EFFECTS OF NUTRIENTS ON MIXED-CULTURE FERMENTATION

A Thesis

by

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ABSTRACT

The MixAlco® process, an example of the carboxylate platform, converts lignocellulosic biomass to hydrocarbon fuels and chemicals using mixing cultures. The performance of mixed-culture fermentation depends on various factors, such as the energy source, nutrient source, and the resulting C/N ratio. It has been proven that co-digestion of two or more substrates has higher acid yields than either substrate fermented on its own. Countercurrent fermentation is employed to increase reaction rates and enhance acid yields and substrate conversion. However, it is time-consuming and labor intensive; it takes months to reach a single steady-state data point for a given liquid retention time (LRT) and volatile solids loading rates (VSLR). To overcome this challenge, the Continuum Particle Distribution Model (CPDM) is a technique that predicts the performance of countercurrent fermentation through mathematical methods using data from batch fermentations conducted at different substrate loadings.

Effects of nutrients were studied using chicken manure (fresh, air-dried, and oven-dried) or sewage sludge (fresh and air-dried) as nutrient sources. Among all chicken manures, the CPDM map showed reduced conversion and acid concentration for oven-dried treatments, which suggests that the drying process damages the nutrient source. At high VSLR, air-dried nutrients have higher acid concentrations than fresh; however, the conversion is low, which adversely affects process economics. In mixed-culture fermentation, fresh nutrients are preferred. At the same conditions, fresh chicken manure and sewage sludge have similar acid concentration; however, in fermentations using sewage sludge, there is a larger portion of caproic acid. At 300 g solids/L liquid, the CPDM map predicts that high acid concentrations (48.2 g/L) and conversions (0.79 g $NAVS_{digested}$/g $NAVS_{feed}$) are obtained at low VSLR (4 g/(L·day)) and high LRT (35 days).

This work is dedicated to my parents, who always believes in me to seek for my dream. Thank you for all your assistance and encouragement. I will forever be grateful for your support.

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TABLES OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Humans cannot live without energy. To prompt global development, there is increasing demand for energy. The EIA (U.S. Energy Information Administration) reported that energy consumption in the United States reached a record high of 101.3 quadrillion Btu in 2018. The energy consumption growth in 2018 is the largest since 2010, in both absolute and percentage terms. Of U.S. energy production, 80% is generated by burning fossil fuels (e.g., petroleum, natural gas, and coal) and it is forecast to constantly increase because of shale gas fracturing.[1] Although fossil fuels reserves have proven sufficient for more than one century, eventually energy demands will outweigh fossil fuel supply. Fossil fuel consumption also escalates the greenhouse effect. The EPA (U.S. Environmental Protection Agency) documented that in 2017, 79% of total U.S. anthropogenic greenhouse gas emissions resulted from burning fossil fuels for electricity, heat, and transportation.[2]

Aiming at sustainability and global warming mitigation, biofuels are an important part of our energy future. Liquid or gaseous biofuels are produced from solid biomass. Biofuels are promising because they can be produced from a wide range of feedstocks (e.g., wood, straw, and food waste). They help humans meet higher energy demands and secure stable energy supplies. Biofuels are also considered carbon neutral. Plants absorb carbon dioxide (CO_2) and water, and convert them to chemical energy via photosynthesis. The amount of CO_2 captured through photosynthesis balances the CO_2 emitted by burning biofuels. This carbon-neutral cycle

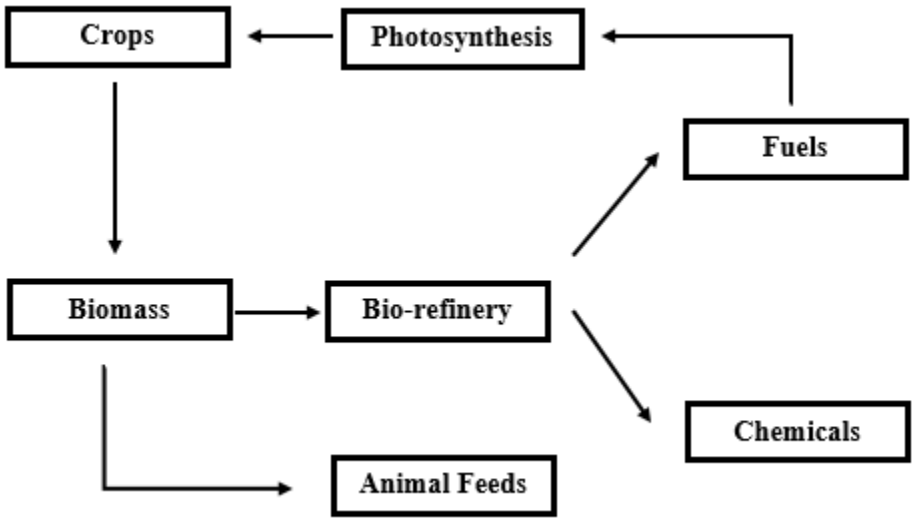

Figure 1-1. Net zero carbon footprint.

maintains constant atmospheric CO_2 concentration and thus mitigates the greenhouse effect (Figure 1-1).[3]

Sugar and starch crops are mainly used as feedstocks for the bioethanol industry, whereas soybeans and other oil seeds are raw materials for biodiesel. However, these feedstocks conflict with their main use as human food. Instead, biofuels can be derived from lignocellulosic biomass. As the world's fourth largest energy source, large quantities of lignocellulose are available as crop residues and it has the potential for high crop yields per acre.[4]

Three platforms are commonly used to convert biomass to liquid fuels: thermochemical, sugar, and carboxylate platforms. In the thermochemical platform, biomass is gasified into syngas ($CO + H_2$), and then is catalytically transformed into liquids. It is considered the least efficient conversion method because thermochemical gasification partially oxidizes the biomass and thus reduces the yields.[5] The sugar platform and carboxylate platforms are biological. Because lignocellulosic biomass has a rigid structure that consists of different portions of cellulose, hemicellulose, and lignin (Figure 1-2), pretreatment is needed to increase accessibility

of cellulase enzyme to cellulose by breaking biomass apart and reducing cellulose crystallinity.[7-8] In the sugar platform, carbohydrate polymers are hydrolyzed to simple sugars that are fermented to ethanol. To guarantee that the desired microorganism dominates, sterile operating conditions are required. Also, the sugar platform is costly because of expensive enzymes added during hydrolysis.[6] Furthermore, because lignin can only be processed thermochemically, it must be gasified for complete utilization of biomass, which is difficult. In contrast, as another biological pathway, the carboxylate platform is more resilient and robust, and requires neither enzyme addition nor sterile operating condition.

The carboxylate platform is an example of consolidated bioprocessing (CBP) where enzyme production, saccharification, and fermentation are integrated in a single process step.[9] Via consolidation, CBP reduces processing cost and meanwhile increases hydrolysis rates.[10] In this anaerobic process, all metabolic products are acids.[11] Because no sterility is required, a mixed culture of microorganisms is introduced as inoculum. As shown in Figure 1-3, the mixed culture of microorganisms digests nearly all lignocellulose components to carboxylic acids, which consequently contributes to the high yields. Through downstream processes, the carboxylate salts are converted to industrial chemicals and fuels. In the carboxylate platform, well-developed gasification technology converts lignin residue into hydrogen.[5]

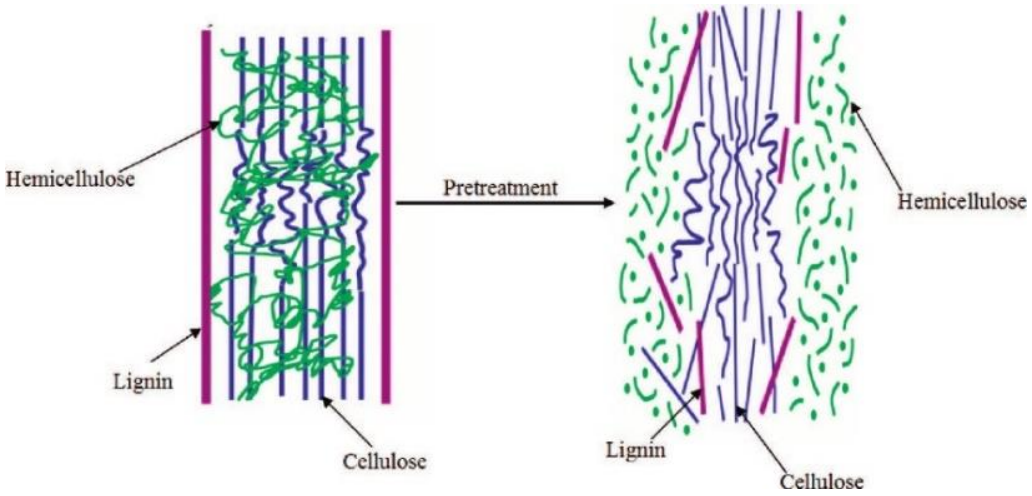

Figure 1-2. Schematic of the lignocellulosic biomass structure before and after pretreatment.[12]

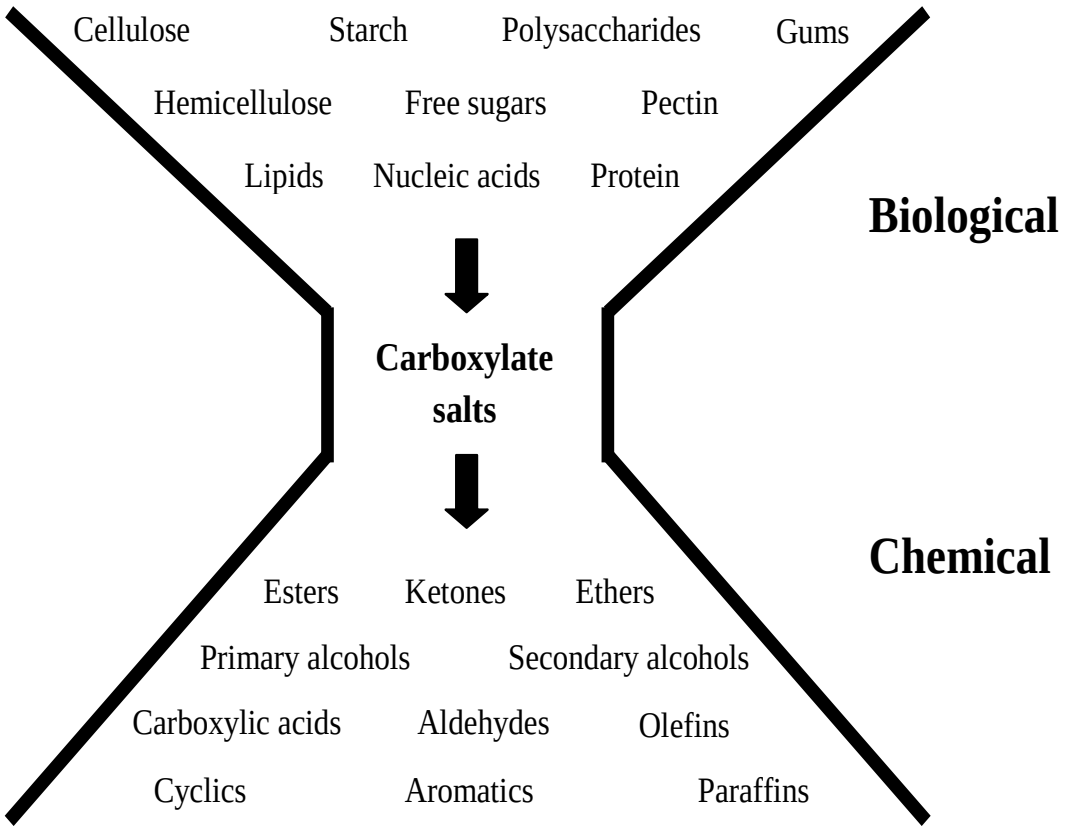

Figure 1-3. Carboxylate salts transformed from biomass via carboxylate platform.[13]

The MixAlco® process is an example of the carboxylate platform developed in Dr. Mark Holtzapple's laboratory at Texas A&M University (Figure 1-4). One version of the MixAlco® process has following steps:

- **Pretreatment** – Lignocellulosic biomass is contacted with lime or sodium hydroxide to remove lignin and increase accessibility to cellulose. Hsu investigated different pretreatment methods on corn stover and proved that shock pretreatment benefitted NaOH-treated corn stover under moderate hydroxide loading and temperature.[14]
- **Fermentation** – As an example of CBP, enzyme production, substrate hydrolysis and mixed-culture fermentation are accomplished in this single step. The original inoculum (Galveston, TX) is a mixed culture of microorganisms from marine soil that have adapted to the fermentor environment. In this controlled anaerobic digestion process, the pretreated biomass undergoes hydrolysis, acidogenesis, and acetogenesis (Figure 1-5). Methanogenesis is constrained by adding a methane inhibitor; thus, products that should have been transformed to methane accumulate as carboxylic acids. Carboxylic acids are present as their corresponding salts because the pH is neutral in the fermentor.
- **Dewatering** – Solids are removed from fermentation broth through centrifugation and water is removed through vapor-compression desalination.
- **Ketonization** – Using thermal processing, carboxylate salts are converted to ketones, and carbon dioxide.
- **Hydrogenation** – Reacting the ketones and hydrogen in the presence of a catalyst produces secondary alcohols.

- **Oligomerization** – Secondary alcohols are dehydrated to olefins that oligomerize to hydrocarbons. Heavy hydrocarbons exit as jet fuels whereas light hydrocarbons are used as gasoline.[6]

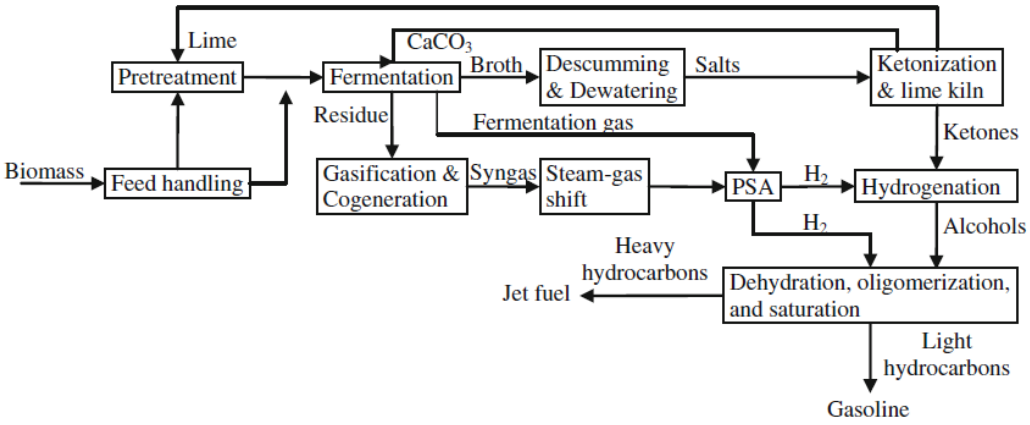

Figure 1-4. Block diagram of the MixAlco® process.

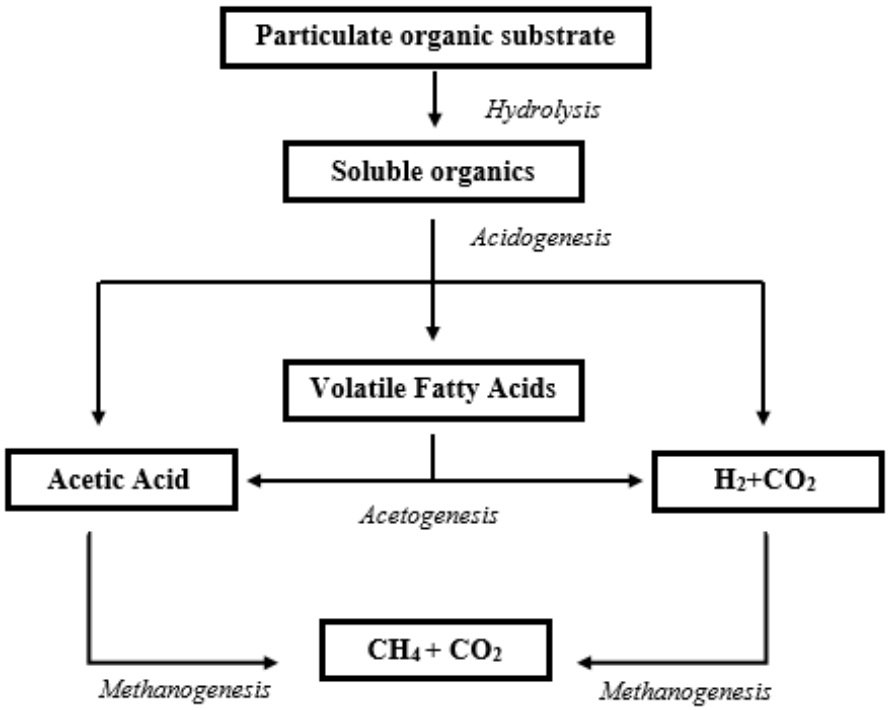

Figure 1-5. Anaerobic digestion process.[15]

The core of the MixAlco® process is the mixed-culture fermentation. Several studies from various perspectives have been conducted to optimize the process and increase acid yields. Yang analyzed the effect of liquid residence time on acid concentration and chain elongation.[16] Roy employed ion-exchange resins for carboxylic acid extraction.[17] Wu started and Hsu and Olokede continued the experiment testing the effect of carbon dioxide-sustained adsorption using ion-exchange resins.[18] Smith and Rughoonundun investigated the optimal carbon-nitrogen ratio for co-digestion.[19-20]

Nutrients are essential for microbial metabolism and reproduction. Lack of nutrients may result in slow reaction rates, an unstable process, and lower product yields.[21] Co-digestion is a productive and economical way to mix different substrates together, adjust the carbon-nitrogen ratio, and provide the nutrients needed by microorganisms. The purpose of this study is to investigate the effects of different nutrient substrates on mixed-culture fermentation and provide suggestions for optimal nutrient storage. The specific objectives of this study follow:

- Examine performance of series of batch fermentation with sewage sludge and chicken manure as nutrients.
- Compare sewage and chicken manure as nitrogen sources for mixed-culture microorganisms.
- Obtain a Continuum Particle Distribution Model (CPDM) map for sewage sludge and chicken manure.
- Determine the optimal storage method for chicken manure and sewage sludge.
- Model the optimal configuration for countercurrent fermentation.

