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Conversion of Municipal Solid Wastes to Carboxylic Acids by Thermophilic Fermentation

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Abstract

The purpose of this research is to generate carboxylic acids from the biodegradable fraction of municipal solid wastes (MSW) and municipal sewage sludge (MSS) by using a thermophilic (55°C), anaerobic, high-solid fermentation. With terrestrial inocula, the highest total carboxylic acid concentration achieved was 20.5 g/L, the highest conversion obtained was 69%, and the highest acetic acid selectivity was 86.4%. Marine inocula were also used to compare against terrestrial sources. Continuum particle distribution modeling (CPDM) was used to predict the final acid product concentrations and substrate conversions at a wide range of liquid residence times (LRT) and volatile solid loading rates (VSLR). "Maps" showing the product concentration and conversion for various LRT and VSLR were generated from CPDM. The predictions were compared to the experimental results. On average, the difference between the predicted and experimental values were 13% for acid concentration and 10% for conversion. CPDM "maps" show that marine inocula produce higher concentrations than terrestrial inocula.

Index Entries: Acetic acid; anaerobic; MixAlco; modeling; municipal sewage sludge; municipal solid waste; thermophilic.

Introduction

Because of increasing energy demand and the decreasing availability of natural resources, such as oil and natural gas, it is necessary to find alternative ways to produce energy. In the past few decades, anaerobic digestion of waste biomass has been proposed to produce alternate energy while simultaneously reducing wastes *(1–4)*. This process will become

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particularly valuable as fossil resources become depleted and more expensive. There have been numerous ideas for using waste biomass and converting negative-value waste into useful chemicals. Some examples of such technologies include simultaneous saccharification and fermentation (SSF) for producing ethanol from biomass using cellulose enzymes and sterile conditions, or gasifying biomass and then combusting the biogas in a turbine to produce electricity *(4)*. One of the more extensive studies is anaerobic fermentation of waste biomass—including agricultural residues, municipal solid wastes, and sewage sludge—to produce methane gas.

Figure 1 shows a variant of the MixAlco process *(5)*, which also uses waste biomass, but instead of producing methane, it produces carboxylic acids. Of these carboxylic acids, acetic acid has the greatest demand. The goal of this experiment is to enhance acetic acid selectivity by using anaerobic thermophilic fermentation. As shown in Fig. 1, lime-treated biomass is fermented using acid-forming micro-organisms under nonsterile, anaerobic conditions to produce carboxylic acids. During fermentation, the carboxylic acids produced are neutralized with calcium carbonate to form calcium carboxylate salts, which maintains the pH at 5.8–6.2. The carboxylate salts produced by fermentation are concentrated and followed by "acid springing" to convert the carboxylate salts back to their corresponding acids *(6)*. Some advantages of the MixAlco process include adaptability to a wide variety of feedstocks, no enzyme requirement, no sterilization requirement, and robustness.

To achieve high product concentration and high substrate conversion, Holtzapple et al. *(5)* recommend countercurrent fermentation (Fig. 2). Biomass becomes more difficult to digest when it spends a longer time in the fermentor, because the easily digested components are used first. Countercurrent fermentation lessens inhibition from carboxylate salts by adding fresh liquid media to the most digested biomass. It allows the fresh (most reactive) biomass to contact the highest carboxylate concentration, which allows for higher product concentrations.

Because they are abundant, collected, and have a negative value, municipal solid waste (MSW) and municipal sewage sludge (MSS) are good feedstocks. MSW has a high carbohydrate content, but lacks nutrients such as vitamins and minerals. On the other hand, MSS is rich in nutrients, but lacks carbohydrates. Both carbohydrates and nutrients are necessary for micro-organisms to perform efficiently, so it is synergistic to combine the two wastes. Rapier *(7)* found that 80% MSW/20% MSS works well.

Lime pretreatment increases digestibility of lignocellulose. Nagwani *(8)* and Karr and Holtzapple *(9)* have shown that the optimum lime loading is 0.1 g Ca(OH)2/g dry biomass, with little benefit above the optimum. Effective pretreatment times and temperatures are 1–3 h at 85–135°C *(10)*. Kaar and Holtzapple *(9)* suggest that water loading does not affect pretreatment, but there should be enough water to cover the biomass.

Methanogenic bacteria can convert carboxylic acids to methane and carbon dioxide *(11)*; therefore, a methane inhibitor is needed, such as meth-

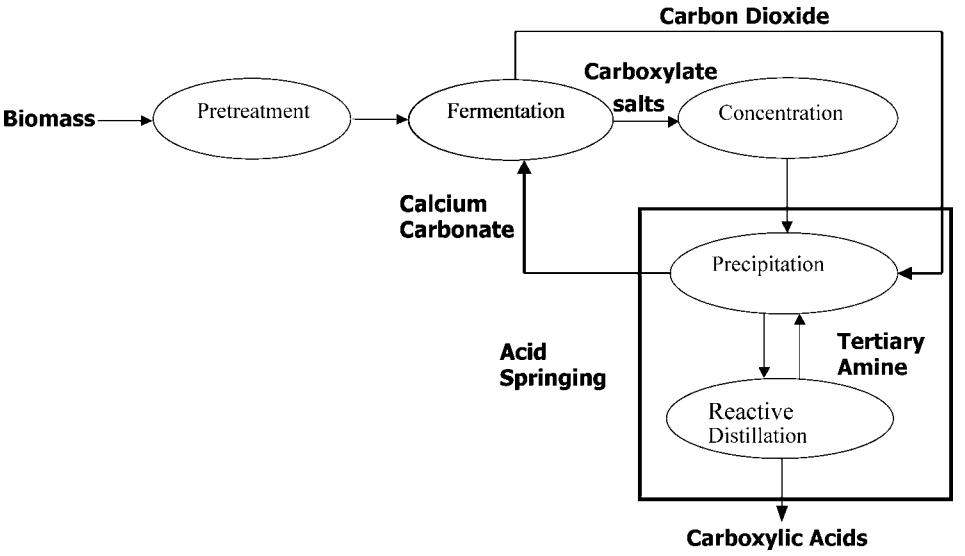

Fig. 1. A variant of the MixAlco process with acid springing.

