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Fermentation of Rice Straw/Chicken Manure to Carboxylic Acids Using a Mixed Culture of Marine Mesophilic Micoorganisms

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Abstract

Countercurrent fermentation of rice straw and chicken manure to carboxylic acids was performed using a mixed culture of marine mesophilic microorganisms. To increase the digestibility of the biomass, rice straw, and chicken manure were pretreated with 0.1 g $Ca(OH)_2$/g biomass. Fermentation was performed for 80% rice straw and 20% chicken manure at various volatile solid loading rates (VSLR) and liquid residence times (LRT). The highest acid productivity of 1.69 g/(L•d) occurred at a total acid concentration of 32.4 g/L. The highest conversion (0.69 g VS digested/g VS fed) and yield (0.29 g total acids/g VS fed) were at a total acid concentration of 25 g/L. A Continuum Particle Distribution Model of the process predicted the experimental total acid concentration and conversion results with an average error of 6.41% and 6.15%, respectively. Results show how total acid concentrations, conversions, and yields vary with VSLR and LRT in the MixAlco process.

Index Entries: Biomass; carboxylic acids; CPDM; digestion; fuels; mixed culture.

Introduction

Currently, rice straw is either burned in open fields or incorporated into the soil. Increasing environmental concerns and government legislation call for a decrease in the quantity of rice straw burned *(1)*. Incorporating rice straw into soil increases foliar disease, reduces crop yield, degrades soil conditions, and produces methane, a greenhouse gas *(2)*. Therefore, a low-cost technology to convert these wastes into useful fuels *(3)* and chemicals is valuable. Significant potential benefits result from fuel derived from cellulosic biomass, a renewable nonfood feedstock *(4)*.

The conversion of rice straw to ethanol has been studied using simultaneous saccharification and fermentation (SSF) *(5,6)* in which lignocellulose is simultaneously hydrolyzed to sugars and fermented to ethanol. Unfortunately, this process requires enzymes that contribute heavily to production costs.

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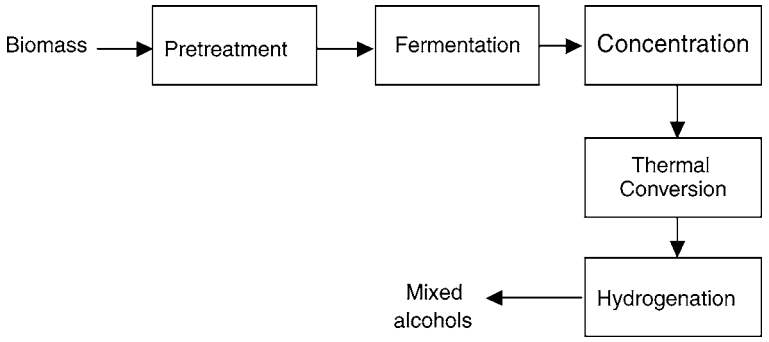

Fig. 1. MixAlco process.

Another option is the MixAlco process, which converts biodegradable materials into mixed alcohols *(7)* (Fig. 1). In the MixAlco process, to increase biomass digestibility, it is treated with lime, an inexpensive chemical. The biomass is then fermented with a mixed culture of microorganisms to produce carboxylic acids under nonsterile, anaerobic conditions. To maintain the pH, calcium carbonate is added as a buffer, which reacts with the acid to form carboxylate salts. These carboxylate salts can be concentrated, followed by "acid springing" to produce the corresponding organic acids *(8)*. Alternatively, fuels can be produced by converting carboxylate salts to ketones, and hydrogenating the ketones to mixed alcohol fuels. The MixAlco process has many benefits, such as no sterility requirement, adaptability to many feedstocks, and no enzyme addition.

To achieve high substrate conversions and product concentrations, countercurrent fermentation is used (Fig. 2). Fresh biomass is added to the fermentor containing the highest carboxylate concentration and fresh water is added to the fermentor with the most digested biomass. This countercurrent flow arrangement addresses two issues: (1) the recalcitrant portion of biomass remains after digestion of the easily converted fraction and (2) product carboxylate salts are extremely inhibitory *(9)*. The countercurrent fermentation allow fresh biomass (most reactive) to contact a high carboxylate acid concentration, leading to a higher product concentration. The most digested biomass (least reactive) contacts fresh water, which is less inhibitory allowing for high conversions.

Chang *(10)* showed that lime pretreatment removes all acetyl groups and a moderate amount of lignin. A lime loading of 0.1 g $Ca(OH)_2$/g dry biomass at 86–135°C for 1–3 h is optimum for pretreatment of biomass *(10)*. Chang *(10)* recommended 10 mL water/g dry biomass and Karr *(11)* recommended 5 g H_2O/g dry biomass. The pretreatment effect was independent of water loading *(11)* but there should be enough water to cover the biomass. Karr *(11)* showed that lime pretreatment increased the conversion of corn stover by about 90%.

