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Ethanol from Lignocellulosic Wastes with Utilization of Recombinant Bacteria

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ABSTRACT

This article presents the advanced technology that has been developed by BioEnergy International of Gainesville, Florida, utilizing novel recombinant strains of bacteria developed by Lonnie Ingram of the University of Florida. The first commercial applications of these unique fermenting organisms convert 5-carbon sugars, as well as 6-carbon sugars, and oligomers of cellulose (e.g., cellobiose and cellotriose) directly to ethanol. The proposed systems that will be utilized for conversion of agricultural wastes, mixed waste papers, and pulp and paper mill waste in forthcoming commercial installations are now under design. This involves the extensive experience of Raphael Katzen Associates International, Inc. in acid hydrolysis, enzyme production, enzymatic hydrolysis, large-scale fermentation engineering, and distillation/dehydration.

Specific examples of this advanced technology will be presented in different applications, namely:

1. Conversion of the hemicellulose content of sugar cane bagasse to 5-carbon sugars by mild-acid prehydrolysis, followed by fermentation of the 5-carbon sugar extract with recombinant *Escherichia coli* in a commercial installation soon to be under construction in Brazil. This unique process utilizes the surplus hemicellulose fraction of bagasse not required for steam and power generation to produce ethanol, additional to that from the original cane juice, which has been converted by conventional sucrose fermentation to ethanol. The process also recovers and converts to ethanol the majority of sucrose normally lost with the bagasse fibers. Resultant beer is enriched in an innovative process to eliminate the need for incremental rectification capacity.

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2. Application of this technology to mixed waste paper in Florida, with a moderate loading of newsprint (85% mechanical wood fiber), will involve a mild-acid prehydrolysis, the partial extraction of the 5-carbon sugars produced from hemicellulose as a feedstock for propagation of the recombinant *Klebsiella oxytoca* bacterium. Included is a facility providing for in-house production of cellulase enzyme, as an active whole broth for direct use in simultaneous saccharification and fermentation (SSF) of the remaining cellulose and residual 5-carbon sugars to ethanol. This is followed by distillation and dehydration in the advanced commercially available low-energy recovery system.
3. Another potential application of this unique technology involves utilization of a variety of wastes from several pulp and paper mills in close proximity, permitting collection of these wastes at low cost and reducing the considerable cost encountered in disposing of such low-energy wet waste. Based on pilot plant experiences with converting such waste by simultaneous enzymatic hydrolysis and fermentation, the same techniques will be applied as in the second case, with use of acid prehydrolysis only if the hemicellulose-derived sugars can be economically recovered. If not, acid hydrolysis will be eliminated and only the simultaneous saccharification and fermentation will be carried out, utilizing in-house-produced enzyme broth and recombinant *Klebsiella oxytoca*.

Index Entries: Ethanol; lignocellulosic wastes; recombinant bacteria.

INTRODUCTION

For many decades, research and development efforts have been made to utilize the so-called nonfermentable sugars obtained from such feed streams as sulfite waste liquor and wood hydrolyzates. Such sugars are primarily the 5-carbon variety, which are not affected by conventional fermentation yeasts. Attempts to modify yeast cells by mutation or genetic means have resulted in fermentations yielding substantial byproducts besides ethanol, with low concentrations and long fermentation times. However, work done during the past decade by L. O. Ingram and coworkers at the University of Florida (*1*) has led to development of genetically modified bacteria, such as *Escherichia coli* and *Klebsiella oxytoca*, which are capable of fermenting C_5 sugars to ethanol with a high degree of selectivity, and at reasonable concentrations and fermentation times. Work is now under way to expand this operation in pilot plant facilities, simultaneously with development of process design for the first commercial installations of this novel technology.

Bagasse Hemicellulose Processing

In most cane sugar mills, there is a surplus of bagasse over that required to produce steam and power for the mill operation. In Brazil, which has had a major fuel ethanol program for more than 15 yr, the ethanol requirements are so large that considerable cane is grown for cane juice-to-ethanol production, without manufacture of sugar. These are the autonomous ethanol plants.

