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Solid-State Production of Ethanol from Sorghum

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ABSTRACT

The main goal of this research is to study the solid-state fermentation of sorghum-sudangrass, Grazex II (F_1 hybrid of *Sorghum vulgare X Sorghum sudanese*), to ethanol. Our research focuses on using a modified method of ensiling to produce ethanol directly in the silo. Thirty-eight liters of ethanol/metric ton (L/MT) on a wet-weight basis were produced from sorghum receiving cellulase compared to 23.4 L/MT for sorghum not receiving cellulase additives. Based on total free sugar content, 101 and 84% of theoretical yield are achieved for cellulase-amended and nonamended sorghum, respectively.

Index Entries: Sorghum; sorghum-sudangrass; ethanol; lactic acid; solid-state fermentation.

INTRODUCTION

In the US, environmental regulations, such as the Clean Air Act of 1990, provide incentives for the use of oxygenated fuels in automobiles *(1)*. Ethanol or methyl tertiary butyl ether (MTBE) boost the oxygen content in gasoline and reduce carbon monoxide emissions. One principal advantage for using ethanol is that the fuel is produced from domestically grown renewable resources *(1–4)*. Atmospheric levels of carbon dioxide, a greenhouse gas, can be decreased by replacing fossil fuels with renewable biofuels *(4,5)*.

Typically, grains, such as barley or corn, are utilized for fuel ethanol production; however, biomass, such as sugar cane, corn stover, wood residues, and waste products, can also serve as substrates *(1,3,4)*. Sweet sorghum has been extensively studied as a substrate for fuel alcohol production for several years *(6–9)*. Sweet sorghum is one of the most promising crops that can be grown for biomass in temperate climates *(6,7)*. It is a genetically diversified and drought-tolerant plant that produces high yields of fermentable sugars and biomass, both of which can be used for ethanol production *(6–9)*. Sweet sorghum has demonstrated an ability to grow in widely diverse temperate zones in both irrigated and nonirrigated environments *(6,7)*. Gross green weight averaged 89.8 $Mg \cdot Ha^{-1}$ for irrigated sor-

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ghum compared to 65.0 $Mg \cdot Ha^{-1}$ for sorghum grown with natural rainfall *(7)*. In this study, total sugars, comprised of sucrose, glucose, and fructose, averaged about 6 $Mg \cdot Ha^{-1}$. Sugar concentrations for sucrose, glucose, and fructose averaged 5.1, 1.8, and 2.4% on a fresh wet-wt basis (dry matter content of 24%) *(7)*.

However, in order for sweet sorghum to be used successfully for ethanol production, three issues need to be addressed:

1. Carbohydrate storage;
2. Accessibility of the lignocellulosic fraction to enzymatic hydrolysis of hemicellulose and cellulose; and
3. A more economical means of producing ethanol from sweet sorghum.

Conversion economics is the key to increased use of biofuel in the open market *(10)*. For biofuels to become economical, means of lowering production costs must be found. The purpose of our research is to investigate the traditional farming method of ensiling with regard to storing the carbohydrates in sweet sorghum on a long-term basis, improving accessibility of structural carbohydrates to hydrolytic enzymes, and solid-state fermentation to ethanol in the silo.

Carbohydrate Storage

Seasonal availability and storability of sweet sorghum are important factors in the use of this renewable biomass. Sugar extraction and storability are two serious problems that have limited the use of sweet sorghum as a substrate for ethanol production *(11)*. Traditionally, juice containing 10–15% sugar has been extracted or pressed from the sweet sorghum pulp. The juice is then either fermented directly to alcohol or evaporated to molasses for storage. Typically, press efficiencies are low, averaging 50–65%. Direct fermentation of the juice to ethanol is a seasonal process, accomplished for only a short time after harvest. In addition, sweet sorghum bagasse containing cellulose and hemicellulose is utilized for fodder, not as a biofuel substrate.

Countercurrent extraction technology is used throughout the sugar industry *(12,13)*. Sugar beet cossetts are moved upward in extractors by means of augers. Water is added at the higher end of the extractor, moving in a countercurrent manner, downward with the force of gravity.

Countercurrent extraction has been used to recover sugars and other components in sweet sorghum silage *(12,13)*. Extracts contain a high solute concentration, depending on the liquid:solid ratio used during the extraction. Temperature also affects product recovery. Over 90% of the sugars and organic acid in sweet sorghum silage were extracted using a 2:1 liquid:solid ratio at 70°C *(12)*.

