue and likely stunted microorganism activity. Limited amounts of fresh biomass were added to F1 during this mode to decrease the solids ratio, which affected acid concentration in the product. However, it is believed that if this operational procedure was performed for a longer time period, the result would become more stable. If solids concentrations were kept constant, a steady state would eventually be achieved. Because of time constraints, the project had to be passed on before further conclusions could be made.

EFFECTS OF SOLIDS RATIO

The procedure for determining the solids ratio in the fermentors is explained in Appendix H. The solids ratio that was compared in each fermentor, was a solids mass to volume ratio. This ratio is defined as grams of dry solids per liters of total slurry volume (dry g/L of slurry). According to the observed substrate behavior, the ideal solids ratio was found to be between 85 and 120 dry g/(L of slurry) (0.7-1.0 dry lb/(gal of slurry)) based on physical handling characteristics. Solids-to-volume ratios in this region seemed to be the maximum ratio that still allowed smooth transfer operations.

The solids ratio was determined after each solid and liquid transfer throughout CM4 and CM5. There were several variables that influenced the validity of the calculated solids ratio. To be accurate, the solid and liquid sample must be representative of the whole fermentor. Channeling sometimes prevented the ability to obtain a representative sample; therefore, the fermentors had to be well mixed before the separation. Assisting mixing with a shovel was common practice. Also, the moisture content of the filter cake depended on the filter pressure reached before the filtration was stopped, as well as the filtration time. Thus, the moisture content used was the average value obtained over several transfers.

Because it was impossible to collect data from a truly representative sample, the solids ratios calculated probably do not represent the real solid to liquid ratios in the fermentors. However, this proved to be a way to compare the amount of solids in the fermentors relative to each other. Figures V-19, V-20, V-21, and V-22 show the solids ratios compared to the total acid concentrations during CM4 and CM5.

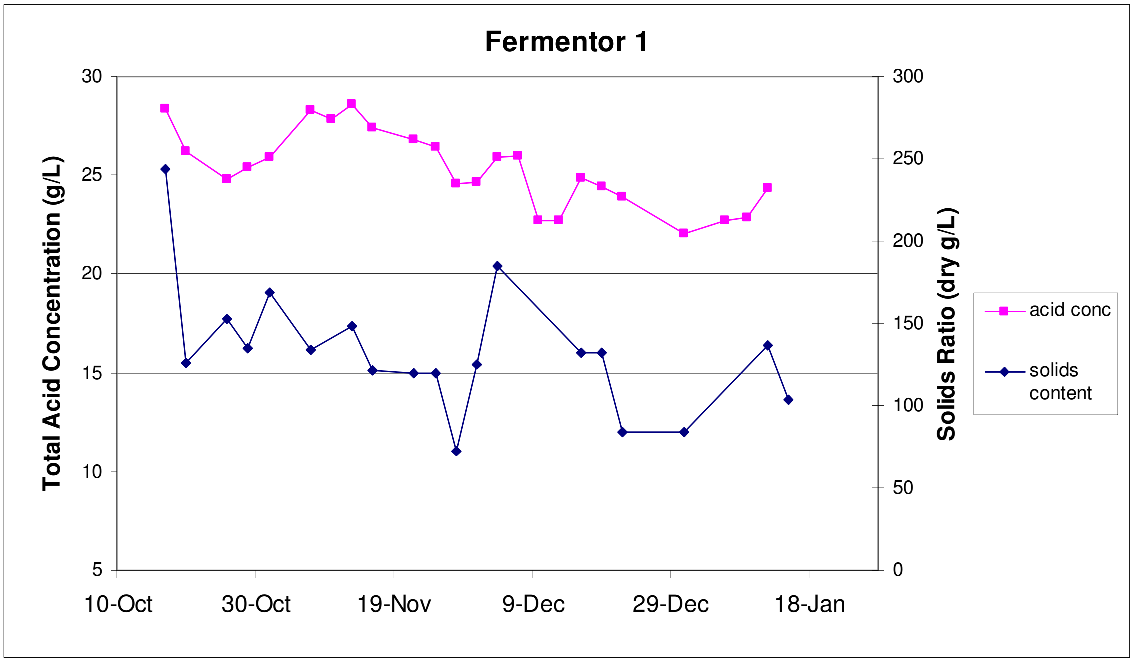


Figure V-19. Effect of Solids Ratio: Fermentor 1 solids ratio and acid concentration.

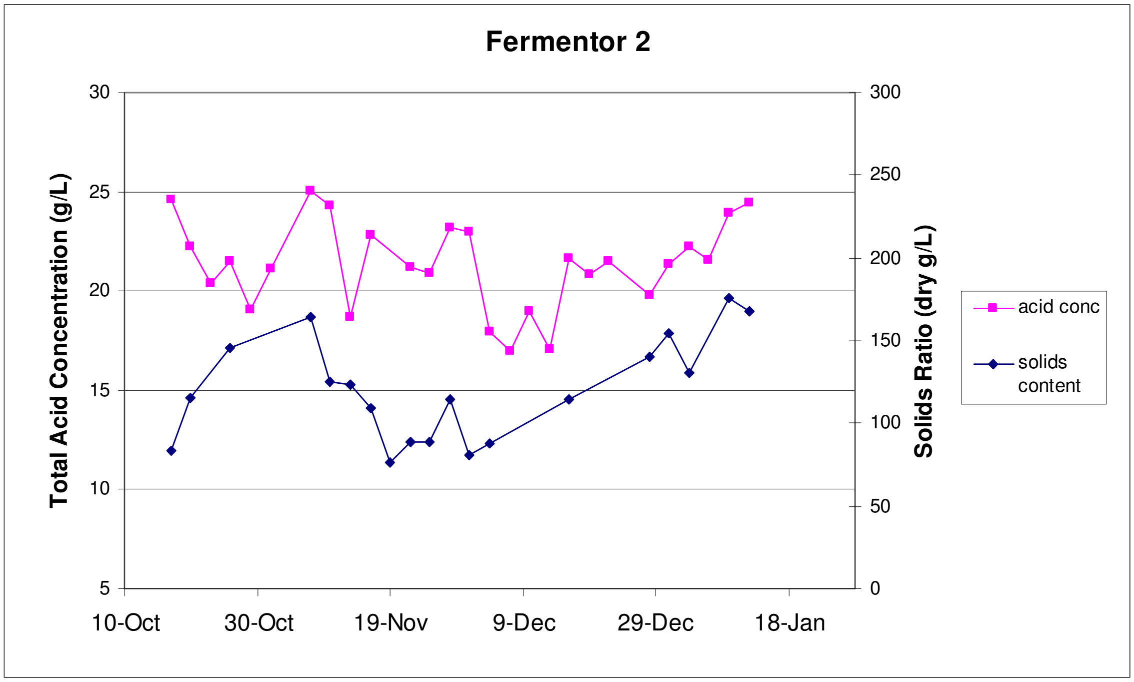


Figure V-20. Effect of Solids Ratio: Fermentor 2 solids ratio and acid concentration.

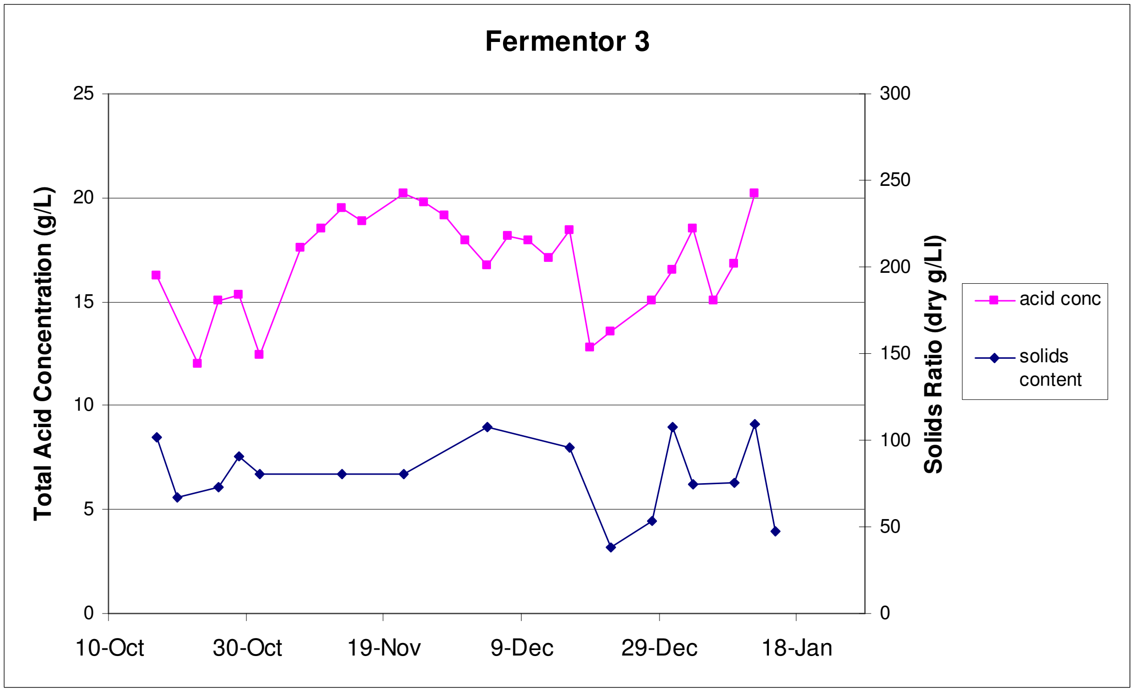


Figure V-21. Effect of Solids Ratio: Fermentor 3 solids ratio and acid concentration.