CHAPTER II

METHODS AND MATERIALS

2.1 Methods

2.1.1 Carbon-to-nitrogen Ratio

Nutrients are critical for microbes to grow and reproduce. Essential nutrients include macro- or micro-nutrients.[24] Carbon, nitrogen, phosphorous, potassium, and sulfur are macro-nutrients whereas cobalt, copper, iron, molybdenum, nickel, selenium, tungsten, and zinc are examples of micro-nutrients. Carbon and nitrogen are used for new cell formation and new cell synthesis, including production of protein, enzymes, RNA, and DNA. In mixed-culture fermentation, the microbial community is fed with substrate consisting of a carbon source (energy source) and a nitrogen source (nutrient source).[20] The carbon source (e.g., office paper or corn stover) is converted into carboxylic acids via controlled anaerobic fermentation. Previous research has been conducted on the effect of energy source on batch fermentation, showing that there is a significant increase in acid concentration and conversion when office paper is added compared to corn stover.[22] The nitrogen source (e.g., chicken manure or sewage sludge) provides nutrients for microorganisms to survive and reproduce. Research led by Kayhanian and Rich concluded that the correct nutrient ratio and concentrations are essential for proper microbial metabolism and stable anaerobic digestion.[25] The carbon-to-nitrogen ratio (C/N ratio) must be in an optimal range to achieve high carboxylic acid concentrations. If the C/N ratio is higher than the ideal (equivalent to nitrogen deficiency), digestion is constrained because fewer cells are active. Excess nitrogen (i.e., low C/N ratio) forms ammonia, an undesired product that can inhibit the microbial community.

In the past two decades, the C/N ratio has been investigated. Kayhanian and Tchobanoglous demonstrated that the optimal range of C/N ratio is 25 to 30 g carbon/g nitrogen based on biodegradable carbon and nitrogen mass.[26] Kalil estimated the maximum acid concentration occurs at approximately 25 g C/g N ratio by observing that the carbon consumption is 25 times faster than nitrogen.[27] Rughoonundun concluded that the ideal C/N ratio is 20 to 40 for mixed-acid fermentation and noted that the C/N ratio impacts the composition of carboxylic acids if a minimum amount of nitrogen is provided.[20] In this study, 31.1 and 25.9 g C/g N are chosen.

2.1.2 Biogas Analysis

Biogas is continuously formed as a by-product of fermentation, which is mainly carbon dioxide. Biogas must be vented periodically to prevent explosion because the fermentor can only tolerate pressures under 2 atm abs. Figure 2-1 shows the biogas measuring apparatus. Biogas measurement helps indicate microorganism metabolism and acid production. Biogas was vented by inserting a needle through the rubber septum on the top of the fermentor. The amount of produced biogas is measured by recording the initial and final scale of the water column. A 300 g/L $CaCl_2$ solution is stored in the water tank to prevent CO_2 adsorption and microbial growth. To control the water level, a vacuum pump was connected to the outlet of the water column. For composition analysis, a 30-mL biogas sample was injected to the gas chromatograph (GC, Agilent 6890 Series) with a thermal conductivity detector (TCD). Appendix A describes the detailed analysis procedure.

Figure 2-1. Biogas measuring apparatus.

2.1.3 Carboxylic Acids Concentration Determination

In mixed-culture fermentation, carboxylic acids are produced as final product. After the fermentor was centrifuged (4000 rpm, 10 min), a 1-mL liquid sample was collected from each fermentor for acid concentration analysis. Samples were stored in the freezer until analysis. Samples were thawed, vortexed, and centrifuged to separate liquid from solids (Beckman Coulter Microfuge® 16, 13,300 rpm, 10 min). Because the supernatant is neutral, carboxylic salts were acidified by adding phosphoric acid for GC analysis.

Supernatant (0.5 mL), 3-M phosphoric acid (H_3PO_4, 0.5 mL), and internal standard solution (isocaproic acid, 1.16 g/L, 0.5 mL) were mixed together in a vial as the intermediate. To

ensure uniform concentration, the intermediate was centrifuged again (13,300 rpm, 10 min) and then 0.5 mL was transferred to glass vials for GC analysis.

The GC system employs an automatic liquid sampler (Agilent 76830), a flame ionization detector (FID), and a 30-m fused-silica capillary column (J&W Scientific, Model # 123-3232). The column head pressure was maintained at 2 atm abs. For each sample injected, the GC program raised the temperature from 40°C to 200°C at 20°C/min. The temperature was subsequently maintained at 200°C for 2 min. Each sample was run for 11 min. Helium was used as carrier gas. For calibration, an external standard carboxylic acids solution (Table 2-1) was injected periodically during the whole run. Appendix B presents the detailed procedure for carboxylic acid concentration analysis.

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
Heptanoic Acid	0.399
Octanoic Acid	0.169

2.1.4 Moisture and Ash Content Measurement

NREL procedures were used to determine moisture and ash content.[28] *Moisture content* (MC) is defined as the fraction of liquid evaporated from the wet sample after 24-h heating in an oven at 105°C. *Volatile solids* are defined as the mass loss from the dry sample after 24-h heating in furnace at 550°C. *Ash content* (AC) is defined as the residue left in the crucible after 24-h combustion in the furnace.[29] Terms defined above are measured to determine the non-acid volatile solids (NAVS):

NAVS = (g total wet weight)(1 – MC)(1 – AC) – (g carboxylic acid in wet weight) (2-1)

At the last day of the experiment, liquid and solid samples were collected from the fermentor and placed in the ceramic crucible. Samples were transferred to the oven using a desiccator to prevent external factors (e.g., moisture adsorption from atmosphere) from impacting the weight. Calcium hydroxide ($Ca(OH)_2$) was added to the liquid sample to convert all volatile acids to their deprotonated form, ensuring that acids are not evaporated and all mass loss in the oven is moisture. Samples were heated in the oven for 24 h at 105°C and then combusted in the furnace for another 24 h at 550°C. Appendix C describes specific procedures for moisture and ash content analysis.

2.2 Materials

2.2.1 Substrate and Drugs

2.2.1.1 Chicken Manure

Chicken manure is rich in nitrogen, carbon, and phosphorous, which are macro-nutrients needed by microorganisms for robust growth.[22] Antibiotic-free chicken manure was obtained from the Poultry Science Department at Texas A&M University and was stored in three different ways:

(1) Wet chicken manure (WCM or FCM) – Store fresh chicken in freezer without any treatment,

(2) Air-dried chicken manure (ADM) – Put fresh chicken manure in a stainless tray, fan dry for 48 h at room temperature (25°C) and store at 4 °C in refrigerator,

(3) Baked chicken manure (BCM) – Dried fresh chicken manure was placed in the oven at 105°C for 48 h and stored in Ziploc bags at room temperature (~25°C).

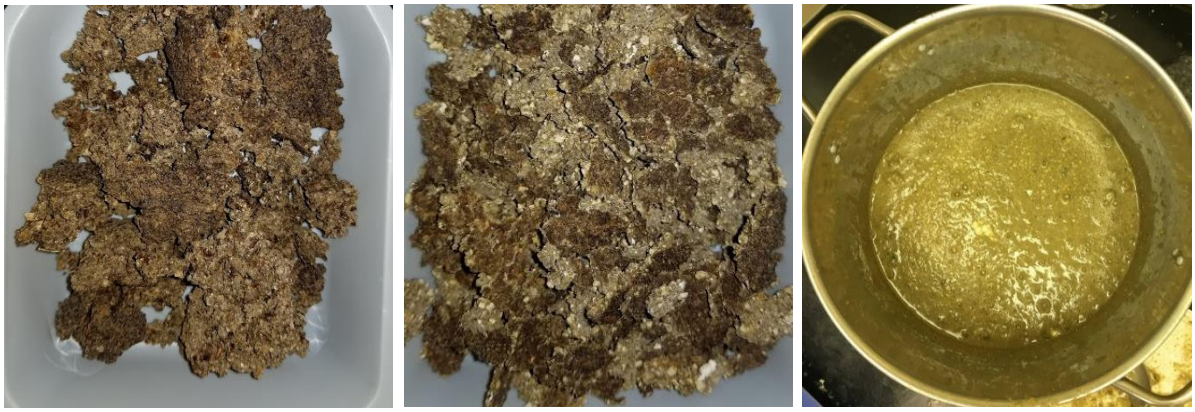

Figure 2-2. Baked, air-dried, and fresh chicken manure.

Figure 2-2 shows the chicken manure processed by the above treatments.

In previous studies, to maintain consistency and avoid degradation, chicken manure was usually processed and stored by Method 3.[18]

2.2.1.2 Sewage Sludge

Sewage sludge can serve as nutrients to the microbial community. As a byproduct of industrial and municipal wastewater, sewage sludge is landfilled, incinerated for electricity production, or applied to agricultural land. However, landfills produce the second largest amount of anthropogenic methane in the United States and incineration for electricity releases pollutants into the atmosphere.[17] Instead, the carboxylate platform is an alternative that uses sewage sludge as substrate for fermentation. Several studies have proven that adding sewage sludge can improve digester performance.[23]

Sewage sludge was collected from the Carter Creek Wastewater Treatment Plant (College Station, TX) and stored in two ways:

(1) Wet sewage sludge (WSS) – Store sewage sludge immediately in the freezer after centrifuge until use.

(2) Air-dried sewage sludge (ADS) – Settle sewage sludge for 1 h and remove supernatant. Distribute the remaining sludge to 1-L polypropylene copolymer (PCCO) bottles and centrifuge for 10 min at 4000 rpm. Pour supernatant again. Fan dry black sediment at the bottom in a metal tray for 48 h at room temperature (Figure 2-3).

To ensure sterilization, supernatant was mixed with 500 mL 6% bleach (Cholorox® Regular Liquid Bleach) per bucket (5 gallons).

Figure 2-3. Air-dried and fresh sewage sludge.

2.2.1.3 Urea

Urea is rich in nitrogen and its nitrogen and carbon contents are 19.35 and 45.16 wt%, respectively. To adjust the C/N ratio, urea was added to each fermentor.

2.2.1.4 Office Paper

Unused office paper (20 pounds, Caliber®) was introduced as an energy source. It has a high C/N ratio; its carbon and nitrogen content are 36.30 and 0.07 wt%, respectively. Normally, lignocellulosic biomass should be pretreated before fermentation; however, office paper is pretreated when produced, thus additional pretreatment is not needed. Office paper was shredded by a Fellows Powershred® W-6C, thus enhancing solid-liquid contact.

Table 2-2 lists substrate contents.

2.2.2 Fermentation Media

Deoxygenated water (D.O. water) serves as fermentation media, providing an anaerobic environment in the fermentor. Deoxygenated water is made by adding 0.275 g/L cysteine hydrochloride and 0.275 g/L sodium sulfide into boiled deionized water (D.I. water). Appendix D describes the procedure for D.O. water preparation.

2.2.3 Inoculum

The original inoculum was a mixed culture of marine microorganism found in biomass-rich beach sediment collected from Galveston Island, TX. Sediments were dug from the bottom of multiple 0.5-m-deep shoreline pits. Samples were immediately collected in airtight plastic bottles filled with deoxygenated water, capped, and frozen at –20°C until use. Before inoculation, samples were thawed, shaken vigorously, and allowed to settle by gravity. The resulting supernatant was homogenized, and aliquots (12.5% of the initial working volume) were used as fermentor inoculum.[20] A typical composition of the bacterial community in the mixed-culture fermentation has been reported elsewhere.[21] Before marine microorganisms can function in a new environment, an adaptation period inoculum adaptation is required before starting the mixed-culture fermentation.

Table 2-2. Substrate contents

Substrate	Office paper	Wet chicken manure	Air-dried chicken manure	Baked chicken manure	Wet sewage sludge	Air-dried sewage sludge	Urea
Moisture Content (g/100 g wet sample)	5.905	83.578	9.952	5.915	89.098	64.990	–
Ash Content (g/100 g dry sample)	14.188	22.989	24.761	28.620	20.110	30.129	–
Volatile solids (g/100 g dry sample)	85.810	77.010	75.239	71.378	79.890	69.77	–
Carbon (g/100 g dry sample)	36.030	35.4	35.4	35.4	42.5	42.5	19.35
Nitrogen (g/100 g dry sample)	0.070	4	4	4	6.94	6.94	45.16
C/N ratio (g carbon/g nitrogen)	514.714	8.85	8.85	8.85	6.124	6.124	0.428

2.2.4 Fermentor

Mixed-culture fermentation is implemented in a 1-L polypropylene copolymer (PPCO) bottle (Nalgene®) capped by a rubber stopper with a septum-sealed glass tube. Screw cap and aluminum crimp seals ensure airtight conditions (Figure 2-4). To mix while the fermentor rotates in the rolling incubator, two 0.25-in stainless steel tubes are inserted.

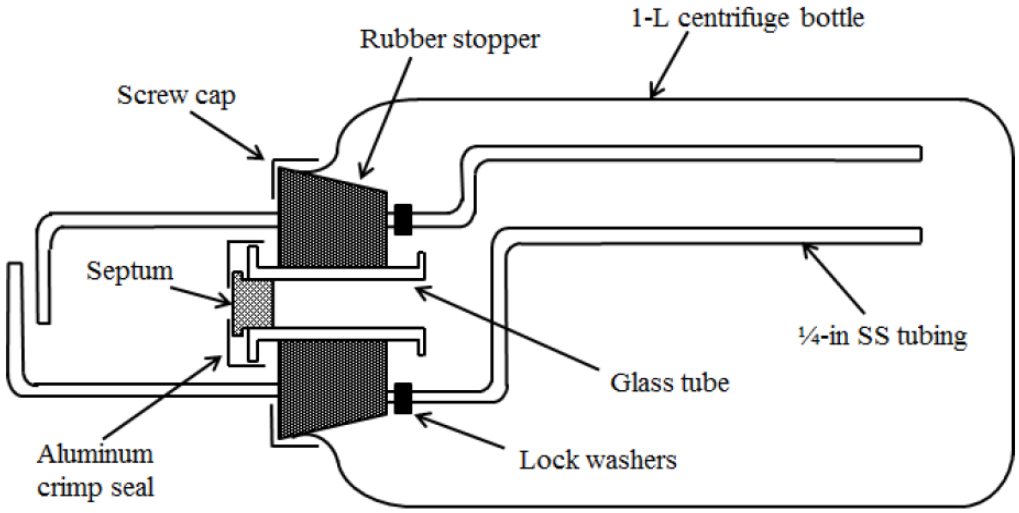

Figure 2-4. Fermentor configuration.

2.2.5 Buffer

The optimal pH range for microorganism metabolism is 6.5–7.2. During fermentation, the pH of the fermentation broth reduces because of produced carboxylic acids. To maintain pH within the desired range, sodium bicarbonate ($NaHCO_3$, Fischer) was introduced periodically (once each 2 days). In previous studies, magnesium carbonate ($MgCO_3$) was used as buffer; however, sodium bicarbonate is preferred because of less precipitate and fouling.

With chicken manure as nutrient source, the initial fermentor pH is usually above 7.0. In this case, carbon dioxide was added to lower the pH to the optimal range.

2.2.6 Methane Inhibitor

Iodoform (CHI_3) is selected as the methane inhibitor. To each batch fermentor, 60 µL iodoform solution (20 g CHI_3/L 200-proof ethanol) was added every 48 h. Because of its light, temperature, and air sensitivity, iodoform is stored in a foil-wrapped amber-colored glass bottle at 4°C. Appendix E describes iodoform solution preparation.

CHAPTER III

CONTINUUM PARTICLE DISTRIBUTION MODEL (CPDM)

3.1 Overview

In mixed-culture fermentation, the reaction kinetics at the interface between solid and liquid phases must be quantified.[30] Such examples include: (1) enzymatic hydrolysis of lignocellulosic biomass, and (2) direct conversion of lignocellulose to volatile fatty acids by microorganisms. The solid phase is often not well defined because liquid-phase homogeneous reactions occur simultaneously. Several methods have been developed to simulate reaction configuration. The conventional method is Residence Time Distribution (RTD), which models solid/liquid reactions; however, it has several disadvantages. First, it is difficult to apply when the interfacial reaction rate depends on solid and liquid phases with nonconstant reactivity and residence time. Second, as a time-parameterized distribution function, one must account for particles with residence times between zero and infinity, which requires that an arbitrary upper bound on time must be assumed.[30] Third, RTD was derived from the zero micromixing case in which fluid elements remain segregated as they pass through the reactor.[31] The difference between simulation and reality can be large when RTD applies to micromixing treatments. Kunii and Levenspiel (1969) described a shrinking-core model where size distribution is used to parameterize solid reactivity;[30] however, it only applies to certain cases where particles are identical spheres.

The performance of mixed-culture fermentation is hard to model because of its complexity. A fermentor contains various feedstocks (e.g., paper and chicken manure), multiple

components (e.g., cellulose, hemicellulose, sugar, protein), multiple microorganisms, and a mixture of carboxylic acids. To overcome these problems, Loescher developed the Continuum Particle Distribution Model (CPDM), which uses a continuous conversion distribution function to model fermentation. Different from a time-parameterized function, it tracks biomass particles as they move through the fermentation train. Such a conversion-parameterized model has following advantages:

- Countercurrent fermentation is time-consuming. It takes months to reach a steady-state data point. The CPDM method can simulate the performance of countercurrent fermentation using batch data sets at different substrate loading.
- The CPDM model can avoid the heavy labor of performing countercurrent fermentation under a wide range of operation conditions.
- Compared to RTD, which is difficult to apply when the relationship between reactivity and residence time is not uniform, the CPDM method separates solid and liquid dependencies explicitly.
- The CPDM method follows particles that are contained in the closed conversion domain from 0 to 1.
- The CPDM method is robust and resilient, and can apply to both linear and nonlinear kinetics.