Because sweet sorghum is harvested seasonally at a high moisture level (70–80%), rapid deterioration of readily available carbohydrates can occur unless the material is stabilized by some process that allows storage of the material on a long-term basis. Sweet sorghum deteriorates after harvest, even when bundled and stored dry at ambient temperatures *(11)*. Solid-state fermentation of sweet sorghum to ethanol has been investigated *(14,15)*, as have agronomic systems for harvesting and processing sweet sorghum for fuel alcohol production *(6,8,16)*. Ensiling has also been investigated as an economical method of long-term storage of potential fermentable carbohydrates in sweet sorghum *(17,18)*.

Ensiling is a traditional agricultural practice that has been used for several centuries *(19,20)*. An acidic environment improves the stability of the silage and allows any remaining fermentable carbohydrates to be stored successfully for a long period of time *(19,20–22)*.

Additives have been used to improve the quality of silage. Additives can be divided into four main categories: stimulants, selective inhibitors, total inhibitors, and bacterial inoculants *(23)*. Amending silage with hydrolytic enzymes to release more fermentable sugars can be considered a stimulant additive. Stable silage results from the addition of cellulolytic and hemicellulolytic enzymes and lactic acid bacteria to forage *(17,18,24–27)*.

Our work involves the utilization of hemicellulases and cellulases as additives at an activity level of 5 International Units (IU) of activity/g of dry sorghum *(28)*. Previous work shows that 5 IU/g dry wt is optimal for the hydrolysis of sweet sorghum cellulose *in situ (18)*. The pH and temperature profiles within the silo provide compatible conditions for fungal cellulase enzymes to hydrolyze hemicellulose and cellulose to release more fermentable sugars *(18)*.

Acid additives, such as formic and sulfuric acids, increase the concentration of soluble sugars and suppress microbial activity *(23–25,29,30)*. The purpose of adding acid is to lower the pH of the freshly chopped material rapidly to a level of 4.0, thus preserving the soluble carbohydrates in the fresh material. Formic acid is a selective inhibitor of certain bacterial populations, particularly clostridial *(23,24)*. The addition of 0.3% formic acid reduces the initial production of lactic acid. More yeasts and fungi are found in formic acid-treated silage than in silages not receiving formic acid *(20,23)*. Increased levels of ethanol have been detected in silages receiving formic acid as an additive *(20)*.

Ethanol is often produced during ensiling fermentations. Naturally occurring yeast and bacteria capable of a heterolactic fermentation are responsible for the presence of ethanol in silage *(20)*. Ethanol has been detected in unamended sweet sorghum silage at a level of approx 8 g/kg dry matter and 5.8 g/kg in cellulase-amended sorghum *(18)*.

Accessibility of the Lignocellulosic Fraction to Enzymatic Hydrolysis

In order for sorghum lignocellulose to serve as a substrate for fuel alcohol production, accessibility of cellulose to enzymatic hydrolysis must be improved through some form of pretreatment that removes lignin and hemicellulose. Our data show that ensiling is a form of dilute-acid hydrolysis *(17,18,29)*. Ensiling improved the reactivity of the lignocellulosic fibers to enzymatic hydrolysis *(17,29)*.

The National Renewable Energy Laboratory in Golden, CO, is currently using an enzymatic technique known as simultaneous saccharification and fermentation (SSF) to produce ethanol from other forms of lignocellulose, i.e., wood, grasses, and municipal solid waste *(10,31)*. SSF incorporates the enzymatic hydrolysis of cellulose to glucose and the fermentation of glucose to ethanol in one reaction vessel, hence the term simultaneous saccharification and fermentation. Our previous work subjected sweet sorghum with or without cellulase additives to SSF *(21)*. Fresh sweet sorghum produced more ethanol than did material that had been ensiled for 60 d, presumably because easily fermentable sugars were so abundant in the fresh material. In addition, product inhibition of the cellulase enzyme system occurred during SSF owing to the high levels of cellobiose and

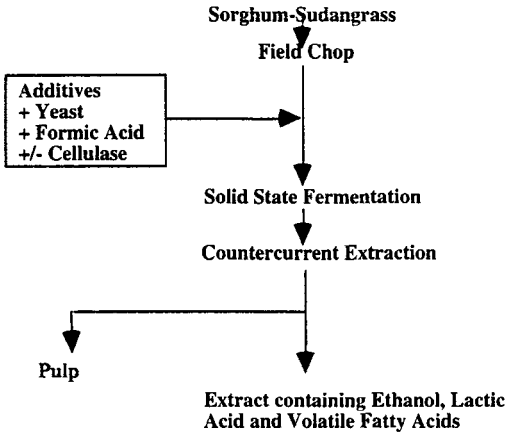

Fig. 1. Flowchart of solid-state fermentation of sorghum-sudangrass to ethanol.

glucose in the cellulase-amended silage. However, it was shown that ensiling enhanced the enzymatic hydrolysis of extracted sweet sorghum silage *(21)*.

