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Conversion of Sugarcane Bagasse to Carboxylic Acids Using a Mixed Culture of Mesophilic Microorganisms

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Abstract

Using the MixAlco process, biomass can be converted into carboxylic acids, which can be chemically converted into mixed alcohol fuels. This study focused on the use of countercurrent fermentation to anaerobically convert sugarcane bagasse and chicken manure to mixed carboxylic acids using a mixed culture of mesophilic microorganisms from terrestrial and marine sources. Bagasse was pretreated with lime to increase digestibility. The continuum particle distribution model (CPDM) simulated continuous fermentors based on data collected from batch experiments. This model saves considerable time in determining optimum operating conditions. For an 80% bagasse/20% chicken manure fermentation with terrestrial inoculum at a volatile solids loading rate (VSLR) of 7.36 g/(L of liquid·d) and a liquid residence time (LRT) of 8.88 d, total carboxylic acid productivity, total acid selectivity, and yield were 2.49 g/(L of liquid·d), 0.581 g of total acid/g of VS digested, and 0.338 g of total acid/g of VS fed, respectively, at a concentration of 18.7 g of total acid/L. At the same VSLR and LRT, fermentation with marine inoculum gave higher total acid productivity, total acid selectivity, and yield than fermentation with terrestrial inoculum. For an 80% bagasse/20% chicken manure fermentation with marine inoculum at a VSLR of 3.83 g/(L of liquid·d) and an LRT of 12.1 d, total carboxylic acid productivity, total acid selectivity, and yield were 1.38 g/(L of liquid·d), 0.667 g of total acid/g of VS digested, and 0.359 g of total acid/g of VS fed, respectively, at a concentration of 16.2 g of total acid/L.

Index Entries: Bagasse; carboxylic acids; lime pretreatment; sugarcane; fermentation.

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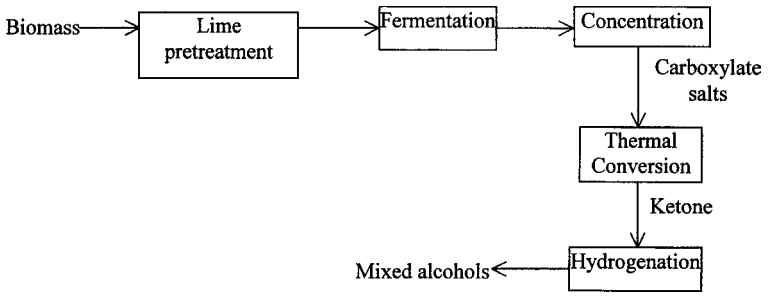

Fig. 1. MixAlco process.

Introduction

In 1995, more than 250,000,000 t of agricultural waste including bagasse was generated in the United States *(1)*. The conversion of waste biomass into liquid fuels has attractive environmental benefits, such as reducing the greenhouse effect and increasing resource availability. The most common method to convert lignocellulosic biomass to useful products is simultaneous saccharification and fermentation, which enzymatically hydrolyzes lignocellulose to sugars that are fermented to alcohol. Currently, the cost of cellulase is too expensive. Moreover, this process requires sterile operation. To solve these problems, Holtzapple et al. *(2)* developed the MixAlco process (Fig. 1).

The MixAlco process converts any biodegradable material into mixed alcohol fuels. The biomass is treated with lime to increase digestibility. Then it is fermented with a mixed culture of acid-forming microorganisms that degrade the major substrates (e.g., polysaccharides, proteins, and lipids) to carboxylic acids under nonsterile, anaerobic conditions. To maintain the pH, calcium carbonate is added, which reacts with the acid to form carboxylate salts. The carboxylate salts can be converted to ketones, which may be hydrogenated to alcohol fuels that are clean burning and do not add net carbon dioxide to the environment *(3,4)*.

Both high conversion and high product concentrations are possible by using a countercurrent fermentation in which solid and liquid pass in opposite directions through a series of fermentors (Fig. 2). The countercurrent flow arrangement is beneficial because the inhibitory effects of high product concentration (in F1 in Fig. 2) is partially offset by the fresh, highly reactive substrate, and the lower reaction rate of highly digested biomass (in F4 in Fig. 2) is partially offset by a lower product concentration *(3,4)*.

In mixed-culture acid fermentations, methane production can be inhibited by methane analogs such as iodoform and bromoform *(5–7)* or the coenzyme M analog, 2-bromoethanesulfonic acid *(6,8,9)*. By inhibiting methane, a potential hydrogen sink is eliminated and the reducing power is used to produce higher carboxylic acids, such as butyric and caproic acids *(4–6)*.

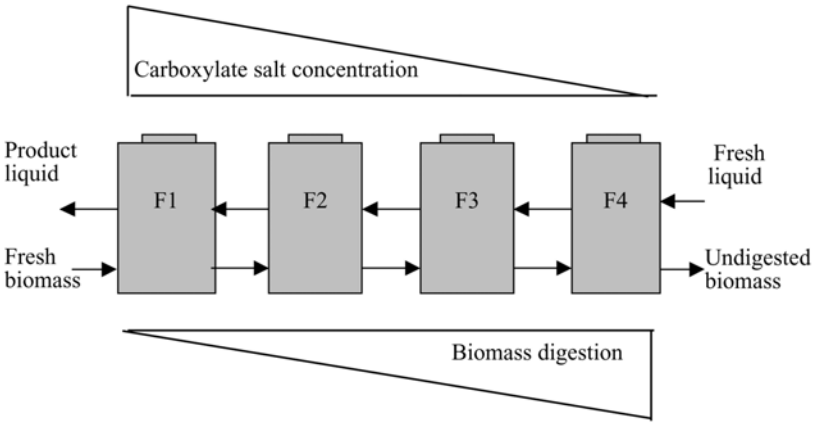

Fig. 2. Four-stage countercurrent fermentation.

Biomass feed consists of volatile solids (VS) and ash. Fig. 3 shows that the biomass VS are converted into gaseous and liquid products, with some remaining solid residues. In the liquid products, VS consist of carboxylic acids and other components, extracellular proteins, and energy storage polysaccharides *(4)*. The following definitions are employed throughout this article:

$$\text{yield} = \frac{\text{total acids produced}}{\text{VS field}} \tag{1}$$

$$\text{conversion} = \frac{\text{VS digested}}{\text{VS fed}} \tag{2}$$

$$\text{total acid selectivity} = \frac{\text{total acids produced}}{\text{VS digested}} \tag{3}$$

$$\text{total acid productivity} = \frac{\text{total acids produced}}{\text{total liquid volume in all fermentors} \cdot \text{time}} \tag{4}$$

$$\text{VSLR} = \frac{\text{VS fed to the fermentor train}}{\text{total liquid volume in all fermentors} \cdot \text{time}} \tag{5}$$

$$\text{LRT} = \frac{\text{total liquid volume in all fermentors}}{\text{flow rate of liquid out of fermentation train}} \tag{6}$$

$$\text{mass balance closure} = \frac{\text{mass out}}{\text{mass in + water of hydrolysis}} \tag{7}$$

To save time determining optimum operating conditions, Loescher *(10)* developed the continuum particle distribution model (CPDM), which can simulate continuous fermentor systems based on the data collected from batch experiments.