Carboxylic acids are intermediates in the fermentation of biomass to methane. Zhang et al. *(12)* studied biogasification of rice straw to produce

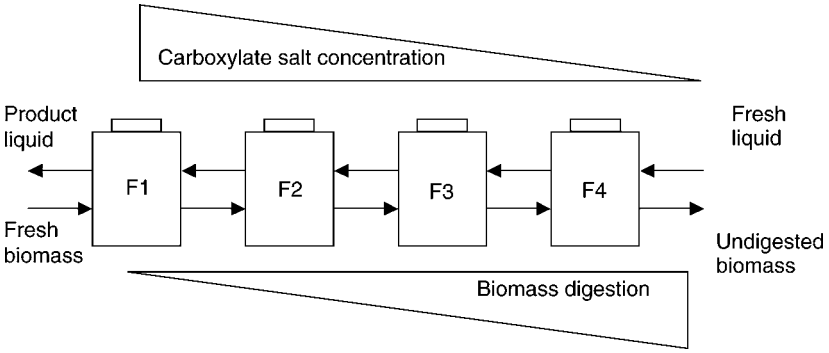

Fig. 2. Four-stage countercurrent fermentation.

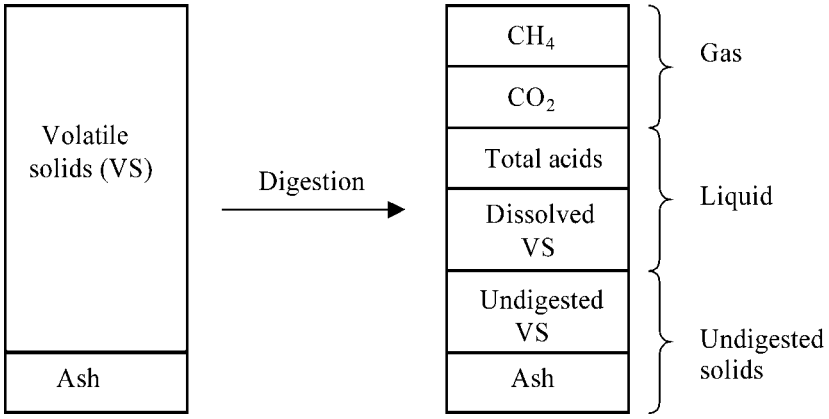

Fig. 3. The digestion of biomass.

biogas (CH_4 50%), however, methane is a low-value product. In the MixAlco process, methane formation from carboxylic acids is inhibited. Methane analogs such as iodoform and bromoform are effective in inhibiting methanogenesis *(13)*, which eliminates a potential hydrogen sink and reducing power is used to produce higher carboxylic acids, such as propionate and butyrate *(9,13)*.

Biomass contains volatile solids (VS) (the major components are cellulose, hemicellulose, and lignin) and ash (Fig. 3). When the biomass is digested, volatile solids convert to gaseous and liquid products, plus solid residues. The gaseous products are principally methane and carbon dioxide, the liquid products are carboxylate salts, extracellular proteins, and energy storage polysaccharides *(9)*, and the solid residue contains ash and undigested VS. The following definitions were used:

$$\text{Volatile solids (VS)} = \text{Dry weight} - \text{Ash weight (g)} \quad (1)$$

$$\text{Conversion } (x) = \frac{\text{VS digested}}{\text{VS fed}} \text{ (g/g)} \quad (2)$$

$$\text{Yield } (y) = \frac{\text{Total carboxylic acids produced}}{\text{VS fed}} \text{ (g/g)} \quad (3)$$

$$\text{Total acid productivity } (p) = \frac{\text{Total carboxylic acids produced}}{\text{Total liquid volume in all fermentors} \cdot \text{time}} \text{ [g/(L·d)]} \quad (4)$$

$$\text{Total acid selectivity} = \frac{\text{Total carboxylic acids produced}}{\text{VS digested}} \text{ (g/g)} \quad (5)$$

$$\text{Liquid residence time (LRT)} = \frac{\text{Total liquid in all fermentors}}{\text{Flow rate of liquid out of the fermentor train}} \text{ (d)} \quad (6)$$

$$\text{Volatile solids loading rate (VSLR)} = \frac{\text{VS fed to the system}}{\text{Total liquid in all fermentors} \cdot \text{time}} \text{ [g/(L·d)]} \quad (7)$$

Countercurrent fermentation requires an extended time to reach steady state (~4 mo). Because of the long residence time, it would be very time-consuming and cost-ineffective to explore a wide variety of operating conditions. To overcome this, Loescher developed the Continuum Particle Distribution Model (CPDM), a mathematical model that predicts the total acid concentration and substrate conversion using batch fermentation data *(14)*. CPDM can save considerable time in determining the optimum operating conditions. The conversion penalty *(15)* was used in CPDM to account for the fact that biomass reactivity reduces at longer residence times because easy-to-digest portions react first.

Nutrients are required to enhance microorganism growth. Rice straw is rich in carbohydrates but low in nutrients. In contrast, chicken manure is rich in nutrients such as nitrogen, vitamins, and minerals. In this study, rice straw (80%) was used as a carbohydrate-rich source whereas chicken manure (20%) was used as a nutrient-rich source. Marine inoculum was used as a source of microorganisms. The volatile solid loading rates (VSLR) and liquid residence times (LRT) were varied to determine their effect on acid concentration, yield, conversion, and total acid productivity. CPDM was used to predict the acid concentrations and conversions, and the results were compared with experimental data.