Silo Fermentation

The main goal of this research is to study the feasibility of solid-state fermentation of sorghum-sudangrass to ethanol. It may be possible to utilize the silo as a bioreactor to produce ethanol *in situ*, thus lowering equipment costs incurred during SSF. By adding yeasts directly to green-chopped sorghum-sudangrass, more of the sugars that are available initially are converted to ethanol. The addition of cellulase will provide a continuous supply of glucose for ethanol production because of continued *in situ* cellulose hydrolysis. Acid-tolerant yeasts should remain metabolically active throughout the ensiling period. Heat generation resulting from the ethanolic fermentation corresponds to the slow rate of fermentation and may be dissipated. Lactic acid and anaerobic conditions in the silo preserve the sorghum until it is processed by countercurrent extraction to recover soluble components of the fermented sorghum-sudangrass *(17,18)*. A flowchart depicting the process is shown in Fig. 1.

Some supporting objectives used to evaluate ethanol formation from sorghum-sudangrass silage in the silo include:

1. Feasibility studies involving *in situ* ethanol production using sorghum amended with formic acid, cellulase, and additional yeast.
2. Evaluation of ethanol separation technology using countercurrent technology.
3. Evaluation of the process economics based on the first two objectives.

MATERIALS AND METHODS

Cultivation of Sorghum

Two varieties of sorghum were grown at the Colorado State University Agricultural Research, Development and Education Center (ARDEC). A variety of sorghum-sudan grass, Grazex II (F_1 hybrid of *Sorghum vulgare X Sorghum sudanese*) (Sharp Seeds, Greeley, CO) was identified as a variety that grows well in northeastern Colorado. The second variety, sweet sorghum (*Sorghum bicolor* [L.] Moench var.

M 81 E) did not reach maturity before the occurrence of a killing frost on September 14, 1993. In addition, M 81 E had a tendency to lodge, thus making harvest with a field chopper difficult. As a result, M 81 E was not used in any further work. M 81 E was used in our earlier studies regarding the SSF of sweet sorghum to ethanol *(21)*.

Laboratory Silo Setup

GrazexII sorghum was green-chopped with a field chopper and transported to laboratory facilities located in ARDEC for application of silage amendments and silo setup.

The following silage conditions were used:

1. Control, no additives (-C, -Y, -F).
2. Formic acid (75 m*M*), **plus** yeast (15 g of Montrachet wine yeast from Red Star/17 kg) **plus** 5 IU of cellulase activity/g dry wt of sorghum (+C, +Y, +F).
3. Formic acid **plus** yeast **minus** cellulase (-C, +Y, +F).

Silos consisted of 5-gal plastic containers with snap-on lids fitted with O-rings that seal the rims. Sorghum (~17 kg, fresh wt at 68% moisture content) was thoroughly mixed with 67 mL of formic acid (85% diluted to 60%, v/v), 210 mL of cellulase (Genencor, South San Francisco, CA), and 15 g of lyophilized Montrachet wine yeast (Universal Foods, Milwaukee, WI). This yeast is an all-purpose wine yeast that is acid-tolerant *(32)*.

The sorghum mixture was packed tightly into the containers and sealed. Weights were recorded for each silo. Silos were set up in triplicate for each sample date (19, 60, 88, 184, and 231 d of ensiling). The silos are stored under ambient conditions in the Food Engineering Laboratory at ARDEC.

Countercurrent Extraction of Sorghum-Sudangrass

A countercurrent diffuser is located in the Food Engineering Laboratory at ARDEC. This equipment has been used for extraction of sweet sorghum silage *(12,13)*. When silos are harvested, approximately one-third of the material is frozen immediately for the analysis. The remaining two-thirds are subjected to countercurrent extraction as described.

