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Development of Bioconversion of Cellulosic Wastes

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ABSTRACT

Improvements during the past decade in cellulolytic enzymes for conversion of cellulosic and lignocellulosic materials to glucose and by fermentation to ethanol and other products have led to development of a practical commercial process. A pilot plant based on utilization of the advanced *Trichoderma reesei* fungal enzyme systems available, utilized in a fed-batch simultaneous saccharification and fermentation system, has been operated successfully at an eastern pulp and paper mill.

Successive improvements in techniques and operating conditions for this pilot plant, with a capacity of 1 t/d feedstock input, has led to production of ethanol with conversions of 80–90% of theoretical, based on cellulose content of the feedstock. With the data and knowledge in hand, this technology is now ready for use in a proposed demonstration facility with a nominal capacity of 50–100 t/d of feedstock. Projected economics are presented for proposed commercial facilities processing up to 500 t/d of cellulosic wastes.

Index Entries: Bioconversion; cellulolytic enzymes; cellulosic wastes.

INTRODUCTION

For many generations, industry has contended with the need to dispose of wastes and to obtain, if feasible, a profit from their utilization. Lignocellulosic and cellulosic wastes have traditionally been utilized primarily as fuel, but in many cases, are not economically viable for such use. This has required other means of disposal, such as burning forest and municipal wastes and landfilling of process wastes.

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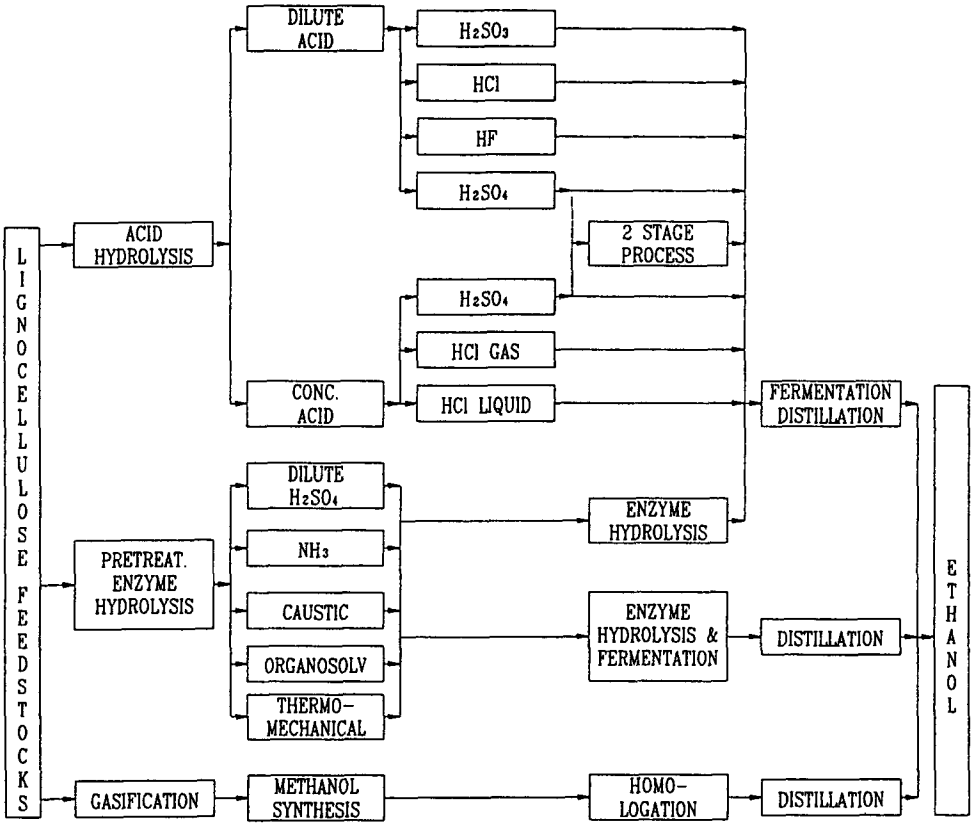

Fig. 1. Ethanol from cellulose and lignocellulose process matrix.

Since the early years of this century, one approach has been to convert the cellulosic fraction of wastes to glucose and subsequently by fermentation to ethanol. Early research, development, and commercialization were based on acid hydrolysis for production of glucose, the primary sugar that is the building block of cellulose (*1*). Since World War II, research has been directed more toward utilization of enzymes to convert such cellulosic wastes to glucose and by fermentation to ethanol. The many alternate approaches to production of ethanol from such wastes are indicated in Fig. 1.

