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Laboratory Method for High-Solids Countercurrent Fermentations

M. Kyle Ross and Mark T. Holtzapple*

Department of Chemical Engineering, Texas A&M University, College Station, TX 77843-3122, E-mail: m-holtzapple@tamu.edu

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Abstract

Equipment and procedures were developed to study the conversion of lignocellulosic biomass to carboxylic acids using high-solids countercurrent fermentations. Countercurrent fermentations of cattle manure yielded a rapid fermentation (maximum 2.98 g of total acid/[L·d]) with high acid concentrations (maximum of 32.5 g of total acid/L), but the acid yield tended to be low (maximum of 0.24 g of total acid/g of volatile solids). Countercurrent fermentations of a mixture of 80% municipal solid waste/20% sewage sludge fermented more slowly (maximum of 1.98 g of total acid/[L·d]) with a lower acid concentration (maximum of 26.5 g of total acids/L), but higher acid yields were achieved (maximum of 0.34 g of total acid/g of volatile solids).

Index Entries: Fermentation; countercurrent; carboxylic acids; volatile fatty acids; MixAlco process; high solids.

Introduction

Cheremisinoff and Ellerbusch *(1)* have summarized waste biomass production in the United States. During the past 30 yr, various ideas have emerged on how to obtain useful products from waste biomass. Sterzinger *(2)* discussed gassifying biomass and then combusting the biogas in a turbine to produce electricity. Simultaneous saccharification and fermentation is a method for producing ethanol from biomass *(3)*. Methane production from the anaerobic fermentation of agricultural residues *(2)*, municipal solid wastes (MSW) *(4)*, and sewage sludge *(5)* has been extensively studied.

Recently, Holtzapple et al. *(6,7)* have described the MixAlco Process for converting waste biomass to mixed alcohol fuels. In the MixAlco Process, the waste biomass is treated with lime to increase digestibility. Then

*Author to whom all correspondence and reprint requests should be addressed.

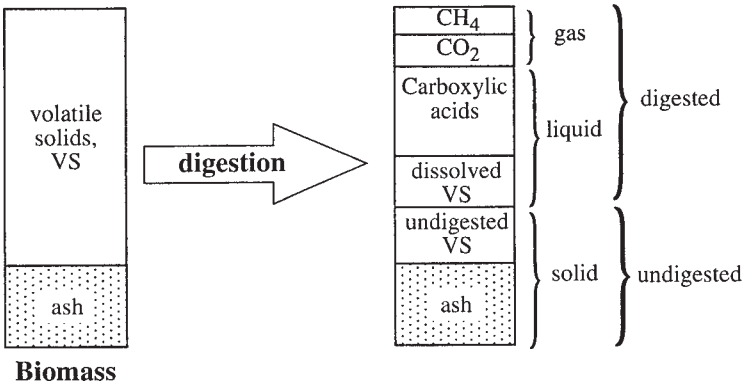

Fig. 1. Definition of terms.

the lime-treated biomass is fed to a mixed-acid fermentation where carboxylic acids are produced. The carboxylic acids are neutralized with calcium carbonate to form calcium carboxylate salts that are concentrated to dryness and thermally converted to ketones. Finally, the ketones are hydrogenated to mixed alcohols. The focus of the present article is on the mixed-acid fermentation that supports the MixAlco Process.

The organic matter in biomass is approximated as volatile solids (VS), the fraction of the biomass that volatilizes on ashing. As shown in Fig. 1, as the biomass digests, VS are converted into gaseous and liquid products (e.g., carboxylic acids, extracellular proteins, storage polysaccharides). Figure 1 and Eqs. 1–4 define terms used throughout this article.

Yield is defined as

$$\text{yield} \equiv \text{total acids produced/VS fed} \tag{1}$$

Conversion is defined as

$$\text{conversion} \equiv \text{VS digested/VS fed} \tag{2}$$

Total acid selectivity is defined as

$$\text{total acid selectivity} \equiv \text{total acids produced/VS digested} \tag{3}$$

Total acid productivity is defined as

$$\text{total acid productivity} \equiv \text{total acids produced/(L of liquid in all reactors} \cdot \text{time)} \tag{4}$$

Playne *(8)* studied carboxylic acid production from bagasse by nonsterile, mixed-culture fermentation. In continuous culture, he achieved a total acid productivity of 4.9 g of total acid/(L of liquid·d) at a concentration of 15.5 g of total acid/L (9.5 g of acetic acid/L), a pH of 6.8, and a yield of 0.25 g of total acid/g of bagasse fed. To compete with traditional technology, he concluded that four improvements would be required: higher fermentor productivity, higher product concentration, higher yield, and better tolerance of low pH (<5.5). Although low pH severely inhibits the fermen-

tation, Playne *(8)* required low pH because his recovery technology required the acid to be in the protonated form. (Note that the MixAlco Process has a proprietary recovery technology that overcomes this obstacle.)

Playne's *(8)* remaining three requirements—high fermentor productivity, high product concentration, and high yield—are thought to be mutually exclusive *(9)*. High product concentrations inhibit cellulose-degrading cultures, and as biomass digests, it becomes less reactive. Both of these effects lower productivity. Using alfalfa, Playne *(8)* showed that a product concentration of 48 g of acetate/L reduced productivity by 82% compared with 9.6 g of acetate/L. Matei and Playne *(10)* showed that at low acid concentrations, high conversions can be obtained with long residence times, but the productivity was low.

