EFFECT OF CARBON DIOXIDE-SUSTAINED ADSORPTION USING ION EXCHANGE RESIN ON MIXED-ACID FERMENTATION

A Thesis

by

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ABSTRACT

The carboxylate platform is a biomass-to-energy process that converts biomass into hydrocarbon fuels or chemicals. The mixed-acid fermentation is the essential unit of the process that uses mixed cultures of microorganisms to anaerobically produce carboxylic acids; however, high acid concentrations in fermentation broth inhibit the microorganisms and negatively affect fermentation performance.

This study employed a weak-base anion-exchange resin (Amberlite IRA-67) to recover inhibitory acid products from countercurrent and propagated fixed-bed mixed-acid fermentations. The ion-exchange resins were employed in a novel fluidized bed that was purged with CO_2. Compared with traditional plug-flow ion-exchange adsorption, fluidized-bed CO_2-sustained ion-exchange resin adsorption increased carboxylic acids recoveries by up to a factor of 4.58 times.

Four countercurrent fermentation trains with an average 1.4 L total liquid volume were established under identical conditions. Different amounts of IRA-67 resin (10–40 g wet $resin_{FB}$) were employed to adsorb the acids produced from fermentation trains in the presence of CO_2. The increases of biomass conversion and acid yield were found out to be 34–128% and 45–107%, respectively. The optimal normalized resin loading for biomass conversion was the 10.9 g wet $resin_{FB}$/ (L_{liq}·d).

One train of propagated fixed-bed fermentation with 1.45 L total liquid volume was run under the same conditions as the countercurrent trains. With CO_2, 30 g wet $resin_{FB}$ was employed to adsorb the acids produced from this train, which caused acid yield to increase by 24%.

DEDICATION

To my parents

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Currently, fossil fuels (oil, coal, natural gas) are the primary sources of energy and chemicals for human society. In 2016, world energy consumption was about 13276.3 million tonnes oil equivalent (16.6 km^3), more than 85% of which came from fossil fuels.[1] Undoubtedly, the economy and energy security of all countries are based on fossil fuels. However, fossil fuels are finite and are a carbon reserve that was naturally formed and accumulated through millions of years. Although global proven fossil fuels reserves can still meet global energy production at 2016 levels for more than one century, at some point in the near future, the demand for fossil fuels will outweigh the available supply.[1] Furthermore, global warming is proceeding at a high rate because of increasing usage of fossil fuels, particularly since the mid-20th century.

Compared with fossil fuels, lignocellulosic biofuels are a more promising alternative energy. First, lignocellulosic biomass is abundant and ranks after oil, coal, and natural gas as a source of energy. It is estimated that photosynthesis annually fixes 2×10^{11} t of carbon, containing energy nearly 3×10^{21} J, which is equivalent to 10 times annual worldwide energy consumption.[2] Second, burning biofuels contributes nearly zero net carbon emission to the atmosphere, which helps relieve global warming.

The major components of lignocellulosic biomass are cellulose and hemicellulose polysaccharides, as well as lignin.[3] To convert lignocellulosic biomass into liquid fuels, three platforms have been developed: thermochemical, sugar, and carboxylate.[4] For the thermochemical pathway, biomass is gasified into syngas ($CO + H_2$), and then is catalytically converted into liquids (e.g., methanol, ethanol, or hydrocarbon).[5] Because of partial combustion of biomass, the thermochemical platform has significantly lower yields compared to the latter two biological platforms. Because lignin can only be processed thermochemically, pretreatment methods are required to improve process performance. After pretreatment, for the sugar platform, polysaccharides are first hydrolyzed to sugars using enzyme catalysts, and then are biologically fermented into ethanol and carbon dioxide. However, the sugar platform is costly because it requires sterile fermentation and addition of expensive enzymes. Likewise, for the carboxylate platform (Figure 1-1), polysaccharides are first hydrolyzed to sugars using naturally present enzyme catalysts, and then are biologically fermented into carboxylic acids. The carboxylate platform is an example of consolidated bioprocessing (CBP) where hydrolysis and fermentation are integrated. A mixed culture is employed that does not require sterile operating conditions because the carboxylate salts are the low-energy products, provided methanogens are inhibited. The carboxylate platform is reported to have the highest yield and lowest cost among the three platforms.[4]

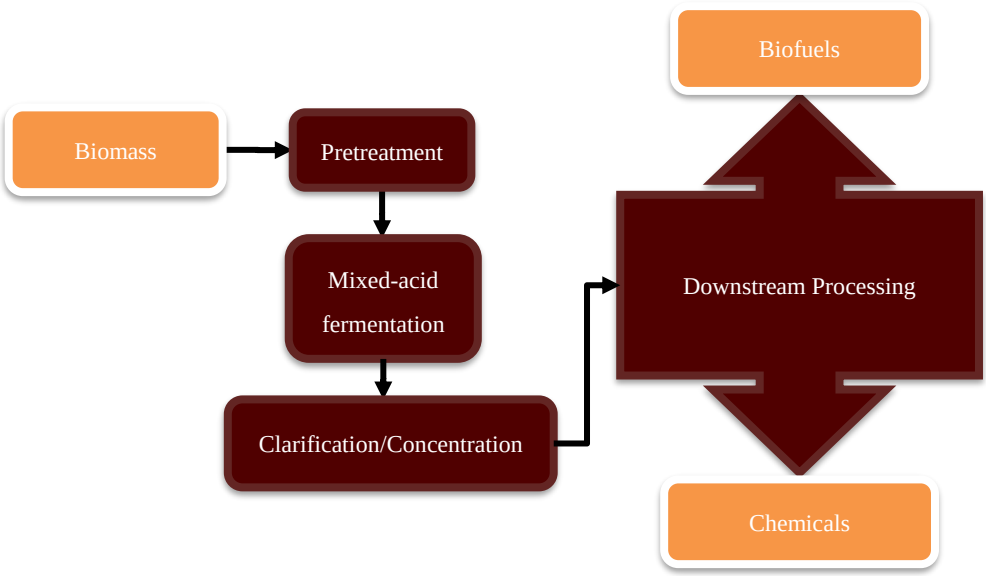


Figure 1-1. Diagram of carboxylate platform.

In industrial practice, sugar or starch crops (e.g., corn, sugarcane) are widely used to produce bioethanol, whereas soybeans and other oil seeds are used to produce biodiesel. Unfortunately, use of these crops as biofuel feedstocks conflicts with their use as food for humans.[6]

The MixAlco process is an example of the carboxylate platform, and it can process both food or non-food lignocellulosic biomass. One version of the process has the following steps: (1) pretreatment with lime, (2) anaerobic mixed-culture methane-inhibited fermentation to obtain carboxylate salts, (3) concentration by dewatering, (4) thermal conversion of salts to ketones, (5) hydrogenation of the ketones to mixed alcohols, (6) oligomerization of alcohols to hydrocarbons.[7] The core of the MixAlco process is the mixed-acid fermentation, which employs a mixed culture of microorganisms to convert biomass components (cellulose, hemicellulose, pectin, fats, and

proteins) into carboxylate salts. Because of this feature, the process has a higher metabolic flexibility than single-culture fermentation system, and it does not require sterile operating conditions. Thus, a wide range of biomass feedstocks from agricultural residues to municipal solid wastes can be used in the MixAlco process. Based on these advantages, the MixAlco process lowers the capital costs and improves operability compared to traditional pathways.

As shown in Figure 1-2, anaerobic digestion occurs in four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.[8,9] Because of microbial syntrophy within the fermentation system, inhibition of one group of microorganisms can result in an inhibition cascade through system, causing digester failure.[10] In the carboxylate platform, iodoform is used to inhibit methanogenesis, which allows carboxylic acids to accumulate and lower the pH. High concentrations of carboxylic acids and low pH negatively affect microorganisms and resulting in low carboxylic acid production rates.[11]

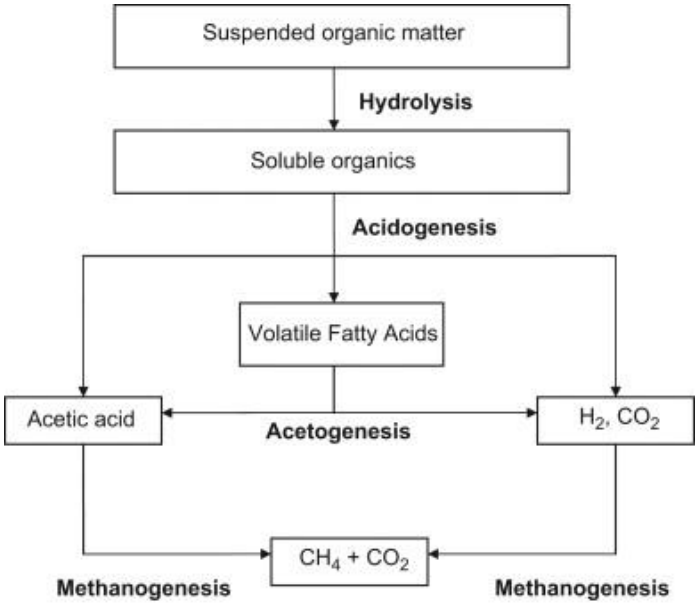


Figure 1-2. Anaerobic digestion process. Reprinted with permission from [8].

To improve fermentation performance, several hypotheses have been proposed and investigated by previous researchers:

(1) Multi-staged countercurrent fermentation (MSCF) was designed to allow solids and liquid to pass in opposite directions through a series of fermentors. Fresh (least-digested) biomass is added into the fermentor with the highest concentration of carboxylic acids and fresh water is added into the fermentor containing the most digested biomass. Because inhibition from accumulated carboxylate salts is minimized, both high conversion of biomass and high product concentration are possible in this configuration.[9,11]

(2) Propagated fixed-bed fermentation (PFBF) is an alternative to countercurrent flow

arrangement where solids are stationary and liquid is transferred. In this pattern, solids and liquid pass in opposite directions through a series of fermentors. Periodically, the fermentor containing fresh biomass is added into the fermentation train. At the same time, the fermentor with the most digested biomass is removed. Fresh water is added to the most-digested fermentor, cascades through fermentor trains, and exits as product liquid from the least-digested fermentor. The advantages of PFBF include minimal solids handling and the potential for high product concentrations and productivities.[9,12]

(3) For mixed-acid fermentation, the selected pH optimizes the chosen fermentation variable and minimizes the production costs. The optimum pH is 5.5–6.5 for acidogens and 7.8–8.2 for the methanogens.[13] For mesophilic methane digestors, optimal pH is 6.7–7.4, and digestors do not function well below 6 or above 8.[14] Because acidogens are significantly less sensitive to low or high pH, the operating pH for mixed-acid fermentation should be controlled to a near-neutral range (6.8–7.2). The desired operating pH of the broth can be achieved either by adding buffer (e.g., calcium or sodium carbonate) or adjusting the C-N ratio of feedstocks.[9]

(4) Extractive fermentation is a type of process intensification in which *in-situ* separation is performed during fermentation to remove products continuously. For mixed-acid fermentation, removing carboxylic acids from fermentors reduces product inhibition and thus increases productivity. Methods for separating carboxylic acids include precipitation, adsorption, liquid-

liquid extraction, electrodialysis, nanofiltration, and reverse osmosis.[15] Many studies of fermentation performance have been conducted to study the effects of product recovery with ion exchange resin. For example, Benjamin[16] shows that lactic acid removal with weak-base ion-exchange resin (Amberlite IRA-67) from homolactic fermentation increases culture productivity by 1.3-fold higher than a control fed-batch process. Also, Roy[17] has proven that using IRA-67 to extract carboxylate anions significantly improves product yield and substrate conversion in mixed-acid batch fermentation.

Based on the former research outcomes, combining mixed-acid fermentation with ion-exchange resin adsorption is a promising pathway to improve fermentation performance. However, while applying resin, fermentation pH would increase dramatically, which limits the acid adsorption ability of resin and negatively affects microorganisms.[17] CO_2-sustained ion-exchange resin adsorption of lactic acid was performed by Husson and King[18], which shows that with the presence of CO_2, acid recoveries are substantially higher than the corresponding recoveries in the absence of CO_2.

In this thesis, the primary goal is to investigate the effects of CO_2-sustained ion-exchange resin adsorption using ion exchange resin on mixed-acid fermentation and provide engineering suggestions to corresponding large-scale applications.

The following is a list of objectives performed to accomplish this primary goal:

- Study the effects of CO_2 on ion-exchange resin (Amberlite IRA-67) uptake capacities for carboxylic acids;
- Design the configuration and operation mode of laboratory-scale adsorption columns;
- Study the effects of ion-exchange resin adsorption on four-stage mixed-acid countercurrent fermentation systems under various resin loadings with the presence of CO_2;
- Determine the optimum resin loading for countercurrent fermentation;
- Study the effects of ion exchange resin adsorption on four-stage propagated fixed-bed fermentation with the presence of CO_2.

CHAPTER II

MATERIALS AND METHODS

2.1 Materials

2.1.1 Substrates

The mixed-acid fermentations were performed using unused office paper, chicken manure, and urea as feedstocks. These substrates served as energy source, nutrient source, and supplemental nitrogen source, respectively.

Unused office paper (Caliber®) was shredded before adding into fermentors using Fellowes Powershred® W-6C. No pretreatment was required. Previous studies found that the content of carbon and nitrogen was approximately 36.3% and 0.07% by weight, respectively.[17]

Fresh chicken manure was collected from the Department of Poultry Science, Texas A&M University (College Station, TX). Because fresh chicken manure would degrade over time, dried and homogenized chicken manure was used to maintain consistency during the entire experiment. Fresh chicken manure was air dried in the oven at 105 °C for 48 h and kept in Ziploc bags at room temperature (~25 °C). Carbon and nitrogen content in chicken manure was 28.2% and 2.2% by weight, respectively. To determine the acids concentrations in the dry chicken manure, the dry

manure was resuspended in distilled water at different concentration for 48 h and the supernatant was analyzed by gas chromatograph (GC).[9] The total carboxylic acids concentration was measured to be 0.0006 ± 0.0005 g acid/g dry chicken manure.

Urea (Fisher Scientific®, Product no. U15-500) was added as a source of supplemental nitrogen to adjust the carbon-nitrogen ratio. To reach the highest conversion, yield, and selectivity, the optimal carbon-nitrogen ratio for the fermentations should be around 25–35 g carbon/g nitrogen.[19]

2.1.2 Fermentor

The fermentor (Figure 2-1) was 1-L polypropylene centrifuge bottle capped by a rubber stopper inserted with a glass tube and two segments of ¼-in stainless steel pipe.[9] These metal bars were used to mix the fermentor contents as it rotated in the rolling incubator (Wheaton®). A rubber septum sealed the glass tube and allowed for gas sampling and release.

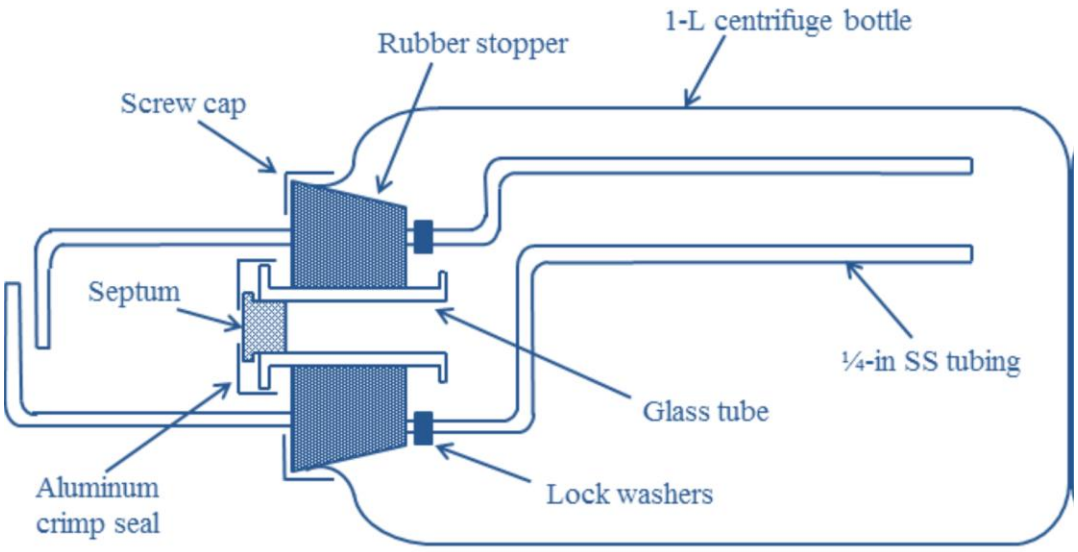


Figure 2-1. Fermentor configuration.

2.1.3 Fermentation media

To prepare deoxygenated water, de-ionized water was boiled to liberate dissolved oxygen. After cooling down, 0.275 g/L cysteine hydrochloride and 0.275 g/L sodium sulfide were added to further reduce the oxygen content.[9] Deoxygenated water was used only in the batch period of fermentation. For the continuous operation, de-ionized water was used as the liquid-phase inlet.

2.1.4 Inoculum

The original inoculum was a mixed-culture of marine microorganisms collected from beach sediment in Galveston Island, TX. The sediment was collected from the bottom of multiple 0.5-m-deep shoreline pits. Samples were immediately placed in airtight plastic bottles filled with deoxygenated water, capped, and frozen at –20°C until use.[9] Before inoculation, samples were

thawed, shaken vigorously, and settled by gravity. The homogenized supernatant was equally taken and used as 12.5% of the initial fermentation working volume. The composition of microorganisms has been reported elsewhere.[20]

In this study, the mixed-acid fermentation systems were operated by using office paper as the main biomass, so inocula adaptation was necessary. To start fermentations, the liquid product from previous paper-consuming mixed-acid fermentation was used as inoculum.[17]

2.1.5 Methane inhibitor

Iodoform (CHI_3) was used as a methane inhibitor for mixed-acid fermentation. Every 48 h, 120 μL iodoform solution (20 g CHI_3/L, 200-proof ethanol) was added into each fermentor. Because iodoform is sensitive to light, high temperature, and air, the solution was kept in an amber-colored glass bottle wrapped in foil and stored at 4 °C until use.

2.1.6 Buffer

During the set-up period of mixed-acid fermentation systems, magnesium carbonate and carbon dioxide were used to buffer the fermentation broth and maintain the pH in a near-neutral range (6.8–7.2). To reach the steady state for countercurrent fermentation and quasi-steady state for propagated fixed-bed fermentation, sodium bicarbonate and carbon dioxide were used as

buffers. Sodium bicarbonate was preferred over magnesium carbonate because less precipitate formed when ion exchange resin adsorption was applied into the fermentation systems.

2.1.7 CO_2-sustained ion-exchange resin adsorption apparatus

Known amounts of Amberlite® IRA-67 ion-exchange resin (Alfa Aesar, Product No. 42253) were loaded into refillable resin cartridge (Max Water®, Part No. 104050) with specified flowrate of carbon dioxide (PRAXAIR, UN1013) for *in-situ* recovery of carboxylic anion produced from fermentation broth.

The refillable resin cartridge was equipped with a fine-mesh screen (210 μm) to keep the resin in the cartridge and allow the liquid to pass through the resin bed without blocking. The procedure to set up the adsorption apparatus is documented in the Appendix C.

Prior to adsorption, ion-exchange resin was washed with de-ionized water to remove impurities and excess amines.

A vacuum filtering system was used to help remove liquid from the adsorption column. A peristaltic pump with standard pump head (Masterflex, Model # 7016) was used to deliver the liquid.

The procedure for CO_2-sustained ion-exchange resin adsorption (Figure 2-2) is documented in the Appendix D.

Sodium hydroxide (1-N NaOH) was used to regenerate ion-exchange resin. The detailed procedure is documented in the Appendix E.

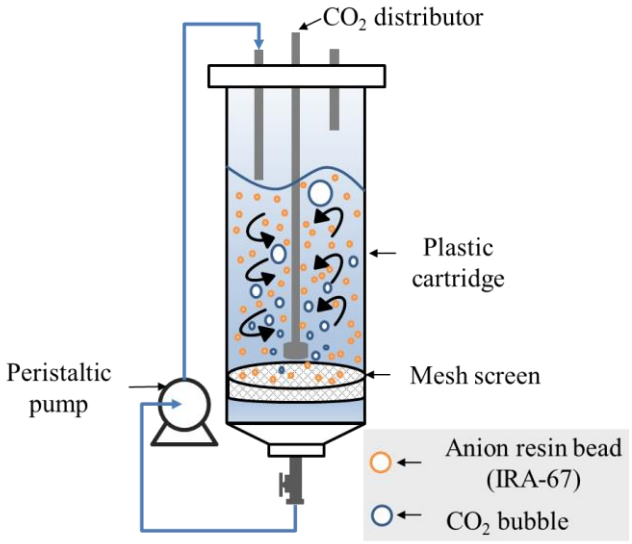


Figure 2-2. CO_2-sustained ion-exchange resin adsorption column configuration**.**

2.2 Methods

2.2.1 Biogas analysis

Biogas was formed continuously during fermentation; however, the fermentor can only tolerate pressures under 2 atm abs.[17] To prevent damage, the fermentors were vented periodically. Every 48 h, biogas was removed by inserting a needle through the fermentor septum, and the biogas volume was measured by using an inverted graduated glass cylinder filled with a 300 g/L $CaCl_2$, which prevented carbon dioxide adsorption and microbial growth.[9]

To analyze the biogas composition, a 30-mL gas sample was taken and manually injected into a gas chromatograph (Agilent 6890 Series) with a thermal conductivity detector (TCD). A 4.6-m-long, 2.1-mm-ID stainless steel packed column (60/80 Carboxen 100, Supelco 1-2390) was used. The inlet temperature was 230°C, the oven temperature was 200°C, and the detector temperature was 200°C. The total run time was 20 min, and helium was the carrier gas.

2.2.2 Carboxylic acid concentration determination

A 1.5-mL sample of centrifuged fermentation liquid (4000 rpm, 10 min) was collected from each fermentor every 48 h for carboxylic acids analysis. The frozen samples were thawed, vortexed, and centrifuged (13,300 rpm, 10 min). Supernatant liquid (0.5 mL) from each sample was mixed with 0.5 mL of 3-M phosphoric acid (H_3PO_4, 3 mol/L) and 0.5 mL of internal standard solution (isocaproic acid, 1.16 g/L), respectively. The 3-M phosphoric acid was added to acidify the sample liquid, and thus convert carboxylate salts into carboxylic acid. Then, the intermediate solution was centrifuged (13,300 rpm, 10 min) and transferred into a glass vial and capped.

The concentrations of carboxylic acids were measured by using a gas chromatograph (Agilent 6890 Series) equipped with an automatic liquid sampler (Agilent 76830) and a flame ionization detector (FID). A 30-m fused-silica capillary column (J&W Scientific, Model # 123-3232) was used. The column head pressure was maintained at 2 atm abs. After each sample injection, the GC temperature program raised the temperature from 40°C to 200°C at 20°C/min. The temperature

was subsequently held at 200°C for 2 min. The total run time per sample was 11 min. Helium was the carrier gas.[9] The external standard was a volatile acid mix (Table 2-1), used to calibrate the samples against the internal standard solution.

The detailed carboxylic acid analysis is documented in Appendix F.

Table 2-1. Carboxylic acid concentration in external standard solution.

