EFFECT OF LIQUID RESIDENCE TIME, EXTRACTION, AND CHAIN ELONGATION ON COUNTERCURRENT MIXED-ACID FERMENTATIONS

A Thesis

by

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ABSTRACT

The carboxylate platform is one of three recognized processes (sugar, thermochemical, and carboxylate) that converts biomass into chemicals and hydrocarbon fuels. One example of the carboxylate platform is the MixAlco process. Using mixed-culture countercurrent fermentation, biomass is digested into carboxylate salts. Using chemical reactions (e.g., hydrogenation), a variety of products are produced such as alcohols, esters, ketones, and ethers. In this process, methane production is blocked using an inhibitor, which enhances product value. To enhance the conversion of biomass and increase product yields, the following experiments were done.

To extend the liquid residence time, the liquid maintenance target was adjusted to increase the total liquid volume of the system. The average liquid residence time was 88.9 days compared with the traditional 20–30 days. The results show that the concentration of liquid product is 34.3 g/L, conversion is 64.2%, yield is 11.2%, acetic acid equivalent yield (aceq) is 16.6%, selectivity is 17.5%, and acetic acid equivalent selectivity is 25.9%. Also, this countercurrent system shown comparably high selectivity to butyric acid.

Using the established steady-state countercurrent fermentation system described above, all the conditions are the same except for the usage of resin extraction. The average concentration of liquid product is 28.4 g/L which is lower because of the extraction. The conversion is 70.3%, total acid yield (including resin extraction) is 16.5%, aceq total acid yield is 27.0%, selectivity is 23.4%, and aceq selectivity is 38.5%.

The conversion, yield, and selectivity all were enhanced. The extraction enhances the selectivity of liquid product, especially the selectivity of medium-chain carboxylic acids (C5–C7), which means extraction using a resin promotes chain elongation in the secondary fermentation. One explanation is that extraction reduces product inhibition.

Introducing ethanol to a countercurrent fermentation system shows different phenomenon compared with a previously investigated batch system. In countercurrent fermentation, primary fermentation was promoted, but chain-elongation was inhibited. In contrast, batch fermentation enhanced chain-elongation.

DEDICATION

To my parents, they always try hard

to give me a better life.

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CHAPTER I

INTRODUCTION

Currently, we depend on fossil fuels (oil, natural gas, and coal) to provide most of our energy. Globally, fossil fuels account for more than 80% of energy consumption [1] (Figure 1.1). The advantages of fossil fuels are obvious: they are inexpensive, reliable, energy dense, and so on. However, with great usage of fossil fuels, some negative side effects appear: they are non-renewable, their combustion pollutes the air, and products are greenhouse gas. These impacts are dangerous to human health and the environment. [2]

Compared with fossil fuels, biofuels have many advantages. Firstly, they are environmentally friendly and no extra greenhouse gas (CO_2) are produced. Secondly, it has high potential to make an impact because large amounts are available. Currently, 95% to 97% of bio-energy in the world is produced by directly combusting biomass. [3]

From biomass feedstocks, there are several routes to useful fuels (Figure 1.2).

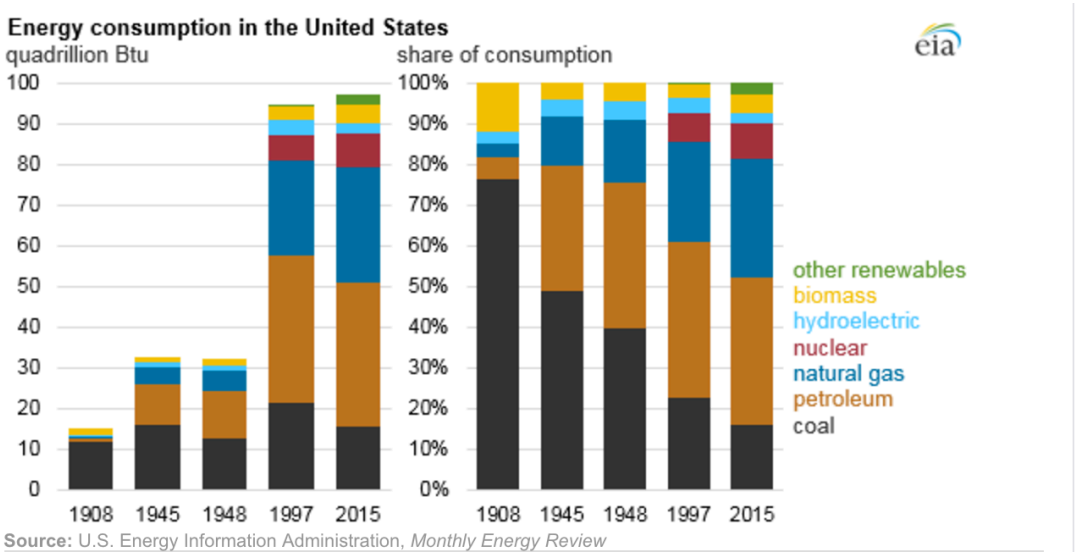

Figure 1.1 Energy consumption in the United States.

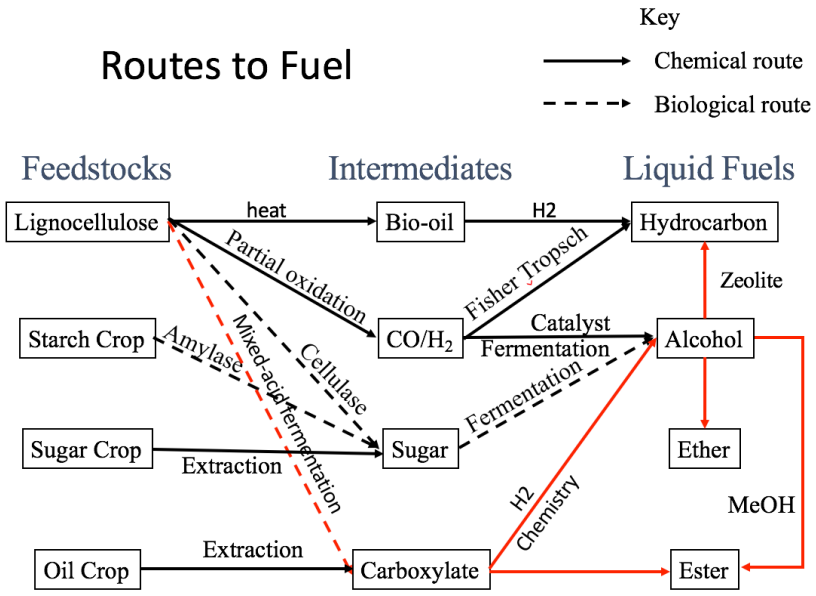

Figure 1.2 Different biomass routes to fuels. [4]

Among all the three major routes to produce biofuels from lignocellulose, three platforms are commonly considered: sugar, thermochemical, and carboxylate. The carboxylate platform has the highest yields. [4]

For the thermochemical platform, common steps follow: (1) partially oxidize lignocellulose to produce syngas (CO, H_2). (2) Use rhodium-based catalyst to transform the syngas to alcohols or iron-based catalysts to produce hydrocarbons.

For the sugar platform, common steps follow: (1) hydrolyze the feedstock to sugars with catalyst (acid or enzyme). (2) Convert sugars to ethanol and CO_2.

The carboxylate platform is similar to the sugar platform and employs the following steps: (1) hydrolyze the feedstock to sugars. (2) Ferment the sugars to mixed acids. (3) Transform the acids to alcohols or hydrocarbon fuels.

The MixAlco^{TM} process (Figure 1.3) is one example of carboxylate platform and has following advantages [5]: (1) no enzyme addition; (2) no contaminates; (3) although it is anaerobic digestion process, some contact of oxygen is acceptable; [6] (4) low operating and capital costs; (5) feedstock flexibility [7]; and (6) non-aseptic operation. However, the main limitation of the carboxylic platform is the low rate of fermentation.

For the carboxylate fermentation, multi-staged countercurrent fermentations are commonly used. More stages would increase the acid concentration and selectivity whereas fewer stages would increase conversion. [8] Commonly, four-stage countercurrent fermentations are used (Figure 1.4). Solids are transferred from F1 to F4 and liquids are transferred from F4 to F1.

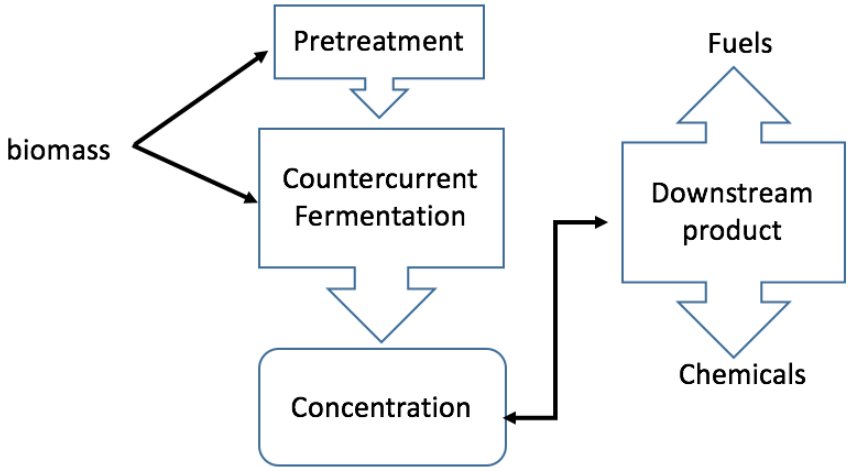

Figure 1.3 MixAlco process.

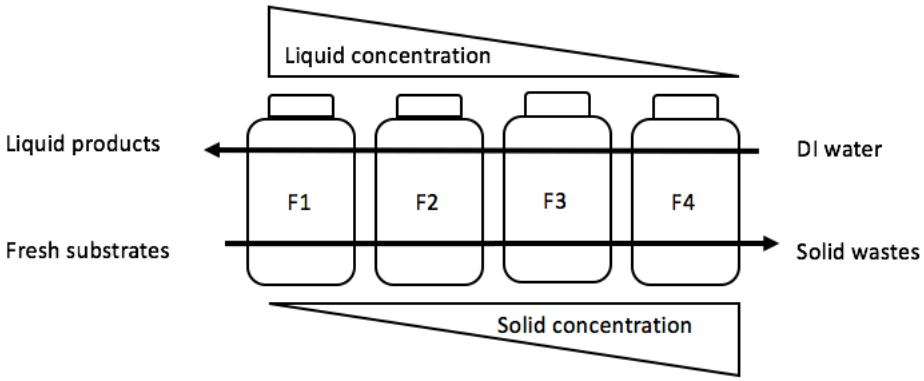

Figure 1.4 Four-stage countercurrent fermentation.

To improve conversion and yield, as well as the economic value of products, the following three hypotheses are proposed:

First, longer liquid residence time would result in higher product concentrations. Thus, the Total Liquid Volume (TLV) is specified to maintain an comparably longer liquid residence time (LRT).

Second, using an ion-exchange resin has proven to significantly improve product yield and substrate conversion in mixed-culture batch system [5]. The objective is to prove that it also works for continuous countercurrent fermentations.

Third, to enhance economic value, Sagar Lonkar recommends adding alcohol for chain elongation [9]. Medium-chain fatty acids production is improved by adding 5–10 g/L of ethanol. Thus, one series of experiment will be performed to prove that it also works for continuous countercurrent fermentations.

CHAPTER II

MATERIALS AND METHODS

2.1 Materials

2.1.1 Substrates and nutrients

Office paper (Caliber Multipurpose Paper) is the substrate, which is shredded before filling the bottles using Fellowes Powershred® W-6C. Also, samples were taken to determine the moisture content. Shredded office paper serves as energy source for the bacteria.

Chicken manure provides nutrients for bacteria, much like vitamins for humans. Fresh chicken manure was taken from the Department of Poultry Science, Texas A&M University (College Station, TX). Because wet chicken manure can readily spoil and is smelly, it was dried in oven (105 °C) for 48 h and kept in Ziploc bags at room temperature.