Such facilities yield a substantial surplus of bagasse over the ethanol distillery requirements. In a project now being designed and built in Brazil (Fig. 1), a major part of the bagasse formerly used for fuel is treated by acid hydrolysis (dilute sulfuric acid) at appropriate temperature, pH, and time to result in essentially complete hydrolysis of hemicellulose to sugars. These are predominately xylose. Control of the acid hydrolysis process provides essentially complete hydrolysis and dissolution of hemicellulose sugars, while avoiding secondary reactions producing furfural from the xylose and phenolic fractions from lignin degradation.

The hemicellulose sugars are then pressed from the moist hydrolyzed bagasse, followed by a dilution-washing stage for additional extraction of these sugars. The desugared hydrolyzed bagasse is then pressed to approx 50% moisture content, mixed with hydrated lime to neutralize residual acidity, and is sent to the boilers for production of steam and electric power. The balance between hydrolyzed and unhydrolyzed bagasse is maintained in such proportion that there is sufficient energy available, in the form of steam and electric power, to operate both the sucrose ethanol and hemicellulose sugar ethanol facilities.

The hemicellulose sugar solution pressed and washed from the bagasse is neutralized with hydrated lime slurry. The precipitated calcium sulfate and organic impurities are filtered off, washed, and transferred to landfill.

The clarified sugar solution, at appropriate pH for the recombinant *E. coli* fermentation, is fed to batch fermenters at a concentration sufficient to produce ethanol beer at 4–4.5 wt% concentration, a practical level for the recombinant bacteria. Fermentations are carried out under optimum pH and temperature conditions within 48 h. The fermented beer is then blended with a measured portion of the more concentrated fermented ethanol from the sucrose fermentation. The resultant beer is enriched by way of a new stripper, with the overhead condensate being fed to an appropriate point in the existing rectifying tower. The enrichment process is designed to optimize both the energy and hydraulic balance of the system, and in so doing, eliminating the need for additional distillation capacity and boiler capacity.

The stillage from the new stripper is sterile and is used as make-up water insofar as possible for dilution of sulfuric acid for acid hydrolysis, for the sugar extraction operation, for lime slurry production, and for miscellaneous uses in other operations of the combined facility.