3.2 Principles

CPDM can quantitatively account for liquid-phase dependencies and effects of particle conversion, while allowing for generalized reaction-rate models to be used for specific reaction

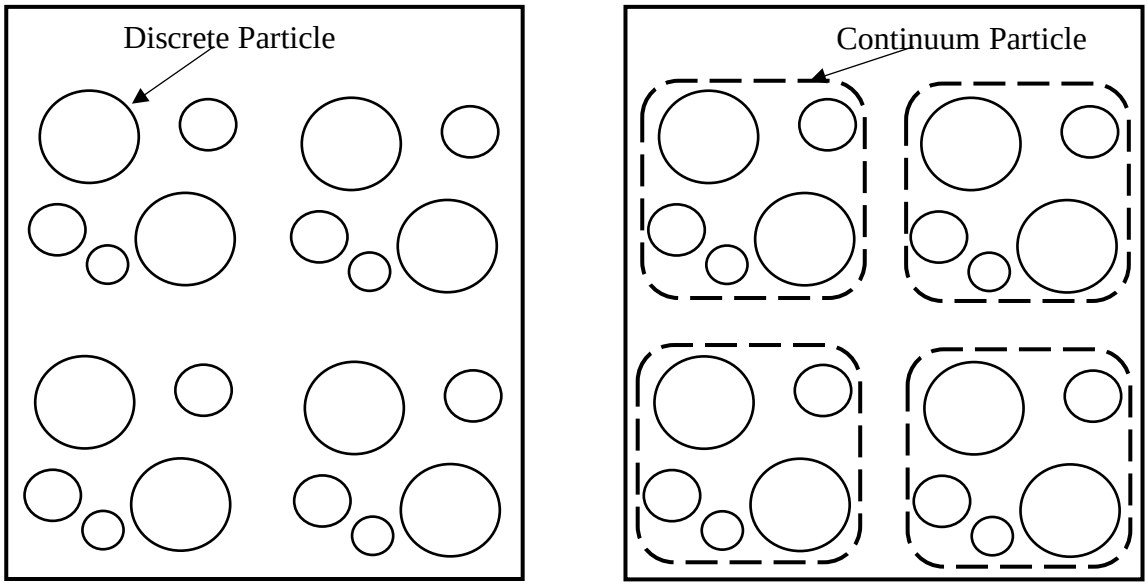

Figure 3-1. Illustration of a discrete particle and continuum particle.

systems.[30] A *continuum particle* is defined 1.0 gram of solids in the initial unreacted state, which represents 1.0 gram of non-acid volatile solids (NAVS) in the study (Figure 3-1).[32]

The particle concentration n_0 (particles/L) depends on the particle distribution:

$$n_0 = \int_0^1 \hat{n}(x)dx \tag{3-1}$$

The total reaction rate *r* is related to the specific rate $\hat{r}$ as a function of particle conversion and product concentrations *A*:

$$r = \int_0^1 \hat{r}(x, A)\hat{n}(x)dx \tag{3-2}$$

where $\hat{r}(x, A)$ reflects reacting system and products and $\hat{n}(x)$ specifies substrate concentrations and conversions.[33]

For a batch reaction, all continuum particles have the same conversion, x' and therefore $\hat{n}(x) = 0$ everywhere except at x'. Equation 3-1 changes to

$$n_0 = \int_0^1 \hat{n}(x)dx = \lim_{g \to 0} \int_{x'-g}^{x'+g} \hat{n}(x)dx \tag{3-3}$$

The Dirac delta function δ satisfies the above equation:

$$\hat{n}(x) = n_0 \delta(x - x') \tag{3-4}$$

Substituting Equation 3-4 into Equation 3-2:

$$r = \int_0^1 \hat{r}(x, A)\hat{n}(x)dx = \int_0^1 \hat{r}(x, A)\, n_0 \delta(x - x')dx = \hat{r}(x', A)S_0 \tag{3-5}$$

As shown in Equation 3-5, the specific reaction rate can be determined directly and is proportional to the overall reaction rate if the proposed weighting system is utilized.[32] Therefore, the CPDM method can simulate the performance of fermentations in multiple operating conditions and determine the optimal conditions for countercurrent fermentation (e.g., volatile solids loading rates and liquid retention time).

3.3 Simulating Countercurrent Fermentation

Data from batch fermentation at five different substrate loadings are used to simulate countercurrent fermentation. Substrate loadings are designed as follows: 20, 40, 70, 100, and 100+ g dry substrate/L liquid.[34] The 100+ and 100 groups have the same substrate loading. The only difference is that the 100+ condition uses an additional 20 g carboxylic acid/L in the fermentation media, which helps elucidate product inhibition. Table 3-1 lists the contents of

Table 3-1. Contents of carboxylic acids added to the 100+ group

Acid	Concentration (g/L)
Acetic acid	16
Propionic acid	1
Butyric acid	3

manually added carboxylic acids. To provide the optimal environment for microbial metabolism and growth, prior to inoculation, pH is neutralized to 7 using sodium bicarbonate.

The mixture of carboxylic acids is quantified as "acetate equivalents" (Aceq):

$$\begin{aligned}\text{Aceq}(\text{mol/L}) = {} & 1.00 \times (\text{acetic})\left(\frac{\text{mol}}{\text{L}}\right) + 1.75 \times (\text{propionic})\left(\frac{\text{mol}}{\text{L}}\right) \\ & + 2.50 \times (\text{butyric})\left(\frac{\text{mol}}{\text{L}}\right) + 3.25 \times (\text{valeric})\left(\frac{\text{mol}}{\text{L}}\right) \\ & + 4.00 \times (\text{caproic})\left(\frac{\text{mol}}{\text{L}}\right) + 4.75 \times (\text{heptanoic})\left(\frac{\text{mol}}{\text{L}}\right) \\ & + 5.5 \times [\text{octanoic}]\left(\frac{\text{mol}}{\text{L}}\right) \end{aligned} \quad (3\text{-}7)$$

To ensure accuracy and reproductivity, each reaction condition was conducted in triplicate. Once the specific rate and conversion are known, the governing equation of specific reaction rate, $\hat{r}(x, \text{Aceq})$, can be fit by the least-square method (Equation 3-6).

$$\hat{r} = \frac{e(1-x)^f}{1 + g(\varphi \cdot \text{Aceq})^h} \tag{3-7}$$

where x (conversion) $= \dfrac{\text{NAVS}_{\text{feed}} - \text{NAVS}_{\text{exit}}}{\text{NAVS}_{\text{feed}}}$

e, *f*, *g*, and *h* are empirical constants

φ is the ratio of total grams of carboxylic acid to total grams of Aceq.

Using a least-square model, empirical constants *e*, *f*, *g*, and *h* are determined by fitting the model to data collected from batch fermentations. Other performance variables needed to determine empirical constants are listed below:

$$\text{Exit yield } (Y_E) = \frac{\text{g total acid output from solid and liquid streams}}{\text{g NAVS}_{\text{feed}}} \tag{3-8}$$

$$\text{Product yield } (Y_P) = \frac{\text{g total acid output in liquid stream}}{\text{g NAVS}_{\text{feed}}} \tag{3-9}$$

$$\text{Feed yield } (Y_F) = \frac{\text{g total acid entering with feed}}{\text{g NAVS}_{\text{feed}}} \tag{3-10}$$

$$\text{Selectivity } (s) = \frac{\text{g total acid produced}}{\text{g NAVS}_{\text{feed}} - \text{g NAVS}_{\text{exit}}} = \frac{Y_E}{x} \tag{3-11}$$

Aceq(t) is the acetic acid equivalents at each instant during the entire batch fermentation, where t is the time in days.[27] Using least-square regression, Aceq(t) is fit by Equation 3-12.

$$\text{Aceq}(t) = \text{a} + \frac{bt}{1+ct} \tag{3-12}$$

The reaction rate is determined by differentiating Aceq(t) (Equation 3-13).

$$r = \frac{d(\text{Aceq}(t))}{dt} = \frac{b}{(1+ct)^2} \tag{3-13}$$

The specific reaction rate ($\hat{r}$, the reaction rate per particle) is calculated by the reaction rate in Equation 3-14.[33] S_0 represents the initial substrate concentration and is determined by Equation 3-15, where m_0 is the mass of initial substrate (g volatile solid) and V is the fermentor working volume (L).

$$\hat{r} = \frac{r}{S_0} \tag{3-14}$$

$$S_0 = \frac{m_0}{V} \tag{3-15}$$

Conversion $x(t)$ is calculated through Equation 3-16, which is a time-dependent parameter for the CPDM model determination.

$$x(t) = \frac{\text{Aceq}(t) - \text{Aceq}(0)}{S_0 \cdot \sigma} \tag{3-16}$$

where σ is selectivity (g Aceq produced/ g VS digested).

Selectivity σ is assumed constant throughout all substrate concentrations and is derived from selectivity s (g total acids produced/g VS digested). The relationship between σ and s is shown in Equation 3-17.

$$\sigma = \frac{s}{\varphi} \tag{3-17}$$

Once described parameters are calculated, MatLab code (Appendix H and I from Darvekar[34]) can be used to simulate four-stage countercurrent fermentation with different volatile solids loading rates (VSLR) and liquid retention times (LRT).[33]

CHAPTER IV

EFFECTS OF NUTRIENTS ON MIXED-CULTURE FERMENTATION

4.1 Overview

Biomass can be converted to liquid fuels through anaerobic fermentation. The MixAlco® process is a biorefinery that ferments lignocellulosic biomass to carboxylic acids using a mixed culture of microorganisms. The carboxylic acids are separated from fermentation broth, converted to ketones, and eventually processed to mixed alcohols. Anaerobic fermentation is the core of the MixAlco process. Several studies have proven that co-digesting mixed substrates offer more ecological, technological, and economic advantages than fermenting a single substrate.[35-38] *Co-digestion* is defined as the anaerobic fermentation of a mixture of at least two different types of waste.[35] With co-digestion, potential toxic compounds are diluted and nutrient balance is improved. Mixtures of substrates prompt synergistic effects of microorganisms and increase the load of biodegradable organic matter. To optimize co-digestion, the main focus of this study is to balance several parameters: C/N ratio, pH, inhibitors, biodegradable organic matter, and dry matter.[39]

In co-digestion, optimal combinations of substrates are investigated to increase product yield. Rughoonundun investigated carbon-to-nitrogen ratio using mixtures of wastewater sludge and pretreated bagasse whereas Smith blended chicken manure and office paper.[19-20] Golub mixed office paper with chicken manure stored in different conditions testing the effect of additional microbial community on anaerobic fermentation.[40] She showed that microorganisms in the substrate provide additional inocula and can benefit fermentation performance. The above

studies all concluded that acid yields are significantly increased in co-digestion compared to mono-digestion of a single substrate.

Because of their economic feasibility, chicken manure and sewage sludge are often used as nutrients in fermentation. They provide the microbial community with fundamental elements for growth and reproduction. To meet the rising demand for animal protein, poultry farming has become increasingly intense. It produces large amounts of waste, especially poultry manure. When applied in excess as fertilizer, poultry manure causes serious environmental concerns.[41] Instead, anaerobic digestion is another option for manure disposal and exploits poultry manure as a rich source of nitrogen and phosphorus. Sewage sludge has been mainly studied as nitrogen-rich source in the context of anaerobic digestion for methane production.[20] Several studies proved that adding sewage sludge to municipal solid waste yields the highest biogas production and minimizes reactor upsets.[41] Rughoonundum operated fermentors containing wastewater sludge and pretreated bagasse. She concluded that co-digestion of wastewater sludge and pretreated bagasse had higher acid yields and the loading ratio affects the composition profile of the acid products.

To improve reaction rates and enhance yields and conversion, countercurrent fermentation was developed to replace batch fermentations. Golub studied the effects of one to six stages in countercurrent fermentation and found that more stages increase acid concentration and selectivity, whereas fewer stages increase conversion.[43] Four-stage countercurrent fermentations are usually used in the study. As shown in Figure 4-1, fresh substrate and nutrients enter the fermentation train at the opposite direction against fresh water. It allows the least reactive substrate to contact the lowest acid concentration media and the most reactive substrate to contact the highest acid concentration media, thus minimizing inhibition from

accumulated acids. To reduce product inhibition, extraction can be employed. Wu combined countercurrent fermentation and ion-exchange resin adsorption to improve product yield and substrate conversion.[18] However, countercurrent fermentation is labor intensive and time-consuming; it takes months and heavy labor to reach steady state.

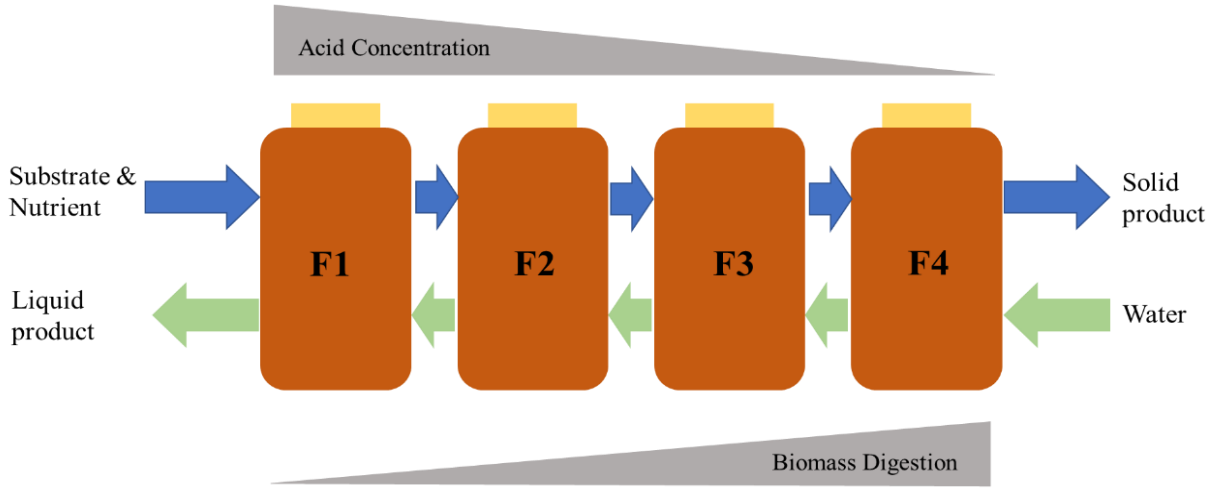

Figure 4-1. Schematic of countercurrent fermentation.

CPDM is a widely used technique that predicts countercurrent fermentation through mathematical modeling. It avoids heavy labor and lengthy time to perform countercurrent fermentation. To predict conversion and acid concentration at a range of VSLR and LRT, the CPDM method utilizes data from sets of batch fermentations.

This chapter investigates effects of nutrients on mixed-culture fermentation by analyzing data from multiple sets of batch fermentation. Each data set has its own substrate conversion, acid concentration, and selectivity. Chicken manure and sewage sludge – each with different storage methods – were selected as nitrogen-rich sources.

This chapter is a collaborative effort with Opeyemi Olokede, a PhD candidate in the Department of Chemical Engineering, Texas A&M University.

4.2 Experimental Methods

4.2.1 Inoculum Adaptation

It takes the microbial community two to three weeks to function in a newly introduced environment. To ensure high-quality data, inoculum adaptation is started prior to the batch fermentations, where quantitative measurements are taken. During inoculum adaption, conditions (e.g., substrate, pH, temperature, and inhibitor) are the same as those used in the CPDM experiment.

In this study, 20 wt% nitrogen-rich source (i.e., chicken manure or sewage sludge) and 80 wt% shredded office paper were added as substrate into a 1-L PCCO fermentor detailed in Paragraph 2.2.4. D.O. water was mixed with soils collected from Galveston followed by the treatment described in Paragraph 2.2.3. Supernant was collected as fresh incolumn. D.O. water served as fermentation media and was added to the fermentor with fresh inoculum. In inoculum adaptation, fresh inoculum was 12.5% of the working volume and dry substrate concentration was 50 g/L. To prevent methane production, methane inhibitor was added periodically. After the substrate and fermentation were loaded, the fermentor was purged with nitrogen for anaerobic condition and kept in the incubator. Every 48 h, the fermentor was vented to prevent fermentor upset. Every 48 h, methane inhibitor was added to prevent methane production. The whole adaptation process ran for approximately two to three weeks. Appendix F describes specific procedures of inoculum adaptation.

4.2.2 Substrate Treatment

Office paper served as the carbon source whereas chicken manure and sewage sludge served as the nutrient source. Additional pretreatment is not required for office paper because it is already pretreated when produced. Chicken manure and sewage sludge were treated and stored in different ways prior to substrate loading (Paragraph 2.2.1).

4.2.3 Batch Fermentation

The substrate concentrations were 20, 40, 70, 100, and 100+ g dry substrate /L liquid. The 100 and 100+ fermenters have the same substrate concentration, but 20 g carboxylic acids/ L liquid was added to the D.O. water in the 100+ group. The carboxylic acid composition of the added solution is described in Paragraph 3.3. Each concentration was measured in triplicate (Figure 4-2).

This study employed a mixture of 80 wt% carbon source and 20 wt% nutrient sources, which Rapier determined is the optimal combination for a mixed-culture of acid-forming microorganism.[44] Prior to initializing the batch fermentation, specific amounts of substrate, inoculum, and fermentation media were calculated for each loading (Tables 4-1, 4-2, and 4-3). Chicken manure employed 31.1 and 25.9 g carbon/g nitrogen. Sewage sludge employed 25.9 g carbon/g nitrogen. Nutrient source (i.e., chicken manure or sewage sludge), office paper, D.O. water, inoculum, urea (if applicable), methanogen inhibitor, and buffer (if applicable) were added and completely mixed in the fermentor. Prior to start-up, 1-L PCCO fermentors were autoclaved. Fermentors were purged with nitrogen after substrate loading and placed in the incubator (Figure 4-3).