2.1.2 Fermentor and gas release system

The fermentors are 1-L centrifuge bottles. A septum is penetrated by a syringe needle connected to a gas-release system (Figure 2.2). Two pieces of stainless tubing function as a handle to help pull the stopper out of the bottle. The bottle cannot tolerate pressures above 2 atm absolute. [10]

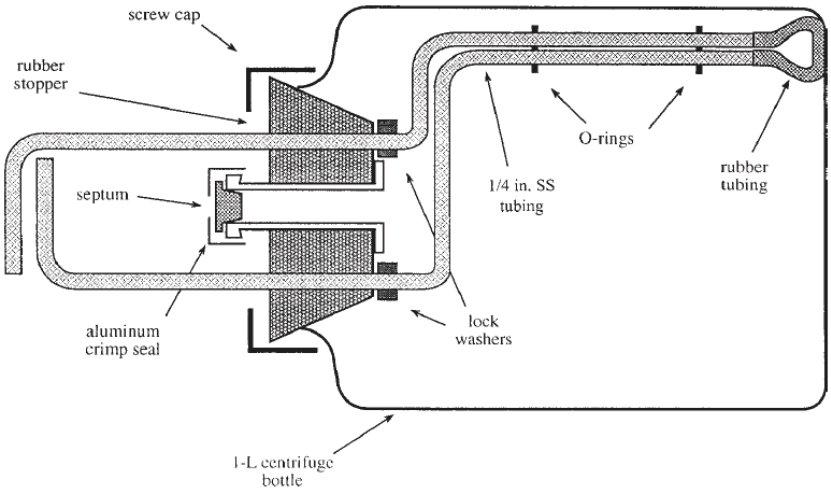

Figure 2.1 Centrifuge bottle fermentor. [10]

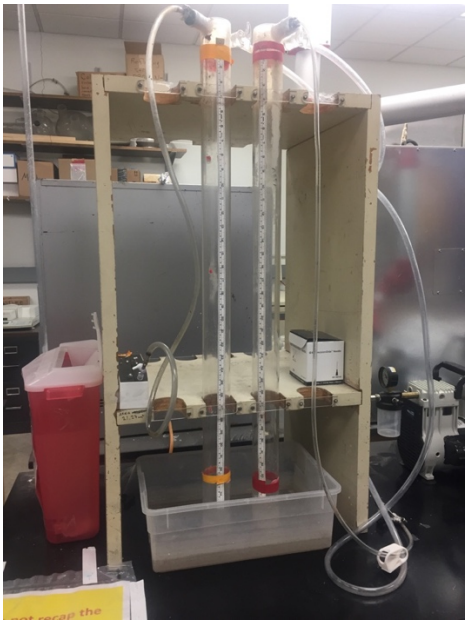

Figure 2.2 Gas-release system.

2.1.3 Fermentation media

Deoxygenated water was prepared and used in the set-up period following the method of Appendix A. Then for the daily operation, de-ionized water was used.

2.1.4 Inoculum

The inoculum of mixed-culture was from the sediment in the beach of Galveston, TX. The sediment was collected from several half a meter-deep shoreline holes. Samples were taken and kept in a sealed bottle immediately, filling with deoxygenated water under about 4 °C until use. Before using, samples should be warmed to room temperature, shaken vigorously. The inoculum is 12.5% of working volume.

2.1.5 Methane inhibitor

Iodoform (CHI_3) is a great inhibitor to prevent production of methane. Every 48 h, 120 μL CHI_3 solution (20 g Iodoform /L, 200-proof ethanol) was used in each bottle. The iodoform solution was kept in a aluminum foil wrapped glass bottles and kept in the refrigerator under 4 °C because of its sensitivity to air, light, and high temperature. [11]

2.1.6 Buffer

Magnesium carbonate ($MgCO_3$) and hydrochloric acid (HCl) were used to balance the pH in a near-neutral range (6.8–7.2). $MgCO_3$ (0.05–0.5 g) was added when the pH is lower than 6.8. When the pH is higher than 7.2, 0.05–2 mL of HCl (5 mol/L) was added to balance it. Ideally, minimal HCl is used, which prevents accumulation of salts.

2.1.7 Ion-exchange resins

Amberlite® IRA-67 ion-exchange resin (Alfa Aesar, Product No. 42253) was used to extract acid product from the fermentation. Before use, resin beads were washed with DI water to remove impurities and unattached amines. A vacuum filtering system was used to help pass liquid through.

A column (Figure 2.3) was used to keep the resin and separate the liquid from the resin.

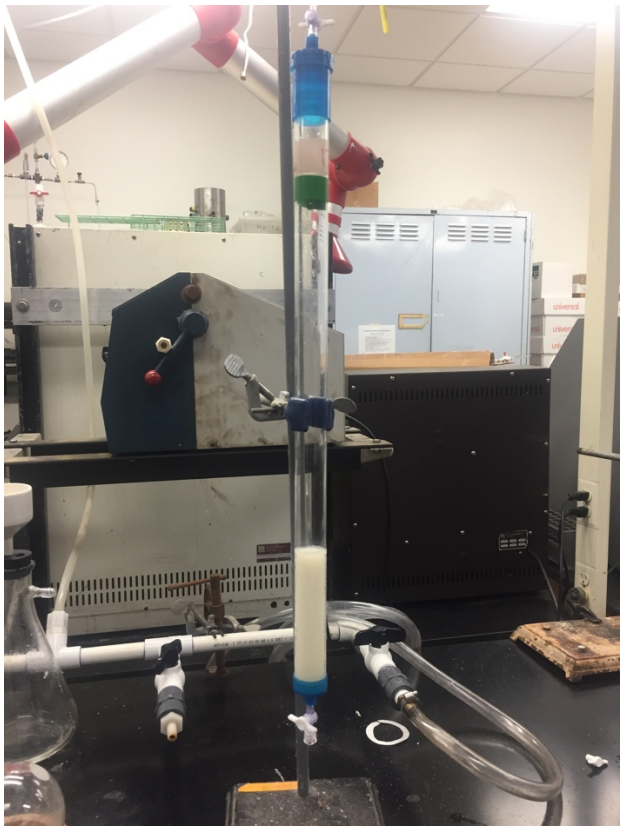

Figure 2.3 Resin column.

2.2 Methods

2.2.1 Biogas analysis

Every 48 h, 30 mL of each biogas sample was collected by puncturing a needle through the septum. The remaining gas was measured by connecting to a well-sealed inverted cylinder using a polypropylene tube. To prevent adsorption of CO_2 and microbial growth, the cylinder was filled with 300 g calcium chloride/L solution. [8] The gas sample was passed through the Gas chromatography (Agilent 6890 Series).

2.2.2 Carboxylic acid concentration determination

Before liquid transfer, liquid product from the fermentation was collected for acid analysis. After centrifugation (4,000 rpm, 10 min), 0.5 mL fermentation liquid was mixed with 0.5 mL phosphoric acid (H_3PO_4, 3 mol/L) and 0.5 mL internal standard (4-methyl-*n*-valeric acid, 1.16 g/L). Then, the solution was centrifuged (13,000 rpm, 10 min). Concentrations of carboxylic acids were measured with the gas chromatography (Agilent 6890). Carrier gas was helium. A mixed volatile acid was used as the external standard, with IC-6 as the internal standard. Table 2.1 shows the concentration of carboxylic acids in the external standard.

Table 2.1 Carboxylic acid concentration in external standard

Acid	Concentration (g/L)
Acetic Acid	3.980
Propionic Acid	3.015
Iso-Butyric Acid	0.997
Butyric Acid	1.989
Iso-Valeric Acid	0.803
Valeric Acid	1.562
Iso-Caproic acid	1.158
Caproic acid	0.808
Enanthic acid	0.397
Caprylic acid	0.169

2.2.3 Moisture content analysis

Moisture content of the substrates were determined according to NREL procedures [8, 12]. To avoid loss of volatile solids (e.g., unionized acids) from evaporation, 0.3 g Calcium hydroxide was added to the liquid sample before placing into the oven (105 °C).

Moisture content (MC) of biomass is defined by the loss of mass in a 105 °C oven. Volatile Solids (VS) is defined as dry solid material (g) lost from combustion under 550 °C. Non-Acid Volatile Solids (NAVS) is defined as difference between VS (g) and carboxylic acid (g).

$$\text{NAVS} = [(1 - \text{MC}) \times (1 - \text{ash}) \times \text{Total biomass (g)}] - (\text{g total carboxylic acid})$$

2.3 Fermentation performance parameters

The production of acid, biogas, and the moisture content were tested and calculated in the steady-state period. The slope method [5] was used with accumulated feed, waste, and production respecting to time. Parameters like yield, conversion, and selectivity can be calculated with the slope.

$$\text{Conversion} = \frac{\text{Non-acid volatile solids}_{\text{digested}}\ (\text{g})}{\text{Non-acid volatile solids}_{\text{feed}}\ (\text{g})}$$

$$\text{Yield} = \frac{\text{Total carboxylic acid produced (g)}}{\text{Non-acid volatile solids}_{\text{feed}}\ (\text{g})}$$

$$\text{Selectivity} = \frac{\text{Yield}}{\text{Conversion}}$$

The volatile solid loading rate (VSLR) and the liquid residence time (LRT) could be calculated as follows:

$$\text{VSLR} = \frac{\text{Non-acid volatile solids}_{\text{feed}}}{\text{Total liquid volume in all fermentors} \cdot \text{time}}$$

$$\text{LRT} = \frac{\text{Total liquid volume in all fermentors}}{\text{Flow rate out of fermentation train}}$$

Figure 2.4 shows the conversion of various components of biomass to final products in solid, liquid, or gas phase. The desired product of this process is carboxylic acids; hence, non-acid volatile solids (NAVS) was used as measure of biomass when calculating fermentation parameters. Chicken manure contains some carboxylic acids and this amount is considered when calculating the total acids produced.

During fermentation, a mixture of acids is produced. The concentration of each acid can be expressed as acetic acid equivalents (Aceq), defined as the potentially reduced acetic acid equivalent amount. [8]

$$\text{Aceq (mol/L)} = \text{Acetic (mol/L)} + 1.75 \times \text{Propionic (mol/L)} + 2.5 \times \text{Butyric (mol/L)} + 3.25 \times \text{Valeric (mol/L)} + 4.0 \times \text{Caproic (mol/L)} + 4.75 \times \text{Heptanoic (mol/L)}$$

$$\text{Aceq (g/L)} = \text{Aceq (mol/L)} \times 60.05 \text{ (g/mol)}$$

The advantage of using acetic acid equivalents is that the various carboxylic acid fractions can be represented as a single concentration.

As the fermentation reaction proceeds and biomass gets digested, its reactivity reduces, and rate of production of carboxylic acids decreases.

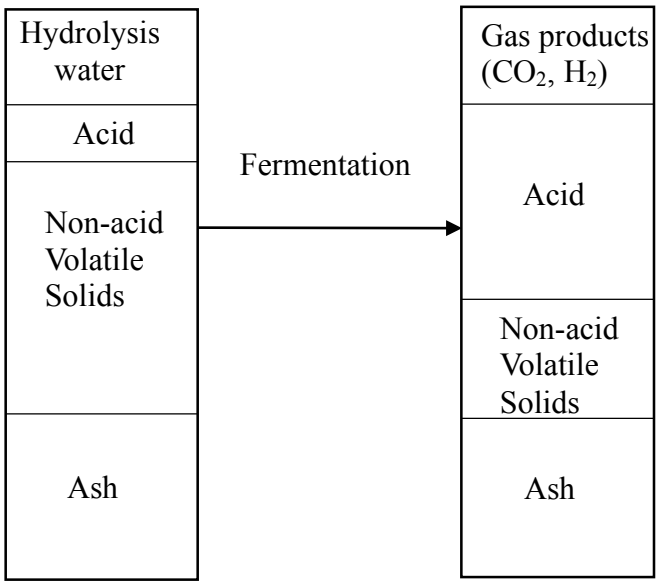

Figure 2.4 Biomass conversion.

2.4 Slope method

When performing calculations for semi-continuous fermentations, three methods were tested: average method, accumulation method, and slope method. [5] According to the research of Smith [5], the slope method has minimal error (less than 2%). This method is used to calculate fermentation parameters like yield, conversion, and selectivity.

The detailed steps of the slope method are shown in Appendix E.

CHAPTER III

INVESTIGATION OF LIQUID RESIDENCE TIME ON COUNTERCURRENT FERMENTATION

3.1 Introduction

Liquid residence time (LRT) is an important parameter that affects product concentration, conversion, yield, and selectivity. It is usually controlled by the transfer frequency (T); however, longer frequency can potentially negatively affect the system because acid production will acidify the solution. Low pH would inhibit the growth of bacteria, which are most prolific at neutral pH (~7). To overcome this potential problem, frequent addition of buffer ($MgCO_3$) is required.

3.2 Experimental design

3.2.1 Hypothesis

To increase the liquid residence time, a longer liquid maintenance target was set for each bottle. The hypothesis is that with liquid contacting the biomass for a longer time, a higher product concentration will be reached. Higher concentrations will be easier to separate and purify. The key question is whether the yield would decrease significantly because of greater product inhibition.

3.2.2 Operation

During countercurrent fermentation, solids are transferred from F1 to F4 while liquid is transferred from F4 to F1.

The traditional countercurrent fermentation progress is described in Appendix B. This experiment has the following differences:

After centrifuging, wet solid and liquid were separated (Figure 3.1). Liquid in F1 was moved to Beaker 1 (B1), F2 to B2, F3 to B3, and F4 to B4. After calculating how much liquid should be moved, some amount of liquid was removed from B4 to B8, B3 to B7, B2 to B6, and B1 to B5. Then the liquid was moved from B8 to B3, B7 to B2, and B6 to B1. The liquid in B5 is the liquid product whereas 60 mL of DI water was added to B8 as the fresh liquid. Before pouring liquid back, the pH of B1, B2, B3, and B4 was neutralized to 6.8–7.2.