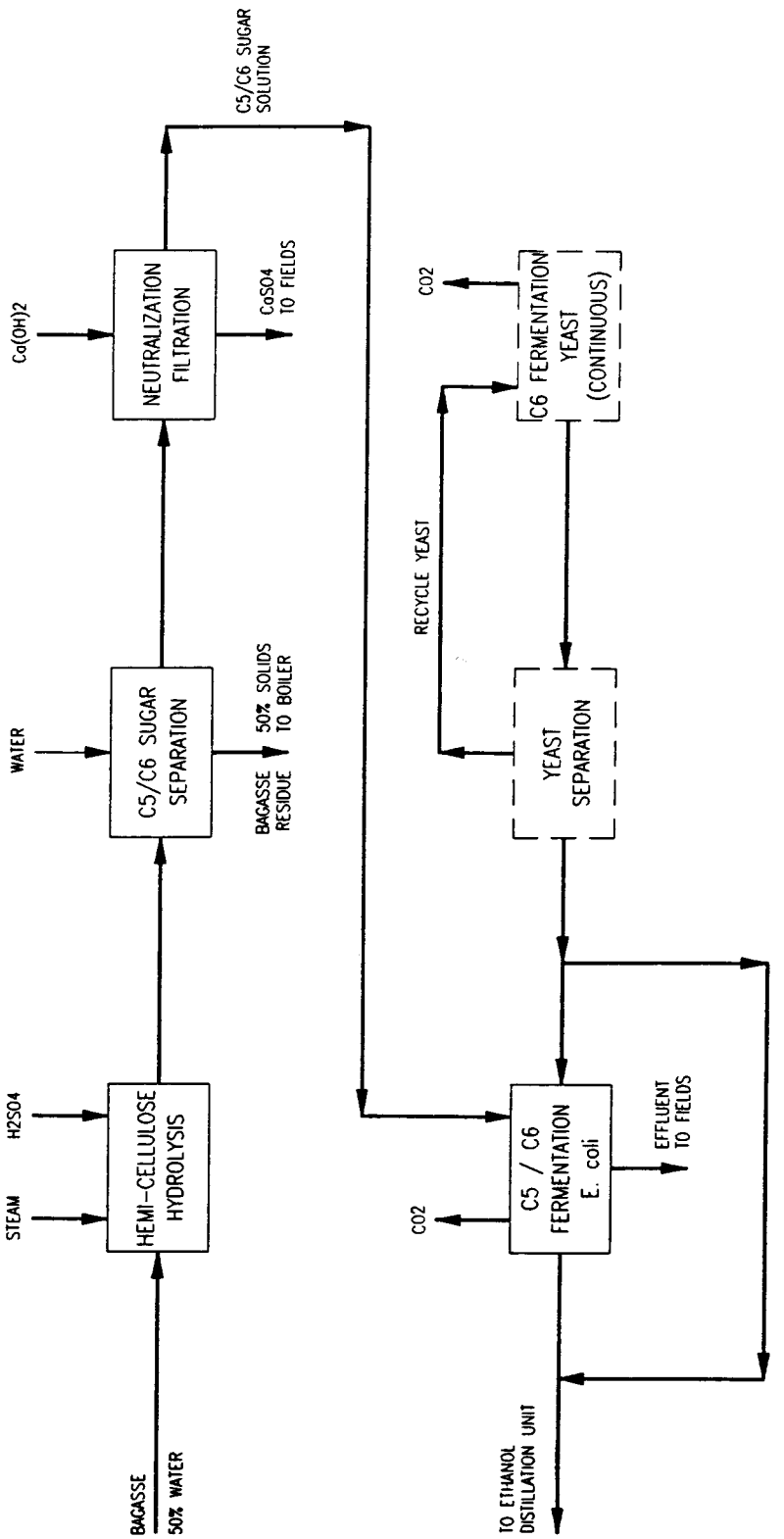

Fig. 1. Bagasse hemicellulose ethanol.

An inherent advantage of this process application is the ability to provide incremental ethanol capacity while minimizing capital and operating costs. The process, trademarked the Bio-Bagasse Process, also lends itself to modularity in design and short construction periods. The full-scale plant designed for Brazil is projected to have a capital cost of < $1.50 (US)/annual gal ethanol production and will have a total operating cost (including depreciation) of < $0.40/gal ethanol.

Mixed-Waste-Paper Conversion

In Florida, limitations on landfills and public resistance to mass burning (incineration) have led to the collection of a wide variety of waste papers at depots in many parts of the state. Office waste and newsprint are major components of this mixed-waste-paper stream. Because of the varying nature of this feedstock, which can be removed with a reasonable tipping fee for its disposal, a flexible design is required for the ethanol facility. The process that is now under design is indicated in Fig. 2. Mixed waste paper (MWP) and newsprint (NP) are brought into the plant site and stored in a bulk warehouse to provide an inventory sufficient to avoid interruption of process operations by any transport difficulties. Reasonably constant proportions of mixed waste paper (office waste) and newsprint are transferred by front-end loader and conveyors to a conventional hydrapulper operation. Proportions of MWP and NP will be maintained as constant as possible to yield a reasonably uniform feed to the subsequent process. The hydrapulping operation will not only reduce the waste material to fibrous form, but will permit separation of dirt, heavy metal objects, and plastics, with conventional rock trap and roper mechanisms. Following the hydrapulper, a pressure screen will be used to separate remaining plastic materials from the fibrous fraction. Use of a densifying screen and screw press will provide for dewatering and expression of surplus water from the fiberized material, to a moisture level of < 60%. Such water removed by screening and pressing will return to the hydrapulper, along with make-up water from other streams in the process.