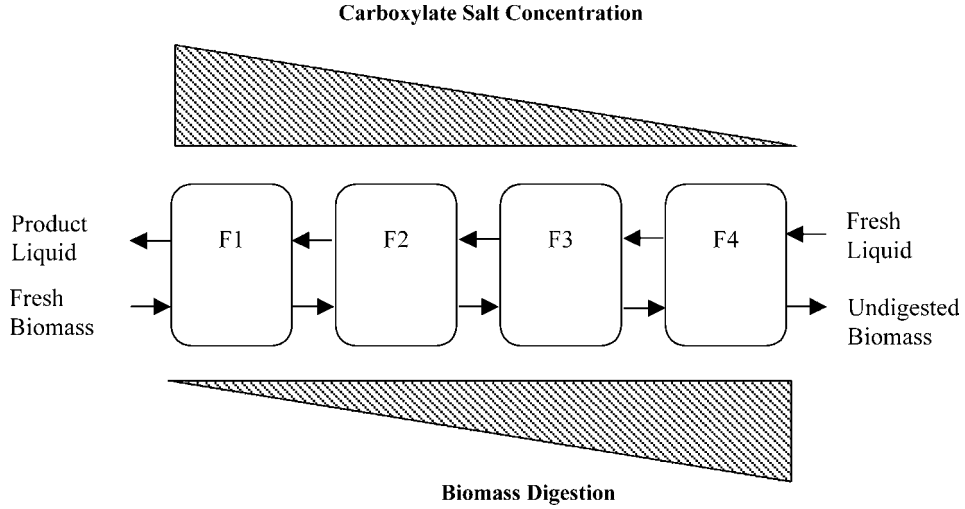

Fig. 2. Countercurrent fermentation.

ane analogs (e.g., iodoform or bromoform), or coenzyme M analogs (2-bromoethane-sulfonic acid [BES]). The methane analogs successfully inhibit methane formation *(12,13)*. Ross *(13)* showed that adding 4 mg of iodoform to each fermentor for each liter of fresh liquid fed to the fermentors caused a fourfold decrease in methane production, but at the expense of lower acetate selectivity. This occurred because inhibiting methane generates excess reducing power by eliminating methane as a potential hydrogen "sink," which then goes to produce more reduced products, such as propionate and butyrate *(13,14)*.

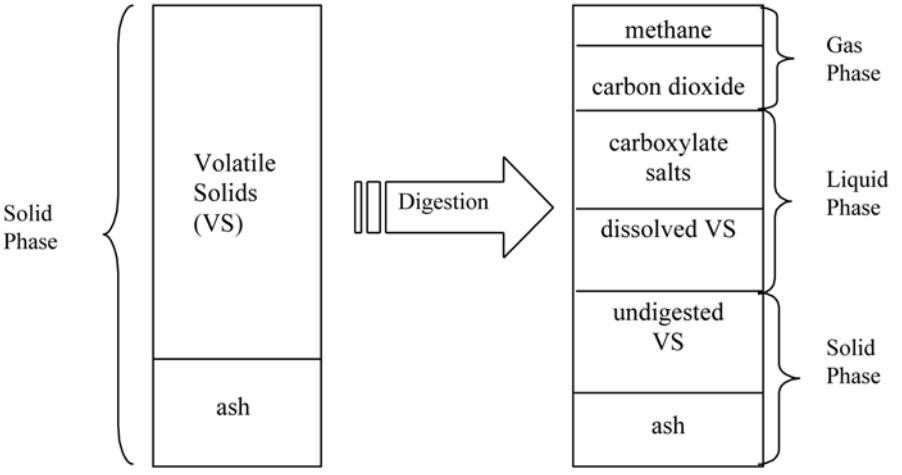

Fig. 3. Biomass feed digestion and definition.

Temperature controls the acetic acid selectivity and fermentation activity. Thermophilic temperatures (50–60°C), as opposed to mesophilic temperatures (30–40°C) increase acetic acid selectivity.

Figure 3 illustrates that biomass feed is composed of volatile solids (VS) and ash. Volatile solids, except for lignin, are the reactive part of the biomass. After digestion, VS are converted to gas (methane, carbon dioxide), liquid [carboxylate acids, extracellular proteins, energy storage polysaccharides *(13)*], and solid phases (undigested VS, ash). The data collected throughout the experiment were used to calculate the following terms:

$$\text{Volatile solids (VS)} \equiv \frac{\text{Dry weight} - \text{Ash weight}}{\text{Dry weight}} \tag{1}$$

$$\text{Conversion} \equiv \frac{\text{VS digested}}{\text{VS fed}} \tag{2}$$

$$\text{Yield} \equiv \frac{\text{Total carboxylic acids produced}}{\text{VS fed}} \tag{3}$$

$$\text{Total acid productivity} \equiv \frac{\text{Total carboxylic acids produced}}{\text{Liter of liquid in all reactors} \times \text{time}} \tag{4}$$

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

$$\text{Acetic acid selectivity} \equiv \frac{\text{Acetic acid produced}}{\text{Total carboxylic acids produced}} \tag{6}$$

$$\text{Liquid residence time (LRT)} \equiv \frac{\text{Total liquid in all fermentors}}{\text{Flow rate of liquid out of the fermentor train}} \tag{7}$$

$$\text{Volatile solids loading rate (VSLR)} \equiv \frac{\text{VS fed to the system}}{\text{Total liquid in all fermentors} \times \text{time}} \quad (8)$$

Because countercurrent fermentation requires extensive experimental time to reach steady state (3–12 mo, depending on the system), it would be very time-consuming and cost-ineffective to perform an array of experiments to find the optimum operating conditions. To overcome this, Loescher *(15)* developed continuum particle distribution modeling (CPDM), a mathematical model that predicts the total acid concentration and substrate conversion using data from batch fermentation experiments. He defined the *continuum particle* to be a representative collection of biomass particles that weighed 1 g upon entering the fermentation system. Ross *(13)* modified his definition by specifying 1 g of volatile solids, rather than total solids. By using CPDM, the optimum volatile solid loading rate and liquid residence time for countercurrent fermentation can be determined in only 15–25 d.

This article presents the results of continuous countercurrent fermentation of 80% MSW/20% MSS as the substrate. The VSLR and LRT were varied to determine their effect on acid concentration, yield, conversion, and total acid selectivity. We compared the effect of terrestrial and marine inocula. Finally, CPDM predicted the total acid concentration and conversion, which were compared against experimental data.