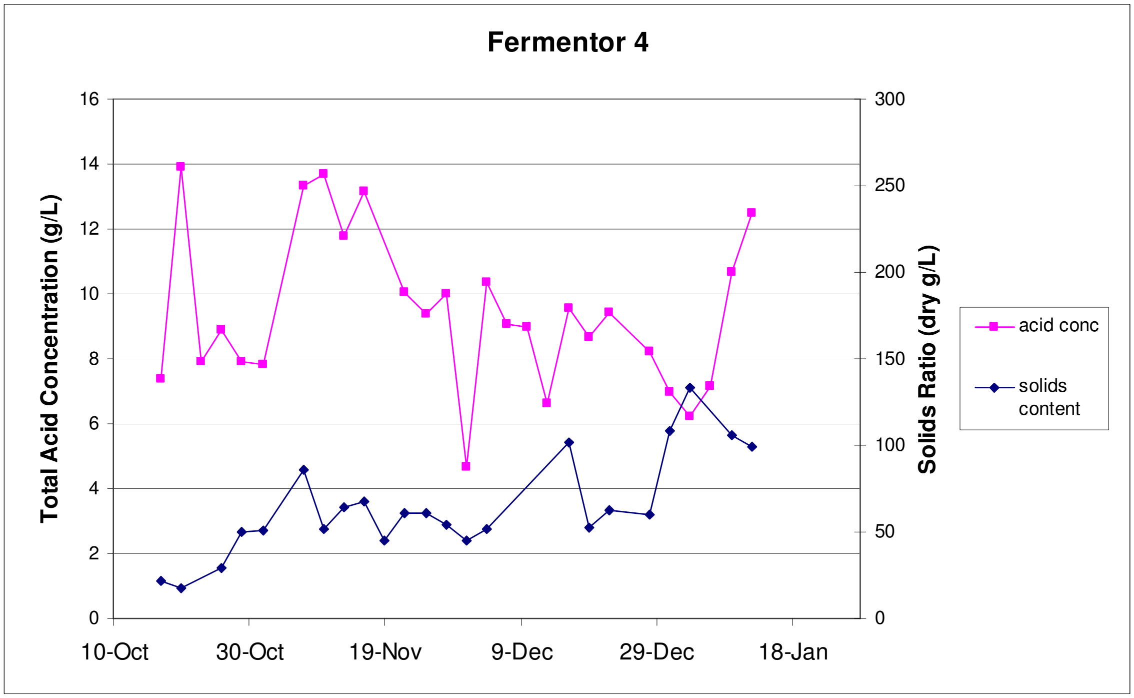


Figure V-22. Effect of Solids Ratio: Fermentor 4 solids ratio and acid concentration.

In the beginning of CM4, solids ratios decreased when evaluated from F1 to F4. This effect was expected because the biomass was more digested from F1 to F4. To increase the solids ratios biomass was added to F2, F3, and F4. If too much biomass was added at one time, the solids ratio climbed over 120 dry g/(L of slurry) and made operations difficult. To reduce the solids ratio, biomass additions were stopped until the ratio decreased. The sludge pump for F3 was down from November 7 to December 1, 2005, thus solids ratios for F3 were not determined for this time period. The solids ratio for this time was assumed to stay constant.

In general, the solids ratios correlate well with total acid concentrations. In most cases, when the solids ratio increased, the acid concentration also increased, and when the solids ratio decreased, the acid concentration also declined. There was a time delay in the response of acid concentrations to changes in solids ratios. This was expected because the microorganisms did not immediately digest the freshly added biomass. Thus, data simply prove that higher acid concentrations are achieved when there is more biomass available to digest.

OVERALL RESULTS

The overall results were not optimal, but operating on a pilot-scale level was successful. Much was learned about the processes limitations and how to overcome these limitations. To put the duration of the fermentation process in perspective, Figure V-23 shows the total product (F1) acid concentration over time.

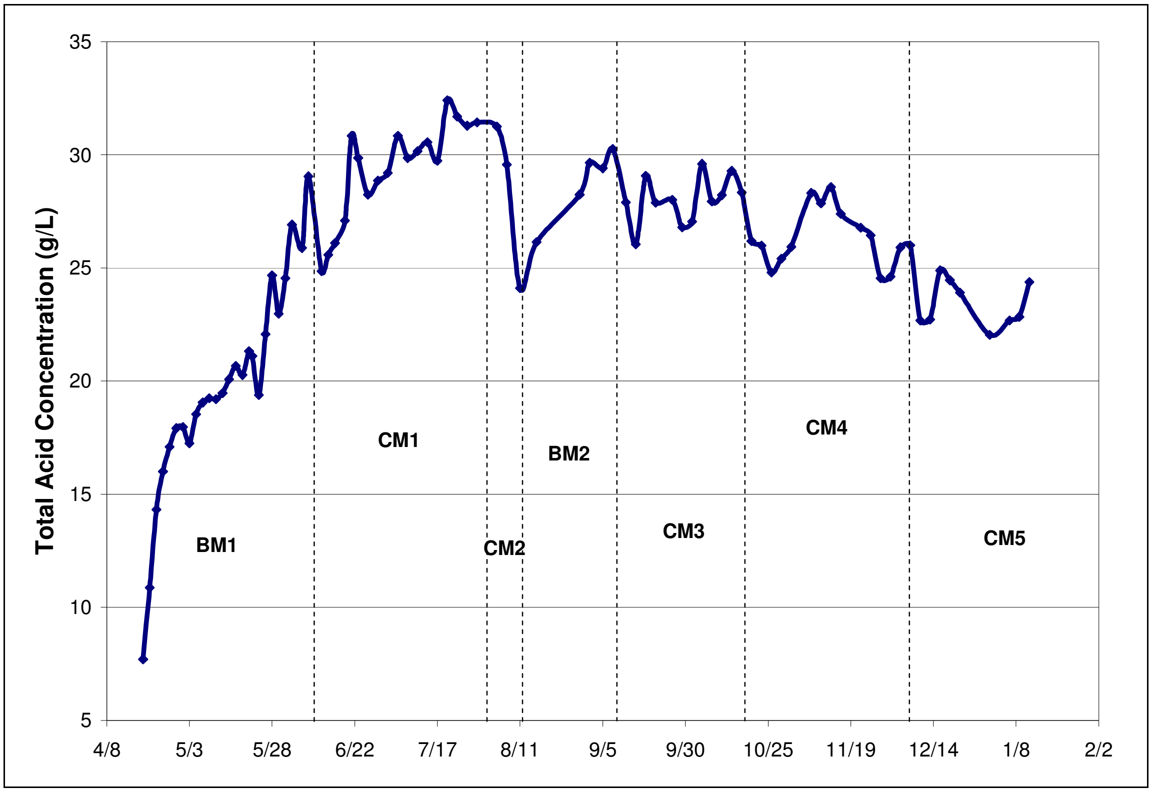


Figure V-23. Overall Results: Fermentor 1 total acid concentration.

Figure V-23 is divided into sections of the different operational modes. In CM1, the acid concentration grew to a maximum of 32.4 g/L. The acid concentration decreased in CM3, but stayed steady. The drop in acid production was due to a lower VSLR in CM3. In CM4 and CM5, temperature became an issue in the colder months. The solids handling also became a problem and limited fresh biomass additions in F1. These factors caused fluctuations in the acid concentrations in CM4 and CM5. Steady state was never achieved because of the many operational changes, thus biomass conversions and productivities could not be evaluated.

Results on the laboratory scale were not matched on the pilot plant-scale. The results were dissimilar because of different operating parameters and methods. The dissertation, “Fermentation of Industrial Biosludge, Paper Fines, Bagasse, and Chicken Manure to Carboxylate Salts” by Susan Domke, will be used to compare results. Domke (1999) used office paper and chicken manure with a ratio of 80/20 in one experiment. The primary difference between Domke’s experiment and the pilot plant project was the VSLR and LRT used. Table V-14 shows the results of Domke’s experiment as well as results for CM1, CM3, CM4, and CM5.

TableV-14. Overall Results: Comparing results.

	Units	Lab	CM1	CM3	CM4	CM5
VSLR	g /(L liquid · d)	2.1	1	0.25	0.92	0.43
LRT	days	24	80	318	318	318
Transfer Frequency	days	3	3	3	3	3
Avg. F1 Solids Conc.	g dry solids/(L liq)	153	ND	ND	151	133
Avg. F2 Solids Conc.	g dry solids/(L liq)	213	ND	ND	118	168
Avg. F3 Solids Conc.	g dry solids/(L liq)	338	ND	ND	92	82
Avg. F4 Solids Conc.	g dry solids/(L liq)	355	ND	ND	54	99
Total Avg. Acid Conc.	g/L	20.7	30.5	28	26	23
Acetic Acid	wt%	39.0	39.6	46.2	48.4	49.5
Propionic Acid	wt%	13.0	8.9	9.7	11.8	10.6
Butyric Acid	wt%	23.0	26.8	21.8	18.5	13.6
Valeric Acid	wt%	10.0	3.4	3.3	3.3	3.9
Caproic Acid	wt%	4.0	18.1	15.4	13.6	15.7
Other Higher Acids	wt%	4.0	3.2	3.7	4.3	6.7

In the laboratory the VSLR was much higher, and the LRT was much lower. Also the amount transferred between each fermentor is done based on maintaining a constant solid concentration to establish steady state. At the pilot plant, it was much harder to transfer enough material to maintain a constant solids concentration, thus it was more difficult to reach steady state. In general, the acid concentrations are lower in the laboratory because the LRT is much shorter, although CPDM predicts that total acid concentrations can reach 35 g/L at a VSLR of 4 g /(L liquid · d) and a LRT of 30 days (Domke, 1999). In the product, the acetic acid composition was similar in CM1, but increased in the other three modes of operation, as shown in Table V-11.

The average solids concentrations in F3 and F4 were also much lower at the pilot plant than in the laboratory. The solids concentrations in the lab were kept constant to achieve steady state, whereas at the pilot plant, the solids concentrations in F2, F3, and F4 seemed to continuously decrease. Efforts were made in CM4 and CM5 to increase the solids content, but it proved to be much more difficult than in the laboratory.

Being able to achieve practical VSLRs and LRTs are crucial for countercurrent fermentation success. The pilot-scale fermentors were limited because of the inability to transfer large amounts of material. The transfer of material depended chiefly on the sludge pumps capability to move the fermentor contents to achieve solid and liquid separation. The substrate, even after the paper was further shredded, continually caused a congested state in F1. When F1 was congested material transfers were very difficult. F1 could not handle a solids feed rate over 6.7 lb/day (VSLR of 0.25 g /(L liquid · d)) without becoming congested. Operations with a VSLR and LRT that were comparable to what was achieved in the laboratory was not possible.

Despite limited success on achieving data comparable to the laboratory, the ability to operate this process on a pilot-scale level was proven. Importantly, high carboxylic acid concentrations were achieved (32.4 g/L). The solids handling issues are now recognized to be of utmost importance when an industrial design is considered. The process proved to be very robust and durable; specifically, the system did not require special care and was not overly sensitive to changes in environmental conditions. The fermentation of biomass into carboxylic acids never ceased regardless of the numerous operational complications encountered; this is what makes this process unique.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

The following was learned about the pilot plant fermentation process:

1. When office paper is used as a substrate, the particles form a heavy suspended layer in the liquid.
2. Material cannot be moved without the use of a sludge pump.
3. The fermentation design limits high VSLRs and low LRTs.
4. VSLRs over 0.25 g /(L liquid · d) (~7 lbs VS/ day) will congest F1 if the solids ratio was maintained at a high level (120 dry g/(L of slurry)).
5. Lower LRTs (> 300 days) were hard to achieve if the solids ratio was above120 dry g/(L of slurry) because the sludge pump would clog when liquid was removed.
6. Higher product concentration can be achieved with higher solids ratios.
7. Methane cannot be effectively controlled less than 5 wt% without proper mixing to distribute the inhibitor.
8. Poor mixing can also lead to lower total acid production.
9. Shredding the fresh paper was extremely important.
10. The most digested biomass is the easiest to pump; therefore, it is primarily what is transferred out of F1. The freshest biomass will accumulated and further add to the congested state of F1.