Materials and Methods

Substrates

Rice straw (RS) was obtained from Lee Tarpley of the Texas A&M Agricultural Research and Extension Center. The substrate was milled in a

Thomas Wiley Laboratory mill and was passed through a 2-mm screen. Rice straw was pretreated with 0.1 g $Ca(OH)_2$/g dry biomass at 100°C for 1 h. The average moisture content of the pretreated RS was 0.028 g water/g raw RS, the average ash content was 0.274 g ash/g dry RS, and the VS was 0.726 g VS/g dry RS. Chicken manure was obtained from the Poultry Science Center, Texas A&M University, College Station, Texas. The manure was air dried and then pretreated with 0.1 g $Ca(OH)_2$/g dry biomass at 100°C for 1 h. The average moisture content of pretreated chicken manure was 0.052 g water/g raw chicken manure, the average ash content was 0.34 g ash/g dry chicken manure, and the volatile solids was 0.660 g VS/g dry chicken manure. Pretreated rice straw (80%) and pretreated chicken manure (20%) were used as substrates in all experiments.

Media and Nutrient

The liquid medium was deoxygenated water prepared by boiling distilled water under nitrogen purge for 5 min. After cooling the media to room temperature, 0.275 g/L sodium sulfide and 0.275 g/L cysteine hydrochloride were added under continuous nitrogen purge. Sodium sulfide and cysteine hydrochloride were added to further reduce the oxygen content of the media.

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) *(8)*. The dry nutrients were added during the fermentation.

Inoculum

Marine inoculum was used from a previous fermentation of sugarcane bagasse/chicken manure *(16)*. The original inoculum was previously collected from the sediments of three coastal swamps at Galveston, Texas. The sediment was collected from 0.5-m-deep holes and placed into bottles filled with deoxygenated media, consisting of 0.275 g/L sodium sulfide and 0.275 g/L cysteine hydrochloride.

Inhibitor

Iodoform (CHI_3) solution containing 20 g CHI_3/L ethanol was used as a methanogen inhibitor in this experiment. Owing to light and air sensitivity, the solution was kept in a tinted bottle and capped immediately after use.

pH Control

Calcium carbonate ($CaCO_3$) was added as a neutralizing agent to control the pH between 5.7 and 6.4.

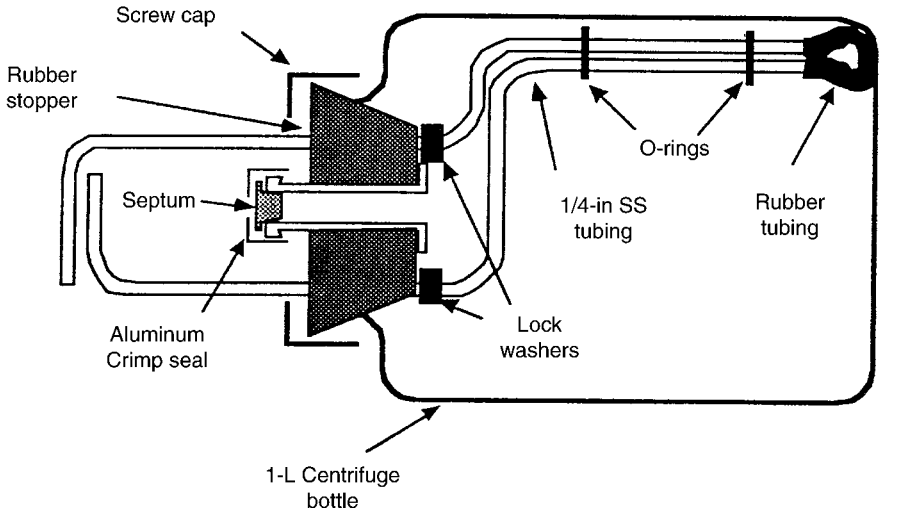

Fig. 4. Centrifuge bottle bioreactor *(8)*.

Fermentor

The fermentors (Fig. 4) were made from Beckman 1-L polypropylene centrifuge bottles (98×169 mm^2), Nalgene brand NNI 3120-1010. The bottles were closed with a size 11 rubber stopper with a hole drilled in the middle. A glass tube was inserted through the hole and capped with a rubber septum for gas sampling and release. The release of gas from the fermentors was necessary to prevent explosions because the fermentors could only withstand a pressure of 2 atm. The rubber septum was replaced when there was a visible hole owing to frequent gas venting. Two 0.25-in. stainless steel tubes with welded ends were also inserted into holes in the stopper. The tubes were used as stirrers to mix the components inside the fermentors. The fermentors were placed in a Wheaton Modular Cell Production Roller Apparatus (Model III) located in a 40°C incubator and were rotated horizontally at 1 rpm.

Experimental Procedure

The fermentations were performed at 40°C. Anaerobic conditions were maintained by purging with high-pressure nitrogen whenever fermentors were opened. Four fermentors were started as a batch fermentation with 80% pretreated rice straw and 20% pretreated chicken manure, calcium carbonate, urea, dry nutrients, and deoxygenated water. To establish the culture, a batch fermentation was maintained for 10 d, then the countercurrent fermentation was started. Every 2 d, liquid/solid were transferred (Fig. 2) and 2 g of $CaCO_3$ were added to each fermentor to neutralize the carboxylic acids.

