THE ACIDIC AND ENZYMATIC SACCHARIFICATION OF RICE STRAW

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Introduction

The energy crisis which started in 1972 spurred the Philippine government to embark on a long-term and ambitious program of developing its indigenous energy resources. These included the geothermal, dendrothermal, hydro and coal resources as well as agricultural wastes. The latter included such materials as sugarcane bagasse, rice straw and hull, sawdust, coconut coir dust and coconut expeller residues, and fruit processing wastes among others. These local energy resources have been tapped at such a fast rate as to reduce to about 50% the share of imported oil on the total energy consumed for 1984 from a high of 90+ % in the early 1970s.

The utilization of agricultural wastes also solves the problem of their disposal. These wastes are generally starchy or cellulosic in composition and are converted into more adaptable forms either by biological or thermal degradation. In the Philippines, some of the process technologies have been developed or adapted and are being field-tested or under commercial application. Prime examples are bio-gas digesters utilizing animal wastes, pyrolysis of rice hulls,* ethanol from molasses and cassava, and gasification of wood and other cellulosic materials.

Of particular focus in this report is the bioconversion of rice straw into ethanol using two processes: acidic and enzymatic saccharification. A comparative processes on the technical and economic levels is presented in Section 4.0. No attempt has been made to discuss fermentation except when necessary and relevant.

Production Estimates of Rice Straw.

Being primarily a rice-producing country, the Philippines produces large amounts of rice straw after each harvest season. These are generally used as animal feed, burned or left to decompose into organic fertilizer in the field.

Production estimates of rice straw in 1982 are presented in Table 1. Other agricultural wastes are also included for comparison.

If, on the average, rice straw contains 9% moisture, has a saccharification efficiency of 26%, 43% hexose content and 8% ethanol conversion efficiency*, then 6.6 million metric tons of wet rice straw would be equivalent to 6440 barrels of oil per year (corrected for differences in the heating values). At $28/barrel, this means an annual savings of $180,320 from crude oil importation.

Proximate Analysis of Rice Straw.

Table 2 next page shows the proximate analysis of rice straw. Again, other cellulosic materials are included for comparison. Take note that the lignin and hemicellulose content of rice straw varies from 40-50%. This makes bioconversion to ethanol difficult. Its high ash content makes it an ideal component or substitute in cement mix for hollow-blocks manufacture and general construction.

Table 1. Production Estimates of Agricultural Wastes in 1982

Waste Material	Annual Production (In million metric tons)
Rice straw	6.6[a]
Rice hull	1.2[a]
Sugarcane bagasse	7.1[a]
Corn cobs	1.1[a]
Molasses	0.7[b]
Cococnut endosperm residue	0.4[c]
Banana fruit wastes	0.02[d]
Coconut water	2.2

Ref: del Rosario & Gonzales (1982)

[a]Based on 9% moisture content.

[b]Based on 89% solids.

[c]Fat-free and dry basis; potential amount calculated on dry basis of aqueous process yield of 180 g dry, fat-free, less than 8% protein residue per kg. of coconut meat.

[d]Based on 80% moisture.

Table 2. Composition of Rice Straw and Selected Cellulosic Materials

Materials	Cellulose, %	Hemicellulose, %	Lignin %	Ash %
Rice straw & rice hull	35–45	20	20–30	15–20
Sugarcane bagasse	40–50	20–30	18–20	4
Coconut expeller residue	25–34	60–69	6.5	1
Coconut coir dust	24.2	27.3	54.8	6.2
Wood	40–50	20–30	18–20	4

Ref: del Rosario (1978)

Cellulose is an insoluble polymer made up of glucoside (6 carbon sugars) and xylan (5 carbon sugars) units. It is crystalline in form thereby inhibiting reaction with water. During hydrolysis, the chain is broken up through the attachment of water molecules.

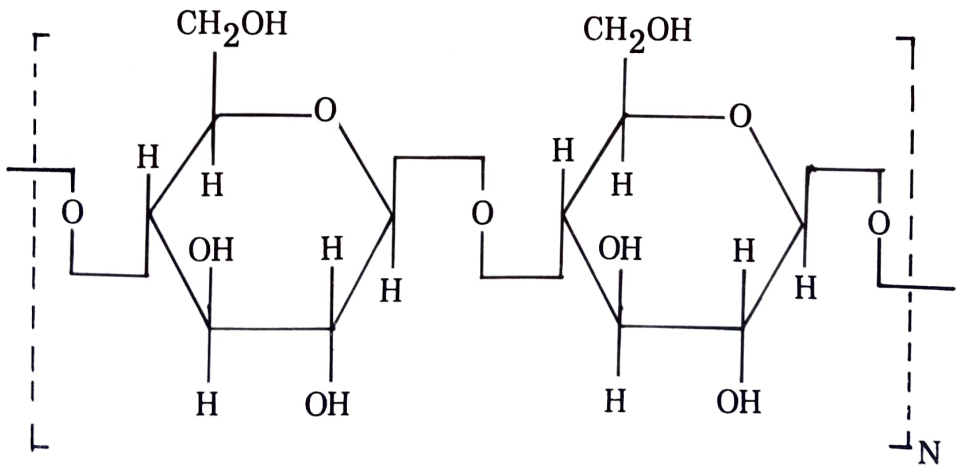


Figure. 1. Cellulose

In hemicellulose, xylan is the predominant saccharide. Lignin is a highly aromatic polymer which in living plants serves as the "glue" which holds cellulose fibers together and lends structural integrity to the stems or stalks. The presence of both hemicellulose and lignin tends to protect cellulose from hydrolytic attack. Thus, any method of reducing cellulose to sugars must include some form of pre-treatment which will, either chemically or mechanically, lessen the inhibiting effect of lignin and hemicellulose and which will destroy the crystallinity of cellulose.

A Generalized Ethanol Production Scheme.

The technology for converting cellulose to ethanol is relatively simple and, for some materials, economically promising. Fig. 2 shows a generalized process scheme.

