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Evaluation of Sugar Cane Bagasse and Rice Straw as Process Substrates for the Production of Ethyl Alcohol

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EVALUATION OF SUGAR CANE BAGASSE AND RICE STRAW AS PROCESS SUBSTRATES FOR THE PRODUCTION OF ETHYL ALCOHOL

During the past ten years, a great deal of attention has been given to the production of liquid fuels, particularly ethyl alcohol, from renewable resources. A major portion of the research in this area has centered around the utilization of non food-chain resources such as waste lignocellulosics. Within this grouping municipal solid waste (MSW) has received the greatest consideration due to its availability in large, collected quantities on a daily basis. The future of ethyl alcohol production from renewable resources, however, may lie in the conversion of agricultural wastes such as sugar cane bagasse and rice straw due to their availabilities on a world-wide basis, especially in many developing countries where these are the major agricultural crops. This study reports the evaluation of both sugar cane bagasse and rice straw as potential substrates for the production of ethyl alcohol.

Microorganisms

Trichoderma reesei, QM 9414G, obtained originally from the American Type Culture Collection, Rockville, Maryland, was used to produce a full complement cellulase system consisting of endoglucanase, cellobiohydrolase, and cellobiase activities (Emert et al., 1974) for use in simultaneous saccharification fermentation (SSF) Boltkamp et al., 1978; Takagi et al., 1977). Permanent stock cultures were maintained as lyophilized spores and working stock cultures were allowed to sporulate on Difco potato dextrose agar, Difco, Detroit, Michigan, and then were maintained at 4°C. Both seed cultures and cellulase production cultures were run according to the method of Gracheck et al. (1981).

Candida brassicae IFO 1664, obtained originally from the Institute for Fermentation, Osaka, Japan, was the yeast of choice for SSF. Permanent stock cultures were lyophilized, and working stock cultures were maintained at 4°C following growth on Difco YM agar. Seed cultures were according to the method of Rivers (1983).

Simultaneous Saccharification Fermentation

Simultaneous saccharification fermentations were run according to the method of Rivers (1983) using F2 medium. Samples were aken at 24 and 48 hours and analyzed for ethanol and residual glucose.

Substrates

Bagasse, Sugar Cane Growers Cooperative of Florida, Bell Glade, Florida, and rice straw, University of Arkansas Agricultural Experiment Station, Stuttgart, Arkansas, were selected as prominent agricultural crop wastes.

Pretreatments

Substrates were subjected to two pretreatments, one mechanical and one thermochemical. Each substrate was ball milled in a laboratory scale ball mill using 1 inch diameter balls as the grinding medium for 4 hours. The substrates were also subjected to caustic pulping in 0.5N NaOH at 60°C for 24 hours. Following pulping the substrate was washed in 0.005M citrate buffer to equilibrate the pH to 5.0.

Substrate Composition

Substrate components including hemicellulose, lignin, cellulose, and insoluble ash were determined by the method of Van Soest and Wine (1968).

Substrate Composition

Following pretreatment, a compositional analysis was completed for each substrate case (Table 1). Characteristically, for agricultural wates, cellulose content was found in the range of 35-45% of dry weight in the native state. Hemicellulose was also determined to be at characteristic levels for native agricultural wastes, 35-45% of dry weight. Bagasse also had typical levels of both lignin and insoluble ash; however, rice straw was found to contain lower than average lignin and higher than average insoluble ash. This variance from the agricultural waste norm may be explained by the fact that rice straw is known to contain from 15-30% silica by dry weight. Following ball milling, no compositional changes were observed; however, following pretreatment in 0.5N NaOH, changes were evident. In both substrate cases, the cellulose content was increased significantly through the semiselective extraction of primarily hemicellulose, and partially ash. The basis for this extraction lies in the type bonds found between lignin and hemicellulose. In grasses, ester bonds are the predominant linkage and are susceptible to the action of caustic whereas the ether bonds which are predominant in woody plants are not.