The following are recommendations for future work:

1. Lower solids ratios should be used in F1. This will allow more biomass to be fed per day. If the solids ratio is kept low, the sludge pump can handle the material even with higher VSLRs. Reducing the solids ratio in F1 to 50 dry g/(L of slurry) (0.36 dry lb/(gal of slurry)) and increasing the VSLR to the initial 1 g /(L liquid · d), used in CM1, is recommended. The solids ratio in the other three fermentors should be held constant (120 dry g/(L of slurry)). Maintaining constant solids ratios in the other three fermentors will require separate fresh biomass additions because not enough can be transferred to account for consumption.
2. Better mixing should be achieved. The simplest way to do this would be to construct a basic device to help evenly distribute the fluid as it circulates to the top of the fermentor; this will prevent channeling. The unused internal pipe, should also be removed to reduce channeling.
3. A better solid and liquid separation method is recommended to overcome solids handling issues. Separation with a screw press could be one such method.
4. The addition of urea as nitrogen supplement is recommended.
5. A more convenient way to collect gas samples would be beneficial. A portable gas detector pump could be used.
6. A more responsive temperature control system should be considered. A feedback control loop could be beneficial.

7. All material in the fresh paper that can be abrasive should be removed.
8. To improve the fermentation process at an industrial scale, a new fermentor design should be considered that is not limited by material handling issues and can achieve better mixing.

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APPENDIX A

SOLIDS AND LIQUID TRANSFERRING PROCEDURE

The following is the method used to transfer solids and liquid.

1. Transferring solids and liquid is done every 3 days.
2. First the paper needs to be further shredded before adding to the fermentors. It is recommended that this is done the day before transferring because it can take up to 1 h.
3. Mix tanks for 30 min before transfer, making certain the correct valves are open for circulation.
4. Fermentor 1 transfer (liquid to product tank and solids to Fermentor 2)
    a. Prepare filter for transfer.
        i. Add inhibitor inside the filter or in one of the hoses so it mixes with the product on its way to the product tank.
        ii. Bolt up filter lid with gasket.
        iii. Close vent valve.
    b. Hook up hose from fermentor to filter.
        i. Use green hose for pump discharge.
    c. Hook up hose from discharge of filter to product tank.
        i. Leave hose to top of product tank disconnected.
        ii. Leave valve on top of product tank closed until ready to pump.
    d. Turn off pump.

e. Make note of the level in the product tank.
f. Make certain valves are in the correct position.
g. Turn on pump to filter.
h. Connect hose to product tank when liquid gets close to filling up the hose; this will prevent air from getting into the tank. Oxygen from the air will degrade product.
i. Turn the pump off when the filter pressure gets over 30 psig (filter is full), or the necessary volume of liquid is obtained in product tank.
j. When the filter is full (20 – 30 psig):
    i. Shut off pump.
    ii. Close filter discharge valve.
    iii. Put pump in reverse to decrease pressure.
    iv. When pressure reaches 0 psig, open vent on filter.
    v. Attach filter supply hose to filter drain to suck liquid from the filtrate side of the filter to remove excess liquid in solids.
    vi. While performing Step v. unbolt filter lid and help drain liquids with putty knife by making a small hole in solids on the perimeter of the basket so the liquids will escape.
    vii. Shut off the pump when all liquids are removed; it is not good to run the pump dry.
    viii. Empty basket into buckets.
k. Weigh solids and set them aside.

        i. Separate solids to be transferred to Fermentor 2 and excess solids that need to be put back into Fermentor 1.

l. Repeat process if more liquid or solids are needed.
    i. If more solids are needed.
        1. Hook the filter discharge to the measuring tank and pump liquids there until enough solids are acquired.
        2. When finished, hook the hose from Fermentor 1 to the measuring tank and put the pump in reverse to suck out excess liquid back into Fermentor 1.
    ii. If more liquid is needed
        1. Empty filter and proceed, but make sure the excess solids are put back into the fermentor they came from.

m. When finished acquiring solids and liquids for Fermentor 1.
    i. Drain hoses back into Fermentor 1 by putting the pump in reverse and walking the liquid back to the fermentor.
    ii. Take a sample of liquid from Fermentor 1 from residue in the bottom of the filter.

n. Add fresh biomass and calcium carbonate to Fermentor 1.
    i. Biomass: 80 wt% shredded office paper ; 20 wt% chicken manure
    ii. Calcium carbonate: 0.4 lbs. $CaCO_3$/lb of biomass

o. Record data on solid mass, solid volume, liquid volume, solids transferred, and liquid transferred.

5. Fermentor 2 transfer (liquid to Fermentor 1 and solids to Fermentor 3)
    a. Repeat steps for Fermentor 1 except:
        i. Solid free liquid from filter goes into measuring tank so it can be measured and then transferred to Fermentor 1.
        ii. When enough liquid is acquired, the liquid must be transferred to Fermentor 1.
            1. When hoses are drained, attach Fermentor 1 to the measuring tank and turn the pump in reverse to suck out liquid.
        iii. Solids are transferred to Fermentor 3 by buckets.
6. Fermentor 3 transfer (liquid to Fermentor 2 and solids to Fermentor 4)
    a. Same as Fermentor 2
7. Fermentor 4 transfer (liquid to Fermentor 3 and solids to waste)
    a. Same as Fermentor 3 transfer except:
        i. Only 20 wt% of the normal solids transferred are taken out for waste. This is because it assumed that 80 wt% is digested by the time it gets to Fermentor 4.
        ii. Also, fresh water is introduced here. The amount of water transferred is the same as the volume of liquid transferred to the other fermentors in most cases.
            1. The water is pumped from the water tank by a red centrifugal pump through a 1-in PVC pipe to the reactor.

Sometimes the PVC becomes plugged just as it enters Fermentor 4. There is a valve and hose adaptor along the fence where you can attach a hose to bypass the plug, or the water can be sucked out by the sludge pump on Fermentor 4.

8. Inhibitor can be added at anytime after solids and liquid are removed from the fermentor.
9. Each fermentor should be circulated for at least 1 h after transfer and inhibitor addition to mix reactor contents.

APPENDIX B

SLUDGE PUMPS

The following should be considered when using the sludge pumps:

1. They should never be dead-headed, because it can damage the pumps and pressure can build and burst pipes.
2. It is important to prevent the pumps from running dry because it causes substantial wear to the pump internals.
3. The pumps will leak a few drops per minute; it helps cool the drive shaft.
4. If the pumps leak excessively, tighten the packing nuts. If the packing nuts cannot be tightened any more, replace the packing (see pump manual).
5. If the pumps start making unusual noises, it probably means that the pump is not moving any material. It could be a clogged pipe or a shut valve, but it should be immediately investigated.

APPENDIX C

INHIBITOR ADDITION

Iodoform was dissolved in ethanol to improve inhibitor dispersion in the fermentors. The solution concentration was 20 g iodoform/L of ethanol. The solution was made before every addition to ensure the integrity of the inhibitor. The addition rate was 200 to 400 mL per fermentor. This was equivalent to 4 to 8 mg of iodoform/L of reactor volume per day. If high methane concentrations were noticed a higher dose of inhibitor is required. The inhibitor must be evenly distributed and, in most cases, required manual mixing with a shovel.

APPENDIX D

GAS SAMPLES

Approximately every week, two gas samples per fermentor were taken with 5-mL syringes, which were sealed with a small ball valve. The samples were taken from the sample ports in the fermentor gas vent system and were transported to the laboratory in the Brown Building to be analyzed by the gas chromatograph (GC). Two samples were taken to ensure reproducibility of the results. Getting the GC to register the gas samples was difficult. The main problem occurred when the samples were injected into the GC. Often, it would take several attempts to obtain gas composition data for all four fermentors, thus gas sample data were able to be obtained only every couple of weeks making it difficult to effectively manage methane production. If the methane concentrations elevated above 5 wt%, a double dose of inhibitor was added and mixing time was extended to ensure even distribution.

APPENDIX E

LIQUID SAMPLES

To monitor acid production, liquid samples were taken every 2 or 3 days. The fermentors were well mixed before one sample per fermentor was taken. The samples were collected in 15-mL centrifuge tubes and stored in the freezer in the office next to the pilot plant. The samples were frozen to stop biomass digestion by microorganisms in the sample. Once 24 samples were accumulated, they were taken to the laboratory to be analyzed with the gas chromatograph (GC). Due to the number of sample slots in the GC, 24 samples was a convenient number.

APPENDIX F

DETERMINING PILOT SCALE OPERATING PARAMETERS FOR COUNTERCURRENT MODE I

In the laboratory, a typical volatile solids loading rate (VSLR) was approximately 6 g /(L liquid · d) and a typical liquid residence time (LRT) was approximately 13 days. Because of the large scale, it is more convenient to work with units of pounds and gallons.

VSLR = 6 g /(L liquid · d) = 0.05 lbs /(gal liquid · d)

To operate at a VSLR of 0.05 lbs /(gal liquid · d), 160 lb of biomass would be added per day. If transfers were done every 3 days, this would be equivalent to adding about 477 lb every 3 days. This was not achievable, so it was decided that a reasonable addition rate would be 80 lbs every 3 days.

A LRT of about 13 days would require a liquid flow rate out of the fermentor train to be approximately 245 gal/day. This is equivalent to a liquid transfer rate of 735 gallons every 3 days, which was also not feasible. A transfer rate of 120 gallons every 3 days was actually used.

During Countercurrent Mode 1, the following parameters were used.

VSLR = 1 g /(L liquid · d) = 0.0084 lb/(gal liquid · day)

LRT = 80 days = 120 gal/day

The VSLR was about 1/6 of a typical laboratory VSL, and the LRT was about 6 times a typical laboratory LRT.

APPENDIX G

DETERMINING SOLID MOISTURE CONTENT

To determine the moisture content (*MC*) of the solid sample taken from the filter cake the following procedure was followed.