Study of the "green fungus among us" by Reese at the US Army Natick Laboratory after World War II (2), showed that the fungus *Trichoderma reesei* (named after him) produced a basic three-enzyme system; endoglucanases, exocellobiohydrolase, and β-glucosidases. Additional enzymes secreted by the fungus have since been indicated. Thus, nature provided the enzyme system necessary to degrade cellulose in the form of fallen trees and other vegetation. Various mutations of the fungus were developed to improve the desired proportion of the three basic enzymes particularly the β-glucosidase, and to increase the yield and activity of the

enzymes secreted by the fungus (*3*). This combination of cellulolytic enzymes can be produced in an operating facility and will probably have to be incorporated in plant facilities for economic reasons. They are also available commercially from major enzyme suppliers.

Among the various groups carrying on cellulolytic enzyme research, the partnership of Gulf Oil (now Chevron) and Nippon Mining, in the Bio-Research Corporation of Japan, led not only to advances in technology for production of the cellulolytic enzymes, but also the critical and major advance of simultaneous saccharification and fermentation (SSF) (*4,5*).

A 1 t/d pilot plant built and operated by Gulf Oil Chemicals at Pittsburg, KS, starting in the late 1970s, operated with both batch and continuous fermentation. Feedstocks included wood waste, pulp and paper mill waste, municipal solid waste, and various agriculture residues. The key results from application of SSF were a major decrease in retention time required for both the cellulolytic and the fermentation operations, and a major increase in yield (*6*). This was the result of the fact that as fast as the cellulolytic enzymes produced glucose, the fermentation organism converted the glucose to ethanol. Thus, the inhibitory action of glucose on enzyme activity was essentially eliminated. Also, the Gulf/University of Arkansas researchers developed a continuous 48-h (retention time) process for production of the *Trichoderma* fungus and the secreted cellulolytic enzymes, from the same feedstocks. Coupled with a novel enzyme recycle process (*7*), enzyme cost was minimized. More recent work sponsored and carried out by the National Renewable Energy Laboratory of the Department of Energy has led to improvements in the SSF process (*8*).

DEVELOPMENT

Although the Gulf pilot plant was operated for several years, there were shortcomings that limited its application to continuous SSF operation. In 1981, Gulf turned over this program to the University of Arkansas for further research and development. This included the improved enzyme production process, recovery and recycling of cellulolytic enzymes, and continued evaluation of different cellulosic feedstocks. Considerable emphasis was placed at this time on pretreatment methods for breaking the lignin-hemicellulose-cellulose linkages to permit ready access of the enzymes to the cellulose. Such pretreatments included organosolv delignification, and caustic, acid, and thermo-mechanical processing.

Another opportunity developed to advance this technology when a major pulp and paper producer decided to apply this process to its cellulosic waste, which had become a major landfill and waste-disposal (by incineration) problem. A 1 t/d pilot plant was designed by our firm and erected by the company at their pulp mill in Pennsylvania. This included a 2500-gal (9500-L) fermenter.

Table 1
Ethanol from Lignocellulose SSF Fermentation Data

Conditions	Yeast strains		
	Candida brassicae	*Saccharomyces cerevisiae*	
Pilot plant yeast tests[a]			
Total fiber, g (dry basis)	6804	1600	1000
Enzyme dose, g	183.7	43.2	27.0
Enzyme/fiber ratio	0.027	0.027	0.027
Vacuum-recovered ethanol, g	1427	None	None
Final ethanol concentration, g/L	17.3	36.3	32.7
Final volume, L	24.00	11.42	8.12
Total ethanol produced, g	1569	415	265
Ethanol yield, gal/t	69.8	78.5	80.2
Enzyme/ethanol ratio	0.117	0.104	0.102
Pilot plant runs[a]			
Fermentation time, h	192	168	188
Volumetric production rate, g/L/h	0.34	0.22	0.17
Average glucose concentration, g/L	2.40	1.05	0.82
Aeration rate, $ft^3/min/ft^3$	0.020	None	0.002
Temperature, °C	40	37	37

[a] 10,000-L fermenter, fed-batch operation.

Six months of intensive experimentation in this pilot plant, with both primary pulping waste and paper-making waste, resulted in substantial improvement in the efficiency of conversion of cellulose to glucose and ethanol (*9*).