Fig. 2. Generalized Ethanol Production Scheme

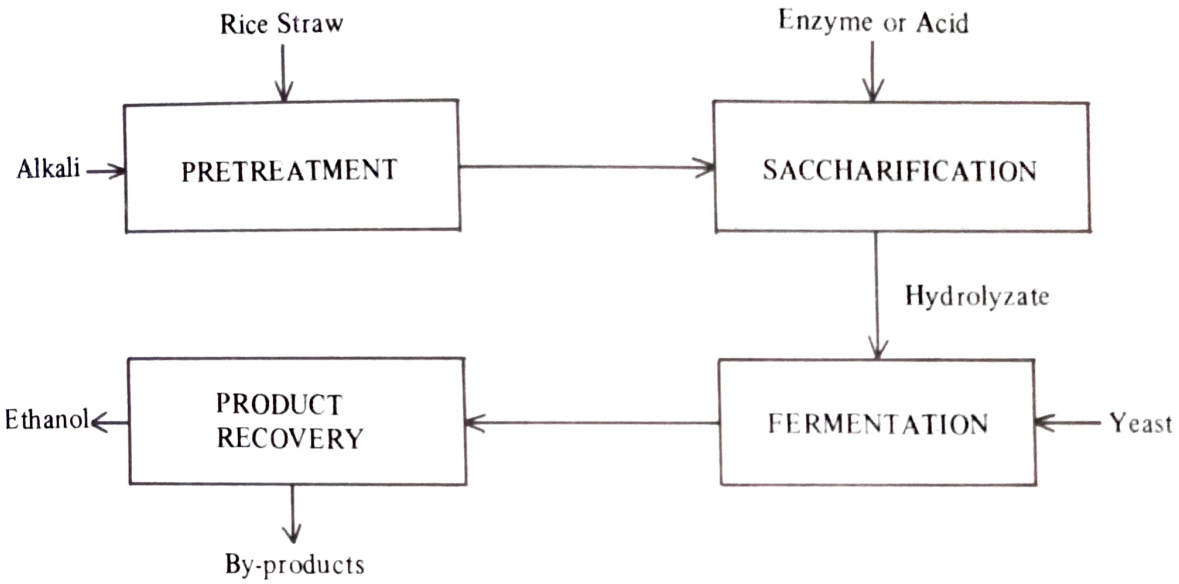
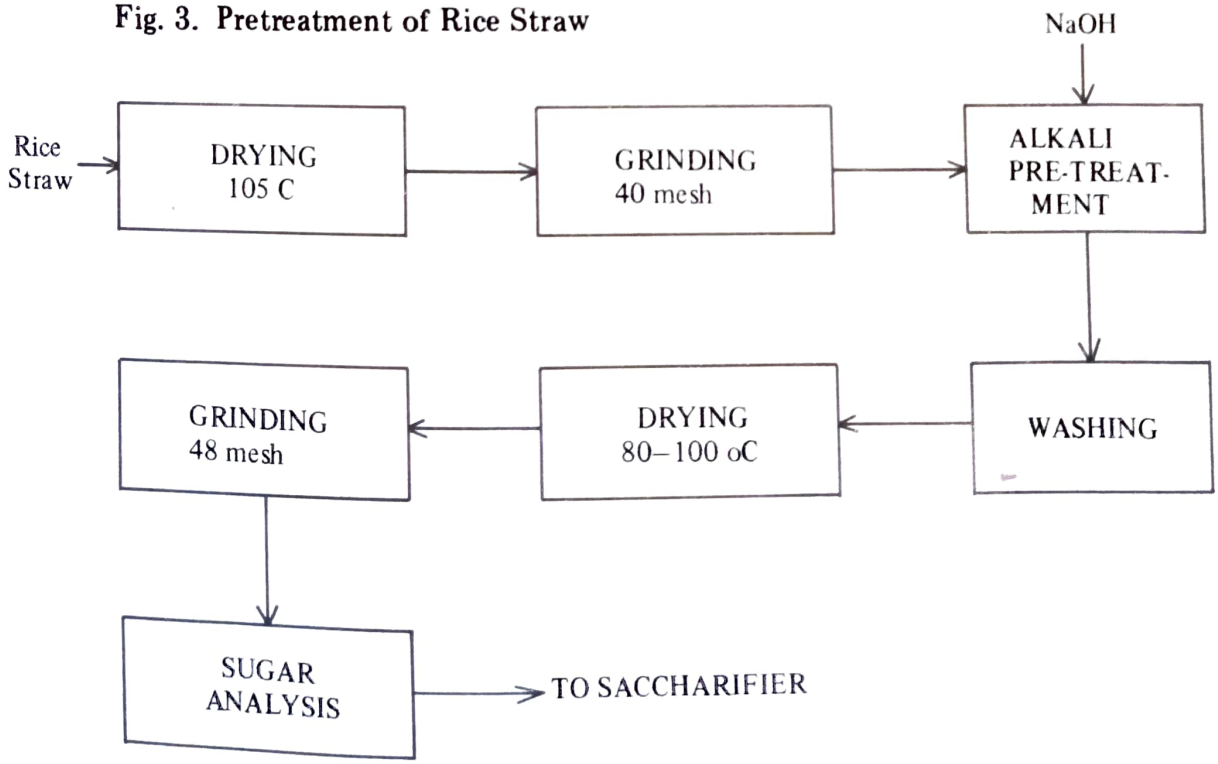


Fig. 3. Pretreatment of Rice Straw



Pretreatment of the raw material results in decrystallization and partial delignification thus rendering the cellulose polymer susceptible to hydrolytic attack. Hydrolysis may be carried out using either an acid or an enzyme. Simple sugars are produced from the breakdown of the polymer chain. It is for this reason that the term *hydrolysis* is interchangeably used with *saccharification.* For starchy materials, glucose is produced; for cellulosic materials, the reducing sugars are composed of pentoses, hexoses, cellobiose and oligomers. Hexose, a 6-carbon sugar, is the basic feedstock in ethanol production. Pentose, a 5-carbon sugar, is not easily fermentable into ethanol. Therefore, it is important that the hydrolyzate should contain a significant amount of hexose. Del Rosario, Gonzales, et.al. (1977) reported that the maximum hexose content in the hydrolyzate should be 1.5%. In certain instances, then, it may be necessary to adjust first hexose concentration to the desired level before feeding into the fermenter. Details of the pretreatment and saccharification operations will be separately discussed in this report.

Fermentation of hexose into ethanol is enzymatically catalyzed. Experiments conducted by UPLB and NIST made use of a local strain of *Saccharomyces cerevisiae* (papaya strain L 15 Kr). Conversion efficiency, unfortunately, is low at 16% (actual alcohol yield/theoretical amount based on the initial hexose level of the hydrolyzate), particularly when compared with results obtained for sugarcane bagasse and coconut expeller residue (sapal). Research thrusts on this particular phase of the conversion process should focus on improved fermentation techniques and fermenter design, and identification or development of better microorganisms and strains. Recovery of ethanol from the product mix is generally done by distillation or solvent extraction.

Current Status.

The conversion of rice straw to ethanol is still in its stage of infancy in the Philippines. It appears that only two institutions — U.P. Los Banos, particularly the Institute of Biotechnology and Applied Microbiology, and the National Institute of Science and Technology (NIST) — are doing pioneering research on this field. Preliminary results have not been very encouraging, both technically and economically. Further research is needed in practically all phases of the bioconversion process, from pretreatment techniques to new fermenter designs and better strains of yeasts which have high specificity towards ethanol production. Extensive use of indigenous materials should also be explored (i.e. culture medium for microbial growth) in order to drastically reduce total production cost.

Pretreatment of Rice Straw

A schematic representation of pretreatment of rice straw is shown in Fig. 3. Pretreatment of rice straw is very important to the success of hydrolysis (saccharification). Some approaches which are applicable to any cellulosic material involve an acid pretreatment which dissolves the hemicellulose and loosens the lignin thereby exposing the cellulose (Huff, 1981). This method, however, has the disadvantage of corrosion and inconvenience of acid handling and subsequent neutralization, and

is not effective in cellulose decrystallization. Other processes use solvents in the dissolution of lignin but these are very complex and uneconomical.

Mechanical pretreatment in the form of fine grinding seems to be the simplest and most practical pretreatment technique. Grinding increases the surface area and renders cellulose more susceptible to hydrolytic attack. The main problem with fine grinding, however, is the intensive energy requirement. Improvement of attrition mill designs may reduce energy consumption drastically.

Alkali pretreatment increases the cellulose content and partially reduces the lignin content of rice straw as shown in Table 3. The mechanism involved here is similar to acid pretreatment. Thus, a pretreatment scheme involving fine grinding and alkali pretreatment, done serially, will serve the purposes of decrystallizing cellulose and delignifying the raw materials.

Table 3. Mositure, Cellulose and Lignin Contents of Treated and Untreated Cellulosic Materials

Material	% Moisture	% Cellulose*	% Lignin*
Rice Straw			
Untreated	9.8	34.7	36.6
Treated	5.5	78.9	16.5
Rice Hulls			
Untreated	9.8	28.8	35.8
Treated	4.4	63.8	32.9

Ref: del Rosario, Gonzales, et. al. (1977)

* Dry basis.

Saccharification Processes

The processes described and data presented in the succeeding text were culled from results of experiments conducted by the UPLB-NIST team. The two process schemes — acidic saccharification and enzymatic saccharification — are shown in Figs. 4a and 4b. Having been done on an experimental scale and still very preliminary in approach, no attempt has been made by the researchers to project parameters such as saccharifier dimensions and design, retention time, filtration rates, etc. into a bigger scale via dimensional analysis. Operating conditions used here are by no means final and conclusive and are thus subject to further verification.

Enzymatic Saccharification.

Two fundamental unit operations are involved and these are enzyme preparation and saccharification (Fig. 4).

Enzyme preparation.

Enzyme preparation was done through the aerobic fermentation of a mold called *Trichoderma viride* strain ITCC—1433. The fungus was first precultured on potato dextrose agar for 6 days and then aseptically grown in rum bottles using 2:1 rice bran-rice hull mix as substrate. The enzyme produce is known as cellulose and crude extracts of this were used in saccharification.

In a related experiment done by Vilela, et.al. (1976), it was shown that cellulase could be produced in semisolid cultures of rice bran, rice straw and rice hulls. *Trichoderma viride* QM 9414 produced higher cellulolytic activity on carboxymethyl (CM) cellulose (Cx activity) than ITCC—1433. One Cx activity unit is defined as the amount of enzyme which produces 4 mg. of reducing sugars from CM—cellulose in 1 hour at 50 C. Also, a higher Cx activity was obtained from a rice straw-rice bran mixture of 30% rice straw. Significant cost reduction could be realized with this method.