1. Collect solid sample after filtration.
2. Take the samples to the laboratory.
3. Weigh the sample in a glass beaker (*WSM* [g]).
4. Dry the samples in an oven at 100 ^{o}C for 24 hours.
5. Weigh the samples in the beakers (*DSM* [g]).
6. The following calculation was performed to determine the moisture content (*MC*).

$$MC = 100 \cdot \left[\frac{WSM - DSM}{WSM} \right]$$

APPENDIX H

DETERMINING FERMENTOR SOLIDS RATIO

The solids ratio is defined as the ratio of the mass of dry solids to the total slurry volume. This ratio was based on how much liquid and solids were separated during filtration. During each solids and liquid transfer, data were collected to determine the solids to volume ratio. After filtration, the following data were collected.

1. Wet solids mass (M_{wet}) = total filter cake mass collected (wet lb).
2. Wet solids volume (V_{sol}) = total filter cake volume collected (gallon).
3. Liquid volume (V_{liq}) = total liquid volume collected (gallon).
4. Solids moisture content (MC) = liquid in filter cake (lb H_2O/100 lb wet solids) .

For the calculation the following terms are also defined:

5. Liquid density (ρ) = fermentation liquid density (lb/gallon)
6. Solids liquid volume (V_{SL}) = volume of liquid in filter cake (gallon)
7. Total sample volume (V_{tot}) = Solids liquid volume + Liquid volume (gallon)
8. Dry solids mass (M_{dry}) = mass of dry solids (lb)
9. Solids ratio = mass dry solids per total volume (dry lb/gallon)

The solids ratio was determined as follows.

$$V_{SL} = M_{wet} \cdot \frac{MC/100}{\rho}$$

$$V_{tot} = V_{liq} + V_{SL}$$

$$M_{dry} = \left[1 - MC/100\right] \cdot M_{wet}$$

solids ratio = M_{dry} / V_{tot} [dry lb/gal]

APPENDIX I

ANALYZING SAMPLES

The following is the procedure for preparing liquid samples:

1. Thaw samples and mix with vortex.
2. Centrifuge the samples for 10 minutes at 3,500 rpm in DPR-6000 (Industrial Equipment Co., Needham Hts., MA).
3. Pipette (Rainin Instrument Co., Woburn, MA) 1 mL of the liquid sample into a 15-mL round-bottom ultracentrifuge tube.
4. To the same tube, pipette 1 mL of 10-mM 4-methyl-valeric acid and 1mL of 3-M phosphoric acid.
5. Cap and vortex tube.
6. Centrifuge the mixture at 15,000 rpm in the IEC B-20A centrifuge (Industrial Equipment Co., Needham Hts., MA).
7. Pipette 1 mL of centrifuged sample to a glass GC vial and cap (Fishcer Cat. No. 03-395C and 03-396A).

The following is the procedure for operating the gas chromatograph (GC):

1. Check the supply gas cylinders to ensure a pressure of at least 100 psig.
2. Establish gas flow to the GC by setting the regulator to 50 psig for air, 60 psig for helium, and 40 psig for hydrogen.
3. Check the needle, and rinse methanol and waste vials on the automatic injector.

4. Make sure the column head pressure on the GC is at 15 psig and replace septum if pressure is too low.
5. Setting conditions should be:
    a. Oven temperature = 50 ^{o}C
    b. Inlet temperature = 230 ^{o}C
    c. Det temperature = 250 ^{o}C
    d. Ramp = 20 ^{o}C/min
    e. Hydrogen flow = 40 mL/min
    f. Air flow = 400 mL/min
    g. Helium flow = 179 mL/min
6. Place acid standard at the beginning and end of every 24 samples for calibration (Matreya, Inc., #1075).
7. Run GC.

The gas samples were analyzed by following steps 1, 2, 4, and 5 but setting the GC to analyze gas. The gas samples were then injected into the gas sample port. The samples were run manually and could not be sampled automatically. A more detailed GC operational guide is described by Loescher (1996).

APPENDIX J

CARBOXYLIC ACID PRODUCTION DATA

Table J-1. Fermentor 1 acid concentrations (g/L).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
19-Apr-05	2.48	1.51	0.08	3.46	0.11	0.06	0.00	0.00	7.70
21-Apr-05	3.05	2.01	0.11	5.24	0.16	0.11	0.16	0.00	10.86
23-Apr-05	4.06	2.34	0.16	6.47	0.23	0.28	0.80	0.00	14.32
25-Apr-05	4.54	2.36	0.19	6.48	0.27	0.40	1.70	0.06	16.00
27-Apr-05	5.00	2.19	0.20	5.20	0.29	0.53	3.46	0.22	17.09
29-Apr-05	5.30	2.26	0.22	5.28	0.31	0.57	3.74	0.25	17.92
1-May-05	5.34	2.24	0.22	5.30	0.31	0.58	3.74	0.24	17.97
3-May-05	5.18	2.15	0.21	5.09	0.30	0.56	3.53	0.22	17.25
5-May-05	5.58	2.30	0.23	5.47	0.33	0.60	3.80	0.24	18.54
7-May-05	5.77	2.34	0.24	5.58	0.34	0.67	3.86	0.24	19.05
9-May-05	5.91	2.36	0.25	5.63	0.35	0.60	3.89	0.24	19.24
11-May-05	5.96	2.35	0.25	5.60	0.36	0.59	3.86	0.23	19.20
13-May-05	6.06	2.37	0.25	5.71	0.36	0.61	3.89	0.23	19.47
15-May-05	6.32	2.41	0.26	5.88	0.38	0.63	3.98	0.23	20.08
17-May-05	6.63	2.45	0.27	6.03	0.39	0.65	4.05	0.22	20.67
19-May-05	6.58	2.37	0.26	5.87	0.37	0.65	3.97	0.21	20.27
21-May-05	7.00	2.48	0.27	6.19	0.39	0.68	4.11	0.21	21.32
22-May-05	6.95	2.43	0.26	6.08	0.38	0.67	4.11	0.21	21.10
24-May-05	6.43	2.21	0.24	5.55	0.34	0.62	3.79	0.19	19.38
26-May-05	7.61	2.45	0.27	6.31	0.38	0.72	4.14	0.19	22.06
28-May-05	8.62	2.63	0.30	7.13	0.44	0.84	4.55	0.16	24.67
30-May-05	7.92	2.42	0.28	6.68	0.41	0.80	4.32	0.14	22.97
1-Jun-05	8.82	2.63	0.31	7.04	0.45	0.83	4.32	0.15	24.54
3-Jun-05	10.10	2.92	0.36	7.50	0.50	0.87	4.51	0.16	26.91
6-Jun-05	9.91	2.68	0.34	7.11	0.47	0.85	4.38	0.15	25.89
8-Jun-05	11.19	2.97	0.36	8.05	0.54	0.97	4.80	0.17	29.05
12-Jun-05	9.58	2.38	0.29	7.00	0.40	0.84	4.22	0.15	24.86
14-Jun-05	9.77	2.39	0.30	7.34	0.40	0.86	4.38	0.15	25.58
16-Jun-05	9.55	2.50	0.29	7.77	0.39	0.89	4.55	0.16	26.09
19-Jun-05	10.02	2.53	0.29	8.01	0.40	0.92	4.77	0.16	27.09
21-Jun-05	11.83	2.73	0.32	8.46	0.42	1.09	5.81	0.19	30.84
23-Jun-05	11.46	2.62	0.32	7.93	0.42	1.09	5.84	0.19	29.86
26-Jun-05	10.81	2.41	0.30	7.83	0.39	0.99	5.35	0.17	28.24
29-Jun-05	10.73	2.62	0.30	8.12	0.39	1.03	5.49	0.18	28.85
2-Jul-05	10.98	2.56	0.30	8.11	0.40	1.04	5.63	0.18	29.20
5-Jul-05	11.83	2.73	0.32	8.46	0.42	1.09	5.81	0.19	30.84
8-Jul-05	11.46	2.62	0.32	7.93	0.42	1.09	5.84	0.19	29.86
11-Jul-05	11.68	2.64	0.33	8.06	0.43	1.08	5.75	0.19	30.16
14-Jul-05	11.94	2.63	0.34	8.11	0.44	1.09	5.80	0.20	30.55

Table J-1. (continued).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
17-Jul-05	11.69	2.53	0.34	7.88	0.44	1.06	5.59	0.20	29.73
20-Jul-05	12.96	2.74	0.37	8.52	0.49	1.13	5.98	0.21	32.40
23-Jul-05	14.33	2.97	0.38	7.36	0.51	0.98	4.96	0.20	31.69
26-Jul-05	13.67	2.86	0.42	7.78	0.56	0.97	4.84	0.19	31.28
29-Jul-05	14.77	2.74	0.35	7.35	0.47	0.93	4.63	0.19	31.43
4-Aug-05	14.61	2.71	0.36	7.27	0.46	0.95	4.70	0.19	31.25
7-Aug-05	13.80	2.55	0.34	6.85	0.43	0.91	4.50	0.19	29.57
11-Aug-05	10.22	2.22	0.26	5.97	0.36	0.82	4.07	0.19	24.11
16-Aug-05	11.44	2.30	0.29	6.35	0.41	0.86	4.30	0.18	26.14
29-Aug-05	12.81	2.39	0.32	6.71	0.45	0.89	4.49	0.18	28.24
1-Sep-05	13.55	2.53	0.34	6.97	0.47	0.93	4.65	0.19	29.65
5-Sep-05	13.61	2.49	0.33	6.85	0.46	0.91	4.57	0.18	29.40
8-Sep-05	14.03	2.54	0.34	7.06	0.47	0.93	4.69	0.19	30.25
12-Sep-05	11.90	2.74	0.41	6.62	0.47	0.95	4.63	0.20	27.89
15-Sep-05	11.03	2.61	0.39	6.05	0.43	0.91	4.45	0.19	26.05
18-Sep-05	13.22	2.84	0.43	6.43	0.49	0.96	4.51	0.19	29.06
21-Sep-05	12.28	2.73	0.41	6.39	0.45	0.94	4.50	0.19	27.88
26-Sep-05	12.78	2.66	0.39	6.27	0.45	0.91	4.37	0.18	28.01
29-Sep-05	12.63	2.42	0.35	5.81	0.41	0.87	4.13	0.18	26.79
2-Oct-05	12.46	2.83	0.43	5.68	0.47	0.95	4.04	0.18	27.04
5-Oct-05	14.31	2.70	0.40	6.25	0.47	0.94	4.36	0.18	29.60
8-Oct-05	13.40	2.58	0.39	5.91	0.46	0.90	4.14	0.17	27.94
11-Oct-05	13.33	2.78	0.40	5.93	0.47	0.93	4.20	0.18	28.22
14-Oct-05	14.22	2.78	0.40	6.06	0.47	0.92	4.26	0.18	29.29
17-Oct-05	13.64	2.89	0.38	5.88	0.45	0.88	4.04	0.18	28.33
20-Oct-05	11.67	2.89	0.44	5.60	0.47	0.92	4.01	0.19	26.18
23-Oct-05	11.95	2.78	0.37	5.55	0.42	0.87	3.86	0.19	25.99
26-Oct-05	11.48	2.56	0.38	5.23	0.42	0.84	3.69	0.19	24.80
29-Oct-05	11.92	2.72	0.37	5.25	0.41	0.85	3.70	0.19	25.41
1-Nov-05	12.25	3.02	0.39	5.16	0.43	0.87	3.62	0.19	25.93
7-Nov-05	14.00	3.54	0.45	5.32	0.48	0.87	3.47	0.20	28.31
10-Nov-05	13.84	3.63	0.44	5.11	0.47	0.85	3.31	0.20	27.85
13-Nov-05	14.29	3.62	0.42	5.32	0.47	0.85	3.40	0.20	28.57
16-Nov-05	13.56	3.52	0.44	5.03	0.47	0.86	3.31	0.20	27.39
22-Nov-05	13.19	3.54	0.45	4.70	0.48	0.88	3.29	0.25	26.78
25-Nov-05	12.93	3.42	0.46	4.46	0.49	0.92	3.42	0.33	26.44
28-Nov-05	12.16	2.73	0.37	3.93	0.41	0.85	3.65	0.44	24.54
1-Dec-05	12.20	2.74	0.38	3.96	0.41	0.87	3.62	0.44	24.61
4-Dec-05	12.82	2.93	0.40	4.24	0.44	0.92	3.75	0.40	25.91
7-Dec-05	12.84	2.94	0.40	4.26	0.44	0.94	3.76	0.41	25.99
10-Dec-05	11.20	2.38	0.34	3.37	0.37	0.86	3.57	0.61	22.68
13-Dec-05	11.21	2.38	0.34	3.38	0.37	0.87	3.57	0.61	22.73
16-Dec-05	12.57	2.54	0.41	3.32	0.45	0.94	3.89	0.78	24.89
19-Dec-05	12.32	2.71	0.42	3.10	0.43	0.93	3.77	0.77	24.45
22-Dec-05	11.70	2.63	0.38	3.31	0.43	0.96	3.71	0.78	23.91