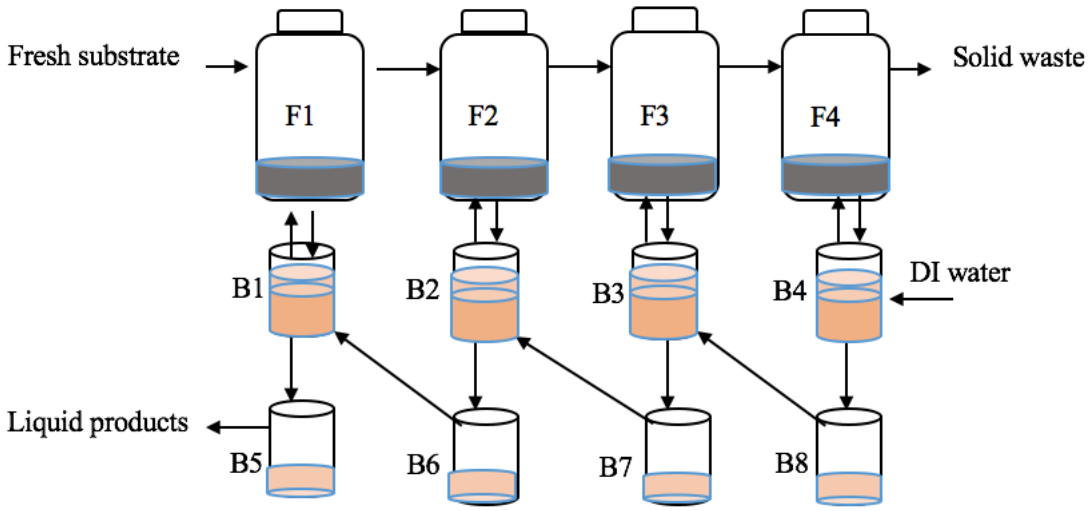

Figure 3.1 Four-stage countercurrent fermentation process with liquid target.

3.2.3 C/N ratio and pH

Aiming at reach the highest productivity, conversion, and culture yield, an optimal C/N ratio of around 25–35 g C/g N is employed. [5]

To keep the C/N ratio in the right range, urea was selected as the nitrogen source. Chicken manure contains 28.2% carbon and 2.20% nitrogen (data from Feather Crest Farms, Inc. Bryan, TX).

Table 3.1 Initial C/N ratio

Mass of dry paper (g)	Mass of dry chicken manure (g)	C in paper (%)	C in chicken manure (%)	N in chicken manure (%)	Initial C/N ratio
32	8	36.0	28.2	2.2	78.3

In contrast, paper contains 36% of carbon. Table 3.1 shows the initial C/N ratio. After calculation, 0.8 g of urea is added during each transfer to reach the optimum C/N ratio (Table 3.2).

The pH was maintained neutral (6.8–7.2). When the pH was lower than 6.8, magnesium carbonate ($MgCO_3$) was added. When the pH was higher than 7.2, hydrochloric acid was added. To ensure that salts did not accumulate in the fermentors, only rarely was HCl added. If a large amount of hydrochloric acid is added, $MgCl_2$ will be produced, which makes the fermentors become salty. This influence will be discussed later.

Table 3.2 Final C/N ratio after adding urea

Mass of urea (g)	C in Urea (g)	N in urea (g)	Total C (g)	Total N (g)	Final C/N ratio
0.8	0.16	0.368	13.936	0.544	25.62

3.3 Results and discussions

The volatile solid loading rate (VSLR) is an important parameter. Previous research shows that 1.5–5.2 g VS/(L·d) achieves adequate conversion and yield.

According to Table 3.3, the acid concentration in this work is the highest among all the countercurrent fermentations previously reported in the literature. Also, conversion is comparably very high. For all the input parameters, the obvious difference is that this work used a much higher liquid residence time.

Table 3.3 Comparison of acid productivity, concentration, LRT, VSLR, transfer frequency, substrate feed rate, chicken manure feed rate, liquid transfer rate, conversion, yield, materials, and pH among different countercurrent fermentation groups

	Douglas [5]	Ross [10]	Domke [13]	Aiello [14]	Golub [8]	Darvekar [15]	Yang
Acid productivity (g/(L·d))	0.211	1.17	0.8	0.835	0.799	0.16	0.386
Acid concentration (g/L)	27	17.2	20.2	19.5	22.1	14.19	34.32
LRT (days)	171	14.7	25	24	29.6	16	88.9
VSLR (g VS/ (L·d))	1.6	N	2	1.9	5.2	1.875	3
Transfer frequency (h)	48	48	72	72	72	48	48
Substrate feed rate (dry /T)	100 lb	20 g	10.5 g	10.7 g	16.7 g	4.8 g	4.8 g
Chicken manure feed rate (dry /T)	25 lb	4 g			16.7 g	1.2 g	1.2 g
Liquid transfer rate	50 gallons	200 mL	150 mL	200 mL	300mL	60 mL	60 mL
Conversion	0.521	N	0.5	0.871	0.454	0.49	0.642
Yield	0.104	0.17	0.39	0.419	0.179	0.285	0.112
Substrate	paper	MSW/ SS	paper	Paper	Paper	OLP corn stover	paper
pH	6.2–6.4	6–6.2	5.8	5.8	6.8–7.2	6.8–7.2	6.8–7.2

Note: N stands for “not reported”; T stands for transfer.

Compared with Golub, longer liquid residence time should result in higher acid concentration, which improves downstream separation and purification. [8]

With long liquid residence time, the acid product composition shows obvious selectivity to butyric acid (Figure 3.2).

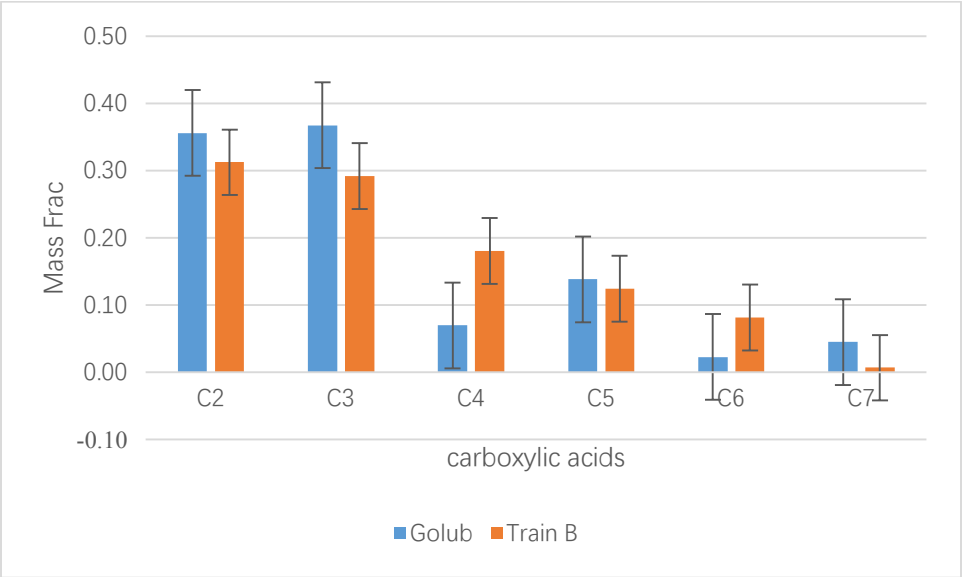

Figure 3.2 Carboxylic acid composition profile.

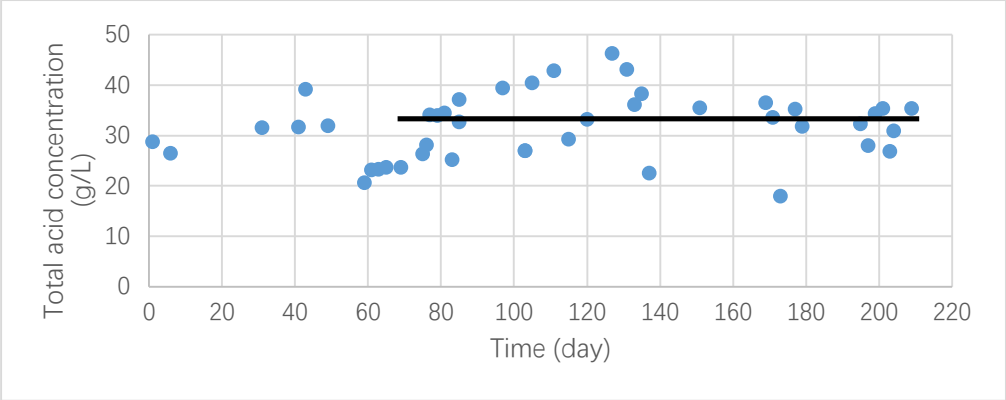

Figure 3.3 Total carboxylic acid concentrations of Train B.

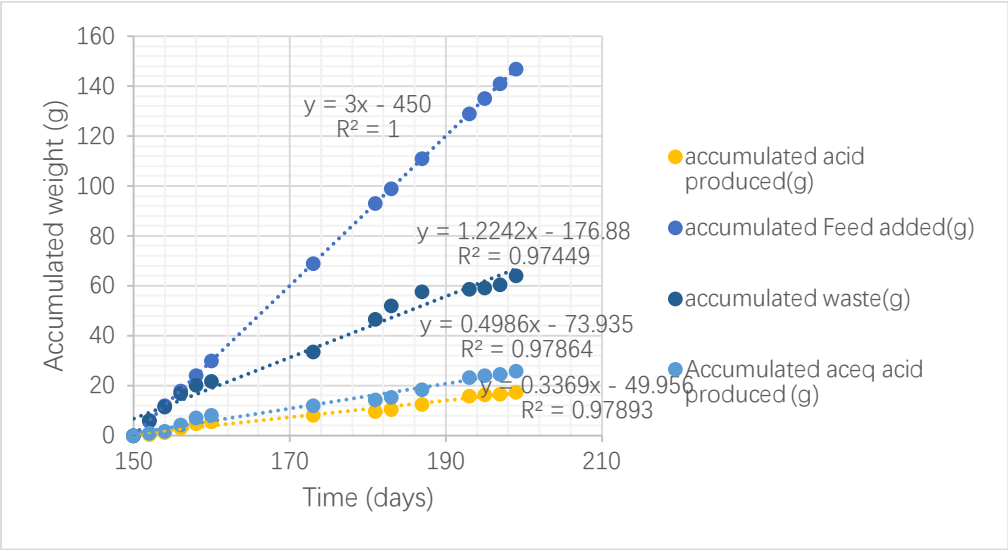

Figure 3.4 Slope method under Train B at steady state.

According to the slope method, the yield can be calculated as follows:

$$\text{Yield} = \frac{0.3369\ \text{g acid product/d}}{3\ \text{g feed}_{\text{feed}}/\text{d}} = 0.112$$

$$\text{Yield(aceq)} = \frac{0.3369\ \text{g acid product/d}}{3\ \text{g feed}_{\text{feed}}/\text{d}} = 0.166$$

The conversion can be calculated as follows:

$$\text{Conversion} = \frac{3\ \text{g non–acid volatile solids}_{\text{feed}}/\text{d}\ - 1.224\ \text{g dry solid waste/d}}{3\ \text{g non–acid volatile solids}_{\text{feed}}/\text{d}}$$

$$= 0.642$$

$$\text{Selectivity} = \frac{0.112}{0.642} = 0.175$$

$$\text{Selectivity (aceq)} = \frac{0.166}{0.642} = 0.259$$

3.4 Conclusion

To extend the liquid residence time, the liquid maintenance target was set to increase the total liquid volume of the system. The average liquid residence time reached 88.9 days compared with the traditional 20–30 days. The results show that the concentration of liquid product is 34.3 g/L, the conversion is 64.2%, yield is 11.2%, aceq yield is 16.6%, total acid selectivity is 18.4%, and aceq selectivity is 25.9%. Also, this countercurrent system shown comparably high selectivity toward butyric acid.

CHAPTER IV

EFFECT OF EXTRACTION USING ION-EXCHANGE RESIN

4.1 Overview

Ion-exchange resins have a matrix framework that consists of macromolecular, irregular and three-dimensional hydrocarbon network chains (Figure 4.1). For cation resins, the matrix carries ionic groups like SO_3^- ,COO^-, PO_3^{2-}, AsO_3^{2-}. For anion resins, the matrix carries ionic groups like NH_3^+, $=NH_2^+$, $=N^+=$, $=S^+-$ and so on.