Acid	Concentration (g/L)
Acetic Acid	4.000
Propionic Acid	3.030
Isobutyric Acid	1.002
Butyric Acid	1.999
Isovaleric Acid	0.807
Valeric Acid	1.570
Isocaproic Acid	1.160
Caproic Acid	0.812
Enanthic Acid	0.399
Caprylic Acid	0.169

2.2.3 Moisture and ash content analysis

Moisture and ash contents were determined by NREL procedures.[21] *Ash content* (AC) was calculated on a dry basis. To minimize the mass loss of volatile solids from evaporation, 30 mg

$Ca(OH)_2$/(g sample) was added into the liquid sample before placing into the oven. Samples were dried in a 105°C oven for at least 24 h, and subsequent combustion in a 575°C furnace for at least 24 h.

Moisture content (MC) is defined as the percentage of liquid evaporated from the sample after 24 h in 105°C oven. *Volatile solids* (VS) are defined as the mass of combusted dry solid materials after 24 h in 575°C furnace. *Ash* is defined as the mass of residuals after 24 h combustion in 575°C furnace.

The detailed procedures for moisture and ash content are documented in Appendix G.

2.3 Fermentation performance parameters

2.3.1 Operation parameters

The desired product of mixed-acid fermentation is carboxylic acids; thus, the concept of non-acid volatile solids (NAVS) was used to calculate the mass of digestible biomass. *NAVS* is defined as the difference between mass of VS and mass of carboxylic acid present in biomass.

$$\text{NAVS} = (\text{g total biomass})(1 - \text{MC})(1 - \text{AC}) - (\text{g carboxylic acids in biomass}) \quad (2\text{-}1)$$

The volatile solid loading rate (VSLR) and liquid residence time (LRT) were regulated by controlling the transfer frequency (*T*), feed rates of NAVS and fresh water, and the amounts of centrifuged solid cake and centrifuged liquid retained in each fermentor.

$$\text{VSLR} = \frac{\text{NAVS}_{\text{FEED}}\ (\text{g})}{\text{total liquid volume in all fermentors (L)} \cdot T(\text{day})} \tag{2-2}$$

$$\text{LRT} = \frac{\text{total liquid volume in all fermentors (L)}}{\text{flow rate out of fermentor train (L/day)}} \tag{2-3}$$

2.3.2 Performance parameters

For countercurrent operation, fermentation performance was analyzed during the steady-state period. For propagated fixed-bed operation, the quasi-steady-state period was taken for performance analysis. The average rates of feedstocks, products, and wastes were calculated using the Slope Method.[22] The accumulation of each component is plotted with respect to time, and the slope of the (quasi-) steady-state portion of this line is the average rate. Figure 2-3 shows the biomass conversion in mixed-acid fermentation.

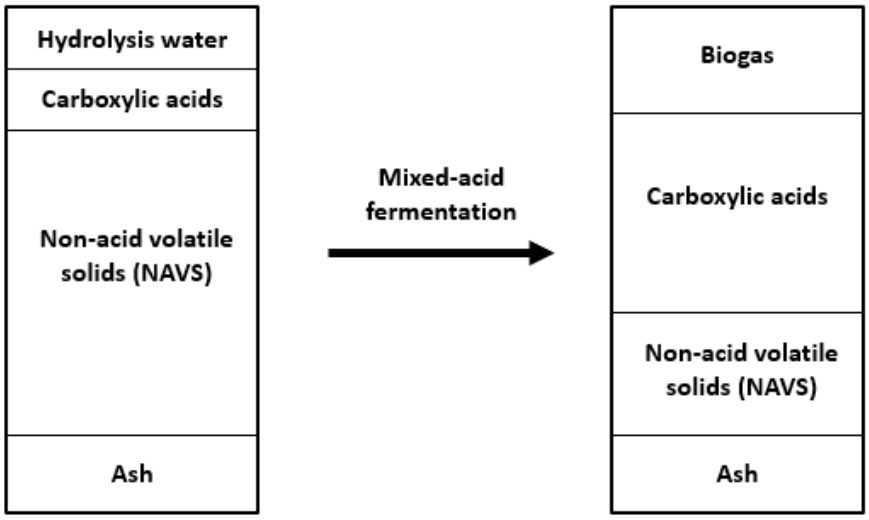


Figure 2-3. Biomass conversion.

To analyze fermentation performance, conversion, yield, and selectivity are the most important parameters. These variables were calculated using the average rates of components, as described below:

$$\text{Conversion}(x) \equiv \frac{\text{rate}\ [\text{NADS}_{\text{feed}}(\text{g/d}) - \text{NADS}_{\text{exit}}(\text{g/d})]}{\text{rate}\ [\text{NAVS}_{\text{feed}}(\text{g})]}$$
$$= \frac{\text{rate}\ \left[(\text{NAVS}_{\text{feed}}\ (\text{g/d}) + \text{Ash}_{\text{feed}}\ (\text{g/d}) - \text{NAVS}_{\text{exit}}\ (\text{g/d}) - \text{Ash}_{\text{exit}}\ (\text{g/d})\right]}{\text{rate}[\ \text{NAVS}_{\text{feed}}\ (\text{g/d})]}$$
$$= \frac{\text{rate}\ [\text{NAVS}_{\text{consumed}}\ (\text{g/d})]}{\text{rate}\ [\text{NAVS}_{\text{feed}}\ (\text{g/d})]} \tag{2-4}$$

$$\text{Yield}\ (Y) \equiv \frac{\text{rate}\ [\text{Total acid produced}\ (\text{g/d})]}{\text{rate}[\text{NAVS}_{\text{feed}}\ (\text{g/d})]} \tag{2-5}$$

$$\text{Yield } (Y_{Aceq}) \equiv \frac{\text{rate [Total Aceq produced (g/d)]}}{\text{rate[NAVS}_{\text{feed}}\text{ (g/d)]}} \tag{2-6}$$

$$\text{Selectivity } (\sigma) \equiv \frac{\text{rate[Total acid produced (g/d)]}}{\text{rate[NAVS}_{\text{consumed}}\text{ (g/d)]}} = \frac{Y_E}{x} \tag{2-7}$$

$$\text{Productivity } (P) \equiv \frac{\text{g (Total acid produced)}}{\text{Total liquid volume in all fermentors (L)}\cdot\text{Time(day)}} \tag{2-8}$$

where *NADS* is defined as the ash and volatile-solid component of biomass, $NADS_{feed}$ is the NADS fed, $NADS_{exit}$ is the NADS removed from the fermentation train. $NAVS_{feed}$ is the NAVS fed, $NAVS_{exit}$ is the NAVS removed from the fermentation. Ash_{feed} is the inert solids fed in feedstocks and buffer, and Ash_{exit} is the inert solids exiting from fermentation train. Ash_{feed} is assumed to be equal to Ash_{exit}.

The mixture of carboxylic acids can be expressed via acetic acid equivalent (Aceq), which is the reducing potential of an equivalent amount of acetic acid.[23] By using Aceq, the various concentration of carboxylic acids can be represented as a single acid concentration.

$$\begin{aligned}\text{Aceq (mol/L)} = {} & 1.00 \times \text{acetic(mol/L)} + 1.75 \times \text{propionic(mol/L)} \\ & + 2.50 \times \text{butyric(mol/L)} + 3.25 \times \text{valeric(mol/L)} \\ & + 4.00 \times \text{caproic(mol/L)} + 4.75 \times \text{heptanoic(mol/L)}\end{aligned} \tag{2-11}$$

$$\text{Aceq (g/L)} = 60.05\ \text{g/mol} \times \text{Aceq (mol/L)} \tag{2-12}$$

CHAPTER III

ION-EXCHANGE RESIN

3.1 Overview

Ion-exchange resin is a cross-linked porous polymer containing active functional groups with mobile ions, such as $–SO_3H$, $–SO_3Na$, –COOH, $–NH_3OH$. Cation-exchange resin carries exchangeable positively charged ions, whereas anion-exchange resin carries exchangeable negative charged ions. Based on the chemical behavior of functional groups, resins can be categorized as strong acid, weak acid, strong base, weak base ion-exchange resins. Based on the porous structure, resin can be categorized as microporous (gel) and microporous resins.

Amberlite® IRA-67 ion-exchange resin is a weak-base anion-exchange resin with a gel-type structure and acrylic matrix with divinylbenzene as copolymer. The functional group of IRA-67 is the tertiary amine group ($:NR_3$) in freebase (conjugate base) form.

The acid adsorption (Eq. 3-1) (Eq. 3-2) and desorption (Eq. 3-3) mechanism of weak-base anion-exchange resin can be described as follows:[24]

$$\mathrm{H+ : NR_3 \leftrightarrow H^+NR_3} \tag{3-1}$$

$$\mathrm{H^+NR_3 + Ac^- \leftrightarrow H^+NR_3(Ac^-)} \tag{3-2}$$

$$\mathrm{H^+NR_3(Ac^-) + OH^- \leftrightarrow : NR_3 + H_2O + Na^+ + Ac^-} \tag{3-3}$$

IRA-67 has proven to efficiently adsorb carboxylic acids formed during mixed-acid fermentation, but the acid uptake capacity of resin would dramatically decrease when the pH increased.[17]

In this chapter, carbon dioxide was introduced into adsorption to maintain liquid pH and sustain the resin capacity. The adsorption performance was studied with various CO_2 flowrates under plug-bed and fluidized-bed operating modes.

3.2 Experimental methods

3.2.1 Moisture content analysis of IRA-67

The moisture content of IRA-67 is 56–64% according to the product data sheet. Two methods were conducted to measure the moisture content of IRA-67. In the Method 1, about 3 g of wet resin was placed in a crucible, weighed, and inserted into an oven at 105 ˚C. After 24 h, the crucible was removed from the oven, cooled in a desiccator, and its final weight was noted. In the Method 2, the moisture content of resin samples was measured using a moisture content analysis device (Denver Instruments, IR 120). In this equipment, a weighed amount of resin was heated to 105˚C and the mass percentage of moisture in resin was measured.[17] The average of moisture content of resins was used to calculate the dilution effect in resin-related experiments.

3.2.2 Effects of CO_2 flowrate and operation mode on total acid adsorption performance with IRA-67

To study the effects of carbon dioxide flowrate (L/min) and operation mode on total acid adsorption performance with IRA-67, fermentation broth gained from countercurrent mixed-acid fermentation systems was used. The initial concentration of total carboxylic acid was tested according to the analytical method described in Chapter II. IRA-67 was loaded onto the adsorption column without further treatment. Different flowrates of CO_2 were used. For all experiments, fermentation broth was firstly poured into the adsorption column. Upon pouring, the stop watch was started to count the time. The retention time for all experiments was 10 min. Samples were taken at 10 min. All experiments were performed in triplicate.

For plug-flow adsorption (PFA), the solution was circulated through the adsorption column using a peristaltic pump. CO_2 was added into the adsorption column, but did not fluidize the resin bed. For fluidized-bed adsorption (FBA), the solution was circulated through the adsorption column using a peristaltic pump. CO_2 was added directly into the adsorption column to fully fluidize resin beads within the column. The experiment without resin loading was also performed as a control group. Table 3-1 lists the experimental settings.

Table 3-1. Experimental settings for the adsorption of carboxylic acids onto IRA-67 at different CO_2 flowrates and operational modes.

No.	CO_2 flowrate (L/min)	Operation mode	Initial conc., C_0 (g/L)	Fermentation broth volume (mL)	Amount of IRA-67 (g)
1	0	PFA	17.57 ± 0.34	80	20
2	1.5	PFA	17.57 ± 0.34	80	20
3	0.5	FBA	17.57 ± 0.34	80	20
4	1	FBA	17.57 ± 0.34	80	20
5	1.5	FBA	17.57 ± 0.34	80	20
6	2	FBA	17.57 ± 0.34	80	20
7	2.5	FBA	17.57 ± 0.34	80	20
8	1.5	FBA	17.57 ± 0.34	80	0

3.3 Result and discussion

3.3.1 Moisture content of IRA-67

The average moisture content of IRA-67 gained from two methods with standard deviation was 56.87 ± 0.24% and 56.12 ± 0.09%. To calculate the percentage of total acids adsorbed onto resin, the moisture content from Method 1 was used.

3.3.2 Effect of CO_2 flowrate on adsorption performance

The average percentage of total acids adsorbed onto resin during the retention time for each group was calculated. Figure 3-1 compares the results of groups No. 1, 2, 3, 4, 5, 6, and 7. Based on the t-test (Table 3-2), the adsorption performance was significantly increased by using CO_2 with FBA (p = 0.0008 between No. 1 and No. 3). For PFA and FBA, although there is no significant difference among No. 2 and No. 5, the average percentage of total acids adsorbed increased when FBA was used.

For different CO_2 flowrates, there was no significant difference between each group, which means that 0.5 L/min should be sufficient to sustain the resin uptake capacity for this amount of resin loading. However, for mixed-acid fermentation, more resin loadings were required. Thus, 1.5 L/min were selected as a fixed CO_2 flowrate for both countercurrent and propagated fixed-bed fermentations.

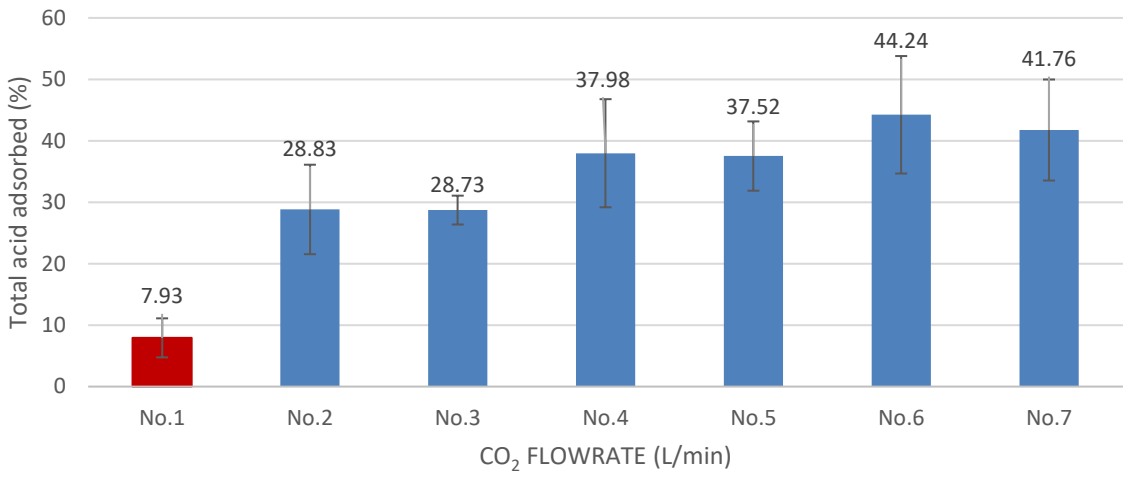


Figure 3-1. Effect of CO_2 flowrate on total acids uptake performance, error bar = ± 1σ.

Table 3-2. *p* values for adsorption performance between groups (α = 0.05).

Group	*p* values
No. 1 vs. No. 2	0.0041
No. 1 vs. No. 3	0.0008
No. 2 vs. No. 5	0.1585
No. 3 vs. No. 4	0.2204
No. 3 vs. No. 5	0.0906
No. 3 vs. No. 6	0.1121
No. 3 vs. No. 7	0.1184
No. 4 vs. No. 5	0.9373
No. 4 vs. No. 6	0.4512
No. 4 vs. No. 7	0.6155
No. 5 vs. No. 6	0.4385
No. 5 vs. No. 7	0.6187
No. 6 vs. No. 7	0.7509

3.4 Conclusion

This study indicates that adding CO_2 as a pH buffer is a promising pathway to sustain resin adsorption capacity. Combined with fluidized-bed operating mode, CO_2-sustained resin adsorption boosted percentage of total acids adsorption up to a factor of 5.58 of the plug-flow resin adsorption in absence of CO_2. Further investigation should be performed to find the effect of fouling caused by fermentation broth on resin beads.

CHAPTER IV

EFFECT OF CARBON DIOXIDE-SUSTAINED ADSORPTION USING ION-EXCHANGE RESIN ON COUNTERCURRENT MIXED-ACID FERMENTATION

4.1 Overview

Mixed-acid fermentation is the core of the MixAlco process. The carboxylic acids are anaerobically produced from lignocellulosic biomass by using a mixed culture of microorganism. However, accumulation of carboxylic acids negatively affects the microorganisms via acid products inhibition.

The Continuum Particle Distribution Modeling (CPDM) of mixed-acid fermentation developed by Loescher and Ross[25] depicts the reaction rate of acid production (r) using the following empirical equation:

$$r = \frac{e(1-x)^f}{1+g(\varphi \cdot \mathrm{Aceq})^h} \tag{4-1}$$

where x represents volatile solids conversion, ϕ is the ratio of total acids to acetic acid equivalents, Aceq is the product concentration expressed as acetic equivalent acid, e, f, g, h are empirical constants. Eq. 4-1 shows that both high conversion and concentration of carboxylic acids can slow the production rate.

To overcome slow reaction rates in mixed-acid fermentations, multi-stage countercurrent fermentation has been investigated.[9] Figure 4-1 shows fresh biomass is added into the fermentor with highest concentration of carboxylic acids and fresh water is added into the fermentor containing the most digested biomass. By using this arrangement, inhibition of accumulated acids is minimized, and thus better fermentation performance can be achieved.

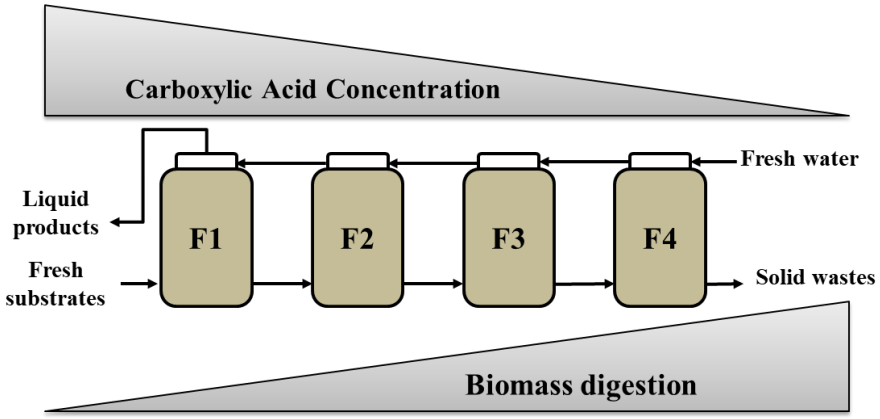


Figure 4-1. Four-stage countercurrent fermentation.

In-situ separation of products is a promising detoxification method that can help reduce product inhibition. Roy has demonstrated that using weak-base ion-exchange resin (Amberlite IRA-67) to adsorb carboxylate anions from fermentation broth significantly improves product yield and substrate conversion in mixed-acid batch fermentation.[17] Also, Yang[26] employed the same type of ion exchange resin to adsorb carboxylate anions from mixed-culture countercurrent fermentation systems and found that fermentation performance was enhanced by adsorption.

However, the relationship between the loading of resin and the fermentation performance of countercurrent systems still needs further investigation. Based on former research, several technical issues must be addressed:

(1) Maintain the pH of the fermentation broth during ion-exchange resin adsorption

When carboxylic acids are removed from an unbuffered solution, the pH will rise sharply. This pH rise will largely reduce the adsorption capacity of ion-exchange resin, and also detrimentally affect the microorganisms; thus, the fermentation broth should be acidified or properly buffered during adsorption.

Carbon dioxide was chosen as buffer during adsorption in this study for five reasons:

a) Carbon dioxide can be directly added into fermentation broth without negatively affecting the microorganisms;

b) Carbon dioxide has a pKa (pKa_1 = 6.37 at 25 ºC) similar to the pH of mixed-acid fermentation, and thus prevent the pH swing associated with acid recovery;

c) Carbon dioxide and its corresponding ions have lower affinity to basic sites in ion-exchange resin compared with carboxylate anions;

d) Carbon dioxide is one of the byproducts of mixed-acid fermentation, and thus lowers operating costs;

e) The carbon dioxide-sustained ion exchange resin adsorption can be recycled, and thus minimize chemical consumption and waste salt generation.

(2) Prevent column from becoming clogged

Mixed-acid fermentation is a heterogeneous reaction, and thus the fermentation broth contains fine solids particles. In Yang's experiments, the adsorption column was clogged by residues, and had to be unplugged with strong acid (HCl). Also, white particles were formed when CO_2 was employed in adsorption. Here are two hypotheses for the plugging:

a) By the ion exchange mechanism, OH^- is released from resins. According to J.P. Durham and R.O. Lopez-Solis[27], this blinding is caused by cellular protein that precipitates from solution upon contact with OH^-;

b) Prior to applying ion exchange resin in fermentors, $MgCO_3$ is used as a buffer to convert carboxylic acids into carboxylate salts. When fermentation broth is contacted with resins, $Mg(OH)_2$ might be formed from the high pH and with the presence of CO_2, $MgCO_3$ or $Mg(HCO_3)_2$ could form.

Countercurrent mixed-acid fermentation is operated semi-continuously and resin is supposed to be reused in the system. Thus, the blocking would cause system failure. To overcome the problem and minimize operating costs, a reusable fine-mesh screen was

explored and used in the adsorption column, which could prevent column blocking and resin leaking.

(3) Regenerate and decontaminate ion-exchange resin

Weak-base ion-exchange resin cannot only adsorb carboxylic acids, but also be regenerated for a next capture cycle if desorption is performed with a base such as NaOH[15]:

$$\text{Adsorption: } HA(aq1) + B(resin) \leftrightarrow BH^{+}A^{-}(resin) + (aq1) \quad (4\text{-}2)$$

$$\text{Desorption: } BH^{+}A^{-}(resin) + NaOH(aq2) \leftrightarrow B(resin) + NaA(aq2) + H_2O \quad (4\text{-}3)$$

$$\text{Overall: } HA(aq1) + NaOH(aq2) \leftrightarrow (aq1) + NaA(aq2) + H_2O \quad (4\text{-}4)$$

However, resin beads are prone to fouling because of the impurities in fermentation broth, which could deactivate the ion-exchange resin and limit its operational life; thus, proper decontamination procedures were considered. Additionally, the resin column reloading period was determined by monitoring the adsorption capacity of the resin column.

(4) Determine operation mode of adsorption column

Although mixed-acid fermentation is an anaerobic process, periodical air exposure (every 48 hours in this study) is unavoidable for laboratory-scale fermentation because of the need for mass transfer and adsorption. To minimize its influence on fermentation performance, a certain operation time for adsorption was set. Within a limited time, the

same adsorption column in different operation modes showed different adsorption capacities.

With better adsorption performance and greater operability, carbon dioxide-sustained fluidized-bed ion-exchange resin column with fermentation broth recycle was used as an innovative operation mode for adsorption in countercurrent mixed-acid fermentation.

In this study, different loadings of weak-base ion-exchange resin (IRA-67) were used in the presence of CO_2 to adsorb carboxylate anions from four identical countercurrent mixed-acid fermentation systems under semi-continuous conditions. The effects of various loadings of resin on fermentation performance were evaluated at near-neutral pH and optimal carbon-nitrogen ratio.

4.2 Experimental methods

4.2.1 Countercurrent mixed-acid fermentation

Four continuous four-stage countercurrent mixed-acid fermentation trains were run under the same operation conditions (Figure 4-1).