Tabla 1. Substrate Composition

Substrate	Cellulose	Lignin	Insoluble Ash
Bagasse	.	.	
Native	44.4	9.6	1.2
Ball Mill	44.4	9.6	1.2
0.5N NaOH	61.3	9.9.	1.0
Rice Straw			
Native	36.1	3.3	14.8
Ball Mill	36.1	3.3	14.8
0.5N NaOH	52.9	4.6	6.3

Table 2. Ethyl Alcohol Production

Bagasse[1]	24 Hour			48 Hour		
Pretreatment	g/l	% Conversion	Gallons/Dry Ton	g/l	% Conversion	Gallons/Dry Ton
Native	0.1	0.4	0.3	0.1	0.5	0.4
Ball Mill	0.0	0.2	0.1	0.0	0.0	0.0
0.5N NaOH	10.3	61.1	63.9	11.4	67.6	70.7
Rice Straw[2]	**24 Hour**			**48 Hour**		
Pretreatment	g/l	% Conversion	Gallons/Dry Ton	g/l	% Conversion	Gallons/Dry Ton
Native	7.1	21.0	12.9	7.1	21.0	12.9
Ball Mill	10.9	32.4	19.4	10.2	30.4	18.7
0.5N NaOH	21.6	64.2	57.9	24.9	74.0	66.7

1. 3% w/v cellulose
2. 6% w/v cellulose

Simultaneous Saccharification Fermentation

Following hydrolysis and ethanol production in SSF, a number of observations were made with respect to each substrate (Table 2). First, neither bagasse nor rice straw were readily hydrolyzed in the native state. In fact, bagasse was only negligibly hydrolyzable. Following mechanical pretreatment in the ball mill, conversions of bagasse were again negligible. Rice straw, on the other hand, produced a 50% increase in ethanol following the same pretreatment. Finally, following chemical pretreatment in 0.5N NaOH, both bagasse and rice straw showed dramatic increases in conversion when compared with the native state. Bagasse increased from negligible conversions in both the native and ball milled forms to greater than 60% of theoretical. At the same time rice straw increased by 350% over the native state and 200% over the ball milled case. In each case the corresponding increase in gallons of ethanol produced/dry ton of substrate is evident (Table 2) where 170.5 gallons of ethanol/dry ton of substrate is theoretical yield.

The explanation for the conversions observed are basic to lignocellulose hydrolysis. First, in the native state, the individual components of the substrate are in their most resistant form. Second, the relatively large particle size of the native substrate is prohibitive in allowing cellulase access to the β-1,4-bonds even if they were not highly unsusceptible to hydrolysis. Third, ball milling results in no alteration of the substrate components from their native, resistant state even though particle size is reduced significantly. Finally, the caustic pretreatment, which is similar to the old caustic pulping process once used in the paper industry, resulted in dramatic increases in product yield as a result of significantly disrupting the highly resistant nature of the native substrate. As previously mentioned, this may be attributed to the fact that grassy plants contain ester linkages between the lignin and the hemicellulose. This allows the resistant nature of the substrate to be sufficiently altered in a manner which provides β-1,4-bonds which are not only accessible but also susceptible to enzymatic hydrolysis.

Agricultural wastes have great potential as process substrates for the production of ethyl alcohol and other chemicals currently produced from petroleum feedstocks. In order to effectively hydrolyze these lignocellulosic wastes, they must be pretreated in order to increase both accessibility and susceptibility to cellulases. Mechanical pretreatment in the form of ball milling is ineffective in the cases of bagasse and rice straw; however, caustic pretreatment did result in significant increases in product yields. Theoretical conversions attained were 67.6% for bagasse and 74.0% for rice straw which represent 70.7 and 66.7 gallons of ethanol produced/dry ton of the respective substrates.

LITERATURE CITED

BLOTKAMP, P. J., M. TAKAGI, M. S. PEMBERTON, and G. H. EMERT. 1978. Enzymatic Hydrolysis of Cellulose and Simultaneous Fermentation to Alcohol. AIChE Symp. Ser. No. 181, 74:85-90.

EMERT, G. H., E. K. GUM, JR., J. A. LANG, T. H. LIU, and R. D. BROWN, JR. 1974. Cellulases. Adv. Chem. Ser. 136:79-100.

GRACHECK, S. J., K. E. GIDDINGS, L. C. WOODFORD, and G. H. EMERT. 1981. Continuous Enzyme Production as Used in the Conversion of Lignocellulosics to Ethanol. ASAE Natl. Energy Symp., Ag Energy. 2:305-310.

RIVERS, D. B. 1983. Effects of Intrinsic Physical and Chemical Factors on the Enzymatic Hydrolysis of Lignocellulosics. Dissertation, University of Arkansas.

TAKAGI, M., S. ABE, SUZUKI, G. H. EMERT, and N. YATA. 1977. A Method for Production of Alcohol Directly from Cellulose Using Cellulase and Yeast. 551-571. Proc. Biocon. Symp. IIT Delhi.