Bagasse is low in nutrients, whereas animal manure contains larger amounts of nutrients such as nitrogen, vitamins, and minerals *(11)*. Conse-

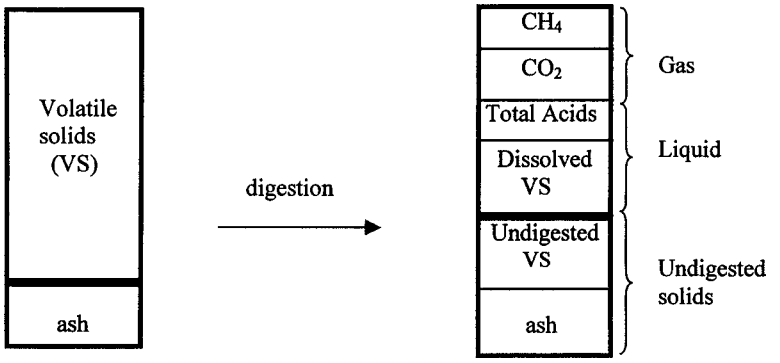

Fig. 3. Digestion of biomass.

quently, our research combined two substrates: sugarcane bagasse (a carbohydrate-rich biomass) and chicken manure (a nutrient-rich biomass). From previous studies, Playne *(12)* studied carboxylic acid production from bagasse by nonsterile, mixed-culture fermentation in continuous fermentation. Total acid productivity was 4.9 g/(L of liquid·d) and yield was 0.25 g of total acid/g of bagasse fed at a concentration of 15.5 g of total acid/L.

To improve total acid productivity and yield, we studied the use of 80% bagasse/20% chicken manure countercurrent fermentation with terrestrial and marine microorganisms. The effect of volatile solids loading rate (VSLR) and liquid residence time (LRT) on total acid productivity, yield, conversion, and total acid selectivity were investigated. In addition, CPDM was used to predict total acid concentration and conversion for countercurrent concentration based on batch fermentation.

Materials and Methods

Substrates

Sugarcane bagasse (80%) and chicken manure (20%) were used as substrates. Bagasse was obtained from a sugar facility in Raceland, LA. It was stored in a shed and allowed to dry. Using conditions recommended by Chang et al. *(13)*, the bagasse was pretreated with 0.1 g of $Ca(OH)_2$/g of dry biomass at 65°C for 24 h and then hammer milled. The average moisture content of treated bagasse was 0.070 g of water/g of wet bagasse, and the average ash content was 0.140 g of ash/g of dry bagasse. The treated bagasse consisted of 0.860 g of VS/g of dry bagasse (0.651 g of carbohydrate/g of dry bagasse, 0.029 g of crude protein/g of dry bagasse, and 0.180 g of lignin/g of dry bagasse).

Chicken manure was collected from the Poultry Science Center, Texas A&M University, College Station, TX. The manure was air-dried and then pretreated with 0.1 g of $Ca(OH)_2$/g of dry biomass at 100°C for 1 h. The treated chicken manure consisted of 0.052 g of water/g of wet chicken manure and 0.340 g of ash/g of dry chicken manure. The average moisture

content of treated chicken manure was 0.052 g of water/g of wet chicken manure, and the average ash content was 0.340 g of ash/g of dry chicken manure. The treated chicken manure consisted of 0.660 g of VS/g of dry chicken manure (0.482 g of carbohydrate/g of dry chicken manure, 0.178 g of crude protein/g of dry chicken manure, and no lignin). The chicken manure was collected in three batches throughout the research; unfortunately, the quality varied from batch to batch.

Medium

The liquid medium used in the bagasse/chicken manure system was the modified Caldwell & Bryant (C&B) medium deoxygenated water, which consists of a dry nutrient mixture in deoxygenated water at a concentration of 1.4 g of dry nutrient mixture/L of deoxygenated water *(11)*.

Deoxygenated water was prepared by boiling distilled water under a nitrogen purge for 5 min. The medium was allowed to cool to room temperature, and then 0.275 g/L of cysteine hydrochloride and 0.275 g/L of sodium sulfide was added under a nitrogen purge. The medium solution was stirred and poured into storage bottles with a nitrogen purge.

Dry nutrient mixture contained (g/100 g of mixture) K_2HPO_4 (16.3), KH_2PO_4 (16.3), $(NH_4)_2SO_4$ (16.3), NaCl (32.6), $MgSO_4 \cdot 7H_2O$ (6.8), $CaCl_2 \cdot 2H_2O$ (4.4), HEPES (0.86), hemin (0.71), nicotinamide (0.71), *p*-aminobenzoic acid (0.71), Ca-pantothenate (0.71), folic acid (0.35), pyridoxal (0.35), riboflavin (0.35), thiamine (0.34), cyanocobalamin (0.14), biotin (0.14), EDTA (0.35), $FeSO_4 \cdot 7H_2O$ (0.14), $MnCl_2$ (0.14), H_3BO_3 (0.021), $CoCl_2$ (0.014), $ZnSO_4 \cdot 7H_2O$ (0.007), $NaMoO_4$ (0.0021), $NiCl_2$ (0.0014), and $CuCl_2$ (0.0007) *(4)*.

Inoculum

Two primary sources of inoculum were used: terrestrial and marine. The terrestrial source included the rumen fluid from a forage-fed fistulated steer located at the Meat Center, Department of Animal Science, Texas A&M University, College Station, TX. In addition to rumen fluid, compost from Dr. Mark Holtzapple's residence and swamp material from Wolf Pen Creek, College Station, were used. They were collected in bottles filled with deoxygenated medium, which consisted of distilled water, 0.275 g/L of sodium sulfide, and 0.275 g/L of cysteine hydrochloride.

Another source was marine microorganisms, a salt-resistant inoculum, from sediments at East Beach, Harborside Street, 51st Street, and Sportsman Road on Galveston Island, TX. The sediment was collected from 0.5-m-deep holes and placed in bottles filled with deoxygenated medium, which consisted of distilled water, 0.275 g/L of sodium sulfide, and 0.275 g/L of cysteine hydrochloride.

Inhibitors

The inhibitor in this experiment was iodoform (CHI_3) prepared in a solution containing 20 g of inhibitor/L of ethanol. The inhibitor was kept

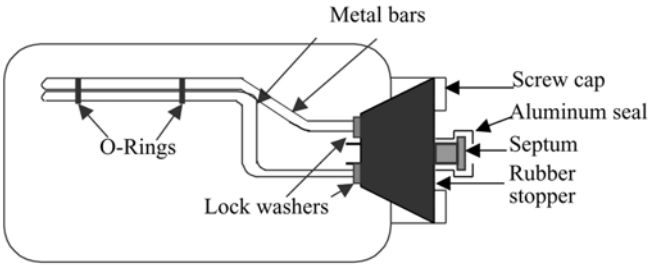

Fig. 4. Assembled fermentor.

in a tinted bottle. The cap was replaced immediately after use owing to light and air sensitivity.

pH Control

To control the pH between 6.0 and 6.5, excess $CaCO_3$ was added to the fermentors. In addition, as a nitrogen supplement, urea was added if the pH was <6.5.