To grow the microflora, four fermentation trains were initiated as batch cultures under anaerobic conditions at 40 ºC. The substrates consisted of 80% dry paper and 20% dry homogenized chicken manure. The initial concentration of non-acid volatile solids (NAVS) in

batch fermentors were achieved at 97.15 g NAVS/ L deoxygenated water by adding substrates, urea, inoculum, buffer, and liquid medium to each fermentor. Table 4-1 shows the detailed feed description of batch cultures.

Table 4-1. Feed composition for batch cultures.

Feed composition	Dry paper (g)	Dry chicken manure (g)	Deoxygenate water (mL)	Inocula (mL)	Urea (g)
All Trains	32	8	350	50	0.8

The fermentors in Trains 1, 2, 3, and 4 were operated in batch mode for the first 38, 38, 40, and 8 days, respectively. After initial batch operations, the trains were operated in countercurrent mode. These differences in the length of the batch growth on countercurrent fermentation were thus investigated and found to be negligible.

After batch growth, solid and liquid transfers were performed every 48 h in countercurrent fermentation to reach steady state. To regulate the fermentation volumes in all trains, the mass of solids and liquid were kept at a specific setpoint. The residence times of solid and liquid were determined by the chosen transfer frequency (48 h).

For countercurrent fermentation, fermentors were removed from the incubator every 48 h. After cooling down to room temperature, gas samples from fermentors were collected, and gas

volume was measured. The vented fermentors were centrifuged at 4000 rpm (4050 × g) for 10 min to separate the solid and liquid phases. The centrifuged liquid of each fermentor was decanted into a beaker and was weighted. Liquid samples (1.5 mL) were taken from each beaker, and then the initial pH of liquid phase was measured by a calibrated pH meter. The weight of remaining solid in each fermentor was measured. The retained solid and liquid weights in fermentors were controlled by mass setpoints, and the solid/liquid mass removed was determined by the following material balance:

$$\text{Mass remove} = \text{Initial mass} + \text{mass added from previous fermentor (or fresh feeding)} - \text{mass setpoint} \quad (4\text{-}5)$$

In each train, the solids were transferred from the least-digested fermentor (F1) to the most-digested fermentor (F4), while liquid was transferred from the most-digested fermentor to the least-digested fermentor. For each train, the fresh substrates (paper and dry chicken manure) were added into F1 at a constant amount, and fresh deionized water and urea were added into F4 at a constant amount.

After liquid transfer, the pH in each fermentor was then measured and buffer was added to neutralize the carboxylic acids and maintain the liquid-phase pH in a near-neutral range (6.8–7.2). To inhibit methane production, 120 μL methane inhibitor (20 g CHI_3/L, 200-proof ethanol) was added into each fermentor at the end of operation. Before returning the fermentors to the incubator, they were purged with nitrogen and properly resealed to create an anaerobic environment for microorganisms. The detailed countercurrent mixed-acid fermentation procedure is listed in Appendix A. Table 4-2 provides operating parameters and the normalized parameters for the mean of steady-state values.

Table 4-2. Operating parameters for countercurrent mixed-acid fermentations, (a) controlled parameters, (b) normalized parameters during S-S-1, (c) normalized parameters during S-S-2, error bar = ± 1σ.

(a)

	Train	1	2	3	4
Controlled	Solid and liquid transfer frequency, *T* (h)	48	48	48	48
	NAVS feed rate (gNAVS/*T*)	9.71	9.71	9.71	9.71
	Paper (g/*T*)	10.2	10.2	10.2	10.2
	Chicken manure (g/*T*)	2.4	2.4	2.4	2.4
	Urea added (g/*T*)	0.2	0.2	0.2	0.2
	Liquid feed rate (mL/*T*)	120	120	120	120
	Solid retained in each fermentor (g)	200 (250 for F4)	200 (250 for F4)	200 (250 for F4)	200 (250 for F4)
	Centrifuge liquid retained in each fermentor (g)	200	200	200	200
	Methane inhibitor (μL/(*T*·Fermentor))	120	120	120	120

Table 4-2 Continued

(b)

	Train	1	2	3	4
Normalized (S-S-1)	Volatile solid loading rate, VSLR (g NAVS/(L_{liq}·d))	3.42 ± 0.05	3.42 ± 0.07	3.44 ± 0.05	3.42 ± 0.05
	Liquid residence time, LRT (d)	28.26 ± 2.51	28.02 ± 1.82	28.20 ± 1.71	28.10 ± 2.18
	Total liquid volume, TLV (L)	1.42 ± 0.02	1.42 ± 0.03	1.41 ± 0.02	1.42 ± 0.02
	Total NAVS (g)	137.42 ± 12.62	133.58 ± 13.15	143.60 ± 10.56	137.21 ± 15.63
	Volatile solid, VS, concentration (g NAVS/L_{liq})	96.78 ± 8.99	94.07 ± 9.47	101.84 ± 7.63	96.63 ± 11.09
	Carbon-nitrogen ration in feed, C-N ratio (g OC_{NA}/g N)	30.31 ± 0.00	30.31 ± 0.00	30.31 ± 0.00	30.31 ± 0.00
	Urea addition rate (g urea/(L_{liq}·d))	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00

S-S-1 stands for steady state without resin adsorption.

Table 4-2 Continued

(c)

	Train	1	2	3	4
Normalized (S-S-2)	Volatile solid loading rate, VSLR (g NAVS/(L_{liq}·d))	3.49 ± 0.12	3.46 ± 0.05	3.51 ± 0.05	3.54 ± 0.05
	Liquid residence time, LRT (d)	29.20 ± 2.23	29.72 ± 1.58	30.11 ± 2.84	30.13 ± 1.71
	Total liquid volume, TLV (L)	1.39 ± 0.05	1.40 ± 0.02	1.38± 0.02	1.37± 0.02
	Total NAVS (g)	149.71 ± 18.26	151.95 ± 14.68	156.64 ± 20.11	174.30 ± 15.94
	Volatile solid, VS, concentration (g NAVS/L_{liq})	107.70 ± 13.69	108.54 ± 10.60	113.51 ± 14.66	127.23 ± 11.78
	Carbon-nitrogen ration in feed, C-N ratio (g OC_{NA}/g N)	30.31 ± 0.00	30.31 ± 0.00	30.31 ± 0.00	30.31 ± 0.00
	Urea addition rate (g urea/(L_{liq}·d))	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00

S-S-2 stands for steady state with resin adsorption.

4.2.2 CO_2-sustained fluidized-bed ion exchange resin adsorption

4.2.2.1 Ion-exchange resin loading

The theoretical amount of resin loading needed for countercurrent fermentation was determined by Eq. 4-6:

$$\text{Theoretical Resin Loading (g wet resin}_{FB}\text{/day)} = \frac{\text{Acids production rate}\cdot\rho_r}{M_w \cdot C_r} \cdot \frac{1\text{ eq.}}{1\text{ mol}} \quad (4\text{-}6)$$

According to the product data sheet for IRA-67, the total adsorption capacity (C_r) is 1.60 eq./L wet resin (free-base form) and the resin density (ρ_r) is 700 g/L wet resin. For countercurrent fermentation during S-S-1, the carboxylic acid production rate was analyzed to be 0.55 ± 0.006 g/day. The average molecular weight of acid products based on mass fraction (M_w) was calculated to be 72.5 g/mol. For carboxylic acids produced in mixed-acid fermentation, 1 mole of acids equals to 1 equivalent (eq.).

To adsorb all carboxylic acids produced each day in a 1.4 L_{liq} fermentation train, the calculated theoretical amount of resin loading is 3.319 g wet $resin_{FB}$/day. Because the transfer frequency of countercurrent trains in this study is 2 days (48 h) and resin are supposed to be reused, the theoretical resin loading in adsorption column should be 6.638 g wet $resin_{FB}$. Unless specified otherwise, wet resin in free-base form are described as “wet resin.”

However, Roy[17] showed that the adsorption efficiency of ion-exchange resins could be tremendously affected by the equilibrium pH of the solution. When contacting resin with fermentation broth, a sharp rise of solution pH would occur within a short time period, which would largely inhibit acid adsorption.

To maintain resin adsorption capacity, CO_2 was used as a buffer during adsorption. The pKa_1 of CO_2 is 6.37 at 25 ºC, and can sustain fermentation pH within 6.5 to 7.5 during adsorption, and thus it allows the resin to have around 70–85% adsorption capacity. To adsorb all acids at the S-S-1 production rate, the minimum resin loading amount should be 9.48 g wet resin (for S-S-1, TLV = 1.4 L).

According to Eq. 4-1, the acid production rate should increase when acid concentrations are lowered; thus, it is reasonable to question if different amounts of resin loading will variously affect countercurrent mixed-acid fermentation.

To answer this question, four sets of resin loading (Table 4-3) were chosen for these four countercurrent fermentation trains. To provide suggestions for industrial applications, the wet resin loading should be normalized with total liquid volume in fermentation system (TLV) and transfer frequency. The detailed procedures for getting normalized wet resin loading are documented in Appendix H.

Table 4-3. Wet resin loading and normalized wet resin loading, error bar = ± 1σ.

Train	1	2	3	4
Wet Resin (g)	10	20	30	40
Total Liquid Volume (L)	1.39 ± 0.05	1.40 ± 0.02	1.38± 0.02	1.37± 0.02
Normalized Wet Resin Loading (g wet resin/(L_{liq} · day))	3.60 ± 0.13	7.14 ± 0.10	10.87 ± 0.16	14.60 ± 0.21

Normalized basis = total liquid volume in each train during S-S-2

4.2.2.2 Adsorption operation

After countercurrent fermentation trains reached the steady-state region, CO_2-sustained ion exchange resin adsorption was applied to fermentation broth to adsorb carboxylate anions produced by microorganisms (Figure 4-2).

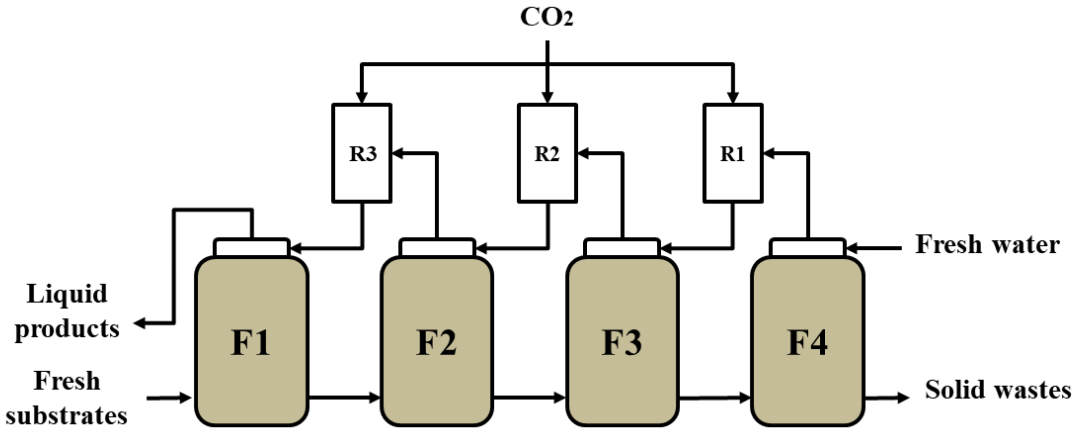


Figure 4-2. Diagram of CO_2-sustained ion exchange resin adsorption.

For one specific countercurrent fermentation train, the specified amount of resin was loaded into one adsorption column (R). Every 48 h, removed fermentation broth from F4, F3, and F2 was passed through the adsorption column (R) in the presence of CO_2, ordered respectively as R1, R2 and R3. To be clear, only one adsorption column was used for this adsorption process, and R1, R2, R3 only denote the order of adsorption.

The mass of removed fermentation broth from each fermentor was determined by Eq. 4-5. To reduce the length of periodic air exposure, the operation time for unit adsorption recycle was set to be 10 min. To make full use of the resin adsorption capacity within the limited operation time, the flowrate of CO_2 was set to 1.5 L/min to fully fluidize the resin bed. The circulation of fermentation broth in the adsorption column was set to 1.34 mL/s. After each adsorption, 1.5-mL liquid sample was taken from the effluent. The detailed procedure of resin adsorption is described in Appendix D.

The amount of carboxylic acids adsorbed onto the resin column was calculated by Eq. 4-7:

$$\text{Acid adsorbed (g)} = (C_{initial} \times V_{initial}) - (C_{final} \times V_{final}) \quad (4\text{-}7)$$

where $C_{initial}$ and C_{final} are carboxylic acid concentrations (g/L), $V_{initial}$ is the initial volume of fermentation broth passed through the adsorption column (L), and V_{final} is the volume of fermentation broth collected after it passed through the adsorption column (L).

4.2.2.3 Resin regeneration

Ion-exchange resin was regenerated with 1-N sodium hydroxide (NaOH) solution after three times adsorption (R1, R2, R3). The regeneration method was determined as suggested in the IRA-67 product data sheet. One bed volume (BV) is defined as 1 m^3 solution per m^3 resin. The saturated resin was rinsed with 8 BV of NaOH solution for more than 30 min under 1.34 mL/s circulation rate. After regeneration, 1.5-mL liquid sample was taken from the effluent. The detailed procedure of resin adsorption is described in Appendix E.

The acids recovered from regeneration was calculated by Eq. 4-8:

$$\text{Acid recovered (g)} = C_{r\text{-}final} \times V_{r\text{-}final} - C_{r\text{-}initial} \times V_{r\text{-}final} \quad (4\text{-}8)$$

where $C_{r\text{-}initial}$ and $C_{r\text{-}final}$ are carboxylic acid concentrations (g/L) in NaOH solution, $V_{r\text{-}initial}$ is the initial volume of NaOH solution passed through the adsorption column (L), and $V_{r\text{-}final}$ is the volume of NaOH solution collected after it passed through the adsorption column (L).

4.2.3 Statistical analysis

In this study, statistical analysis was performed using Microsoft Excel. For countercurrent mixed-acid fermentation, the steady-state region (S-S-1) was the period during which the product total acid and acetic acid equivalent (Aceq) concentration, dry solids exiting, liquid volume and solid weight of each fermentor, pH, and mass of acid exiting did not vary by more than 2.2 standard deviations from the average for a period of at least one liquid residence time.[9] Based on the data,

the average time required for countercurrent trains to reach the steady-state region was around 4 months. However, the new steady-state region (S-S-2) could be readily achieved within 1 week after applying the resin adsorption.

The means and standard deviations of the total acid concentration, Aceq concentration, non-acid volatile solids (NAVS) in and out were determined from steady-state region data. With the Slope Method, these values were then used to calculate the fermentation performance, such as acid productivity (P), selectivity (σ), yield (Y), and conversion (x). All error bars are reported as one standard deviation. The two samples t-test (two-tailed, unequal variance, $\alpha = 0.05$) was used to compute the p values between the performance variables of the trains. To control Type I Error (familywise error rate), the Bonferroni correction was used.

4.3 Results and discussion

4.3.1 Biogas analysis

The biogas production increased after applying resin adsorption (Figure 4-3). At S-S-1, the amounts biogas produced for Trains 1, 2, 3, 4 were as follows: 0.42 ± 0.05, 0.40 ± 0.06, 0.42 ± 0.05, 0.43 ± 0.10 L gas/d. For all trains, biogas compositions were similar (Table 4-4). At S-S-2, the amounts biogas produced for Trains 1, 2, 3, 4 were as follows: 0.57 ± 0.03, 0.62 ± 0.10, 0.61 ± 0.09, 0.62 ± 0.05 L gas/d. For all trains, biogas compositions were similar (Table 4-4). For all

fermentors, the production of CO_2 and H_2 increased, which could result from two reasons: (1) biomass conversion increased after applying resin adsorption; (2) because CO_2 was used in the adsorption process, it would release from liquid phase during fermentation. By adding iodoform as methane inhibitor, no methane was detected in any fermentors during each steady-state region.

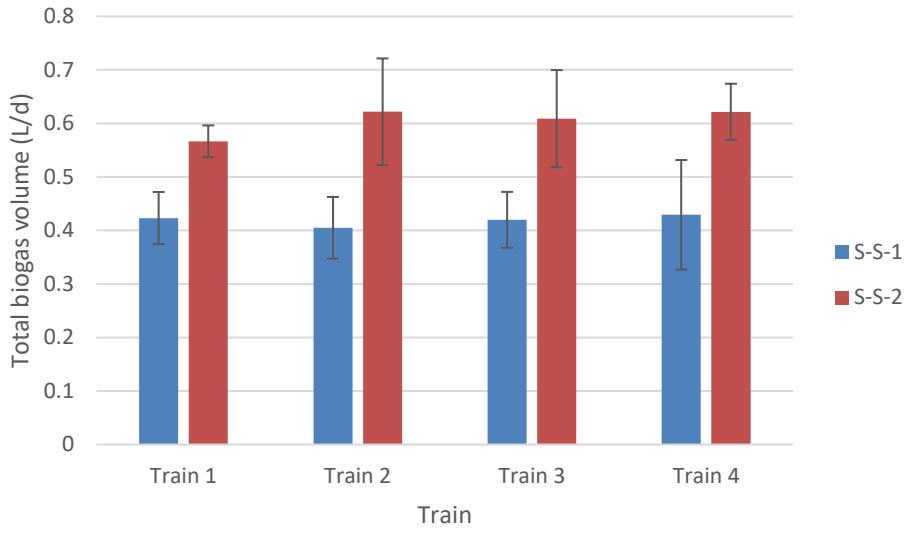


Figure 4-3. Total biogas production in trains, error bar = ± 1σ.

Table 4-4. Biogas composition*, error bar = ± 1σ.

		F1	F2	F3	F4
S-S-1	CO_2	21.53 ± 11.67%	14.92 ± 9.40%	13.02 ± 5.05%	9.62 ± 4.88%
	N_2	75.94 ± 11.34%	82.14 ± 8.54%	85.20 ± 5.57%	88.59 ±5.83%
	H_2	1.94 ± 1.05%	1.61 ± 0.70%	1.40 ± 0.69%	0.69 ±0.75%
S-S-2	CO_2	32.32 ± 1.83%	25.61 ± 4.34%	23.03 ± 2.19%	16.24 ± 1.29%
	N_2	65.31 ± 1.83%	71.61 ± 4.77%	74.63 ± 2.23%	81.84 ± 1.56%
	H_2	2.03 ± 0.30%	2.43 ± 0.63%	1.99 ± 0.46%	1.52 ± 0.36%

* Biogas composition is based in mole percentage

4.3.2 pH

Table 4-5 lists the average of pH measured before mass transfer (pre-transfer pH) in each fermentor. For S-S-1, sodium bicarbonate and carbon dioxide were used as pH buffers, and the average of pre-transfer pH in each fermentor was 5.97 to 6.98. For S-S-2, CO_2-sustained ion-exchange resin adsorption was used to remove the carboxylate anions and maintain the pH. In this case, the average of pre-transfer pH in each fermentor was 5.79 to 6.40.

For all fermentors (except Trains 4-F1 and 4-F2), the averages of pre-transfer pH decreased after resin adsorption (Figure 4-4). In each train, Fermentor F4 achieved the largest pre-transfer

pH decline. This pH decline may result from higher acid production after applying resin adsorption. In semi-continuous countercurrent fermentation, to mitigate the pH drop, sodium bicarbonate should be added into fermentors as a buffer after mass transfer and resin adsorption.

Table 4-5. The average of pre-transfer pH in each fermentor for S-S-1 and S-S-2, error bar = ± 1σ.

	S-S-1				S-S-2			
	F1	F2	F3	F4	F1	F2	F3	F4
Train 1	6.03 ± 0.17	6.51 ± 0.28	6.71 ± 0.38	6.90 ± 0.65	5.91 ± 0.14	6.33 ± 0.11	6.33 ± 0.10	6.21 ± 0.11
Train 2	6.03 ± 0.17	6.52 ± 0.23	6.61 ± 0.25	6.67 ± 0.44	5.79 ± 0.12	6.20 ± 0.13	6.22 ± 0.07	6.16 ± 0.07
Train 3	5.98 ± 0.13	6.48 ± 0.19	6.63 ± 0.20	6.86 ± 0.50	5.89 ± 0.23	6.28 ± 0.41	6.29 ± 0.31	6.25 ± 0.15
Train 4	5.97 ± 0.21	6.38 ± 0.20	6.66 ± 0.37	6.98 ± 0.75	6.13 ± 0.14	6.40 ± 0.29	6.28 ± 0.15	6.13 ± 0.11

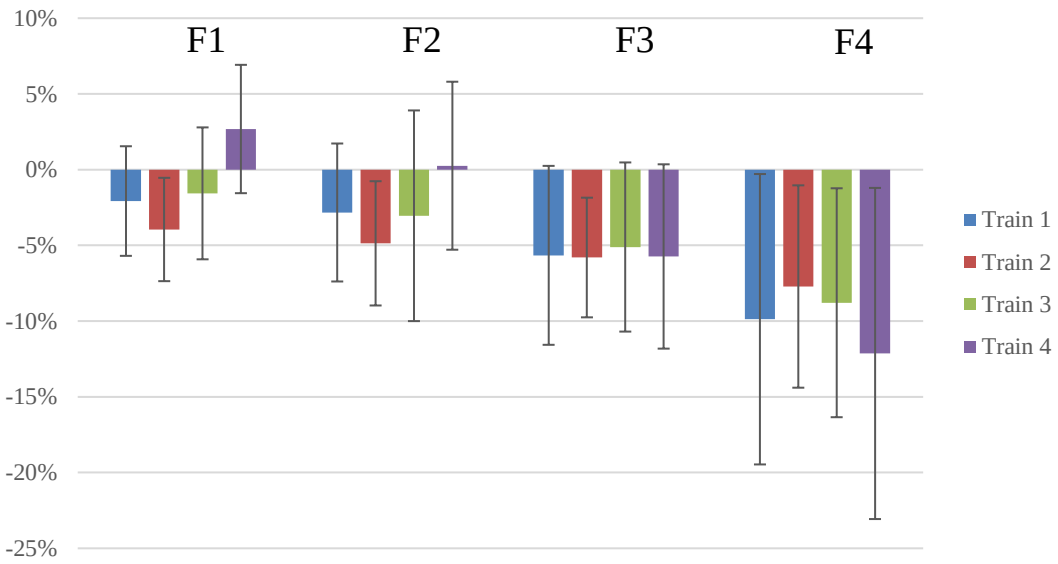


Figure 4-4. The percentage changes of average pre-transfer pH between S-S-1 and S-S-2 in fermentors, error bar = ± 1σ.

4.3.3 Acid production

For all trains, Figure 4-5 shows total carboxylic acid concentration in each stage with time. In all fermentors, acid concentrations were maintained in a narrow range during steady states. By applying resin adsorption, new steady states were readily achieved. Figure 4-6 shows the average total acid concentrations in each fermentor during S-S-1 and S-S-2. For all trains, total acid concentrations decreased in F1, F2, F3, but increased in F4. This phenomenon could result from higher microorganism density in the fermentation systems after resin adsorption.

Respectively, Figures 4-7, 4-8, and 4-9 show the total carboxylic acid concentrations, Aceq concentrations, and carboxylic acid composition profiles in liquid products for both S-S-1 and S-S-2.

For S-S-1, total acid and Aceq concentrations in liquid products were similar among Trains 2, 3, 4, whereas Train 1 exhibited a slightly higher acid concentration. The carboxylic acid composition profiles in liquid products were similar among all trains, with acetic acid (C2), propionic acid (C3) and butyric acid (C4) as primary components. For S-S-2, total acid and Aceq concentrations in liquid products decreased from resin adsorption, but the mass fractions of propionic acid (C3), valeric acid (C5), and caproic acid (C6) increased.