A series of six countercurrent fermentation experiments were performed at various combinations of volatile solid loading rates (VSLR) and

Table 1
Operating Parameters for Rice Straw/Chicken Manure Countercurrent Fermentation With Marine Inoculum

Fermentation trains	A	B	C	D	E	F
VSLR (g VS/[L of liquid in all fermentors·d])	6.32	3.45	6.47	9.99	5.87	8.06
LRT (days)	23.4	23.7	24.0	24.1	19.2	26.3
VS fed at each transfer (g VS)	13.9	6.96	13.9	22.2	11.1	18.1
Liquid volume in all fermentors (L)	1.1	1.01	1.08	1.11	0.944	1.12

liquid residence time (LRT). The operating parameters for the fermentation trains are shown in Table 1. On each transfer, 0.2 g of dry nutrients, 40 µL of iodoform solution (20 g/L of iodoform dissolved in ethanol), and 0.1 g of nutrients (if pH < 6.0) were added. Deoxygenated water (0.1 L) was fed to F4 on each transfer. The single-centrifuge procedure, in which liquids are transferred in a single step, was used *(9,17,18)*. After the system reached a steady state (±5 g/L average total acid concentration), fermentation data was collected for at least 10 transfers to determine acid productivity, carboxylic acid concentration, yield, selectivity, conversion, biotic CO_2 productivity, and CH_4 productivity.

Analytical Methods

The volume of gas produced during fermentation was measured using an inverted graduated glass cylinder apparatus (water displacement apparatus) that was filled with a solution of 300 g calcium chloride/L solution. A gas chromatograph (Agilent 6890 series Agilent Technologies, Palo Alto, CA) with thermal conductivity detector was used to determine the methane and CO_2 composition of the fermentation gas. Samples were taken directly from the fermentors using a 5-mL syringe. To calibrate the samples, a standard gas mixture of carbon dioxide (29.99 mol%), methane (10.06 mol%), and the balance nitrogen was used.

CO_2 produced during fermentation is the sum of biotic and abiotic CO_2. Abiotic CO_2 is produced by neutralizing the carboxylic acids with calcium carbonate and biotic CO_2 is produced from the fermentation. It is assumed that for every 2 mol of acid produced in the fermentor, 1 mol of abiotic CO_2 is produced. The biotic CO_2 produced directly from the fermentation was calculated by subtracting abiotic CO_2 from total CO_2. Only biotic CO_2 was used in the mass balance calculations.

During each transfer schedule, liquid from Fermentor 1 and solids from Fermentor 4 were collected. A liquid sample (~3 mL) was taken from Fermentor 1 and analyzed for carboxylic acid concentration. The remaining liquid collected from Fermentor 1 was analyzed for VS. Although every attempt was made to obtain solid-free liquid stream from Fermentor 1,

the liquid contains some carryover solids. The solids collected from Fermentor 4 were analyzed for undigested volatile solids. Acid analysis was performed using an Agilent 6890 gas chromatograph with capillary column (J & W Scientific, model DB-FFAP, Agilent Technologies, CA). It was operated with a flame ionization detector and an Agilent 7683 Series Injector. The oven temperature in the gas chromatograph (GC) increased from 50°C to 200°C at 20°C/min and was held an additional 1 min at 200°C. Liquid samples were mixed with 1.162 g/L of internal standard (4-methyl-n-valeric acid) and acidified with 3 *M* phosphoric acid. Volatile solids in all samples were determined by first drying the samples at 105°C and then ashing at 550°C in an oven *(18)*.

Mass balance closure on the entire system was calculated over the steady-state period.

The mass balance closure was calculated as:

$$\text{Closure} = \frac{\text{Mass out}}{\text{Mass in} + \text{Water of hydrolysis}} \ (\text{g/g}) \tag{8}$$

$$\text{Closure} = \frac{\text{Undigested VS} + \text{Dissolved VS} + \text{Acids} + \text{Biotic } CO_2 + CH_4}{\text{VS in} + \text{Water of hydrolysis}} \ (\text{g/g}) \tag{9}$$

Dissolved VS is the carryover solids in the liquid from Fermentor 1 and solubilized lignin and they were measured by drying at 105°C and then ashing at 550°C in an oven. Theoretically, the system should have a 100% closure; in practice, human errors in measurement and transfer processes caused some discrepancies. During cellulose hydrolysis, a mole of water is gained per mole of monomer resulting in mass increase. Ross *(9)* suggested that biomass could be represented as cellulose, with a monomer weight of 162 g/mol. The water of hydrolysis was calculated as:

$$\text{Water of hydrolysis} = \text{VS digested} \times \frac{18}{162} \ [\text{g}] \tag{10}$$

Continuum Particle Distribution Modeling

CPDM was used to simulate data for the countercurrent fermentation using data collected from batch fermentations. Batch experiments at varying initial substrate concentrations (20, 40, 70, 100, and 100$^+$ g dry substrate/L liquid) were used to obtain the data. 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% calcium butyrate) at a concentration of 20 g carboxylic acids/L of liquid to better simulate the high acid concentration and low conversion existing in Fermentor 1. The inoculum for batch fermentations was taken from a steady-state countercurrent

fermentation on the same substrates. Deoxygenated water was used for this fermentation and other components, such as urea (0.25 g/L), dry nutrient (0.5 g/L), and calcium carbonate (5 g/L), were added initially to the fermentors. To prevent methane production, iodoform (40 μL of iodoform solution) was added continuously. Liquid samples were taken daily from the five batches. Reaction rates at varying acid concentrations and biomass digestion were determined from the batch data.