The pressed wet fiber will be fed by a screw feeder into a continuous acid prehydrolysis reactor. Dilute sulfuric acid will be sprayed onto the material as it is fed into the reactor. Direct steam admission will maintain the required temperature and pressure. A variable-speed drive on the conveyor within the reactor will permit adjustment of retention time. The major control variables will be acid concentration (pH) and temperature (by control of steam pressure). The hydrolyzed pulp is then discharged from the reactor through a pressure lock to minimize steam loss from the system. Flash steam from the hydrolyzed fiber goes to the feed bin to preheat incoming material. With countercurrent hot-water extraction in a two-stage belt-filter operation, the major part of the hydrolyzed hemicellulose sugars and toxic substances is removed from the fibrous residue.

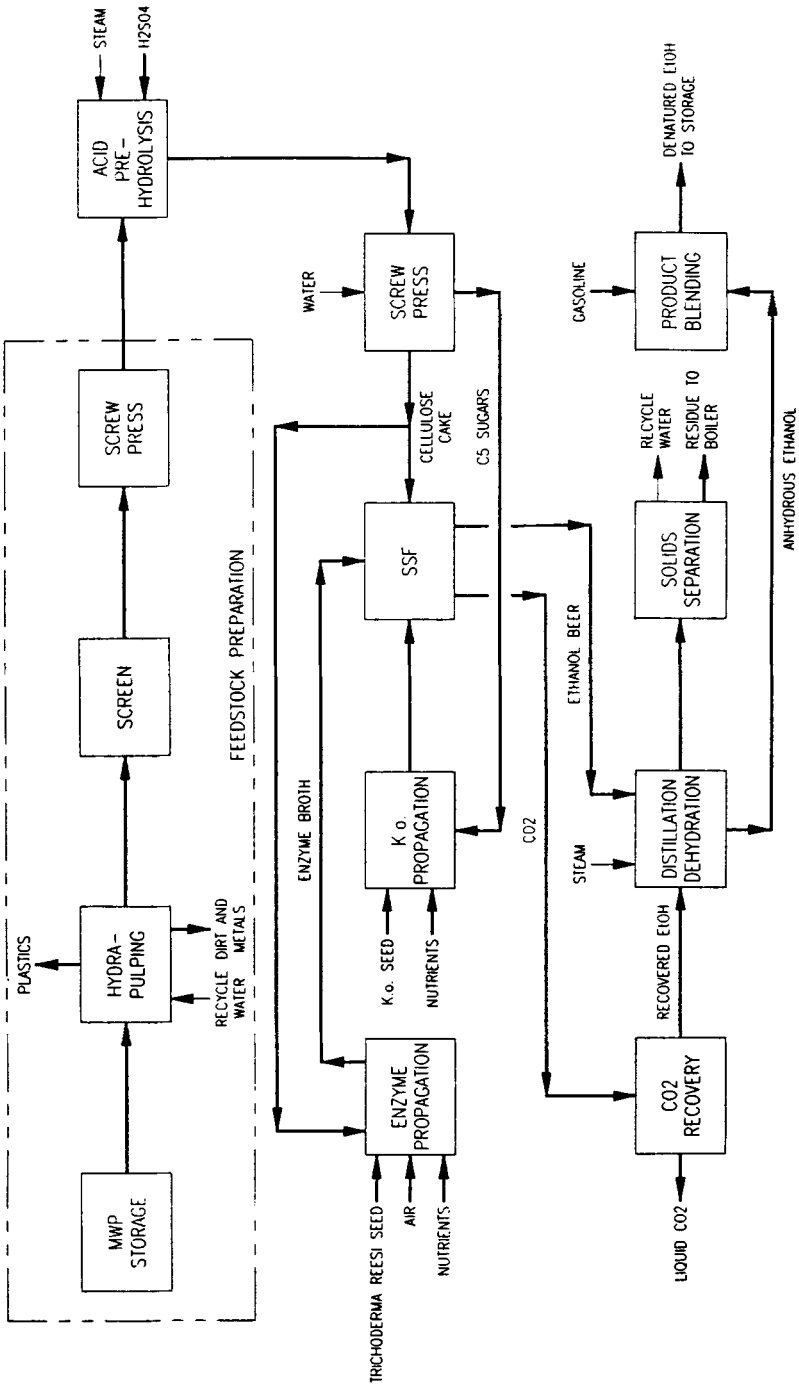

Fig. 2. Mixed-waste-paper ethanol.