Based on statistical analysis, acid productivities in all trains were similar in S-S-1, and significantly increased after resin adsorption (Tables 4-6, 4-7 and 4-10). Figure 4-10 also shows that the increase of normalized wet resin loading can boost acid production.

Table 4-6. Fermentation performance for all trains during S-S-1, error bar = ± 1σ.

Fermentation performance	Train 1	Train 2	Train 3	Train 4
Total carboxylic acid concentration (g/L)	18.50 ± 0.32	17.58 ± 0.36	17.06 ± 0.26	17.15 ± 0.23
Aceq concentration (g/L)	24.32 ± 3.23	22.94 ± 2.95	22.59 ± 2.37	22.55 ± 2.70
Aceq/Acid ratio	1.32 ± 0.18	1.30 ± 0.17	1.32 ± 0.14	1.31 ± 0.16
Conversion, *x* (g NAVS digested/g NAVS fed)	0.174 ± 0.009	0.179 ± 0.008	0.179 ± 0.004	0.181 ± 0.005
Yield, *Y* (g total acid/g NAVS fed)	0.120 ± 0.001	0.112 ± 0.005	0.114 ± 0.004	0.114 ± 0.001
Selectivity, σ (g total acid produced/g NAVS consumed)	0.693 ± 0.037	0.623 ± 0.027	0.638 ± 0.014	0.637 ± 0.020
Productivity, *P* (g total acid/(L_{liq} · d))	0.412 ± 0.130	0.386 ± 0.116	0.387 ± 0.097	0.392 ± 0.107

Table 4-7. Fermentation performance for all trains during S-S-2, error bar = ± 1σ.

Fermentation performance	Train 1	Train 2	Train 3	Train 4
Total carboxylic acid concentration (g/L)	15.10 ± 0.37	14.18 ± 0.34	14.67 ± 0.35	13.38 ± 0.24
Aceq concentration (g/L)	19.95 ± 2.73	18.95 ± 2.38	19.53 ± 2.66	18.20 ± 2.38
Aceq/Acid ratio	1.32 ± 0.18	1.34 ± 0.17	1.33 ± 0.18	1.36 ± 0.18
Conversion, x (g $NAVS_{digested}$/g $NAVS_{fed}$)	0.233 ± 0.009	0.308 ± 0.008	0.409 ± 0.013	0.399± 0.009
Yield, Y (g total acid/g $NAVS_{fed}$)	0.174 ± 0.000	0.195 ± 0.002	0.217 ± 0.001	0.236 ± 0.001
Selectivity, σ (g total acid produced/g $NAVS_{consumed}$)	0.747 ± 0.030	0.633 ± 0.017	0.536 ± 0.017	0.591 ± 0.014
Productivity, P (g total acid/($L_{liq} \cdot$ d))	0.605 ± 0.148	0.679 ± 0.203	0.755 ± 0.179	0.847 ± 0.117

Table 4-8. *p* values for fermentation performance for all trains during S-S-1 (α=0.05).

Trains	Conversion	Yield	Selectivity	Productivity
1 and 2	0.007	0.000	0.000	0.323
1 and 3	0.001	0.000	0.000	0.284
1 and 4	0.000	0.000	0.000	0.384
2 and 3	1.000	0.047	0.003	0.967
2 and 4	0.165	0.018	0.007	0.796
3 and 4	0.022	1.000	0.760	0.800

p value < 0.008 statistically different, with the Bonferroni correction

Table 4-9. *p* values for fermentation performance for all trains during S-S-2 (α=0.05).

Trains	Conversion	Yield	Selectivity	Productivity
1 and 2	0.000	0.000	0.000	0.112
1 and 3	0.000	0.000	0.000	0.001
1 and 4	0.000	0.000	0.000	0.000
2 and 3	0.000	0.000	0.000	0.124
2 and 4	0.000	0.000	0.000	0.000
3 and 4	0.001	0.000	0.000	0.018

p value < 0.008 statistically different, with the Bonferroni correction

Table 4-10. *p* values for fermentation performance between S-S-1 and S-S-2 (α=0.05).

Trains	Conversion	Yield	Selectivity	Productivity
1	0.000	0.000	0.000	0.000
2	0.000	0.000	0.065	0.000
3	0.000	0.000	0.000	0.000
4	0.000	0.000	0.000	0.000

p value < 0.012 statistically different, with the Bonferroni correction

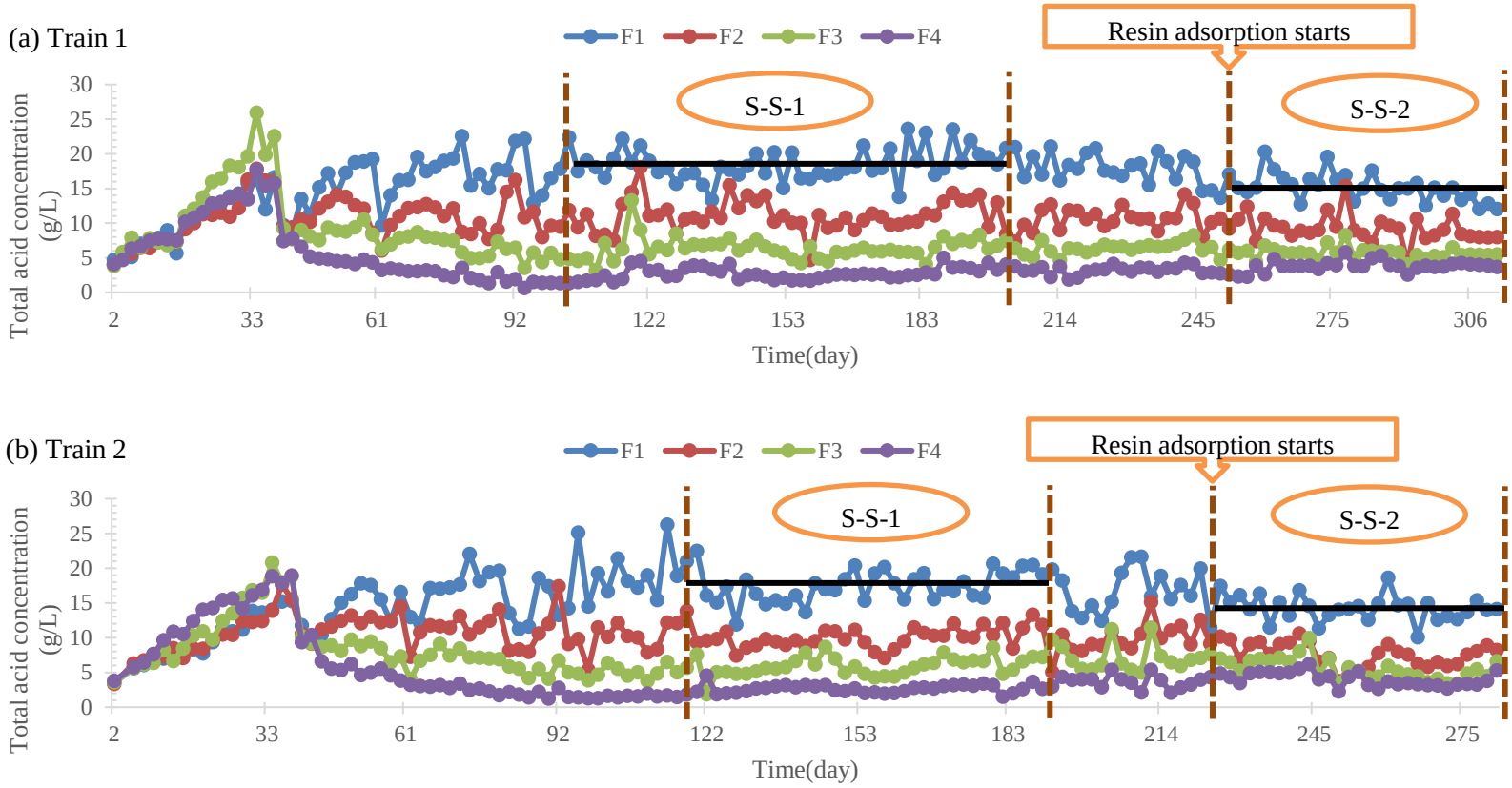


Figure 4-5. Total carboxylic acid concentration in each stage of four countercurrent train

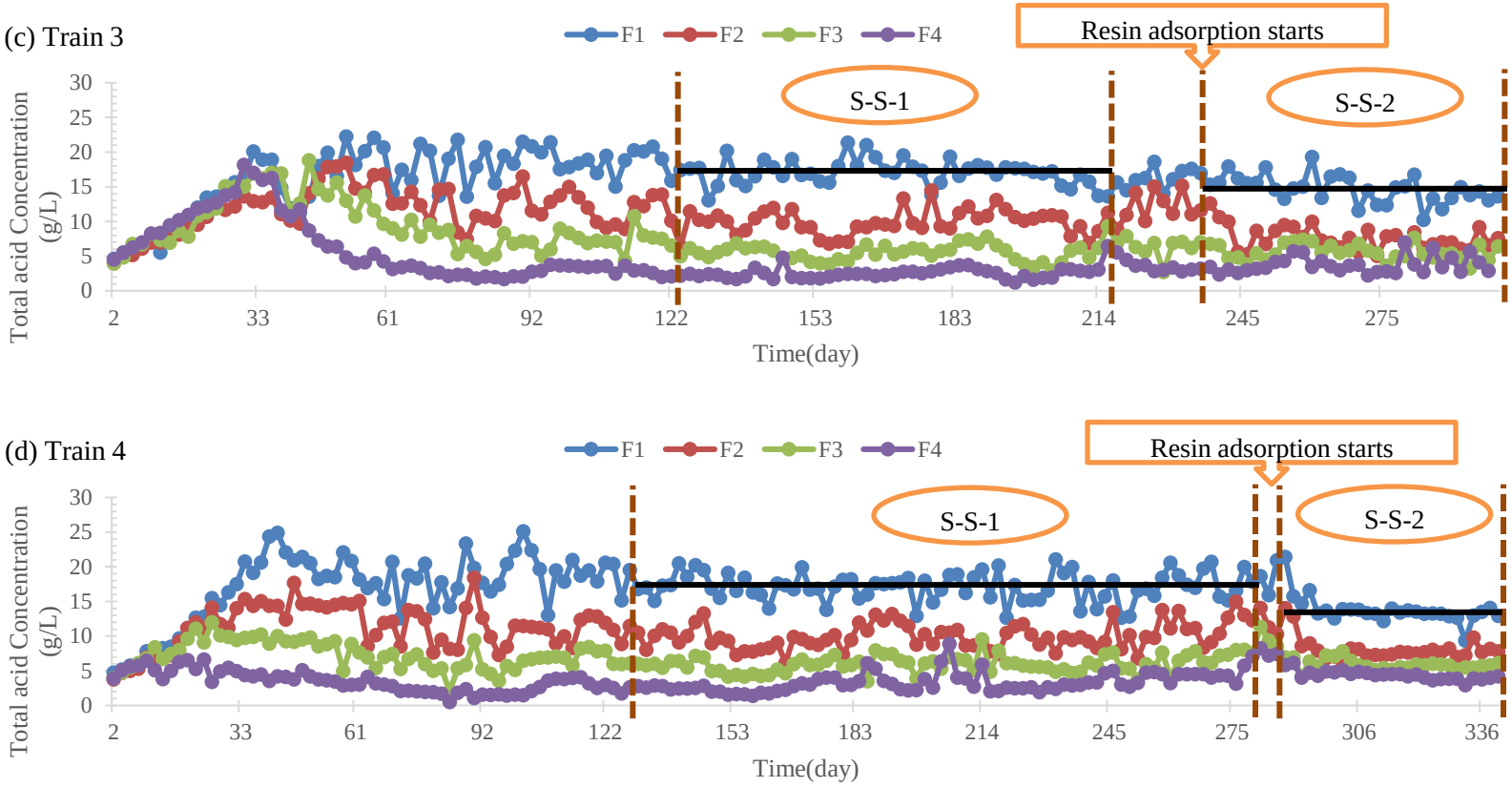


Figure 4-5 Continued

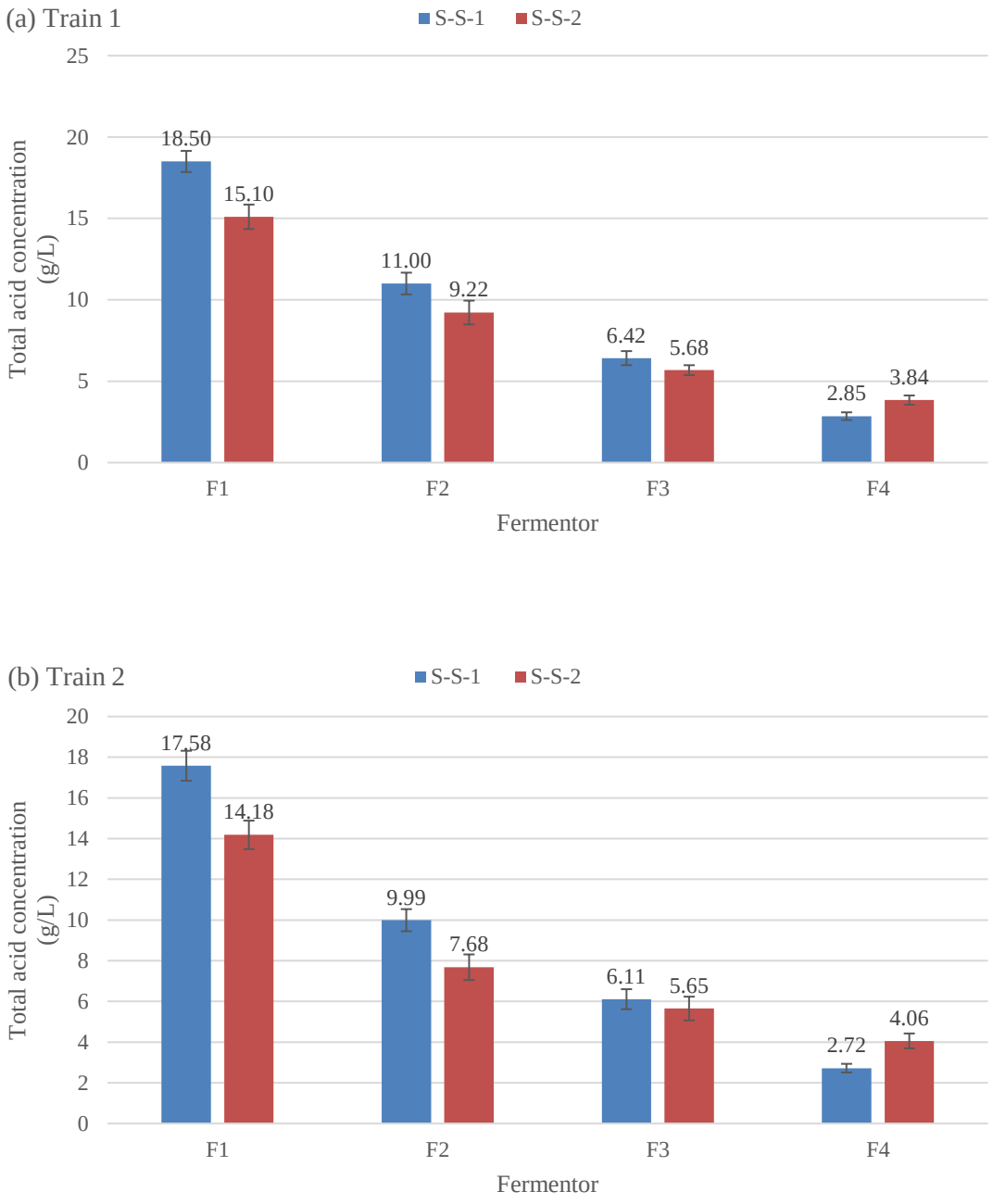
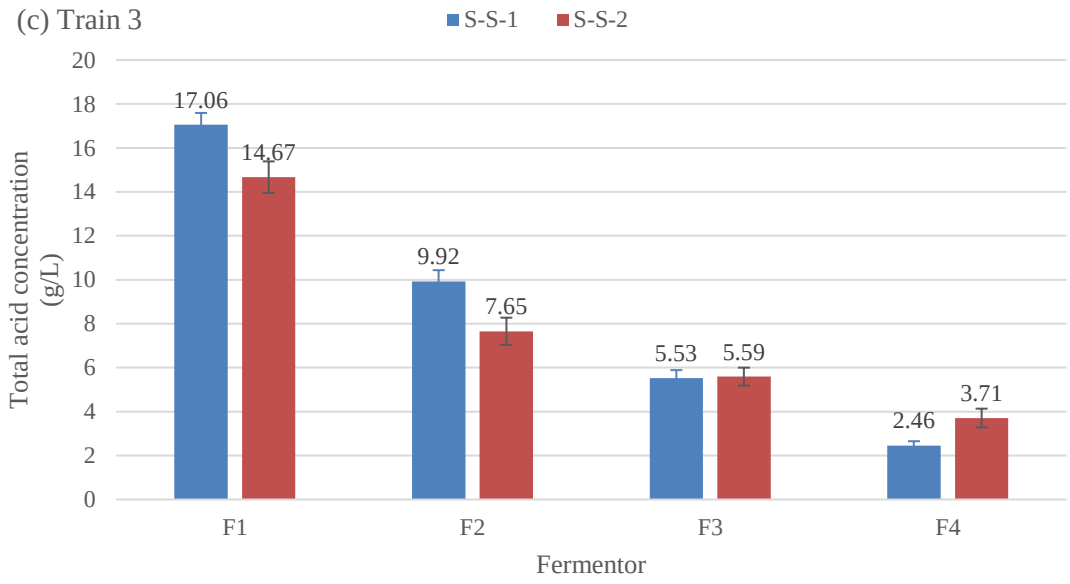


Figure 4-6. The average total acid concentration in each fermentor of four countercurrent trains, error bar = ± 1σ.



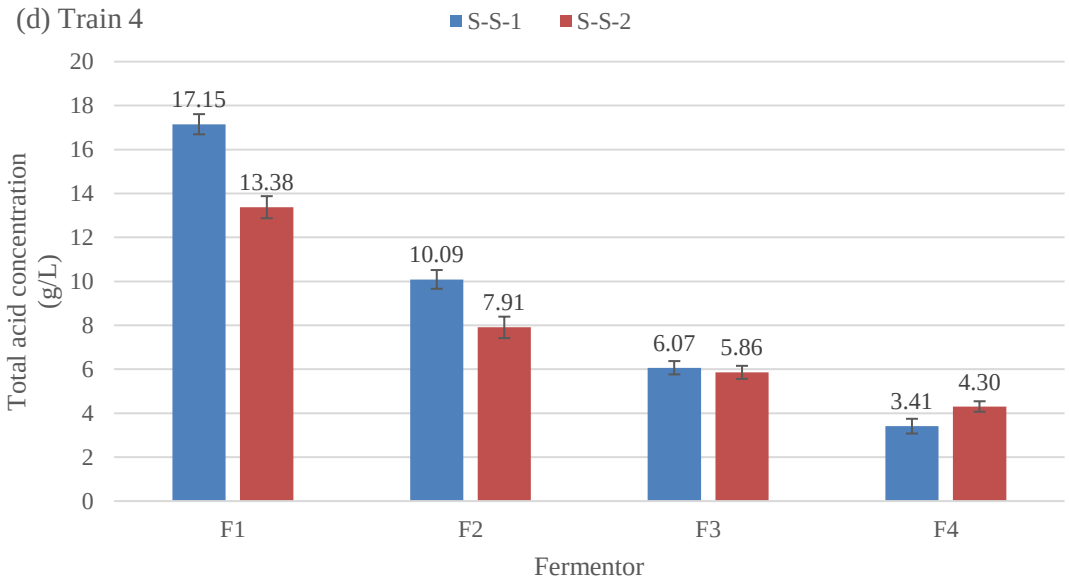


Figure 4-6 Continued

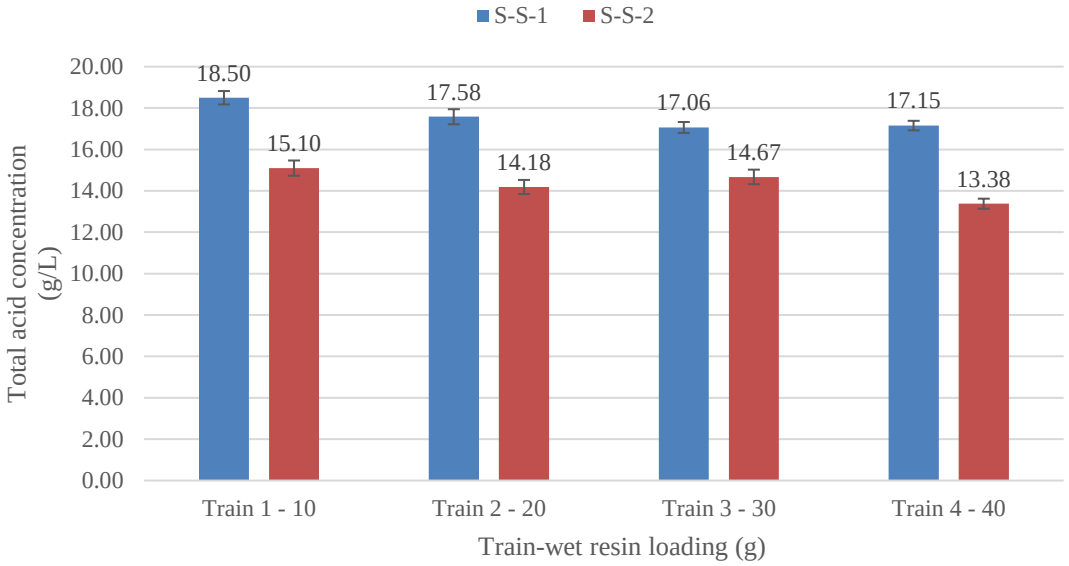


Figure 4-7. Total carboxylic acid concentration in liquid product of four countercurrent trains, error bar = ± 1σ.