Fermentor

Figure 4 shows the assembled fermentor used in this research. The fermentor was a 1-L polypropylene copolymer centrifuge bottle (98 × 169 mm), Nalgene brand NNI 3120-1010, 7100*g* maximum force. The centrifuge bottle was placed on a Wheaton Modular Cell Production Roller Apparatus (Model III). The apparatus, which was located in an incubator, consists of rollers that rotated the fermentors horizontally at 1 rpm. The bottles were capped with a size 11 rubber stopper with a glass tube inserted through it. The glass tube was capped with a rubber septum for gas sampling and release. The septum was replaced when there was a visible hole resulting from frequent gas venting. Two pieces of 0.25-in. stainless steel tubing, with ends welded shut, were inserted into holes in the stopper. The tubes formed a stir bar to assist in mixing the components inside the bottle; thus, this fermentor could use a high solids concentration. The fermentor could not stand a pressure >2 atm (15 psig); above this pressure, it would leak or explode if the gases were not released.

Experimental Procedure

Continuous countercurrent fermentations were performed in anaerobic fermentors at 40°C. The transfer process occurred every 1 or 2 d, depending on the system. To maintain anaerobic conditions, a nitrogen purge was used during transferring, or whenever the fermentors were open. Two grams of calcium carbonate were added every 1 or 2 d to each fermentor to neutralize the carboxylic acids.

Countercurrent fermentations were conducted at varying LRT and VSLR, as described in Tables 1 and 2. The operating parameters for each

Table 1
Operating Parameters for Bagasse/Chicken Manure Countercurrent Fermentation with Terrestrial Inoculum

Fermentation train	A	B	C	D	E	F	G	H	I	J	K	L	M
LRT (d)	11.7	11.4	13.5	9.70	10.8	13.1	8.87	8.88	12.1	20.5	19.0	20.0	19.7
VSLR (g VS/[L of liquid in all fermentors·d])	10.1	10.3	17.9	11.2	14.4	10.1	10.9	7.36	3.81	2.13	2.00	4.82	6.54
VS feed to F1 at each transfer (g VS)	9.3	7.3	19.6	12.2	15.7	7.3	11.7	7.40	3.90	3.90	3.90	12.4	16.5
Liquid feed to F4 at each transfer (L)	0.1	0.08	0.15	0.15	0.15	0.08	0.15	0.15	0.10	0.10	0.15	0.15	0.15
Frequency of transfer[a]	1/d	1/d	1/d	1/d	1/d	1/d	1/d	1/d	1/d	E 2 d	E 2 d	E 2 d	E 2 d
Liquid volume in all fermentors (L)	0.920	0.708	1.10	1.10	1.04	0.728	1.08	1.00	1.02	0.916	0.976	1.28	1.26
Iodoform addition rate (mg iodoform added to each fermentor/L of liquid fed to F4)	16.0	20.0	10.7	5.3	10.7	20.0	10.7	10.7	24.0	24.0	16.0	10.7	10.7
Urea addition rate (g urea added to each fermentor/L of liquid fed to F4) (if pH < 6.5)	1.00	1.25	0.67	0.67	0.67	1.25	0.67	0.67	1.0	1.0	0.67	0.67	0.67

[a]1/d, once a day; E 2 d, every 2 days.

Table 2
Operating Parameters for Bagasse/Chicken Manure Countercurrent Fermentation with Marine and Terrestrial Inoculum

Fermentation train	N	P	Q	R	I	J
LRT (d)	12.1	12.1	20.5	20.5	12.1	20.5
VSLR (g VS/[L of liquid in all fermentors·d])	3.83	3.84	2.13	2.13	3.81	2.13
Marine inoculum (wt%)	100	40	100	40	0	0
Terrestrial inoculum (wt%)	0	60	0	60	100	100
VS feed to F1 at each transfer (g VS)	3.9	3.9	3.9	3.9	3.9	3.9
Liquid feed to F4 at each transfer (L)	0.1	0.1	0.1	0.1	0.1	0.1
Frequency of transfer	Daily	Daily	Every 2 d	Every 2 d	Daily	Every 2 d
Liquid volume in all fermentors (L)	1.02	1.02	0.916	0.916	1.02	0.916
Iodoform addition rate (mg iodoform added to each fermentor/L of liquid fed to F4)	24.0	24.0	24.0	24.0	24.0	24.0
Urea addition rate (g urea added to each fermentor/L of liquid fed to F4) (if pH < 6.5)	1.0	1.0	1.0	1.0	1.0	1.0

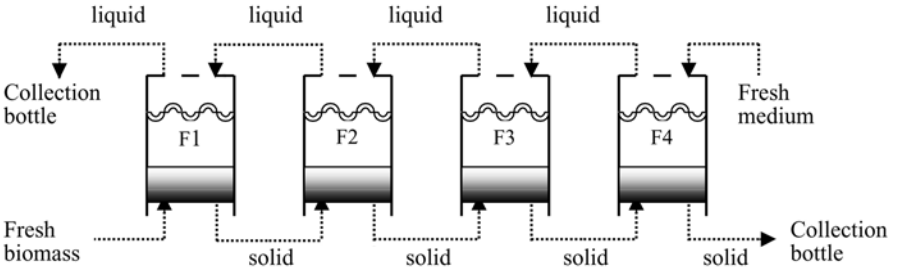

Fig. 5. Single-centrifuge procedure.

fermentation train with 100% terrestrial inoculum are given in Table 1. The operating parameters for each fermentation train with 40% and 100% marine inoculum, compared with 100% terrestrial inoculum, are given in Table 2. All fermentation trains consisted of four countercurrent stages and the single-centrifuge procedure (Fig. 5).

After the system reached steady state, fermentation data were collected for 40 d to determine carboxylic acid concentration, acid productivity, selectivity, yield, conversion, and CH_4 productivity.

Analytical Methods

The total gas volume from each fermentor was measured using an inverted, graduated glass cylinder apparatus (water displacement apparatus) filled with an aqueous solution of 30 wt% $CaCl_2$ *(14)*. The methane content was determined using a gas chromatograph (Agilent Model 6890) with a thermal conductivity detector and packed column (Carboxen1004, Supelco1-2390; J&W Scientific). Samples were taken directly from the fermentors using a 5-mL syringe. To ensure calibration of the column and instrument, a standard consisting of 10.06 mol% methane, 29.99 mol% carbon dioxide, and the balance nitrogen was periodically run through the column before running the gas samples. Methane and carbon dioxide are both products of microbial digestion. Knowledge of the amount of total gas and methane allowed CO_2 production to be calculated. CO_2 production is of two kinds: biotic and abiotic. The abiotic CO_2 is produced by neutralizing the carboxylic acids with calcium carbonate. It is assumed that 1 mol of abiotic CO_2 is produced for every 2 mol of acid produced. The biotic CO_2 produced directly from the fermentation can be calculated from the total CO_2 subtracted from the abiotic CO_2.