To increase rates and conversion, biomass can be made more easily digestible. Rapier *(11)* lime-treated municipal solid waste with $Ca(OH)_2$, which increased the rate and extent of *in situ* digestion by 27%.

Much research has been conducted to increase the volumetric productivity of methane-producing digesters. Because carboxylic acids are intermediates in methane fermentation, much of that research applies to carboxylic acid fermentations. It has been proposed that methane productivity is proportional to the solids concentration *(12)*, which is logical because cellulose hydrolysis is usually rate limiting *(13)*. De Baere et al. *(4)* increased the solids concentration in a methanogenic digester and increased the volumetric productivity. A problem with these "drier" fermentations is that conventional mixing systems are inadequate; incomplete dispersion of intermediates and microorganisms slows the reaction *(12)*. Conventional mixing systems normally employ shakers (laboratory scale) or impellers (industrial scale). Although these conventional systems produce very rapid and complete mixing for very fluid systems, they perform poorly in slurries above 5–10% total solids.

Our analysis of Wujcik and Jewell's data *(14)* shows that in a trickle-bed fermentor with leachate recycle, methane productivity was proportional to solids concentration (up to 20% solids). Mixing in a trickle bed is slow, but Wujcik and Jewell's residence time was weeks or months, so rapid mixing was not necessary. In general, for solid-substrate fermentations, thorough—but not necessarily rapid—mixing is needed. This article describes a laboratory-scale fermentor that thoroughly mixes high-solids (>15 dry wt%) fermentations.

As biomass digests, the remaining fraction is less reactive *(13)*, which is logical because it is heterogeneous. Biomass contains solubles, cellulose, hemicellulose, lignin, ash, and so forth *(15)*; some components are highly digestible whereas others are inert. The solubles rapidly ferment and do not remain in the solid residue, whereas the intractable components (e.g., lignin) concentrate in the solid residue. In a conventional batch or continuous stirred-tank reactor (CSTR) fermentor, the biomass and liquid enter together. The easily fermented solubles quickly ferment to carboxylic acids

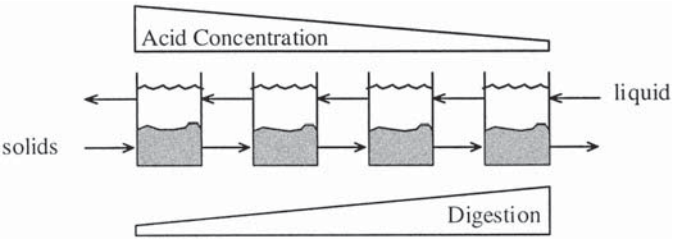

Fig. 2. Countercurrent biomass fermentation.

at a relatively low acid concentration, leaving the more recalcitrant components; to attain a high final product concentration and high conversion, the remaining recalcitrant components must ferment for an extremely long time.

To obtain both high conversions and high product concentrations, a countercurrent fermentation may be employed (Fig. 2). The most digested biomass contacts the lowest acid concentration, allowing for more complete digestion. The freshest biomass contacts the highest acid concentration, providing the best chance for further acid production.

Materials and Methods

High Solids Fermentors

Our development of a laboratory fermentor that provides adequate mixing of a high-solids countercurrent fermentation was largely a trial-and-error process that moved from the complex to the simple. Two designs that were used in the experiments are described next.

Centrifuge Bottle Fermentor I

The Centrifuge Bottle Fermentor I (CBF I) is described in Fig. 3. The 1-L centrifuge bottle (no. #355676; Beckman) was placed horizontally in a Wheaton® modular cell culture roller bottle apparatus with multiple decks of parallel rollers that rotated the bottles at approx 2.0 rpm. A hole was cut in the centrifuge bottle cap to allow the exhaust tube to pass through. The cap retained a no. 11-1/2 EPDM stopper modified to contain a lip. The lip was formed by placing the stopper in a lathe and cutting away rubber with an Exacto knife mounted as the cutting tool. Two pieces of 1/4-in. stainless steel tubing were welded together and inserted into holes in the stopper to form a stir bar. As the bottle rotated, the stir bar passed through the biomass and turned it over. The rotating seal on the exhaust tube consisted of two $1/4 \times 3/8 \times 1/2$ in. (id × od × length) nylon bushings that sandwiched an EPDM $1/4 \times 3/8$ in. O-ring. The exhaust tube remained vertical using a fixed external support and was connected to a gas collection manifold. To ensure a tight seal, the holes in the stopper were undersized as much as possible.

The use of centrifuge bottles significantly reduced the time required to operate the countercurrent fermentation. The countercurrent transfer pro-