The matrix is hydrophobic, but incorporates ionic groups like HSO_3^- that are hydrophilic. The insoluble resins are connected by crosslinks that connect different hydrocarbon chains. Resins are a single macromolecule. Because the matrix is elastic, resins swell by taking up solvent. [16]

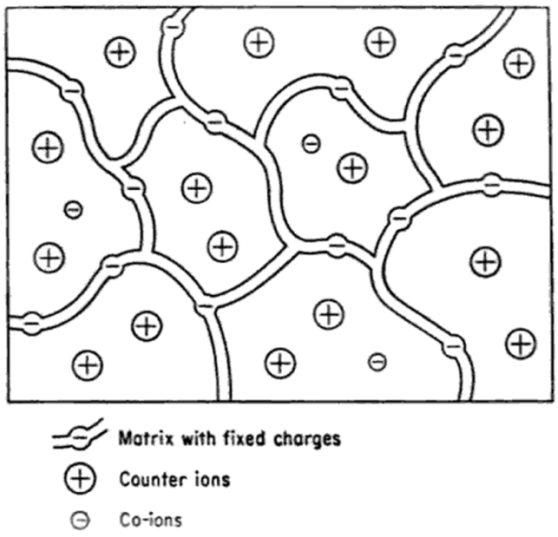

Figure 4.1 Structure of an ion-exchange resin. [16]

Table 4.1 Main types of ion-exchange resins

resins	Functional groups
Strongly acidic resin	Sulfonic acid groups
Strongly basic resin	Quaternary amino groups
Weakly acidic resin	Carboxylic acid groups
Weakly basic resin	Primary, secondary, and/or tertiary amino groups

Table 4.1 shows there are four types of resins based on their functional groups. Because carboxylic acids are the target products, weakly basic resins are the best choice. According to Roy [17], Amberlite IRA-67 is a preferred choice for the MixAlco process.

Weak-base resins have a high capacity for absorbing strong acids and can be regenerated with caustic. Strong-base resins have affinity for weak acids, but the regeneration efficiency is lower. Weak-acid resins have a high affinity to hydrogen ion and are regenerated with strong acids. Strong-acid resins are used for exchange cations or split neutral salts. [18]

The principle of anion exchange resin can be explained by acid adsorption mechanism [19]

$$\mathrm{RNH_2 + HX = RNH_3X}$$

or by the exchange mechanism. [20]

$$\mathrm{RNH_3^+ + OH^- + X^- = RNH_3X + OH^-}$$

$$\mathrm{OH^- + H^+ = H_2O}$$

For regeneration, strong alkalis (e.g., caustic soda (NaOH) or lime ($Ca(OH)_2$)) can supply OH^- ions to replace the carboxylate and neutralize the H^+:

$$RNH_3\ X + OH^- = RNH_2 + H_2O + X^-$$

Table 4.2 Properties of Amberlite IRA-67

Characteristics	Properties
Form	Translucent white spherical beads
Ionic form as shipped	Free base (FB)
Capacity (eq./L)	1.6 (FB form)
Moisture holding capacity (%)	56–64 (FB form)
Particle size (mm)	0.500–0.750
Functional group	Tertiary amine
Shipping weight (g/L)	700

Amberlite IRA-67 is a weak-base gel-type acrylic matrix. According to Roy [17], periodic extraction of carboxylate salts in batch fermentations increase production using Amberlite IRA-67 resin. Table 4.2 shows the properties of this resin. Ion-exchange resin improves microbial activity by removing product and reducing product inhibition. [21]

4.2 Experimental method

4.2.1 Acid adsorption capability

According to the product data sheet of Rohm and Haas (Table 4.2), [6] the total adsorption capability is about 1.6 eq/L (FB form), which could be calculated through the following formula:

$$\text{Acid adsorption capacity}_{\text{FB}} = \frac{\text{Acid adsorbed (g)}}{\text{Resin mass}_{\text{FB}}\text{(g)}}$$

The units are transformed to eq./g resin using the resin density of 700 g/L,

$$\frac{1.6\ \text{eq.}}{\text{L}} \times \frac{\text{L}}{700\ \text{g}} = 0.00228\ \frac{\text{eq.}}{\text{g resin}}$$

The fermentation adds 6 g of fresh dry biomass every 2 days. According to Figure 4.2, the yield of acids could be 0.37 g acids/g biomass. Countercurrent fermentation system has proven to be more effective than batch system [8], thus product yield could be 0.4 g acids /g biomass. Thus, acid production can be estimated as followed:

$$\frac{6\ \text{g biomass}}{2\ \text{days}} \times \frac{0.4\ \text{g acids}}{\text{g biomass}} = \frac{2.4\ \text{g acid}}{2\ \text{days}}$$

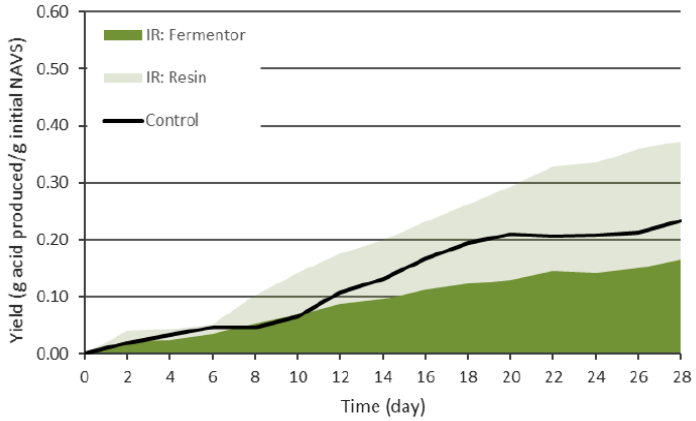

Figure 4.2 Yield of resin group compared with control group in batch system. [17]

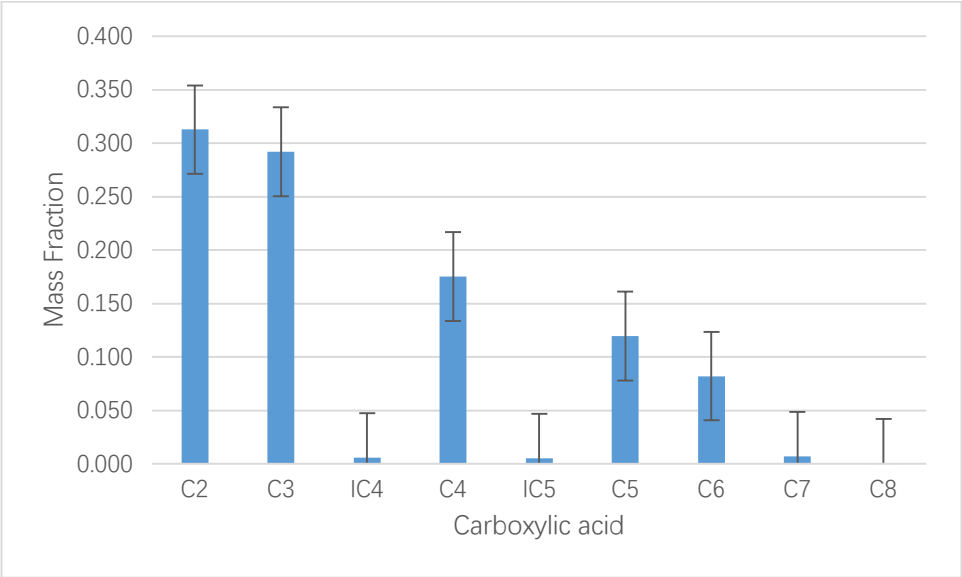

Figure 4.3 Carboxylic acid product distribution. [17]

Figure 4.3 shows the carboxylic acid composition. Based on the molecular weight of each acid, the average molecular weight of acid products is about 82.55 g/L; thus, an equivalent can be calculated as follows:

$$\frac{2.4\text{ g acid}}{2\text{ days}} \times \frac{\text{mol acid}}{82.55\text{ g}} \times \frac{1\text{ eq.}}{1\text{ mol}} = 0.029\ \frac{\text{eq.}}{2\text{ days}}$$

the following formula specifies x expressed as g/2 days.

$$\frac{0.04\ \frac{\text{eq.}}{2\text{ days}}}{x\text{ g resin}/2\text{ days}} = 0.00228\ \frac{\text{eq.}}{\text{g resin}}$$

Thus $x = 12.7$ g, and the minimum amount of resin is 12.7 g.

By adsorbing carboxylic acids, the liquid pH raises from 7 to 10. The adsorption capability decreases at high pH. According to Figure 4.4, because the liquid sample concentration varies from 20 to 30 g/L, thus the adsorption capability would be 10–20%. To adsorb enough acids, 80 g resin is used.

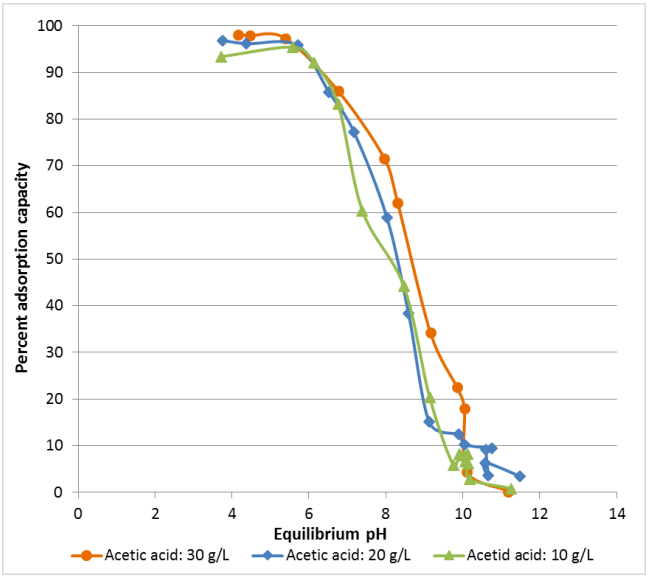

Figure 4.4 Percent adsorption capacities achieved for acetic acid. [17]

4.2.2 Ion-exchange resin operation

In a four-stage countercurrent fermentation system, the liquid transferred between F4 and F3, F3 and F2, F2 and F1, F1 and the liquid product storage bottle is passed through resin column (Figure 4.5). The liquid samples are taken from both the entrance and the exit of the resin column to quantify the adsorption capability. The IRA-67 resin actually used is 81.4 g (FB).

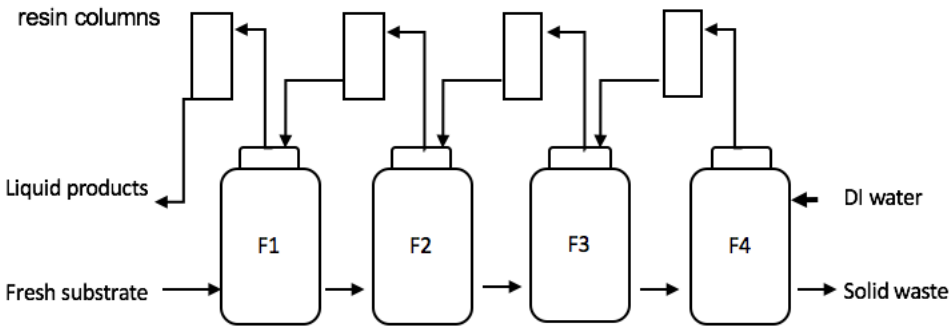

Figure 4.5 Four-stage countercurrent fermentation with resin column.

To collect practical data for industrial use, adsorption of acids from the liquid must reach equilibrium. Trial experiments were performed to see how much time is required to reach equilibrium. It has been proved that 1 min is enough to reach the equilibrium (Figure 4.6).

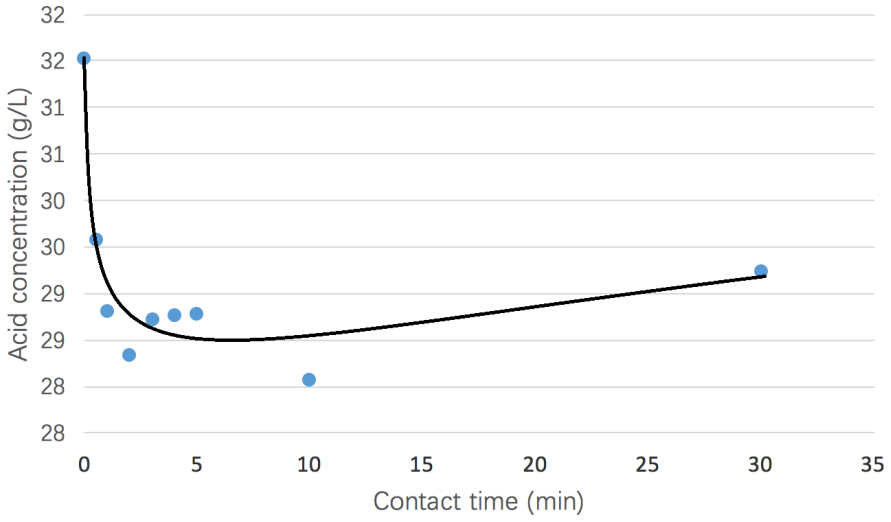

Figure 4.6 Equilibrium time experiment.