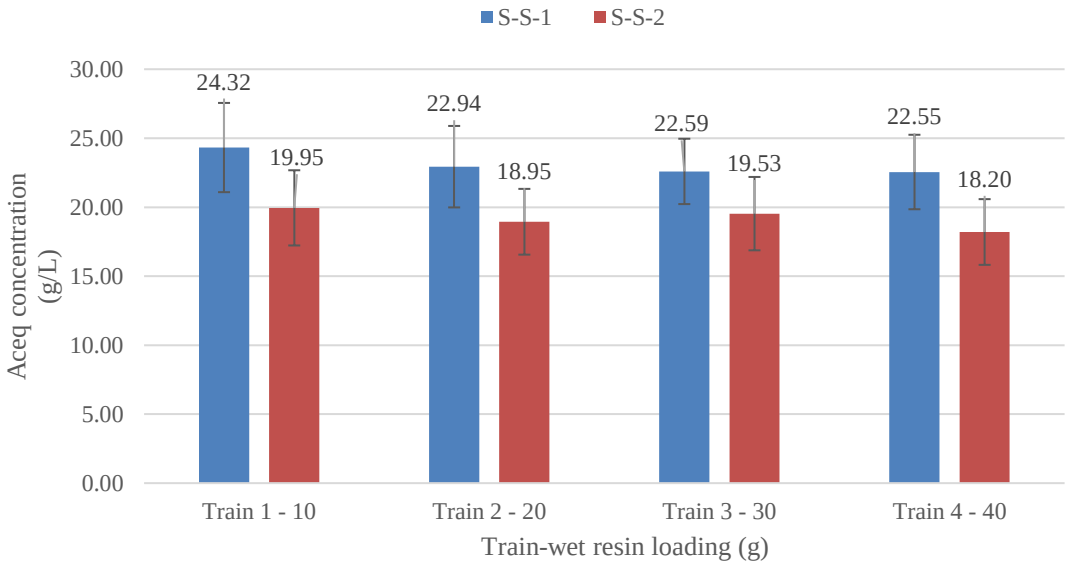


Figure 4-8. Aceq concentration in liquid product of four countercurrent trains, error bar = ± 1σ**.**

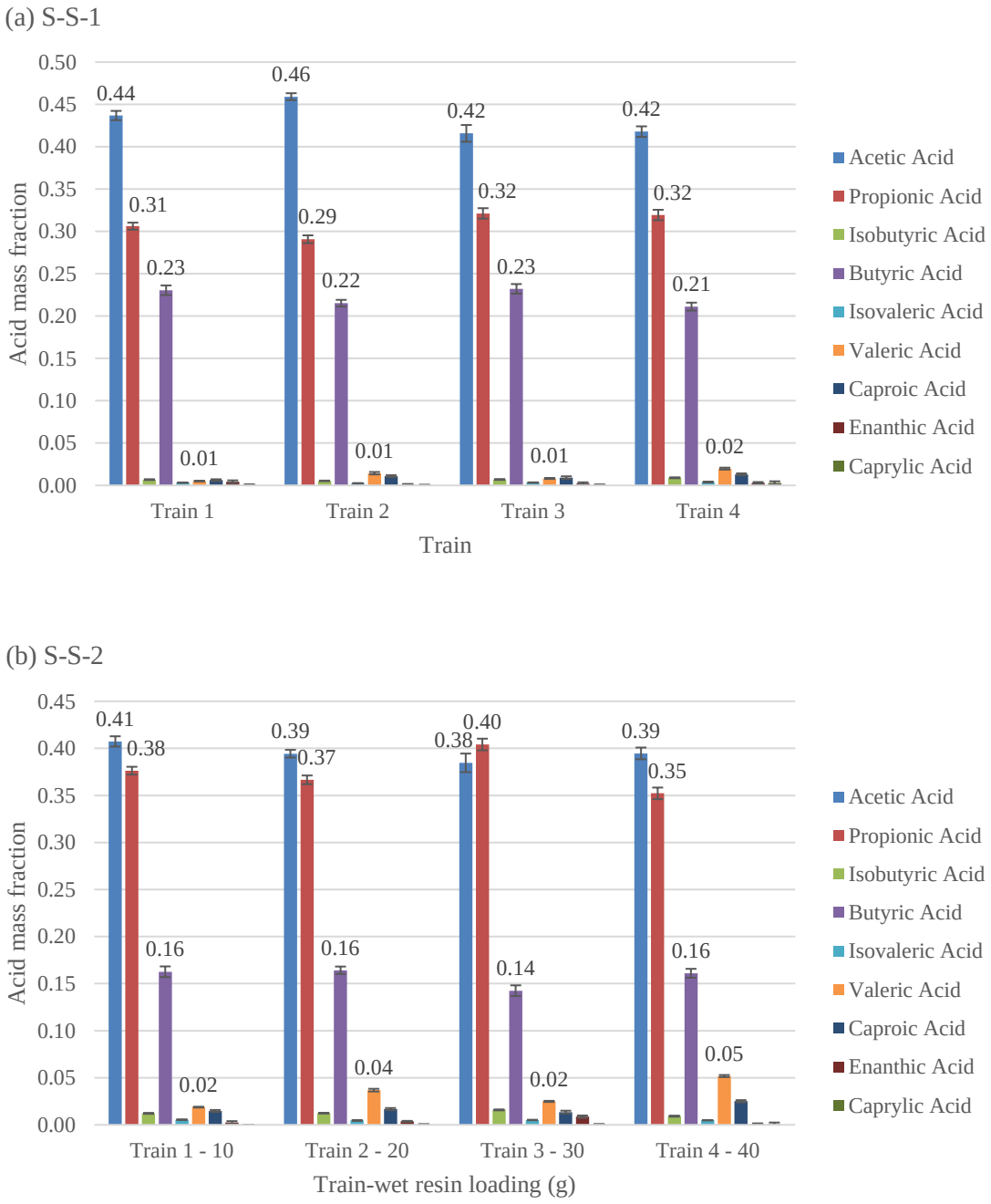


Figure 4-9. Carboxylic acid composition profiles in liquid product of four countercurrent trains, (a) S-S-1, (b) S-S-2, error bar = ± 95%CI.

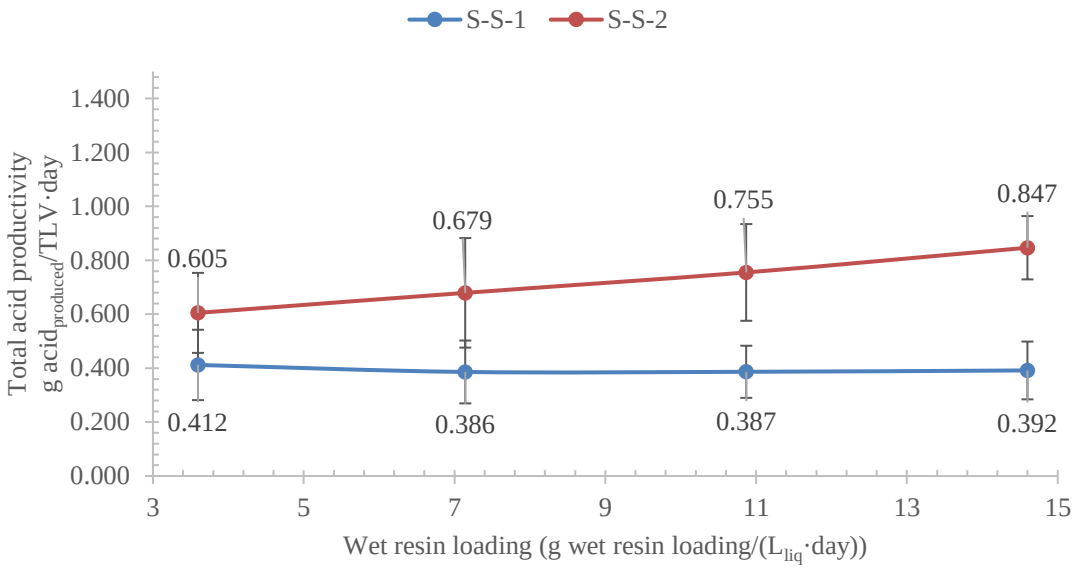


Figure 4-10. Carboxylic acid productivity with normalized wet resin loading, error bar = ± 1σ. (The issue of error bar overlapping was caused by fluctuations of mass transfer and could be solved by using the Slope Method when calculating acid yield.)

4.3.4 Fermentation performance

For countercurrent mixed-acid fermentation, the Slope Method was used to measure the fermentation performance. Figures 4-11 and 4-12 show the accumulations of the $NAVS_{feed}$, dry solid waste exit ($NADS_{exit}$), and total acid exit from two steady states in all trains. The slopes of linear regression models were then used to calculate biomass conversion, acid yield, and selectivity. Tables 4-6 and 4-7 list all fermentation parameters.

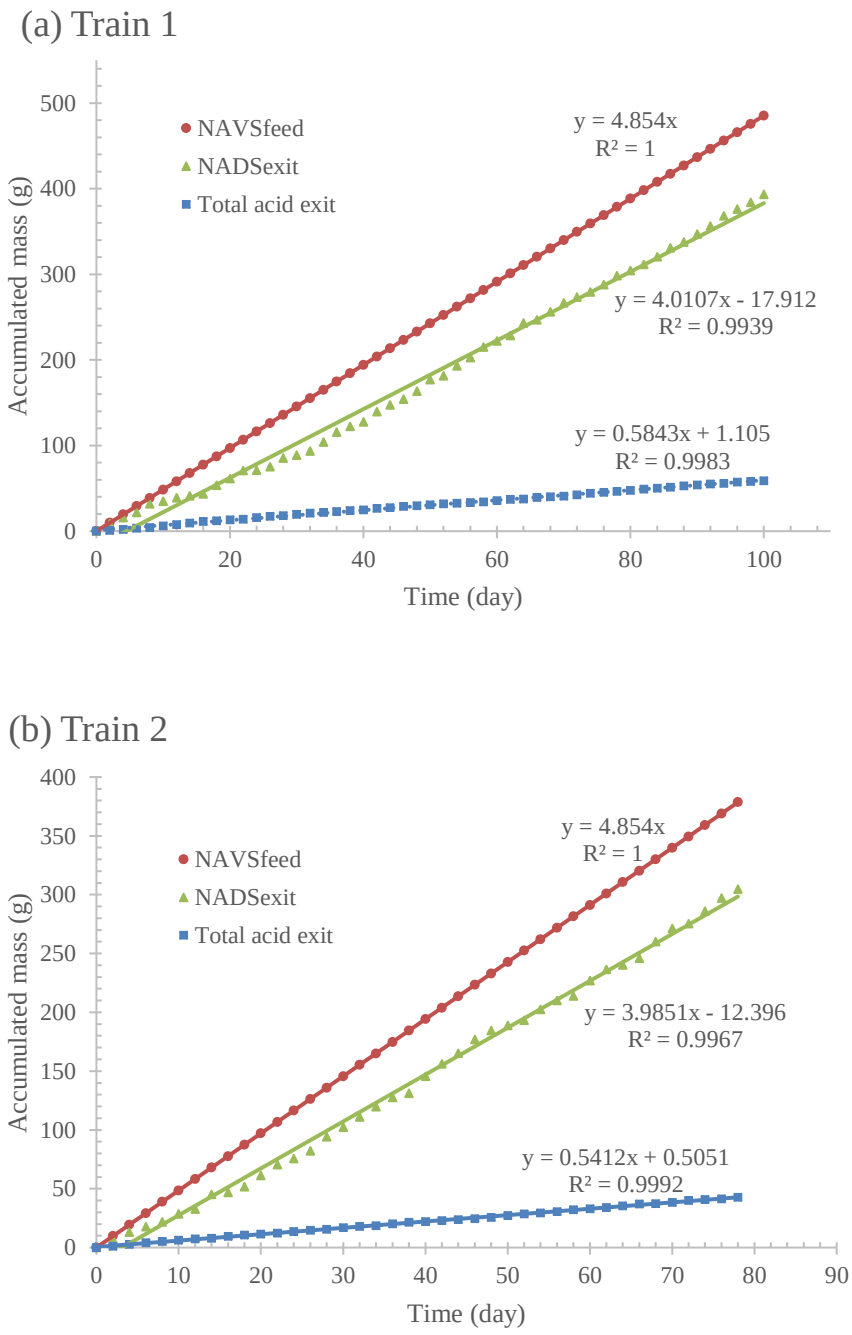
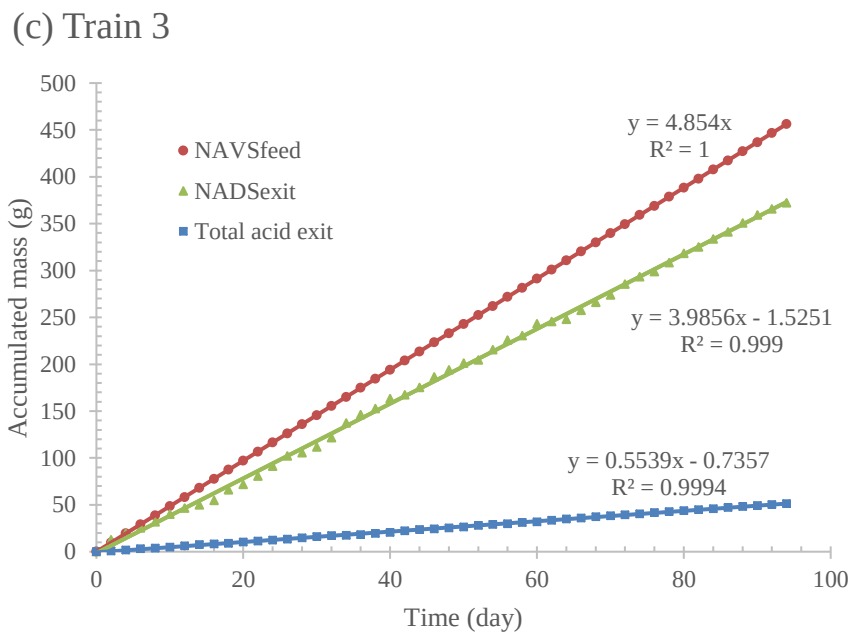


Figure 4-11. Accumulations of the $NAVS_{feed}$, $NADS_{exit}$, and total acid exit for countercurrent mixed-acid fermentation trains, 'Day 0' marks the Steady State 1 (S-S-1).



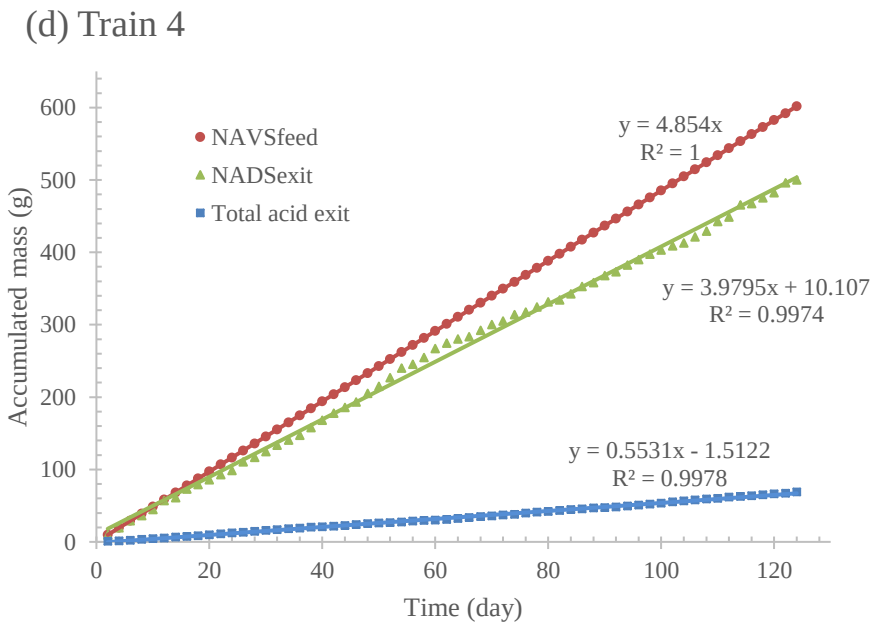


Figure 4-11 Continued

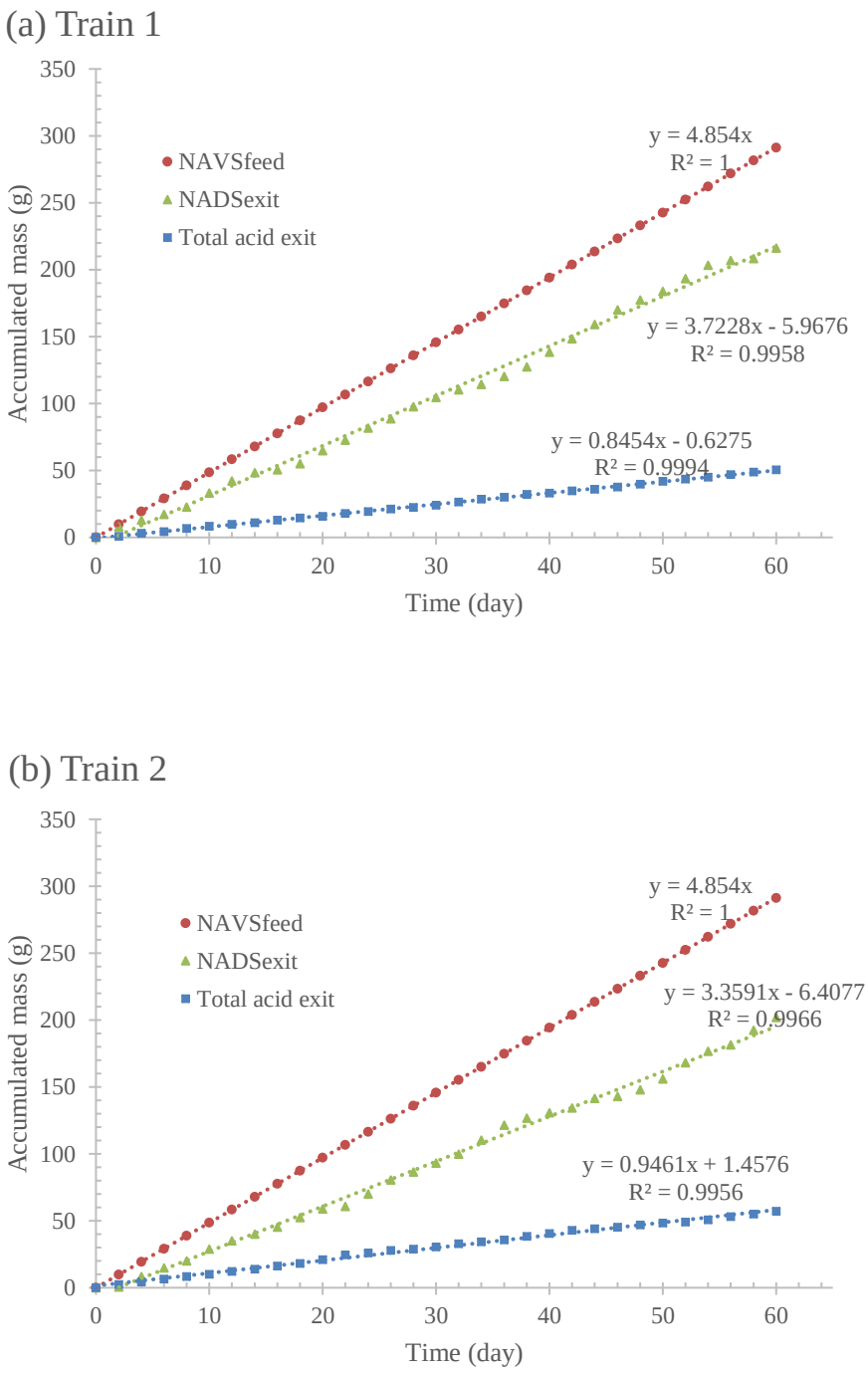
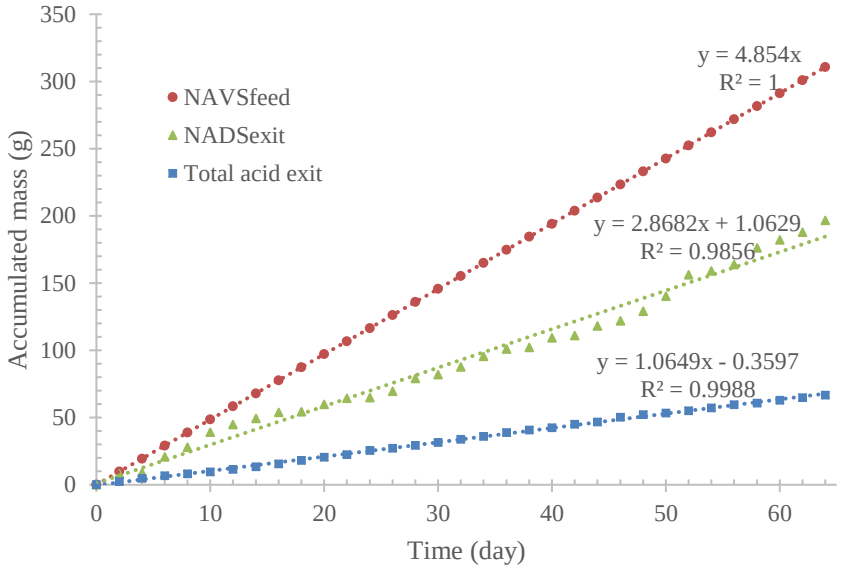


Figure 4-12. Accumulations of the $NAVS_{feed}$, $NADS_{exit}$, and total acid exit for countercurrent mixed-acid fermentation trains, 'Day 0' marks the Steady State 2 (S-S-2).

(c) Train 3



(d) Train 4

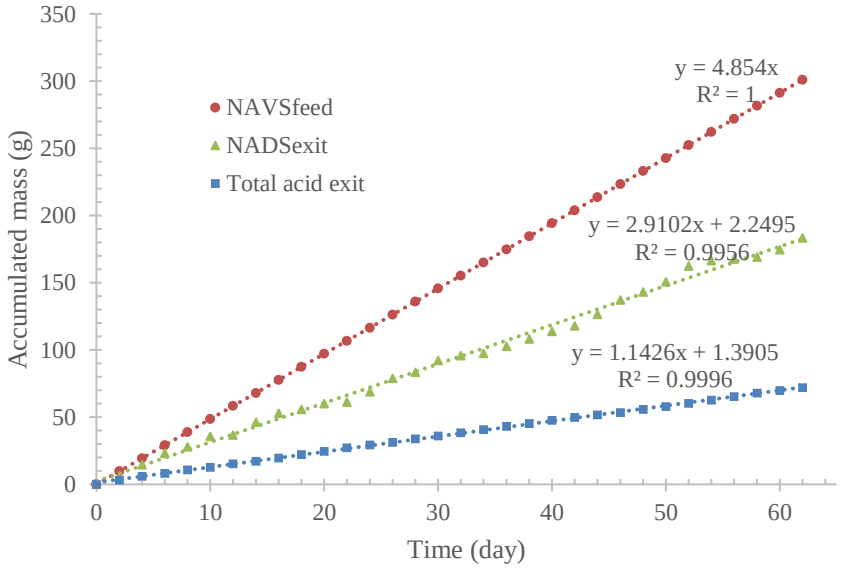


Figure 4-12 Continued

Biomass conversion, carboxylic acids yield, and selectivity of four trains during the two steady states were calculated by using Eqs. 2-4, 2-5, 2-6, and 2-7. For S-S-1, because they operated under the same condition, four countercurrent fermentation trains achieved similar conversion (17.80 ± 0.30%), yield (11.49 ± 0.38%) and selectivity (64.75 ± 3.10%). Thus, these parameters were used as controls to compare with fermentation performance gained from S-S-2 under various amount of normalized wet resin loading.

When *in-situ* acid adsorption was used during S-S-2, biomass conversion significantly increased by 34 to 128% compared to S-S-1. For all trains, the highest conversion was achieved in Train 3 with 10.9 g wet resin/ (L_{liq}·d) normalized wet resin loading (Figure 4-13). Because Train 4 (14.60 g wet resin/ (L_{liq}·d)) did not exhibit a higher conversion compared to Train 3, and Train 3 is significantly different with Train 4 by statistical analysis, it is reasonable to conclude that the optimal amount of wet normalized resin loading is 10.9 g wet resin/ (L_{liq}·d) provided the goal is to maximize the biomass conversion.

During S-S-2, carboxylic acid yield also significantly increased by 45 to 107%. Among all trains, the highest yield was achieved in Train 4 (Figure 4-14). Based on this graph, a steady increase in yield with increase normalized wet resin loading is observed. To find the optimum normalized resin loading for yield, countercurrent mixed-acid fermentation with higher normalized wet resin loading should be conducted.