Liquid from the first reactor and solid from the last reactor were collected during each transfer. Liquid samples were analyzed for carboxylic acids and VS. Solid samples were also collected to determine the mass of undigested VS. Acid analysis was performed using an Agilent 6890 gas chromatograph with capillary column (J&W Scientific, model DB-FFAP). Liquid samples were combined with 1.162 g/L of internal standard (4-methyl-*n*-valeric acid) and acidified with 3 *M* phosphoric acid before injection into the gas chromatograph. VS in the fed substrates

and from liquid and solid samples were determined by drying at 105°C and then ashing at 550°C.

A complete mass balance for the fermentation systems was obtained on the entire train over a steady-state period. The mass balance closure determined the difference between the mass entering the system and the mass exiting the system.

The mass balance closure was calculated as follows:

$$\text{mass balance closure} = \frac{\text{undigested VS} + \text{non-acid dissolved VS} + \text{acids out} + \text{biotic } CO_2 + CH_4}{\text{VS in} + \text{water of hydrolysis}} \quad (8)$$

The system should theoretically have 100% closure; any discrepancies in the closure result from errors in the measurements and transfer process. Ross *(4)* suggested that the biomass could be represented as cellulose, which has a monomer weight of 162 g/mol. When cellulose is hydrolyzed to sugars, it gains a molecule of water (mol wt of 18 g/mol) per monomer that accounts for the increase in mass. An approximation for the water of hydrolysis is

$$\text{water of hydrolysis} = \text{VS digested} \times \frac{18 \text{ g/mol}}{162 \text{ g/mol}} \quad (9)$$

Continuum Particle Distribution Modeling

Ross *(4)* describes a *continuum particle* as a collection of particles that has a VS wt of 1 g upon entering a fermentor. To apply the CPDM method, batch experiments were used at a variety of initial substrate concentrations.

The batch experiments consisted of five fermentors at varying initial substrate concentrations (20, 40, 70, 100, and 100$^+$ g of dry substrate/L of liquid). The 100 and 100$^+$ fermentors had the same initial substrate concentrations, but the 100$^+$ fermentor contained a medium with a mixture of carboxylate salts (70 wt% calcium acetate, 20 wt% calcium propionate, and 10 wt% calcium butyrate) at a concentration of 20 g of carboxylic acids/L of liquid. The inoculum for the CPDM batch experiment was 20% of liquid that was taken from countercurrent fermentation operating with the same substrate. The medium for the CPDM batch experiments was modified C&B medium, the same as the countercurrent fermentation. Initially, 2.0 g of calcium carbonate and 0.1 g of urea were added to each fermentor. Daily samples of the liquid were taken from each batch fermentor. From these batch experiments, the reaction rate at varying acid concentrations and biomass digestion were determined.

After analyzing the liquid, the carboxylic acid concentration was converted to acetic acid equivalents (A_e) by the following equations:

$$\begin{aligned}\alpha \text{ (mol/L)} = {} & \text{acetic (mol/L)} + 1.75 \times \text{propionic (mol/L)} + \\ & 2.5 \times \text{butyric (mol/L)} + 3.25 \times \text{valeric (mol/L)} + \\ & 4.0 \times \text{caproic (mol/L} + 4.75 \times \text{heptanoic (mol/L)}\end{aligned} \quad (10)$$

This can be expressed on a mass basis as follows:

$$A_e\,(\text{g/L}) = 60.05\,(\text{g/mol}) \times \alpha\,(\text{mol/L}) \tag{11}$$

The acetic acid equivalent unit is based on the reducing power of the acids produced in the fermentation. From the batch experiments, the data were fit to an empirical equation by least-squares analysis. The concentration of acetic acid equivalents in each batch experiment was fit to Eq. 12:

$$A_e = a + \frac{bt}{1 + ct} \tag{12}$$

in which a, b, and c are constants fit by least-squares analysis. The equation derived from the curve fit was then differentiated to give the rate of acid production, r, by Eq. 13:

$$r = \frac{dA_e}{dt} = \frac{b}{(1 + ct)^2} \tag{13}$$

This rate in Eq. 13 was then converted into specific rate, $\hat{r}$ (g of A_e produced/g of VS·d), by dividing it by the initial substrate concentration, S_0 (g of VS/L), in the respective batch fermentation.

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

The specific rate equation in the mixed-acid fermentation is empirical. Equation 15 can be determined by using least-squares analysis:

$$\hat{r}_{pred} = \frac{e\left(1 - x\right)^f}{1 + g\left(\phi A_e\right)^h} \tag{15}$$

in which x is the fraction conversion of volatile solids; e, f, g, and h are empirical constants; and ϕ is the ratio of total grams of actual acid to grams of A_e.

Ross *(4)* included ϕ to decrease the inhibitory effects of the higher acids that would overestimate the specific rate. The $(1 - x)^f$ term in the numerator is the conversion penalty function described by South and Lynd *(15)*. The conversion, x, is calculated using

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

in which σ is the selectivity (g of A_e produced/g of VS digested), and S_0 is the initial substrate concentration (g of VS/L).

In the CPDM model, the selectivity σ was taken as a constant. The selectivity σ for CPDM was calculated from the selectivity s from the countercurrent experiment (g of total acid produced/g of VS digested) by dividing by ϕ (the ratio of total grams of actual acid to grams of A_e). The relationship between s and σ follows:

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

Equation 15 was used in a Mathematica program *(14)* to predict acetic acid equivalent concentration (A_e), which could be converted back to total acid concentration, and conversion (x) for the countercurrent fermentations at various LRT and VSLR. Other system-specific parameters needed for the Mathematica program were the average selectivity (σ), holdup (ratio of g of liquid to g of VS wet solid), moisture (ratio of g of liquid to g of wet solid in feed), solids concentration (g VS/L of liquid), and liquid volume (L).

Results and Discussion

Countercurrent Fermentations

Table 3 presents the results of the countercurrent fermentations with terrestrial inoculum. The highest acid productivity, yield, and acid selectivity were 2.49 g/(L of liquid·d), 0.338 g of total acid/g of VS fed, and 0.581 g of total acid/g of VS digested, respectively, at a concentration of 18.7 g of total acid/L in fermentation train H (LRT = 8.88 d and VSLR = 7.36 g/[L of liquid·d]). Fermentation train J (LRT = 20.5 d and VSLR = 2.13 g/[L of liquid·d]) had the highest conversion (0.60 g of VS digested/g of VS fed). Its yield of 0.338 g of total acid/g of VS fed (0.336 g of total acid/g of dry bagasse fed) at a concentration of 18.7 g of total acid/L was higher than the yield from Playne *(12)* (0.25 g of total acid/g of bagasse fed at a concentration of 15.5 g of total acid/L). The difference could result from the benefits of countercurrent fermentation and addition of chicken manure in this research.