4.2.3 Regeneration

After adsorption, the ion-exchange resin can be returned to a fresh state by regeneration. Strong alkalis (1-M NaOH solution) was used to supply OH^- and replace the acetate ions on the resins. The quantity of caustic soda used is 120% to 140% of the operating capacity.

To explore how long it takes to reach equilibrium, an experiment was done (Figure 4.7). Detailed experimental steps are shown in Appendix G. According to the results, 1 min is enough to reach equilibrium.

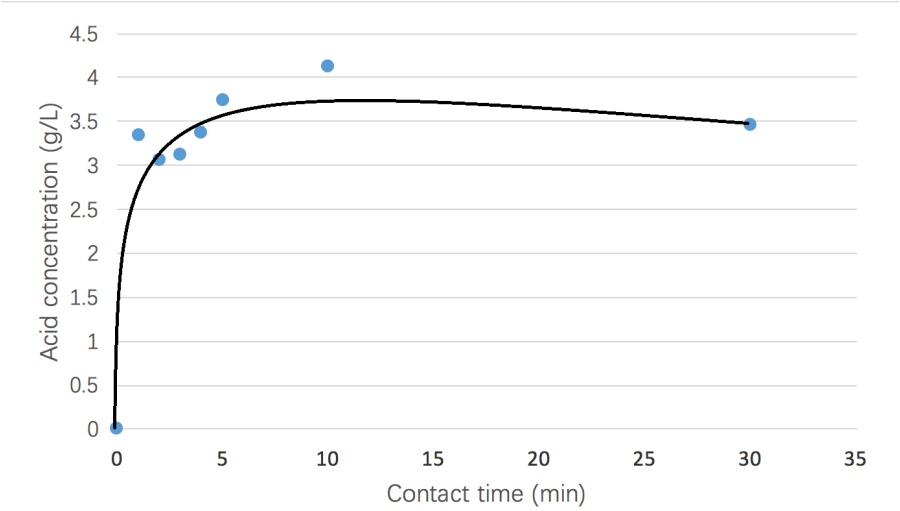

Figure 4.7 Equilibrium time experiment.

Table 4.3 shows that in addition to the acid adsorbed by resin, some liquid also remains among the pores of resin. It has great impact, so the remaining liquid is part of the acid extracted from the system. Also, the data show that most of the product (98.8%) can be extracted from the resin.

Table 4.3 Resin adsorption and regeneration

	Resin	Regeneration (NaOH)
Original concentration (g/L)	31.52	0
Equilibrium concentration (g/L)	28.67	3.46
Initial volume (L)	0.240	0.2
Volume after extraction (L)	0.215	N/A
Absorbed acid (g)	0.68	1.38
Acid in remain liquid (g)	0.72	
Recovery (%)	98.8	

Note: The NaOH has been neutralized and diluted with 1:1 of 1-M hydrochloride acid.

4.3 Results and discussions

4.3.1 Biogas analysis

The resin experiment was run for about 1 month. Initially biogas production increased, and then decreased evenly (Figure 4.8). Because methane inhibitor (iodoform) was added, no methane was detected (Figure 4.9). Only 0.29% of oxygen remained, which is lower than the 0.4% that anaerobes can tolerate (Table 4.4).

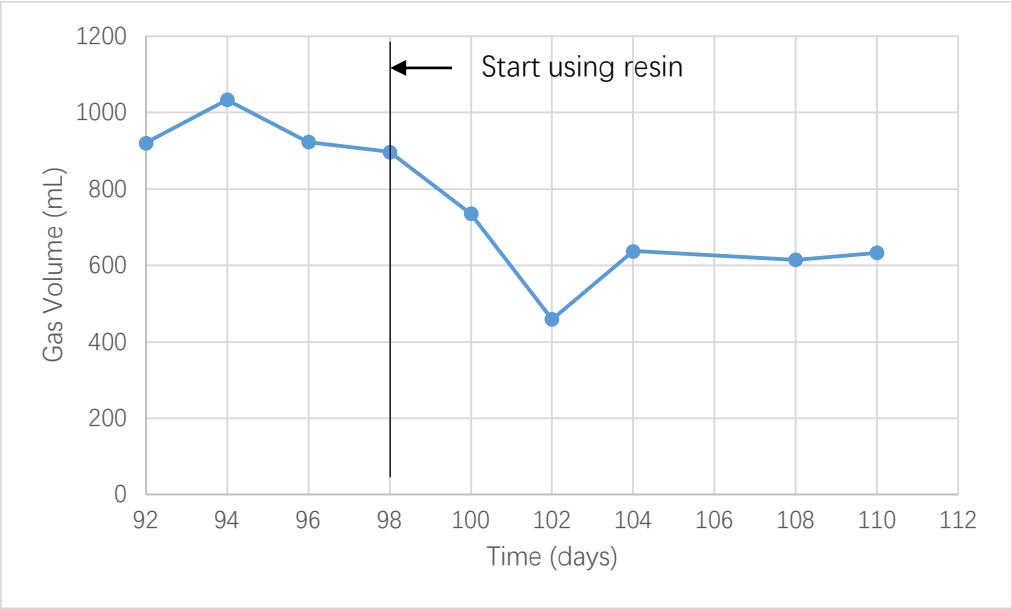

Figure 4.8 Total gas production of Train A.

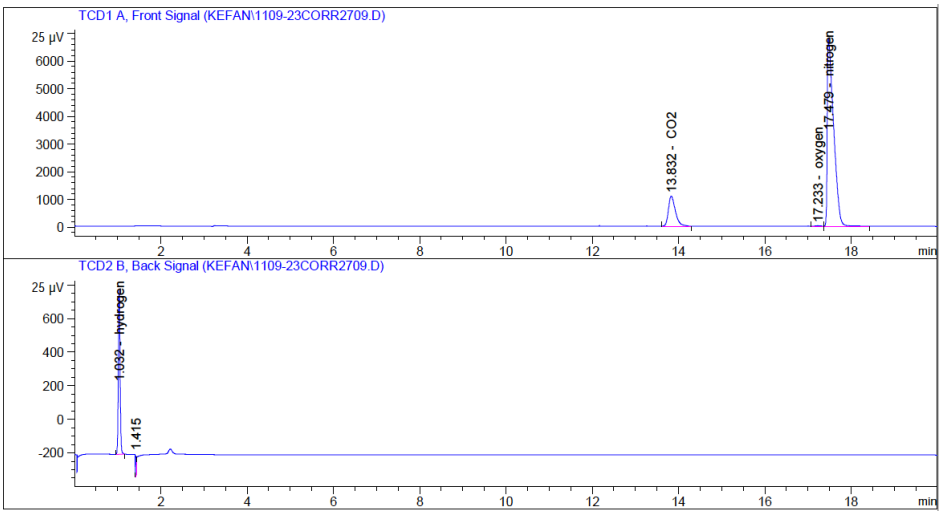

Figure 4.9 Gas chromatography front/back signals for gas product.

Table 4.4 Gas product composition of Train A

Name	CO_2	O_2	N_2	H_2
Composition (volume %)	13.37	0.29	82.54	3.80

4.3.2 Acid concentrations

Using the slope method (Figures 4.10 and 4.13), yield, conversion, and selectivity were calculated (Table 4.5). Compared with the control group, the productivity of medium-chain carboxylic acids increased, which means that the extraction of products helps the chain elongation. However, Figure 4.12 shows that ion-exchange resin has a similar adsorption percentage (average 29%) for short- and medium-chain carboxylic acids, which means the product inhibition of medium-chain carboxylic acids is greater than short-chain carboxylic acids.

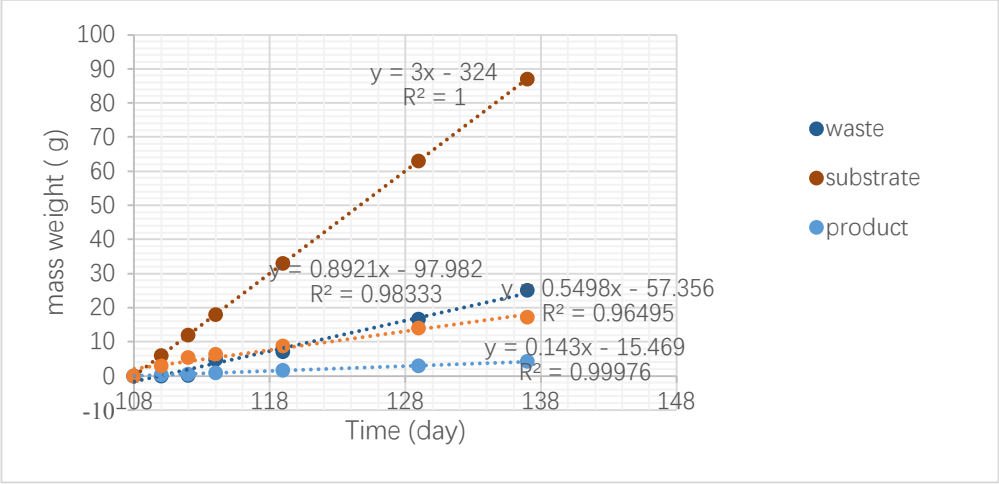

Figure 4.10 Slope method analysis.

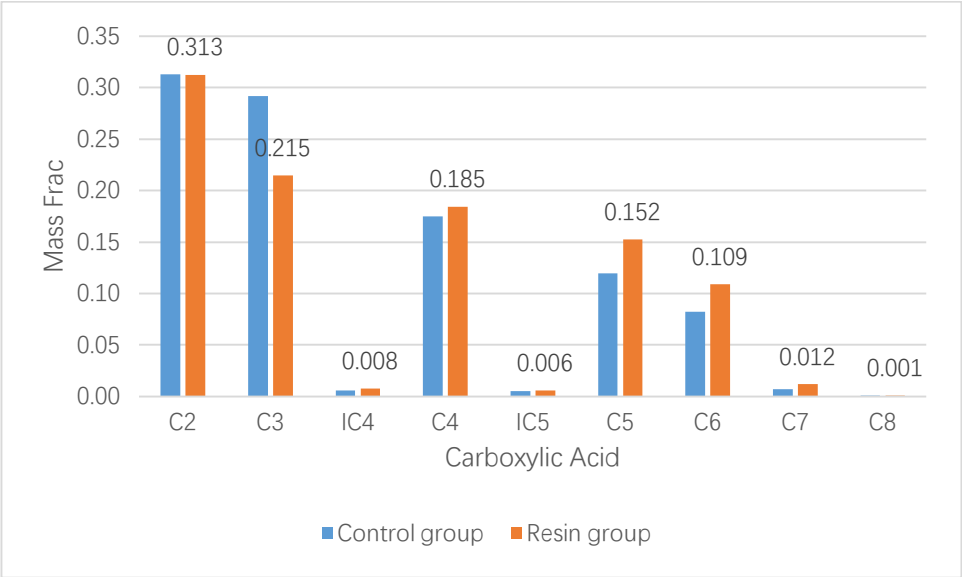

Figure 4.11 Carboxylic acid composition in liquid products compared with control group.

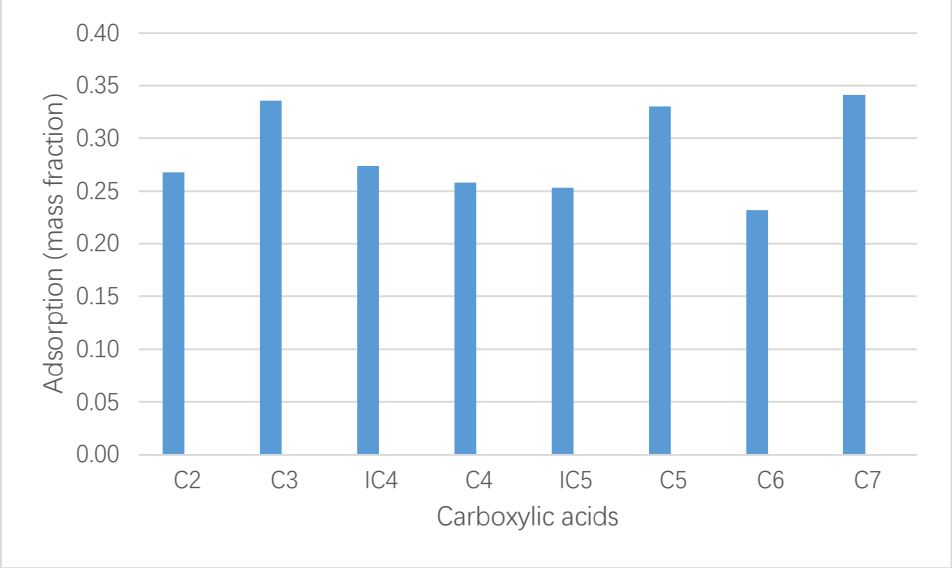

Figure 4.12 Adsorption percentage for countercurrent acid products.