VAN SOEST, P. J., and R. H. WINE. 1968. Determination of Lignin Cellulose in Acid-Detergent Fiber with Permanganate. J. Ass. Offic. Anal. Chem. 51(4):780-785.

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THE INFLUENCE OF DeGRAY RESERVOIR ON ZOOPLANKTON POPULATIONS IN THE CADDO AND OUACHITA RIVERS

Potamoplankton populations are usually rather limited (Hynes 1970). However, reservoirs with significant water retention time have extensive zooplankton communities and large populations can greatly influence the tailwater plankton community via releases. In 1978, at the request of the Arkansas Game and Fish Commission, MORS began sampling zooplankton populations in the DeGray Reservoir tailwater on the Caddo River (R3), in the Ouachita River above the confluence with the Caddo (R2), and in the Ouachita below the confluence (R5) (Figure).

Zooplankton samples were duplicate five minute horizontal tows with a Clarke-Bumpas sampler equipped with a No. 10 mesh (160 micron) net. Samples were taken at 4 to 6 week intervals at all three stations; all stations being sampled within a week. Sampling occurred from April through October. Sampling was not event dictated so some samples were collected during high water, some during moderate flow, and some during low flow. Cladocerans and rotifers were identified to species when possible, while copepods were identified to suborder.

Twelve cladoceran species, eleven rotifier species (Table 1), and two orders of copepods were found at the upper Ouachita station (R2). Twenty cladoceran species, twenty-one rotifer species, and two copepod suborders were found at the Caddo River station (R3). Thirteen cladoceran species, thirteen rotifer species, and two suborders of copepoda were found at the lower Ouachita (R5). Mean densities for each year were greater by two orders of magnitude at the Caddo station than at the Ouachita River stations (Table 2). Abundant cladocerans at the Caddo station (R3) were *Bosmina longirostris, Ceriodophnia lacustris, Chydorus sphaerius, Daphnia ambigua, D. galeata, D. catawba, Diaphansoma leuchtenbergianum,* and *Holopedium amazonicum*. Abundant rotifers were *Asplanchna priodonta* and *Conochilus unicornus*. Common rotifers were *Kellicottia bostoniensis, Keratella cochlearis,* and *Synchaeta stylata*. All of the preceding forms were frequently encountered in DeGray Reservoir. Littoral cladocerans such as *Latona parviremis, Macrothrix rosea, Eurycercus lamellatus, Camptocercus oklahomensis,* and *Alona sp.* were also found at the Caddo station. The only forms found at the Ouachita stations but not the Caddo station were *Scapholebris kingi, Leydigia acanthocercoides,* and *Kellicottia longispina,* which had not been found in the DeGray Reservoir. Forms found at the Caddo station but not at the Ouachita stations included *Bosminopsis deitersi, Latona parviremis, Camptocercus oklahomenses, Ceriodaphnia reticulata, Eurycercus lamellatus, Lecane luna, Platyis quadricornis,* and *Proalinopsis sp.* Some Ouachita River samples (especially during high water periods) had no zooplanktors, while many high water samples had extremely low numbers.

Cyclopoids dominated the copepod segment of the community in 96 percent of the samples at the upper Ouachita station (R2), 53 percent of the samples at the Caddo station (R3), and 85 percent of the samples at the lower Ouachita station (R5). Calanoid densities at the Ouachita River stations were always very low but were occasionally very important in the Caddo River sample.

The Caddo River station was more diverse than the Ouachita stations. This station community is composed of reservoir produced zooplankton and also those forms associated with a riverine or littoral situation. Numerically the reservoir-produced organisms dominate the population at this point but the other forms are not excluded. Hynes (1970) summarized the findings of several workers that found reservoirs and lakes greatly influence the plankton populations of their immediate tailwaters and contribute the vast majority of the constituents of the population. Edmondson (1959) states "The limnetic region of the inland lakes has a cladoceran population large in number of individuals but not rich in species." Thus this tailwater area is dominated by a relatively small group of species but not limited to just these forms. Shallow, weedy areas produce a greater variety of species (Edmondson, 1959) than any other habitat. Therefore the weedy river margins, shallow shoals areas, and the flow retarding influence of the reregulating pool account for the presence of a substantial number of littoral and/or riverine forms at the Caddo River station, even though the densities are dominated by reservoir produced limnetic zooplanktors.