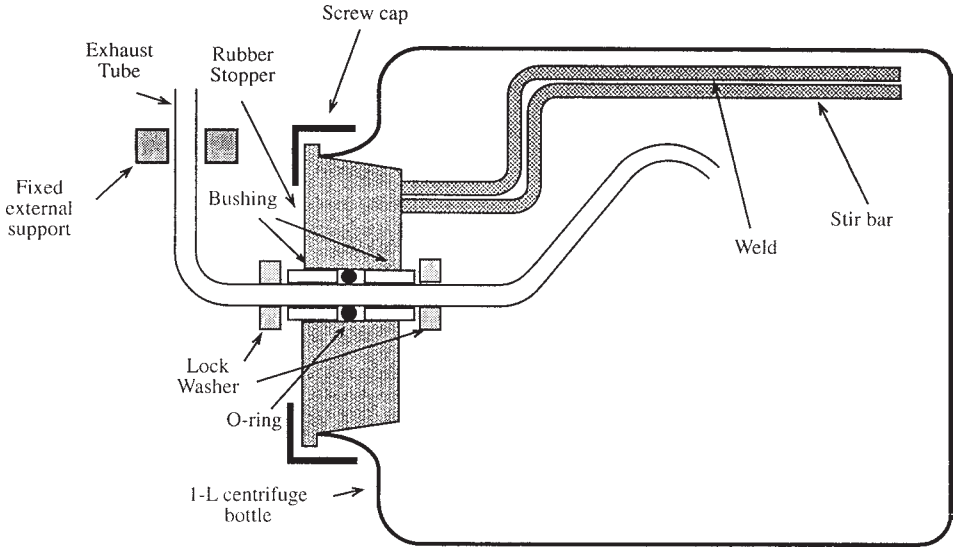

Fig. 3. Centrifuge Bottle Fermentor I.

cedure involved removing the stopper from the bottle, capping it, and centrifuging the fermentor contents to separate liquid from the solids; solids were transferred in one direction and liquid in the other.

Two problems arose with this apparatus. The rotating seal around the exhaust tube in the stopper failed within 2 mo. Also, the exhaust tube would often become plugged with biomass. Both of these problems did not allow gas production measurements.

Centrifuge Bottle Fermentor II

To address the problems with CBF I, Centrifuge Bottle Fermentor II (CBF II) was developed (Fig. 4). It also used a centrifuge bottle for the fermentor body, but the exhaust tube was replaced with a septum. Rather than continuously collecting the evolved gasses, the gasses were contained within CBF II until measured. A syringe needle connected to a gas collection device was inserted through the septum to measure the evolved gasses, which alleviated problems of leaking rotary seals. The only maintenance required was to replace the septum after about a hundred 22-gage needle insertions. The 18-mm od glass tube that held the septum was a modified aluminum crimp seal tube (no. 2048-00150; Bellco) that was originally designed for studying methanogenic bacteria by Balch and Wolfe *(16)*. The tube was cut 5.0 cm long and the bottom was flared to approx 30 mm in diameter.

The stir bar in CBF II consisted of two pieces of 1/4-in. stainless tubing inserted through the no. 11 stopper to form a handle to assist in pulling the stopper out of the bottle. No modifications were made to the stopper other than to drill the holes for the crimp seal tube and the stainless steel tubing. Rubber O-rings held the pieces of tubing together inside the fermentor.

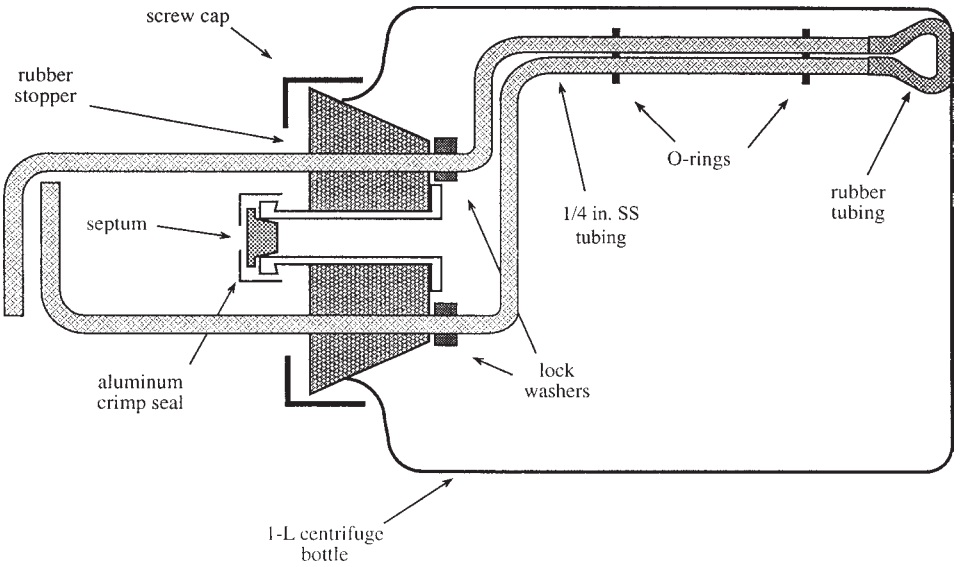

Fig. 4. Centrifuge Bottle Fermentor II.

For sealing purposes, a short piece of rubber tubing was placed over both ends of the stainless steel tubing. In retrospect, it would have been better to weld the ends closed.

The only problem experienced with CBF II fermentors was that pressures greater than approx 2 atm absolute tended to permanently deform the bottles so that they would not fit in the centrifuge. Centrifuge bottles made by Beckman (no. 355676) tended to have heavier walls and resisted deformation much better than any other brand used, so they are recommended.