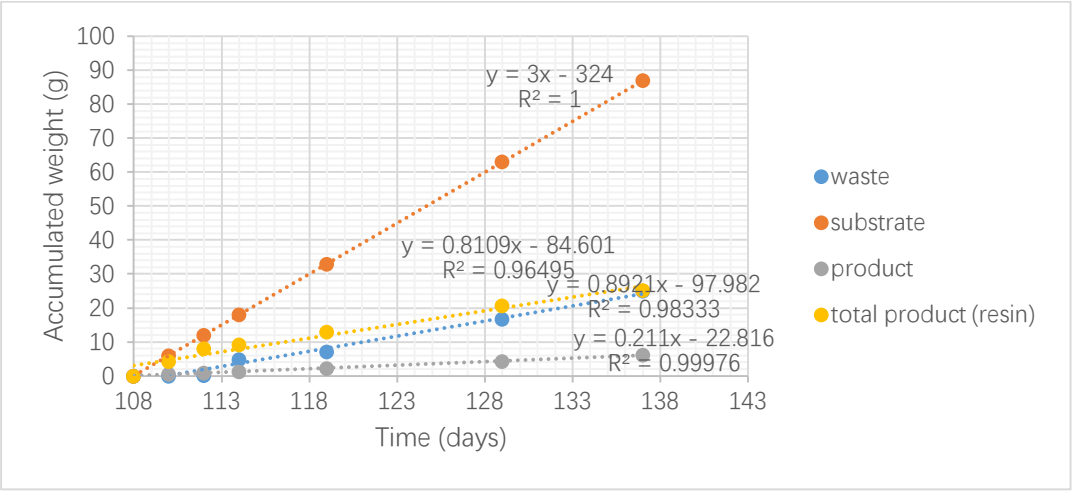

Figure 4.13 Slope method analysis (aceq).

Table 4.5 Comparison of total acid products, conversion, yield, and selectivity between batch group and countercurrent with/without resin

	Control	Resin
Total acid in 30 days (g)	10.08	14.85
Conversion (g NAVS digested/g NAVS fed)	0.622	0.703
Yield liquid only (g total carboxylic acid produced/ g NAVS fed)	0.112	0.049
Yield (g total carboxylic acid produced/ g NAVS fed)	—	0.165
Aceq yield liquid only (g total carboxylic acid produced/ g NAVS fed)	0.166	0.070
Aceq yield with resin (g total carboxylic acid produced/ g NAVS fed)	—	0.270
Selectivity (g total carboxylic acid produced/g NAVS digested)	0.180	0.234
Aceq selectivity (g total carboxylic acid produced/g NAVS digested)	0.259	0.385

4.3.3 Challenges and future work

Several problems have been found during the experiments, including the following:

When regenerating the resin with NaOH, while passing NaOH solution through the filter disk, the column always became plugged. According to J.P. Durham and R.O. Lopez-Solis [21], this blinding is caused by cellular protein that is released upon contact with NaOH. This problem can be solved by flushing strong acids (e.g., hydrochloric acid) through the column. However, strong acids also cause protein denaturation. Thus, the practical solution is to remove the liquid and resin beads, wash the column with strong acids, and then pass the mixed solution through the column again. Liquid and resin will inevitably be lost during this process; thus, a new solution is required. In the future, to retain the ion exchange beads, students should explore the use of a fine-mesh screen rather than a filter.

Although Roy [17] tested regeneration with NaOH, she used new resin beads for her experiment. In contrast, this experiment used old resin beads that were regenerated with NaOH; however, not all acids could be removed. The remaining acid has some effect on the results. Thus, the remain liquid in the resin is defined as “absorbed” and considers the volume remaining in the resin.

4.4 Conclusion

Using ion-exchange resin, the average concentration of liquid product is 28.4 g/L which is lower because of the extraction. The conversion is 70.3%, the total acid yield (including resin extraction) is 16.5%, aceq total acid yield is 27.0%, selectivity is 23.4%, and aceq selectivity is 38.5%. The data show that the conversion has increased a little, the yield was enhanced, and the selectivity was raised. Extraction enhances selectivity of liquid product, especially medium-chain carboxylic acids (C5–C7), which means using resin promotes chain-elongation secondary fermentation. The explanation might be the reduced because of product inhibition.

CHAPTER V

CHAIN ELONGATION

5.1 Introduction

In mixed-culture anaerobic environment, there are several microbial pathways that elongate carbon chains, such as homoacetogenesis, succinate formation, and reverse beta oxidation (RBO). [22]

Normally, biomass is converted to mainly C_2–C_4 short-chain fatty acids (SCFA), H_2, and CO_2. Through reverse beta oxidation (RBO), SCFA can be converted to C_5–C_8 medium-chain fatty acids (MCFA).

Many bacteria have enzymes to catalyze the RBO pathway; *Clostridium kluyveri* is the best-known microbe. It performs chain-elongation reactions similar to the following reaction: [22]

$$\text{Ethanol} + n\text{–butyrate}^- \rightarrow n\text{–caproate}^- + H_2O$$

Clostridium kluyveri requires carbon dioxide for its metabolism. [23]

To improve the productivity of MCFA, it is proposed to create a co-culture of microorganisms with *Clostridium kluyveri* or cultivate a mixed culture that contains bacteria related to *Clostridium kluyveri.*

The MixAlco process has many options that include are several pathways from cellulose to mixed acids. The organic substrates are digested to short-chain carboxylates (acetate, propionate, lactate, and *n*-butyrate) through primary fermentation. Then, the

short-chain carboxylates will be used as substrates for secondary fermentation to produce medium-chain carboxylates (Figure 5.1). [24]

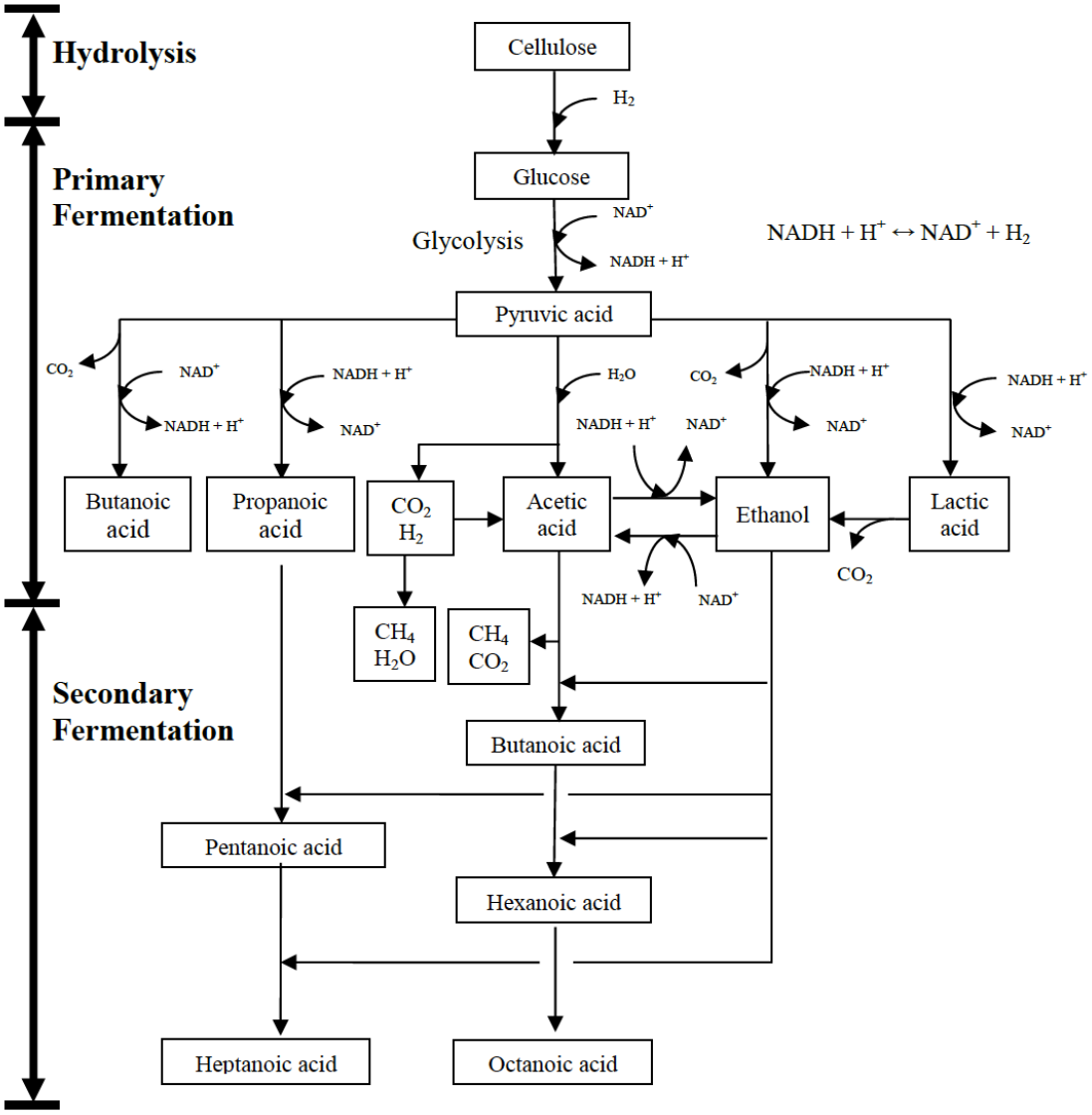

Figure 5.1 Biological pathways in the mixed culture anaerobic fermentation. [9][24]

5.2 Material and methods

5.2.1 Ethanol addition method

To enhance economic value, Sagar Lonkar recommends adding alcohol for chain elongation [9]. Medium-chain fatty acids production is improved by adding 5–10 g/L of ethanol. Thus, one series of experiment was performed to determine if it also works for mixed-culture countercurrent systems.

According to Lonkar's research [9], adding 10 g/L ethanol increases the production of caproic acid. Two steady states have been achieved, so two experiment groups will be performed compared with their own steady state as control groups. Because 5–10 g/L of ethanol is recommended by Lonkar [9], thus same concentration will be added to each bottle (Figures 5.2 and 5.3). Our experimental plans follow:

Add 1 g of ethanol to each bottles of Train B first. When the detected concentration reaches 10 g/L, ethanol addition of each bottle stopped; instead, 0.6 g of ethanol is added to F4 along with 60 mL of DI water.

Add 2 g of ethanol to each bottles of Train C first. When the detected concentration reaches 5 g/L, ethanol addition of each bottle stopped; instead, 0.3 g of ethanol is added to F4 along with 60 mL of DI water.

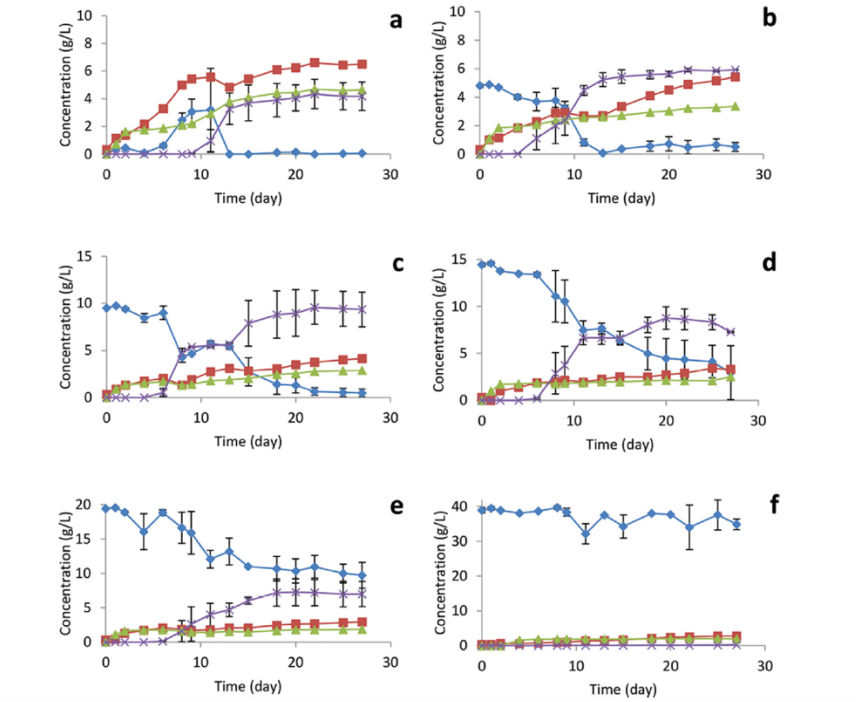

Figure 5.2 Concentration profiles of different amount of ethanol addition. [9]

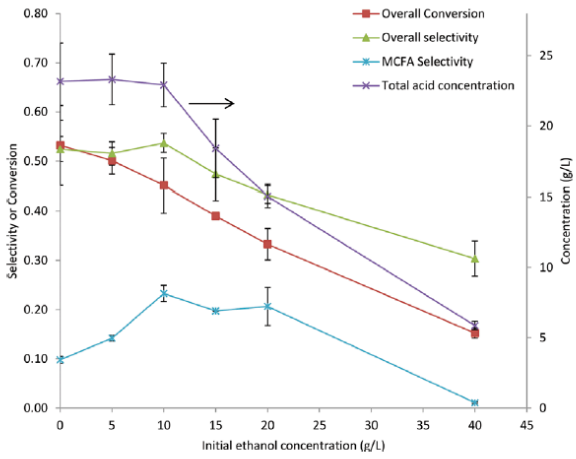

Figure 5.3 Effect of different initial ethanol addition on overall conversion, selectivity, and concentration. [9]

5.3 Results and discussion

Two countercurrent groups (Trains B and C) reached steady state. Two different doses of ethanol were added to each group. To maintain the ethanol concentration in a steady range, some ethanol was added to the fresh DI water. Figures 5.4 and 5.5 show the influence of ethanol to the system. Generally, adding ethanol inhibits the secondary fermentation or chain-elongation reactions. With long contact time, it promotes the primary fermentation, especially the production of acetic acid and butyric acid.