Extraction of ~12 kg of silage takes approx 3 h. Subsets of silage weighing 600 g each are continuously fed into the countercurrent extractor over the period of 10-min intervals. Water is delivered at a rate of 2 to 1 for the mass of sorghum on a wet-wt basis (1200 mL water delivered to 600 g sorghum silage in 10 min). This ratio was optimized for the extraction of sugars *(12,13)*. After the first nine subsets of 600 g silage are delivered to the diffuser, liquid samples are taken every 20 min and are taken for 90–120 min thereafter. These liquid samples are immediately frozen. In addition, a sample of pulp (extracted silage) is also frozen for further analysis. Extractions are conducted under ambient conditions to avoid the possible volatilization of ethanol. No experiments were conducted to quantify the amount of volatilization. Rather, extraction efficiencies were determined by comparing the amount of ethanol present in countercurrent extracts to blender extracts.

Analyses

Moisture Content

Moisture content of solids was determined by conventional oven dry wt (80°C, vacuum, overnight).

Ethanol

Glucose, fructose, xylose, lactic acid, acetic acid, and ethanol are evaluated by HPLC using a Bio-Rad (Hercules, CA) HPX-87H cation-exchange column. The solvent used is 0.008*N* H_2SO_4 at a flow rate of 0.6 mL/min. The column temperature is maintained at 65°C. Peaks are detected by refractive index *(17,18)*.

Lactic Acid

Countercurrent extracts were analyzed for lactic acid, volatile fatty acids, and sugars by HPLC *(17,18)* and enzymatically using a Lactate Diagnostic Kit (Sigma Chemical, St. Louis, MO).

Sugars

Countercurrent extracts and blender extracts were analyzed for sugars by HPLC as follows *(17,18)*. To prepare the blender extracts, 10 g of wet sorghum were added to 90 mL of deionized water, and a final weight was recorded. The mixture was kept on ice until blended at high speed in five short bursts of 10–15 s with periods of cooling (no blending for approx 1 min). This procedure was done in order to avoid heating of the blender blades, which may cause volatilization of ethanol from the solids. Prior to filtering, the blended extracts were returned to the ice bath and allowed to cool.

Both countercurrent extracts and blender extracts were prepared for analysis by first filtering the liquid:solid mixture through Whatman 541 filter papers lined with glass wool. Aliquots of filtrate (1.5 mL) were centrifuged at 18,000*g* in an Eppendorf microcentrifuge, and the supernatant was filtered through a 0.45-μ nylon membrane filter (Gelman Sciences, Ann Arbor, MI). Monosaccharides are analyzed using a Bio-Rad HPX-87P column with degassed, filtered high-purity water (Baxter, McGraw Park, IL) as the solvent at a flow rate of 0.6 mL/min. The column is maintained at 85°C, and sugars are detected by refractive index.

RESULTS AND DISCUSSION

Ethanol Production

Adding yeast, formic acid, and cellulase as silage amendments results in significant ethanol concentrations from 76 to 103 kg/MT on a dry-wt basis during the ensiling fermentation (Fig. 2). The addition of cellulase provides a continuous supply of glucose for ethanol production because of continued *in situ* cellulose hydrolysis. Acid-tolerant yeasts remain metabolically active throughout the ensiling period. As seen in Fig. 2, ethanol levels increase throughout the 231-d fermentation period, thus proving that the ethanolic material is stable over a long period of time.

Data regarding ethanol yields are shown in Table 1 for the day 231 fermentation of sorghum-sudangrass. Theoretical ethanol yield, based on soluble sucrose, glucose, and fructose present at day 0 is obtained from sorghum treated with cellulase, yeast, and formic acid (+C, +Y, +F) because of cellulose hydrolysis *in situ*. Without cellulase, but with formic acid as an additive (-C, +Y, +F), an ethanol yield that is 84% of theoretical is obtained. Total sugar available for fermentation in the unfermented sorghum-sudangrass equals 63.5 kg/MT of wet sorghum. The amount of sugar consumed to support yeast growth was not considered in the calculation of ethanol yield.