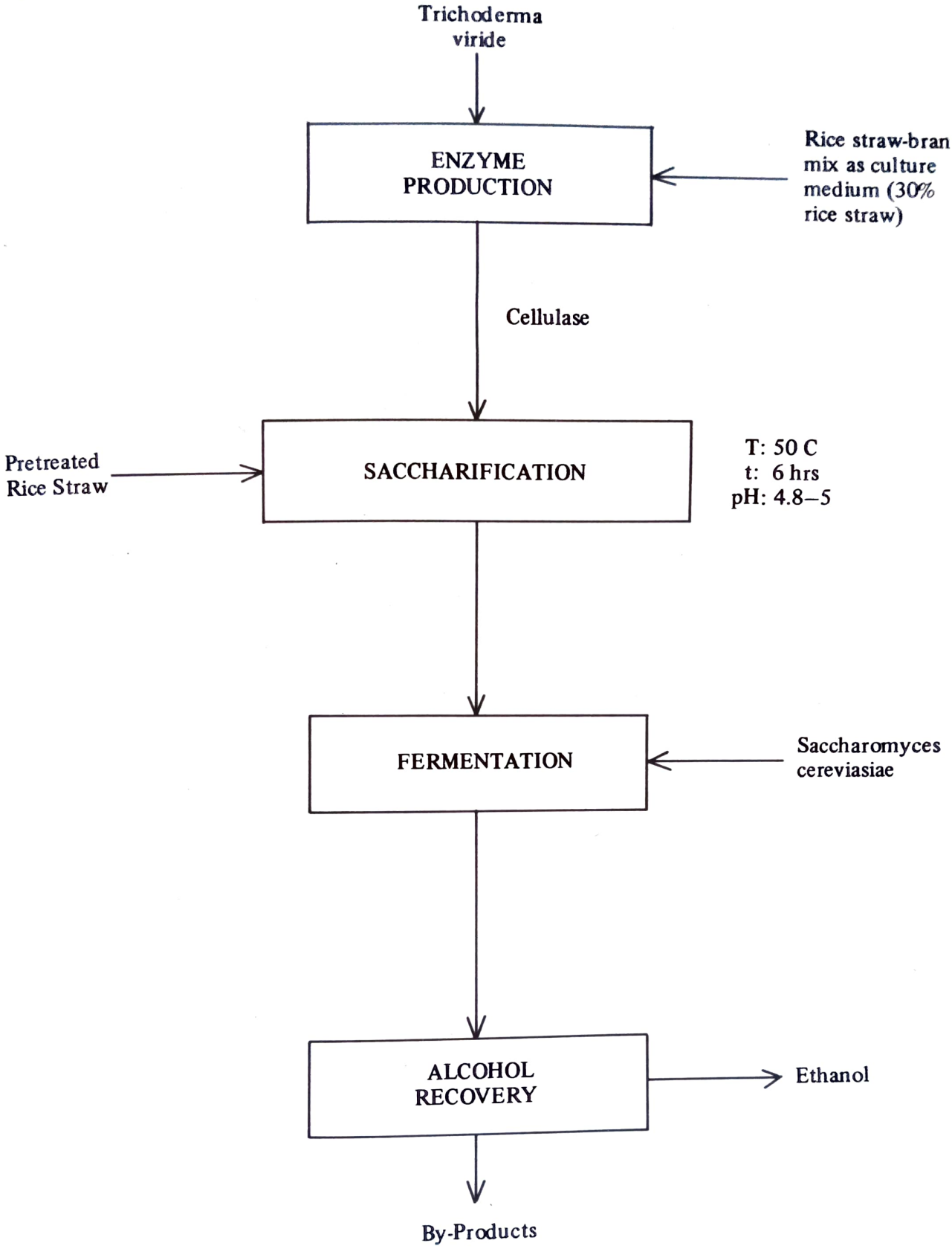


Fig. 4a. Enzymatic Saccharification of Rice Straw

Enzymatic Saccharification.

The pretreated substrate was incubated with the cellulase extract at a pH 4.8—5 at 50 C for 6 hours. The reaction was stopped by placing the incubation mixture in boiling water for 15 minutes. The mixture was quickly filtered and the amount of reducing sugars was determined in the filtrate (hydrolyzate) using the dinitrosalicylic acid or Shaffer-Somogyi method. The hydrolyzate thus obtained was used as substrate for fermentation.

Results.

Sugar composition and sugar yield are presented in Tables 4 and 5, respectively. Results show that alkali pretreatment improved the saccharification of rice straw. The specificity of enzyme cellulase for hexose is evident in Table 4 where the percentage of hexose is largest among the various reducing sugars. It is interesting to note that the hexose fraction in untreated rice straw is greater than in treated straw, however. Also, no pentose is present in untreated rice straw. This is because hemicellulase, which predominantly contains the 5-carbon sugars, was rendered insusceptible to hydrolytic attack in the absence of both chemical and mechanical pretreatment. This was not the case with pretreated rice straw where a considerable fraction of xylan was converted into pentoses, thereby reducing the fraction of hexose in reducing sugars composition.

Table 4, however, does not give the absolute sugar content. Sugar yields, expressed in terms of saccharification efficiency (wt reducing sugar/wt original

Table 4. Sugar Composition of Enzyme Hydrolyzates

	Cellobiose *Oligomers, %	Hexoses %	Pentoses %
Untreated rice straw	37.4	68.8	9
Alkali-treated (soaked 18 hrs)	30.8	52.6	16.7
Alkali-treated (boiled 1 hr)	23.1	43.3	33.6

Ref: del Rosario, Gonzales, et. al. (1977)

Table 5. Enzymatic Saccharification Efficiency

Treatment	Reducing Sugars in Hydrolyzate, %	Sacchar. Eff. %	Col. 1 x Col. 2
Boiled for 30 min*	2.9	27.1	78.6
Boiled for 1 hr*	2.8	26.4	73.9
Boiled for 1½ hr*	2.6	25.2	65.5
Boiled for 2 hrs*	2.7	25.3	68.3
Soaked for 18 hrs*	2.3	21.5	49.4
Untreated	0.8	6.8	5.4

Ref: del Rosario, Gonzales, et.al. (1977)

Conditions: 48-100 mesh size; 10% substrate (w/v);
pH 4.8; 24 hrs at 50 C; Cx = 6.5 units/ml

*Delignified with NaOH.

dry weight sample) are shown in Table 5. In the first column it is observed that untreated rice straw has a low sugar content in hydrolyzate. Thus, even with a higher hexose fraction, the absolute hexose content in untreated rice straw is still lower for equal volumes of the hydrolyzate in comparison with treated rice straw (i.e. by a factor of (0.688) (O.008)/(0.526) (0.023) = 0.455 relative to alkali-treated straw, soaked for 18 hrs).

Column 2 in Table 5 gives the degree of conversion of polysaccharides into simple reducing sugars. Saccharification efficiency for treated rice straw at 25% is much higher than for untreated rice straw at 6.8%.

A measure of the absolute amounts of reducing sugars should take into consideration both reducing sugar content and saccharification efficiency. Substantial discussion of this is presented in Section 3.2.

Table 6 presents the alcohol yields for the enzymatic hydrolyzate. On the average, lower alcohol yields based on hexose content were obtained. Untreated rice hull hydrolyzate passed through an anion exchanger, however, gave exceptionally high alcohol yields. This seems to indicate that higher fermentation efficiency may be obtained by passing pretreated rice straw hydrolyzate through an anion exchanger considering that it has even a higher initial sugar content. This is an area where further research may be pursued.