Materials and Methods

Substrates

Municipal Solid Waste

Rapier *(7)* describes the percentage of each biodegradable component (dry basis) of the synthetic MSW. Fats and oils were eliminated to prevent spoiling during storage. The components were collected, sun-dried, and ground with a hammer mill fitted with 10-mm mesh screens. The moisture content of each component was determined and then mixed in the right proportion. For homogeneity, the mixture then underwent additional grinding with a 6-mm mesh screen. A total of 150 kg of dry MSW were prepared to ensure enough supply for the experiment. Before the use of the prepared MSW, it was pretreated with lime using 0.1 g $Ca(OH)_2$/g dry MSW at 100°C for 1 h. The VS for the pretreated synthetic MSW were determined to be between 0.81 and 0.82 g VSs/g dry MSW, and the moisture content was between 6 and 8 wt%. The small variations in VS and moisture content of the pretreated MSW resulted from the different batches used when preparing pretreated MSW.

Municipal Sewage Sludge

Municipal sewage sludge was obtained from Bryan Wastewater Treatment Plant Number 3, Bryan, TX, which provided MSS by the activated sludge process. It sends the incoming sewage stream to an aeration basin

for approx 15 d for digestion, followed by aerobic digestion for approx 40 d. The sludge was then removed from the digester and coagulated by adding a cationic polymer. The sludge was air-dried for 10 d and ground in a hammer mill fitted with a 3-mm mesh screen. The aerobically treated MSS was not pretreated with lime for the experiments. The volatile solids for MSS were determined to be 0.59 g VS/g dry MSS.

Inocula

The terrestrial inocula were collected from rumen fluid, commercial and residential compost piles, and lake sediment. Rumen fluid was taken from a forage-fed fistulated steer. The collected rumen fluid was then put in four layers of cheesecloth, and the liquid was filtered out from the solid by squeezing the cheesecloth. The liquid rumen fluid was kept in an airtight polypropylene bottle, and the solid, mostly undigested grass, was discarded. A thermometer was used at the commercial compost pile to measure the temperature in the middle of the pile to ensure that samples were collected from the proper temperature range. The samples taken from the compost pile and lake sediment were immediately placed inside airtight polypropylene bottles filled with deoxygenated water, 0.275 g/L cysteine hydrochloride, and 0.275 g/L sodium sulfide. This was done to minimize exposure to oxygen. The inocula were transported to the lab and used to inoculate the fermentors within 6 h of collection, thus ensuring micro-organism viability.

The marine inocula were sediments collected from three different swamps at Galveston, TX, where the swamps all lead to the Gulf of Mexico. To obtain the samples, 0.5-m-deep holes were dug to ensure anaerobic micro-organisms were obtained. The samples were immediately placed inside airtight polypropylene bottles filled with deoxygenated water, 0.275 g/L cysteine hydrochloride, and 0.275 g/L sodium sulfide. The collected marine inocula were mixed in a fermentor with MSW/MSS as the substrate and batch fermentation was performed as described earlier to maintain the micro-organisms.

Nutrient and Media

Rapier *(7)* determined that the optimum ratio of MSW to MSS is 80:20. The dry nutrient mixture was modified from Caldwell and Bryant medium *(13)*. In addition to dry nutrients, calcium carbonate ($CaCO_3$) was also added as a neutralizing agent to maintain the pH between 5.8 and 6.2.

The liquid medium used throughout the experiments was deoxygenated water prepared by boiling distilled water. It was boiled for an additional 10 min under N_2 purge and then capped with a rubber stopper. After it cooled to room temperature, 0.275 g cysteine–HCl/L deaerated water and 0.275 g hydrated sodium sulfide/L deaerated water were added under continuous N_2 purge. Because the fermentations were anaerobic, cysteine and sodium sulfide were added to further eliminate any oxygen in the liquid. $CaCO_3$ was added to control the pH between 5.8 and 6.2.

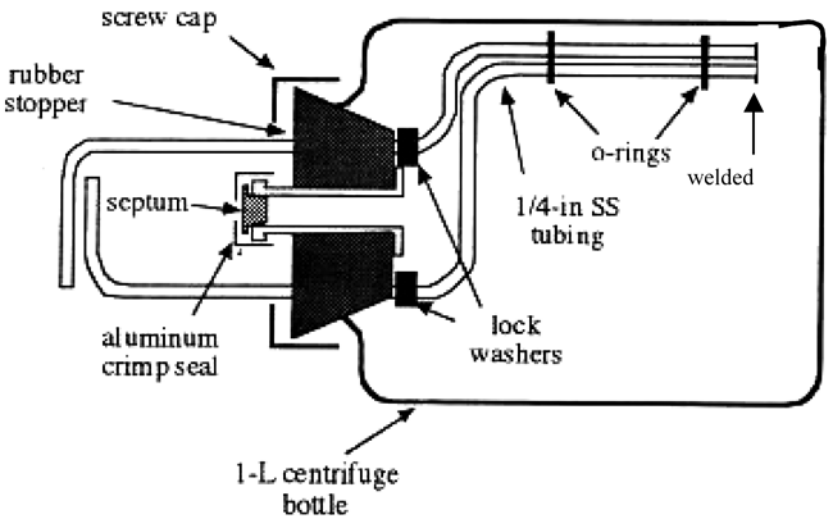

Fig. 4. Design of the fermentor.

Inhibitor

The methane inhibitor used throughout the experiment was an iodoform (CHI_3) solution containing 20 g CHI_3/L alcohol. Because of its light sensitivity, the solution was kept in a tinted brown bottle, capped immediately after use, and stored in the refrigerator.

Fermentor Design

The fermentors were Beckman 1-L polypropylene centrifuge bottles (98 × 169 mm), Nalgene brand NNI 3120-1010 (*see* Fig. 4 *[13]*). Each bottle was closed with a size 11 rubber stopper with a hole drilled in the middle. A glass tube was cut to a length of 1.5 in. with an added flare at the end. The glass tube was inserted through the hole and was capped with a rubber septum for gas release and sampling purposes. It was then sealed with an aluminum crimp seal. Gases that accumulated in the bottles were released through the septum and recorded every other day or every 3 d, depending on the gas-release schedule. Periodically releasing the gas is important to prevent leaks or explosions of the fermentors because the fermentors could withstand only 103 kPa. The rubber septum was replaced as soon as there was visible tearing or holes as a result of needle insertion. The stopper was further secured with the centrifuge bottle cap. Two 0.25-in. stainless-steel tubes with welded ends were also inserted on two sides of the stopper. These tubes were as long as the bottles and were used as stirrers to mix the components inside the fermentors.

The fermentors were placed in a Bellco® incubator. The incubators consisted of rollers that rotated the fermentors horizontally at 2 rpm and the temperature was set at 55°C.