Carboxylic acid selectivity was determined by biomass conversion and acid yield. Based on t-test, selectivity is significantly changed by adsorption among each train except Train 2. Figure

4-15 shows three periods of selectivity change with normalized wet resin loading. One possible hypothesis to describe the phenomenon is as follows:

1. When the amount of normalized resin loading was small (0–3.6 g wet resin/ (L_{liq}·d)), acid inhibition was lowered, and more biomass was used to produce acids than microorganism reproduction; thus, the yield increase was larger than the conversion increase, and selectivity improved.
2. When more resin was used in the system (3.6–10.9 g wet resin/ (L_{liq}·d)), the low acid concentration in fermentors allowed microorganism to reproduce, so biomass conversion largely increased. However, microorganisms were washed out during the adsorption process, but not severely; thus, yield did not increase as fast as conversion, and selectivity decreased.
3. When even higher amount of resin was used (10.9–14.6 g wet resin/ (L_{liq}·d)), microorganism washed out was severe. Even though acid inhibition was further lowered, yield only increased slightly and conversion started to decrease. As a result, selectivity improved again.

To fully understand the fluctuating selectivity, further studies with measurement of microorganism density in the fermentation-adsorption system should be performed.

Figure 4-16 shows the fermentation performance with normalized wet resin loading.

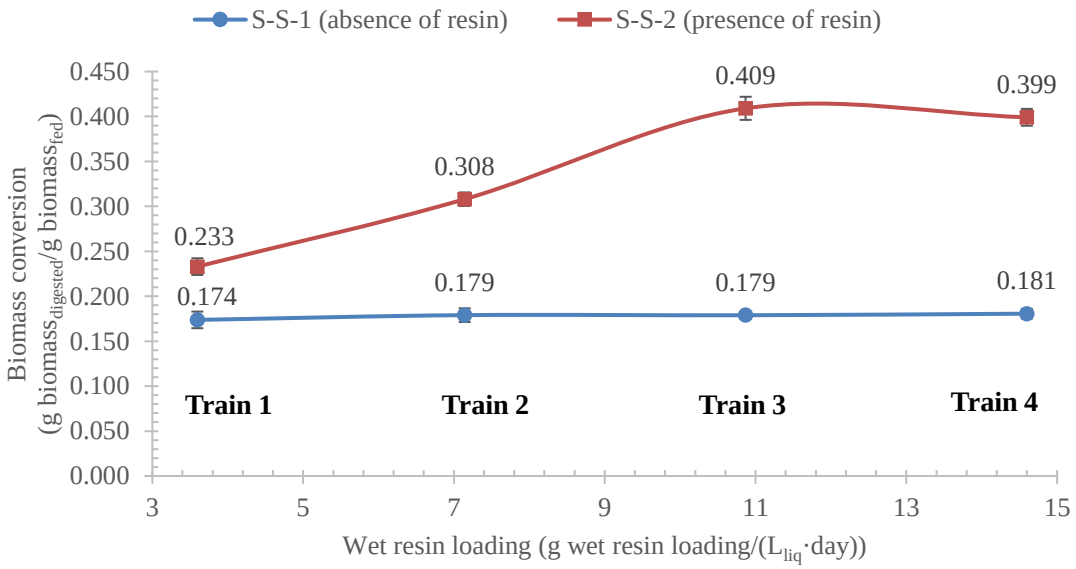


Figure 4-13. Conversion with normalized wet resin loading, error bar = ± 1σ.

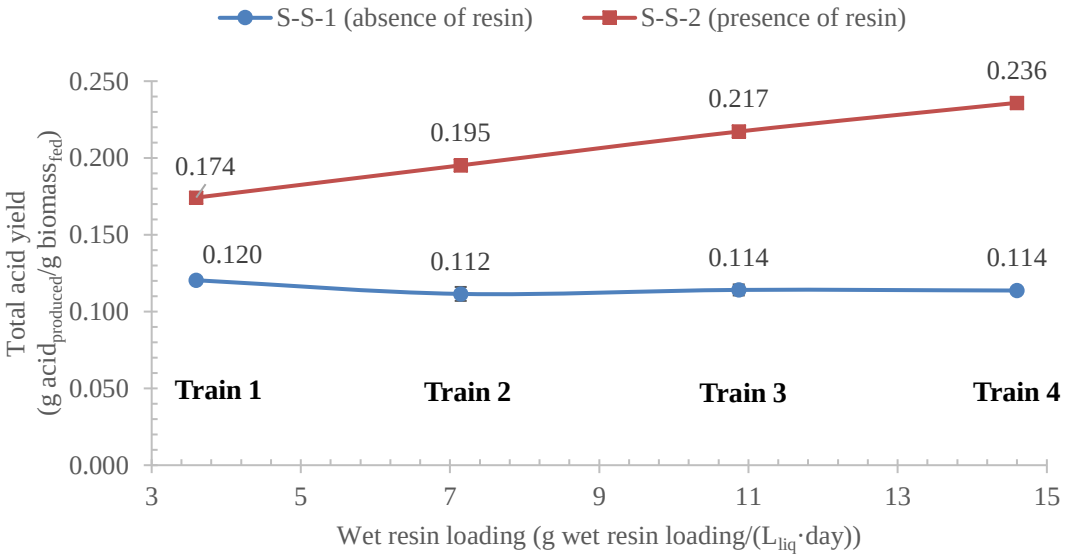


Figure 4-14. Yield with normalized wet resin loading, error bar = ± 1σ.

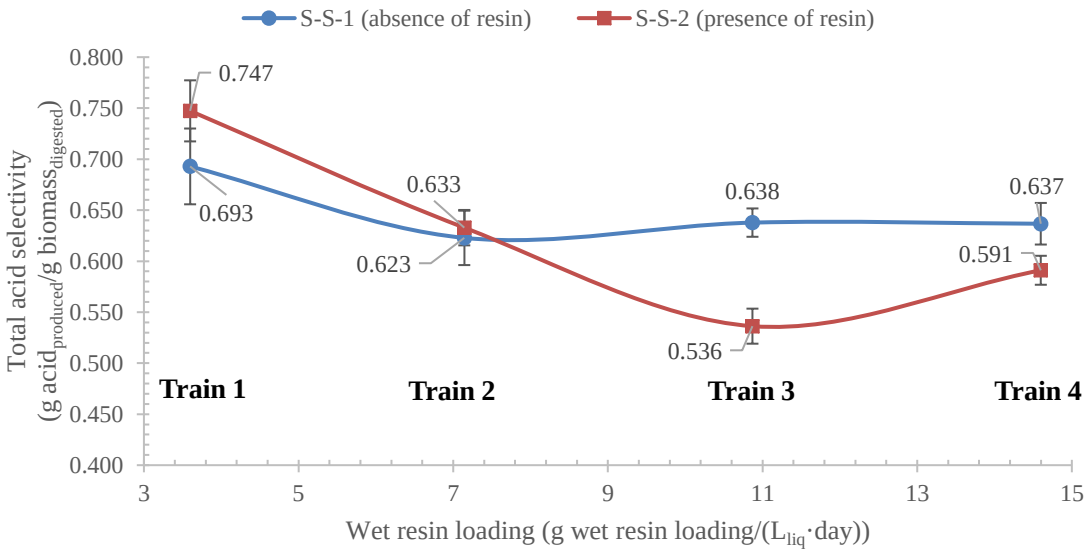


Figure 4-15. Selectivity with normalized wet resin loading, error bar = ± 1σ.

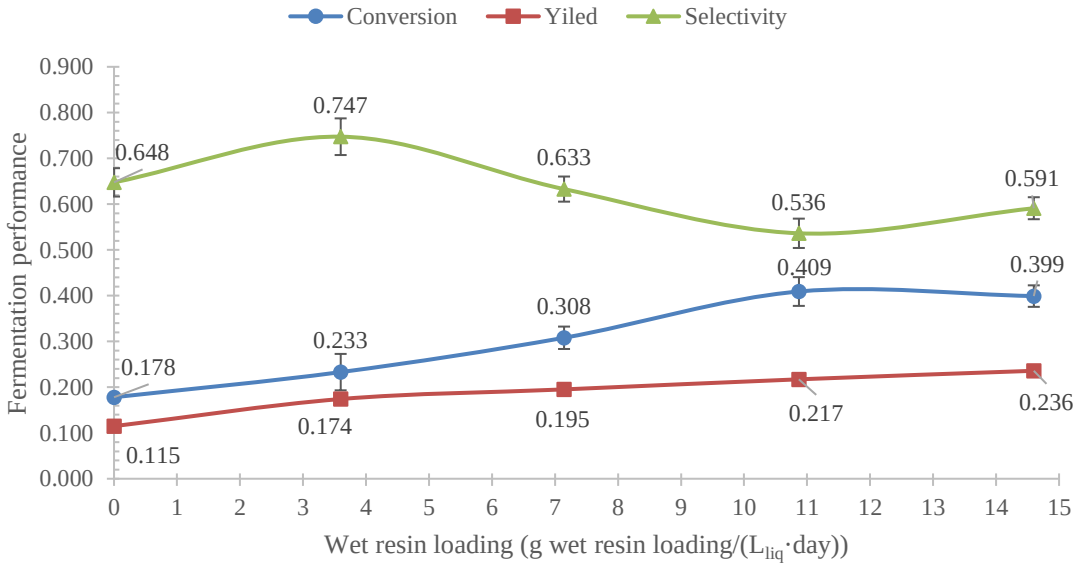


Figure 4-16. Fermentation performance with normalized wet resin loading, error bar = ± 1σ.

4.3.5 Regeneration performance

The resin columns were regenerated by using sodium hydroxide (1-N NaOH). Figure 4-17 compares the quantities of total acid initially adsorbed and later recovered. More than 80% total acid adsorbed on resin could be recovered. To calculate fermentation performance, total acid adsorbed onto the resin was used instead of the amount of acid recovered from the resin column.

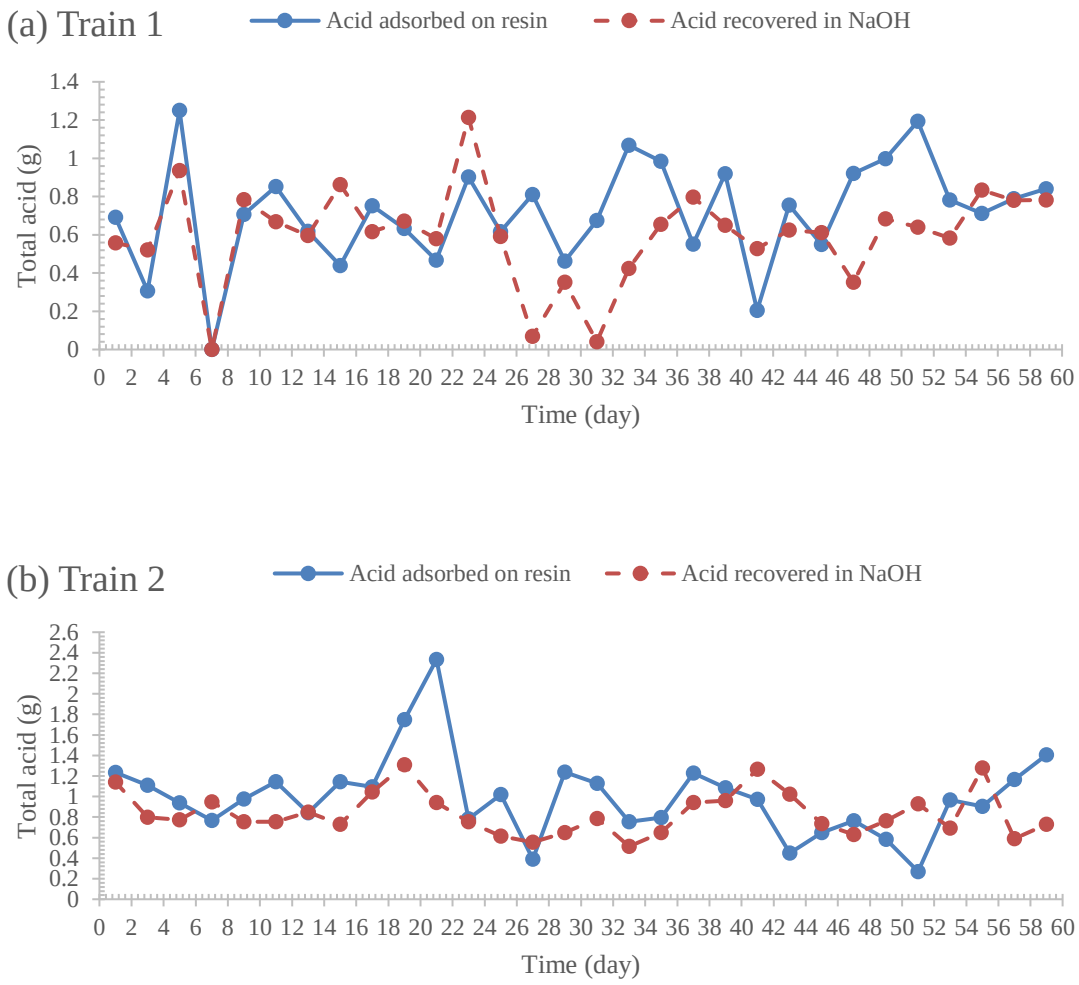
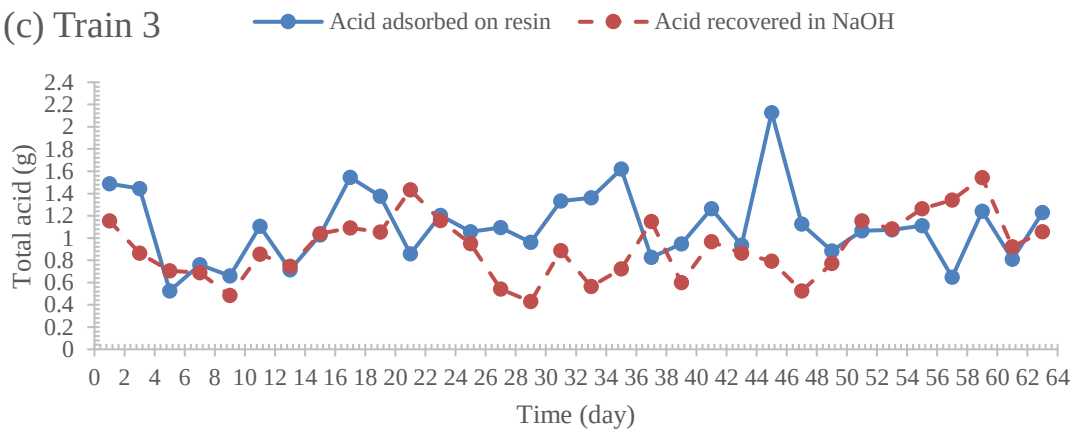


Figure 4-17. Acid adsorption and recovery in all trains.



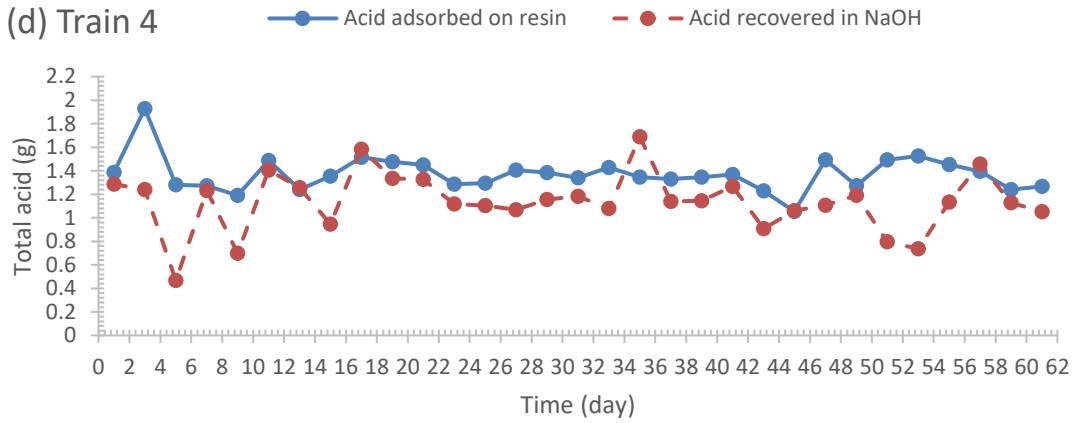


Figure 4-17 Continued

4.4 Conclusion

The procedure for countercurrent mixed-acid fermentation with *in-situ* CO_2-sustained ion-exchange resin adsorption was developed. The fermentation performance of countercurrent mixed-acid fermentation was significantly enhanced by using this newly developed adsorption system. In general, the amounts of resin loading affected production of both biogas and carboxylic acid. Based on this study, the recommended normalized wet resin loading is 10.9 g wet resin/(L_{liq}·d) for optimum biomass conversion. However, because the acid yield had not reached a maximum, further studies with higher resin loadings should be performed to fully understand the effect of resin adsorption on countercurrent fermentation and to determine the optimum normalized wet resin loading for acid production.

CHAPTER V

PROPAGATED FIXED-BED FERMENTATION WITH CO_2 SUSTAINED ION-EXCHANGE RESIN

5.1 Overview

The countercurrent strategy for mixed-acid fermentation achieves both high carboxylic acids concentrations and high substrate conversion.[28] However, at industrial scale, solid transportation in countercurrent mode is expensive and prone to failure, and thus is a major contributor of equipment costs and energy consumption. On the contrary, liquid handling is easily accomplished by using pumps and pipes.[19]

To overcome the shortcomings of the countercurrent strategy, fixed-bed fermentation was used in mixed-acid fermentation, which only involved liquid phase transportation between fermentors in series.[12] This configuration could achieve minimal solids handlings with high productivity and acids concentrations. However, unsteady acid concentrations are major concerns of fixed-bed fermentation, because biomass becomes less reactive as digested with time.

To solve the unsteady-state issue, propagated fixed-bed fermentation was then introduced into mixed-acid fermentation.[9] This fermentation configuration allows the fresh liquid to cascade through four fermentors from the most-digested one to the least-digested one, while the solid phase remains stationary. The fermentor with the most-digested biomass is periodically replaced by a fermentor with fresh biomass. Quasi-steady-state and near-constant acids concentrations could be achieved because of the propagation of fermentors.

Accumulation of carboxylic acids in fermentation broth have been reported to have an important toxic effect on the anaerobic processes.[29] To achieve better fermentation performance, proper methods of detoxification should be employed into the fermentation systems. Ion-exchange resin adsorption can be used as an *in-situ* separation method to adsorb carboxylic anions from the fermentors, and thus reduce acid inhibition. To maintain the fermentation pH in a near neutral range (6.8–7.2), CO_2 can be used as a buffer during adsorption.

In this study, one propagated fixed-bed mixed-acid fermentation was operated with the same NAVS loading as the countercurrent mixed-acid fermentation systems from Chapter IV. When quasi-steady-state operation of the propagated fixed-bed system was achieved, weak-base ion-exchange resin (IRA-67) was used in presence of CO_2 to adsorb carboxylate anions from fermentors. The acid concentrations and fermentation performance were analyzed and compared with the corresponding countercurrent mixed-acid fermentation using the same resin loading.

5.2 Experimental methods

5.2.1 Propagated fixed-bed fermentation

One continuous four-stage propagated fixed-bed mixed-acid fermentation train (Train P) was run using the same operation conditions as countercurrent trains (Figure. 5-1).

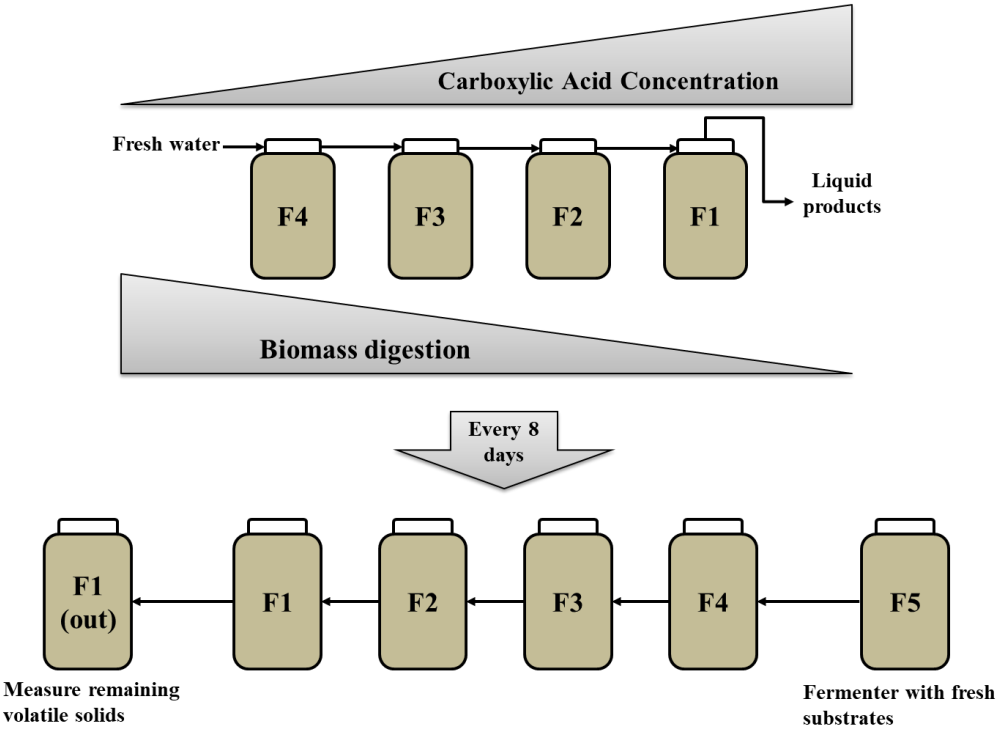


Figure 5-1. Diagram of propagated fixed-bed mixed-acid fermentation.

To grow the microflora, the fermentors in the train were initiated as batch cultures under anaerobic conditions at 40ºC. The substrates consisted of 80% dry paper and 20% dry homogenized chicken manure. The initial concentration of non-acid volatile solids (NAVS) in batch fermentors were achieved at 96.5 g NAVS/ L deoxygenated water by adding substrates, urea, inoculum, buffer, and liquid medium to each fermentor. Table 5-1 shows the detailed feed description of batch cultures.

Table 5-1. Feed composition for batch cultures.

Feed composition	Dry paper (g)	Dry chicken manure (g)	Deoxygenate water (mL)	Inocula (mL)	Urea (g)
All trains	32	8	350	50	0.8

After batch growth, liquid transfers were operated every 2 days in propagated fixed-bed fermentation from the most-digested fermentor (F1) to the least-digested fermentor (F4). In addition, every 8 days a new fermentor (F5) with fresh substrates (paper and chicken manure) was propagated into the train as new F4, and old F4, F3, F2 became new F3, F2, F1, respectively. The most-digested fermentor (F1 out) was removed for performance analysis. Table 5-2 lists the feeding parameters of propagated fixed-bed fermentation. The mass of liquid was kept according to a specified setpoint. The residence times of solid and liquid were normalized by choosing the transfer frequency (8 days for solid and 2 days for liquid).