Table J-1. (continued).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
31-Dec-05	10.99	1.96	0.38	2.85	0.41	0.85	3.73	0.89	22.04
6-Jan-06	10.57	2.62	0.42	3.05	0.45	1.02	3.71	0.83	22.68
9-Jan-06	11.33	2.54	0.41	3.00	0.43	0.92	3.47	0.74	22.84
12-Jan-06	12.38	2.59	0.46	3.17	0.48	0.94	3.57	0.76	24.37

Table J-2. Fermentor 2 acid concentrations (g/L).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
19-Apr-05	2.83	1.60	0.10	4.35	0.13	0.24	1.29	0.05	10.59
21-Apr-05	3.94	2.08	0.00	5.31	0.18	0.32	1.86	0.07	13.77
23-Apr-05	4.40	2.30	0.14	6.01	0.20	0.39	2.30	0.09	15.84
25-Apr-05	4.68	2.43	0.15	6.43	0.21	0.48	2.77	0.11	17.26
27-Apr-05	4.82	2.50	0.16	6.58	0.21	0.54	3.08	0.12	18.01
29-Apr-05	4.96	2.58	0.17	6.84	0.23	0.60	3.33	0.13	18.83
1-May-05	5.02	2.61	0.17	7.06	0.23	0.62	3.42	0.13	19.28
3-May-05	4.44	2.28	0.16	6.30	0.21	0.56	3.16	0.13	17.24
5-May-05	5.61	2.87	0.20	7.98	0.27	0.71	3.88	0.15	21.68
7-May-05	5.14	2.58	0.19	7.19	0.25	0.62	3.49	0.13	19.59
9-May-05	5.20	2.60	0.19	7.25	0.26	0.63	3.52	0.13	19.79
11-May-05	5.42	2.66	0.20	7.38	0.27	0.64	3.59	0.14	20.30
13-May-05	5.66	2.70	0.20	7.49	0.28	0.66	3.68	0.14	20.79
15-May-05	5.80	2.67	0.20	7.42	0.28	0.66	3.69	0.14	20.87
17-May-05	5.84	2.60	0.20	7.25	0.27	0.66	3.67	0.13	20.64
19-May-05	6.14	2.67	0.21	7.44	0.28	0.69	3.80	0.14	21.37
21-May-05	6.34	2.73	0.21	7.51	0.28	0.70	3.85	0.14	21.77
22-May-05	6.16	2.67	0.21	7.34	0.28	0.69	3.80	0.14	21.29
24-May-05	6.32	2.72	0.21	7.47	0.29	0.71	3.89	0.14	21.75
26-May-05	6.21	2.64	0.20	7.25	0.28	0.69	3.81	0.14	21.22
28-May-05	6.00	2.53	0.20	7.10	0.27	0.69	3.80	0.13	20.71
30-May-05	5.37	2.24	0.17	6.29	0.24	0.62	3.51	0.12	18.56
1-Jun-05	5.56	2.29	0.18	6.51	0.24	0.65	3.59	0.12	19.14
3-Jun-05	6.46	2.60	0.20	7.46	0.28	0.76	4.16	0.14	22.05
6-Jun-05	6.70	2.64	0.21	7.57	0.28	0.77	4.25	0.14	22.57
8-Jun-05	7.22	2.79	0.23	7.95	0.30	0.81	4.40	0.15	23.85
10-Jun-05	7.38	2.82	0.23	8.11	0.31	0.83	4.51	0.15	24.34
12-Jun-05	7.50	2.83	0.23	8.17	0.31	0.84	4.54	0.16	24.58
14-Jun-05	7.60	2.83	0.23	8.18	0.32	0.85	4.59	0.16	24.76
16-Jun-05	8.32	2.70	0.25	8.13	0.32	0.90	4.89	0.17	25.69
21-Jun-05	8.21	2.75	0.25	8.35	0.33	0.92	5.02	0.18	26.01
23-Jun-05	8.82	2.89	0.26	8.78	0.35	0.97	5.23	0.19	27.48
26-Jun-05	8.58	2.79	0.26	8.40	0.35	0.92	4.95	0.18	26.44
28-Jun-05	8.47	2.73	0.26	8.19	0.34	0.91	4.91	0.18	25.98
29-Jun-05	9.56	2.58	0.27	8.32	0.36	1.02	5.51	0.21	27.83
2-Jul-05	9.27	2.58	0.27	8.14	0.36	0.99	5.25	0.19	27.05
5-Jul-05	10.09	2.63	0.30	8.07	0.39	1.02	5.27	0.21	27.96

Table J-2. (continued).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
8-Jul-05	10.56	2.71	0.31	8.11	0.41	1.05	5.45	0.21	28.80
11-Jul-05	10.33	2.51	0.30	7.81	0.40	1.04	5.39	0.22	27.99
14-Jul-05	10.23	2.42	0.30	7.40	0.39	1.01	5.14	0.21	27.11
17-Jul-05	10.31	2.39	0.30	7.20	0.39	1.00	5.07	0.21	26.85
20-Jul-05	10.10	2.30	0.29	6.84	0.37	0.96	4.85	0.21	25.91
23-Jul-05	10.16	2.31	0.30	6.42	0.34	0.92	4.47	0.21	25.13
26-Jul-05	10.35	2.33	0.30	6.29	0.34	0.90	4.35	0.20	25.05
29-Jul-05	9.89	2.21	0.25	5.85	0.31	0.84	4.03	0.19	23.58
4-Aug-05	9.78	2.17	0.24	5.54	0.28	0.81	3.92	0.19	22.94
7-Aug-05	9.90	2.17	0.24	5.64	0.28	0.83	4.03	0.20	23.30
11-Aug-05	9.48	2.07	0.23	5.43	0.28	0.83	4.02	0.19	22.53
16-Aug-05	10.38	2.15	0.24	5.78	0.30	0.87	4.28	0.19	24.20
29-Aug-05	10.82	2.07	0.24	5.44	0.28	0.89	4.51	0.20	24.45
1-Sep-05	11.78	2.21	0.26	5.83	0.30	0.96	4.67	0.19	26.20
5-Sep-05	11.37	2.08	0.24	5.61	0.29	0.93	4.76	0.20	25.50
8-Sep-05	12.03	2.17	0.25	5.70	0.29	0.95	4.77	0.20	26.37
12-Sep-05	12.17	2.18	0.25	5.59	0.29	0.97	4.77	0.20	26.41
15-Sep-05	10.59	1.95	0.23	4.89	0.26	0.86	4.36	0.20	23.32
18-Sep-05	11.38	2.21	0.24	5.30	0.27	0.91	4.49	0.19	24.99
21-Sep-05	10.88	2.12	0.23	5.03	0.26	0.89	4.52	0.20	24.12
26-Sep-05	11.16	2.07	0.24	5.04	0.27	0.89	4.50	0.20	24.37
29-Sep-05	11.25	2.12	0.24	5.00	0.27	0.89	4.37	0.19	24.34
2-Oct-05	11.06	2.10	0.24	4.87	0.27	0.87	4.22	0.19	23.82
5-Oct-05	10.77	2.05	0.23	4.72	0.26	0.86	4.19	0.19	23.27
8-Oct-05	10.97	2.12	0.25	4.78	0.28	0.84	4.07	0.19	23.51
11-Oct-05	10.88	2.14	0.25	4.72	0.27	0.84	3.96	0.19	23.24
14-Oct-05	10.90	2.15	0.24	4.62	0.27	0.83	3.97	0.19	23.18
17-Oct-05	12.04	2.32	0.25	4.85	0.28	0.82	3.87	0.18	24.61
20-Oct-05	10.48	2.23	0.24	4.43	0.27	0.78	3.64	0.18	22.25
23-Oct-05	9.74	2.02	0.21	3.90	0.23	0.66	3.45	0.18	20.39
26-Oct-05	10.11	2.22	0.24	4.32	0.26	0.75	3.44	0.18	21.51
29-Oct-05	8.93	1.99	0.21	3.78	0.23	0.65	3.06	0.17	19.02
1-Nov-05	9.98	2.26	0.24	4.23	0.26	0.72	3.23	0.18	21.10
7-Nov-05	12.40	2.71	0.28	4.81	0.30	0.80	3.50	0.21	25.02
10-Nov-05	11.86	2.67	0.27	4.87	0.30	0.78	3.37	0.21	24.33
13-Nov-05	8.71	1.96	0.20	3.70	0.22	0.62	3.07	0.21	18.69
16-Nov-05	10.94	2.60	0.26	4.71	0.28	0.74	3.11	0.21	22.84
22-Nov-05	10.00	2.41	0.23	4.33	0.25	0.71	3.03	0.25	21.20
25-Nov-05	9.85	2.34	0.23	4.07	0.25	0.71	3.12	0.30	20.87
28-Nov-05	11.41	2.45	0.35	3.50	0.38	0.88	3.60	0.59	23.17
1-Dec-05	11.31	2.43	0.35	3.46	0.38	0.88	3.56	0.59	22.97
4-Dec-05	8.15	2.02	0.21	3.35	0.25	0.67	2.95	0.32	17.92
7-Dec-05	7.84	1.87	0.20	3.11	0.23	0.64	2.79	0.30	16.99
10-Dec-05	8.77	2.10	0.22	3.51	0.25	0.71	3.08	0.32	18.97
13-Dec-05	7.97	1.88	0.17	3.25	0.19	0.63	2.68	0.27	17.04