The extract fraction is neutralized with lime to a controlled pH. Precipitated calcium sulfate and precipitated organic materials are filtered off, and washed in a rotary vacuum filter. The filtrate, primarily hemicellulose sugars, is used as a growth medium for recombinant *Klebsiella oxytoca*. The remainder is mixed with hydrolyzed residue for the SSF operation. The hydrolyzed residue is adjusted to appropriate pH with lime in a closed hot conveyor system to maintain sterility, and is then transferred on a regular basis to a sequence of fed-batch SSF fermenters.

A small portion of the hydrolyzed residue is fed to the second stage of a continuous *Trichoderma reesei* enzyme production system, the first stage being fed with C_5 or C_6 sugars for *Trichoderma* growth, whereas the second-stage feed of hydrolyzed material supplies a more resistant material to force secretion of the desired cellulase enzymes. Stagewise propagation vessels are also provided to develop the *Trichoderma* from laboratory flasks to sufficient quantities for the continuous enzyme production system.

The whole broth produced in the continuous enzyme production system is fed to the SSF fermenters on initiation of the fed-batch operation.

Because of the absorptive nature of the cellulose in the hydrolyzed residue, it is not feasible to feed the required quantity of this material into a fermenter batchwise. This would require about 16% suspended solids, which when absorbing water would yield an extremely viscous mass. Therefore, the SSF fermentation is initiated with a relatively small amount of hydrolyzed residue (2–3% concentration), but with a full charge of a batch of enzyme broth fed from the continuous propagation system. Thus, the fermentation is initiated with a high ratio of enzyme to cellulose fiber, resulting in rapid enzymatic hydrolysis, reduction in solids content, and thinning of the fermenting slurry. Then, by small incremental additions of prehydrolyzed residue, production of ethanol is continued, but at relatively low-solids slurry concentration with moderate viscosity conditions.

After feeding solids on a fed-batch basis for 24–48 h, with total solids fed being approx 16% on an accumulated basis, the fermentation is allowed to continue until an ethanol concentration of 4–4.5% is achieved with conversion of 80% or more of the cellulose to soluble sugars and then to ethanol. The charge of recombinant *Klebsiella oxytoca* added to the fermenter with the initial charging of enzyme and feedstock results in rapid conversion of the cellulose sugars and C_5 sugars to ethanol, so that ethanol inhibition of the SSF fermentation is minimized until the latter hours of the fed-batch fermentation, which may run on the order of 72–96 h.

After completion of a given fed batch operation, the fermented material is pumped in slurry form (containing residual lignin and unconverted cellulose) to a baffle tray beer still stripper designed to accommodate the flow of suspended solids, while ethanol is stripped from the fermented beer.

The beer still operates at atmospheric pressure, utilizing heat from the vapors from a pressurized stripper/rectifer to minimize thermal energy consumption. The overhead vapors from the beer still, after being used to