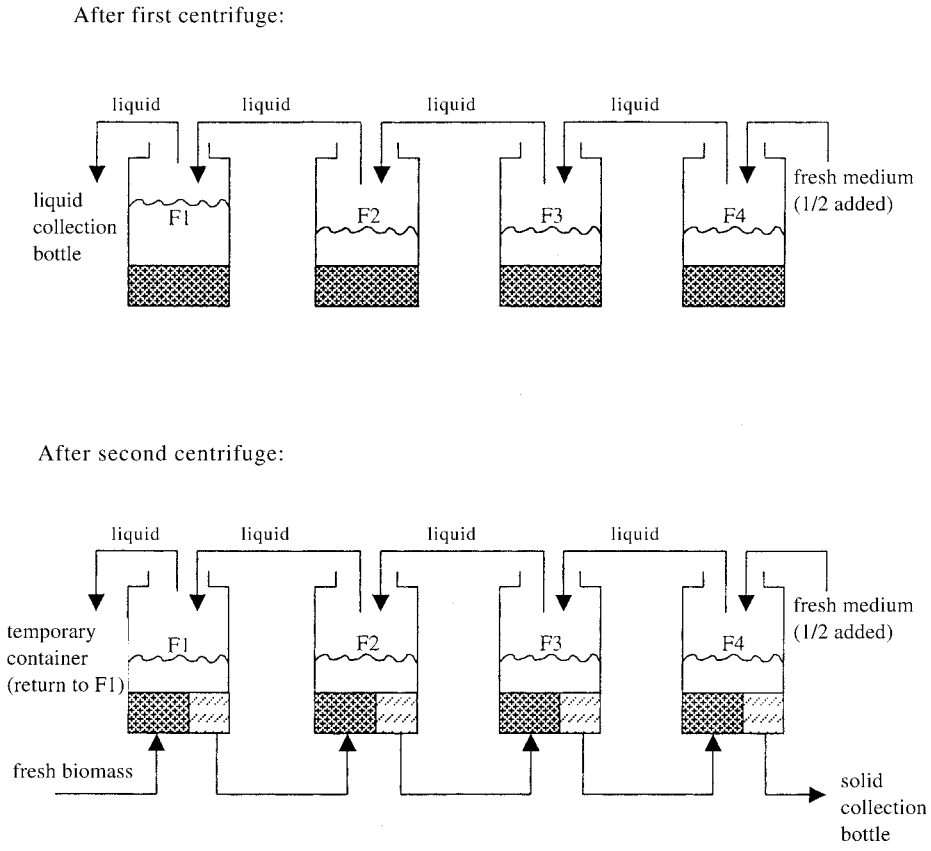

Fig. 5. Double-centrifuge countercurrent transfer procedure.

Double-Centrifuge Countercurrent Fermentation

For this research, all of the fermentors transferred the solids and liquids using the countercurrent double-centrifuge method *(5)*. Four-stage countercurrent solid and liquid transfer is depicted in Fig. 5. The transfer period varied between 2 and 3 d, depending on the desired liquid residence time. To keep the fermentors under anaerobic conditions, whenever the fermentor was opened to the atmosphere, it was purged with nitrogen.

Experimental Procedure

To establish the culture, four fermentors with the same amount of inocula, ratio of pretreated MSW and MSS, calcium carbonate, urea, dry nutrient, and deoxygenated media were started as batch fermentations for 14 d. After the culture was established, the countercurrent double-centrifuge procedure with transfers of solid and liquid followed.

A series of eight countercurrent fermentation experiments (MS1–MS8) were performed with various combinations of volatile solid loading rate (VSLR) and liquid residence time (LRT). The operating parameters are given in Table 1. The purpose of these countercurrent experiments was to

Table 1
Operating Conditions for MSW/MSS Countercurrent Fermentation Experiments, MS1–MS8

	Experiment							
	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8
Temperature (°C)	55	55	55	55	55	55	55	55
LRT (d)	14	21	23	19	19	21	25	21
VSLR (g/[L of liquid in all four fermentors × d])	6.2	6.8	3.4	1.3	4.0	2.1	2.6	2.8
VS feed/liquid feed ratio (g VS/g liquid)	0.08	0.11	0.06	0.02	0.06	0.04	0.06	0.06
TLV (L)	1.2	1.1	1.14	0.98	0.96	0.97	0.97	1.1
Liquid feed to F4 at each transfer (L)	0.2	0.14	0.2	0.16	0.12	0.14	0.12	0.16
Total solid feed at each transfer (g dry)	20	20	15	5	10	8	10	12
Pretreated MSW feed (g dry)	16	16	12	4	8	6.4	8	9.6
MSS feed (g dry)	4	4	3	1	2	1.6	2	2.4
Iodoform addition rate (mg iodoform added to each fermentor/L liquid feed to F4)	12	12	12	12	12	12	12	12
Urea addition rate (g urea added to each fermentor/L liquid feed to F4) (if pH < 6.2)	0.5	0.7	0.5	0.63	0.7	0.6	0.6	0.6
Nutrient addition rate (g dry nutrient added to each fermentor/L liquid feed to F4)	1.0	1.4	1.0	1.25	1.4	1.25	1.25	1.25
Free-liquid volume in F1 (L)	0.14	0.11	0.15	0.16	0.13	0.14	0.12	0.15
Free-liquid volume in F2–F4 (L)	0.11	0.09	0.11	0.09	0.07	0.08	0.08	0.10
Frequency of transfer (d)	2	2	3	3	2	3	3	3

Abbr: LRT, liquid residence time; VS, volatile solid; VSLR, volatile solid loading rate; TLV, total liquid volume.

examine the effect of VSLR and LRT on carboxylic acid concentrations and substrate conversions and to use these data to compare results from CPDM. The data were taken after a steady state had been established for a period of at least 75 d. Steady state was assumed when the total acid concentration did not vary more than ±5 g/L from the average value.

Analytical Methods

Gas Chromatography for Determining Carboxylic Acid Concentration

At the end of each transfer schedule, a liquid sample of the fermentor broth of approx 5 mL from Fermentor 1 was taken and analyzed for the carboxylic acid concentration. If the sample was not used immediately, it was stored in the freezer at about –15°C. An Agilent 6890 Series gas chromatography (GC) was used for this analysis. It was operated with a flame ionization detector (FID) and an Agilent 7683 Series Injector. The column was a 30-m fused-silica capillary column with a 0.32-mm inner diameter and 0.25-µm film thickness (J&W Scientific, model DB-FFAP no. 123-3232). For optimum performance, the column pressure was maintained at a gage pressure of 103 kPa. The temperature of the oven in the GC increased from 50°C to 200°C at 20°C/min and was held an additional 1 min when it reached 200°C. To analyze the liquid samples using the GC, liquid samples were added with equal amounts of 1.162 g/L internal standard (4-methyl-*n*-valeric acid) and acidified with 3-*M* phosphoric acid.