The liquid samples were analyzed for carboxylic acid concentrations using the GC and the results were converted to acetic acid equivalent (α):

$$\alpha = \text{acetic (mol/L)} + 1.75 \times \text{propionic (mol/L)} + 2.5 \times \text{butyric (mol/L)} + 3.25 \times \text{valeric (mol/L)} + 4 \times \text{caprioc (mol/L)} + 4.75 \times \text{heptanoic (mol/L)} \text{ [mol/L]} \quad (11)$$

On mass basis, the acetic acid equivalent can be expressed as:

$$A_e = 60.05 \text{ (g/mol)} \times \alpha \text{ (mol/L) [g/L]} \quad (12)$$

Acetic acid equivalents are based on the reducing power of the acids produced from the fermentation and allows the various acid products to be expressed on a common basis *(19)*. The acetic acid equivalents (A_e) from each of the five batch experiment was fit to the equation:

$$A_e = a + \frac{bt}{1+ct} \text{ [g/L]} \quad (13)$$

where t is the time (d) of fermentation, and a, b, and c are constants fit by least squares analysis. The rate r was obtained from Eq. 14.

$$r = \frac{d(A_e)}{dt} = \frac{b}{(1+ct)^2} \text{ [g/(L·d)]} \quad (14)$$

The specific rate, $\hat{r}$ (g A_e produced/(g VS·d)) was determined from Eq. 14 by dividing it by the initial substrate concentration, S_o (g VS/L), in each of the five fermentors:

$$\hat{r} = \frac{r}{S_o} \text{ [g/(g·d)]} \quad (15)$$

The predicted rate, $\hat{r}_{pred}$, was obtained from Eq. 16, where the rate of acid production depends on volatile solids conversion (x) and product concentration (A_e):

$$\hat{r}_{pred} = \frac{e(1-x)^f}{1+g[\phi A_e]^h} \text{ [g/(g·d)]} \quad (16)$$

where, $\hat{r}_{pred}$ is the g acetic acid equivalents produced/(g VS·d); x the dimensionless; ϕ the ratio of g total acid to g acetic acid equivalents; and A_e the g acetic acid equivalent produced.

Least square analysis was used to determine the empirical parameter constants e, f, g, and h for $\hat{r}_{pred}$ (Eq. 16) from the specific rate $\hat{r}$ (Eq. 15). A_e was converted back to carboxylic acid concentration by ϕ (the ratio of total grams of actual acids to grams of A_e). The $(1 - x)$ term in the numerator of Eq. 16, is the conversion penalty function by South and Lynd *(15)*. The conversion term $(1 - x)$ in the numerator (Eq. 16) shows that as the biomass is digested, the reaction rate decreases because the less reactive components remain. The acid concentration in the denominator (A_e) shows the inhibitory effect on the microorganisms when product concentration is high.

The conversion, x was calculated using

$$x(t) = \frac{A_e(t) - A_e(t=0)}{S_o \cdot \sigma} \text{ (g/g)} \tag{17}$$

where, σ is the selectivity (g A_e produced/g VS digested); and S_o is the initial substrate concentration (g VS/L).

The selectivity σ, for Eq. 17 was calculated from the selectivity s (g total acids produced/g VS digested) determined in the countercurrent experiment.

$$s = \phi\sigma \text{ (g/g)} \tag{18}$$

Equation 16 was used in a Mathematica program *(16)* to predict acetic acid equivalent concentration (A_e) and conversion (x) for the countercurrent fermentation at various VSLR and LRT. A_e was converted back to carboxylic acid concentration by multiplying by ϕ. Other system-specific parameters needed for the Mathematica program are selectivity, holdup (ratio of liquid–solid in wet solids), and moisture (ratio of liquid–solid in feed) (*see* Table 4).

Statistical Methods

The "solver" in Microsoft Excel was used to obtain the model fit parameters in Eqs. 13 and 16. The sum of the mean-square error between experimental and predicted values was minimized to obtain the model parameters.

Results and Discussion

Countercurrent Fermentation Using Marine Inocula

The results for the countercurrent fermentation (data collected for at least 10 transfers at steady state) are shown in Table 2. The highest acid productivity of 1.69 g/(L·d) occurred at a concentration of 32.4 g/L in Fermentation Train E (LRT = 19.2 d and VSLR = 5.87 g/[L·d]). This fermentation train had the shortest LRT; Fermentation Train F (LRT = 26.3 d and VSLR = 8.06 g/[L·d]) had the longest LRT. The highest selectivity of 0.57 g total acids/g VS digested was in Fermentation Train C (LRT = 24 d and VSLR = 6.47 g/[L·d]). The acid productivity, total acid concentration, conversion, and yield depend on the VSLR and LRT.