The correlations between VSLR and acid productivity (p), selectivity (s), yield (y), and conversion (x) are shown in Figs. 6–9, respectively. Acid productivity increased with increasing VSLR. Moreover, the selectivity, yield, and conversion decreased with increasing VSLR. These correlations can be described by the following equations:

$$p = 0.0497\ \text{VSLR} + 1.06 \tag{18}$$

$$s = -0.0077\ \text{VSLR} + 0.483 \tag{19}$$

$$y = -0.0088\ \text{VSLR} + 0.253 \tag{20}$$

$$x = -0.0142\ \text{VSLR} + 0.578 \tag{21}$$

At higher solid loading rates, the microorganisms have a higher acid productivity; however, the conversion is lower. Moreover, the mixed cultures of microorganisms digest primarily the "easy" portions and produce non-acid soluble VS that presumably are energy-storage products *(11)*. Consequently, the ratio of digested to undigested VS (conversion) was low. With the higher VS loadings, the selectivity (g acid/g of VS digested) and yield (g acid/g of VS fed) were lower. On the other hand, at smaller VS loading rates, the acid productivity was lower. Furthermore, the microorganisms must consume both the easy and difficult portions and produce less of the soluble energy-storage products. With the lower VS loading, the

Table 3
Results for Bagasse/Chicken Manure Countercurrent Fermentation with Terrestrial Inoculum[a]

Fermentation train	A	B	C	D	E	F	G
Average pH in all fermentors	6.2 ± 0.41	6.4 ± 0.19	6.4 ± 0.31	6.1 ± 0.38	6.3 ± 0.25	6.4 ± 0.20	6.1 ± 0.21
Total acid productivity (g/[L of liquid in all fermentors·d])	1.22	1.41	1.55	1.56	1.84	1.51	2.12
Total acid concentration (g/L)	14.2 ± 0.76	16.6 ± 1.06	21.0 ± 0.48	15.2 ± 0.73	20.1 ± 0.80	20.0 ± 0.36	19.0 ± 0.87
Acetic acid (wt%)	35.7 ± 5.10	35.4 ± 3.21	39.6 ± 2.48	35.6 ± 4.09	39.3 ± 3.15	40.1 ± 1.68	40.2 ± 3.06
Propionic acid (wt%)	26.9 ± 3.25	20.2 ± 2.74	18.1 ± 1.82	26.5 ± 2.91	17.2 ± 3.44	18.3 ± 1.68	18.7 ± 1.71
Butyric acid (wt%)	17.0 ± 2.91	19.0 ± 2.96	19.3 ± 2.28	15.9 ± 2.16	20.1 ± 3.22	18.6 ± 1.34	17.5 ± 2.28
Valeric acid (wt%)	13.6 ± 3.06	13.2 ± 2.31	10.2 ± 2.14	14.0 ± 1.68	11.7 ± 2.66	10.3 ± 1.05	11.6 ± 1.18
Caproic acid (wt%)	5.54 ± 1.84	9.61 ± 2.41	9.82 ± 1.82	6.76 ± 0.77	9.29 ± 3.10	9.86 ± 1.91	8.24 ± 1.06
Heptanoic acid (wt%)	1.26 ± 0.29	2.63 ± 0.99	2.87 ± 0.91	1.18 ± 0.36	2.36 ± 1.38	2.86 ± 0.74	3.76 ± 1.81
VS digested (g VS/d)	4.00	3.30	8.10	4.70	4.30	3.20	4.50
Yield (g total acid/g VS fed)	0.118	0.137	0.087	0.139	0.127	0.151	0.197
Selectivity (g total acid/g VS digested)	0.275	0.303	0.210	0.362	0.465	0.344	0.511
Non-acid dissolved VS (g VS/g VS digested)	0.078	0.100	0.091	0.074	0.216	0.100	0.180
Conversion (g VS digested/g VS fed)	0.430	0.452	0.413	0.385	0.274	0.438	0.385
Biotic CO_2 productivity (g CO_2/[L of liquid in all fermentors·d])	1.38	N/A	0.567	1.27	0.645	N/A	0.765
CH_4 productivity (g CH_4/[L of liquid in all fermentors·d])	0	N/A	0	0.0028	0	N/A	0
Mass balance closure (g VS out/g VS in)	0.819	N/A	0.707	0.860	0.929	N/A	0.913

[a]All errors are ± 1 SD. N/A, not available.

(Continued)

Table 3 *(Continued)*
Results for Bagasse/Chicken Manure Countercurrent Fermentation with Terrestrial Inoculum[a]

Fermentation train	H	I	J	K	L	M
Average pH in all fermentors	6.3 ± 0.19	6.4 ± 0.29	6.3 ± 0.31	6.4 ± 0.21	6.2 ± 0.29	6.2 ± 0.28
Total acid productivity (g/[L of liquid in all fermentors·d])	2.49	0.791	0.547	0.615	0.623	0.634
Total acid concentration (g/L)	18.7 ± 0.49	9.6 ± 1.05	13.2 ± 0.64	11.1 ± 0.79	13.1 ± 0.88	15.2 ± 0.68
Acetic acid (wt%)	40.4 ± 2.63	35.4 ± 4.10	35.1 ± 4.95	44.5 ± 3.99	38.5 ± 3.88	35.8 ± 3.66
Propionic acid (wt%)	19.9 ± 1.91	20.2 ± 2.50	25.0 ± 3.31	24.3 ± 5.08	22.5 ± 3.14	21.8 ± 2.41
Butyric acid (wt%)	17.1 ± 2.02	10.7 ± 2.06	16.5 ± 3.01	9.6 ± 3.43	17.8 ± 3.58	18.8 ± 2.15
Valeric acid (wt%)	11.5 ± 1.52	14.1 ± 2.51	13.1 ± 2.10	12.2 ± 2.94	11.9 ± 2.68	12.7 ± 2.27
Caproic acid (wt%)	8.27 ± 1.09	8.38 ± 1.35	5.60 ± 0.64	5.10 ± 1.35	6.83 ± 1.71	8.17 ± 1.77
Heptanoic acid (wt%)	2.84 ± 0.65	11.2 ± 2.66	4.66 ± 1.16	4.34 ± 1.16	2.45 ± 0.92	2.72 ± 1.05
VS digested (g VS/d)	4.30	2.10	1.20	1.06	3.10	3.50
Yield (g total acid/g VS fed)	0.338	0.208	0.250	0.300	0.125	0.094
Selectivity (g total acid/g VS digested)	0.581	0.386	0.417	0.566	0.258	0.229
Non-acid dissolved VS (g VS/g VS digested)	0.133	0.157	0.225	0.226	0.181	0.160
Conversion (g VS digested/g VS fed)	0.581	0.538	0.600	0.530	0.484	0.412
Biotic CO_2 productivity (g CO_2/[L of liquid in all fermentors·d])	0.809	0.628	0.601	0.435	0.466	0.515
CH_4 productivity (g CH_4/[L of liquid in all fermentors·d])	0	0.0001	0.0001	0.0001	0.0004	0.0008
Mass balance closure (g VS out/g VS in)	0.886	0.863	1.01	1.02	0.786	0.791

[a]All errors are ± 1 SD. N/A, not available.