Table 4-1. Initial loadings to start fermentation using chicken manure at 31.1 C/N ratio

Code	Label	Substrate concentration (g/L)	Working volume (mL)	Inoculum (mL)	Dry office paper (g)	Dry chicken manure (g)	Carboxylic acids (g/L)	Total water volume (mL)
WCM **(wet chicken manure)**	20-WCM	20	200	25	3.2	0.8	0	175
	40-WCM	40	200	25	6.4	1.6	0	175
	70-WCM	70	200	25	11.2	2.8	0	175
	100-WCM	100	200	25	16	4	0	175
	100^{+}-WCM	100	200	25	16	4	20	171.1
ACM **(air-dried chicken manure)**	20-ACM	20	200	25	3.2	1.2	0	175
	40-ACM	40	200	25	6.4	2.4	0	175
	70-ACM	70	200	25	11.2	4.2	0	175
	100-ACM	100	200	25	16	6	0	175
	100^{+}-ACM	100	200	25	16	6	20	171.1
BCM **(baked chicken manure)**	20-BCM	20	200	25	3.2	0.8	0	175
	40-BCM	40	200	25	6.4	1.6	0	175
	70-BCM	70	200	25	11.2	2.8	0	175
	100-BCM	100	200	25	16	4	0	175
	100+-BCM	100	200	25	16	4	20	171.1

Table 4-1. Initial loadings to start fermentation using chicken manure at 31.1 C/N ratio

Code	Label	Wet office paper (g)	Wet chicken manure (g)	Urea (g)	Total carbon (g)	Total nitrogen (g)	Water in feed (g)	D.O. water (mL)
WCM **(wet chicken manure)**	20-WCM	3.401	4.872	0.027	1.445	0.056	4.272	170.73
	40-WCM	6.802	9.743	0.053	2.891	0.112	8.545	166.46
	70-WCM	11.903	17.050	0.093	5.059	0.195	14.953	160.05
	100-WCM	17.004	24.358	0.133	7.227	0.279	21.362	153.64
	100^{+}-WCM	17.004	24.358	0.133	7.227	0.279	21.362	149.74
ACM **(air-dried chicken manure)**	20-ACM	3.402	0.888	0.027	1.445	0.056	0.289	174.71
	40-ACM	6.802	1.777	0.053	2.891	0.112	0.578	174.42
	70-ACM	11.903	3.109	0.093	5.059	0.195	1.012	173.99
	100-ACM	17.004	4.442	0.133	7.227	0.279	1.446	173.55
	100^{+}-ACM	17.004	4.442	0.133	7.227	0.279	1.446	169.65
BCM **(baked chicken manure)**	20-BCM	3.401	0.850	0.027	1.445	0.056	0.251	174.749
	40-BCM	6.802	1.700	0.053	2.891	0.112	0.502	174.489
	70-BCM	11.903	2.976	0.093	5.059	0.195	0.879	174.121
	100-BCM	17.004	4.251	0.133	7.227	0.279	1.255	173.744
	100+-BCM	17.004	4.251	0.133	7.227	0.279	1.255	169.844

(*Note*: D.O. water stands for de-oxygenated water, and the densities of acetic acid, propionic acid, and butyric are 1.05, 0.99, and 0.96 g/cm^3. For the D.O. water required for 100^{+} group: $171.1 = 200 - 25 - 0.2 \cdot \left(\frac{16}{1.05} + \frac{1}{0.99} + \frac{3}{0.96}\right)$. Codes are described in Paragraph 2.2.1. C/N ratio is set to 31.1 g carbon/g nitrogen)

Table 4-2. Initial loadings to start fermentation using sewage sludge at 25.9 C/N ratio

Code	Label	Substrate concentration (g/L)	Working volume (mL)	Inoculum (mL)	Dry office paper (g)	Dry sewage sludge (g)	Carboxylic acids (g/L)	Total water volume (mL)
WSS **(wet sewage sludge)**	20-WSS	20	200	25	3.2	0.8	0	175
	40-WSS	40	200	25	6.4	1.6	0	175
	70-WSS	70	200	25	11.2	2.8	0	175
	100-WSS	100	200	25	16	4	0	175
	100^{+}-WSS	100	200	25	16	4	20	171.1
ADS **(air-dried sewage sludge)**	20-ADS	20	200	25	3.2	1.2	0	175
	40-ADS	40	200	25	6.4	2.4	0	175
	70-ADS	70	200	25	11.2	4.2	0	175
	100-ADS	100	200	25	16	6	0	175
	100^{+}-ADS	100	200	25	16	6	20	171.1

Table 4-2. Initial loadings to start fermentation using sewage sludge at 25.9 C/N ratio

Code	Label	Wet office paper (g)	Wet sewage sludge (g)	Total carbon (g)	Total nitrogen (g)	Water in feed (g)	D.O. water (mL)
WSS **(wet sewage sludge)**	20-WSS	3.401	7.338	1.493	0.058	4.970	170.03
	40-WSS	6.802	14.677	2.986	0.115	9.940	165.06
	70-WSS	11.903	25.685	5.225	0.202	17.395	157.605
	100-WSS	17.004	36.693	7.465	0.289	24.850	150.149
	100^{+}-WSS	17.004	36.693	7.465	0.289	24.850	146.249
ADS **(air-dried sewage sludge)**	20-ADS	3.402	2.285	1.493	0.058	1.686	173.314
	40-ADS	6.802	4.570	2.986	0.115	3.372	171.628
	70-ADS	11.903	7.998	5.225	0.202	5.901	169.099
	100-ADS	17.004	11.425	7.465	0.289	8.429	166.571
	100^{+}-ADS	17.004	11.425	7.465	0.289	8.429	162.571

(*Note*: D.O. water stands for de-oxygenated water, and the densities of acetic acid, propionic acid, and butyric are 1.05, 0.99, and 0.96 g/cm^3. For the D.O. water required for 100^{+} group: $171.1 = 200 - 25 - 0.2 \cdot \left(\frac{16}{1.05} + \frac{1}{0.99} + \frac{3}{0.96}\right)$. Codes are described in Paragraph 2.2.1. C/N ratio is set to 25.9 g carbon/g nitrogen)

Table 4-3. Initial loadings to start fermentation using chicken manure at 25.9 C/N ratio

Code	Label	Wet office paper (g)	Wet chicken manure (g)	Urea (g)	Total carbon (g)	Total nitrogen (g)	Water in feed (g)	D.O. water (mL)
FCM (fresh chicken manure)	20-WCM	3.401	4.872	0.048	1.445	0.056	4.272	170.73
	40-WCM	6.802	9.743	0.096	2.891	0.112	8.545	166.46
	70-WCM	11.903	17.050	0.168	5.059	0.195	14.953	160.05
	100-WCM	17.004	24.358	0.240	7.227	0.279	21.362	153.64
	100^{+}-WCM	17.004	24.358	0.240	7.227	0.279	21.362	149.74

Code	Label	Substrate Concentration (g/L)	Working volume (mL)	Inoculum (mL)	Dry office paper (g)	Dry chicken manure (g)	Carboxylic acids (g/L)	Total water volume (mL)
FCM (fresh chicken manure)	20-FCM	20	200	25	3.2	0.8	0	175
	40-FCM	40	200	25	6.4	1.6	0	175
	70-FCM	70	200	25	11.2	2.8	0	175
	100-FCM	100	200	25	16	4	0	175
	100^{+}-FCM	100	200	25	16	4	20	171.1

(*Note*: D.O. water stands for de-oxygenated water, and the densities of acetic acid, propionic acid, and butyric are 1.05, 0.99, and 0.96 g/cm^3. For the D.O. water required for 100^{+} group: $171.1 = 200 - 25 - 0.2 \cdot \left(\frac{16}{1.05} + \frac{1}{0.99} + \frac{3}{0.96}\right)$. Codes are described in Paragraph 2.2.1. C/N ratio is set to 25.9 g carbon/g nitrogen)

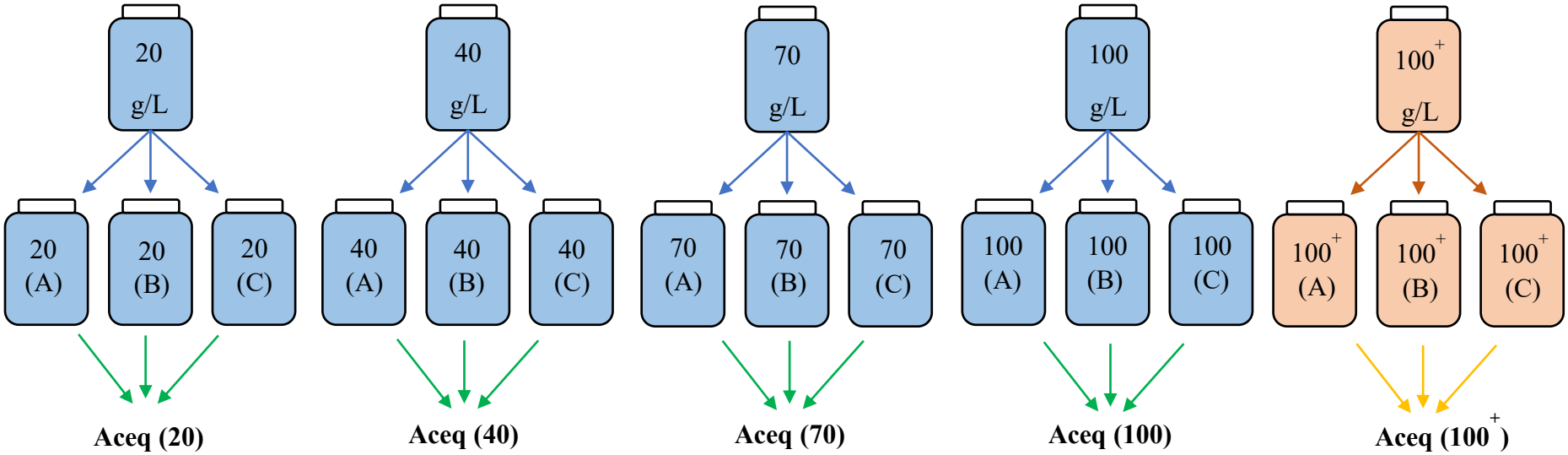

Figure 4-2. Schematic of batch fermentation set-up.

Figure 4-3. Thermostatic incubator equipped with rollers.

At the beginning, because large amounts of biogas were produced, fermentors were removed from the incubator every 48 h and vented. As the experiments proceeded, due to less reactive substrate and greater product inhibition, the interval was extended to 3, 4, and 5 days. Using the graduated water column, biogas was measured as a reaction indicator. The biogas measuring apparatus is described in Paragraph 2.1.2. To reflect inhibitor efficacy and fermentation activity, biogas was randomly sampled from two fermentors and was analyzed in the gas chromatograph (Agilent 6890 series). After rubber stoppers were removed, fermentors were capped with plastic lids and then centrifuged at 4000 rpm for 10 min to separate liquid from solids. The supernatant of each fermentor was collected in a beaker and a 1-mL liquid sample was taken from each beaker to determine the carboxylic acids concentration. The pH was measured and buffer was added if the pH was out of the optimal range (i.e., 6.5–7.2). To prevent methanogenesis, 60 µL iodoform solution (20 g CHI_3/L 200-proof ethanol) was added to each fermentor after sampling and pH adjustment. To ensure anaerobic conditions, nitrogen

purging followed. All fermenters were homogenized by vigorous shaking and returned to the incubator. Appendix G describes detailed procedures.

4.2.4 Acid Concentration Determination

Liquid samples taken from fermentors were analyzed in a gas chromatograph (Agilent 6890 series) with a flame ionization detector (FID) and autosampler (Agilent 7683 series).[27] Paragraph 2.1.3 and Appendix B describe the details of acid concentration determination.

4.2.5 pH Control

The pH was measured after the fermentors were centrifuged. In the batch fermentations, carboxylic acids accumulated and lowered the pH. Buffer was added to maintain pH within the optimal range (i.e., 6.5–7.2). In previous studies, magnesium carbonate and ammonium bicarbonate were used as buffers.[45] However, controlling the C/N ratio becomes more complicated using ammonium bicarbonate, and magnesium carbonate causes precipitation and fouling. Instead, in this study, sodium bicarbonate ($NaHCO_3$, Fischer) was introduced as buffer.

4.3 Results and Discussion

4.3.1 Acid Yield

Figures 4-4, 4-5, 4-6, 4-7, and 4-8 show the Aceq concentrations. Because each substrate concentration was performed in triplicate, the reported data are average Aceq concentration with 95% confidence intervals.

The batch fermentations were run for 45 days. After 34 days, Aceq yields tended to stabilize. Yield is defined as *exit yield*, which quantifies the total acid exiting the fermentation. Generally, higher substrate concentrations result in greater Aceq yields. Because of initial product inhibition, in the 100+ groups, yield grows more slowly than other groups. Among all the chicken manures at 31.1 C/N ratio, WCM has the highest Aceq concentration. This result suggests that the microorganism in raw wet chicken manure may be beneficial, or that drying removes or damages some essential components. Air-dried and oven-dried nutrient sources had poorer results either because it killed a desirable portion of the microbial community, or some essential components were damaged or volatilized. Golub studied the effect of sterilizing, drying, and freezing on chicken manure and found that the best-performing fermentations were run with chicken manure that was wet and never frozen; the worst-performing fermentations used chicken manure that was dried and previously frozen.[43]

In contrast, at high substrate loadings, ADS had higher Aceq concentration than WSS whereas WSS performed better at low substrate loading. Although sludge used in ADS underwent air drying, it still contained 65.0% moisture.

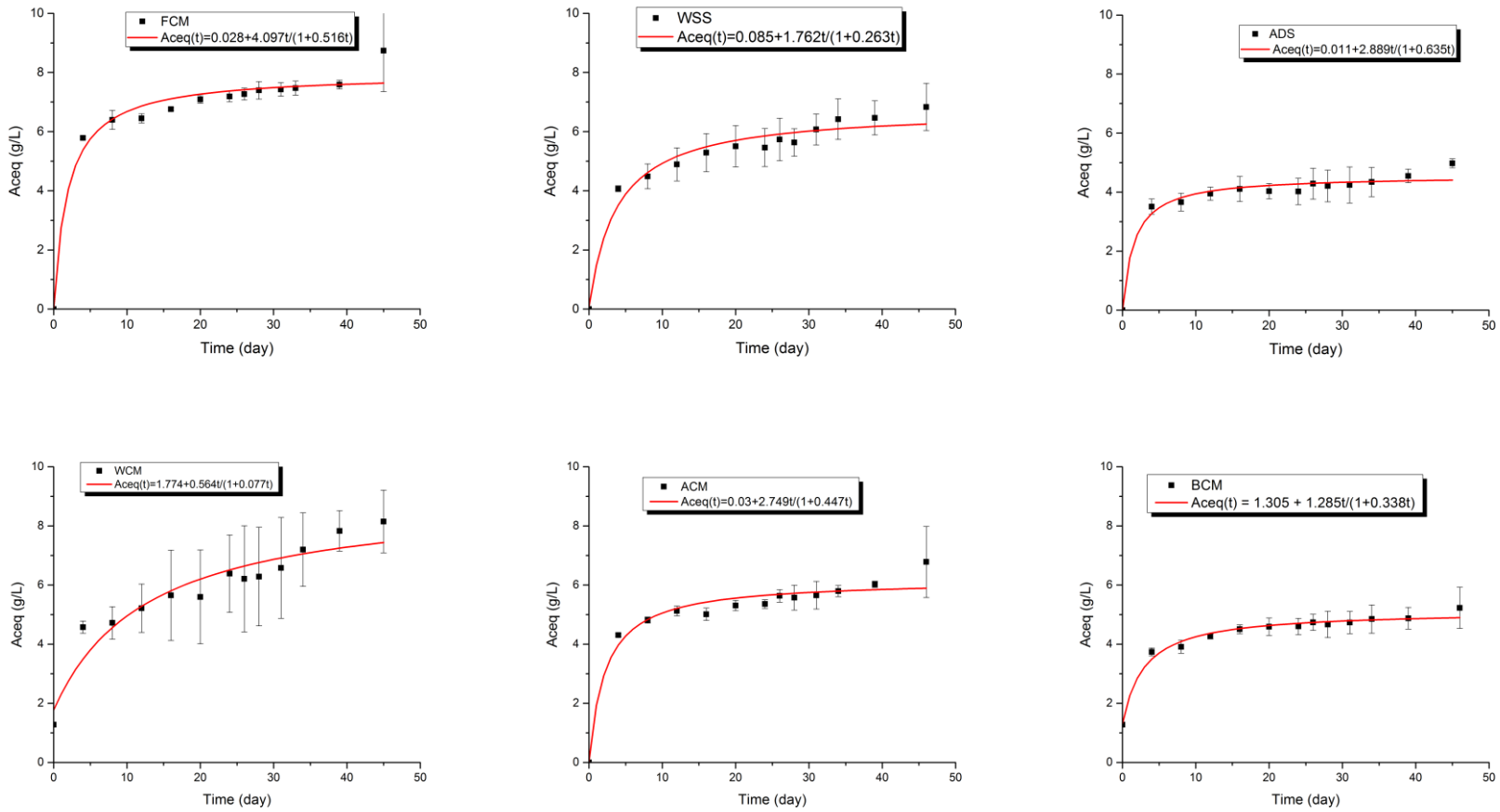

Figure 4-4. Aceq concentration profiles for different nutrients based on 20 g/L substrate concentration.

(*Note*: Error bars are derived from 95% confidence intervals.)

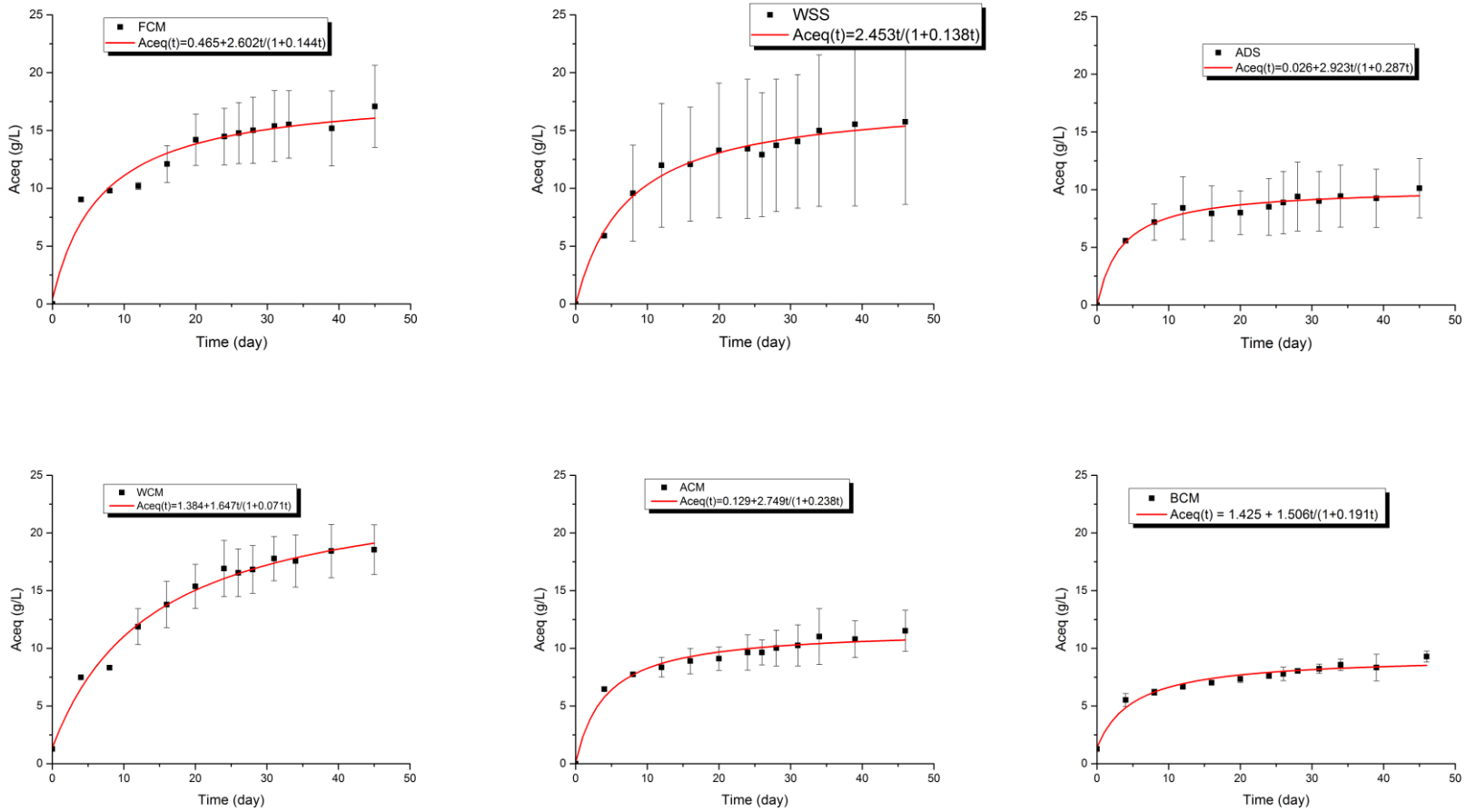

Figure 4-5. Aceq concentration profiles for different nutrients based on 40 g/L substrate concentration.