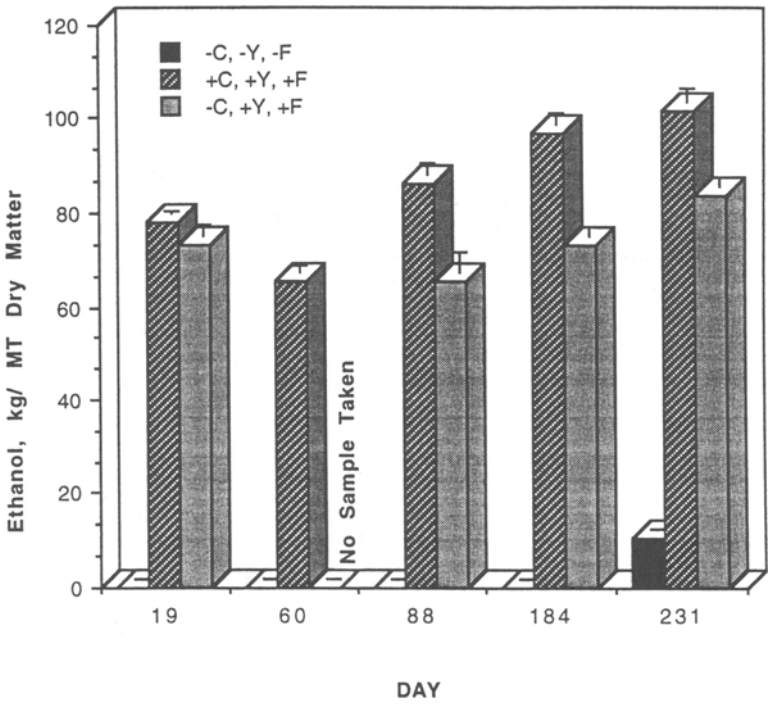

Fig. 2. Ethanol content of sorghum-sudangrass silage as determined by blender extraction. Sorghum-sudangrass control is designated as -C,-Y,-F. Forage receiving yeast, 75 m*M* of formic acid, and cellulase (5 IU/ g dry matter) is designated as +C,+Y,+F, whereas forage receiving yeast and formic acid, but no cellulase is designated as -C,+Y,+F.

The solid-state fermentation (231 d) of sorghum-sudangrass receiving both yeast and formic acid additives can be compared to previous fermentation studies using sweet sorghum *(21)*. The solid-state fermentation produces 64% of the ethanol found in SSF of sweet sorghum ensiled for 60 d (102 g ethanol/kg dry wt compared to 159 g/kg dry matter for SSF).

Lactic Acid Production

Lactic acid is produced during an ensiling fermentation and is responsible for regulating the acidity of the silage *(19,20,33)*. Over 61,000 silage samples preserved with formic acid (4.6 $g \cdot kg^{-1}$ wet wt) were analyzed for pH, lactic acid, and other silage components *(33)*. The potential for continuous lactic acid production exists when there is a minimum of 10 $g \cdot L^{-1}$ reducing sugar present in the silage. The average pH of these samples was 3.87, and the average lactic acid concentration was 57 $g \cdot kg^{-1}$ dry matter.

In our work, 3.0 $g \cdot kg^{-1}$ wet wt of 60% formic acid were added to freshly field chopped sorghum-sudangrass. The pH of sorghum-sudangrass prior to the addition of formic acid was 5.3; after formic acid addition, the pH value was 4.6. In addition to ethanol, lactic acid was produced during the solid-state fermentation of sorghum-sudangrass. More lactic acid was produced in the control than in the forage receiving formic acid, yeast, and with or without cellulase (Fig. 3). L-lactic

Table 1
Ethanol Yield as Determined by Dividing Ethanol Obtained from Blender Extraction by the Total Sugars Available for Fermentation in Sorghum-Sudangrass[a]

Trial	Ethanol, kg/MT, wet wt	Total sugars, kg/MT, wet wt	Yield based on total sugars present at d 0	Percent of theoretical yield based on 0.510
-C,-Y,-F	0.0	63.5	0.000	0%
+C,+Y,+F	32.7	63.5	0.515	101%
-C,+Y,+F	27.1	63.5	0.427	84%

[a]The control is denoted by -C,-Y,-F. Sorghum-sudangrass receiving yeast, formic acid, and cellulase additives is +C,+Y,+F, whereas forage receiving formic acid and yeast additives but no cellulase is -C,+Y,+F.