Substrates

Two different substrate formulations were tested in this research: feedlot manure and a mixture of MSW and municipal SS. Feedlot manure from cattle fed a finishing ration (60% corn, 12% crude protein, 28% cottonseed hull) was obtained in two batches from the Texas A&M University Research Center in MacGregor, TX. The manure was collected within 1 to 2 h after elimination. It was quickly transported to a vented drying oven where it dried for 2 d at 105°C. Once dried, it could be stored indefinitely. MSW was combined with SS in an 80:20 ratio by weight. The MSW was prepared according to the composition described by Holtzapple et al. *(17)* with the fats and oils omitted to prevent spoilage. The components were dried, combined, and then ground in a hammer mill (Forest Science Research Lab, Texas A&M University) fitted with a 3-mm screen. SS was obtained from Bryan Wastewater Treatment Plant Number 3 (Bryan, TX). The sewage had undergone activated sludge treatment for approx 15 d and then was sent to an anaerobic digester for approx 40 d. On removal from the digester, a

cationic polymer was added to coagulate the sludge. The sludge was dried for 10 d and ground in a hammer mill fitted with a 3-mm screen.

Both the MSW/SS and the manure were pretreated with lime as described by Rapier *(11)*. For the manure, 0.05 g of $Ca(OH)_2$ and 5 g of distilled water were added per 1 g of dry manure. For MSW, which tended to absorb much more liquid than manure, 0.1 g of $Ca(OH)_2$ and 10 g of distilled water were added per gram of dry MSW/SS. The substrates were well mixed with the lime and water and then autoclaved for 1 h at 121°C. The substrates were allowed to cool, and the lime was neutralized by bubbling CO_2 through the mixture until the pH was below 7.0.

Media and Nutrients

Deaerated water was prepared by boiling distilled water for 5 min under continuous N_2 purge. The deaerated water was then allowed to cool under N_2 purge to room temperature. Deoxygenated water contained (g/L of deaerated water): cysteine-HCl (0.275) and $Na_2S{\cdot}9H_2O$ (0.275). Modified Caldwell and Bryant (C&B) medium contained (mL/L of deoxygenated water) hemin solution (10), Mineral 1 solution (38), Mineral 2 solution (38), vitamin solution (10), and Pfennig's heavy metal solution (10). The preparation of these solutions is described by Loescher *(9)*.

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). The proportions of the mixture corresponded to that of modified C&B medium.

pH Control

To control the pH between 5.8 and 6.3, $CaCO_3$ was added in excess to the fermentors.

Batch Fermentations

For both substrates, batch fermentations at 40°C were performed at varying initial substrate concentrations. The manure fermentation was initiated under anaerobic conditions by adding substrate to rumen fluid in one of the CBF I fermentors. The MSW/SS fermentation was initiated in an identical manner, except that there was an additional 0.2 g of dry nutrient mixture/L of liquid. The rumen fluid was collected from a forage-fed fistulated steer. After collection, the rumen fluid was centrifuged at 300*g* for 5 min to separate plant material from the fluid. The supernatant was then harvested and used in the experiments. A volume of the supernatant was also incubated to determine the acid produced from the residual plant matter in the supernatant. (This residual acid production has been deleted in the reported results.)

Table 1
Operating Parameters for MSW/SS Countercurrent Fermentation Experiments

	Fermentation		
	A	B	C
Temperature (°C)	40	40	40
No. of fermentors	4	4	4
Liquid residence time (d)	13.4	14.7	19.3
SLR (g VS/[L of liquid·d])	6.8	6.8	3.3
Fermentor 1 solids conc. (g VS/L of liquid)[a]	95	94	97
Fermentors 2–4 solids conc. (g VS/L of liquid)[a]	131	125	134
VS/liquid feed ratio (g/g)	0.070	0.070	0.052
Fermentor 1 retained weight (wet g)[b]	192	192	228
Fermentors 2–4 retained weight (wet g)[b]	212	212	228
Fermentor design	CBF I	CBF II	CBF II
Liquid feed at each transfer period (mL)	200	200	200
Solid feed at each transfer period (g dry wt)	20	20	15
Frequency of transfer procedure	Every 2 d	Every 2 d	Every 3 d
Liquid medium	[c]	[g]	[g]
Nutrients	[c,d,e]	[d]	[d,e]
Centrifuge procedure	Double	Double	Double
Centrifuge conditions	[f]	[f]	[f]

[a]The double centrifuge procedure increased the solids concentration in Fermentors 2–4 relative to Fermentor 1.
[b]Net weight of fermentor contents.
[c]Modified C&B medium.
[d]To each fermentor 0.15 g of urea was added at each transfer period.
[e]To each fermentor 0.2 g of dry nutrients was added at each transfer period.
[f]Fermentors 1 and 2 at 5040*g* for 20 min, 3 and 4 at 3500*g* for 20 min.
[g]Deoxygenated water.

The volume of liquid in all experiments was 300 mL. For MSW/SS, the initial VS concentrations were 35, 62, and 88 g/L of liquid; and for manure, 42, 75, and 105 g/L of liquid. Samples were collected by removing the fermentor from the incubator, opening the fermentor, collecting a 3-mL liquid sample while purging the fermentor with N_2, resealing the fermentor, and placing it back in the roller apparatus. The N_2 was industrial-grade N_2 from a 1585-kPa (abs.) liquid N_2 cylinder (Praxair; Bryan, TX).