(*Note*: Error bars are derived from 95% confidence intervals.)

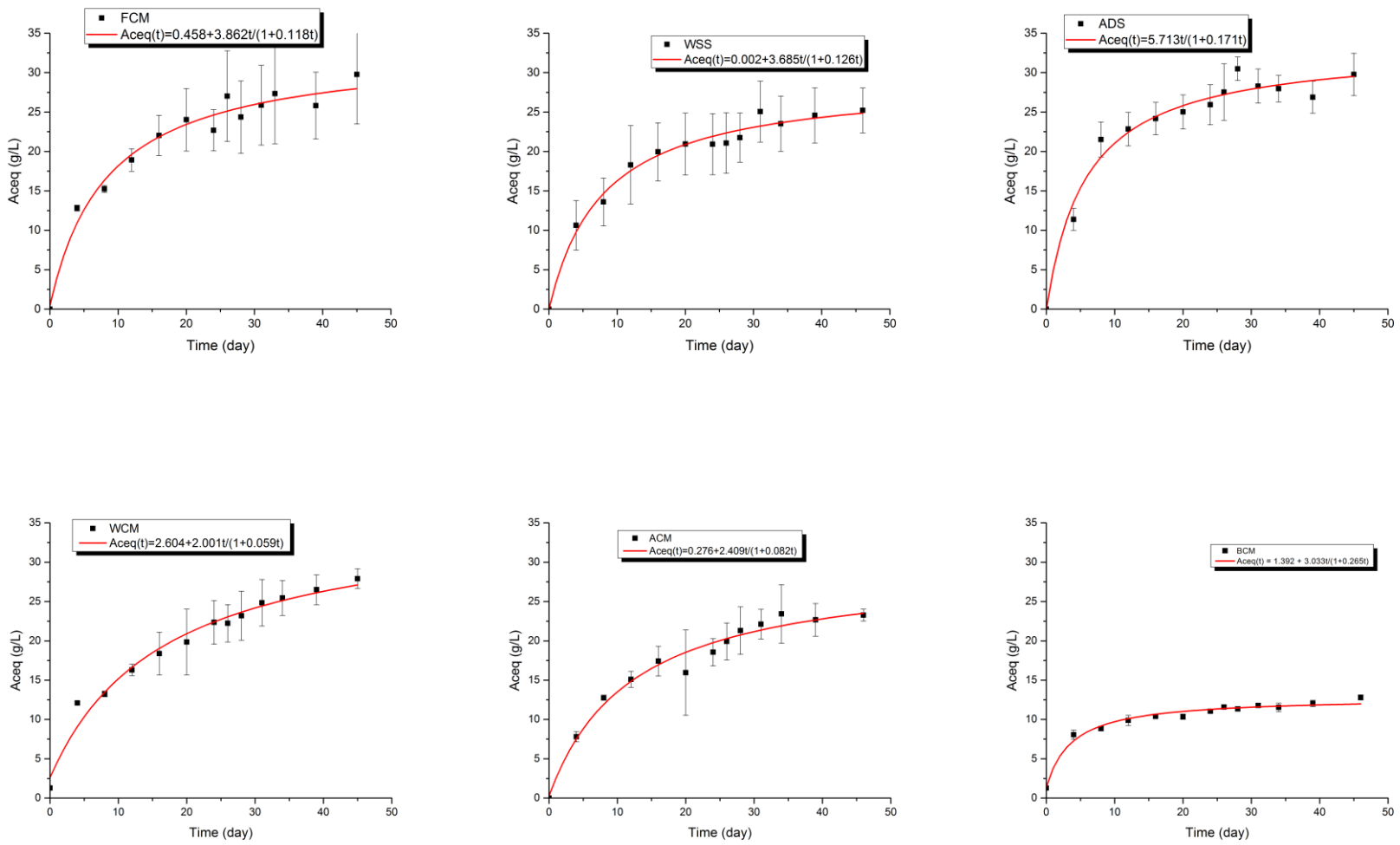

Figure 4-6. Aceq concentration profiles for different nutrients based on 70 g/L substrate concentration.

(*Note*: Error bars are derived from 95% confidence intervals.)

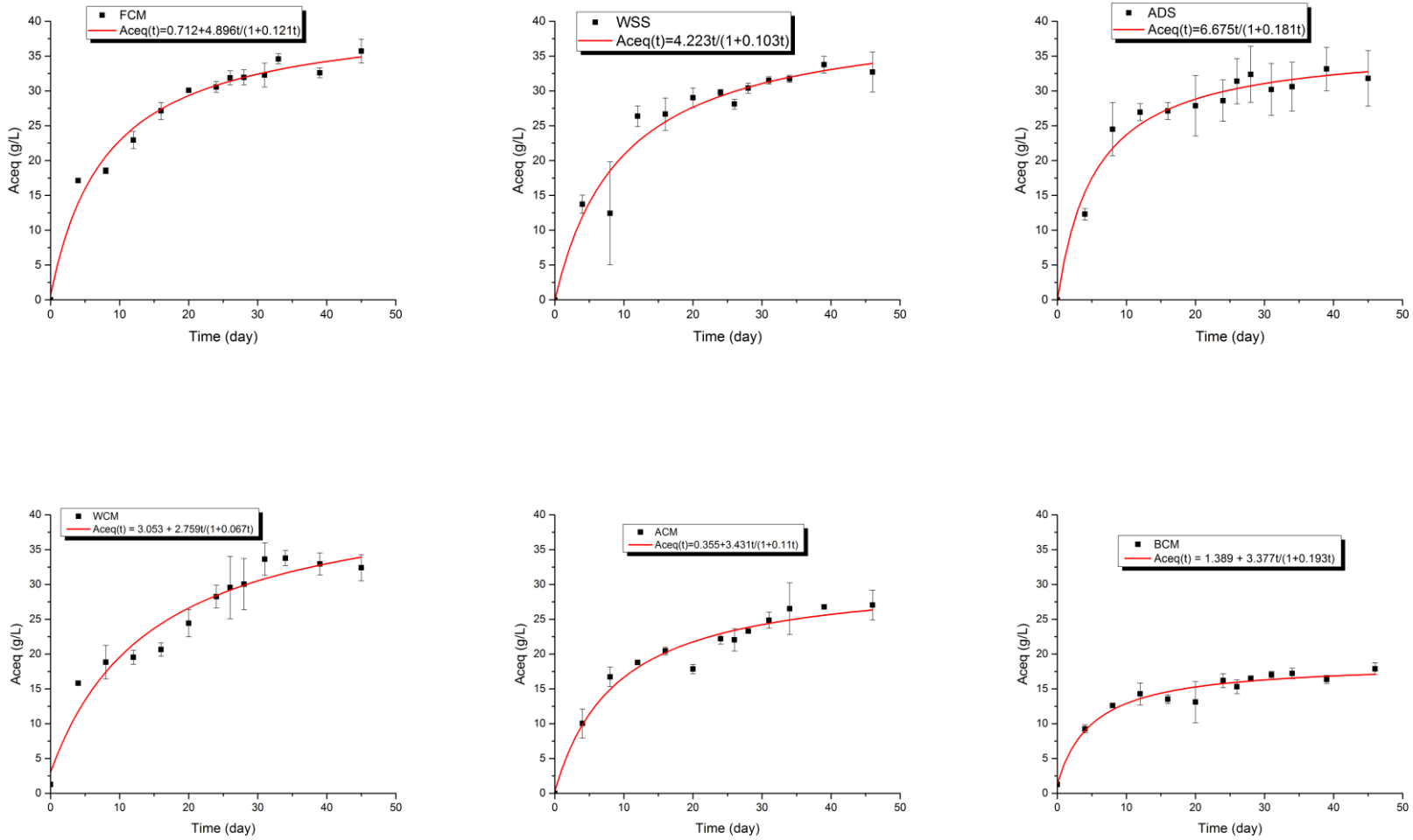

Figure 4-7. Aceq concentration profiles for different nutrients based on 100 g/L substrate concentration.

(*Note*: Error bars are derived from 95% confidence intervals.)

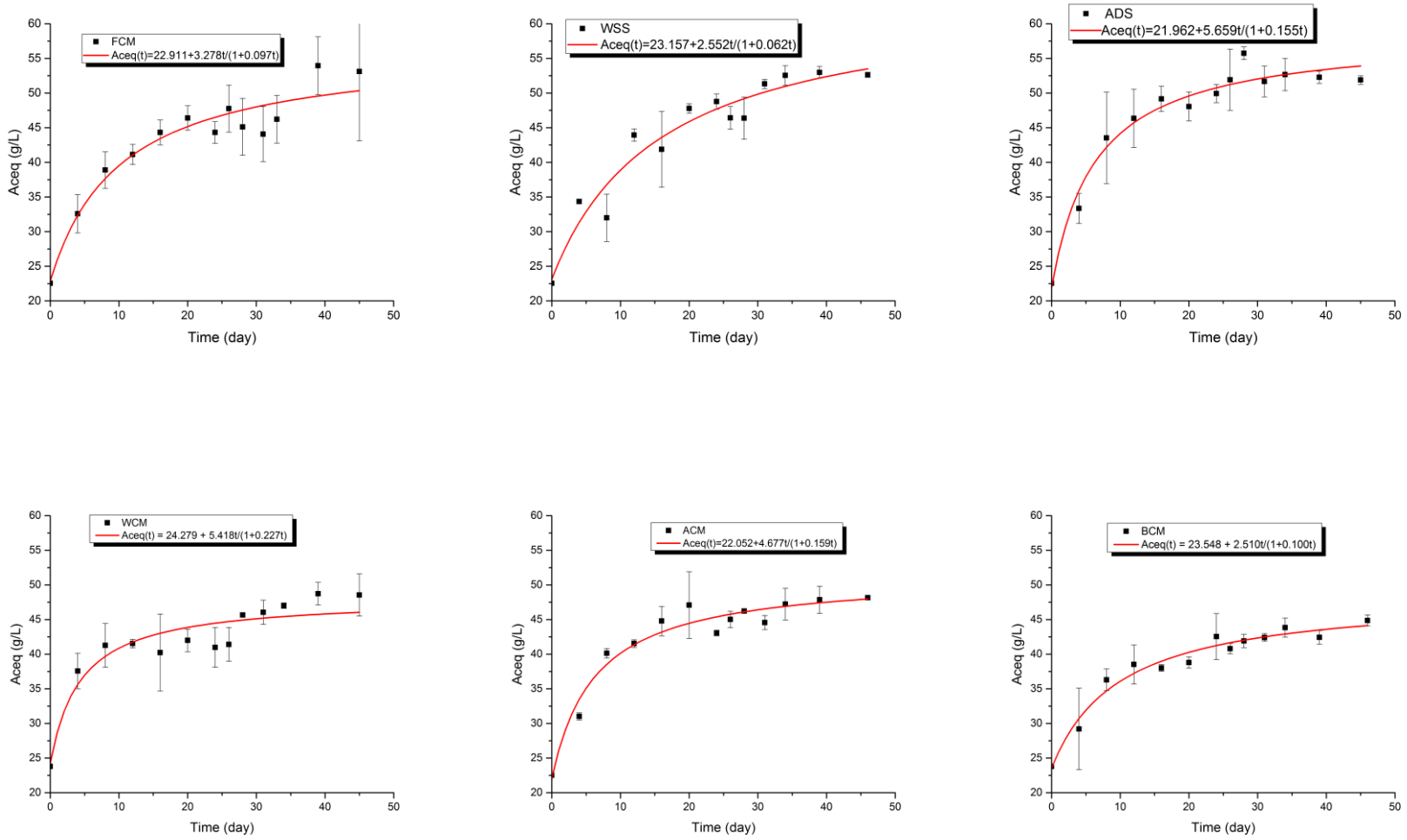

Figure 4-8. Aceq concentration profiles for different nutrients based on 100+ g/L substrate concentration.

(*Note*: Error bars are derived from 95% confidence intervals.)

4.3.2 Selectivity

Table 4-4 shows the selectivity of ACM, BCM, FCM, WCM, WSS, and ADS. Eq. 3-11 and 3-17 define selectivity *s* (g total acid produced/g digested solid) and σ (g total Aceq produced/ g digested solid). Selectivity of experiments at 25.9 C/N ratio is greater than at 31.1 C/N ratio. Comparing *s* and σ, more high-molecular-weight acids were produced in ADS, WSS, and FCM.

Table 4-4. Selectivity of ACM, BCM, FCM, WCM, WSS, and ADS

Name	ACM	BCM	WCM	FCM	ADS	WSS
s	0.50 ± 0.10	0.47 ± 0.06	0.40 ± 0.10	0.68 ± 0.13	0.57 ± 0.09	0.59 ± 0.07
σ	0.65 ±0.02	0.60 ± 0.03	0.52 ± 0.02	0.91 ± 0.03	0.81 ± 0.06	0.84 ± 0.07

(*Note*: Unit of *s* is g total acid produced/g digested solid whereas unit of σ is g total Aceq produced/ g digested solid.)

4.3.3 CPDM map

Using data from batch fermentations, empirical constants *e*, *f*, *g*, and *h* (Eq. 3-6) were calculated by minimizing the sum of square error between the experimental and predicted Aceq. For ADS and WCM, to obtain a better fit, the inhibition term (*h*) was set within a suggested range ($0.3 < h < 5$). Selectivity (σ) and ratio of carboxylic acids to Aceq (ϕ) are assumed constant. The specific rate ($\hat{r}$) models are listed as follow:

$$\hat{r}_{ACM} = \frac{0.0819(1 - x)^{4.6842}}{1 + 0.0808(0.7587 \cdot \text{Aceq})^{0.6500}} \quad \text{(ACM)}$$

$$\hat{r}_{BCM} = \frac{0.0602(1 - x)^{7.6939}}{1 + 0.1202(0.7808 \cdot \text{Aceq})^{0.6730}} \quad \text{(BCM)}$$

$$\hat{r}_{WCM} = \frac{0.0435(1 - x)^{2.1645}}{1 + 0.001(0.7669 \cdot \text{Aceq})^{1.7600}} \quad \text{(WCM)}$$

$$\hat{r}_{FCM} = \frac{0.1112(1 - x)^{4.1429}}{1 + 0.009(0.7501 \cdot \text{Aceq})^{1.8100}} \quad \text{(FCM)}$$

$$\hat{r}_{ADS} = \frac{0.1104(1 - x)^{6.5190}}{1 + 0.1677(0.7017 \cdot \text{Aceq})^{0.4000}} \quad \text{(ADS)}$$

$$\hat{r}_{WSS} = \frac{0.075(1 - x)^{3.6381}}{1 + 0.0690(0.6990 \cdot \text{Aceq})^{1.0000}} \quad \text{(WSS)}$$

Based on the above specific rate ($\hat{r}$) models, the CPDM maps were subsequently acquired to predict total acid concentrations and conversions for four-stage countercurrent fermentation with VSLR from 4 to 12 g/(L·day) and LRT from 5 to 35 days. In general, as VSLR increases, conversion declines whereas acid concentration increases. Similarly, as LRT increases, conversion drops but acid concentration increases.

The CPDM simultaneously discussed here all correspond to 100 g solids/L liquid.

4.3.3.1 Chicken Manure at 31.1 C/N Ratio

Figure 4-9 shows predicted total acid concentrations and conversion at different VSLR and LRT using 80 wt% office paper and 20% different-treated chicken manure. In general, the CPDM map gradually shifted towards the upper right from BCM to ACM, and then from ACM to WCM. These results are consistent with the batch data and show that drying negatively affects substrate digestibility, particularly when the nutrient source is oven baked. At each condition, BCM has the worst performance. At high VSLR, the difference between air-dried chicken manure and wet chicken manure is not clear; however, WCM has higher acid concentration and conversion at low VSLR of 2 g/(L·day). When wet chicken manure serves as a nutrient source, the highest acid concentration (32.3 g/L) is acquired at VSLR of 4 g/(L·day)

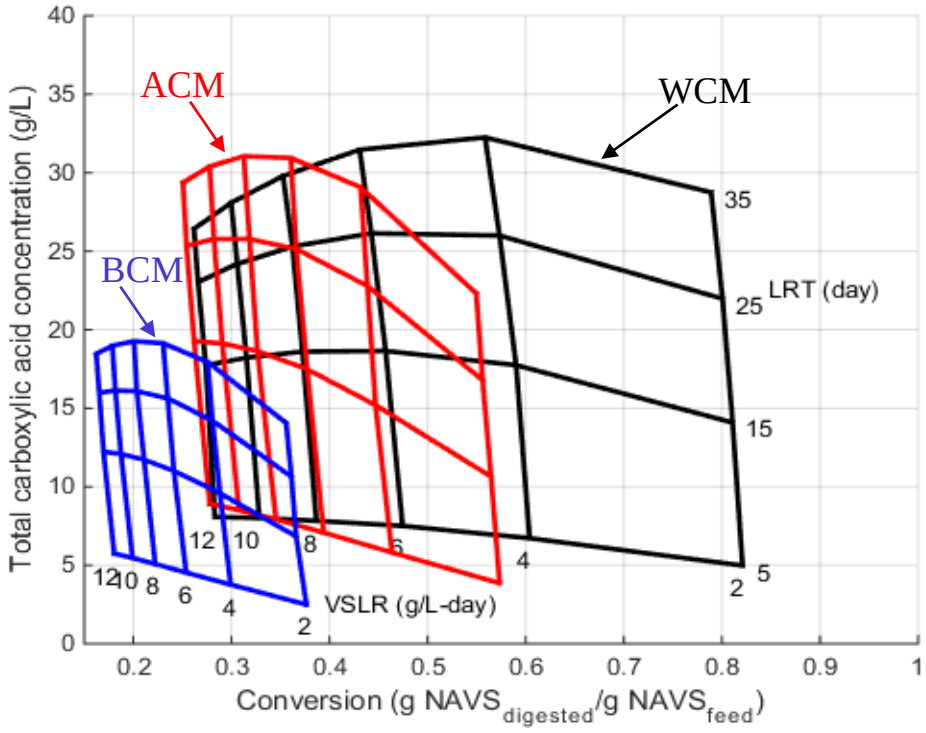

Figure 4-9. CPDM map for countercurrent fermentation at 100 g solids/L liquid using 80 wt% office paper and 20 wt% chicken manure at 31.1 g carbon/g nitrogen. (*Note*: *y*-axis is actual acid concentration, not Aceq.)

and LRT of 35 days, whereas the highest conversion (0.82 g $NAVS_{digested}$/g $NAVS_{feed}$) is acquired at VSLR of 2 g/(L·day) and LRT of 5 days.