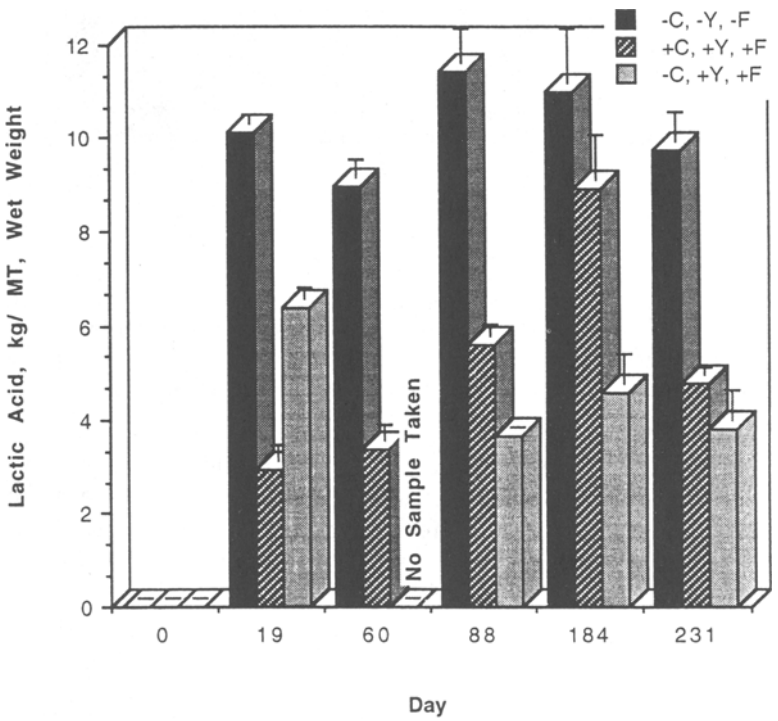

Fig. 3. Lactic acid concentrations in countercurrent extracts of fermented sorghum-sudangrass. Extracts were analyzed for lactic acid by HPLC using a Bio-Rad HPX-87H cation-exchange column. Sorghum-sudangrass control is designated as -C,-Y,-F. Forage receiving yeast, 75 m*M* of formic acid, and cellulase (5 IU/g dry matter) is designated as +C,+Y,+F, whereas forage receiving yeast and formic acid, but no cellulase, is designated as -C,+Y,+F.

acid concentrations are determined enzymatically. The concentrations and distribution of lactic acid at d 231 are given in Table 2. Currently, there is industrial interest in both D- and L-lactic acid for the production of poly-lactic acid *(34,35)*. In addition, the solid-state fermentation of sugar cane bagasse for the production of lactic acid has been investigated *(36)*.

Table 2
Distribution of Lactic Acid After D 231 of Fermentation in Countercurrent Extracts[a]

Trial	L-Lactic acid, kg/MT, wet wt	Total lactic acid, kg/MT, wet wt	L-Lactic acid, %
-C,-Y,-F	4.40	9.72	45.3
+C,+Y,+F	2.78	4.78	57.9
-C,+Y,+F	2.56	3.82	66.6

[a]The control is denoted by -C,-Y,-F. Sorghum-sudangrass receiving yeast, formic acid and cellulase additives is +C,+Y,+F, whereas forage receiving formic acid and yeast additives but no cellulase is -C,+Y,+F.

Sugars

Initially, 63.5 g sucrose/kg wet wt were present in the freshly chopped sorghum. Sucrose was hydrolyzed rapidly to glucose and fructose once sorghum was packed into the silos. These sugars were consumed in all three series throughout the fermentation. In the control series, 49.8% of the sugars were consumed in the first 19 d, as is seen in a typical lactic acid fermentation. Using the value of 11 g glucose/kg wet wt as the glucose remaining in the sorghum after 231 d of ensiling, 17.7% of the sugar remains in the control. The remainder of the glucose was consumed by the lactic acid bacteria over the 231-d fermentation period.

In both series receiving yeast and formic acid, the sugar content drops below levels seen in the control (Fig. 4). Total sugars are the summation of the concentrations of sucrose, glucose, fructose, and xylose at each respective time-point. Yeast consumes fermentable sugars present in the sorghum more rapidly and completely than the lactic acid bacteria utilize the sugars in the control series. This effect is seen at d 19 and remains constant until d 184. At this point, the glucose levels in the sorghum receiving cellulase surpass those seen in the control, indicating that the cellulase enzymes remain active through the 231 d of fermentation. Fructose is not detected in any of the three series after d 19. An increase in the xylose content in the series receiving cellulase may be owing to the continued action of hemicellulases, present in the Genencor cellulase, throughout the 231-d fermentation period (data not shown).