Volume of Gas Measurement

In addition to liquid products, gas was also produced in the fermentations. Gas was released by piercing a needle through the rubber septum on the fermentor. The released gases displaced liquid (300 g $CaCl_2$/L aqueous solution) in an inverted glass cylinder.

Gas Chromatography for Methane Measurement

An Agilent 6890 Series gas chromatograph with thermal conductivity detector was used to measure the amounts of methane and carbon dioxide in the gas. The oven temperature was set at 200°C. The inlet temperature was fixed at 230°C, and the detector temperature was set at 250°C. Helium was used as the carrier gas. The total run for a sample was 5 min. The packed column was J&W Scientific, Carboxen 1004, Supelco (no. 1-2390). A standard gas mixture of carbon dioxide (29.99 mol%), methane (10.06 mol%), and nitrogen was used to calibrate the samples.

The carbon dioxide obtained from the fermentors is the sum of biotic and abiotic carbon dioxide. Abiotic CO_2 is produced by neutralizing the carboxylic acids with calcium carbonate. It is assumed that for every 2 mol of acid produced in the fermentor, there is 1 mol of abiotic CO_2 produced. The abiotic CO_2 was subtracted from the total CO_2, leaving only biotic CO_2. In the mass balance, only the biotic CO_2 was included in the calculation.

Volatile Solids Determination

Waste biomass consisted of volatile solids (VS) and ash. Except for lignin, the VS are the reactive part of the biomass. During digestion, some VS were converted into gas and liquid, with undigested solids remaining. VS are defined as the fraction of biomass that volatilizes upon ashing.

At each transfer period, the liquid from Fermentor 1 (after the first centrifuge) was saved in a 1-L bottle and the solids removed from Fermentor 4 (after the second centrifuge) were saved in another 1-L bottle (*see* Fig. 5). To determined the VS in the substrate and solid residue from fermentation, the material collected in the bottle (liquid bottle or solid bottle) was first dried in the oven at 105°C for 2 d, followed by ashing at 550°C in the furnace for 3 h. To determine the VS of the liquid broth from the fermentation, small amounts of calcium hydroxide (lime) were added to the liquid to prevent carboxylic acids from volatilizing, which would lead to inaccurate measurement.

Mass Balance Calculation

Mass balance closure on the entire system was calculated over the steady-state period. Theoretically, the system should have 100% closure; in practice, human errors during measurements and transfer process caused some discrepancies.

For mass balance closure,

$$\text{Closure} = \frac{\text{Mass out}}{\text{Mass in} + \text{Water of hydrolysis}} \tag{9}$$

$$\text{Closure} = \frac{\text{Undigested VS} + \text{Dissolved VS} + \text{Acids} + \text{Biotic } CO_2 + CH_4}{\text{VS in} + \text{Water of hydrolysis}} \tag{10}$$

The water of hydrolysis was calculated as VS digested × 18/162, where 162 is the molecular weight of cellulose monomer, which represents biomass. Acid is the total carboxylic acids produced. All of the variables in the equation have units of grams. Figure 4 illustrates the biomass digestion.

A more detailed description of analytical methods can be found in ref. *16*.

Continuum Particle Distribution Modeling

To obtain the data needed for CPDM, five batch fermentors at varying initial substrate concentrations were run simultaneously for a period of 15–25 d, depending on when the carboxylic acids concentration started to decrease. The inocula for the batch fermentors were obtained from countercurrent fermentors operated with the same target substrates. The advantage of using these inocula is that the mixed culture had already been adapted to the feedstock. Substrate concentrations for the five batches were 20, 40, 70, 100, and 100A g dry substrate/L liquid. The 100A fermentor also contained a mixture of carboxylate salts (70 wt% calcium acetate, 20 wt% calcium propionate, and 10 wt% calcium butyrate) at a concentration of

20 g carboxylic acids/L liquid. Other materials, such as urea, dry nutrient, iodoform, and calcium carbonate, were added the same rate as the target countercurrent fermentation systems. Liquid samples were taken every day for the five batches. Carboxylic acid concentrations were analyzed by GC and converted to acetic acid equivalent (α):

$$\begin{aligned}\alpha\ (\text{mol/L}) = {} & \text{acetic (mol/L)} + 1.75 \times \text{propionic (mol/L)} + \\ & 2.5 \times \text{butyric (mol/L)} + 3.25 \times \text{valeric (mol/L)} + \\ & 4.0 \times \text{caproic (mol/L)} + 4.75 \times \text{heptanoic (mol/L)}\end{aligned} \tag{11}$$

It can also expressed on a mass basis as follows:

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

The model uses Eq. (12) to account for the various carboxylic acid concentrations as one single concentration. Acetic acid equivalent concentrations from each of the five batch experiments were then fit to the equation

$$\text{Aceq} = a + \frac{bt}{1 + ct} \tag{13}$$

where t is the time (d) of fermentation, and a, b, and c are constants fit by least squares analysis. The constants obtained were then used to determine the rate r as

$$r = \text{Rate} = \frac{d(\text{Aceq})}{dt} = \frac{b}{(1 + ct)^2} \tag{14}$$

The specific rate, r, is determined from Eq. (14) by dividing it by the initial amount of substrate concentration (S_0) in each of the five fermentors:

$$r = \frac{r}{S_0} \tag{15}$$

where S_0 is defined as $S_0 = m_o/V$. For a continuous countercurrent fermentor, m_o is the mass of volatile solids in the fresh substrate added to Fermentor 1 and V is the liquid volume added to Fermentor 4.

Volatile solids conversion, x, was calculated using

$$x = \frac{(\text{Aceq})V}{m_0\sigma} \tag{16}$$

where Aceq is the acetic acid equivalents produced (g/L) from Eq. (12), V is the liquid volume (L), m_o is the initial substrate mass (g volatile solids), and σ is the selectivity (g acetic acid equivalents produced/g VS digested).