Table 2
Results for Rice Straw/Chicken Manure Countercurrent Fermentation With Marine Inoculum

Fermentation trains	A	B	C	D	E	F
Average pH in all fermentors	6.0 ± 0.18	6.4 ± 0.37	5.7 ± 0.06	5.7 ± 0.18	5.8 ± 0.16	5.8 ± 0.17
Total acid productivity (g/[L of liquid in all fermentors·d])	1.47	1.00	1.53	1.63	1.69	1.57
Total acid concentration (g/L)	35.1 ± 1.61	25.0 ± 2.80	36.7 ± 0.85	39.8 ± 4.30	32.4 ± 3.35	40.8 ± 3.37
Acetic acid (wt%)	42.8 ± 2.21	36.1 ± 3.18	48.6 ± 1.28	48.4 ± 4.36	44.0 ± 4.72	50.9 ± 2.56
Propionic acid (wt%)	8.55 ± 2.85	21.3 ± 2.78	3.64 ± 0.18	7.32 ± 4.1	5.46 ± 2.15	8.13 ± 2.8
Butyric acid (wt%)	24.8 ± 1.55	26.7 ± 3.46	27.2 ± 0.49	25.4 ± 2.55	29.9 ± 3.54	23.9 ± 3.41
Valeric acid (wt%)	8.44 ± 1.37	8.84 ± 0.99	4.45 ± 0.45	5.17 ± 1.55	5.59 ± 1.1	4.79 ± 0.8
Caproic acid (wt%)	13.4 ± 2.65	6.11 ± 0.81	15.6 ± 0.72	13.2 ± 2.48	14.5 ± 1.76	11.9 ± 1.65
Heptanoic acid (wt%)	2.01 ± 0.33	0.95 ± 0.43	0.56 ± 0.04	0.47 ± 0.14	0.55 ± 0.11	0.41 ± 0.05
VS digested (g VS/d)	3.54	2.41	2.91	4.13	3.39	3.77
Yield (g total acid/g VS fed)	0.23	0.29	0.24	0.16	0.29	0.19
Selectivity (g total acid/g VS digested)	0.46	0.42	0.57	0.44	0.47	0.47
Conversion (g VS digested/g VS fed)	0.51	0.69	0.42	0.38	0.61	0.42
Biotic CO_2 productivity (g CO_2/[L of liquid in all fermentors·d])	0.43	0.61	0.34	0.17	0.22	0.25
CH_4 productivity (g CH_4/[L of liquid in all fermentors·d])	0.003	0.043	0.015	0.001	0.001	0.001
Mass balance closure (g VS out/g VS in)	1.19	0.93	1.08	0.96	1.15	1.16

All errors are ±1 standard deviation.

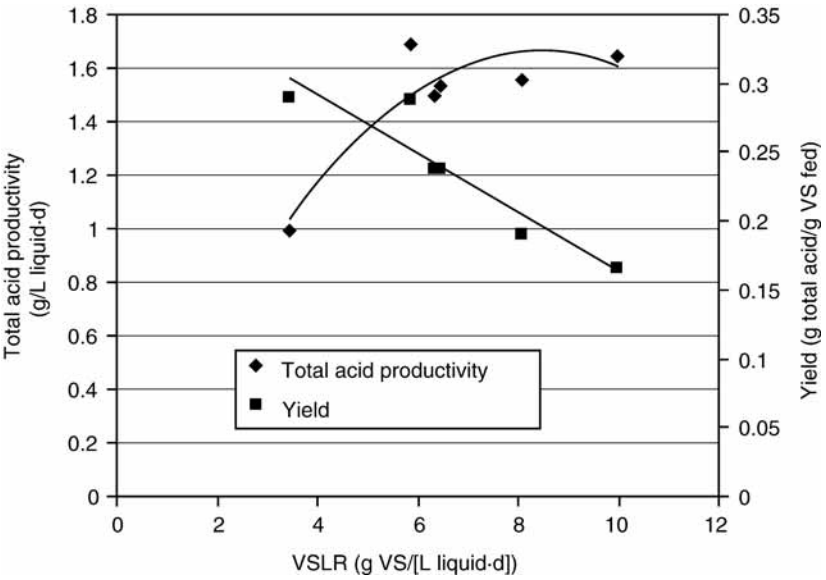

Fig. 5. Correlation of total acid productivity and yield with volatile solid loading rate.

The highest acid concentrations 40.8 g/L (Train F), and 39.8 g/L (Train D) were obtained at high VSLR of 8.06 g/(L·d) and 9.99 g/(L·d), for Trains F and D, respectively. This is because at high VSLR, the microorganisms have more substrates available for acid production. However, the conversion for Trains F and D were 0.42 g/g and 0.38 g/g, respectively, which are low. The low conversions resulted because the VSLR was so high that there was insufficient time to digest all of the solids entering the fermentors.

Fermentation Train B (LRT = 23.7 d and VSLR = 3.45 g/[L·d]), had the lowest total acid concentration of 25 g/L and the highest conversion (0.69 g VS digested/g VS fed) and yield (0.29 g total acids/g VS fed). At low VSLR, less substrate is available to the microorganisms and therefore the total acid concentration is low. However, the conversion is high because the microorganisms do not only digest the easily digestible fractions, but also the difficult portions of the biomass. The MixAlco process requires the selection of VSLR and LRT that is optimum for both high total acid concentration and conversion.