Countercurrent Fermentations

Countercurrent fermentations at 40°C for both manure and MSW/SS were conducted at varying liquid residence times (LRTs) and solids loading rates (SLRs), as described in Tables 1 and 2. Fermentations were initiated under anaerobic conditions by adding substrate, nutrients, and rumen fluid to deoxygenated water in high-solids fermentors. The fermentors were

Table 2
Operating Parameters for Countercurrent Manure Fermentations

	Fermentation		
	D	E	F
Temperature (°C)	40	40	40
No. of fermentors	4	4	4
Liquid residence time (d)	8.3	13.1	12.3
SLR (g VS/[L liquid·d])	14.7	14.4	4.3
Fermentor 1 solids conc. (g VS/L liquid)[a]	123	178	123
Fermentors 2–4 solids conc. (g VS/L liquid)[a]	123	178	178
VS/liquid feed ratio (g/g)	0.091	0.122	0.046
Fermentor 1 retained weight (wet g)[b]	198	198	228
Fermentors 2–4 retained weight (wet g)[b]	228	228	228
Fermentor design	CBF I	CBF II	CBF II
Liquid feed at each transfer period (mL)	200	100	200
Solid feed at each transfer period (g dry wt)	30	20	15
Frequency of transfer procedure	Daily	Daily	Every 2 d
Liquid medium	[c]	[e]	[e]
Nutrients	[c]	None	None
Iodoform addition rate (mg iodoform added to each fermentor/ L liquid fed to Fermentor 4)	0	0	2
Centrifuge procedure	Single	Single	Double
Centrifuge conditions	[d]	[d]	[d]

[a]The double centrifuge procedure increased the solids concentration in Fermentors 2–4 relative to Fermentor 1.
[b]Net weight of fermentor contents.
[c]Modified C&B medium.
[d]All at 3500*g* for 20 min.
[e]Deoxygenated water.

then incubated in batch-mode for approx 1 wk to develop the anaerobic mixed culture. After the culture developed, the countercurrent transfer of liquid and substrate was begun.

All fermentations consisted of four fermentors operated countercurrently. Industrially, this would be a continuous operation, but in the laboratory, the transfer of liquid and solids occurred once every 1, 2, 3, or 4 d, as indicated in Tables 1 and 2. At each transfer session, the following steps were taken:

1. The fermentors were removed from the incubator, purged with N_2, capped with a centrifuge bottle cap, and centrifuged to separate some broth from the solids. After centrifugation, the compacted cake tended to reabsorb the supernatant; therefore, the centrifuge bottles were inverted on removal from the centrifuge and held in that position until needed. The different substrates required different centrifugation times and forces, as noted in Tables 1 and 2.

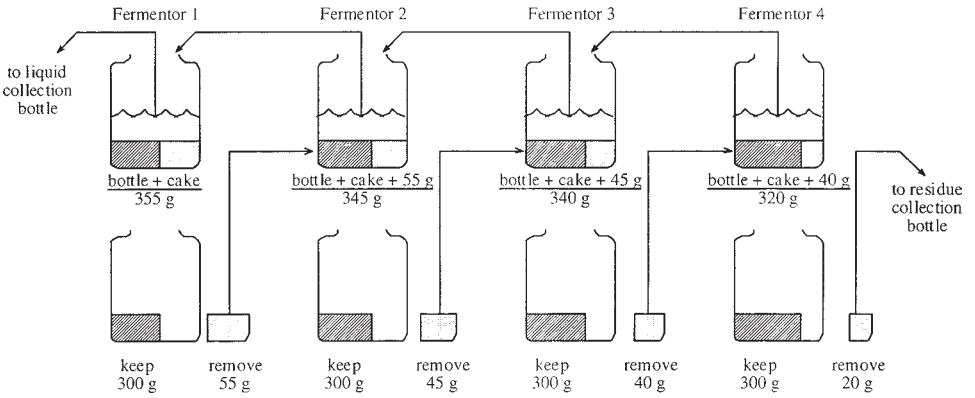

Fig. 5. Countercurrent transfer procedure.

2. When needed, 3-mL samples of the supernatant liquid were taken and stored at –18°C.
3. Solids and liquid were transferred countercurrently. The technique for solids and liquid transfer is described next and in Fig. 5 for some hypothetical conditions. Liquid and solids were transferred between fermentors under continuous nitrogen purge.
4. The supernatant liquid of Fermentor 1 was decanted into a collection bottle.
5. To achieve a pseudo–steady state, a constant wet cake weight was maintained in each fermentor. The predetermined retained weight for each fermentor in the example shown in Fig. 5 was 300 g (wet wt of cake + fermentor). The mass of the bottle + cake in Fermentor 1 was 355 g. Therefore, 55 g was removed from Fermentor 1 and set aside.
6. The liquid from Fermentor 2 was decanted into Fermentor 1.
7. The mass of the Fermentor 2 cake + bottle was 290 g. Adding the 55 g from Fermentor 1 gave a total of 345 g; thus, 45 g was removed from Fermentor 2 and set aside.
8. The 55 g removed from Fermentor 1 was added to Fermentor 2.
9. The procedure follows similarly for Fermentors 3 and 4.
10. The solids removed from Fermentor 4 were stored at –18°C in a collection bottle.
11. Fresh medium was added to Fermentor 4 and fresh solids were added to Fermentor 1. All additions of urea, dry nutrients, and $CaCO_3$ were made at this time.
12. The fermentors were closed and placed back in the incubator.

A modified "double centrifuge procedure" *(18)* allowed for slightly higher (15–20%) solids concentrations in the fermentors.

The weight of the centrifuge bottles was approx 72 g. The retained weights given in Tables 1 and 2 are net weights with the weight of the bottle subtracted. In the case of one manure fermentation, iodoform was added to inhibit methanogens (*see* ref. *18* for further details).