Table J-2. (continued).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
16-Dec-05	11.07	2.32	0.00	3.72	0.27	0.69	3.20	0.35	21.64
19-Dec-05	10.31	2.22	0.22	3.62	0.25	0.69	3.19	0.34	20.84
22-Dec-05	10.77	2.41	0.24	3.66	0.25	0.70	3.10	0.33	21.47
28-Dec-05	9.32	2.22	0.22	3.19	0.25	0.74	3.37	0.47	19.77
31-Dec-05	10.68	2.29	0.23	3.34	0.25	0.73	3.39	0.47	21.38
3-Jan-06	11.07	2.22	0.35	3.04	0.37	0.86	3.59	0.74	22.24
6-Jan-06	11.11	2.25	0.00	3.27	0.27	0.75	3.45	0.49	21.59
9-Jan-06	12.44	2.37	0.27	3.64	0.32	0.77	3.69	0.46	23.96
12-Jan-06	12.56	2.44	0.27	3.82	0.31	0.84	3.74	0.47	24.45

Table J-3. Fermentor 3 acid concentrations (g/L).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
19-Apr-05	4.05	1.67	0.15	4.86	0.17	0.48	1.86	0.09	13.34
21-Apr-05	4.24	1.70	0.15	5.00	0.18	0.50	1.95	0.09	13.82
23-Apr-05	5.33	2.07	0.20	6.18	0.24	0.65	2.75	0.13	17.56
25-Apr-05	6.12	2.28	0.22	6.78	0.26	0.73	3.21	0.17	19.76
27-Apr-05	6.34	2.26	0.21	6.70	0.25	0.76	3.84	0.24	20.59
29-Apr-05	6.72	2.36	0.20	7.22	0.24	0.79	4.04	0.24	21.82
1-May-05	7.00	2.38	0.20	7.33	0.23	0.81	4.17	0.24	22.36
3-May-05	7.36	2.43	0.20	7.52	0.23	0.84	4.36	0.24	23.18
5-May-05	7.64	2.43	0.20	7.66	0.24	0.86	4.52	0.24	23.79
7-May-05	7.73	2.35	0.20	7.53	0.23	0.83	4.55	0.22	23.65
9-May-05	8.05	2.34	0.20	7.63	0.23	0.86	4.79	0.23	24.34
11-May-05	8.40	2.34	0.21	7.68	0.23	0.90	5.09	0.23	25.07
13-May-05	8.20	2.21	0.20	7.30	0.23	0.89	5.27	0.23	24.52
15-May-05	8.82	2.31	0.21	7.68	0.24	0.94	5.40	0.23	25.83
17-May-05	8.19	2.13	0.20	7.09	0.23	0.88	5.13	0.22	24.06
19-May-05	9.05	2.32	0.22	7.76	0.25	0.98	5.54	0.23	26.33
21-May-05	9.19	2.34	0.22	7.91	0.25	1.00	5.65	0.23	26.78
22-May-05	9.11	2.33	0.22	7.87	0.25	0.99	5.66	0.22	26.65
24-May-05	8.73	2.21	0.21	7.51	0.24	0.95	5.44	0.21	25.51
26-May-05	8.61	2.15	0.21	7.36	0.24	0.94	5.38	0.21	25.09
28-May-05	7.27	1.78	0.17	6.28	0.20	0.83	5.06	0.21	21.80
30-May-05	7.81	1.91	0.19	6.79	0.22	0.90	5.25	0.20	23.25
1-Jun-05	8.51	2.05	0.21	7.33	0.24	0.97	5.54	0.21	25.06
3-Jun-05	8.55	2.02	0.21	7.28	0.24	0.97	5.64	0.21	25.12
6-Jun-05	7.22	1.70	0.17	6.22	0.21	0.83	5.01	0.18	21.54
8-Jun-05	9.42	2.20	0.23	8.01	0.27	1.05	6.05	0.21	27.45
10-Jun-05	9.44	2.16	0.23	7.96	0.27	1.05	6.08	0.21	27.41
12-Jun-05	9.48	2.16	0.23	8.01	0.28	1.05	6.09	0.21	27.51
14-Jun-05	9.75	2.21	0.24	8.20	0.29	1.09	6.25	0.22	28.25
16-Jun-05	9.71	2.05	0.26	7.16	0.31	1.05	5.22	0.25	26.00
19-Jun-05	10.02	2.16	0.26	7.74	0.32	1.09	5.70	0.24	27.52
21-Jun-05	9.99	2.20	0.27	8.07	0.32	1.12	5.99	0.23	28.21

Table J-3. (continued).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
23-Jun-05	10.25	2.26	0.27	8.29	0.34	1.14	6.18	0.23	28.97
26-Jun-05	9.46	2.08	0.25	7.66	0.32	1.07	6.05	0.23	27.11
28-Jun-05	10.14	2.23	0.27	8.13	0.34	1.12	6.16	0.23	28.61
2-Jul-05	9.91	2.02	0.28	6.93	0.34	1.06	5.02	0.25	25.81
5-Jul-05	9.65	2.01	0.28	6.67	0.34	1.00	4.90	0.23	25.09
8-Jul-05	9.44	1.99	0.28	6.47	0.34	0.97	4.74	0.23	24.47
11-Jul-05	9.10	1.94	0.27	5.90	0.33	0.91	4.32	0.21	22.98
14-Jul-05	8.84	1.88	0.25	5.61	0.30	0.86	4.07	0.20	22.01
17-Jul-05	8.47	1.85	0.24	5.43	0.29	0.82	3.88	0.20	21.17
20-Jul-05	7.98	1.85	0.21	5.11	0.25	0.75	3.49	0.19	19.84
23-Jul-05	8.73	2.05	0.22	5.08	0.24	0.75	3.45	0.19	20.72
26-Jul-05	7.76	1.97	0.18	4.73	0.20	0.69	3.23	0.18	18.94
29-Jul-05	7.60	1.94	0.18	4.57	0.20	0.69	3.27	0.18	18.63
4-Aug-05	7.45	1.77	0.16	4.20	0.18	0.66	3.18	0.18	17.77
7-Aug-05	7.28	1.73	0.15	4.04	0.17	0.65	3.05	0.18	17.25
11-Aug-05	6.43	1.42	0.13	3.61	0.15	0.59	2.88	0.17	15.39
16-Aug-05	6.91	1.50	0.14	3.87	0.16	0.64	3.13	0.17	16.52
29-Aug-05	7.43	1.63	0.16	4.11	0.19	0.67	3.28	0.18	17.65
1-Sep-05	7.42	1.62	0.17	4.00	0.20	0.66	3.25	0.18	17.49
5-Sep-05	7.30	1.60	0.17	3.91	0.20	0.65	3.19	0.17	17.19
8-Sep-05	10.56	1.96	0.22	4.82	0.25	0.85	4.22	0.19	23.07
12-Sep-05	11.64	2.15	0.24	5.16	0.27	0.93	4.55	0.20	25.15
15-Sep-05	6.62	1.24	0.08	3.05	0.08	0.63	3.02	0.18	14.91
18-Sep-05	7.12	1.57	0.00	3.50	0.19	0.61	2.87	0.16	16.02
21-Sep-05	7.04	1.63	0.16	3.54	0.18	0.60	2.88	0.16	16.20
26-Sep-05	7.61	1.66	0.00	3.66	0.21	0.62	2.84	0.16	16.76
29-Sep-05	6.90	1.49	0.16	3.25	0.18	0.57	2.65	0.15	15.37
2-Oct-05	6.94	1.55	0.17	3.22	0.19	0.57	2.54	0.15	15.34
5-Oct-05	6.39	1.48	0.17	2.92	0.18	0.53	2.47	0.15	14.30
8-Oct-05	7.02	1.60	0.18	3.32	0.19	0.58	2.62	0.15	15.65
11-Oct-05	7.07	1.64	0.19	3.11	0.19	0.56	2.39	0.14	15.28
14-Oct-05	6.21	1.38	0.15	2.76	0.15	0.47	2.16	0.13	13.43
17-Oct-05	7.78	1.68	0.18	3.40	0.18	0.53	2.37	0.13	16.25
23-Oct-05	5.47	1.28	0.12	2.53	0.12	0.41	1.93	0.13	12.00
26-Oct-05	7.14	1.61	0.16	3.13	0.15	0.50	2.21	0.14	15.05
29-Oct-05	7.18	1.74	0.16	3.15	0.17	0.51	2.26	0.14	15.31
1-Nov-05	5.84	1.41	0.13	2.58	0.12	0.41	1.77	0.13	12.39
7-Nov-05	8.59	2.30	0.17	3.61	0.17	0.52	2.04	0.16	17.57
10-Nov-05	9.11	2.27	0.17	3.76	0.17	0.57	2.26	0.18	18.48
13-Nov-05	9.48	2.54	0.18	4.02	0.18	0.59	2.32	0.17	19.48
16-Nov-05	9.43	2.23	0.17	3.84	0.17	0.58	2.29	0.18	18.88
22-Nov-05	10.10	2.38	0.18	4.10	0.17	0.63	2.46	0.19	20.22
25-Nov-05	9.87	2.27	0.17	3.97	0.17	0.62	2.49	0.20	19.77
28-Nov-05	9.33	2.21	0.16	3.85	0.16	0.64	2.57	0.21	19.15
1-Dec-05	8.74	2.04	0.15	3.58	0.15	0.60	2.45	0.20	17.92