Correlation for Productivity, Selectivity, Conversion, and Yield

The correlations between VSLR and total acid productivity (p), and yield (y) are shown in Fig. 5. The data for the six fermentation trains were fit with a second order polynomial and linear regression and the following correlations were obtained:

$$p = -0.0255\ \mathrm{VSLR}^2 + 0.4301\ \mathrm{VSLR} - 0.1456 \quad (19)$$

$$y = -0.0213\ \mathrm{VSLR} + 0.3763 \quad (20)$$

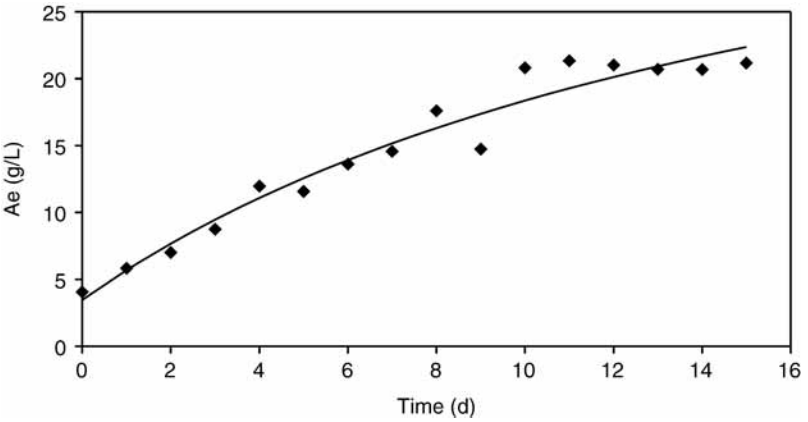

Fig. 6. Acetic acid equivalent of rice straw/chicken manure batch fermentor (70 g dry substrate/L liquid).

From Fig. 5, the acid productivity (p) increases from 1 g/(L·d) (VSLR = 3.45 g/[L·d]) to 1.6 g/(L·d) (VSLR = 6 g/[L·d]) with no apparent increase in acid productivity after VSLR of 6 g/(L·d). Selectivity (s) is essentially constant and does not depend on VSLR (Table 2). From Fig. 5, yield (y) decrease as VSLR increases.

At a high VSLR, the microorganisms digest more biomass leading to a high acid productivity; however, yield, and conversion are lower because only a small fraction of the total biomass fed is digested. On the other hand, at low VSLR, the microorganisms are limited by the amount of digestible biomass available. Therefore, the acid productivity is low but yield, and conversion are higher because the microorganisms consume both the reactive and recalcitrant biomass components.

CPDM Verification

Batch fermentations at different initial substrate concentrations were performed. Figure 6 shows the acetic acid equivalent profile in 70 g dry substrate/L liquid. The values for a, b, and c at the different initial substrate concentrations are shown in Table 3. The values of e, f, g, h, and other parameter constants used in the Mathematica program are shown in Table 4. The rate equation obtained for 80% rice straw/20% chicken manure fermentation with marine inocula is:

$$\hat{r}_{\text{pred}} = \frac{1.06(1-x)^{3.18}}{1+3.00[\phi A_e]^{0.917}} \tag{21}$$

Table 5 compares the experimental total carboxylic acid concentration and conversion to the CPDM predictions. The average error between the experimental and predicted total acid concentrations is 6.41%, and the average error between the experimental and predicted conversion is 6.15%. The average error in conversion is very similar to results from corn

Table 3
The Value of *a*, *b*, *c* in CPDM for Rice Straw/Chicken Manure Fermentation

Initial substrate concentration (g/L)	*a* (g/L liquid)	*b* (g/[L liquid·d])	*c* (/d)
20	3.44	1.33	0.061
40	2.49	1.83	0.067
70	3.47	2.34	0.057
100	4.51	2.04	0.023
100^{+}	16.48	3.83	0.148

Table 4
Parameter Constant Values in CPDM for Rice Straw/Chicken Manure Fermentation

Parameter constant	Values
Hold up (g liquid/g VS wet cake)	4.4
Moisture (g liquid/g wet solid)	0.051
Selectivity (g Ae/g VS digested)	0.497
ϕ (g total acid/g Ae)	0.704
Liquid volume (L)	0.269
e (g Ae/g VS·d)	1.06
f (dimensionless)	3.18
g (L/g total acid)$^{1/h}$	3
h (dimensionless)	0.917

stover fermentation *(20)*, when the selectivity was maintained as a constant. This shows that a constant selectivity in the fermentation improves the predictability of the CPDM model. The highest error in total acid total carboxylic acids is 16.2% and the highest error in conversion is 23.8%. Further improvements in the CPDM can be made by making replicate measurements of the CPDM batch data so as to obtain a more accurate rate equation.