Table 6. Alcohol Fermentation of Enzyme Hydrolyzates

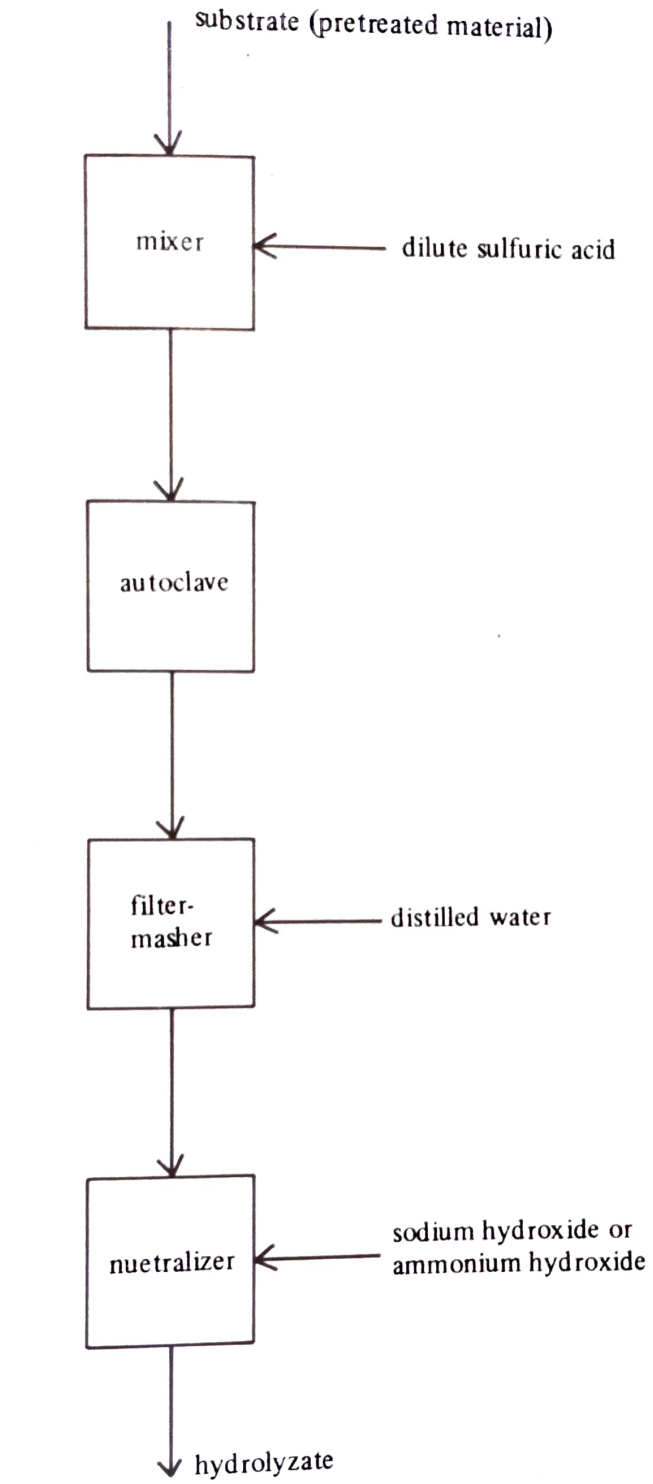
	Initial Sugar %	Residual Sugar %	% Alcohol by Wt. of Hydro. After 48 hrs	Eff. of Alcohol Ferment.
Alkali-treated				
Soaked overnight	9.9	6.5	0.1	3.0
Boiled 1 hr	11.5	10.2	0.2	8.0
Untreated rice hull (Anion exchanger)	6.3	1.4	2.0	81.7
Untreated rice hull (Cation exchanger)	9.4	7.8	0.2	5.6

Ref: del Rosario, Gonzales, et. al. (1977)

Acidic Saccharification.

Another biological method of saccharification of rice straw is through acid hydrolysis. The high cellulose content of rice straw causes it to have high crystallinity and, hence, a greater resistance to hydrolytic attack. Therefore, any method of reducing cellulose to sugars must include pretreatment which will destroy crystallinity.

Acid hydrolysis has been studied for many years and one of the most extensive investigations on the acid hydrolysis of rice straw was done in 1977 (del Rosario, Gonzales, et.al.). The study involved a pretreatment process which was also used in enzymatic saccharification after which dilute sulfuric acid was added to the substrate in a desired ratio, thoroughly mixed and placed in an autoclave or cooker. The pressure and time of autoclaving was regulated. The acid-treated material was then filtered under suction and washed sparingly with distilled water. The combined filtrates were then neutralized using NaOH or NH_4OH. The flow diagram for the process is shown in Fig. 5.



FLOW DIAGRAM OF
ACID SACCHARIFICATION

Fig. 4b.

Optimization of operating conditions.

Several parameters were used in acid hydrolysis of rice straw and these are indicated below:

Parameters	Range of Values
Substrate: acid ratio (SAR)	1:3, 1:4, 1:5
H_2SO_4 concentration, %	1, 3, 5
Autoclaving time, min	30, 60
Pressure, psi	20,24

Each of these parameters was varied so as to determine a combination of parameters that would give the optimum result. At the optimum condition, both saccharification efficiency and reducing sugar content are optimized in order to obtain the highest level of fermentable sugars in the hydrolyzates.

Results are shown in Table 7 and graphically presented in Figs. 5a to 5d.

Discussion of results.

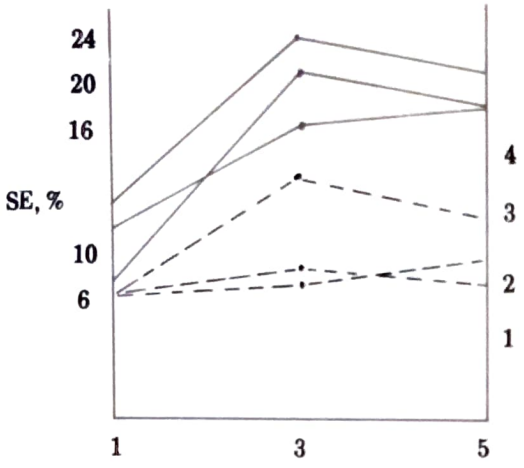
The tabulated results give a maximum saccharification efficiency at 23.8% at 20 psi, autoclaving time of 60 minutes, SAR of 1:5 and 1% acid concentration. However, this combination of parameters did not result in a maximum reducing sugar concentration. In effect, therefore, maximum reducing sugar concentration does not necessarily occur at the point where saccharification efficiency is also maximum. This observation is supported by the tabulated results which shows that the maximum sugar concentration of 3.8% occurs at a saccharification efficiency of only 23.6%.

SE

substrate acid ratio I:3
substrate acid ratio I:4
substrate acid ratio I:5

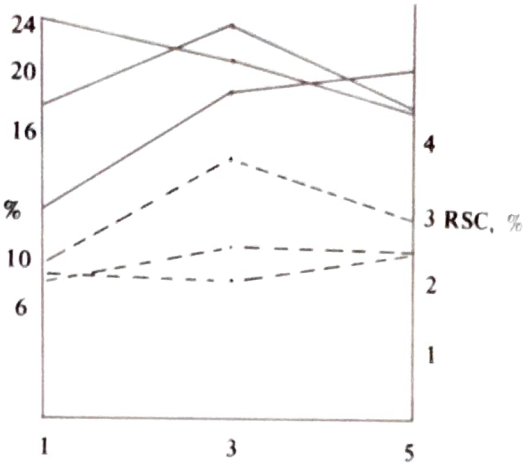
RSC

Fig. 5a.



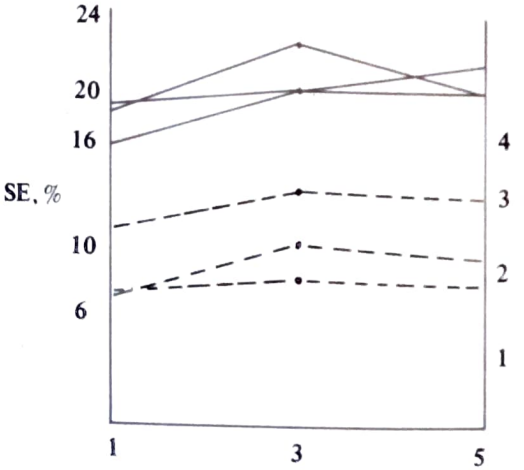
sulfuric acid conc., %
pressure = 20 psi
time = 30 min.

Fig. 5b.



sulfuric acid conc., %
pressure = 20 psi
time = 60 min.

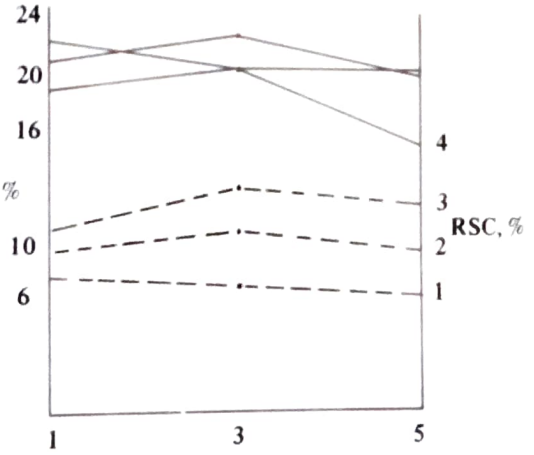
Fig. 5c.



sulfuric acid conc., %

pressure =24 psi
time = 30 min.