Table J-3. (continued).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
4-Dec-05	8.06	1.87	0.15	3.33	0.15	0.59	2.41	0.21	16.77
7-Dec-05	9.17	1.89	0.15	3.48	0.16	0.58	2.48	0.21	18.14
10-Dec-05	8.98	1.89	0.16	3.47	0.16	0.59	2.47	0.22	17.94
13-Dec-05	8.43	1.80	0.15	3.32	0.15	0.56	2.43	0.22	17.07
16-Dec-05	9.20	1.96	0.18	3.39	0.17	0.59	2.66	0.27	18.41
19-Dec-05	6.05	1.33	0.10	2.34	0.10	0.42	2.17	0.23	12.75
22-Dec-05	6.52	1.65	0.11	2.52	0.11	0.44	2.02	0.22	13.59
28-Dec-05	7.38	1.78	0.12	2.54	0.12	0.49	2.32	0.30	15.05
31-Dec-05	8.11	1.90	0.14	2.62	0.15	0.57	2.64	0.41	16.54
3-Jan-06	9.33	1.90	0.00	2.77	0.22	0.62	3.22	0.46	18.53
6-Jan-06	7.32	1.60	0.12	2.26	0.12	0.51	2.68	0.40	15.02
9-Jan-06	8.53	1.73	0.13	2.51	0.14	0.56	2.77	0.41	16.79
12-Jan-06	10.41	2.08	0.18	3.01	0.18	0.66	3.23	0.47	20.22

Table J-4. Fermentor 4 acid concentrations (g/L).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
19-Apr-05	8.26	2.72	0.29	6.08	0.35	0.92	1.64	0.16	20.42
23-Apr-05	8.09	2.52	0.29	5.91	0.36	1.06	2.61	0.30	21.14
25-Apr-05	8.15	2.50	0.29	5.89	0.37	1.09	2.85	0.35	21.48
27-Apr-05	7.32	2.22	0.27	5.24	0.33	0.97	2.70	0.35	19.41
29-Apr-05	7.87	2.36	0.29	5.64	0.36	1.04	2.98	0.40	20.93
3-May-05	7.94	2.34	0.30	5.92	0.36	1.02	2.92	0.40	21.19
5-May-05	8.71	2.53	0.33	6.66	0.39	1.11	3.16	0.43	23.32
7-May-05	8.61	2.46	0.32	6.70	0.38	1.04	3.13	0.41	23.06
9-May-05	8.89	2.49	0.33	6.98	0.38	1.06	3.21	0.41	23.76
11-May-05	8.47	2.31	0.31	6.65	0.35	1.00	3.09	0.39	22.57
13-May-05	10.22	2.72	0.37	7.93	0.42	1.19	3.68	0.44	26.96
15-May-05	9.46	2.42	0.33	7.17	0.37	1.09	3.51	0.39	24.74
17-May-05	9.97	2.49	0.35	7.40	0.39	1.15	3.76	0.41	25.92
19-May-05	9.70	2.35	0.33	7.00	0.36	1.11	3.67	0.39	24.91
21-May-05	9.95	2.36	0.33	7.05	0.37	1.13	3.78	0.39	25.35
22-May-05	10.17	2.38	0.34	7.14	0.37	1.15	3.89	0.39	25.83
24-May-05	9.95	2.27	0.32	6.86	0.35	1.13	4.00	0.40	25.28
26-May-05	10.75	2.41	0.34	7.29	0.38	1.21	4.19	0.40	26.97
28-May-05	10.82	2.39	0.34	7.26	0.38	1.21	4.18	0.40	26.98
30-May-05	9.63	2.10	0.30	6.40	0.33	1.08	3.88	0.37	24.10
1-Jun-05	10.73	2.30	0.33	7.04	0.38	1.20	4.30	0.40	26.69
3-Jun-05	10.36	2.18	0.32	6.72	0.35	1.15	4.14	0.38	25.61
6-Jun-05	10.95	2.27	0.34	7.01	0.37	1.21	4.37	0.39	26.92
8-Jun-05	11.93	2.42	0.36	7.52	0.39	1.31	4.76	0.42	29.10
10-Jun-05	12.11	2.41	0.37	7.51	0.40	1.30	4.75	0.41	29.25
12-Jun-05	12.03	2.39	0.36	7.46	0.40	1.29	4.74	0.40	29.08
14-Jun-05	12.29	2.41	0.37	7.54	0.41	1.31	4.79	0.40	29.52
16-Jun-05	4.80	0.92	0.14	2.92	0.16	0.52	1.97	0.17	11.58
19-Jun-05	7.48	1.43	0.22	4.50	0.24	0.81	3.08	0.26	18.01

Table J-4. (continued).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
21-Jun-05	8.11	1.56	0.25	5.02	0.27	0.91	3.45	0.28	19.84
23-Jun-05	8.54	1.63	0.26	5.24	0.28	0.94	3.54	0.28	20.70
26-Jun-05	7.11	1.35	0.21	4.37	0.23	0.79	3.15	0.26	17.47
28-Jun-05	7.75	1.45	0.23	4.73	0.25	0.85	3.48	0.28	19.03
29-Jun-05	4.39	0.81	0.13	2.66	0.14	0.48	1.94	0.16	10.72
2-Jul-05	5.70	1.05	0.17	3.42	0.19	0.61	2.39	0.20	13.73
8-Jul-05	5.64	1.09	0.17	3.55	0.19	0.59	2.46	0.19	13.87
11-Jul-05	5.09	1.10	0.15	3.42	0.16	0.53	2.21	0.17	12.84
14-Jul-05	4.56	1.20	0.13	3.55	0.14	0.49	2.02	0.16	12.26
17-Jul-05	4.51	1.45	0.13	4.03	0.13	0.50	2.08	0.18	13.01
20-Jul-05	4.04	1.43	0.11	3.34	0.12	0.44	1.81	0.16	11.44
23-Jul-05	3.80	1.67	0.09	2.82	0.08	0.37	1.54	0.14	10.51
26-Jul-05	3.98	1.60	0.08	2.73	0.09	0.39	1.64	0.15	10.67
29-Jul-05	3.90	1.39	0.08	2.44	0.09	0.37	1.59	0.13	10.00
4-Aug-05	3.79	1.25	0.07	2.10	0.08	0.36	1.42	0.13	9.19
7-Aug-05	3.69	1.11	0.06	2.01	0.07	0.35	1.40	0.13	8.82
11-Aug-05	3.75	1.08	0.06	2.09	0.07	0.38	1.53	0.13	9.10
16-Aug-05	4.83	1.20	0.07	2.55	0.07	0.46	1.95	0.16	11.30
29-Aug-05	6.17	1.28	0.00	2.94	0.08	0.58	2.66	0.18	13.89
1-Sep-05	6.24	1.22	0.00	2.92	0.07	0.59	2.79	0.18	14.00
5-Sep-05	6.59	1.22	0.00	3.04	0.08	0.62	3.01	0.19	14.76
8-Sep-05	7.43	1.76	0.00	3.79	0.20	0.64	3.04	0.17	17.04
12-Sep-05	4.87	0.90	0.07	2.20	0.07	0.44	2.11	0.13	10.79
15-Sep-05	5.02	0.94	0.07	2.28	0.07	0.46	2.22	0.14	11.20
18-Sep-05	4.99	0.97	0.07	2.19	0.08	0.43	2.03	0.13	10.89
21-Sep-05	5.34	0.99	0.07	2.43	0.07	0.50	2.40	0.15	11.96
26-Sep-05	6.62	1.23	0.09	3.06	0.09	0.63	3.06	0.19	14.97
29-Sep-05	4.57	0.89	0.07	2.01	0.07	0.39	1.86	0.12	9.97
2-Oct-05	4.29	0.82	0.06	1.85	0.07	0.36	1.75	0.11	9.32
5-Oct-05	3.49	0.68	0.05	1.50	0.06	0.29	1.54	0.10	7.72
8-Oct-05	4.12	0.76	0.06	1.70	0.07	0.32	1.56	0.10	8.69
11-Oct-05	4.24	0.77	0.06	1.74	0.07	0.33	1.55	0.10	8.88
14-Oct-05	4.26	0.83	0.07	1.75	0.08	0.32	1.51	0.10	8.92
17-Oct-05	3.63	0.66	0.00	1.42	0.06	0.25	1.27	0.09	7.39
20-Oct-05	6.37	1.52	0.14	2.83	0.14	0.48	2.28	0.14	13.92
23-Oct-05	3.77	0.83	0.07	1.61	0.08	0.27	1.20	0.10	7.91
26-Oct-05	4.17	0.96	0.08	1.89	0.09	0.30	1.30	0.11	8.90
29-Oct-05	3.53	0.90	0.07	1.87	0.08	0.26	1.10	0.10	7.90
1-Nov-05	3.14	1.00	0.06	2.18	0.07	0.25	1.04	0.10	7.84
7-Nov-05	4.60	2.03	0.10	4.45	0.10	0.39	1.49	0.16	13.32
10-Nov-05	4.93	2.14	0.09	4.32	0.10	0.40	1.55	0.15	13.69
13-Nov-05	4.52	1.72	0.08	3.39	0.08	0.37	1.47	0.14	11.77
16-Nov-05	5.42	1.76	0.10	3.33	0.12	0.44	1.85	0.17	13.17
22-Nov-05	4.18	1.40	0.00	2.49	0.07	0.35	1.43	0.13	10.07
25-Nov-05	3.96	1.28	0.00	2.24	0.07	0.33	1.36	0.13	9.37
28-Nov-05	4.39	1.29	0.00	2.28	0.08	0.35	1.48	0.14	10.00
1-Dec-05	1.79	0.54	0.00	0.97	0.00	0.18	1.07	0.13	4.67

Table J-4. (continued).