Selectivity can also be expressed as grams of total acids produced per gram of volatile solids digested, denoted s. The relationship between s and σ is

$$s = \phi\sigma \tag{17}$$

In Eq. (17), the term ϕ, introduced by Ross *(13)*, accounts for the inhibition effect and is the ratio of total acids to acetic acid equivalents.

The predicted rate, r_{pred}, is determined from Eq. (18); it describes the effect of conversion and product concentration on the rate:

$$r_{pred} = \frac{e(1-x)^f}{1 + g[(\phi)\,\mathrm{Aceq}]^h} \tag{18}$$

Using least square analysis of specific rate r from Eq. (15) and r_{pred} from Eq. (18), constants e, f, g, and h can be determined by "solver" in Excel. In Eq. (18), x is the volatile solids conversion variable from Eq. (16). The numerator of Eq. (18), $1-x$, is the conversion penalty function by South and Lynd *(17)*. It shows that as the material is converted, the reaction rate decreases. The denominator shows the inhibitory effect on the microorganism when the product concentration is high, which leads to a decreasing rate.

CPDM is a Mathematica program developed by Loescher *(15)*, which uses variables e, f, g, and h from Eq. (18), along with system-specific variables such as selectivity, σ, moisture (ratio of liquid to solid in feed), and holdup (ratio of liquid to solid in wet solids) to predict acetic acid equivalent concentration, Aceq, and substrate conversion for the countercurrent fermentation systems for various LRTs and VSLRs. Aceq was converted back to carboxylic acid concentration by multiplying by ϕ. A "map" of the system was then drawn to show the dependence of substrate conversion and carboxylic acid concentration for various VSLRs and LRTs. Two assumptions made for the CPDM method are that (1) the volume of liquid retained with a given mass of solids was constant and (2) the volatile solids flow rate, S_i, out of a given fermentor is related to the flow rate of solids fed into Fermentor 1, S_f, by

$$S_i = S_f(1 - x_i) \tag{19}$$

where x is VS conversion as described in Eq. (16).

Results and Discussion

Countercurrent Fermentations Using Terrestrial Inocula

The fermentation results for MS1–MS8 using terrestrial inocula are given in Table 2. From this experiment, the highest acid productivity was 1.0 g/(L of liquid × day) at 20.5 g total acid/L in MS2 [LRT = 21 d and VSLR = 6.8 g/(L of liquid × day)]. MS4 [LRT = 19 d and VSLR = 1.3 g/(L of liquid × day)] had the highest substrate conversion, and yield of 0.69 g VS digested/g VS fed, and 0.41 g total acid/g VSs fed, respectively. It also had high acetic acid selectivity (86.4 wt%). The average acetic acid selectivity under mesophilic conditions is only 42% *(18)*. A typical steady-state fermentation is shown in Fig. 6.

Table 2
Results for MSW/MSS Countercurrent Fermentation Experiments, MS1–MS8

	Experiment							
	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8
pH	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2
Total carboxylic acid concentration (g/L)	12.5 ± 1.2	20.5 ± 1.6	13.5 ± 1.4	10.7 ± 0.82	16.9 ± 1.1	13.9 ± 0.79	15.5 ± 1.1	13.5 ± 1.5
Acetic acid (wt%)	74.1 ± 3.9	68.2 ± 2.7	71.1 ± 3.4	86.4 ± 2.5	73.2 ± 3.1	83.9 ± 1.7	80.2 ± 2.4	79.9 ± 3.2
Propionic acid (wt%)	2.63 ± 1.5	3.84 ± 0.41	3.85 ± 1.0	2.62 ± 1.8	5.90 ± 1.6	2.04 ± 0.96	2.25 ± 1.1	2.78 ± 1.1
Butyric acid (wt%)	22.7 ± 4.3	25.3 ± 3.5	22.6 ± 3.6	10.7 ± 2.3	19.5 ± 2.5	14.0 ± 1.6	17.0 ± 2.0	16.5 ± 2.8
Valeric acid (wt%)	0.46 ± 0.70	2.06 ± 0.87	0.92 ± 0.53	0.25 ± 0.40	0.45 ± 0.53	0	0.25 ± 0.36	0.25 ± 0.33
Caproic acid (wt%)	0.096 ± 0.27	0.59 ± 0.57	1.44 ± 0.57	0.089 ± 0.18	1.08 ± 0.93	0	0.21 ± 0.26	0.55 ± 0.38
Heptanoic acid (wt%)	0	0	0.093 ± 0.31	0	0	0	0.079 ± 0.23	0
Conversion (g VS digested/g VS fed)	0.35	0.28	0.38	0.69	0.44	0.44	0.45	0.44
Selectivity (g total acid/g VS digested)	0.35	0.52	0.47	0.63	0.65	0.70	0.53	0.53
Yield (g total acid/g VS fed)	0.12	0.15	0.18	0.41	0.29	0.31	0.24	0.23
Total carboxylic acid productivity [g total acid/(L liquid in all fermentors × day)]	0.78	1.0	0.61	0.58	1.1	0.67	0.64	0.63
CH4 productivity [g CH4/(L of liquid in all fermentors × day)]	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Mass balance closure (g VS out/g VS in)	0.89	0.97	0.94	1.00	0.99	1.01	0.98	0.95

Note: All errors are ± 1 standard deviation.

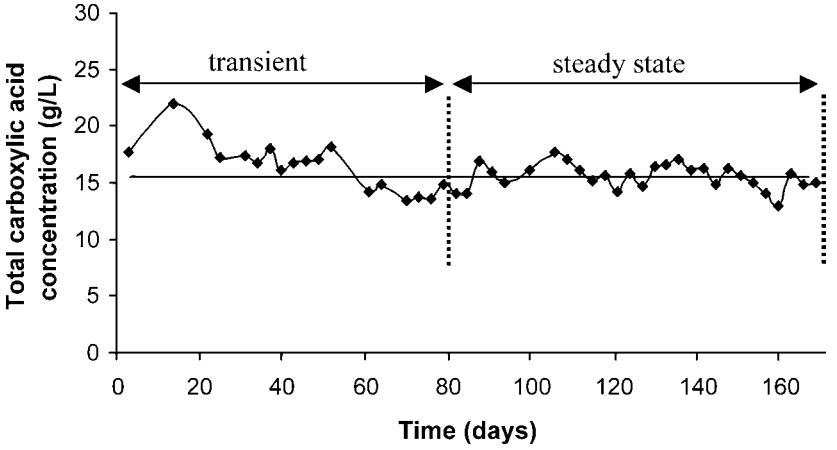

Fig. 6. Typical steady-state fermentation.