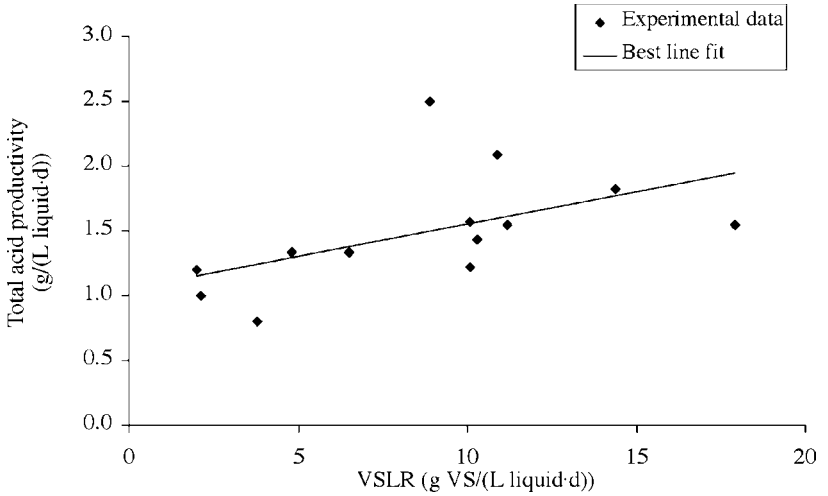

Fig. 6. Correlation of total acid productivity with VSLR ($R^2 = 0.271$).

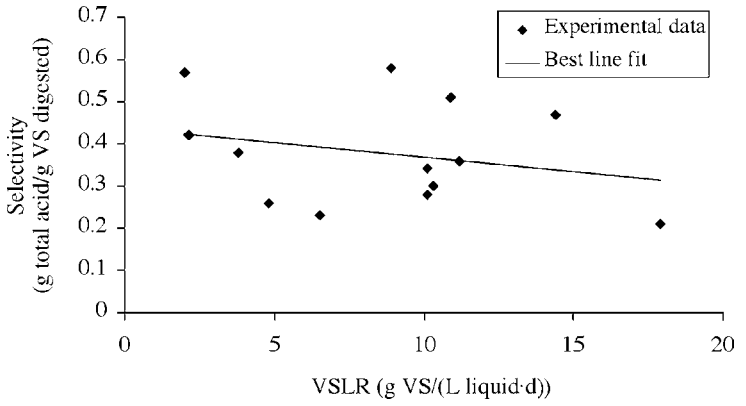

Fig. 7. Correlation of selectivity with VSLR ($R^2 = 0.0677$).

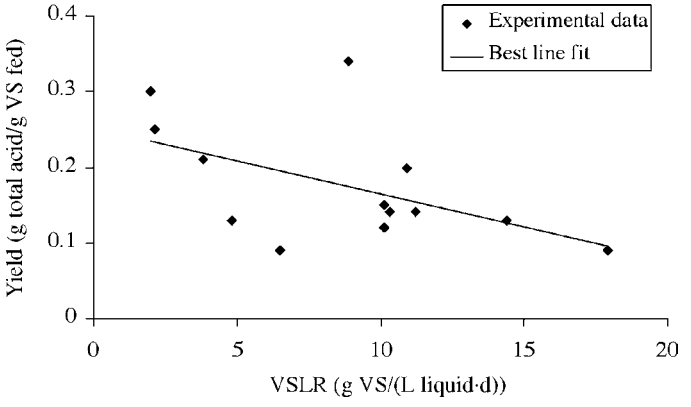

Fig. 8. Correlation of yield with VSLR ($R^2 = 0.275$).

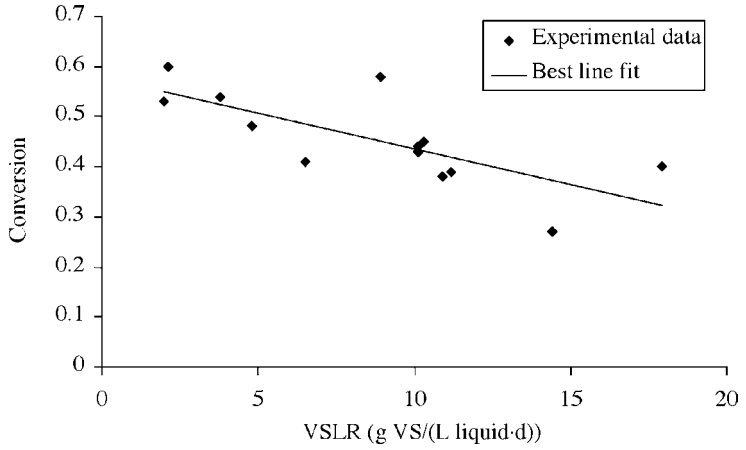

Fig. 9. Correlation of conversion with VSLR (R^2 = 0.540).

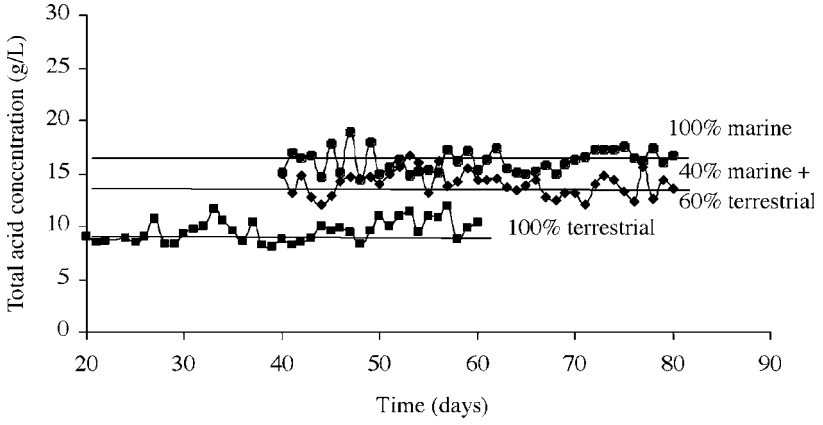

Fig. 10. Total acid concentration for baggasse/chicken manure fermentation with different inoculum sources at LRT = 12.1 d and VSLR = 3.8 g/(L·d).

selectivity (g of acid/g of VS digested) and yield (g of acid/g of VS fed) were higher. Moreover, the ratio of digested to undigested VS (conversion) was higher.

Figures 10 and 11 show the steady-state total acid concentration for the fermentations with 100% marine, 40% marine + 60% terrestrial, and 100% terrestrial inocula. The total acid concentration for the fermentation with 100% marine inoculum was higher than the fermentations with 40% marine + 60% terrestrial and 100% terrestrial inocula at VSLR = 2.13 and 3.8 g/(L·d). Other results are given in Table 4. The acid productivity, selectivity, and yield in the fermentations with marine inoculum (trains N, P, Q, and R) were higher than fermentation trains I and J with solely terrestrial inoculum. Fermentation trains N and Q with 100% marine