Date	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
4-Dec-05	4.45	1.34	0.00	2.31	0.08	0.38	1.61	0.16	10.33
7-Dec-05	3.77	1.15	0.00	2.05	0.07	0.34	1.54	0.16	9.08
10-Dec-05	3.74	1.12	0.06	2.01	0.07	0.34	1.49	0.16	8.99
13-Dec-05	2.78	0.82	0.00	1.50	0.00	0.26	1.14	0.13	6.63
16-Dec-05	4.09	1.27	0.07	1.97	0.08	0.35	1.56	0.19	9.58
19-Dec-05	3.58	1.13	0.06	1.84	0.07	0.33	1.49	0.18	8.68
22-Dec-05	4.08	1.31	0.07	1.85	0.08	0.34	1.50	0.19	9.42
28-Dec-05	3.50	1.31	0.00	1.49	0.07	0.29	1.37	0.20	8.23
31-Dec-05	2.91	1.08	0.00	1.19	0.00	0.26	1.28	0.24	6.96
3-Jan-06	2.41	0.91	0.04	1.03	0.05	0.25	1.32	0.24	6.24
6-Jan-06	2.91	1.00	0.00	1.20	0.00	0.28	1.52	0.26	7.17
9-Jan-06	4.52	1.51	0.07	1.79	0.08	0.41	2.00	0.31	10.67
12-Jan-06	5.45	1.74	0.08	2.07	0.09	0.48	2.26	0.34	12.51

APPENDIX K

SOLIDS RATIO DATA

Table K-1. Solids ratios (dry lb/gal of fermentor volume).

date	F1	F2	F3	F4
17-Oct-05	2.03	0.70	0.85	0.18
20-Oct-05	1.05	0.96	0.55	0.14
26-Oct-05	1.27	1.21	0.61	0.25
29-Oct-05	1.12	0.47	0.76	0.42
1-Nov-05	1.40	0.49	0.67	0.43
7-Nov-05	1.12	1.37	0.67	0.71
10-Nov-05	0.71	1.04	0.67	0.43
13-Nov-05	1.24	1.03	0.67	0.53
16-Nov-05	1.01	0.91	0.67	0.57
22-Nov-05	1.00	0.74	0.67	0.51
25-Nov-05	1.00	0.74	0.67	0.51
28-Nov-05	0.60	0.96	0.67	0.45
1-Dec-05	1.04	0.67	0.67	0.38
4-Dec-05	1.54	0.74	0.90	0.43
16-Dec-05	1.10	0.95	0.80	0.85
19-Dec-05	1.10	0.52	0.09	0.44
22-Dec-05	0.70	0.59	0.32	0.52
28-Dec-05	0.70	1.17	0.45	0.50
31-Dec-05	0.70	1.29	0.89	0.90
3-Jan-06	0.70	1.09	0.62	1.11
9-Jan-06	1.76	1.46	0.63	0.88
12-Jan-06	1.14	1.40	0.91	0.83
15-Jan-06	0.86	0.50	0.40	0.51

APPENDIX L

FERMENTOR TEMPERATURE DATA

Table L-1. Fermentor temperature (^oC).

Date	F1	F2	F3	F4	Average
27-Apr-05	36.5	33.0	34.5	34.5	34.6
28-Apr-05	37.0	35.0	35.0	36.0	35.8
29-Apr-05	37.0	37.0	35.5	37.0	36.6
1-May-05	39.0	37.0	35.0	37.0	37.0
3-May-05	40.0	39.0	36.0	37.0	38.0
4-May-05	40.0	39.0	36.0	37.0	38.0
5-May-05	41.0	38.0	36.0	38.0	38.3
6-May-05	41.0	38.0	36.0	38.0	38.3
7-May-05	41.0	38.0	36.0	38.0	38.3
9-May-05	41.0	39.0	36.0	37.0	38.3
10-May-05	41.0	39.0	36.0	39.0	38.8
11-May-05	40.0	40.0	37.0	40.0	39.3
15-May-05	42.0	42.0	38.0	42.0	41.0
16-May-05	42.0	42.0	38.0	42.0	41.0
17-May-05	42.0	42.0	38.0	40.0	40.5
18-May-05	41.0	40.5	37.5	38.0	39.3
19-May-05	40.0	39.0	37.0	37.0	38.3
20-May-05	39.0	39.0	36.0	38.0	38.0
22-May-05	38.0	38.0	37.0	41.0	38.5
23-May-05	37.0	37.0	38.0	44.0	39.0
24-May-05	36.0	36.0	41.0	43.0	39.0
25-May-05	36.0	35.0	41.0	42.0	38.5
26-May-05	36.0	35.0	41.0	40.0	38.0
30-May-05	37.0	37.0	41.0	38.0	38.3
31-May-05	38.0	37.0	41.0	38.0	38.5
1-Jun-05	39.0	38.0	40.0	38.0	38.8
2-Jun-05	39.0	38.0	40.0	39.0	39.0
3-Jun-05	39.0	38.0	40.0	39.0	39.0
6-Jun-05	39.0	38.0	40.0	40.0	39.3
7-Jun-05	39.0	38.0	40.0	40.0	39.3
8-Jun-05	40.0	39.0	39.0	41.0	39.8
10-Jun-05	35.0	39.0	39.0	41.0	38.5
15-Jun-05	38.0	39.0	40.0	41.0	39.5
16-Jun-05	39.0	39.0	40.0	39.0	39.3
17-Jun-05	39.0	39.0	40.0	40.0	39.5
19-Jun-05	39.0	39.0	40.0	40.0	39.5
21-Jun-05	40.0	39.0	39.0	40.0	39.5
22-Jun-05	39.0	40.0	39.0	40.0	39.5
23-Jun-05	40.0	40.0	39.0	41.0	40.0

Table L-1. (continued).

Date	F1	F2	F3	F4	Average
28-Jun-05	40.0	41.0	39.0	41.0	40.3
29-Jun-05	40.0	40.0	38.0	40.0	39.5
1-Jul-05	40.0	42.0	39.0	40.0	40.3
2-Jul-05	40.0	41.0	38.0	42.0	40.3
7-Jul-05	40.0	32.0	38.0	41.0	37.8
8-Jul-05	37.0	35.0	38.0	40.0	37.5
9-Jul-05	38.0	37.0	37.0	38.0	37.5
10-Jul-05	38.0	38.0	37.0	38.0	37.8
11-Jul-05	38.0	39.0	37.0	38.0	38.0
12-Jul-05	38.0	40.0	37.0	38.0	38.3
13-Jul-05	39.0	40.0	37.0	38.0	38.5
16-Jul-05	38.0	39.0	37.0	37.0	37.8
18-Jul-05	39.0	37.0	37.0	37.0	37.5
19-Jul-05	39.0	37.0	37.0	37.0	37.5
22-Jul-05	39.0	39.0	36.0	38.0	38.0
25-Jul-05	39.0	38.0	37.0	37.0	37.8
26-Jul-05	39.0	40.0	38.0	38.0	38.8
27-Jul-05	40.0	40.0	38.0	38.0	39.0
29-Jul-05	40.0	41.0	39.0	40.0	40.0
1-Aug-05	41.0	43.0	40.0	40.0	41.0
2-Aug-05	41.0	42.0	40.0	40.0	40.8
3-Aug-05	41.0	42.0	40.0	41.0	41.0
4-Aug-05	41.0	41.0	40.0	42.0	41.0
5-Aug-05	42.0	42.0	40.0	40.0	41.0
7-Aug-05	42.0	38.0	40.0	42.0	40.5
8-Aug-05	42.0	38.0	40.0	42.0	40.5
9-Aug-05	42.0	39.0	40.0	41.0	40.5
10-Aug-05	41.0	39.0	39.0	41.0	40.0
16-Aug-05	39.0	42.0	39.0	41.0	40.3
17-Aug-05	39.0	41.0	39.0	41.0	40.0
29-Aug-05	41.0	38.0	41.0	37.0	39.3
30-Aug-05	40.0	38.0	41.0	36.0	38.8
31-Aug-05	40.0	38.0	40.0	40.0	39.5
1-Sep-05	40.0	38.0	40.0	40.0	39.5
2-Sep-05	38.0	37.0	41.0	41.0	39.3
5-Sep-05	39.0	38.0	40.0	43.0	40.0
6-Sep-05	40.0	38.0	40.0	43.0	40.3
8-Sep-05	39.0	39.0	40.0	41.0	39.8
9-Sep-05	40.0	40.0	40.0	43.0	40.8
12-Sep-05	40.0	40.0	35.0	40.0	38.8
15-Sep-05	40.0	40.0	39.0	40.0	39.8
16-Sep-05	40.0	40.0	39.0	40.0	39.8
18-Sep-05	40.0	40.0	40.0	43.0	40.8
21-Sep-05	40.0	42.0	40.0	40.0	40.5
24-Sep-05	40.0	40.0	40.0	38.0	39.5

Table L-1. (continued).

Date	F1	F2	F3	F4	Average
26-Sep-05	40.0	40.0	40.0	37.0	39.3
29-Sep-05	40.0	39.0	41.0	40.0	40.0
2-Oct-05	40.0	39.0	41.0	40.0	40.0
5-Oct-05	40.0	39.0	39.0	40.0	39.5
6-Oct-05	40.0	40.0	39.0	41.0	40.0
9-Oct-05	36.0	38.0	36.0	38.0	37.0
12-Oct-05	37.0	36.0	36.0	37.0	36.5
19-Oct-05	37.0	37.0	36.0	37.0	36.8
25-Oct-05	37.0	36.0	33.0	36.0	35.5
29-Oct-05	35.0	32.0	32.0	35.0	33.5
30-Oct-05	35.0	33.0	32.0	35.0	33.8
1-Nov-05	34.0	32.0	30.0	34.0	32.5
7-Nov-05	38.0	36.0	37.0	38.0	37.3
8-Nov-05	38.0	36.0	39.0	39.0	38.0
10-Nov-05	39.0	37.0	36.0	39.0	37.8
15-Nov-05	38.0	37.0	36.0	39.0	37.5
16-Nov-05	38.0	37.0	34.0	39.0	37.0
28-Nov-05	42.0	42.0	32.0	35.0	37.8
1-Dec-05	40.0	41.0	36.0	39.0	39.0
4-Dec-05	37.0	40.0	33.0	35.0	36.3
6-Dec-05	34.0	40.0	34.0	35.0	35.8
8-Dec-05	31.0	34.0	30.0	30.0	31.3
11-Dec-05	30.0	35.0	30.0	31.0	31.5
13-Dec-05	32.0	36.0	28.0	31.0	31.8
19-Dec-05	30.0	33.0	25.0	27.0	28.8
22-Dec-05	34.0	34.0	20.0	27.0	28.8
27-Dec-05	35.0	35.0	25.0	29.0	31.0
29-Dec-05	35.0	35.0	30.0	33.0	33.3
31-Dec-05	35.0	39.0	31.0	34.0	34.8
3-Jan-06	34.0	39.0	33.0	36.0	35.5
5-Jan-06	34.0	39.0	33.0	35.0	35.3
9-Jan-06	31.0	42.0	40.0	37.0	37.5
10-Jan-06	31.0	42.0	40.0	37.0	37.5
12-Jan-06	35.0	40.0	37.0	36.0	37.0
15-Jan-06	34.0	35.0	38.0	37.0	36.0
16-Jan-06	36.0	35.0	36.0	38.0	36.3

VITA

Andrew Garret Moody was born and grew up in Houston, Texas. He graduated from Texas A&M University in May 2003 with a B.S. in chemical engineering. He then studied under Dr. Mark Holtzapple at Texas A&M University and received a M.S. in chemical engineering. His area of research was the MixAlco process, specifically the fermentation of biomass to carboxylic acids. He then accepted a position with SBM-IMODCO in Houston, Texas as a process engineer. His permanent address will be 11902 Normont Dr., Houston, TX 77070.