Countercurrent Extraction Efficiencies

Countercurrent technology provides an efficient way to extract sugars from sweet sorghum silage *(12,13)*. Countercurrent extraction also extracts byproducts (lactic acid, acetic acid, and ethanol). In our preliminary work, ethanol was extracted under ambient conditions and a solid:liquid ratio of 2:1. Extraction efficiencies are calculated by dividing the amounts obtained from countercurrent extraction by the amounts obtained from blender extraction (Table 3). A complete extraction would have an extraction efficiency of 1.00. It is assumed that blender extraction is complete and that the quantities found from blender extraction are representative of the total amount found in the solid substrate. Extraction efficiencies for L-lactic acid from sorghum-sudangrass fermented for 184 d are 0.41 and 0.50 for material with and without cellulase, respectively. Extraction efficiencies for

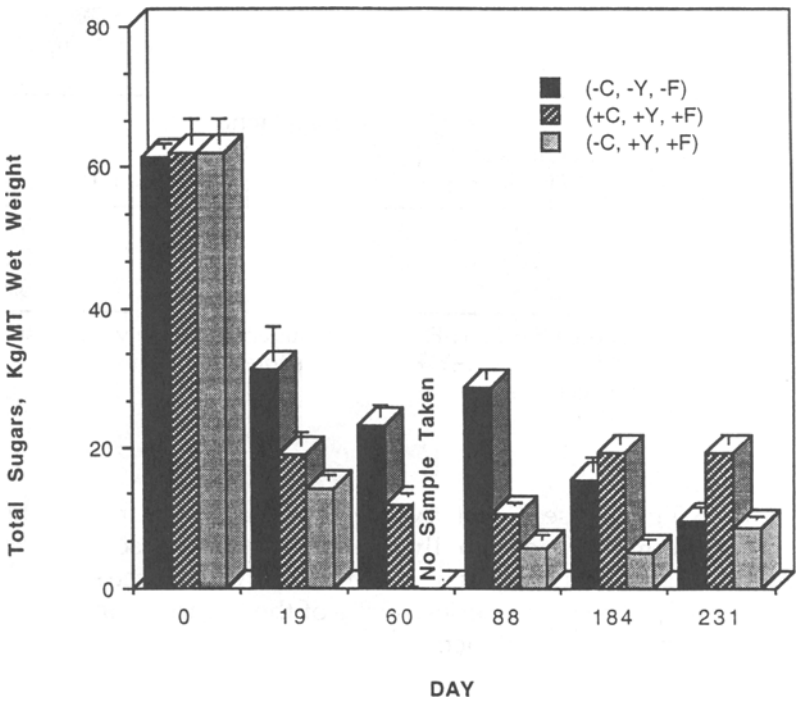

Fig. 4. Changes in sugar content with time suring the solid-state fermentation of sorghum-sudangrass to ethanol. Extracts were analyzed for sugars by HPLC using a Bio-Rad HPX-87P column. Sorghum-sudangrass control is designated as -C,-Y,-F. Forage receiving yeast, 75 m*M* of formic acid, and cellulase (5 IU/g dry matter) is designated as +C,+Y,+F, whereas forage receiving yeast and formic acid, but no cellulase, is designated as -C,+Y,+F.

Table 3
Extraction Efficiencies for the Extraction of Ethanol from Sorghum-Sudangrass Receiving Additives[a]

Day	-C,-Y,-F	+C,+Y,+F	-C,+Y,+F	Extraction temperature, °C
19	(No	0.69	0.75	19.5
60	ethanol	0.65		16.5
88	produced	0.66		16.3
184	until d 231)	0.76	0.72	16.5
231	0.80	0.45	0.63	20.0

[a]The control is denoted by -C,-Y,-F. Sorghum-sudangrass receiving yeast, formic acid, and cellulase additives is +C,+Y,+F, whereas forage receiving formic acid and yeast additives, but no cellulase is -C,+Y,+F. Extraction efficiencies are not available for d 60 and 88 for sorghum-sudangrass without cellulase.

ethanol and lactic acid were lower than those previously achieved at 70°C *(12)*. Average recovery efficiencies were 0.64 for ethanol (Table 3) and 0.45 for lactic acid. By varying temperature and liquid:solid ratios, ethanol and lactic acid should be extracted more efficiently.

Table 4
Estimated Product Value of Ethanol and Lactic Acid
from the Solid-State Fermentation of Sweet Sorghum[a]

Treatment	Estimated product value for ethanol, $/MT, wet	Estimated product value for lactic acid $/MT, wet	Estimated cost of production, $/MT, wet	Estimated net product value for both ethanol and lactic acid, $/MT wet
-C,-Y,-F	$2.89	$53.48	$27.56	$28.81
+C,+Y,+F	29.74	24.92	32.48	22.18
-C,+Y,+F	24.67	21.56	30.88	15.35

[a]The control is denoted by -C,-Y,-F. Sorghum receiving yeast, formic acid, and cellulase additives is +C,+Y,+F, whereas forage receiving formic acid and yeast additives, but no cellulase, is -C,+Y,+F.