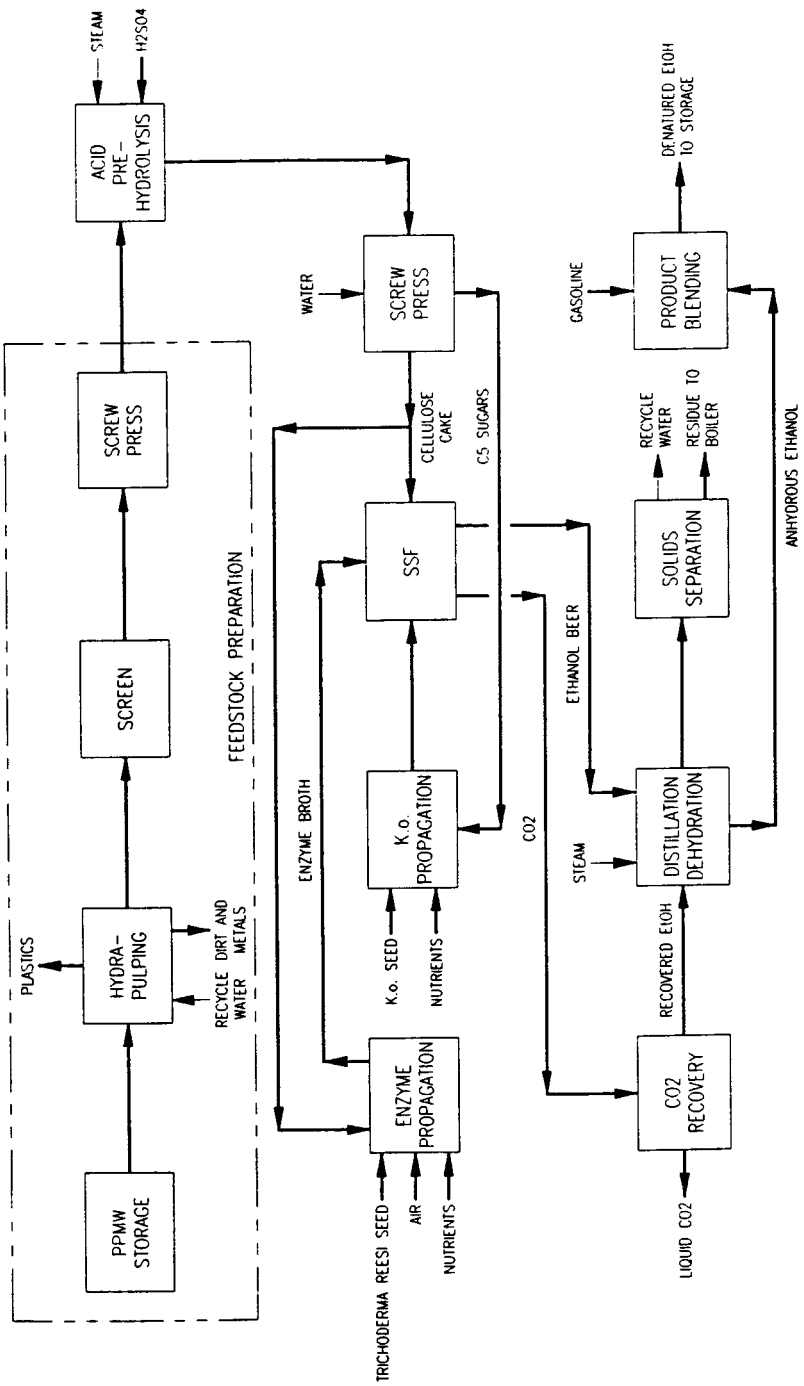

Fig. 3. Pulp, paper, and mill waste ethanol.

preheat incoming feed, pass as condensate to the stripper/rectifier. This is fitted with a steam-driven reboiler, at sufficient steam pressure to permit operation of the stripper/rectifier at a pressure of about 5 Bars. Most of the 95 vol% ethanol vapors overhead from the stripper/rectifier are condensed in the primary reboiler of the beer still. An auxiliary reboiler using steam and one using anhydrous ethanol vapors are also used to boil up the beer still.

Approximately one-third of the rectifier overhead vapors are not condensed, but are sent through a steam-heated superheater to the dual molecular sieve beds for dehydration. Operated with conventional switching controls, the beds are regenerated by use of part of the superheated anhydrous ethanol vapor to remove the water and ethanol content therein. The recovered ethanol water stream is condensed and returned to the rectifier for ethanol recovery. The anhydrous ethanol vapors are condensed in one of the auxiliary reboilers on the beer still. The condensed anhydrous ethanol then passes through a cooler to day tanks for checking, and then to bulk storage with the addition of gasoline denaturant.