For propagated fixed-bed fermentation, fermentors were removed from the incubator every 48 h. After cooling down to room temperature, gas samples from fermentors were collected, and gas volume was measured. The vented fermentors were centrifuged at 4000 rpm (4050 × g) for 10 min to separate the solid and liquid phases. The centrifuged liquid of each fermentor was decanted into a beaker and weighted. Liquid samples (1.5 mL) were taken from each beaker, and then the initial pH of liquid phase was measured by a calibrated pH meter. The weight of remaining solid in each fermentor was measured. The retained liquid weights in fermentors were controlled by mass setpoint, and the liquid mass removed was determined by a material balance (Eq. 4-5).

Fresh deionized water and urea were added into F1 at a constant amount. The pH after liquid transfer in each fermentor was then measured and buffer was added to neutralize the carboxylic acids and maintain the liquid phase pH in a near-neutral range (6.8–7.2). To inhibit methane production, 120 μL methane inhibitor (20 g CHI_3 /L, 200-proof ethanol) was added into each fermentor at the end of operation. Before returning to the incubator, each fermentor was purged with nitrogen and properly resealed to create an anaerobic environment for microorganisms. Appendix B lists the detailed propagated fixed-bed mixed-acid fermentation procedure. Table 5-2 provides operation parameters and the normalized parameters for the mean of quasi-steady-state values.

Table 5-2. Operating parameters for propagated fixed-bed mixed-acid fermentations, error bar = ± 1σ.

	Train P	S-S-1	S-S-2
Controlled	Solid and liquid transfer frequency, T_{solid} (day)	8	8
	Liquid transfer frequency, T_{liquid} (h)	48	48
	NAVS feed rate (gNAVS/T_{Solid})	38.83	38.83
	Paper (g/T_{solid})	10.2	10.2
	Chicken manure (g/T_{Solid})	2.4	2.4
	Urea added (g/T_{liquid})	0.2	0.2
	Liquid feed rate (mL/T_{liquid})	120	120
	Fermentor retention time, FRT (day)	28	28
	Centrifuge liquid retained in each fermentor (g)	200	200
	Methane inhibitor (μL/ T_{liquid} ·Fermentor)	120	120
Normalized	Volatile solid loading rate, VSLR (g NAVS/(L_{liq}·d))	3.35 ± 0.10	3.42 ± 0.11
	Liquid residence time, LRT (d)	28.10 ± 0.86	27.54 ± 0.88
	Total liquid volume, TLV (L)	1.45 ± 0.04	1.42 ± 0.04
	Total NAVS (g)	136.72 ± 6.76	131.16 ± 6.90
	Volatile solid, VS, concentration (g NAVS/L_{liq})	94.48 ± 5.45	92.49 ± 5.66
	Carbon-nitrogen ration in feed, C-N ratio (g OC_{NA}/g N)	30.31 ± 0.00	30.31 ± 0.00
	Urea addition rate (g urea/(L_{liq}·d))	0.07 ± 0.00	0.07 ± 0.00

5.2.2 CO_2-sustained fluidized-bed ion exchange resin adsorption

5.2.2.1 Ion exchange resin loading

To compare the effect of resin adsorption on fermentation performance between countercurrent strategy and propagated fixed-bed strategy, 30 g wet resin was used in propagated fixed-bed fermentation system.

5.2.2.2 Adsorption operation

After the propagated fixed-bed fermentation train reached the quasi-steady-state region, CO_2-sustained ion exchange resin adsorption was applied to fermentation broth to adsorb carboxylate anions produced by microorganisms (Figure 5-2).

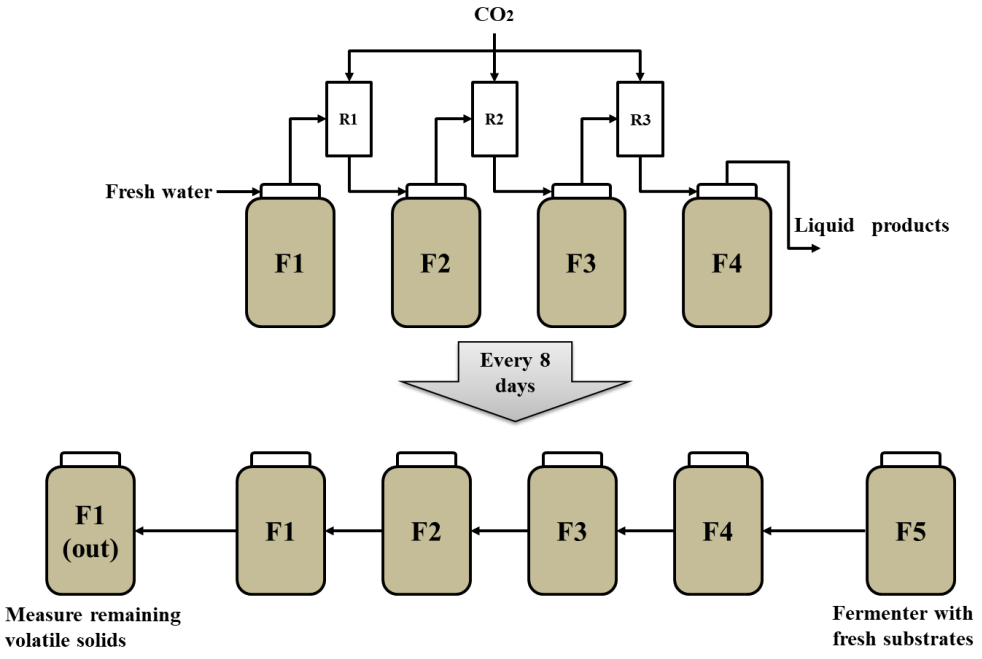


Figure 5-2. Diagram of CO_2-sustained ion exchange resin adsorption with propagated fixed-bed mixed-acid fermentation.

For the propagated fixed-bed fermentation train, a specified amount of resin was loaded into one adsorption column (R). Every 48 h, fermentation broth removed from F1, F2 and F3 was passed through adsorption column in the presence of CO_2, ordered respectively as R1, R2, and R3. To be clear, only one adsorption column was used for this adsorption process, and R1, R2, R3 only denote the order of adsorption.

The mass of removed fermentation broth from each fermentor was determined by Eq. 4-5. To reduce the length of periodic air exposure, the operation time for unit adsorption recycle was set to 10 min. To make full use of the resin adsorption capacity within the limited operation time, the flowrate of CO_2 was set to 1.5 L/min to fully fluidize the resin bed. The circulation of fermentation broth in adsorption column was set to 1.34 mL/s. After each adsorption, 1.5-mL liquid sample was taken from the effluent. Appendix D describes the detailed procedure of resin adsorption.

The amount of carboxylic acids adsorbed onto the resin column was calculated by Eq. 4-7.

5.2.2.3 Resin regeneration

The procedure for regenerating resin was previously described in Section 4.2.2.3.

5.2.3 Statistical analysis

In this study, statistical analysis was performed by Microsoft Excel. For propagated fixed-bed mixed-acid fermentation, the quasi-steady-state region was the period during which the product total acid and acetic acid equivalent (Aceq) concentration, dry solids exiting, liquid volume and solid weight of each fermentor, pH, and mass of acid exiting did not vary by more than 20% from the average for a period of at least one liquid residence time. [9] Based on the data, the time required for propagated fixed-bed train to reach the steady-state region (S-S-1) was around 3

months. However, the new steady-state region (S-S-2) with resin adsorption could be readily achieved within 1 week.

The mean and standard deviations of the total acid concentrations, Aceq concentration, non-acid volatile solids (NAVS) in and out were determined from the quasi-steady-state region data. With the Slope Method, these values were then used to calculate the fermentation performance, such as acid productivity (P), selectivity (σ), yield (Y), and conversion (x). All error bars are reported at one standard deviation. The two samples t-test (two-tailed, unequal variance, $\alpha = 0.05$) was used to compute the p values between the performance variables of the trains. To control Type I Error (familywise error rate), the Bonferroni correction was used.

5.3 Results and discussion

5.3.1 Biogas analysis

After applying resin adsorption, the biogas production increased. Figure 5-3 compares the results with countercurrent fermentation (Train 3). At S-S-1, the amount biogas produced for Train P was 0.44 ± 0.07 gas/d, which was slightly higher than the biogas production found in Train 3. And the biogas compositions were similar between the two trains. At S-S-2, the amounts biogas produced for Train P was 0.57 ± 0.12 L gas/d, which was lower than then biogas production from Train 3 with same wet resin loading (30 g). The biogas compositions were similar between the two trains. By adding iodoform as methane inhibitor, no methane was detected in any fermentors during each steady-state region.

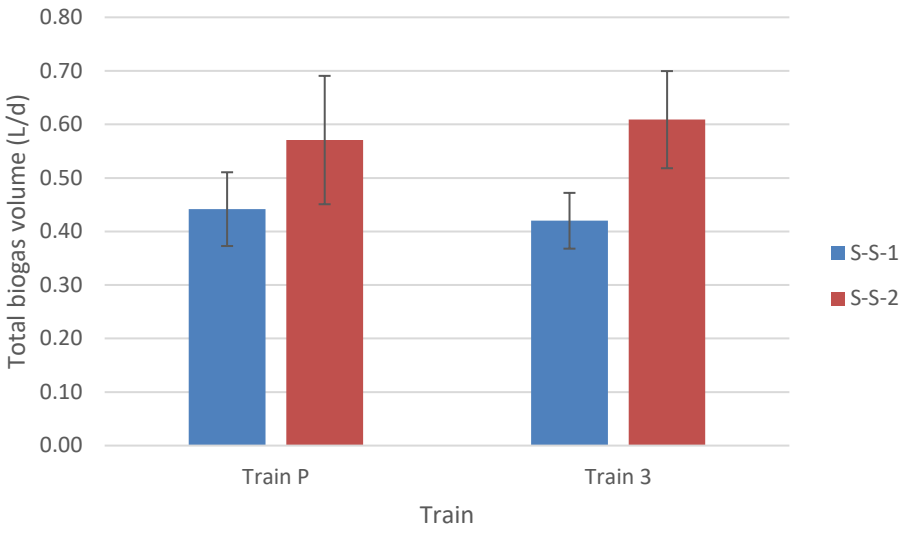


Figure 5-3. Total biogas production in trains, error bar = ± 1σ.

5.3.2 pH

Table 5-3 lists the average of pH measured before mass transfer (pre-transfer pH) in each fermentor. In this study, the pH control process for propagated fixed-bed fermentation was identical to the countercurrent fermentations. The average of pre-transfer pH in each fermentor for S-S-1 and S-S-2 were 6.02 to 6.42 and 6.44 to 6.63, respectively.

Table 5-3. The average of pre-transfer pH in each fermentor for S-S-1 and S-S-2, error bar = ± 1σ.

Fermentor	F1	F2	F3	F4
S-S-1	6.38 ± 0.23	6.42 ± 0.20	6.33 ± 0.21	6.02 ± 0.32
S-S-2	6.44 ± 0.46	6.44 ± 0.46	6.44 ± 0.43	6.63 ± 0.28

5.3.3 Acid production

Figure 5-4 shows the total carboxylic acid concentration in each stage and liquid product with time. In all fermentors, acid concentrations were maintained in a narrow range during steady states. Similar to countercurrent fermentations, a new steady state with resin adsorption was readily achieved. Figure 5-5 shows the average total acid concentrations in each fermentor during S-S-1 and S-S-2. Based on the data, total acid concentrations decreased in all fermentors after applying acid adsorption, which was different from the phenomenon found in countercurrent trains. The possible reason will be discussed later with other fermentation parameters.

Respectively, Figures 5-6 and 5-7 show the comparisons of total carboxylic acid concentrations, and Aceq concentrations in liquid products for both S-S-1 and S-S-2. Countercurrent fermentation exhibited higher total acid and Aceq concentrations in both steady states.

Figure 5-8 shows the carboxylic acid composition profile in liquid product of Train P. Compared with the acid fraction of countercurrent trains in Figure 4-9, Train P had a significantly higher composition toward long-chain carboxylic acids (butyric acid, valeric acid, and caproic acid). Also, although acetic acid, propionic acid, and butyric acid were still the primary components of liquid product, butyric acid became the most abundant acid in Train P liquid product during S-S-2 instead of propionic acid during S-S-1.

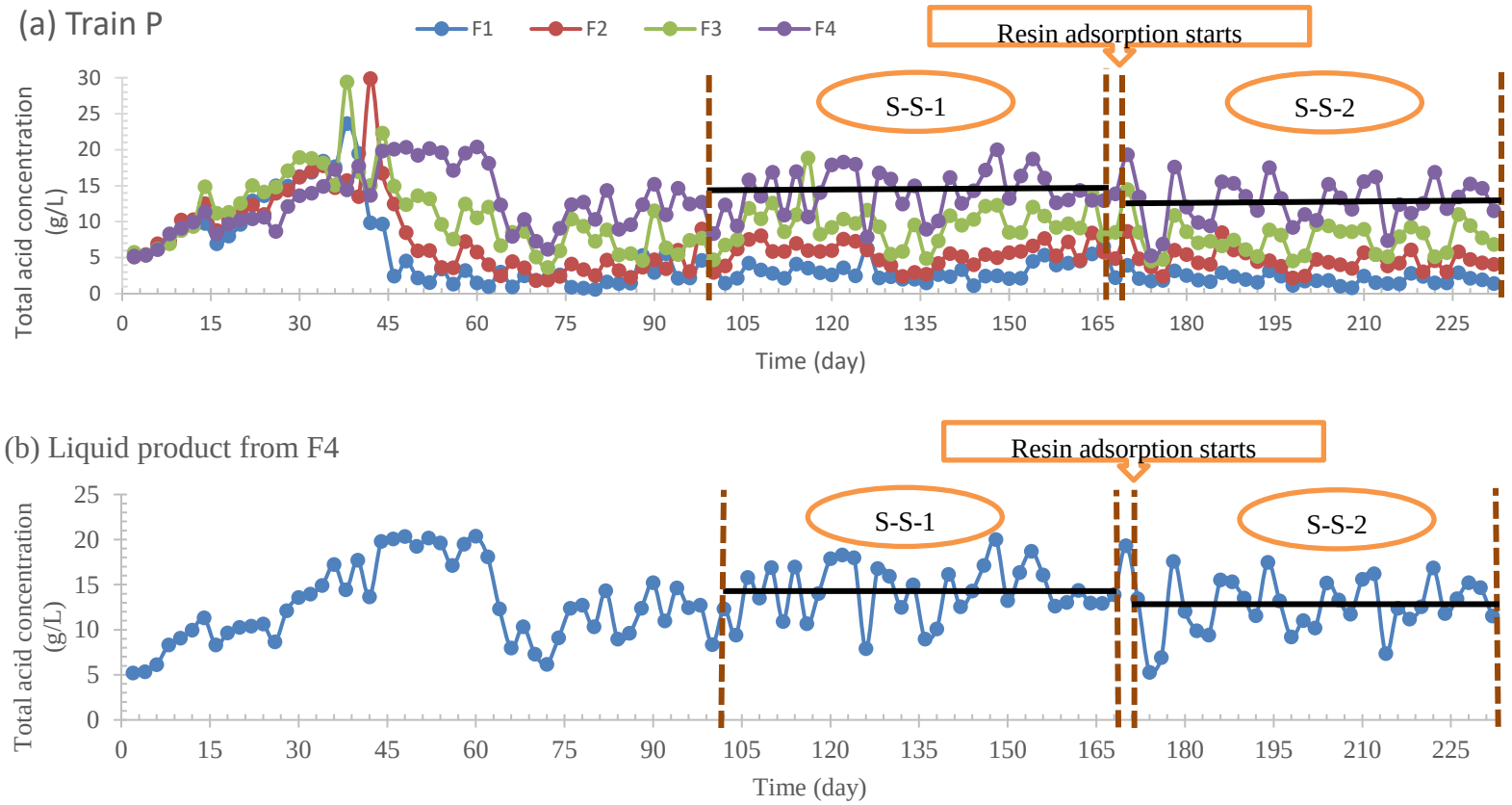


Figure 5-4. Total carboxylic acid concentration in each stage of propagated fixed-bed mixed-acid fermentation and liquid product.

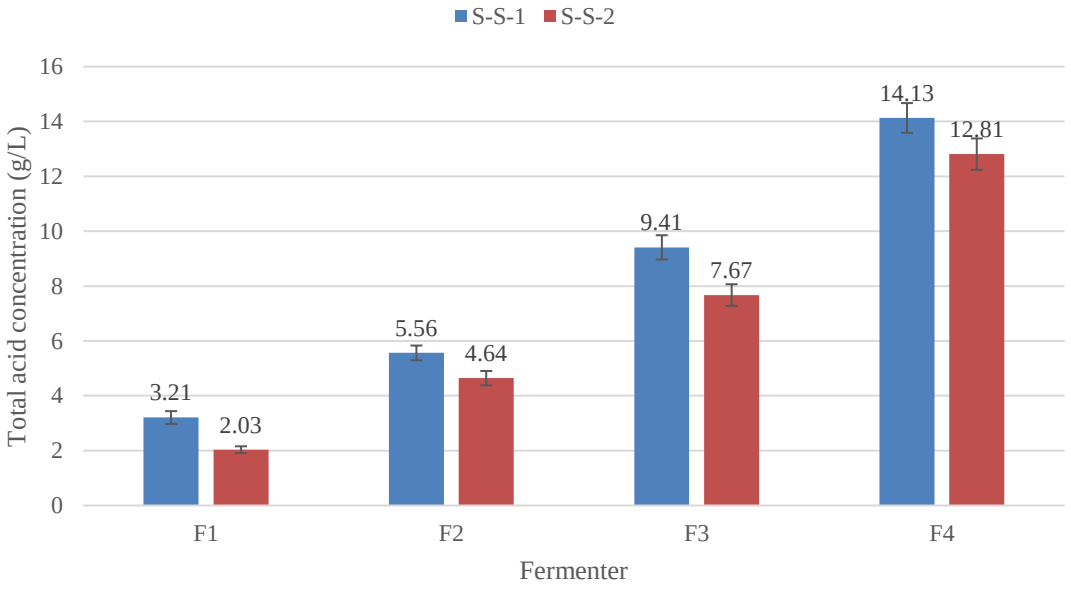


Figure 5-5. The average total acid concentration in each fermentor of propagated fixed-bed fermentation, error bar = ± 1σ.

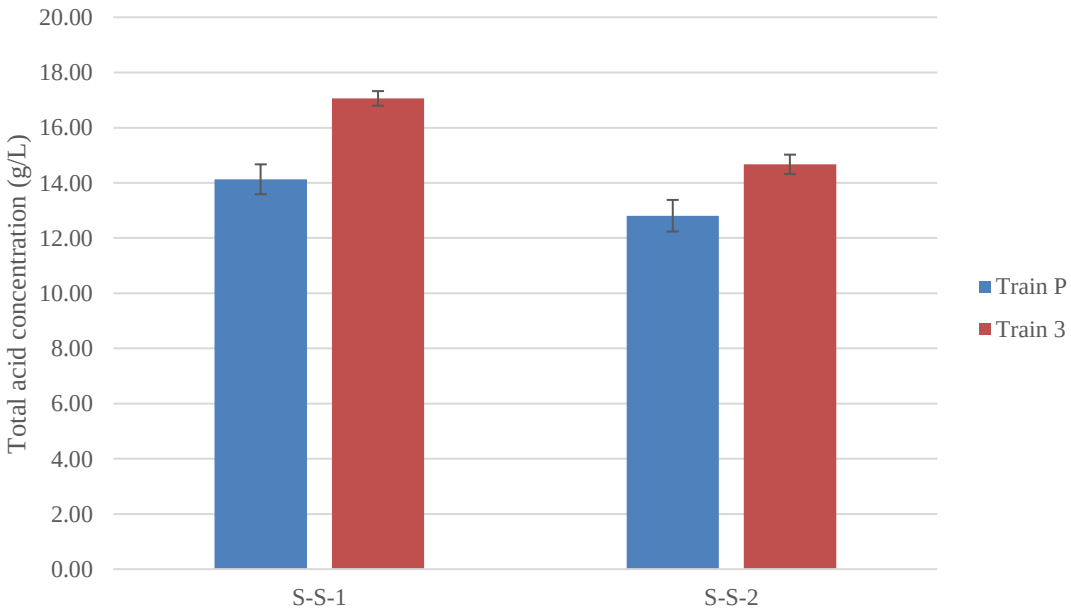


Figure 5-6. Comparison of the averages of total carboxylic acid concentration in liquid products between propagated fixed-bed and countercurrent trains, error bar = ± 1σ.

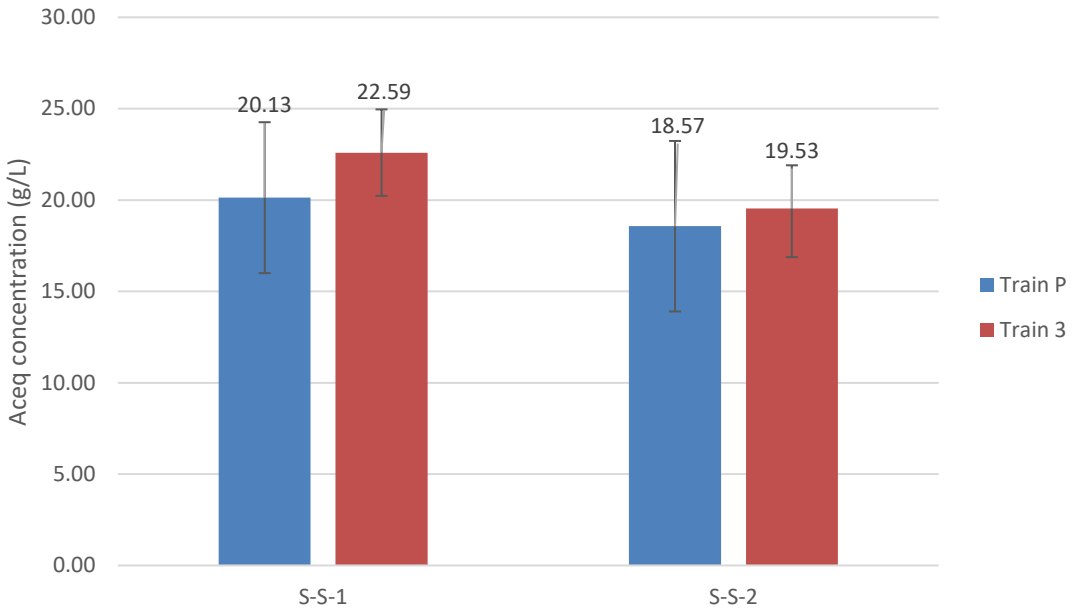
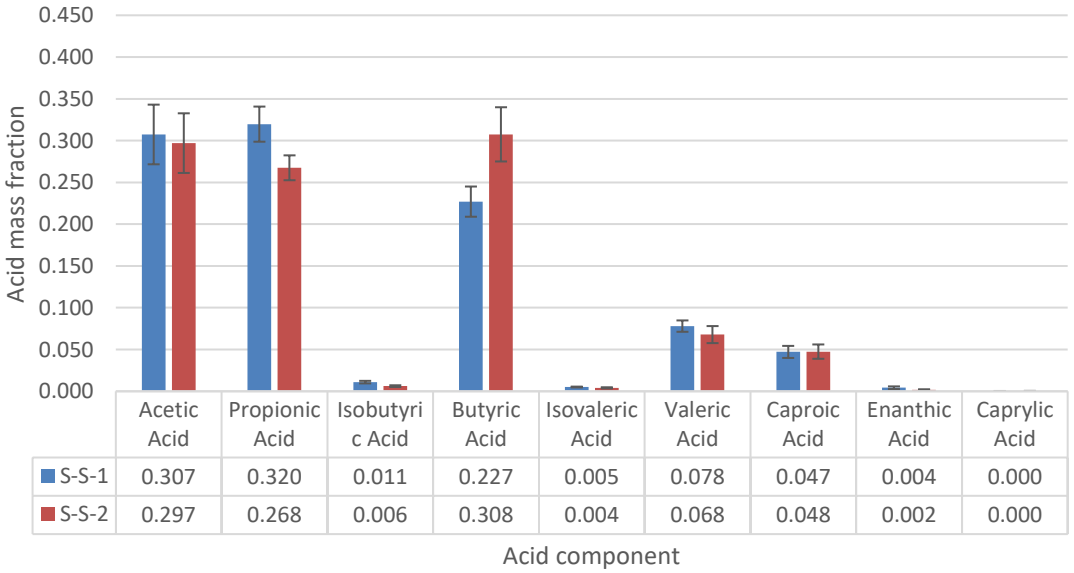


Figure 5-7. Comparison of the averages of Aceq concentration in liquid products between propagated fixed-bed and countercurrent trains, error bar = ± 1σ.