Key improvements that resulted from the relatively short-term operation of this pilot plant related to application of a mild caustic pretreatment combined with steam sterilization (since most of the lignin had been removed by the pulping process) and use of fed-batch techniques in the SSF process. Enzymes from various suppliers were used in the range of 5–15 IU/g cellulose. As indicated in Table 1, runs of up to 190 h were made before a significant reduction in yield was indicated. Aeration was tested and found to have little effect on the SSF process. *Candida brassicae* did not show yields as high as *Saccharomyces cerevisiae*. Total solids processed in the fed-batch pilot runs were in the range of 1000–7000 g/run. Final ethanol concentrations with *Saccharomyces cerevisiae* were in the range of 30–36 g/L.

Yields obtained during the latter two fed-batch pilot plant runs of Table 1 are indicated in Figs. 2 and 3. Yields after each incremental addition of substrate were calculated by measuring increase in ethanol for the total fermenter liquid content, as related in the amount of substrate increment previously added, at 8-h intervals for Fig. 2 and at 4-h intervals for Fig. 3.

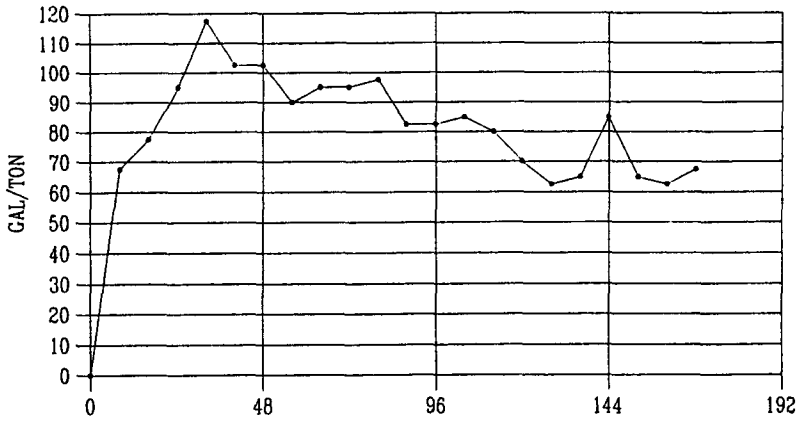

Fig. 2. Ethanol yield base case.

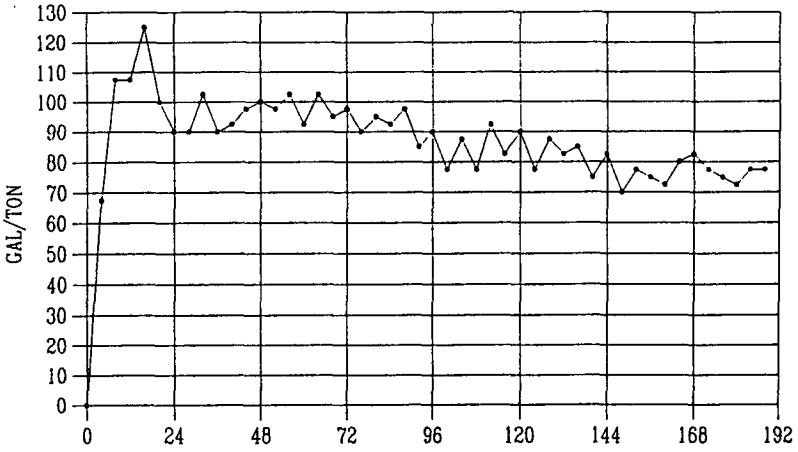

Fig. 3. Ethanol yield with surfactant.

A key item to be noted was the feasibility of operating the SSF system at temperatures as low as 37°C, previously considered too low for optimum cellulolytic enzymatic hydrolysis. Also, this work showed that it is not necessary to use expensive vacuum recovery of ethanol to achieve good yields and reasonable ethanol concentrations.

An interesting development during this pilot plant operation involved the addition of a surfactant, as indicated in Fig. 3, as compared in Fig. 2. This surfactant apparently acts as an agent to facilitate access of the cellulolytic enzymes to the cellulose fiber.

Incidental work carried out by our organization with an industrial client interested in the utilization of bagasse led to substantial pilot plant testing at a 3 t/d rate of a mild-acid prehydrolysis pretreatment. Such pretreatment, carefully controlled with respect to acid use, temperature, and retention time, led to essentially complete hydrolysis of the hemicellulose and recovery of the hemicellulose sugars (primarily five-carbon types) in

an almost quantitative manner without formation of toxic substances, such as furfural. The residue from this mild-acid prehydrolysis was processed in the Gulf SSF system, with theoretical yield of ethanol achieved, based on cellulose content.