Fig. 5d.



sulfuric acid conc., %

pressure = 24 psi
time = 60 min.

note:
SE – saccharification efficiency
RSC – reducing sugar concentration

Table 7. Saccharification Efficiency and Reducing Sugar Concentration of Rice Straw Using Dilute Sulfuric Acid

SAR	Time Min.	Sulfuric Acid Conc., %											
		1.0				3.0				5.0			
		Pressure, psi				Pressure, psi				Pressure, psi			
		20		24		20		24		20		24	
		SE	RSC	SE	RSC	SE	RSC	SE	RSC	SE	RSC	SE	RSC
1:3	30	8.0	1.7	16.6	2.9	20.7	3.6	19.9	3.5	18.3	3.0	20.9	3.3
	60	18.4	2.4	19.3	2.7	23.6	3.8	20.4	3.3	18.3	3.0	20.2	3.1
1:4	30	11.5	1.6	19.0	1.8	17.6	2.0	20.0	2.7	18.3	2.4	19.3	2.4
	60	12.3	2.0	20.9	2.3	19.7	2.6	22.3	2.7	20.9	2.5	20.1	2.3
1:5	30	12.8	1.6	18.4	1.9	22.2	2.2	22.6	2.2	20.6	2.0	19.2	2.0
	60	23.8	2.2	22.0	2.0	21.7	2.1	20.5	1.8	18.2	2.4	15.8	1.7

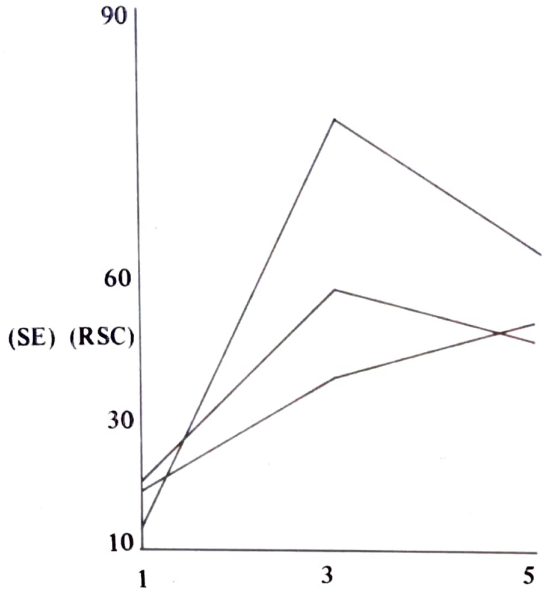
SE = saccharification efficiency, %
RSC = reducing sugar content, %

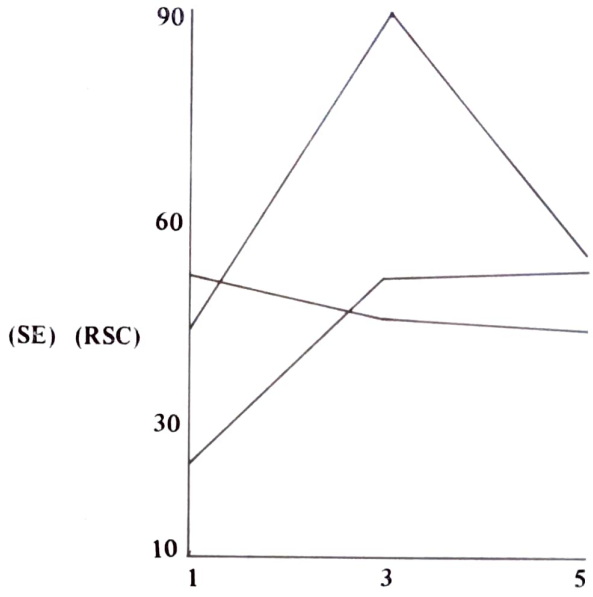
The effects of varying the different parameters are shown in Figs. 5a to 5d. The determination of the optimum conditions takes into consideration the product of saccharification efficiency and reducing sugar content. Since saccharification efficiency represents the degree of conversion of cellulose into simple sugars and reducing sugar content represents the amount of reducing sugar per unit volume of the hydrolyzate, the greater the product is the higher is the degree of saccharification. Computed results are presented in Table 8 and Figs. 6a-6d. From the results, the optimum operating conditions are: SAR, 1:3; sulfuric acid concentration, 3%; autoclave pressure, 20 psi; autocliving time, 60 minutes. Saccharification efficiency is 23.6% and reducing sugar content is 3.8%.

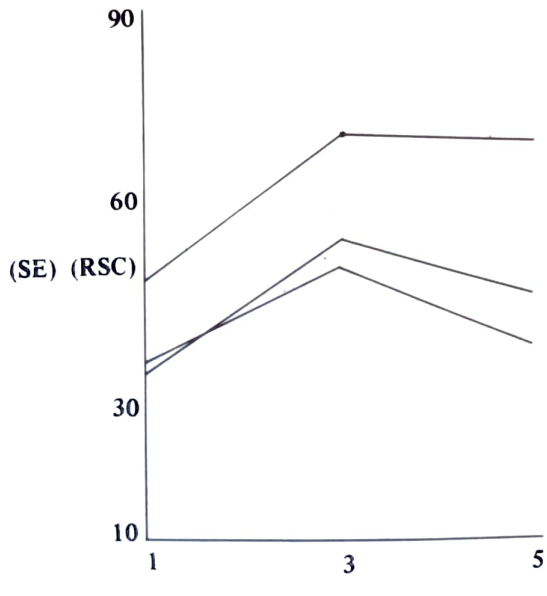
substrate acid ratio I:3

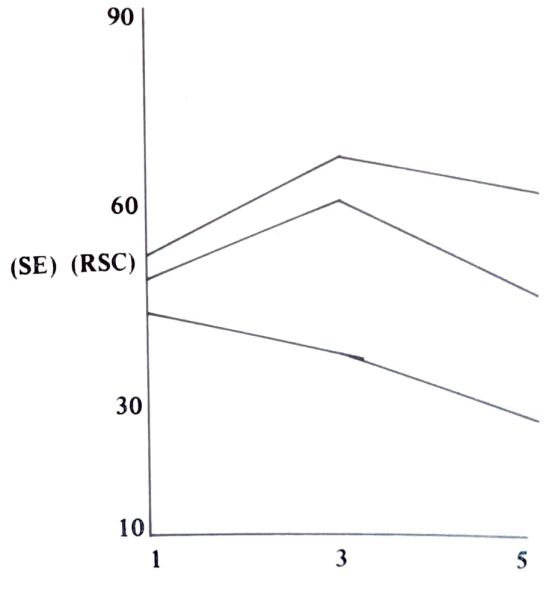
Substrate acid ratio I:4

substrate acid ratio I:5









SACCHARIFICATION EFFICIENCY x REDUCING CONTENT
OF RICE STRAW USING ACID HYDROLYSIS

Table 8. Saccharification Efficiency x Reducing Sugar Content in the Acid Hydrolysis of Rice Straw

SAR	Time min	Saccharification Eff x Reducing Sugar Content					
		Sulfuric Acid Conc. %					
		1.0		3.0		5.0	
		Pressure, psi		Pressure, psi		Pressure, psi	
		20	24	20	24	20	24
1:3	30	13.6	48.14	74.52	69.65	56.40	68.97
	60	44.16	52.11	89.68	67.32	54.90	62.62
1:4	30	18.40	34.20	35.20	54.00	43.92	46.32
	60	24.60	48.07	51.22	60.21	52.25	46.23
1:5	30	20.48	34.96	48.84	49.72	41.20	38.40
	60	52.36	44.00	45.57	36.90	43.68	26.86

Comparative Analysis of the Enzymatic and Acidic Saccharification Processes

Table 9 shows the sugar composition of rice straw and sugarcane bagasse. It could be noted that, in general, enzyme hydrolyzates have higher hexose content than acid hydrolyzates. Since the alcohol yield is dependent on hexose content, it appears that the enzymatic process is better than the acidic process. This is true, however, insofar as sugar composition of the hydrolyzates is concerned.