Economic Evaluation

From the economic analysis of the sorghum-sudangrass control, product values of $27.16, $1.61, and $2.88 could be attributed to lactic acid, ethanol, and wet pulp, respectively, for each metric ton of untreated sorghum-sudangrass. Sorghum-sudangrass has an estimated value of $27.56/wet MT. The (-C, +Y, +F) treatment produced less lactic acid and more ethanol than the control. This series has product values of $10.64, $12.27, and $2.88 for lactic acid, ethanol, and pulp, respectively.

Formic acid additive costs represent $1.60/wet ton at the levels used in the preliminary study. For the (+C, +Y, +F) treatment, product values of $13.44, $14.80, and $2.88 for lactic acid, ethanol, and pulp, respectively, could be reasonably expected. In addition to the raw material costs of $27.56/wet MT of silage, $1.73 for yeast *(32)*, and $1.60 for formic acid *(37)*, enzyme costs currently make the latter option unreasonable at the 5 IU/g dry matter treatment level. However, recent advancements in the solid-state production of fungal cellulases on extracted sweet sorghum pulp in our laboratory *(38)* could reduce the cellulase costs from $375.00/wet ton for commercial enzymes to $1.60/wet ton. After incorporating a value of $1.60 for cellulase, estimated product values, based on yield values for sorghum-sudangrass, are obtained by the solid-state fermentation of sweet sorghum shown in Table 4 are obtained.

Although initially the lactic acid in sweet sorghum ensiled without additives appears to be more valuable than the ethanol and lactic acid in sweet sorghum receiving additives, the cost of product recovery of lactic acid from countercurrent extracts has not been evaluated. Ethanol is recovered by distillation in this process. A complete evaluation of each unit operation in the process will determine the economic potential of our proposed system.

CONCLUSION

Ensiling is an economical means of storing and producing fermentable carbohydrates. We have shown that the silo also serves as a bioreactor for alcohol fuel production. The addition of yeasts and formic acid encourages an ethanolic fermentation within the silo. Ethanol production is 80% complete by d 19 of the fer-

mentation. Since this was the first time-point taken in this study, it may be possible to process ethanol prior to d 19. Ethanolic sorghum silage is stable over a period of at least 230 d, thus potentially producing a low-cost feedstock for continuous ethanol production on a yearly basis.

Yeast additives compete with epiphytic (naturally occurring) microorganisms for the available sugars initially found in sorghum, thus using sucrose, glucose, and fructose for ethanol production. Yeasts are spoilage organisms in silage *(39–44)*. Spoilage occurs because ethanol is not a preservative in silage. When this silage is exposed to aerobic conditions, deterioration occurs in just a few days. However, sorghum-sudangrass receiving yeast and formic acid remained stable over 231 d of storage as long as anaerobic conditions were maintained (Fig. 2). Inhibition studies of acid byproducts and ethanol would provide useful insights into the metabolic activity of the yeasts present during the fermentation.

Separation and recovery of ethanol and lactic acid did not fall within the scope of this research. Countercurrent technology is widely used in the sugar industry; therefore, a broad knowledge base is available. Future work should consider product recovery from the countercurrent liquid extracts. Recovery of lactic acid from these extracts could involve distillation to remove ethanol and volatile fatty acids followed by ion-exchange *(45)* or liquid membrane extraction *(46,47)* to remove lactic acid from the distillery bottoms.

We have addressed the feasibility of *in situ* ethanol production using sorghum-sudangrass as the substrate. Using the silo as a bioreactor in the solid-state fermentation of sorghum as well as for the subsequent storage of the ethanolic material prior to processing is inexpensive. We would expect to double ethanol and lactic acid yields using sweet sorghum instead of sorghum-sudangrass. The utilization of sweet sorghum as an ethanol feedstock will provide renewable biomass equivalent to conventional substrates, such as corn and barley. Sweet sorghum would complement, rather than compete with corn as a substrate for fuel alcohol production, thus adding to the diversity of renewable resources that can be produced for biofuels. Lactic acid may become a valuable byproduct in this system.

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