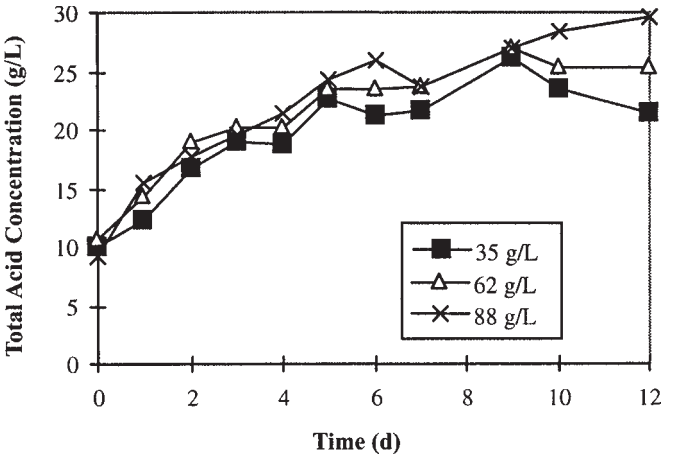

Fig. 6. Batch MSW/SS fermentations.

The fermentations achieved "steady state" when consistent acid concentrations were measured in the fermentors.

Carboxylic Acid Determinations

Liquid samples were analyzed for carboxylic acids by gas chromatography as described be Ross *(18)*.

Gas Production Measurements

Both the volume and composition of fermentor gasses were measured. The only reliable gas production measurements were taken from CBF II fermentations. As described previously, in CBF II fermentors, fermentation gasses accumulated within the fermentor. At each transfer session, the gas volume produced since the last transfer session was measured by displacing liquid in an inverted cylinder filled with an aqueous solution of 30 wt% $CaCl_2$ *(12)*. The gas composition was measured by gas chromatography *(19)*.

Results and Discussion

Figures 6 and 7 and Table 3 highlight the effect of solids concentration on fermentation rate, final product concentration, and yield in batch fermentations. As the solids concentration increased, the rate and final product concentration also increased, but at the expense of yield, indicating end-product inhibition. Attempts to simultaneously achieve high acid concentration in a single-stage CSTR would have required either low rates or low yields. Fortunately, this limitation can be overcome using countercurrent fermentation.

Tables 4 and 5 present the results of the countercurrent fermentations. The highest productivities were obtained with manure, but the highest

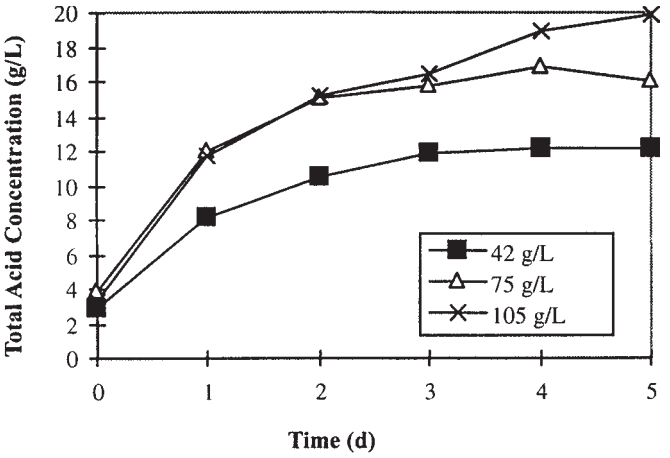

Fig. 7. Batch manure fermentations.

Table 3
Yield of Total Acid from Batch Fermentations

Substrate	Initial substrate concentration (g VS/L)	Yield (g total acid produced/g VS fed)
Manure	42	0.223
	75	0.164
	105	0.158
MSW/SS	35	0.389
	62	0.239
	88	0.219

yields were exhibited in the MSW/SS fermentations. The effect of additional nutrients is seen by comparing MSW/SS Fermentation A with B. With roughly the same SLR and LRT, Fermentation A was nearly 50% higher in productivity, yield, and acid concentration owing to the additional nutrients.

MSW/SS Fermentation C was an attempt to determine the maximum yield attainable from MSW/SS at a moderate acid concentration. Compared with Fermentation B, the SLR was halved and the LRT was extended by roughly 50%. The yield was 0.34 g of total acid/g of VS, and the conversion was 0.47 g of VS digested/g of VS fed. Compared with Fermentation B, the total acid concentration was ~20% lower and the productivity was 40% lower. The deletion of the modified C&B medium should not have affected Fermentation C because the dry nutrient mixture added corresponds to ~3 times the nutrients delivered in the C&B medium.

A mass balance was obtained for Fermentation C. For every 100 g of VS fed to the fermentor system, 34 g of carboxylic acids, 9.1 g of CO_2, and 2.3 g of CH_4 were produced, while 53 g of VS was not digested and was

Table 4
Results from MSW/SS Countercurrent Fermentations[a]

	Fermentation		
	A	B	C
pH	6.0–6.2	6.0–6.2	6.0–6.2
Total acid productivity (g total acid/[L liquid·d])	1.98 ± 0.74	1.17 ± 0.26	1.13 ± 0.14
Product total acid concentration (g total acid/L liquid)	26.5 ± 9.8	17.2 ± 3.8	21.7 ± 2.8
Product acetic acid concentration (g acetic acid/L liquid)	18.1 ± 7.3	11.9 ± 2.8	11.8 ± 2.0
Yield (g total acid/g VS fed)	0.29	0.17	0.34
Conversion (g VS digested/g VS fed)	—	—	0.47
Selectivity (g total acid/g VS digested)	—	—	0.73
CO_2 productivity (g/[L of liquid·d])	—	0.43	0.30
CH_4 productivity (g/[L of liquid·d])	—	0.082	0.077

[a]Error bands represent ± 2 SDs.