4.3.3.2 Chicken Manure at 31.1 C/N Ratio vs. Chicken Manure at 25.9 C/N Ratio

As shown in Figure 4-10, even though two experiments were conducted within the optimal range of C/N ratio, there are still differences in acid concentration and conversion. At 6 g/(L·day) and 35 days LRT, FCM has its peak acid concentration of 29.3 g/L and conversion of 0.41 g $NAVS_{digested}$/g $NAVS_{feed}$. By increasing the C/N ratio from 25.9 to 31.1 g carbon/g nitrogen, the highest acid concentration reached 32.3 g/L. Using wastewater sludge and

pretreated bagasse, Rughoonundun investigated the influence of carbon-to-nitrogen ratio. She showed that even though acid concentration fluctuates when C/N ratio falls within the optimal range, it is relatively constant. In her study, yield was mostly affected at extreme C/N ratio (C/N > 31.8 and C/N ratio < 13.2).[22]

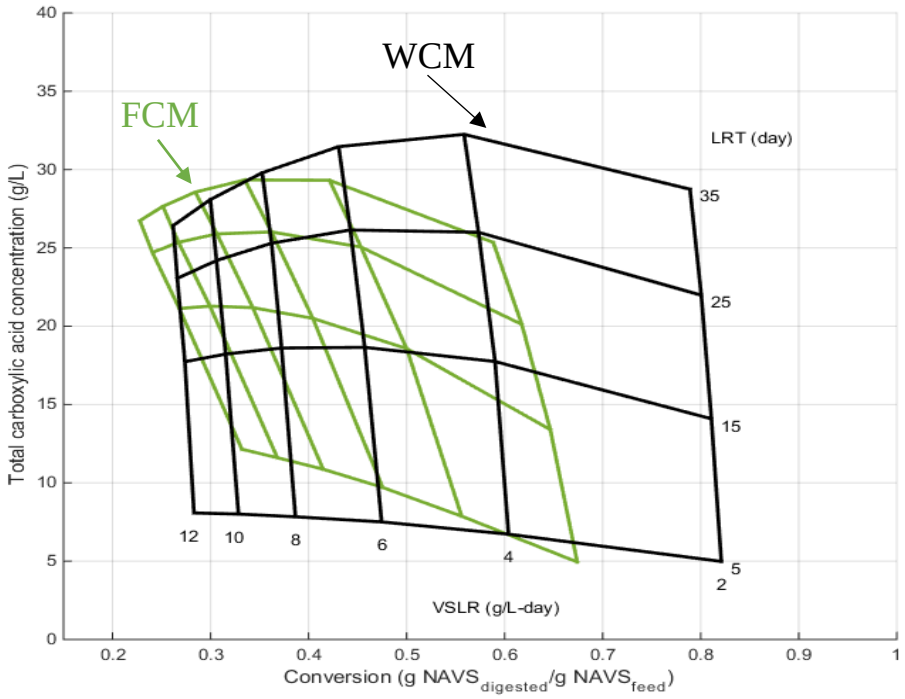

Figure 4-10. CPDM map for countercurrent fermentation at 100 g solids/L liquid using 80 wt% office paper and 20 wt% wet chicken manure at 25.9 and 31.1 g carbon/g nitrogen. (*Note:* FCM stands for experiment run at 25.9 C/N ratio whereas WCM stands for 31.1 C/N ratio. *y*-axis is actual acid concentration, not Aceq.)

4.3.3.3 Chicken Manure at 25.9 C/N Ratio vs. Sewage Sludge at 25.9 C/N Ratio

Figure 4-11 shows the CPDM maps for four-stage countercurrent fermentation for ADS, WSS, and FCM. For all groups, total acid concentration is relatively constant at each LRT. When fermentations were performed using air-dried sewage sludge and office paper at VSLR of 8 g/(L·day) and LRT of 35 days, the highest acid concentration was achieved (29.6 g/L). Compared to ADS, WSS has greater changes in conversion with respect to VSLR, which is from 0.22 to 0.66 g $NAVS_{digested}$/g $NAVS_{feed}$. For FCM and WSS, the CPDM map is generally shifted upper right, from WSS to FCM. At each VSLR and LRT, fermentation is predicted to have better performance using wet chicken manure than wet sewage sludge. Highest conversion (0.67 g $NAVS_{digested}$/g $NAVS_{feed}$) appears when wet chicken manure serves as nutrients and VSLR and LRT are set at 6 g/(L·day) and 5 days.

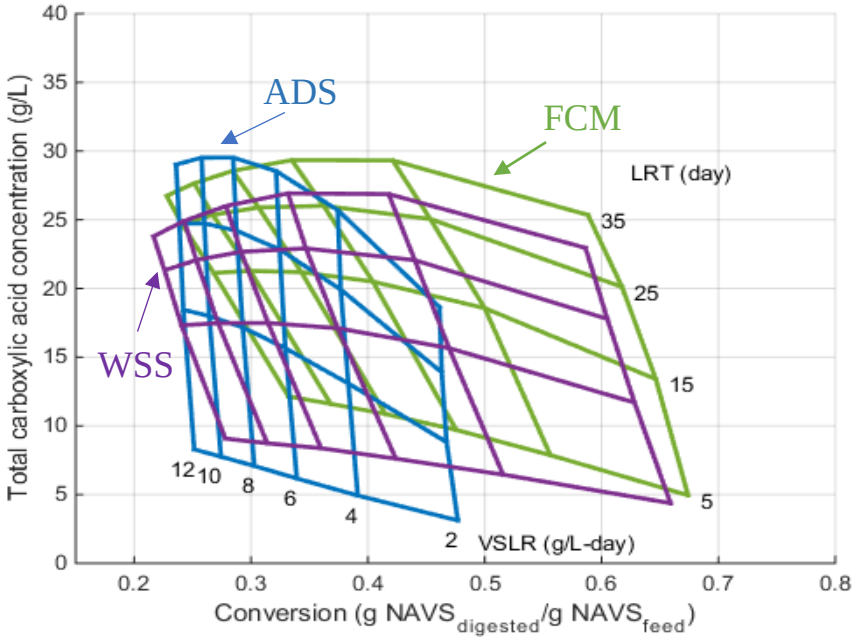

Figure 4-11. CPDM map for countercurrent fermentation at 100 g solids/L liquid using 80 wt% office paper and 20 wt% wet chicken manure or sewage sludge at 25.9 g carbon/g nitrogen. (*Note: y*-axis is actual acid concentration, not Aceq.)

4.3.3.4 CPDM map using chicken manure or sludge as nutrients at 300g solids/L liquid

Figures 4-12, 4-13, and 4-14 show CPDM map for a solid-liquid ratio at 300 g/L, which represents a high substrate concentration that could be employed in industrial-scale operations. The model does not work at VSLR of 2 g/(L·day). This occurs because the consumption rate is faster than the daily loading rate, which fails to maintain the amount of the retained solids required to maintain the substrate concentration. At each condition, because solid retention time increases at low VSLR, conversion and acid concentration improve. In Figure 4-12, when using wet chicken manure as nutrients, highest conversion (0.89 g $NAVS_{digested}$/g $NAVS_{feed}$) and acid concentration (52.8 g/L) are acquired. In Figure 4-13, although FCM and WCM were both conducted within the optimal range, due to increasing substrate concentration, differences in acid concentration and conversion become larger. In Figure 4-14, WSS has higher acid concentration than FCM, which is different from the results in laboratary-scale operations. At VSLR of 12 g/(L·day) and LRT of 35 days, ADS has the highest acid concentration (52.4 g/L). Compared to WSS and FCM, ADS has a narrow conversion range.

In all cases, predictions from CPDM maps must be verified using continuous countercurrent fermentation.

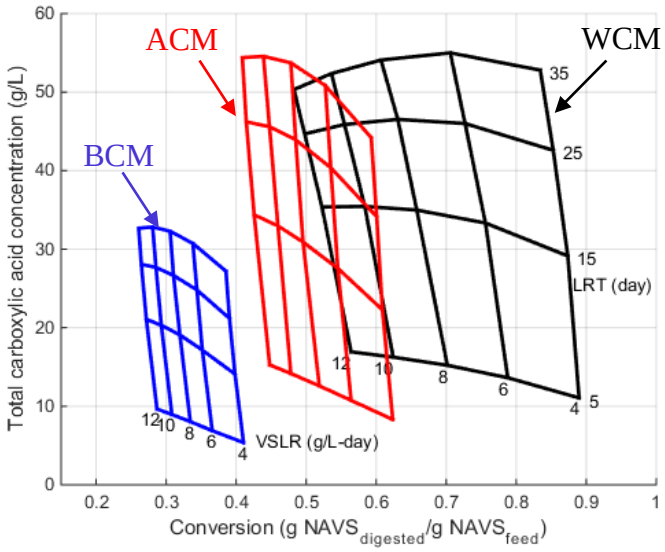

Figure 4-12. CPDM map for countercurrent fermentation at 300 g solids/L liquid using 80 wt% office paper and 20 wt% wet chicken manure at 31.1 g carbon/g nitrogen. (*Note: y*-axis is actual acid concentration, not Aceq.)

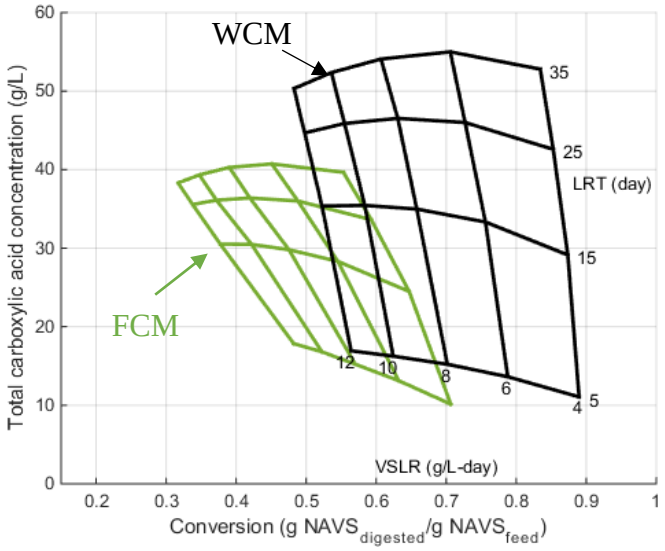

Figure 4-13. CPDM map for countercurrent fermentation at 100 g solids/L liquid using 80 wt% office paper and 20 wt% wet chicken manure at 25.9 and 31.1 g carbon/g nitrogen. (*Note*: FCM stands for experiment run at 25.9 C/N ratio whereas WCM stands for 31.1 C/N ratio. *y*-axis is actual acid concentration, not Aceq.)

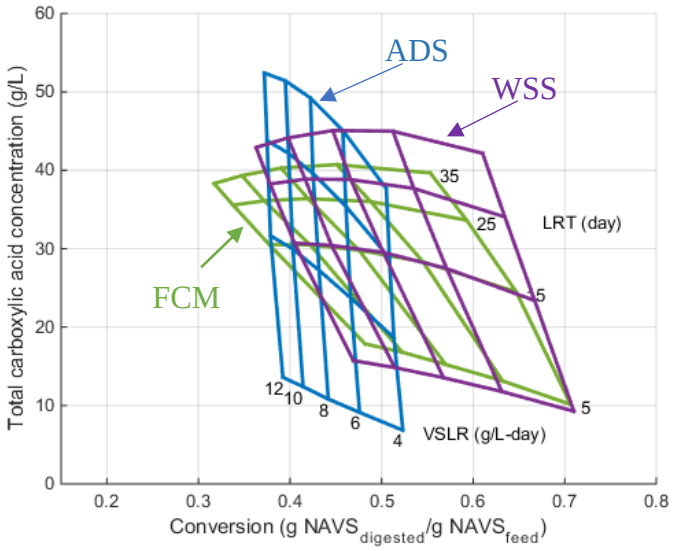

Figure 4-14. CPDM map for countercurrent fermentation at 300 g solids/L liquid using 80 wt% office paper and 20 wt% wet chicken manure or sewage sludge at 25.9 g carbon/g nitrogen. (*Note*: *y*-axis is actual acid concentration, not Aceq.)

4.3.4 Acid Composition

Figure 4-15 shows acid composition of all groups. Acetic and propionic acids are the major fractions of total acid production. Banerjee and Chen had a similar discovery that acetic acid is the chief component among the total volatile fatty acids produced by anaerobic digestion.[46-47] Liu investigated this phenomenon and found that it may occur because high-molecular-weight acids undergo biodegradation, resulting in higher amounts of acetic acid.[48] However, in this study, data do not show a higher caproic acid or caprylic acid concentration at the beginning than then end (Figure 4-16). In Figure 4-15, at high substrate concentration, more caproic acid was produced in fermentation broth with sewage sludge whereas more butyric acid was produced in fermentation broth with chicken manure.

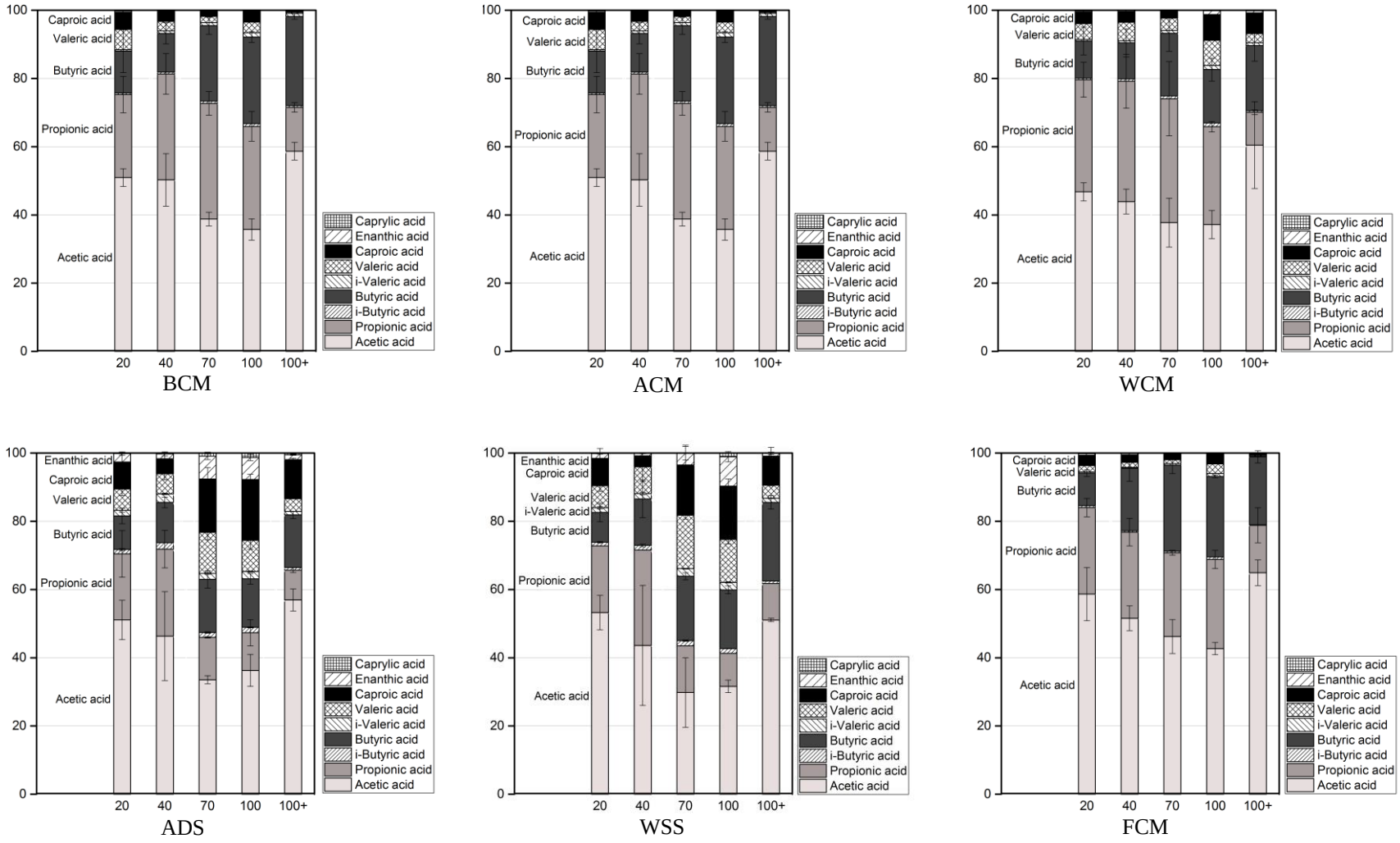

Figure 4-15. Acid profiles of BCM, ACM, WCM, ADS, WSS, and FCM at different substrate concentration.