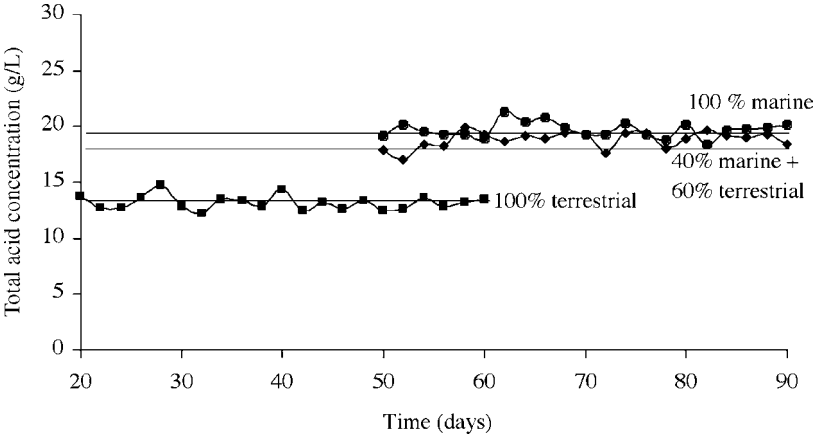

Fig. 11. Total acid concentration for baggasse/chicken manure fermentation with different inoculum sources at LRT = 20.5 d and VSLR = 2.13 g/(L·d).

inoculum had slightly higher acid productivity, selectivity, and yield than fermentation trains P and R with a 40% marine + 60% terrestrial inoculum. Conversion in fermentation trains I, N, and P were similar at LRT = 12.1 d and VSLR = 3.8 g/(L·d). However, conversion increased with increasing percentage of marine inoculum at LRT = 20.5 d and VSLR = 2.13 g/(L·d). The carboxylic acid produced in the fermentation is combined with calcium carbonate and transformed to carboxylate salt. At high salt concentrations, the terrestrial microorganisms could not grow well compared to the marine inoculum. Therefore, the fermentation with 100% marine inoculum resulted in the highest acid productivity, yield, and acid selectivity in this experiment.

Continuum Particle Distribution Modeling

The data from the batch fermentation at varying initial substrate concentrations can be found in Thanakoses *(14)*. The values of *e*, *f*, *g*, and *h* for Eq. 15 and selectivity used in the CPDM Mathematica program are given in Table 5. Other parameters used in the CPDM Mathematica program are presented in Table 6. From the Mathematica program, the predicted total acid concentration and conversion for the countercurrent fermentation at various LRT and VSLR were obtained. Figure 12 shows the CPDM "maps" for the 80% bagasse/20% chicken manure fermentation system (solids concentration = 127 g of VS/[L of liquid]) with different inoculum sources. The results indicated that the fermentation with marine inoculum yielded higher acid concentration and conversion than the fermentation with terrestrial inoculum at any VSLR and LRT. Furthermore, the CPDM "map" for fermentation with marine inoculum showed that 90% conversion is possible and that the carboxylic acid concentrations could reach 23.4 g/L at a VSLR of 2 g/(L·d) and LRT of 20.5 d.

Table 4
Results for Bagasse/Chicken Manure Countercurrent Fermentation with Marine and Terrestrial Inoculum

Fermentation train	N	P	Q	R	I	J
Average pH in all fermentors	6.3 ± 0.18	6.3 ± 0.16	6.3 ± 0.2	6.3 ± 0.2	6.4 ± 0.29	6.3 ± 0.31
Total acid productivity (g/[L liquid in all fermentors·d])	1.38	1.18	0.928	0.841	0.791	0.547
Total acid concentration (g/L)	16.2 ± 1.08	14.0 ± 1.15	19.7 ± 0.70	18.8 ± 0.73	9.6 ± 1.05	13.2 ± 0.64
Acetic acid (wt%)	45.5 ± 3.60	44.9 ± 4.93	45.1 ± 2.12	44.9 ± 0.69	35.4 ± 4.10	35.1 ± 4.95
Propionic acid (wt%)	21.7 ± 4.37	22.2 ± 4.50	15.7 ± 2.12	22.2 ± 0.63	20.2 ± 2.50	25.0 ± 3.31
Butyric acid (wt%)	12.9 ± 2.68	12.9 ± 2.69	14.3 ± 1.32	12.9 ± 0.38	10.7 ± 2.06	16.5 ± 3.01
Valeric acid (wt%)	9.8 ± 1.48	9.5 ± 1.36	11.2 ± 0.87	9.5 ± 0.19	14.1 ± 2.51	13.1 ± 2.10
Caproic acid (wt%)	5.9 ± 2.01	6.6 ± 1.57	8.0 ± 0.87	6.6 ± 0.22	8.38 ± 1.35	5.60 ± 0.64
Heptanoic acid (wt%)	4.2 ± 1.59	3.9 ± 0.98	5.7 ± 2.70	3.9 ± 0.14	11.2 ± 2.66	4.66 ± 1.16
VS digested (g VS/d)	2.20	2.17	1.52	1.39	2.10	1.20
Yield (g total acid/g VS fed)	0.359	0.308	0.425	0.385	0.208	0.250
Selectivity (g total acid/g VS digested)	0.667	0.553	0.559	0.554	0.386	0.417
Non-acid dissolved VS (g VS/g VS digested)	0.286	0.304	0.336	0.281	0.157	0.225
Conversion (g VS digested/g VS fed)	0.538	0.556	0.760	0.695	0.538	0.600
Biotic CO_2 productivity (g CO_2/[L of liquid in all fermentors·d])	0.389	0.576	0.448	0.437	0.628	0.601
CH_4 productivity (g CH_4/[L of liquid in all fermentors·d])	0	0.0002	0.0002	0.0011	0.0001	0.0001
Mass balance closure (g VS out/g VS in)	1.02	1.01	1.03	1.03	0.863	1.01

[a]All errors are ± 1 SD.

Table 5
The Values of e, f, g, h and Selectivity Used in CPDM Mathematica Program for Bagasse/Chicken Manure Fermentation

Inoculum sources	Selectivity (g A_e/g VS digested)	e (g A_e/[g VS·d])	f (dimensionless)	g (L/g total acid)$^{1/h}$	h (dimensionless)
100% Terrestrial	0.512	0.069	2.60	0.003	2.57
40% Marine + 60% Terrestrial	0.675	0.156	2.80	0.015	1.99
100% Marine	0.752	0.153	2.55	0.047	1.38

Table 6
Average Parameter Constant Values for Bagasse/Chicken Manure Fermentation with Terrestrial and Marine Inocula Used in CPDM Mathematica Program

Parameter constant	Value
Holdup (g liquid/g VS wet cake)	4.56 ± 0.828
Moisture (g liquid/g wet solid)	0.066 ± 0.008
F1–F4 solids concentration (g VS/L)	127 ± 17.4
F1–F4 liquid volume (L)	0.250 ± 0.062
ϕ (g total acid/g A_e)	0.815 ± 0.033

[a]All errors are ± 1 SD.

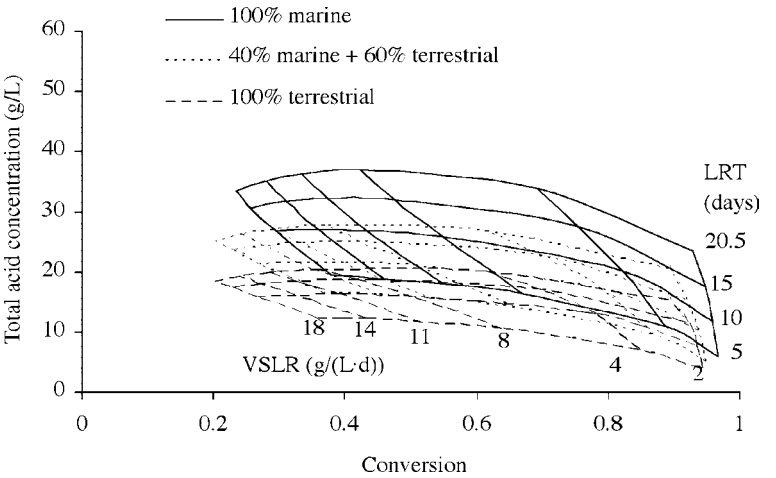

Fig. 12. CPDM maps for bagasse/chicken manure countercurrent fermentation (127 g of VS/L).