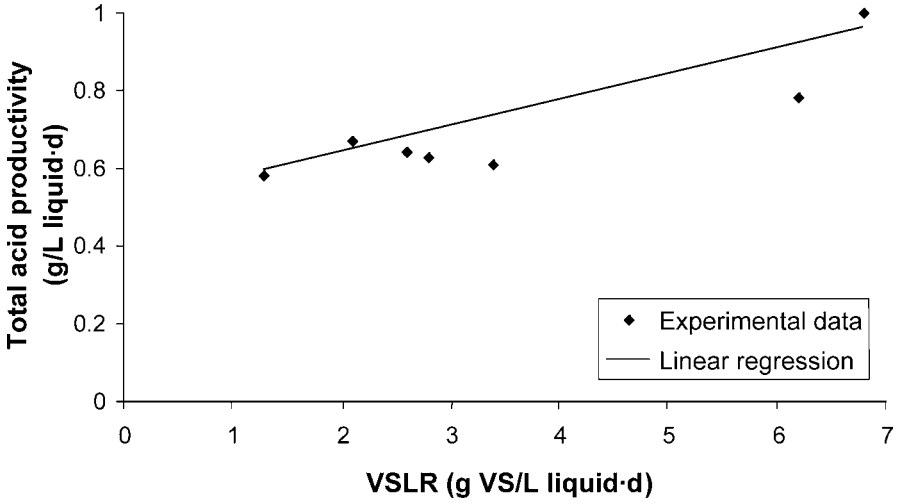

Fig. 7. Correlation between total acid productivity and VSLR.

Correlation Between Variables

For the eight countercurrent fermentations, correlations between VSLR and productivity (p), selectivity (s), conversion (x), and yield (y) are shown in Figs. 7–10. Using linear regression, the following correlations were obtained:

$$p = 0.0669\text{VSLR} + 0.507 \quad (20)$$

$$s = -0.0354\text{VSLR} + 0.677 \quad (21)$$

$$x = -0.0499\text{VSLR} + 0.616 \quad (22)$$

$$y = -0.0401\text{VSLR} + 0.388 \quad (23)$$

Figure 7 shows that as VSLR increases, the total acid productivity also increases. Figures 8–10 show that there is an inverse relationship between

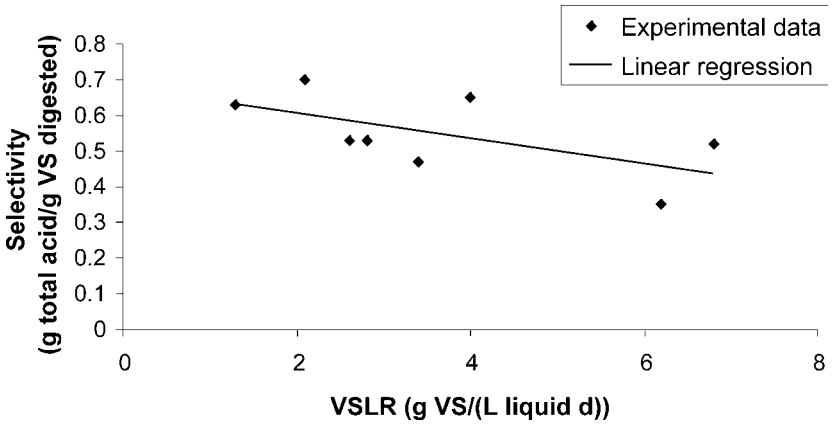

Fig. 8. Correlation between selectivity and VSLR.

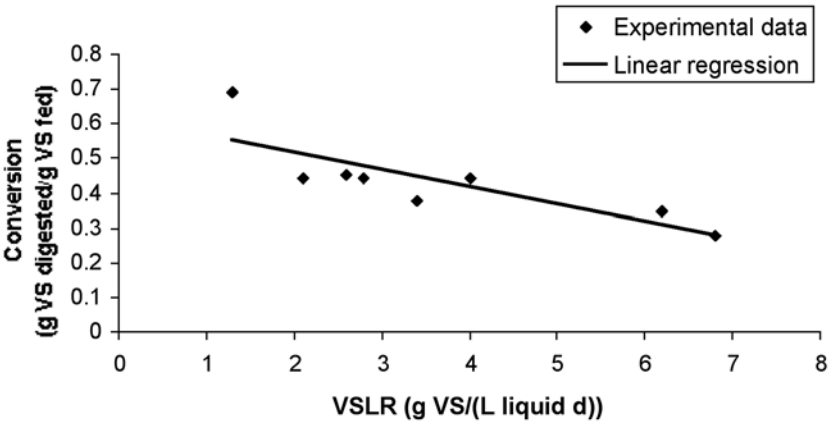

Fig. 9. Correlation between conversion and VSLR.

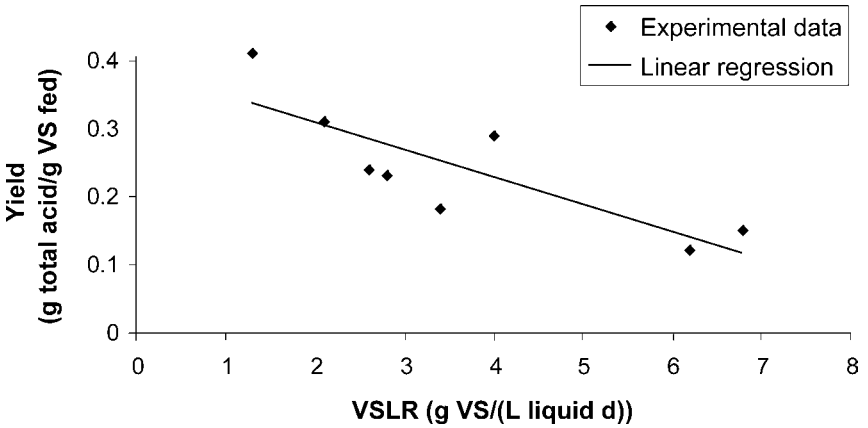

Fig. 10. Correlation between yield and VSLR.