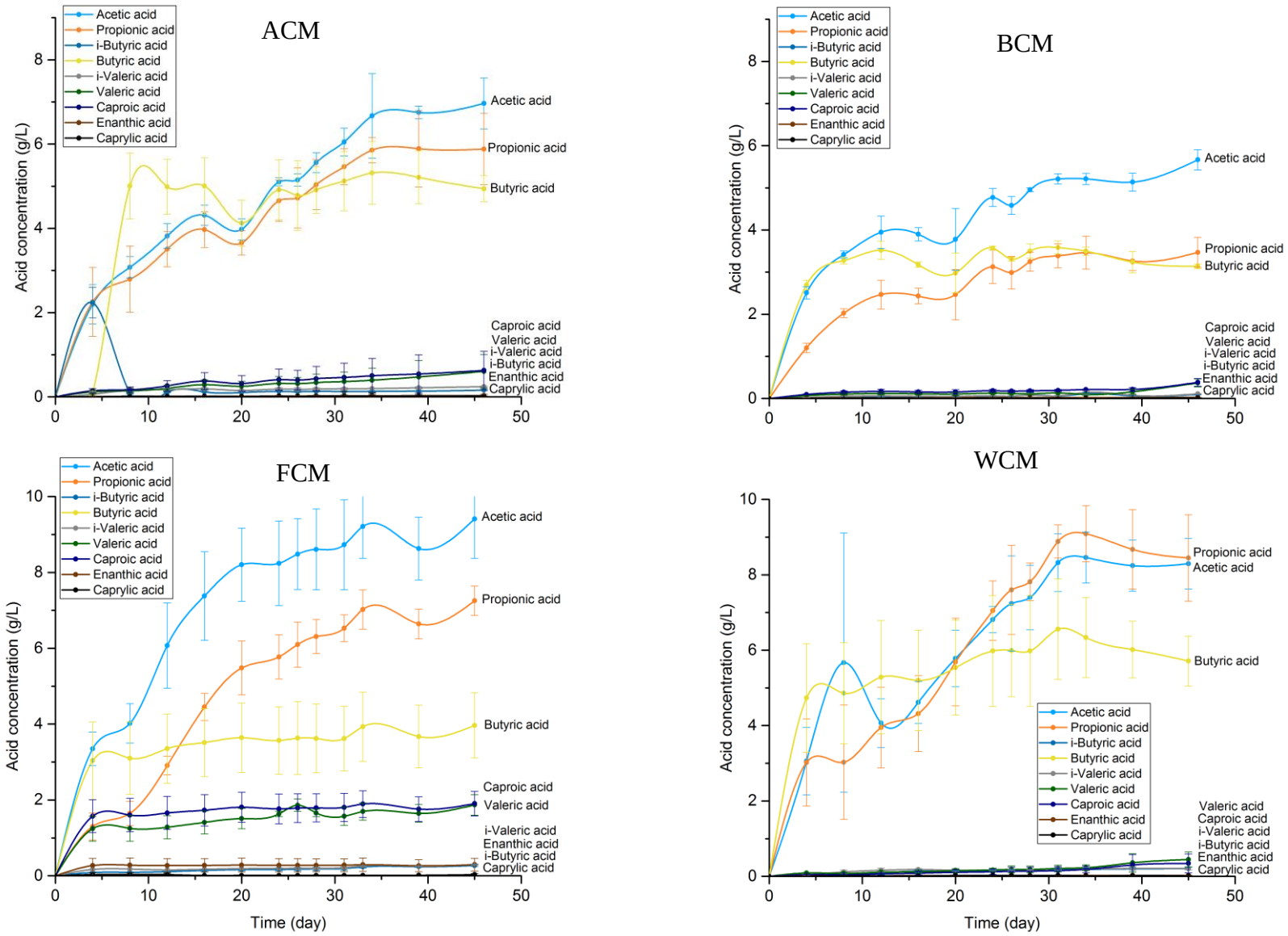

Figure 4-16. Carboxylic acid production for ACM, BCM, FCM, WCM, ADS and WSS.

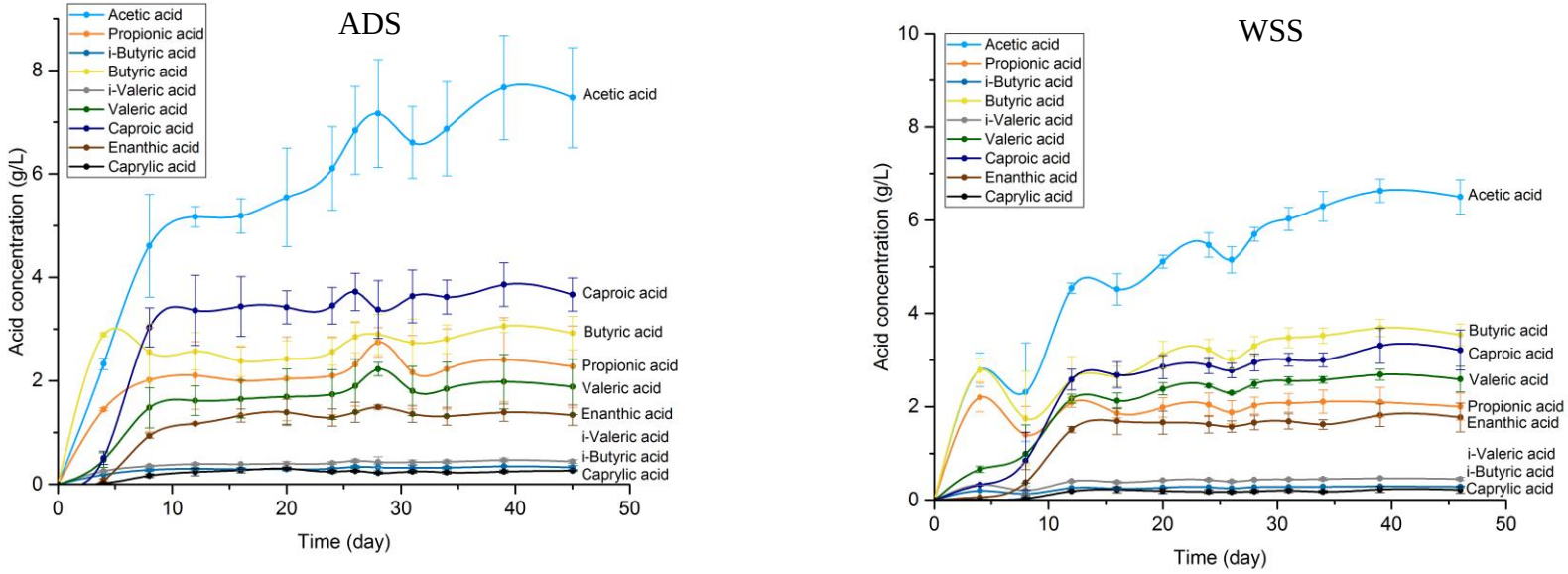

Figure 4-16. Carboxylic acid production for ACM, BCM, FCM, WCM, ADS and WSS.

4.3.5 pH

The pH of all fermenters was maintained in the optimal range (i.e., 6.5–7.2). Fermentors using chicken manure and office paper at low loadings had higher pH than others. This may occur because chicken manure is neutral to moderately alkaline, and acid yield is lowered when fermented at high substrate loadings. Carbon dioxide was used to lower pH below 7.0.

4.3.6 Gas composition and yields

During the experiment, biogas was randomly sampled from two fermentors and was analyzed in the gas chromatograph (Agilent 6890 series). The biogas consists of nitrogen (from purging during sampling) and carbon dioxide. In some samples, some oxygen (< 1%) was detected. This occurs because of either insufficient purging or airtightness of the fermenters. At the beginning, up to 31% carbon dioxide was detected.

4.4 Conclusion

Both chicken manure (without antibiotics) and sewage sludge are valuable nutrients for mixed-culture fermentation. Compared to fermenting a single substrate, acid concentration was significantly improved using carbon and nitrogen sources together.[18-20] Among the chicken manures, wet chicken manure had the best performance, proving that air-drying and oven-drying are not preferred ways to store chicken manure. Although fermentations with air-dried sewage sludge have a relatively high acid concentration, it has a lower conversion, which negatively affects process economics. When fresh nutrients are used, higher conversion and

acid concentration can be acquired. Considering that wet nutrients are difficult to transport, it may be acceptable to air-dry sewage sludge.

CHAPTER V

CONCLUSION AND FUTURE WORK

This study on MixAlco® Process shows that nutrients have a great effect on the performance of mixed-culture fermentation. C/N ratio is an important factor that affects the anaerobic fermentation. Different methods were investigated to store chicken manure and sewage sludge. Among all groups, wet antibiotic-free chicken manure and wet sewage sludge have the best performance. It showed that oven or air drying may remove or damage essential nutrients or kill a desirable portion of microorganisms in the nutrients. When using sewage sludge or chicken manure as nutrients, there is not a clear difference in Aceq concentration; however, when sewage sludge serves as a nutrient source, a larger portion of caproic acid was detected. Using data from sets of batch fermentations, CPDM maps were drawn. It shows that high conversion and total acid concentration is possible at high LRT and low VSLR. Although air-dried sewage sludge has highest acid concentration (42.1 g/L), it has a narrow conversion range, which may adversely affect process economics in industrial-scale operations. When the fermentation is performed far from the nutrient source, air drying is an option to store nutrients. It may lower the transportation cost and meanwhile keep nutrients relatively wet.

Compared to the carbon source, nutrients are wet and more difficult to transport and consolidate. Future work should focus on conducting anaerobic fermentation with lower amounts of nutrients, which could reduce the cost in industrial application. Meanwhile, pretreatment on lignocellulosic biomass increases the production cost. New carbon sources with less lignin (e.g., prickly pear) should be explored as substrate.

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APPENDIX A

BIOGAS ANALYSIS MANUAL

1. Randomly select two fermenters and take around 30 mL gas for each using syringe.
2. Select NGA method in gas chromatograph and wait until temperature reaches 180 °C.
3. In method window, name the gas sample “Date+Group+Number”.
4. Make sure that the gas outlet needle is under the water. Push the piston and insert the gas sample.
5. Press start button on the pad.

APPENDIX B

ACID CONCENTRATION ANALYSIS

1. Centrifuge the liquid sample for 10 min at 13,300 rpm.
2. Prepare a 2-mL plastic microcentrifuge tube. Pipette 0.2 mL internal standard (4-methyl-valeric acid 1.162 g/L, ISTD) and 0.2 mL 3-M phosphoric acid into it.
3. Once centrifuging is completed, pipette 0.2-mL supernatant into its corresponding microcentrifuge tube.
4. Centrifuge the mixture (ISTD + 3-M H_3PO_4 + supernatant) for 10 min at 13,300 rpm for fully mixing.
5. During the centrifuge, prepare four external standards (ESTD).
6. Once centrifuging process is completed, pipette 0.5-mL supernatant into a glass GC vial and cap it properly. Order the samples in the autosampler rack and bring it to the gas chromatograph.
7. Check the solvent and waste bottles on the injection tower. Dispose waste methanol and replenish the storage vial with new methanol. The methanol level must at least above the minimum amount.
8. Replace the septum beneath the injection tower with tweezers or with hand wearing a clean and new glove.
9. Check the gauge pressure of the gas cylinders. Replace it if needed. (Must turn off the machine while replacing.)
10. Purge the GC column with hydrogen flow (40 mL/min) for 15 min without heating.
11. Start the GC on the computer program (on-line mode) by selecting the method with the conditions listed above:

Sequence → New sequence template → Save sequence template → Update the sequence template → Save the sequence template again → Add ESTD in sequence every 10 samples → add STANDYBY at the end of the sequence.

12. Run the sequence.
13. After sequence is completed, go to offline mode for data collection.
14. Batch → Load batch → File of interested → Select all → Unclick the "STANDBY" sample → OK
15. Input the first guesses of the retention time corresponding to each acid. Make sure the retention time of each acid peaks are included in the range specified (blue background).
16. Calibrate the retention time by clicking the "scale sign".
17. Once calibration complete, click "START".
18. Batch → Option → change file name.
19. Batch → Output batch report.

APPENDIX C

MOISTURE AND ASH CONTENTS MEASUREMENTS

1. Take empty crucibles out of oven and put into desiccator only using tongs.
2. Prepare samples by using liquid products and solids waste.
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 crucibles out of oven, cool down and, measure post oven weight.
6. Put the crucibles into furnaces and 24 h later, take it out of furnace, cool down, and measure post furnace weight.
7. Wash crucibles and put into oven to dry.

APPENDIX D

DE-OXYGENATED WATER PREPARATION

1. Fill a large glass flask with D.I. water. Place the flask over a hot plate until boiling.
2. Boil the water for 10 min.
3. Seal the top of the container with aluminum foil and cool down to room temperature.
4. Based on the remaining water volume, add 0.275 g/L cysteine hydrochloride and 0.275 g/L sodium sulfide into the boiled water.
5. Stir the solution overnight.
6. After both chemicals are completely dissolved, pour into storage tank.

APPENDIX E

IODOFORM SOLUTION PREPARATION PROCEDURE

1. Measure the ethanol in the graduated cylinder.
2. Add 20 g/L iodoform to the solvent in the fume hood.
3. Mix the solution and pour it into a jar.
4. Warp up the jar with aluminum foil and store it in the fridge.

APPENDIX F

INOCULUM ADAPTATION PROCEDURE

1. Prepare enough amount of D.O. water.
2. Autoclave fermentor bottle and rubber stopper (with glass tube and septum).
3. Weigh 50 g/L dry solids of substrate into the autoclaved bottle. In this study, 400 mL is the working volume of the fermentor, thus 20 g dry solid is required. The ratio of paper to nutrients is 80:20.
4. Weigh 50 mL of fresh Galveston inoculum.
5. Calculate the volume of D.O. water that maintains the working volume 400 mL.
6. Add all of abovementioned ingredients into the autoclaved fermentor.
7. Add 120 μL methane inhibitor solution into the fermentor.
8. Purge bottle with nitrogen, capped, and place in incubator.