The production efficiency of alcohol using substrates of sugarcane bagasse (included for comparison purposes) and rice straw is shown in Table 10. In this table, the efficiency of alcohol fermentation using acid and enzyme hydrolyzates is indicated. It could be observed that, in general, the efficiency of alcohol fermentation based on hexose content is higher for acid hydrolyzates. It seems that, based on alcohol efficiency alone, acid hydolysis is better than enzyme hydrolysis. It should be kept in mind, however, that the efficiencies indicated are based on the hexose content which was shown to be better for the enzyme hydrolyzate in Table 9. No. definite conclusion could therefore be made regarding the question as to which of the two saccharification processes should be recommended. There are a number of factors that have to be considered, too, for instance, economics. Since the two processes are still in the laboratory stage, no accurate cost estimates are available.

Acid hydrolysis has been studied for many years and was practiced by both the Germans and the Japanese during World War II.It is said that the technology is still being employed in the USSR. In 1947, a demonstration plant was built and operated in the U.S. west coast but this was shortly shut down because of the unfavorable economics.

Table 9. Sugar composition of acid and enzyme hydrolyzates.

Material	Cellubiose & Oligomers %	Hexoses %	Pentoses %
A. Acid Hydrolyzates			
Untreated rice straw	5.2	22.1	72.7
Alkali-treated rice straw	3.4	13.9	82.7
Sugarcane bagasse	2.6	43.3	54.1
B. Enzyme Hydrolyzates			
Untreated rice straw	37.4	68.8	9
Alkali-treated rice straw	30.8	52.6	16.7
Sugarcane bagasse (milled & treated)	24.1	72.3	3.6

Table 10. Alcohol fermentation of acid and enzyme hydrolyzates.

Hydrolyzate	Initial sugar %	Residual sugar %	Alcohol by wt. of hydrolyzate after 48 hours	Efficiency of alcohol fermentation based on hexose content, %
A. Acid Hydrolyzates				
Untreated rice straw	8.1	7.4	0.2	16.0
Untreated sugarcane bagasse	8.0	6.5	0.4	28.6
B. Enzyme Hydrolyzates				
Alkali-treated rice straw	9.9	6.5	0.1	3.0
Untreated sugarcane bagasse	3.3	0	1.1	90.0

Source: del Rosario et. al., 1978.

The acid hydrolysis of cellulosic materials is usually much more severe than the hydrolysis of starch. This involves the use of acid and materials subjected to elevated temperature and pressure. This treatment serves both to overcome the effects of lignin, hemicellulose and cellulose crystallinity and also to hydrolyze the cellulose. In general, however, acid hydrolysis has been found to have the following disadvantages: (a) corrosion problem, (b) difficulty in the separation of sugar from the hydrolyzate and this requires neutralization, (c) lower saccharification efficiency, (d) presence of numerous by-products stemming from further degradation of sugars, and (e) use of specialty alloy and high pressure construction for the equipment. Because of these disadvantages, acid hydrolysis of cellulosic materials has never succeeded as a commercial venture.

Due to these difficulties, enzymatic hydrolysis has of late gained considersiderable interest. Enzymatic hydrolysis of cellulose has the following advantages over acid hydrolysis: (a) mild reaction conditions (temperature and pH), (b) sugar liquor produced by the enzymatic process can be used directly for growing microorganisms or yeasts, or can be separated using ultra-filtration techniques, (c) no corrosion and higher sugar yield and (d) could be operated at ordinary temperature and pressure and does not require high pressure construction and specialty alloy. However, more severe pretreatment is required in enzyme hydrolysis in order to fully expose the cellulose to enzymatic attack.

The Direct Conversion Process: An Improved Enzymatic Saccharification Process

One of the most significant developments in ethanol production from cellulosic materials is the invention of the direct conversion process. Research abroad, particularly in the U.S., has been basically on: (1) development of a simultaneous saccharification-fermentation process and (2) utilization of certain thermophilic and anerobic bacteria.

Wang (1982) studied the direct microbiological conversion of cellulose to ethanol using *Clostridium thermocellum* and *Clostridium thermosaccharolyticum.* The degradation of cellulose is achieved through the use of *C. thermocellum.* This bacterium, however, is quite unique in that it is able to hydrolyze both the cellulosic and hemicellulosic fractions of biomass but is not able to metabolize the pentoses. Therefore, a second bacterium, *C. thermosaccharolyticum* was also studied due to its ability to convert pentoses into ethanol. Strain improvement is done through mutation, selection and adaptation programs, and in order to enhance **ethanol** tolerance and catabolite selectivity. Alcohol yields of 85% of the theoretical maximum have been obtained from solka floc with mixed culture.

On the other hand, the Gulf Oil Corporation has improved on the traditional enzymatic process. This new process has been termed as the "direct ethanol process" and was designed based on theoretical data obtained from a pilot plant which operated between 1975 and 1979.

Fig. 7 shows the schematic diagram for the direct ethanol process. The four principal steps involved are: (a) pretreatment of feedstock, (b) enzyme production,

(c) simultaneous saccharification and fermentation, and (d) product recovery or distillation. The important fermentation ingredient, yeast, is recycled using techniques which are already well established in the fermentation industry.

Pretreatment in the Gulf Oil process is practically similar to what was described in Section 2 except for some modifications. To solve the energy intensiveness problem of fine grinding, the Gulf Oil process has made use of a horizontal attrition mill which consists essentially of a cylindrical chamber filled with small steel balls and containing an internal agitator. The energy requirement of the new attrition mill is said to be about one-tenth that of the traditional attrition mill.

Enzyme production is accomplished by the aerobic fermentation of a mold called *Trichoderma reesei.* The parent strain of this organism is responsible for much of the rotting of plant materials. In nature, *T. reesei* receives its food by secreting a protein called cellulase. This is also the enzyme that hydrolyzes cellulose into sugars. After reducing sugars are formed, they are absorbed by the mold to provide energy and growth. The enzyme produced is harvested and used for the hydrolysis of the main cellulose feedstock. In the Gulf Oil process, a continuous fermentation process was developed which has been refined so that enzyme production can be accomplished in one-seventh of the time required for conventional batch fermentation. Ten per cent of the feedstock stream is diverted to the enzyme fermenter for this purpose. Part of the cellulose produced in the enzyme fermenter is retained.

The other portion of the enzyme reactor production (containing enzyme, mold bodies, unconverted cellulose and nutrients) is then joined with the main feedstock