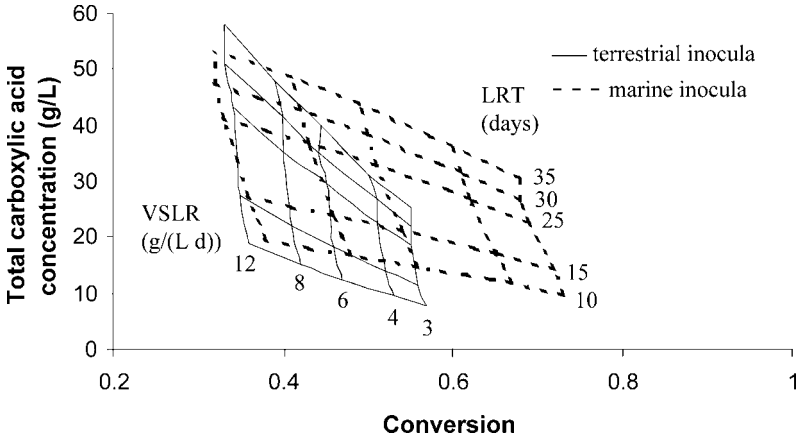

Fig. 11. Comparison of CPDM "maps" for terrestrial-only and marine-only inocula with MSW/MSS countercurrent fermentation at 300 g VS/L liquid.

VSLR and selectivity (s), conversion (x), and yield (y). When the solid feed is high, the micro-organisms only need to digest primarily the "easy" portions of the feedstock, and the digested biomass produces fewer carboxylic acids than "other products," resulting in lower selectivity, conversion, and yield. Conversely, with low solid feed, the micro-organisms must digest the "difficult" portions of the biomass, and more of the carboxylic acids than the soluble energy-storage products are produced, resulting in higher selectivity, conversion, and yield.

CPDM

After the batch experiment (*see* ref. *16* for data), the collected data were applied to Eq. (18), the predicted rate equation. The predicted rate for the 80% MSW/20% MSS fermentation system with terrestrial-only inoculum was

$$r_{\text{pred}} = \frac{0.11(1-x)^{5.48}}{1 + 2.43[(\phi)\,\text{Aceq}]^{0.20}} \tag{24}$$

The system-specific variables for this experiment were selectivity, σ = 0.5 g Aceq/g VS digested, ϕ = 0.89 g total acid/g acetic acid equivalents, moisture = 0.1, and holdup = 1.3.

The predicted rate for the 80% MSW/20% MSS fermentation system with marine-only inocula was

$$r_{\text{pred}} = \frac{0.09(1-x)^{3.2}}{1 + 2.8[(\phi)\,\text{Aceq}]^{0.3}} \tag{25}$$

The system-specific variables for this experiment were selectivity, σ = 0.5 g Aceq/g VS digested, ϕ = 0.87 g total acid/g acetic acid equivalents, moisture = 0.1, and holdup = 1.3. With these data, the CPDM program was able to predict substrate conversion and product concentration for different combinations of VSLR and LRT. Figure 11 compares the CPDM "maps"

Table 3
Comparison of Experimental and Predicted Carboxylic Acid Concentration and Substrate Conversion for MS1–MS8 Fermentation

	Fermentation conditions								
	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8	Avg.[a] (%)
Experimental carboxylic acid concentration (g/L)	12.5	20.5	13.5	10.7	16.9	13.9	15.5	13.5	
Predicted carboxylic acid concentration (g/L)	13.6	20.5	15.8	7.0	14.5	10.8	14.6	13.0	
Error[b] (%)	8.8	0	17.0	34.6	14.2	22.3	5.8	3.7	13.3
Experimental conversion	0.35	0.28	0.38	0.69	0.44	0.44	0.45	0.44	
Predicted conversion (from CPDM)	0.3	0.27	0.38	0.53	0.36	0.46	0.42	0.41	
Error[b] (%)	–14.3	–3.6	0	–23.2	–18.2	4.5	–6.8	–6.8	9.7

[a]Avg. = average absolute error of MS1–MS8.
[b]Error (%) = [(Predicted–Experimental)/Experimental] × 100.

Table 4
Comparison of Experimental and Predicted Carboxylic Acid Concentration and Substrate Conversion for MSW/MSS Fermentation with Marine Inocula

	Marine inocula
Experimental carboxylic acid concentration (g/L)	13.5
Predicted carboxylic acid concentration (g/L) (from CPDM)	14.8
Error[a] (%)	9.6
Experimental conversion	0.50
Predicted conversion (from CPDM)	0.50
Error[a] (%)	0

[a]Error (%) = [Predicted–Experimental]/Experimental × 100.

for the two inocula at 300 g VS/L liquid under the same operating conditions. At the same VSLR and LRT, marine inocula can achieve a higher conversion and higher product concentration than terrestrial inocula. Marine inocula are more tolerant of saline solutions than terrestrial inocula, and thus they adapt better to the high carboxylate salt concentrations in the fermentor.

Comparisons Between Experimental Data and CPDM Prediction

Table 3 compares the carboxylic acid concentration predicted by the "map" with the experimental results for the terrestrial-only inocula fermentation. As shown in Table 3, the total carboxylic acid concentrations from experimental data match well with the predicted values by the CPDM. Experimental and predicted substrate conversions were very close (< 10% difference) for most of the conditions. In the CPDM model, the selectivity was assumed to be constant, but in actuality, it varies with the VSLR. This assumption could account for the deviation between the experimental data and model.

Table 4 compares the carboxylic acid concentration predicted by the "map" with the experimental condition results for marine-only inocula fermentation. As shown in Table 4, the total carboxylic acid concentrations and substrate conversion from experimental data match well (9.6% and 0% differences, respectively) with the predicted values by the CPDM. Only one experimental condition was tested, so future work should include more conditions to further verify the CPDM model.

Conclusions

The fermentation results with 80% MSW/20% MSS using terrestrial inocula from this experiment show that liquid and solid loading rate at LRT = 21 d and VSLR = 6.8 g/(L of liquid × day) achieved the highest acid productivity of 1.0 g/(L of liquid × day) at 20.5 g total acid/L. The highest substrate conversion and yield of 0.69 g VS digested/g VS fed and 0.41 g total acid/g VS fed, respectively, was obtained at LRT = 19 d and

VSLR = 1.3 g/(L of liquid × day). It also had high acetic acid selectivity (86.4 wt%). Thermophilic conditions allow for higher acetic acid selectivity (86%) as opposed to mesophilic conditions (42%). CPDM gave a good prediction for total acid concentration and conversion (13% and 10%, respectively) when compared with experimental results. CPDM can be a useful tool in predicting the optimum conditions for countercurrent fermentation, which saves considerable time and resources. Another finding from this experiment was that the CPDM model predicts that marine inocula give higher conversions and product concentrations than terrestrial inocula.

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