APPENDIX G

MATLAB CODE FOR CPDM

```
%MATLAB Code for CPDM Prediction
%This code is for a standard four-stage countercurrent fermentation
%Program predicts acid concentrations and conversion at varying VSLR and LRT.
%Department of Chemical Engineering, Texas A&M University, College St, TX
clc
clear all
close all
global so taus e1 f1 g1 h1
global holdup moist ratio stages loading tauloverall
global acid nnot factr1
global x_1 nhat_1 x_2 nhat_2 x_3 nhat_3 x_4 nhat_4

%Start Simulation
disp(['Program starts at: ', datestr(now)]);
tic;

VSLR_data= [2,4,6,8,10,12]';
LRT_data= [5,15,25,35]';
ACID = [];
CONVERSION = [];
VSLR_loop=2; %loop is for varying VSLR.
%To make map, set to lowest VSLR, otherwise, set to specific VSLR
while VSLR_loop<12.1 % if want loop, set to highest VSLR (volatile solid
loading rate)
    LRT_loop=5; %loop is for varying LRT (liquid residence time).
    %To make map, set to lowest LRT, otherwise set to specific LRT
    while LRT_loop<35.1 %if want loop, set to highest VSLR

        %%Basic parameters for Fermentation
        stages=4; %Fermentor stages
        so=0.843; %Aeq selectivity (gAEQ/g VS digested)
        %Please note that in older versions of the code (i.e. Loescher's)
        %this term referred to a VS selectivity of g VS/g total solids and
        %was carried over in the differiental equations in Ross and Fu.
        holdup =2.0; %ratio of liq to solid in wet cake (g liq/gVS cake)
        %Note: holdup is the liq in the solid cake NOT the lig of the
        %total slurry
        moist =.07; %ratio of liquid to solid in feed (g liq/gVS cake)
        SQ =1.0;
        ratio=0.7; %phi ratio of g total acid to g AEQ
        loading = VSLR_loop;
        tauloverall = LRT_loop;
        vol=[0.48,0.28,0.28,0.28]'; %Liquid volume in each fermentor
        totvol=sum(vol);
        liquidfeed = totvol/tauloverall;
        nnotreal = [300,300,300,300]'; %VS concentration gVS/L (?in each
fermentor?)
        solidfeed = loading*totvol; %Solid Feed (g dry weight)
        Convrsn = [.1,.2,.3,.4]'; %Initial value for conversion
        nnot = nnotreal./(1-Convrsn);
        taus = nnot.*vol/solidfeed;
```

```
        L =0.1*ones(stages+1,1); %L initial value for liquid flow rate in
every reactor
        taul = tauloverall/stages*ones(stages,1);

        e1=0.075; f1=3.64; g1=0.069; h1=1.00; % CPDM parameters
%         e1=0.103; f1=2.404; g1=3.76e-4; h1=1.725; %CPDM parameters
        %acd=22.3; % acd need to trfer into the Function M file
        rmodel = @(x1,acid) e1.*(1-x1).^f1./(1+g1.*(acid.*ratio).^h1);
        syms x1 acid
        drmodel_1 = diff(e1.*(1-x1).^f1./(1+g1.*(acid.*ratio).^h1),x1);
        drmodel = @(x2,acid2) subs(drmodel_1,{x1,acid},{x2,acid2});

        done = 0; %The index used to trace whether the condtion is satisfied
        liqtoler = 0.05; %tolerance for Liquid flowrate 0.005
        acidtoler = 0.1; %tolerance for acid concentration  0.02
        nnottoler = 1; %tolerance for nnot

        %Initial values for acid, acidold
        %ans=ones(stages,1); % dont use ans it is a matlab variable.
        acid=[35,30,28,25]';
        acidold=ones(stages,1);
        taulnew = 1000*ones(stages,1);  %column vector
        nhatzero =100*ones(stages,1);  %CP concentration
        creation = ones(stages,1);
        destruction = ones(stages,1);
        tauloverallnew = 20;

        disp('Calculation is in progress.......');

        while done < 0.50
            taulnew = 1000*ones(stages,1);  %Obtain Flowrate for each
fermentor
            taulover_error = 0.001;
            while abs(tauloverall-tauloverallnew) > taulover_error
                liquidfeed = liquidfeed*(1+(tauloverallnew-
tauloverall)/tauloverall*0.5);
                L(5) = liquidfeed;
                L(4) = L(5) + solidfeed/1000*holdup*(Convrsn(4)-Convrsn(3));
                L(3) = L(4) + solidfeed/1000*holdup*(Convrsn(3)-Convrsn(2));
                L(2) = L(3) + solidfeed/1000*holdup*(Convrsn(2)-Convrsn(1));
                L(1) = moist*solidfeed/1000 + L(2) -
solidfeed/1000*holdup*(1.0-Convrsn(1));
                tauloverallnew = totvol/L(1);
            end

            taul = vol./L(1:stages);  %vol 4*1, L 5*1
            nnot = nnotreal./(1-Convrsn);
            taus = nnot.*vol/solidfeed;
            scale = ones(stages,1);

            disp([' nnot= ',num2str(nnot','%15.5f')]);

            %parameters for ODE45
            options = odeset('RelTol',1e-3,'AbsTol', 1e-3);
            x_low=0; x_high=0.99;
```

```
            %Reactor 1

            i=1;
            while abs(taulnew(i) - taul(i))> liqtoler  %liqtoler = 0.05
                nhat0 =nhatzero(i);
                [x,nhat]= ode15s(@Chan1,[x_low,x_high],nhat0,options);
                x_1=x;  nhat_1 = nhat;
                F_1 = @(x_1)interp1(x,nhat,x_1);
                factr1 = nnot(i)/quad(F_1,x_low,x_high);  %calculate factor
                F_11 = @(x_1)
factr1*interp1(x,nhat,x_1).*rmodel(x_1,acid(i));
                robs = quad(F_11,x_low,x_high);
                F_12 = @(x_1) interp1(x,nhat,x_1).*x_1;
                Convrsn(i) = quad(F_12,x_low,x_high)/nnot(i)*factr1;
                taulnew(i) = (L(i)*acid(i) + solidfeed/1000*(1-
Convrsn(i))*holdup*acid(i)-L(i+1)*acid(i+1))/(L(i)*robs);
                acid(i) = acid(i) + (taul(i)*robs-
(L(i)*acid(i)+solidfeed/1000*(1-Convrsn(i))*holdup*acid(i)-
L(i+1)*acid(i+1))/L(i))*.4;  %why 0.4 here?
            end
            disp(['  acid(',num2str(i),')=',num2str(acid(i),'%15.5f'),'
taulnew(',num2str(i),')=',num2str(taulnew(i),'%15.5f'),'  robs=',
num2str( robs, '%15.5f')]);

            %Reactor 2

            i=2;
            nnottoler = nnot(i)/50;
            while abs(taulnew(i)-taul(i))>liqtoler;
                ndone = 0;
                while ndone <0.50
                    nhat0=nhatzero(i);
                    options = odeset('RelTol',1e-3,'AbsTol',1e-3);
                    [x,nhat] = ode15s(@Chan2,[x_low,x_high],nhat0,options);
                    x_2=x;  nhat_2=nhat;
                    F_2 = @(x_2)interp1(x,nhat,x_2);
                    nhattot=quad(F_2,x_low,x_high);
                    disp(['  nhatzero= ',num2str(nhatzero(i), '%15.5f'),';
nhattot= ',num2str(nhattot, '%15.5f'),';  nnot(',num2str(i),')=
',num2str(nnot(i), '%15.5f')]);
                    if abs(nhattot - nnot(i))<nnottoler;
                        ndone = 1;
                    end
                    if (nhatzero(i) + (nnot(i) - nhattot)*1.0)>0
                        nhatzero(i)= nhatzero(i) + (nnot(i) - nhattot)*0.7;
                    else
                        nhatzero(i)= nhatzero(i) + (nnot(i) - nhattot)*0.2;
                    end
                end

                F_22 = @(x_2)interp1(x,nhat,x_2).*x_2;
                Convrsn(i)= quad(F_22,x_low,x_high)/nnot(i);
                robs = solidfeed*so/vol(i)*(Convrsn(i)-Convrsn(i-1));
```

```
                taulnew(i) = (L(i)*acid(i) + solidfeed/1000*(1-
Convrsn(i))*holdup*acid(i)-L(i+1)*acid(i+1))/(L(i)*robs);
                acid(i) = acid(i) + (taul(i)*robs-
(L(i)*acid(i)+solidfeed/1000*(1-Convrsn(i))*holdup*acid(i)-
L(i+1)*acid(i+1))/L(i))*.5;
                disp(['  taulnew(',num2str(i),')=',num2str(taulnew(i),
'%15.5f'),'  taul(',num2str(i),')=',num2str(taul(i),'%15.5f'),]);
            end
            disp(['  acid(',num2str(i),')=',num2str(acid(i),'%15.5f'),'
taulnew(',num2str(i),')=',num2str(taulnew(i),'%15.5f'),'  robs=',
num2str( robs, '%15.5f')]);

            %Reactor 3

            i=3;
            nnottoler = nnot(i)/100;
            while abs(taulnew(i)-taul(i))>liqtoler;
                ndone = 0;
                while ndone <0.50
                    nhat0 =nhatzero(i);
                    options = odeset('RelTol',1e-3,'AbsTol',1e-3);
                    [x,nhat] =
ode15s(@Chan3,[x_low,x_high],nhat0,options); %was chan3
                    x_3=x;  nhat_3=nhat;
                    F_3 = @(x_3)interp1(x,nhat,x_3);
                    nhattot=quad(F_3,x_low,x_high);
                    disp(['  nhatzero= ',num2str(nhatzero(i), '%15.5f'),';
nhattot= ',num2str(nhattot, '%15.5f'),';  nnot(',num2str(i),')=
',num2str(nnot(i), '%15.5f')]);
                    if abs(nhattot - nnot(i))<nnottoler;
                        ndone = 1;
                    end
                    if (nhatzero(i) + (nnot(i) - nhattot)*1.0)>0
                        nhatzero(i)= nhatzero(i) + (nnot(i) - nhattot)*0.7;
                    else
                        nhatzero(i)= nhatzero(i) + (nnot(i) - nhattot)*0.2;
                    end
                end

                F_32 = @(x_3)interp1(x,nhat,x_3).*x_3;
                Convrsn(i)= quad(F_32,x_low,x_high)/nnot(i);
                robs = solidfeed*so/vol(i)*(Convrsn(i)-Convrsn(i-1));

                %taulnew(i) = (L(i)*acid(i) + solidfeed/1000*(1-
Convrsn(i))*holdup*acid(i)-solidfeed/1000*(1-Convrsn(i-1))*holdup*acid(i-
1))/(L(i)*robs);
                %acid(i) = acid(i) + (taul(i)*robs-
(L(i)*acid(i)+solidfeed/1000*(1-Convrsn(i))*holdup*acid(i)-solidfeed/1000*(1-
Convrsn(i-1))*holdup*acid(i-1))/L(i))*0.5;
                taulnew(i) = (L(i)*acid(i) + solidfeed/1000*(1-
Convrsn(i))*holdup*acid(i)-L(i+1)*acid(i+1))/(L(i)*robs);
                acid(i) = acid(i) + (taul(i)*robs-
(L(i)*acid(i)+solidfeed/1000*(1-Convrsn(i))*holdup*acid(i)-
L(i+1)*acid(i+1))/L(i))*.5;
                disp(['  taulnew(',num2str(i),')=',num2str(taulnew(i),
'%15.5f'),'  taul(',num2str(i),')=',num2str(taul(i),'%15.5f'),]);
```

```
            end
            disp(['  acid(',num2str(i),')=',num2str(acid(i),'%15.5f'),'
taulnew(',num2str(i),')=',num2str(taulnew(i),'%15.5f'),'  robs=',
num2str( robs, '%15.5f')]);


            %Reactor 4

            i=4;
            nnottoler = nnot(i)/100;
            while abs(taulnew(i)-taul(i))>liqtoler;
                ndone = 0;
                while ndone <0.50
                    nhat0 =nhatzero(i);
                    options = odeset('RelTol',1e-3,'AbsTol',1e-3);
                    [x,nhat] =
ode15s(@Chan4,[x_low,x_high],nhat0,options); %was chan4
                    x_4=x;  nhat_4=nhat;
                    F_4 = @(x_4)interp1(x,nhat,x_4);
                    nhattot=quad(F_4,x_low,x_high);
                    disp(['  nhatzero= ',num2str(nhatzero(i), '%15.5f'),';
nhattot= ',num2str(nhattot, '%15.5f'),';  nnot(',num2str(i),')=
',num2str(nnot(i), '%15.5f')]);
                    if abs(nhattot - nnot(i))<nnottoler;
                        ndone = 1;
                    end
                    if (nhatzero(i) + (nnot(i) - nhattot)*1.0)>0
                        nhatzero(i)= nhatzero(i) + (nnot(i) -
nhattot)*0.7; %25/nnot(i);
                    else
                        nhatzero(i)= nhatzero(i) + (nnot(i) - nhattot)*0.2;
                    end
                end

                F_42 = @(x_4)interp1(x,nhat,x_4).*x_4;
                Convrsn(i)= quad(F_42,x_low,x_high)/nnot(i);
                robs = solidfeed*so/vol(i)*(Convrsn(i)-Convrsn(i-1));

                taulnew(i) = (L(i)*acid(i) + solidfeed/1000*(1-
Convrsn(i))*holdup*acid(i)-solidfeed/1000*(1-Convrsn(i-1))*holdup*acid(i-
1))/(L(i)*robs);
                acid(i) = acid(i) + (taul(i)*robs-
(L(i)*acid(i)+solidfeed/1000*(1-Convrsn(i))*holdup*acid(i)-solidfeed/1000*(1-
Convrsn(i-1))*holdup*acid(i-1))/L(i))*0.5;
                disp(['  taulnew(',num2str(i),')=',num2str(taulnew(i),
'%15.5f'),'  taul(',num2str(i),')=',num2str(taul(i),'%15.5f'),]);
            end
            disp(['  acid(',num2str(i),')=',num2str(acid(i),'%15.5f'),'
taulnew(',num2str(i),')=',num2str(taulnew(i),'%15.5f'),'  robs=',
num2str( robs, '%15.5f')]);
            disp(['  Conversion in each stage (from nhat):
',num2str(Convrsn','%13.5f')]);

            if max(abs(acid-acidold))<acidtoler
                done=1;
            end
```

```
            acidold = acid;
        end

        %Output results section

        disp('Congratulations!  The simulation is successfully finished!')
        toc  %toc is used to check the whole time of the process

        for i3 = 1:(stages+1);
            disp(['  L(',int2str(i3),')= ',num2str(L(i3))]);
        end
        creation(1) = L(1)*acid(1) + solidfeed/1000*(1-
Convrsn(1))*holdup*acid(2)-L(2)*acid(2);
        creation(2) = L(2)/acid(2) + solidfeed/1000*(1-
Convrsn(2))*holdup*acid(3)-L(3)*acid(3)- solidfeed/1000*(1-
Convrsn(1))*holdup*acid(2);
        creation(3) = L(3)*acid(3) + solidfeed/1000*(1-
Convrsn(3))*holdup*acid(4)-L(4)*acid(4)- solidfeed/1000*(1-
Convrsn(2))*holdup*acid(3);
        creation(4) = L(4)*acid(4) - solidfeed/1000*(1-
Convrsn(3))*holdup*acid(4);
        %Calculation of Destruction
        destruction(1) = solidfeed/1000*(Convrsn(1)-0);
        for i3=2:stages;
            destruction(i3)=solidfeed/1000*(Convrsn(i3)-Convrsn(i3-1));
        end
        selectivi = creation./destruction;
        selec = L(1)*acid(1)/(solidfeed*Convrsn(4));

        %output the result and plot the result
        disp(['  Selectivity = ',num2str(selectivi','%15.5f')]);
        disp(['  Creation = ',num2str(creation','%15.5f')]);
        disp(['  Destruction = ',num2str(destruction','%15.5f')]);
        disp(['  selectivity = ',num2str(selec','%15.5f')]);
        disp(['  tauloverall = ',num2str(tauloverall,'%15.5f')]);
        disp(['  taus = ',num2str(sum(taus),'%15.5f')]);
        disp(['  acid levels = ',num2str(acid','%13.5f')]);

        disp(['  VSLR_LOOP = ',num2str(VSLR_loop),'  LRT_loop =
',num2str(LRT_loop)]);

        %Collect data for CPDM map
        ACID = [ACID;acid(1)];
        CONVERSION = [CONVERSION;Convrsn(4)];
        LRT_loop = LRT_loop + 10;
    end
    VSLR_loop = VSLR_loop + 2;
end
disp(['  acid levels = ',num2str(acid','%13.5f')]);
disp(['  convrsn levels = ',num2str(Convrsn','%13.5f')]);
 %disp(['  VSLR = ',num2str(VSLR_data','%13.5f')]);
 %disp(['  LRT = ',num2str(LRT_data','%13.5f')]);
 disp(['  Acid levels = ',num2str(ACID','%13.5f')]);
 disp(['  Conversions = ',num2str(CONVERSION','%13.5f')]);
```

APPENDIX H

MATLAB CODE FOR CPDM MAP

```
clc

VSLR=[2;2;2;2;4;4;4;4;6;6;6;6;8;8;8;8;10;10;10;10;12;12;12;12];
LRT=[5;15;25;35;5;15;25;35;5;15;25;35;5;15;25;35;5;15;25;35;5;15;25;35];
lw = 2;
% PLOTTING FCM
ACID =
[4.9472;13.3959;20.116;25.3483;7.8703;18.5685;25.0529;29.3202;9.7068;20.502;2
6.0179;29.3409;10.8713;21.1865;25.8945;28.5591;11.6029;21.2832;25.3567;27.654
5;12.1473;21.1304;24.7066;26.7323];
CONVERSION =
[0.67400;0.64657;0.61748;0.58839;0.55617;0.50002;0.45344;0.42128;0.47556;0.40
430;0.36209;0.33490;0.41486;0.34306;0.30647;0.28439;0.36813;0.29934;0.26733;0
.25135;0.33196;0.26811;0.24070;0.22786];

mapdata=[VSLR,LRT,CONVERSION,ACID];
VSLR_sorted=sortrows(mapdata,1);
LRT_sorted=sortrows(mapdata,2); %sort
[map_num,map_1]=size(mapdata);
VSLR_sort = sort(mapdata(:,1));
uniqueM = [diff(VSLR_sort);1] > 0;
%count = [VSLR_sort(uniqueM); diff(find([1;uniqueM]))]
VSLR_sort1 = VSLR_sort(uniqueM);
VSLR_number = diff(find([1;uniqueM]));
LRT_sort = sort(mapdata(:,2));
uniqueM = [diff(LRT_sort);1] > 0;
%count = [sortM(uniqueM) diff(find([1;uniqueM]))]
LRT_sort1 = LRT_sort(uniqueM);  %Unique LRT
LRT_number = diff(find([1;uniqueM]));
%plot for VSLR part
temp1=zeros(length(VSLR_sort1)+1,1);
for j1=1:length(VSLR_sort1)
temp1(j1+1)=temp1(j1)+VSLR_number(j1);
mapdata_1=VSLR_sorted(temp1(j1)+1:temp1(j1+1),:) ;
%for VSLR(j1)
F = @(x)interp1(mapdata_1(:,3),mapdata_1(:,4),x,'spline');
hold on;
plot(mapdata_1(:,3),F(mapdata_1(:,3)),'linewidth',lw,'color',[0.4660 0.6740
0.1880]);
if j1==1
for j3=1:length(mapdata_1(:,3))
%text(mapdata_1(j3,3),mapdata_1(j3,4), ['  ',
num2str(mapdata_1(j3,2))] ,'HorizontalAlignment','left');
end
%text(mapdata_1(1,3)-0.17,mapdata_1(1,4)-3, ' VSLR (g/L-day)
' ,'HorizontalAlignment','left');
end
end
%plot for LRT part
temp1=zeros(length(LRT_sort1)+1,1);
for j1=1:length(LRT_sort1)
```

```
temp1(j1+1)=temp1(j1)+LRT_number(j1);
mapdata_2=LRT_sorted(temp1(j1)+1:temp1(j1+1),:) ;
%for LRT(j1)
F2 = @(x)interp1(mapdata_2(:,3),mapdata_2(:,4),x,'spline');
hold on;
plot(mapdata_2(:,3),F2(mapdata_2(:,3)),'linewidth',lw,'color',[0.4660 0.6740
0.1880]);
if j1==1
for j3=1:length(mapdata_2(:,3))
%text(mapdata_2(j3,3)+0.005,mapdata_2(j3,4)-1.5, ['
',num2str(mapdata_2(j3,1))] ,   'HorizontalAlignment','right');
end
%text(mapdata_2(1,3)-0.025,mapdata_2(1,4)+20, 'LRT (day)
' ,'HorizontalAlignment','left');
grid on
end
end
%hold off;
xlabel('Conversion (g NAVS_d_i_g_e_s_t_e_d/g NAVS_f_e_e_d)');
ylabel('Total carboxylic acid concentration (g/L)');
axis([0.15 1.00 0 80]);

% PLOTTING WCM
ACID
=[4.9826;14.0885;21.9806;28.7471;6.7225;17.7461;25.9973;32.2494;7.5063;18.650
2;26.1426;31.4647;7.8335;18.6075;25.3005;29.7938;8.008;18.2208;24.1937;28.083
9;8.0779;17.7344;23.0491;26.4226];
CONVERSION
=[0.82099;0.81089;0.80058;0.78905;0.60394;0.59022;0.57332;0.55854;0.47448;0.4
5724;0.44261;0.43069;0.38658;0.37245;0.36222;0.35265;0.32847;0.31539;0.30601;
0.29974;0.28308;0.27380;0.26606;0.26183]

mapdata=[VSLR,LRT,CONVERSION,ACID];
VSLR_sorted=sortrows(mapdata,1);
LRT_sorted=sortrows(mapdata,2); %sort
[map_num,map_1]=size(mapdata);
VSLR_sort = sort(mapdata(:,1));
uniqueM = [diff(VSLR_sort);1] > 0;
%count = [VSLR_sort(uniqueM); diff(find([1;uniqueM]))]
VSLR_sort1 = VSLR_sort(uniqueM);
VSLR_number = diff(find([1;uniqueM]));
LRT_sort = sort(mapdata(:,2));
uniqueM = [diff(LRT_sort);1] > 0;
%count = [sortM(uniqueM) diff(find([1;uniqueM]))]
LRT_sort1 = LRT_sort(uniqueM);  %Unique LRT
LRT_number = diff(find([1;uniqueM]));
%plot for VSLR part
temp1=zeros(length(VSLR_sort1)+1,1);
for j1=1:length(VSLR_sort1)
temp1(j1+1)=temp1(j1)+VSLR_number(j1);
mapdata_1=VSLR_sorted(temp1(j1)+1:temp1(j1+1),:) ;
%for VSLR(j1)
F = @(x)interp1(mapdata_1(:,3),mapdata_1(:,4),x,'spline');
hold on;
plot(mapdata_1(:,3),F(mapdata_1(:,3)),'linewidth',lw,'color',[0 0 0]);
if j1==1
```

```
for j3=1:length(mapdata_1(:,3))
text(mapdata_1(j3,3),mapdata_1(j3,4)-0.5, ['  ',
num2str(mapdata_1(j3,2))] ,'HorizontalAlignment','left');
end
text(mapdata_1(1,3)-0.3,mapdata_1(1,4)-3, ' VSLR (g/L-day)
' ,'HorizontalAlignment','left');
end
end
%plot for LRT part
temp1=zeros(length(LRT_sort1)+1,1);
for j1=1:length(LRT_sort1)
temp1(j1+1)=temp1(j1)+LRT_number(j1);
mapdata_2=LRT_sorted(temp1(j1)+1:temp1(j1+1),:) ;
%for LRT(j1)
F2 = @(x)interp1(mapdata_2(:,3),mapdata_2(:,4),x,'spline');
hold on;
plot(mapdata_2(:,3),F2(mapdata_2(:,3)),'linewidth',lw,'color',[0 0 0]);
if j1==1
for j3=1:length(mapdata_2(:,3))
text(mapdata_2(j3,3)+0.005,mapdata_2(j3,4)-1.5, ['
',num2str(mapdata_2(j3,1))] ,   'HorizontalAlignment','right');
end
text(mapdata_2(1,3)-0.01,mapdata_2(1,4)+27, 'LRT (day)
' ,'HorizontalAlignment','left');
grid on
end
end
hold off;
xlabel('Conversion (g NAVS_d_i_g_e_s_t_e_d/g NAVS_f_e_e_d)');
ylabel('Total carboxylic acid concentration (g/L)');
axis([0.15 1.00 0 40]);
```