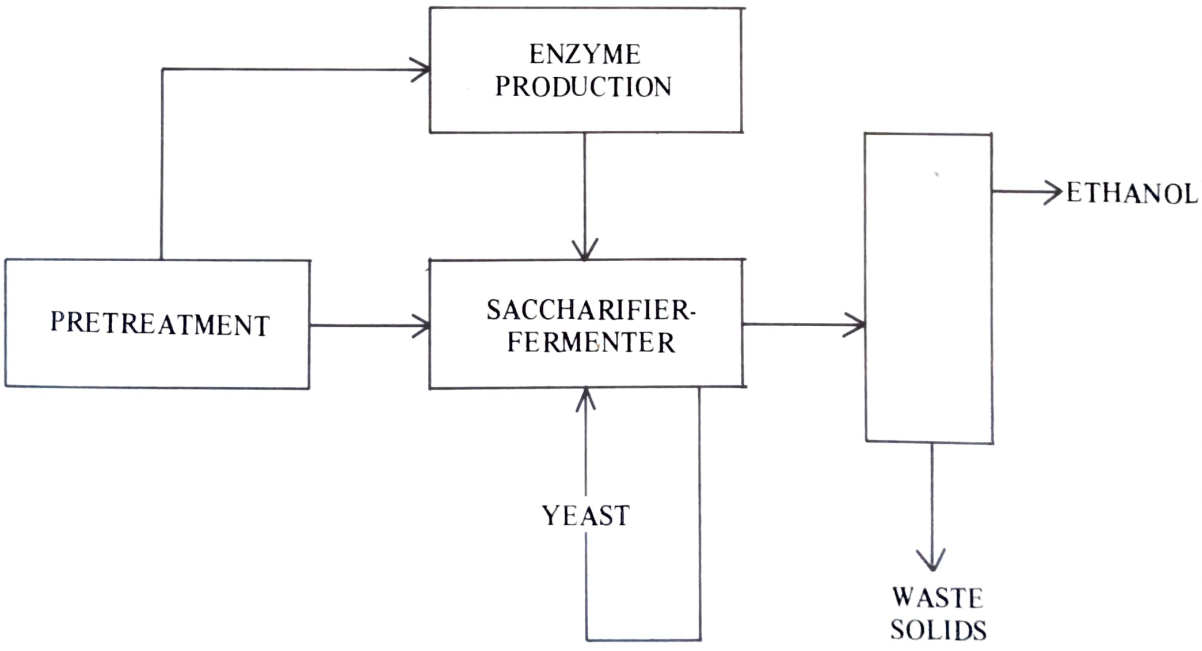


Fig. 7. DIRECT ETHANOL PROCESS

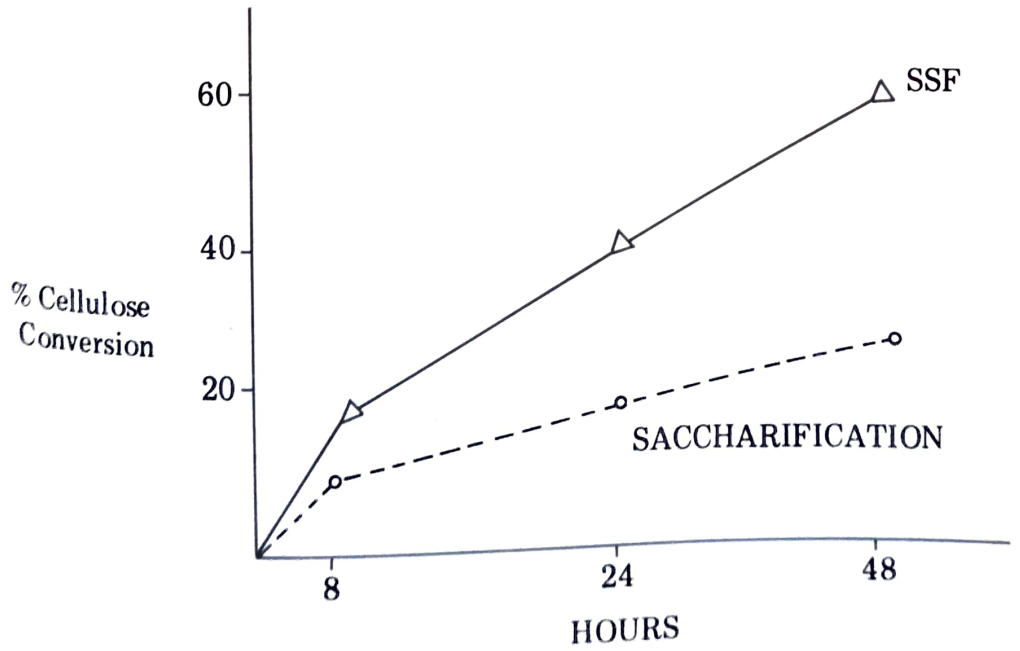
stream. The enzyme is not isolated and purified as done in the conventional process and which simply adds up to the cost and degrades enzyme activity. The continuous process is a significant development in that it permits the economical production of large quantities of enzyme.

In the direct ethanol process, saccharification and fermentation are done simultaneously. Enzyme, yeast and cellulosic feedstock are combined in the same vessel as depicted in Fig. 7. This method cuts down on reactor volume. Another advantage is on the synergistic effect. Hydrolysis is aided by the continuous removal of sugars by the action of yeast. The build-up of sugar concentration which tends to inhibit further sugar production is prevented by the continuous depletion of sugars due to its simultaneous conversion to alcohol.

The synergism in the simultaneous saccharification-fermentation process is proven by results of an experiment shown in Fig. 8. Standard prepared cellulose (Avicel), which is almost pure cellulose and highly crystalline, was used. No pretreatment was done. As shown in the figure, the rate of conversion in simultaneous saccharification-fermentation is more than double the rate in the conventional process where cellulose is first hydrolyzed into fermentable sugars and then followed by fermentation into ethanol.

The strategy of the above described approach involves three principal elements: (1) severe pretreatment (mechanical) to increase susceptibility of the feedstock, (2) use of massive inoculation of enzyme to obtain the highest rate of hydrolysis, and (3) simultaneous saccharification and fermentation to minimize reactor volume.

Fig. 8
COMPARISON OF SIMULTANEOUS SACCHARIFICATION/
FERMENTATION (SSF) AND SACCHARIFICATION



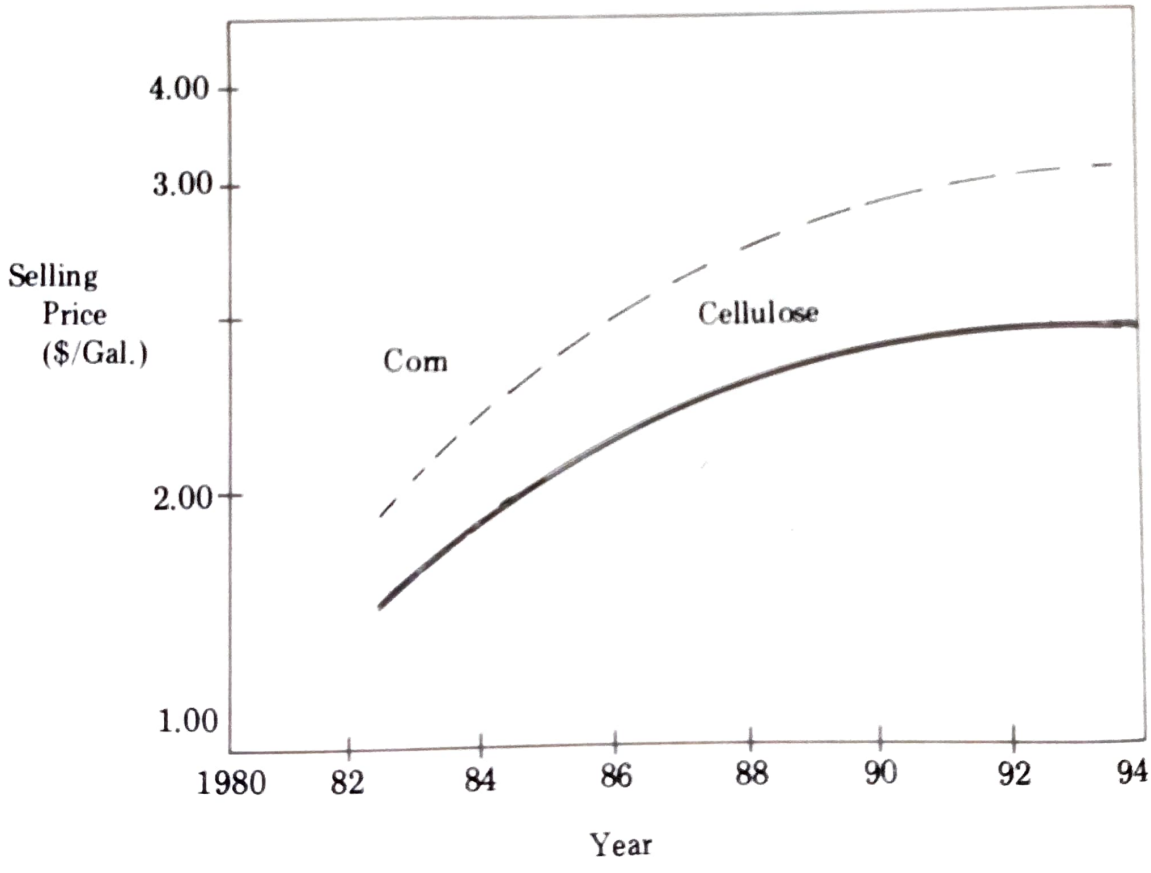
Economic Analysis of the Direct Ethanol Process Using Cellulose Materials

To evaluate the economic viability of the process, a design was made of a commercial plant and economic estimates were made for 1983. Investment was $112 million. Alcohol production was 50 million gallons per year and cellulosic feedstock consumption was about 2,000 tons (oven dry) per day. A summary of the economic study is shown in Table 11. The analysis shows that the process is economically viable at a rate of return (ROI) of 15% and a selling price of alcohol of $1.15. This selling price is competitive with the price of corn alcohol, as shown in Fig. 9.