	Acetic Acid	Propionic Acid	Isobutyric Acid	Butyric Acid	Isovaleric Acid	Valeric Acid	Caproic Acid	Enanthic Acid	Caprylic Acid
S-S-1	0.307	0.320	0.011	0.227	0.005	0.078	0.047	0.004	0.000
S-S-2	0.297	0.268	0.006	0.308	0.004	0.068	0.048	0.002	0.000

Figure 5-8. Carboxylic acid composition profiles in liquid product of Train P, error bar = ± 95%CI.

5.3.4 Fermentation performance

For propagated fixed-bed mixed-acid fermentation, the Slope Method was used to measure the fermentation performance. Figures 5-9 and 5-10 show the accumulations of the $NAVS_{feed}$, dry solid waste exit ($NADS_{exit}$), and total acid exit from the two quasi-steady-state regions. The slopes of linear regression models were then used to calculate biomass conversion, acid yield, and selectivity. Table 5-4 lists all fermentation parameters.

Table 5-4. Fermentation performance for Train P, error bar = ± 1σ.

Fermentation performance	S-S-1	S-S-2
Total carboxylic acid concentration (g/L)	14.13 ± 0.54	12.81 ± 0.57
Aceq concentration (g/L)	20.13 ± 4.13	18.57 ± 4.67
Aceq/Acid ratio	1.42 ± 0.30	1.45 ± 0.37
Conversion, *x* (g $NAVS_{digested}$/g $NAVS_{fed}$)	0.262 ± 0.024	0.210 ± 0.034
Yield, *Y* (g total acid/g $NAVS_{fed}$)	0.092 ± 0.002	0.151 ± 0.004
Selectivity, σ (g total acid produced/g $NAVS_{consumed}$)	0.351 ± 0.032	0.718 ± 0.115
Productivity, *P* (g total acid/(L_{liq}·d))	0.320 ± 0.220	0.528 ± 0.300

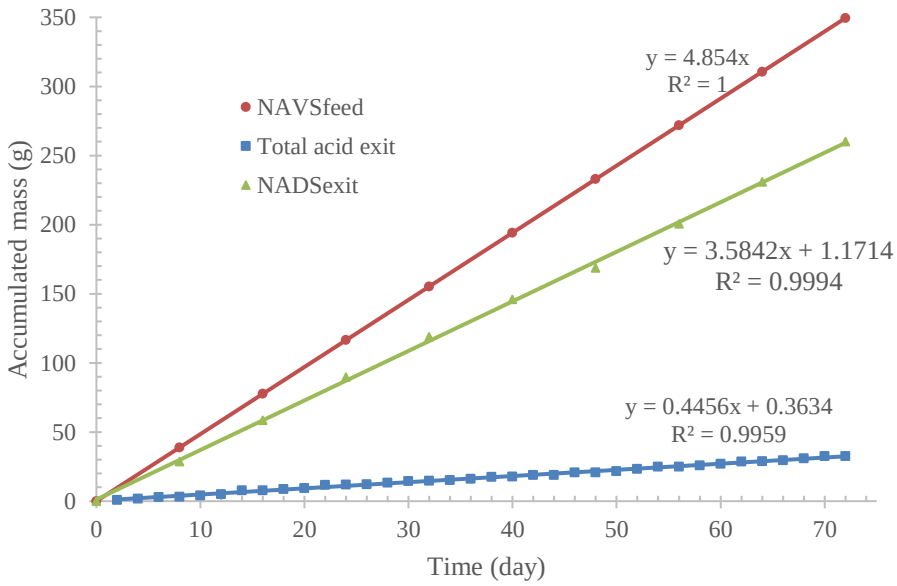


Figure 5-9. Accumulations of the $NAVS_{feed}$, $NADS_{exit}$, and total acid exit for propagated fixed-bed fermentation, 'Day 0' marks the Steady State 1 (S-S-1).

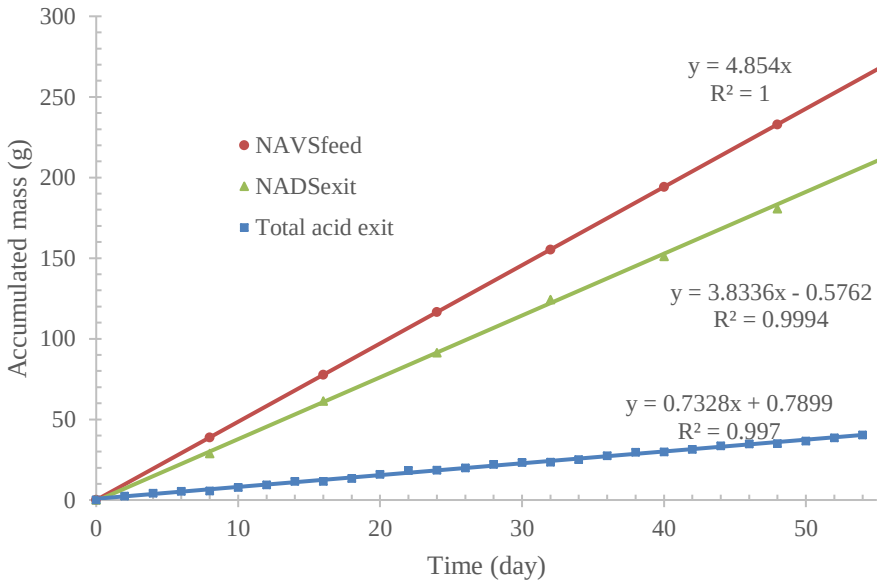


Figure 5-10. Accumulations of the $NAVS_{feed}$, $NADS_{exit}$, and total acid exit for propagated fixed-bed fermentation, 'Day 0' marks the Steady State 2 (S-S-2).

The carboxylic acid yield and selectivity of the fermentation system was enhanced with *in-situ* acid adsorption. However, the biomass conversion was lowered, which was possibly caused by lower microorganism density after applying adsorption. This hypothesis is also supported by the observation mentioned earlier that acid concentration in most-digested fermentor (F1) decreased after using adsorption. In this propagated fixed-bed strategy, solids are transferred every 8 days, but liquid transfer is conducted every 2 days. When resin is applied, the amount of microorganism that are initially supposed to be transferred to the fermentors are eventually kept in resin column and washed out during the regeneration process; thus, the microorganism density tends to reduce.

Table 5-5 compares the fermentation performance between Train P and Train 3. When fermentation systems were operated without adsorption, higher conversion were achieved by Train P; however, the acid yield and selectivity of Train 3 were higher than Train P. When the adsorption process was introduced into the fermentations, the conversion and yield of Train 3 were significantly increased and much higher than Train P.

Table 5-5. Fermentation performance of Train P and Train 3, error bar = ± 1σ.

	S-S-1		S-S-2	
	Train P	Train 3	Train P	Train 3
Conversion, *x* (g $NAVS_{digested}$/g $NAVS_{fed}$)	0.262 ± 0.024	0.179 ± 0.004	0.210 ± 0.034	0.409 ± 0.013
Yield, *Y* (g total acid/g $NAVS_{fed}$)	0.092 ± 0.002	0.114 ± 0.004	0.151 ± 0.004	0.217 ± 0.001
Selectivity, *σ* (g total acid produced/g $NAVS_{consumed}$)	0.351 ± 0.032	0.638 ± 0.014	0.718 ± 0.115	0.536 ± 0.017
Productivity, *P* (g total acid/(L_{liq}·d))	0.320 ± 0.220	0.387 ± 0.097	0.528 ± 0.300	0.755 ± 0.179

Table 5-6. *p* values for fermentation performance for Train P and Train 3, α=0.05.

Trains	Conversion	Yield	Selectivity	Productivity
Train P (S-S-1) and Train P (S-S-2)	0.000	0.000	0.000	0.003
Train P and Train 3 (S-S-1)	0.000	0.000	0.000	0.095
Train P and Train 3 (S-S-2)	0.000	0.000	0.000	0.001

p value < 0.017 statistically different, with the Bonferroni correction

5.3.5 Regeneration performance

The resin columns were regenerated by using sodium hydroxide (1-N NaOH). Figure 5-11 compares the quantities of total acid initially adsorbed and later recovered. More than 80% total acid adsorbed on resin could be recovered. These days with 0 g acid adsorbed or recovered indicate that the adsorption process was not applied because of bottle replacement. To calculate fermentation performance, total acid adsorbed onto the resin was used instead of the amount of acid recovered from the resin column.

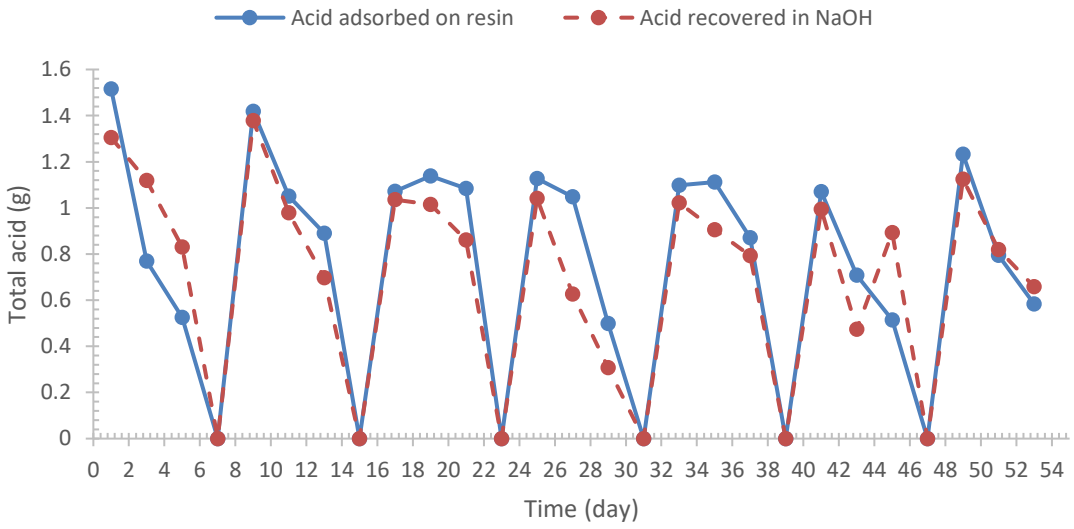


Figure 5-11. Acid adsorption and recovery in Train P.

5.4 Conclusion

The propagated fixed-bed mixed-acid fermentation achieved higher biomass conversion compared to countercurrent fermentation, but the carboxylic acid yield and selectivity were lower.

Applying CO_2-sustained ion-exchange resin adsorption on propagated fixed-bed fermentation was feasible. To address the issue of conversion drop, future work should focus on maintaining microorganism within the system. One possible way to achieve this goal is to combine residual biomass recycle technology into the adsorption-fermentation system. To fully understand the effect of resin loading on fermentation performance and find the optimal operation conditions, propagated fixed-bed fermentation should be operated under different resin loadings.

CHAPTER VI

CONCLUSION

In this project, a novel fluidized bed CO_2-sustained ion-exchange resin adsorption system was developed and the effect of CO_2 on adsorption behavior was investigated. By using weak-base ion-exchange resin IRA-67, the effect of CO_2-sustained ion exchange resin adsorption on mixed-acid fermentation was systematically studied under countercurrent and propagated fixed-bed operation strategies.

In Chapter III, CO_2 was found to be a promising alternative way to sustain resin adsorption capacity. Combined with fluidized bed operation, resin adsorption in the presence of CO_2 had a much higher acid uptake efficiency than the traditional pathway within a limited retention time. Future work should be conducted to study the effect of fouling caused by fermentation broth on resin beads, the adsorption isotherm, and to find the optimal resin regeneration method.

In Chapter IV, the new generation of countercurrent mixed-acid fermentation with *in-situ* CO_2-sustained ion-exchange resin adsorption was developed. Four identical countercurrent trains with different resin loading were studied. For all trains, the fermentation performances were significantly enhanced. The biogas production was increased with an average factor of 1.44, but there was no significant different among trains. The pH in fermentation systems were well controlled in the near-neutral range. For biomass conversion, the optimal normalized wet resin loading was 10.9 g wet resin/ (L_{liq}·d). For carboxylic acid production, the acid yield was significantly increased for all trains and had not reached a maximum in the present range of resin dosages. Further experiments with higher resin loadings should be performed to fully understand

the effect of resin adsorption on countercurrent fermentation and to find the optimum normalized resin loading for acid production.

In Chapter V, the new generation of propagated fixed-bed mixed-acid fermentation with *in-situ* CO_2-sustained ion-exchange resin adsorption was developed. One propagated fixed-bed train was studied under the same operation conditions as the countercurrent trains. With adsorption, the pH in fermentation system was well controlled in the near-neutral range. The biogas production and acid yield were increased by factors of 1.30, 1.64, respectively, but both of them were lower than the countercurrent train with the same resin loading. After applying adsorption, biomass conversion was lowered. A possible explanation is microorganism wash out; thus, future work should be conducted to maintain microorganism within the fermentation. To fully understand the effect of resin loading on fermentation performance and find the optimal operation conditions, propagated fixed-bed fermentation should be operated under different resin dosages.

Mixed-acid fermentation with *in-situ* ion exchange resin adsorption is a feasible technology. To further develop the technology, computer simulation models are necessary to test various reaction conditions and determine the optimal operation condition. Future studies should be performed to combine Continuum Particle Distribution Modeling (CPDM) with adsorption modeling.

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APPENDIX A COUNTERCURRENT FERMENTATION PROCEDURE

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

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 the same size water bottle.

6. Centrifuge the fermentor to separate the solid and liquid phases. 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. Pour the liquid from each fermentor into pre-weighted beakers, record the weight.

9. Take 1.5 mL of liquid from each fermentor for carboxylic acids analysis and measure pre-transfer pH of liquid in each beaker.

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 (10.2 g paper, 2.4g dry chicken manure) to F1.

12. Compare the liquid weight with target liquid weight (assume 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 as the product. Attention, liquid feed in F4 should be considered.

13. Add 120 mL deionized water to the liquid beaker of F4.

14. Balance the after-transfer pH of liquid in F1–F4 beakers at the range of 6.8 to 7.2. If it is higher than 7.2, add carbon dioxide. If it is lower than 6.8, add sodium carbonate.

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

16. Add 0.2 g urea to F4.

17. Add 120 μL iodoform solution (20 g CHI_3/L, 200-proof ethanol) to each fermentor.

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

19. Weight and return fermentors to the incubator.

APPENDIX B PROPAGATED FIXED-BED FERMENTATION PROCEDURE

I. Liquid transfer (No fermentor replacement)

1. Remove the fermentors from the incubator, cool for 10 min at room temperature, and weight.

2. Release and record the gas production.

3. Remove the fermentor caps and using a nitrogen purge line, remove the residual solids adhered to the stopper and metal bars.

4. Cap the fermentor with a regular solid centrifuge cap. Balance each fermentor with the same size water bottle. Pay attention to balance the centrifuge bottles before placing them in the centrifuge.

5. Centrifuge the fermentors to separate the solid and liquid. Centrifuge for 25 min at 4000 rpm and a brake level of 5.

6. After centrifuging, carefully move the bottles to ensure that the solid and liquid do not remix.

7. Pour the liquid from each fermentor into pre-weighted beakers, record the weight.

8. Take 1.5 mL of liquid from each fermentor for carboxylic acids analysis and measure pre-transfer pH of liquid in each beaker.

9. Weigh the fermentor bottles with the remaining solids.

10. Compare the liquid weight with target liquid weight (assume the density is 1 g/mL), remove the difference from beaker of F1 to beaker of F2, beaker of F2 to beaker of F3, beaker of

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

11. Add 120 mL deionized water to the liquid beaker of F1.

12. Balance the after-transfer pH of liquid in F1–F4 beakers at the range of 6.8 to 7.2. If it is higher than 7.2, add carbon dioxide. If it is lower than 6.8, add sodium carbonate.

13. Add 0.2 g urea to F1.

14. Add 120 μL methane inhibitor (20 g CHI_3/L, 200-proof ethanol) to each fermentor.

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

16. Mix all bottles thoroughly, cap, weigh, and return to incubator.

II. Fermentor replacement and liquid transfer

1. Remove the fermentors from the incubator, cool for 10 min at room temperature, and weight.

3. Remove the fermentor caps and using a nitrogen purge line, remove the residual solids adhered to the stopper and metal bars.

4. Cap the fermentor with a regular solid centrifuge cap. Balance each fermentor with the same size water bottle. Pay attention to balance the centrifuge bottles before placing them in the centrifuge.

5. Centrifuge the fermentors to separate the solid and liquid. Centrifuge for 25 min at 4000 rpm and a brake level of 5.

6. After centrifuging, carefully move the bottles to ensure that the solid and liquid do not remix.

7. Pour the liquid from each fermentor into pre-weighted beakers, record the weight.

8. Take 1.5 mL of liquid from each fermentor for carboxylic acids analysis and measure pre-transfer pH of liquid in each beaker.

9. Weigh the fermentor bottles with the remaining solids.

10. Remove the most-digested bottle (old F1) out of the train.

11. Place the new bottle in the train as the least-digested bottle (new F4), and label bottle with correct nomenclature.

12. The order of liquid beakers does not change with bottles. For example, beaker of old F1 still corresponds to new F1 bottle.

13. Compare the liquid weight with target liquid weight (assume the density is 1 g/mL), remove the difference from beaker of F1 to beaker of F2, beaker of F2 to beaker of F3, beaker of F3 to beaker of F4. No liquid product is obtained for this liquid transfer. Attention, liquid feed in F1 should be considered.

11. Add 120 mL deionized water to the liquid beaker of F1.

12. Balance the after-transfer pH of liquid in F1–F4 beakers at the range of 6.8 to 7.2. If it is higher than 7.2, add carbon dioxide. If it is lower than 6.8, add sodium carbonate.

13. Add 0.2 g urea to F1.

14. Add 120 μL methane inhibitor (20 g CHI_3/L, 200-proof ethanol) to each fermentor.

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

16. Mix all bottles thoroughly, cap, weigh, and return to incubator.

17. Take samples from old F1 bottle for moisture content, ash content etc.

APPENDIX C ADSORPTION APPARATUS ASSEMBLY

1. Cut the mesh into right size as the mesh holder from cartridge.

2. Use foil sticker to connect the mesh with holder.

3. Put the mesh-holder into cartridge and make sure to make it well on the button, and the side with mesh should face up.

4. Use a 1/4" FTP fitting for the outlet of cartridge and connect it with a stopcock by using a rubber tube.

5. Connect the outlet of stopcock with inlet of peristaltic pump.

7. Connect the outlet of peristaltic pump with inlet of the cartridge.

8. Insert a stainless tube into cartridge with one side connect with CO_2 flowmeter.

APPENDIX D CO_2-SUSTAINED ION-EXCHANGE RESIN ADSORPTION PROCEDURE

1. Four graduated cylinders, which were specifically numbered and reused for the same batch, were washed, dried, and measured accurately.

2. The resin was rinsed twice with 250 mL of deionized water and vacuum-filtrated to remove any excess water.

3. The peristaltic pump used in the ion exchange is also rinsed with 300 mL of DI water and dried.

4. The calculated amount of liquid is then removed into the dried graduated cylinders and the volume is recorded.

5. Carbon dioxide is then adjusted to 1.5 L/min and the hose is lowered into the dried resin almost touching the bottom of the column.

6. The liquid from the last bottle (F4) is poured into the column first (R1) and the timer is started.

7. Once all the liquid is poured into the column, the pump is started.

8. The liquid circulates from the bottom of the column through the tube and into the top of the column. This circulation is done for 10 minutes.

9. The weight of the graduated cylinder after pouring is recorded to account for loss of liquid throughout the process.

10. After 10 minutes, the valve at the bottom of the column is shut and disconnected from the pump.

11. The liquid is then transferred back to the same graduated cylinder and the weight of the recovered liquid is calculated.

12. A clean, weighed filtration flask is tared, and for 30 seconds, the column is vacuum pumped to remove the remaining liquid.

13. The flask weight with liquid is recorded.

14. A 1.5-mL sample is taken of the liquid that just underwent the adsorption.

15. The liquid in the graduated cylinder and flask is then poured into the liquid from the third bottle.

16. The weights of the final graduated cylinder and final flask are recorded.

17. This process is repeated using the liquid transferred from F3 and then F2.

APPENDIX E ION-EXCHANGE RESIN REGENERATION PROCEDURE

1. After the liquids have been through the resin, the resin is regenerated using sodium hydroxide.

2. The amount varies depending on the resin in the column.

3. The recycle stream as adsorption is used to cycle the sodium hydroxide through the resin for more than 30 minutes.

4. Once completed, the sodium hydroxide is removed through draining and vacuum pumping, weighed, and sampled.

5. The resin in column is washed twice with 250 mL of deionized water and then filled with 100 mL of deionized water to be stored until the next use two days later.

APPENDIX F 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 microcentrifuge 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 microcentrifuge (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. The (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: 20 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 G MOISTURE AND ASH CONTENT ANALYSIS

1. Take empty crucibles out of oven and put into desiccator only using tongs;

2. Prepare samples by using liquid products and solid wastes;

3. For liquid product, add 0.1 g $Ca(OH)_2$ and then add 3 g liquid samples. (No need to tare scale while measuring.);

4. For solids, only add 3 g samples;

5. Put samples into oven and 24 h later, take it, cool down and measure post oven weight;

6. Put the crucibles into furnaces and 24 h later, take it out and cool down and measure post furnace weight;

7. Wash crucibles and put into oven to dry;

APPENDIX H NORMALIZED WET RESIN LOADING

To provide suggestions for industrial applications, wet resin loading for countercurrent fermentation should be normalized. In this appendix, calculation example was present based on Train 1.

1. The transfer period (T) for Train 1 was 2 day;

2. The wet resin loading (WRL) used for Train 1 was 10 g/T;

3. The total liquid volume (TLV) of Train one during Steady-State 2 was 1.39 L using following equation:

$$\text{TLV} = (\text{g in liquid phase})(\text{MC liquid}) + (\text{g in solid phase})(\text{MC solid})$$

where MC liquid is the moisture content in liquid phase and MC solid is the moisture content in solid phase;

4. The normalized wet resin loading (NWRL) for Train 1 was 3.60 g wet resin/$L_{liq} \cdot$ day using equation described below.

$$\text{NWRL} = \frac{WRL}{TLV \cdot T}$$