ECONOMICS

The economic production of ethanol, and other potential fuel or chemical products from lignocellulose wastes depends on two key factors: (1) the cost or credit value of waste disposal and (2) the cost of cellulolytic enzymes. With respect to the lignocellulosic feedstocks, cost may vary from as much as $40.00/t, depending on the method of collection, storage, and shipping distance, to a negative value (tipping fee), based on the concept that the waste is such a problem that the source company is willing to pay to have it removed. Alternatively, the negative cost of displaced landfilling may be considered a credit for disposal of the waste.

With respect to the cellulolytic enzymes, costs are currently based on the type and quality of enzyme being provided from commercial sources, or alternatively, the cost of producing enzyme in-house. In the initial work at the Natick Laboratory, going back several decades, enzyme cost was several dollars per gallon of ethanol. About 10 years ago, Natick indicated that this cost had been reduced to 57¢/gal of ethanol. In the work carried out by Gulf Oil, through a variety of studies, it was indicated that in-house enzyme could be produced for as low as 20¢/gal of ethanol and possibly could be reduced to half that cost by recycling.

To bring economics up to date, we have developed an operating cost estimate for a projected 10 million gal/yr (38.67 MM LPY) commercial facility processing mixed waste papers and woody wastes, utilizing mild acid prehydrolysis as a pretreatment, in-house production of cellulolytic enzymes, and the SSF process. These costs include distillation and dehydration of the ethanol product to motor fuel grade specification and utilization of residues as fuel. For zero or negative cost feedstock, about 20% additional material is brought in to compensate for lower yields, and to provide sufficient fuel to provide all steam and electric power needs. It is assumed that reduced investment in simplified process needs will counterbalance cogeneration power and steam investment. Allowance for waste treatment is also included. Investment would be on the order of $30 million.

With the process adjusted for yields of approx 100 gal of ethanol/t of high-cost feedstock, and 80 gal/t of zero or negative cost feedstock, the total cost is obviously affected primarily by the feedstock charge. This can range from 40¢/gal (10.57¢/L) to a credit of 30¢/gal (7.92¢/L).

On this basis, the bottom line of production costs, before fixed charges, can range from $1.18/gal (31.17¢/L) to $0.27/gal (7.18¢/L). Fixed charges, including pretax return, have been estimated for the proposed facility at $0.82/gal (21.60¢/L), as indicated in Table 2. Potential credits for byproduct fuel value are negligible and for byproduct chemicals value, unproved.

Table 2
Ethanol from Lignocellulose Production Cost
(10 MM GPY; 38.7 MM LPY)
($30 MM Investment)

	Maximum, $40/t		Mid, $0/t		Minimum, $20/t	
	$/Gal	¢/L	$/Gal	¢/L	$/Gal	¢/L
Variable costs						
Feedstock	0.40	10.57	0.00	0.00	(0.30)	(7.92)
Chemicals	0.50	1.30	0.05	1.30	0.04	1.10
Media (*T. reesei* & SSF)	0.15	4.00	0.15	4.00	0.10	2.60
Fuel	0.05	1.30	0.00	0.00	0.00	0.00
Power	1.10	2.60	0.00	0.00	0.00	0.00
Total variable costs	0.75	19.77	0.20	5.30	(0.16)	(4.22)
Fixed costs						
Labor and supervision	0.18	4.80	0.18	4.80	0.18	4.80
Maintenance	0.08	2.10	0.08	2.10	0.08	2.10
Insurance and taxes	0.15	4.00	0.15	4.00	0.15	4.00
G & A costs	0.02	0.50	0.02	0.50	0.02	0.50
Total fixed costs	0.43	11.40	0.43	11.40	0.43	11.40
Total plant gate costs	1.18	31.17	0.63	16.70	0.27	7.18
Fixed charges						
Sales costs	0.02	0.50	0.02	0.50	0.02	0.50
Depreciation (15 yr)	0.20	5.30	0.20	5.30	0.20	5.30
R.O.I.—20% pretax	0.60	15.80	0.60	15.80	0.60	15.80
Total fixed charges	0.82	21.60	0.82	21.60	0.82	21.60
Selling price	2.00	52.77	1.45	38.30	1.09	28.78

CONCLUSIONS

With the accumulated data and knowledge resulting from the Gulf and pulp mill pilot plants, along with recent advances in improved cellulolytic enzyme systems and the research carried out by and for the National Renewable Energy Laboratory, it is now feasible to extend and improve this technology by construction of a process demonstration unit (PDU). It would have a recommended daily capacity of 50–100 t of feedstock (dry basis). Such a PDU would have the capability of treating a variety of lignocellulosic wastes, including municipal (cellulosic fraction), pulp and paper mill, agricultural, and forest residues. With such a PDU, technology can be advanced to permit design and construction of full-scale commercial facilities within the next three years.

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