Continuum Particle Distribution Modeling

Figure 7 shows the CPDM "map" at the average solid concentrations used in this study (129.5 g VS/L of liquid). At high VSLR and LRT, the total acid concentration is high but the conversion is low. This is because the microorganisms have more substrate and the liquids have a high residence time. The low conversions at high VSLR are because only the easily digestible portions of the biomass are consumed, leaving a high fraction of undigested substrate. At low VSLR and LRT, the microorganisms have less substrate to digest and the liquids have a short residence time. Conversion is higher at low VSLR because the microorganisms digest both the easily digestible and recalcitrant portions of the biomass. Both high product

Table 5
Comparison of Experimental and Predicted Carboxylic Acid Concentration and Substrate Conversion for Rice Straw/Chicken Manure Fermentation

Fermentation trains	A	B	C	D	E	F	Average (%)
Experimental carboxylic acid concentration (g/L)	35.1 ± 1.6	25 ± 2.8	36.7 ± 0.85	39.8 ± 4.3	32.4 ± 3.4	40.8 ± 3.4	
Predicted carboxylic acid concentration (g/L) (CPDM)	35.1	29.06	35.7	36.9	30.6	38.26	
Error[a] (%)	0	16.24	2.72	7.29	5.55	6.64	6.41
Experimental conversion	0.52	0.69	0.42	0.38	0.61	0.42	
Predicted conversion (CPDM)	0.52	0.73	0.52	0.38	0.58	0.43	
Error[a] (%)	0	5.8	23.8	0	4.92	2.38	6.15

All errors are ±1 standard deviation.
[a]Error = (Predicted – Experimental) × 100/Experimental.

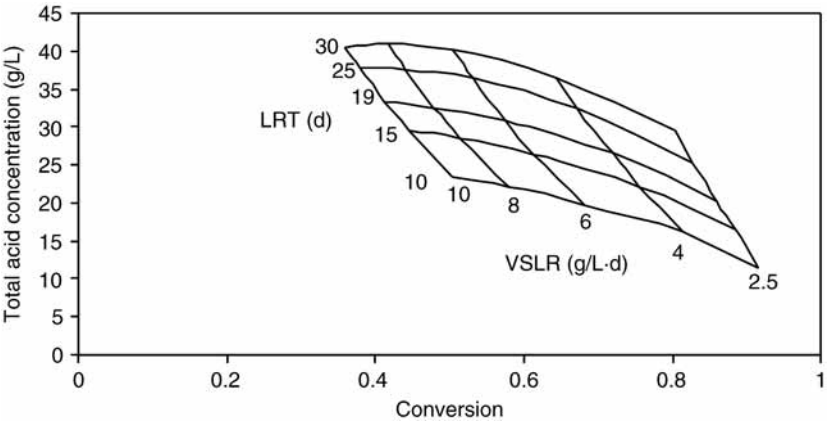

Fig. 7. The CPDM "map" for rice straw/chicken manure countercurrent fermentation (129.5 g VS/L of liquid).

concentrations and conversions are required in the application of this technology. The "map" (Fig. 7) predicts a total acid concentration of 40 g/L at LRT of 30 d, VSLR of 8 g/(L·d) and a conversion of 42%. At a VSLR of 2.5 g/(L(d) and LRT of 30 d, a total acid concentration of 30 g/L could be obtained at 80% conversion. From the map, VSLR of 2.5 g/(L·d) and LRT of 30 d will fit the requirement for both high product concentration and conversion. CPDM helped to determine this operating point, which would have been difficult without the use of the model. Further experimental work is underway to look at the effect of long residence times on total acid concentrations and conversion.

Conclusions

Fermentation results were obtained using a mixed culture of marine microorganisms for fermentation of rice straw (80%) and chicken manure (20%) at various VSLR and LRT. The highest acid concentrations were obtained at high VSLR but at the cost of low conversion. At low VSLR, the total acid concentration was low but the conversion was high. The fermentation had the highest acid productivity of 1.69 g/(L·d) at a total acid concentration of 32.4 g/L. The highest conversion (0.69 g VS digested/g VS fed) and yield (0.29 g total acids/g VS fed) occured at a total acid concentration of 25 g/L. Because it is impossible to experimentally explore all possible VSLR and LRT combinations to obtain both high product concentration and conversion, CPDM was applied. The CPDM predicted the experimental total acid concentration and conversion at an average error of 6.41% and 6.15% respectively. The model was used to predict product concentrations and conversions at other VSLR and LRT. The model predicts that, at a solids concentration of 129.5 g VS/L of liquid, a VSLR of 2.5 g/(L·d),

and LRT of 30 d, a total acid concentration of 30 g/L can be obtained at 80% conversion.

Nomenclature

A_e	acetic acid equivalent concentration (g acetic acid equivalents/L)
a	parameter constant (g acetic acid equivalents/L)
b	parameter constant (g acetic acid equivalents/[L·d])
c	parameter constant (/d)
e	parameter constant (g acetic acid equivalent/[g VS·d])
f	parameter constant (dimensionless)
g	parameter constant (L/g total acid)$^{1/h}$
h	parameter constant (dimensionless)
LRT	liquid residence time (d)
p	total acid productivity (g total acid/[L·d])
r	reaction rate (g acetic acid equivalents/[L·d])
$\hat{r}$	specific rate (g acetic acid equivalents produced/[g VS·d])
$\hat{r}_{pred}$	predicted specific rate (g acetic acid equivalents produced/[g VS·d])
S_o	initial substrate concentration (g VS/L)
s	selectivity (g total acid produced/g VS digested)
t	time (d)
VSLR	volatile solid loading rate (g VS/[L·d])
x	conversion (g VS digested/g VS fed)
α	acetic acid equivalent concentration (mol acetic acid equivalents/L)
ϕ	ratio (g total acid/g acetic acid equivalents)
σ	selectivity (g acetic equivalents produced/g VS digested)

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