Table 5
Countercurrent Fermentation Results for Feedlot Manure[a]

	Fermentation		
	D	E	F
pH	5.7–6.0	5.7–6.0	5.7–6.0
Total acid productivity (g/[L liquid·d])	2.98 ± 0.67	2.48 ± 0.31	1.03 ± 0.28
Product total acid concentration (g/L liquid)	24.8 ± 5.6	32.5 ± 4.1	14.3 ± 3.9
Product acetic acid concentration (g/L liquid)	12.2 ± 3.0	16.5 ± 2.4	7.6 ± 2.2
Yield (g total acid/g VS fed)	0.20	0.17	0.24
Conversion (g VS digested/g VS fed)	—	—	0.34
Selectivity (g total acid/g VS digested)	—	—	0.72
CO_2 productivity (g/[L liquid·d])	—	0.58	0.13
CH_4 productivity (g/[L liquid·d])	—	0.036	0[b]

[a]Error bands represent ± 2 SDs.
[b]Iodoform was used as a methanogen inhibitor in this experiment.

recovered in the residue. (The reported CO_2 production is the total CO_2 collected minus the CO_2 produced from carboxylic acids reacting with $CaCO_3$.)

The manure fermentations were more rapid with higher final acid concentrations, but less complete. The maximum productivity for all experiments was 2.98 g/(L·d) (Manure Fermentation D), and the maximum total acid concentration was 32.5 g/L (Manure Fermentation E). Manure

Table 6
Acid Concentration in Typical MSW/SS and Manure Fermentations

	MSW/SS (B)		Manure (E)	
Fermentor	Total acid concentration (g/L)	Stage 1 total acid concentration (%)	Total acid concentration (g/L)	Stage 1 total acid concentration (%)
1	17.2	100	32.5	100
2	9.7	56	15.4	47
3	6.2	36	7.5	23
4	3.0	17	3.0	9

Table 7
Product Acid Distribution
for Typical MSW/SS and Manure Fermentations

	Wt% of total acids	
Species	MSW (A)	Manure (E)
Acetic (C2)	69.1	51.0
Propionic (C3)	18.9	6.4
Butyric (C4)	7.1	19.1
Valeric (C5)	3.4	7.8
Caproic (C6)	0.9	14.0
Heptanoic (C7)	0.4	1.8

Fermentation F was operated the same as MSW/SS Fermentation C, with a low SLR to obtain the maximum yield. With manure, the maximum yield was 0.24 g of total acid/g of VS fed as compared with 0.34 for MSW/SS. Table 6 gives the average acid concentration in each stage of typical MSW/SS and manure fermentations. The acid concentrations in Fermentors 2–4 were a smaller percentage of the final acid concentration in the manure fermentation, indicating rapid acid production in Fermentor 1. By contrast, in the MSW/SS fermentation, Fermentors 2–4 produced a greater percentage of the total acids. This pattern is consistent with the batch experiments described earlier (Figs. 6 and 7); the manure fermentations were nearly complete after roughly 3 d, whereas the MSW/SS fermentations produced acids for approximately twice as long. It appears that manure has a small fraction of components that can be rapidly fermented, but once these components are consumed, the remaining components are quite resistant.

A mass balance was obtained for Manure Fermentation F. For every 100 g of VS fed to the fermentor system, 24 g of carboxylic acids and 3.1 g of CO_2 were produced while 66 g of VS was not digested and was recovered in the residue. In this case, no methane was produced because iodoform

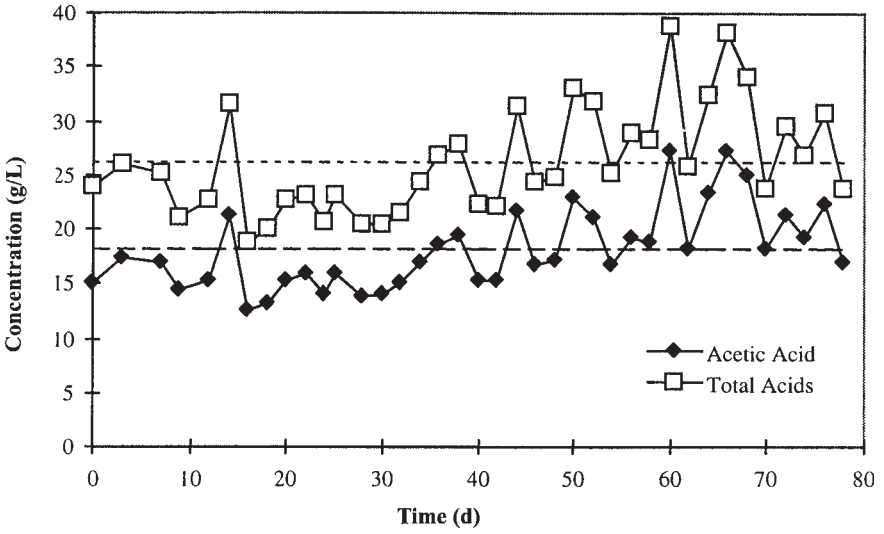

Fig. 8. Typical product concentration for MSW/SS (A).