Verification of CPDM Method

Using Eq. 15 with the parameter constants for each case in the CPDM Mathematica program, the acid concentration and conversion were predicted at varying VSLR and LRT from fermentation trains A to R. Tables 7 and 8 compare the predicted and actual acid concentration and conversion. The average error between the experimental and predicted acid concentration in the fermentations with 100% terrestrial, 40% marine + 60% terrestrial, and 100% marine inocula was 20.1, 25.2, and 33.1%, respectively. The average error between the experimental and predicted conversion in the fermentations with 100% terrestrial, 40% marine + 60% terrestrial, and 100% marine inocula was 24.1, 33.2, and 35.5%, respectively. The model variation could have resulted from variations in chicken manure quality because other studies show much less variation *(14)*.

Table 7
Comparison of Experimental and Predicted Carboxylic Acid Concentration and Conversion for Bagasse/Chicken Manure Fermentation with 100% Terrestrial Inoculum

	Fermentation train													Average
	A	B	C	D	E	F	G	H	I	J	K	L	M	(%)[a]
Experimental carboxylic acid concentration (g/L)	14.2	16.6	21.0	15.2	20.1	20.0	19.0	18.7	9.6	13.1	11.1	13.0	15.1	
Predicted carboxylic acid concentration (g/L) (from CPDM)	17.2	17.0	17.2	16.1	16.6	17.9	15.5	14.9	14.7	16.0	14.5	13.7	20.4	
Error (%)[b]	20.8	2.5	–18.3	5.8	–17.4	–10.6	–18.4	–20.2	53.7	21.9	30.7	5.4	35.2	20.1
Experiment conversion	0.430	0.452	0.413	0.385	0.274	0.438	0.385	0.581	0.538	0.600	0.530	0.484	0.412	
Predicted conversion (from CPDM)	0.393	0.392	0.238	0.395	0.309	0.373	0.420	0.566	0.770	0.877	0.902	0.693	0.431	
Error (%)[b]	–8.6	–13.3	–42.4	2.6	12.8	–14.8	9.1	–2.6	43.1	46.2	70.2	43.2	4.6	24.1
Expected conversion (from Eq. 23)	0.343	0.342	0.220	0.347	0.278	0.325	0.368	0.484	0.641	0.722	0.741	0.581	0.366	
Error (%)[c]	–20.3	–24.3	–46.6	–9.8	1.6	–25.7	–4.3	–16.8	19.2	20.3	39.9	20.1	–11.2	20.0

[a]Average = average absolute error.
[b]Error = (predicted – experimental) × 100/experimental.
[c]Error = (expected – experimental) × 100/experimental.

Table 8
Comparison of Experimental and Predicted Carboxylic Acid Concentration and Conversion for Bagasse/Chicken Manure Fermentation with 40 and 100% Marine Inoculum

	100% Marine inoculum			40% Marine + 60% terrestrial inoculum		
	Train N	Train Q	Average (%)[a]	Train P	Train R	Average (%)[a]
Experimental carboxylic acid concentration (g/L)	16.2	19.7		14.0	18.8	
Predicted carboxylic acid concentration (g/L)	22.9	24.6		19.4	21.0	
Error (%)[b]	41.3	24.9	33.1	38.6	11.7	25.2
Experimental conversion	0.538	0.760		0.556	0.695	
Predicted conversion	0.809	0.917		0.769	0.876	
Error (%)[b]	50.4	20.6	35.5	38.3	21.0	32.2

[a]Average = average absolute error.
[b]Error = (predicted – experimental) × 100/experimental.

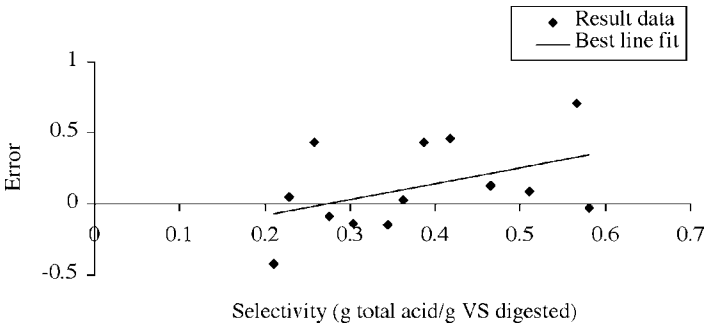

Fig. 13. Correlation of error with selectivity for bagasse/chicken manure countercurrent fermentation:

$$\text{error} = \frac{x_{pred} - x_{exp}}{x_{exp}}$$

Figure 13 shows the error between the predicted conversion, x_{pred}, and the experimental conversion, x_{exp}, as a function of selectivity. Linear regression of the data gives the correlation in Eq. 22.

$$\text{error} = \frac{x_{pred} - x_{exp}}{x_{exp}} = 1.12s - 0.307 \tag{22}$$

Substituting Eq. 19 into Eq. 22 gives the correlation among VSLR, x_{pred}, and x_{exp}:

$$x_{exp} = \frac{x_{pred}}{-0.0086\ \text{VSLR} + 1.23} \tag{23}$$

In Eq. 23, x_{exp} may be interpreted as the "expected conversion." The expected conversions for fermentation trains A–M are presented in Table 7. The results indicate that the average expected conversion error from Eq. 23 (20.0%) was less than the average predicted conversion error from the CPDM map (24.1%). Applying these corrections reduces the error in conversion that was directly obtained from the CPDM model, in which the selectivity was kept constant.

Conclusion

The 80% bagasse/20% chicken manure countercurrent fermentation with terrestrial inoculum operated at LRT = 8.88 d and VSLR = 7.36 g/(L of liquid·d) had the highest acid productivity (2.5 g/[L·d]) with the highest acid selectivity (0.581 g of acid/g of VS digested) and highest yield (0.338 g of acid/g of VS fed). The fermentation operated at a LRT = 20.5 d and a VSLR = 2.13 g/(L of liquid·d) had the highest conversion (0.60 g of VS digested/g of VS fed). Using marine inoculum, instead of terrestrial inoculum, increased the total acid productivity, yield, and acid selectivity. CPDM predicted the total acid concentration and conversion within 20.1 and 24.1%, respectively, of experimental results. By applying a correction for varying selectivity, the experimental conversion could be predicted within 20%.

In summary, 90% conversion of biomass to carboxylic acid at a concentration of 23.4 g/L is possible by using marine inoculum (solids concentration of 127 g/[L of liquid]). Furthermore, applying CPDM could predict the optimum condition for countercurrent fermentation that should be verified at the pilot plant and industrial scales.

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