Stillage is then processed via a centrifuge to provide good separation and concentration of the residual solids from the liquid fraction (thin stillage). The solids are concentrated to approx 50% moisture, and are then burned in a boiler to produce steam and electricity for the facility. Thin stillage is split into two portions: one for the backset and the other for waste treatment. Because of the strict requirement for water conservation in South Florida, the portion of thin stillage, which is directed to waste treatment, is ultimately recycled into the process after receiving treatment in an on-site reverse-osmosis system.

The negative cost of the raw material, coupled with the ability to produce on-site cellulase enzymes and process steam/power, provide for very favorable facility economics. Despite the conservative costs associated with first-of-a-kind facilities like this one, total operating costs (including depreciation) are expected to provide an ethanol at $< \$0.80$/gal[1] with a capital cost of approx \$3.50/annual gal ethanol capacity.

Pulp and Paper Mill Waste Sludge Conversion

Pulp and paper mills yield sludges from waste-water processing that contain a substantial amount of fibers too fine to be retained on fiber screens and paper machines. Depending on the pulping process used and the amount of recycled paper being processed in these mills, the sludge will contain a varying amount of cellulose, hemicellulose, lignin, and inert fillers, as well as inks and dyes. The conversion of these sludges into ethanol is an economically attractive alternative to current landfill and

[1] This does not reflect the contribution made by tipping fees (i.e., negative feedstock cost).

incineration practices. In order to have an economic and profitable facility, it is generally desirable to design these facilities as merchant plants that will serve as a sludge disposer for several pulp/paper mills in a given geographical region. With landfills being limited and incineration being an expensive resort, disposal of these wastes through an ethanol production facility yields a major improvement in waste-abatement technology (2,3).

The sludges from the various mills are proportioned as uniformly as possible into an agitated reslurrying tank, with provision made for separation of metals and dirt. This system does not require as much power as a hydrapulper, but is similar in arrangement. Depending on lignin content of the feed mixture, an acid prehydrolysis step may be installed to break the lignin/hemicellulose bonds and, thus, make the cellulose fiber more accessible for enzymatic conversion. Enzymes and recombinant *Klebsiella oxytoca* are produced in the same manner as in Mixed-Waste-Paper Conversion, the pulp sludge feedstock being the main substrate for enzyme production. Dextrose can be used for the first stage of fungal propagation and also for the *Klebsiella oxytoca* propagation. Here again, the hemicellulose sugars can be pressed and washed from the cellulose fibers for separation of the C_5 sugar-rich steam should this be desirable for the *Klebsiella oxytoca* propagation and for the first stage of enzyme production.

With the SSF fermentation system being run on a fed-batch mode as in Mixed-Waste-Paper Conversion, the pressed fiber from the acid prehydrolysis and hemicellulose sugar-leaching operation can be fed in a sequential system to a series of several fed-batch SSF fermenters. pH adjustment and filtration removal of calcium sulfate will be the same as in the abovementioned section.

With some lignin and unhydrolyzed cellulose residue leaving the baffle tray stripper, the fibers and lignin fractions will be removed by centrifugation and pressed to a water content $< 50\%$, making the residue suitable for burning in the boilers. The residual stillage can be recycled to the process or, if in surplus, sent to an evaporator for concentration for burning or other disposal means. The stripping, rectification, and dehydration of the ethanol from the fermented beer will be done in the same manner as described in Mixed-Waste-Paper Conversion.

Approximately 7 mo of laboratory and pilot fermentation trials have been completed on pulp/paper sludges from over 13 different facilities. All chemical pulp/paper sludges have shown ethanol yields $> 80\%$ of theoretical without any acid prehydrolysis in < 72 h. Sludges derived from ground wood mills (i.e., newsprint) have shown similar performance, but required acid prehydrolysis because of high native lignin contents.

Overall process economics are similar to that projected in Mixed-Waste-Paper Conversion, except that residue disposal will be higher because of a greater quantity of inert filler ($> 20\%$ of original dry wt). However, because tipping fees are paid on wet sludge, which is usually at 60–70% moisture, these incremental costs are more than offset by the high tipping fees.

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