During the Christmas break, when the reactors were kept in the refrigerator (4 ºC) to minimize the action of organisms, the ethanol concentration decreased

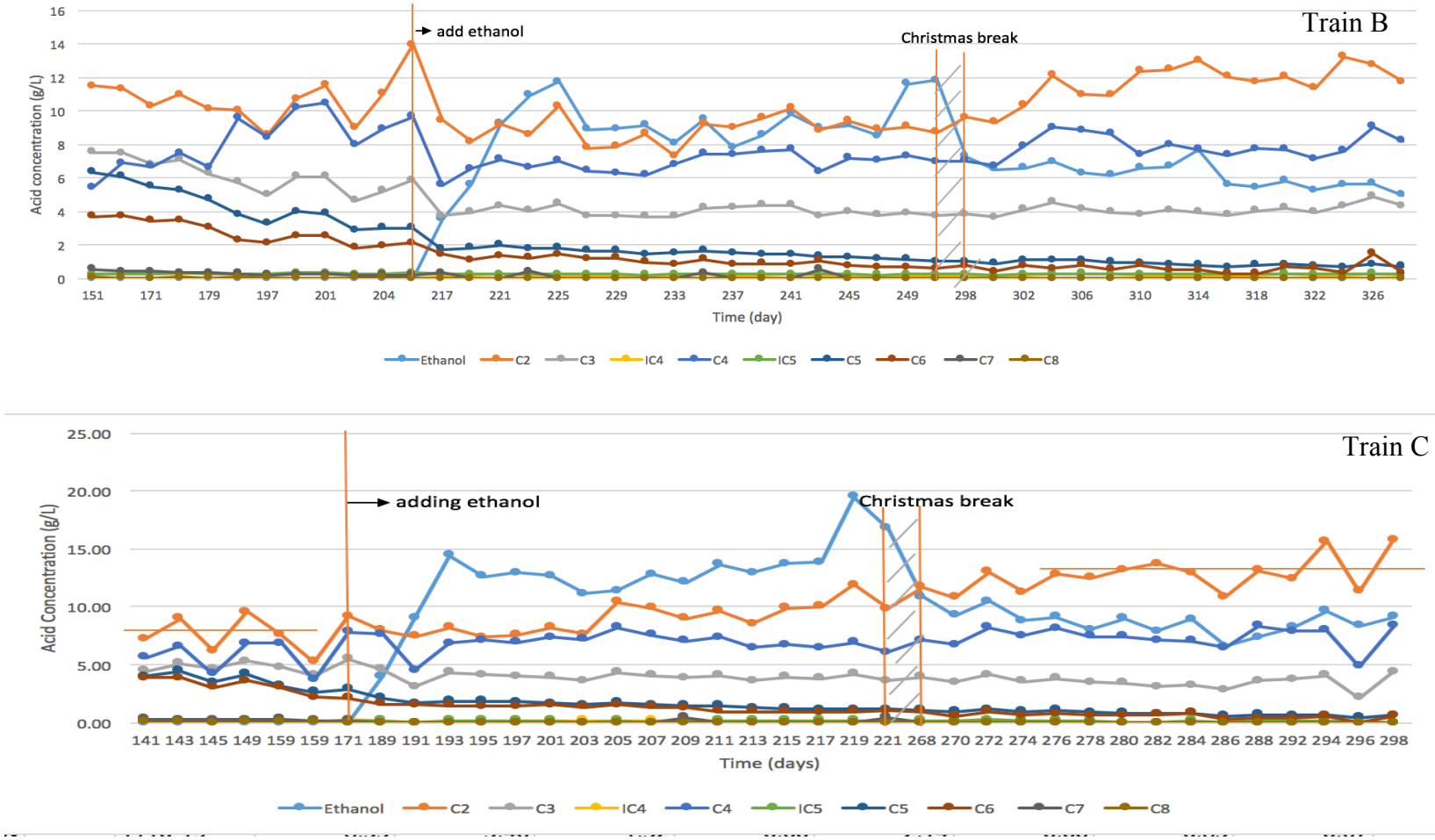

Figure 5.4 Concentration profiles of different additions of ethanol.

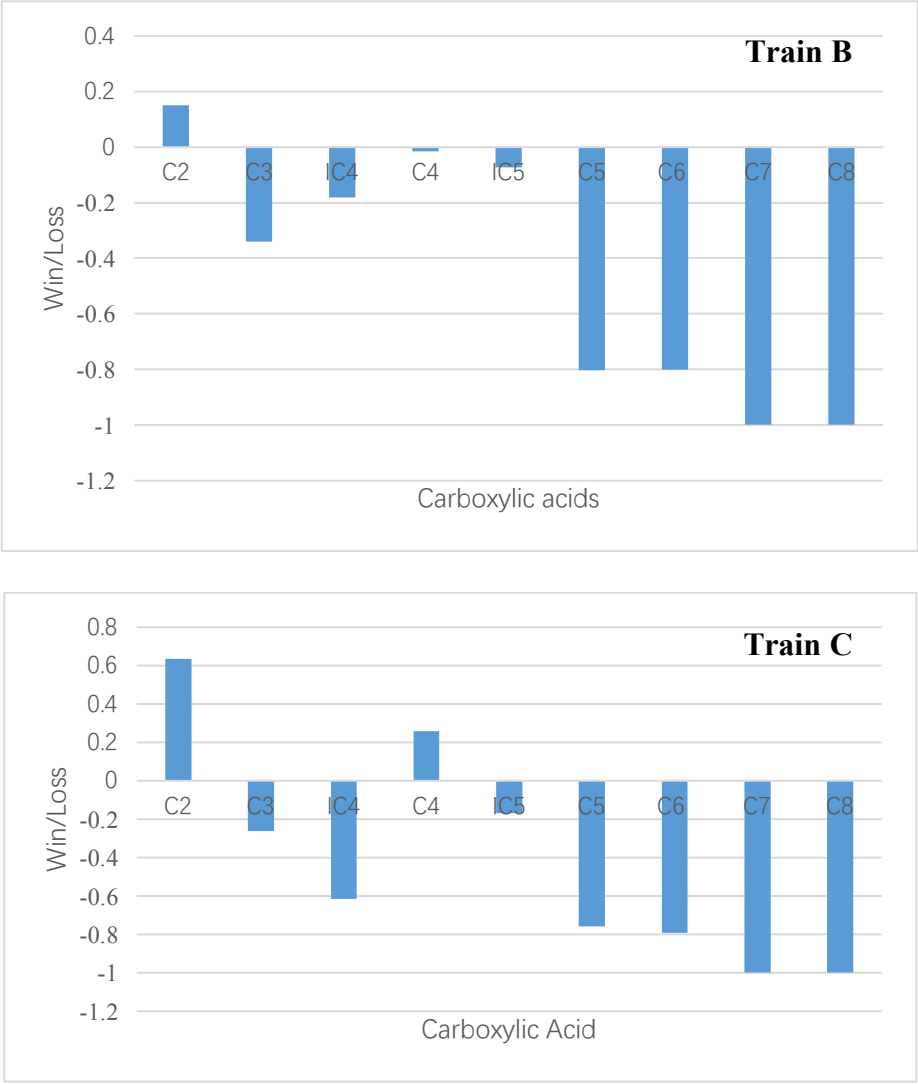

Figure 5.5 Carboxylic acid win/loss between the steady states before and after ethanol addition.

The following are potential reasons why RBO did not occur as in Lonkar's batch fermentations:

(1) Because HCl and $MgCO_3$ were used as buffers to balance the pH, salts ($MgCl_2$) were produced that make the system excessively salty. Chain-elongation microorganisms may not tolerate salty solution.

(2) The population of organisms similar to *Clostridium kluyveri* may not have established in the countercurrent fermentation.

To promote chain elongation, several operations are available:

(1) Add the liquid and wet cake of batch fermentation with ethanol groups into the system to adjust the culture.

(2) Add some fresh marine organisms, to enhance the population of chain-elongation related bacteria.

5.4 Conclusion

According to previous results, adding ethanol can improve chain elongation reactions in batch fermentation system. However, when ethanol was added to continuous countercurrent fermentation, primary fermentation (formation of acetic acid and butyric acid) was enhanced and chain elongation reactions were inhibited.

CHAPTER VI

CONCLUSIONS

The experiments tested the effect of liquid residence time, extraction with ion-exchange resin, and chain elongation.

To extend the liquid residence time, the liquid maintenance target was set to increase the total liquid volume of the system. The average liquid residence time reached 88.9 days compared with the traditional 20–30 days. The results show that the concentration of liquid product is 34.3 g/L, conversion is 64.2%, yield is 11.2%, aceq yield is 16.6%, selectivity is 17.5%, and aceq selectivity is 25.9%. Also, this countercurrent system shows comparably high selectivity to butyric acid.

Ion-exchange resin was incorporated into the established steady-state countercurrent fermentation system described above; all conditions are the same except for the usage of resin extraction. The average concentration of liquid product is 28.4 g/L, which is lower because of the extraction. The conversion is 70.3%, total acid yield (including resin extraction) is 16.5%, aceq total acid yield is 27.0%, selectivity is 23.4%, and aceq selectivity is 38.5%. The conversion, yield, and selectivity have been enhanced. The extraction enhances the selectivity of liquid product, especially the selectivity of medium-chain carboxylic acids (C5−C7), which means extraction using a resin promotes the chain elongation in the secondary fermentation. One explanation is that extraction reduces product inhibition.

In countercurrent system, adding ethanol enhances the primary fermentation (formation of acetic acid and butyric acid) and inhibits the chain-elongation reactions.

These experiment methods may be applied to several conditions. Extending the liquid residence time produces a higher product concentration, which reduces the cost of separation and purification. Resin extraction improves yields and enhances medium-chain carboxylic acids.

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APPENDIX A

DEOXYGENATED WATER PREPARATION

The liquid media used in all fermentation experiments was deoxygenated water with cysteine hydrochloride and sodium sulfide.

1. Fill a large glass container (≥4 L) with distilled water. Place the container over a hot plate to boil.

2. Boil the distilled water for 10 min.

3. Seal the top of the container and cool to room temperature.

4. Add 0.275 g cysteine hydrochloride and 0.275 g sodium sulfide per liter of boiled water.

5. Stir the solution until both chemicals are completely dissolved and pour into storage tank.

APPENDIX B

COUNTERCURRENT TRANSFER PROCEDURE

1. Remove the reactors from the incubator and cool them down at room temperature for 10 min.

2. Release the gas production through vacuum gas release system.

3.Remove the fermentor caps with the help of a nitrogen purge line, blow down the residual solid adhered to the stoppers and metal bars.

4. Cap the fermentor with a regular cap.

5. Balance each fermentor with empty bottles adding water.

6. Centrifuge the fermentor to separate the solid and liquid. Centrifuge for 10 min at 4000 rpm and brake level of 5.

7. After centrifuging, carefully move the bottles to avoid remixing solid and liquid.

8. Place the liquid from F1 into pre-weighted beakers, record the weight.

9. Take 1 mL of liquid from each fermentor for carboxylic acids analysis.

10. Weigh the fermentor bottle with the remaining solids and compare with the target weight. To achieve steady state, a constant wet cake must be maintained in each fermentor. Remove the difference and add to the next fermentor. For F1, fresh biomass should also be taken into calculations.

11. Add fresh biomass to F1.

12. Compare the liquid weight with target liquid weight (200 g, assume that the density is 1 g/mL), remove the difference from beaker of F4 to beaker of F3, beaker of

F3 to beaker of F2, beaker of F2 to beaker of F1, liquid goes out from F1 is the product. Attention, liquid feed in F4 should be considered.