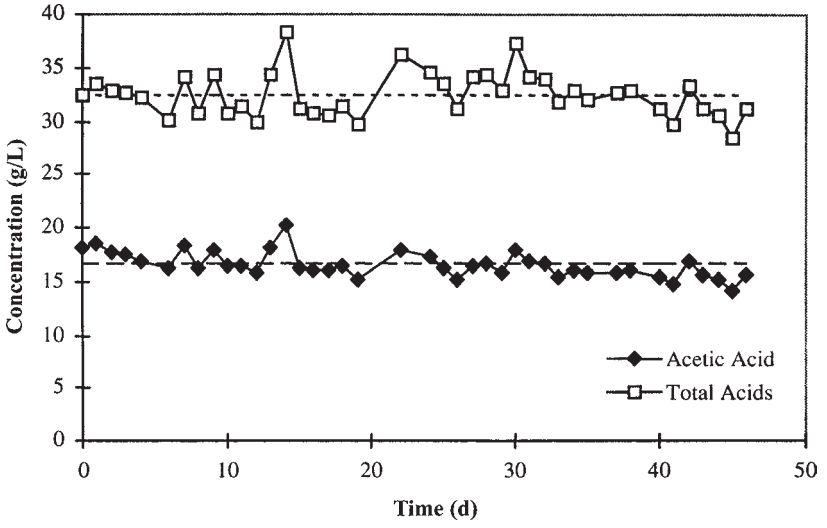

Fig. 9. Typical product concentration for manure (E).

was used as a methanogen inhibitor. Also, the MSW fermentation yielded a higher percentage of acetic acid than the manure (see Table 7).

Figures 8 and 9 show typical time variations in total and acetic acid concentrations for countercurrent MSW/SS and manure fermentations. For MSW/SS, the standard deviation was 4.9 g of total acid/L, and for manure, the standard deviation was 2.1 g of total acid/L.

Holtzapple *(7)* has conducted an economic evaluation of an industrial countercurrent biomass fermentation.

Conclusion

A laboratory procedure for simulating an industrial countercurrent operation has been described. It provides a method to evaluate possible candidate feedstocks. Of the two substrates evaluated, manure fermented faster, but approximately two-thirds of the manure could not be digested. Although the MSW/SS reacted less rapidly, it achieved higher conversion; nearly half of the material could be digested in the MSW/SS fermentation.

References

1. Cheremisinoff, N. P. and Ellerbusch, F. (1980), *Biomass: Applications, Technology, and Production*, Marcel Dekker, New York.
2. Sterzinger, G. (1995), *Technol. Rev.* **98,** 34–40.
3. Krishnan, M. S., Xia, Y., Ho, N. W. Y., and Tsao, G. T. (1997), in *Fuels and Chemicals from Biomass*, Saha, B. C. and Woodward, J., eds., ACS symposium series 666, American Chemical Society, Washington, DC, pp. 75–92.
4. De Baere, L., Verdonck, O., and Verstraete, W. (1985), *Biotechnol. Bioeng. Symp.* **15,** 321–330.
5. Ghosh, S., Conrad, J. R., and Klass, D. L. (1975), *J. Water Pollut. Control Fed.* **47,** 30–45.
6. Holtzapple, M. T., Ross, M. K., Chang, N.-S., Chang, V. S., Adelson, S. K., and Brazel, C. (1997), in *Fuels and Chemicals from Biomass*, Saha, B. C. and Woodward, J., eds., ACS symposium series 666, American Chemical Society, Washington, DC, pp. 130–142.
7. Holtzapple, M. T., Davison, R. R., Ross, M. K., Aldrett-Lee, S., Nagwani, M., Lee, C.-M., Lee, C., Adelson, S., Kaar, W., Gaskin, D., Shirage, H., Chang, N.-S., Chang, V. S., and Loescher, M. (1999), *Appl. Biochem. Biotechnol.* **77–79,** 609–631.
8. Playne, M. J. (1981), *Adv. Biotechnol.* **2,** 85–90.
9. Loescher, M. E. (1996), PhD thesis, Texas A&M University, College Station, TX.
10. Matei, C. H. and Playne, M. J., *Appl. Microbiol. Biotechnol.* **20,** 170–175.
11. Rapier, C. R. (1995), MS thesis, Texas A&M University, College Station, TX.
12. Rivard, C. J., Himmel, M. E., Vinzant, T. B., Adney, W. S., Wyman, C. E., and Grohmann, K. (1989), *Appl. Biochem. Biotechnol.* **20/21,** 461–478.
13. Gizjen, H. J., Zwart, K. B., Teunissen, M. J., and Vogels, G. D. (1988), *Biotechnol. Bioeng.* **32,** 749–755.
14. Wujcik, W. J. and Jewell, W. J. (1980), *Biotechnol. Bioeng. Symp.* **10,** 43–65.
15. Van Soest, P. J. (1982), *Nutritional Ecology of the Ruminant*, O&B Books, New York.
16. Balch, W. E. and Wolfe, R. S. (1976), *Appl. Environ. Microbial.* **32,** 781–791.
17. Holtzapple, M. T., Lundeen, J. E., Sturgiss, R., Lewis, J. E., and Dale, B. E. (1992), *Appl. Biochem. Biotechnol.* **34,** 5–21.
18. Ross, M. K. (1998), PhD thesis, Texas A&M University, College Station, TX.
19. Domke, S. (1999), PhD thesis, Texas A&M University, College Station, TX.