13. Add fresh liquid to the liquid beaker of F4.

14. Balance the pH of F1–F4 liquid beakers at the range of 6.8 to 7.2. If it is higher than 7.2, add hydrochloride acid. If it is lower than 6.8, add magnesium carbonate.

15. Pull the liquid from the beaker to the matched fermentor.

16. Add 0.8 g urea to F4.

17. Add 120 μL iodoform solution to each fermentor.

18. Purge each fermentor with nitrogen and replace fermentor caps.

19. Return fermentors to the incubator.

APPENDIX C

CARBOXYLIC ACID ANALYSIS

For carboxylic acids analysis, at least 3 mL of liquid is sampled from the fermentor, placed in a 15-mL conical centrifuge tube, and stored in the freezer at –10 °C. When analyzed, the samples were defrosted and vortexed. If the acid concentration is high, it may require further dilution before using the method below.

GC LIQUID SAMPLE PREPARATION

1. Centrifuge the liquid sample for 5 min at 4000 rpm.

2. Pipette 0.5 mL of clear liquid broth into a 2.0-mL micro centrifuge tube.

3. Add 0.5 mL of internal standard 4-methyl-valeric acid (1.162 g/L internal standard, ISTD).

4. Add 0.5 mL of 3-M phosphoric acid to convert all salts to acid form.

5. Cap and vortex the tube.

6. Centrifuge the mixture in a micro centrifuge (8000 × g) for 10 min.

7. Remove the tube and decant the mixture into a glass GC vial and cap. The centrifuged sample in the vial is ready to be analyzed now.

8. If the prepared sample will not be analyzed immediately, it can be frozen. Before GC analysis, make sure to thaw and vortex the sample.

GC OPERATION

1. Before starting the GC, check the gas supply cylinders (compressed hydrogen, compressed helium and compressed air from Praxair Co., Bryan, TX) to insure at least 200 psig pressure in each gas cylinder. If there is not enough gas, switch cylinders. Make sure to place an order for new ones.

2. Check the solvent and waste bottles on the injection tower. Fill up solvent vials with methanol. Empty the waste vials in designated waste container.

3. Before starting the GC, replace the septum beneath the injection tower.

4. Up to 150 samples can be loaded in the autosampler tray in one analysis batch. Place the samples in the autosampler racks. Include a vial with the volatile acid standard.

5. Check the setting conditions in the method:

a. Inlet Conditions:

i. Temperature: 230 °C

ii. Pressure: 15 psig

iii. Flow rate: 185 mL/min

b. Detector conditions:

i. Temperature: 230 °C

ii. Air flow rate: 400 mL/min

iii. H_2 flow rate: 40 mL/min

iv. He (makeup) flow rate: 45 mL/min

c. Oven conditions:

i. Initial temperature: 40 °C

ii. Initial hold time: 2 min

iii. Ramp rate: 20 °C/min

iv. Final temperature: 200 °C

v. Final hold time: 1 min

d. Total run time per vial: 11 min

6. Start the GC on the computer by selecting the method with the setting conditions mentioned above. Load the sample sequence.

7. For quality control, run the standard mix every 15–25 samples. At the end of the sequence table, set the GC into standby mode to save gas.

APPENDIX D

MOISTURE AND ASH CONTENT ANALYSIS

This procedure was modified from NREL Standard Procedures (2004). If volatile acids are present in sample, lime may be added to retain all acids for more thorough measurement of moisture content (Meysing, 2011). However, when lime is added, the ash content cannot be measured as directed below. In this case, a separate sample must be dried with no lime addition, and subsequently ashed.

1. Record the label and weight of a clean, dry crucible (W1).

2. Place a representative sample of the material (liquid or solid) into the crucible and record the weight (W2).

3. Dry the crucible at 105 °C for 1 day in the drying oven. In a desiccator, allow to cool to room temperature before weighing. Record the dry weight (W3).

4. Ash the crucible at 575 °C for at least 12 h. Remove and allow to cool to room temperature in a desiccator. Record the ash weight (W4).

5. The moisture content (MC) of the sample is calculated as

$$MC = (W2 – W3)/(W2 – W1)$$

6. The ash content (AC) of the sample is calculated as

$$AC = (W4 – W1)/(W3 – W1)$$

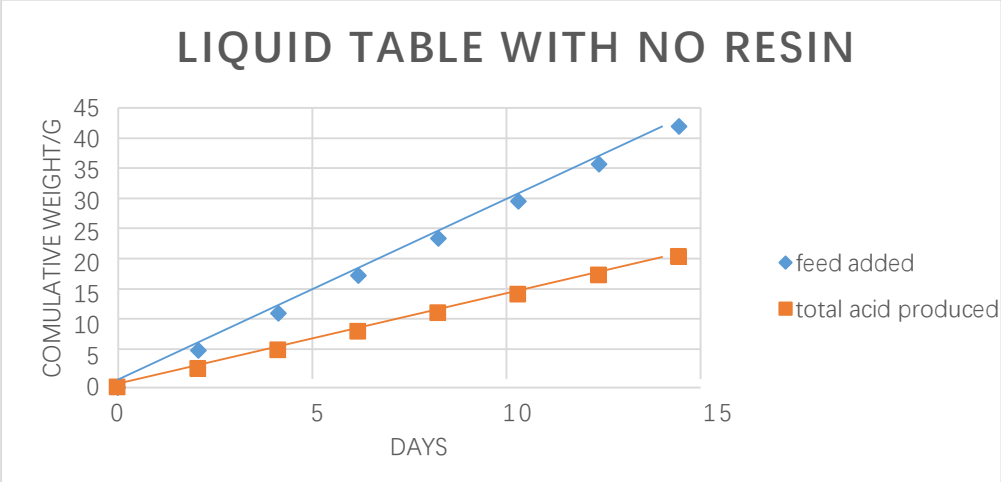
APPENDIX E

SLOPE METHOD

1. Countercurrent Fermentation

Yield

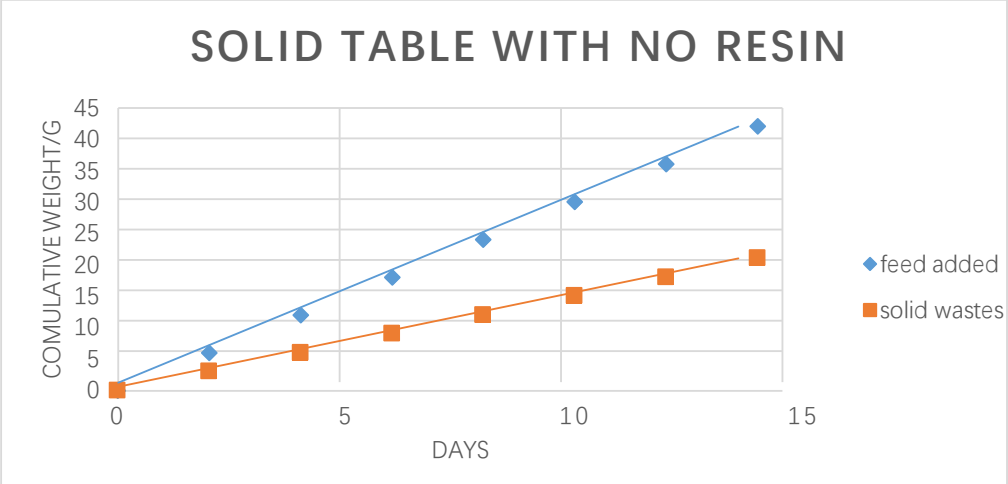
Suppose this figure comes from the liquid product.



Set the slope of feed added as m1, set the slope of total acid produced as m2.

Yield = m2/m1.

Conversion



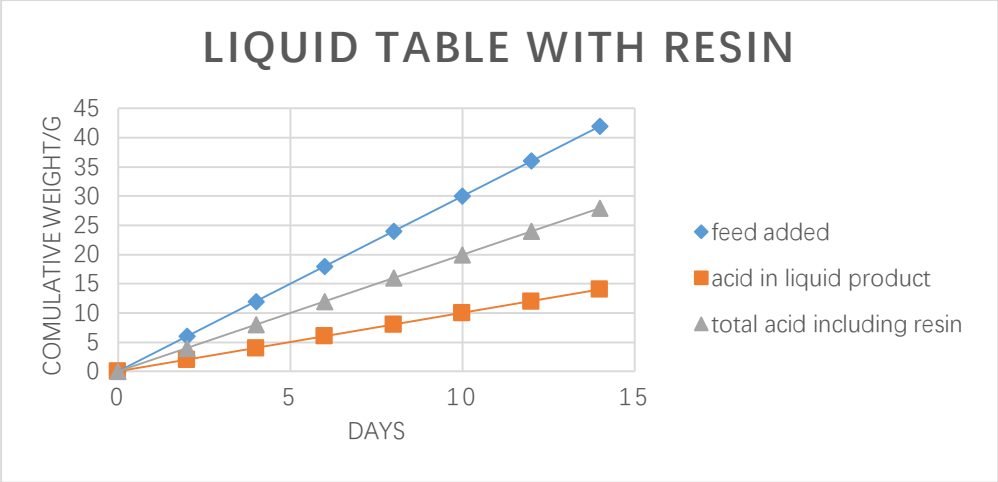
Set the slope of feed added as m1, set the slope of solid wastes as m2.

Conversion = (m1 – m2)/m1;

2. Countercurrent Fermentation with resin

Yield

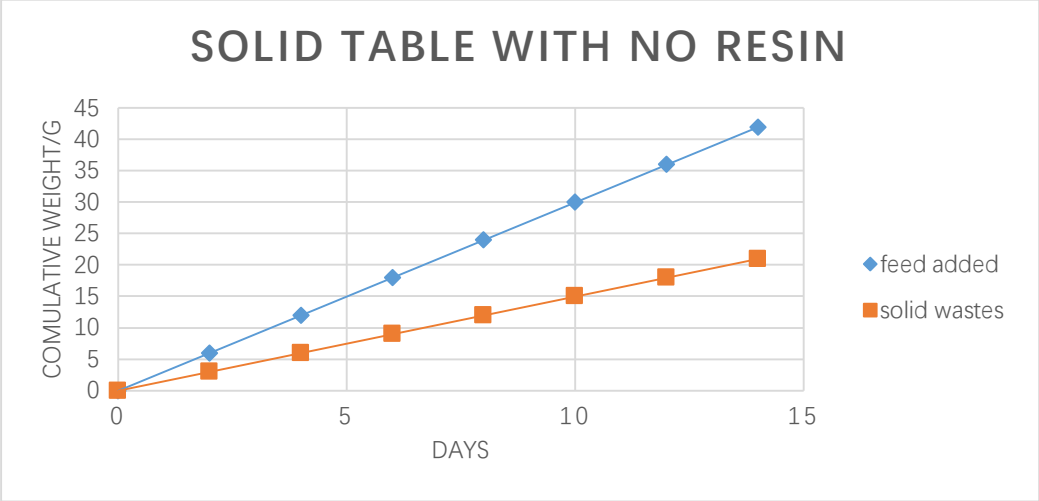
Suppose this figure comes from the liquid product.



Set the slope of feed added as m1, set the slope of acid in liquid as m2, set the slope of total acid including resin as m3.

Yield = m3/m1.

Conversion



Set the slope of feed added as m1, set the slope of solid wastes as m3.

Conversion = (m1 – m2)/m1;

APPENDIX F

CALCULATION OF TOTAL ACID PRODUCTS

Daily acid produced

$$= (V4 + V5) \times C4 + V3 \times C3 + V2 \times C2 + V1 \times C1 + M \times R \times C1$$

Where V1 is the volume of liquid sample from bottle 1 (which produce solid wastes);

V2 = volume of liquid sample from Bottle 2

V3 = volume of liquid sample from Bottle 3

V4 = volume of liquid sample from Bottle 4

V5 = volume of liquid products

C1–C4 are the concentrations of these bottles

M = mass of solid wastes

R = ratio of liquid product/dry solid

APPENDIX G

CONTACT TIME EXPERIMENT FOR RESIN

To test the contact time of resin adsorption and NaOH regeneration, the following experiment has been done:

1. Take 240 mL of previous liquid product. Take 1 mL sample (L0).
2. Take about 80 g of free base resin.
3. Mix resin and liquid product, stir for 30 s, take 1 mL sample (L1).
4. Continue stirring, take samples (L2, L3, L4, L5, L6, L7, L8, L9) when the time comes to 1, 2, 3, 4, 5, 10, and 30 min.
5. Separate liquid and resin with resin column and vacuum system, measure the volume of liquid goes out.
6. Take 200 mL 1-M NaOH solution.
7. Mix adopted resin with NaOH solution, stir for 1 min, take 1-mL sample (N2).
8. Continue stirring, take samples (N3, N4, N5, N6, N7, N8) when the time comes to 2, 3, 4, 5, 10, and 30 min.