Inspection of Table II reveals that sensitivity to feedstock price is low. The dominant factors are capital cost, fixed charges and gross profit. Further process development should concentrate on the reduction of capital cost. There is heavy dependence on the animal feed by-product credit. For rice straw, there may be a

Fig. 9

ETHANOL ECONOMICS



Alcohol From Corn Grains vs. Alcohol From Waste Cellulose. (Source: Huff, 1981)

market for the by-product. Otherwise, the by-product may be burned right in the plant and used to generate electricity. In such a case, a cost penalty of $0.30/gallon is incurred and for an ROI of 15% to be maintained, the selling price of alcohol would have to be increased to $1.45/gallon, which is still competitive.

The Energy Efficiency of the Direct Ethanol Process.

Even if a process is economically viable, it may not still be desirable if its energy efficiency is low. In energy production, the energy efficiency is as important as its economics. The energy balance for the direct ethanol process is shown in Fig. 10. In this process, the energy input represents the heat of combustion of the feedstock as if it has an alternative energy use as boiler fuel.

Table II. CELLULOSE ALCOHOL PLANT

50 MILLION GALLONS PER YEAR
FIXED INVESTMENT $91.59 MILLION
WORKING CAPITAL $8.00 MILLION

Cost Item	Annual Cost $	$/Gallon
Fixed Charges	14.65	0.293
Raw Materials	15.75	0.315
Utilities	10.25	0.205
Labor	2.20	0.044
Total Production Cost	42.85	0.875
By-Products Credits	(21.00)	(0.420)
Freight, Sales, GA&O	5.74	0.115
Total Operating Cost	27.59	0.552
Taxes	14.95	0.299
Net Profit After Tax	14.95	0.299
Selling Price for 15% Return After Tax	—	1.150

Selling Price x 50 = Total Operating Cost + Taxes + Net Profit After Tax

$$\text{Rate of Return} = \frac{14.95}{91.50 + 8.00} \times 100\% = 15\%$$

Source: Huff, 1981

The energy required to produce the waste material should be assigned by any realistic energy accounting system of the primary use of this material (not the wastes) and should not enter into the calculus of energetics of the cellulose to ethanol process. So, in this case, the energy content of the cellulosic materials is the one being considered alone.

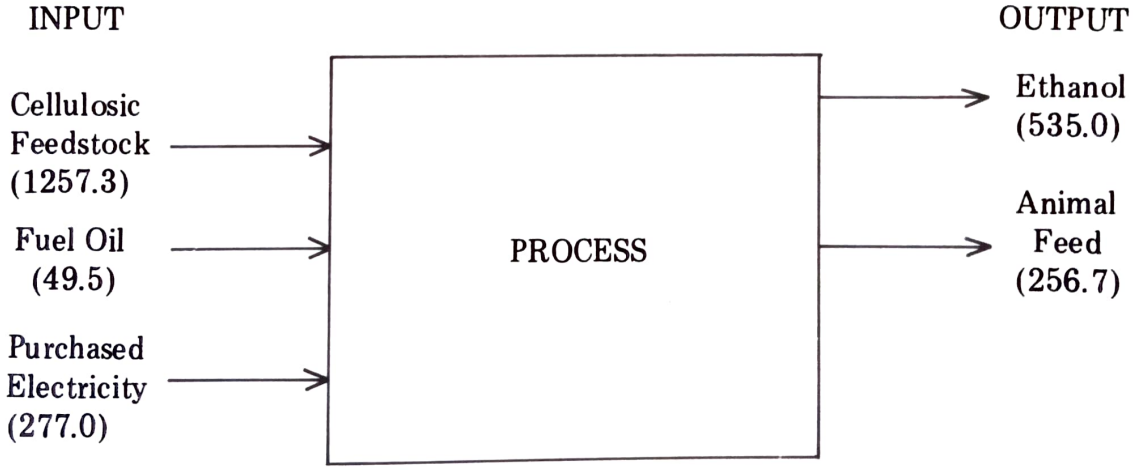
Fig. 10 indicates that the gross efficiency of this process is 50%. If one corrects the inputs and outputs for combustion efficiency (i.e. ethanol burns with 85% efficiency and the cellulosic material with 67% efficiency), the net energy efficiency is 54%. If the cellulose feedstock is waste material like rice straw, the energy value of the feedstock can be considered to be zero. If so, the energy efficiency will be 192%.

The information in Fig. 10 is based on the requirement to purchase 27,700 kw of electric power, 2,000 oven-dry tons of feedstock per day, and 194 barrels of fuel oil per day. The outputs are 150,000 gallons of ethanol and 534 tons of animal feed per day. The purpose of the fuel oil is to aid in the combustion of solid or insoluble residues obtained from the fermentation step in the boiler which, through cogeneration, supplies all the steam requirements of the plant as well as 3,400 kw of electric

Fig. 10

DIRECT ETHANOL PROCESS

Energy Balance For Ethanol From Cellulose
50 Million Gallons Per Year
(in million Btu/hour)



$$\text{Gross Efficiency} = \frac{\text{Total Energy Output}}{\text{Total Energy Input}} = \frac{791.7}{1583.8} \times 100\%$$

$$= 50\%$$

Net Efficiency (Corrected for boiler efficiency differences)

$$= \frac{626.7}{1165.9} \times 100\% = 54\%$$

$$\text{Net Efficiency (If feedstock valued at zero)} = \frac{626.7}{326.5} \times 100\%$$

$$= 192\%$$

power. The soluble residue from the fermentation step is the animal feed. The energy associated with the feed is its heat of combustion. The energy associated with electric power is the amount of coal required to produce this amount of power — 10,000 BTU/KWH.

If the animal feed cannot be sold, it must be burned as fuel in the plant, and the plant will be nearly self-sufficient in electricity. In this case, the energy efficiency is 70% where the feedstock is valued for it heat of combustion, and 1266% where its value is zero. Thus, the use of by-products as fuel improves the energetics of the plant even though the economics suffer.

Related Developments

Since preliminary results on the bioconversion of rice straw to ethanol were not really encouraging, other possible uses of rice straw were investigated. Cayabyab, Uyenco and del Rosario (1977) studied the production of yeast biomass from rice straw. Rice straw was 25% saccharified by 0.6M HC1 in an autoclave for 1 hour at 24 psi. The hydrolyzate was then used as culture substrate for *Candida tropicalis* under various conditions of pH, temperature and reducing sugar concentration. Results showed that the efficiency of conversion of sugar into yeast biomass was maximum (46.8%) at pH 5.5, 29 C and 1.6% sugar concentration. Under these conditions, the harvested yeast contained 51% protein, dry basis, whose amino acid composition compared favorably with the FAO reference protein.

Conclusions/Recommendations

Two factors are of vital importance in the development of an efficient biochemical exploitation of waste cellulose like rice straw using enzyme hydrolysis. These are: (a) development of process for the production of highly active cellulose enzyme system, and (b) increasing the susceptibility of the cellulosic substrate to enzyme attack.

Although the biochemical conversion of waste cellulose consists of two basic processes, saccharification and fermentation, it must be pointed out that the success of converting cellulosic materials to alcohol is dependent on the optimization of the enzymatic saccharification since fermentation of reducing sugars into ethanol is already a well-established process.

Several areas of research investigation which are expected to have a high payout in bioconversion are identified. First, new microorganisms should be developed or discovered through certain techniques such as genetic engineering, and which can operate efficiently at higher temperatures. The use of these microorganisms can have a dual effect of increasing rates, thus overcoming one of the drawbacks of the fermentation process, and of discouraging the existence of contaminating organisms which pose a constant hazard to any fermentation process. Wang (1982) has studied *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* on solka floc and corn stovers; since the results are very encouraging, it may be interesting to investigate the efficiency of these microorganisms on the bioconversion of rice straw.

Second, means or microorganims should be sought which convert xylans or five carbon to either simple sugars or to ethanol.directly. Such capability would increase the yield of ethanol from cellulosic wastes, particularly rice straw, since rice straw contains a significant amount of hemicellulose for which xylan is the dominant component.

Third, improvement on the pretreatment process should be made which would further decrease the energy requirement of mechanical pretreatment. Fourth, research, in the area of the use of lignin for its valuable chemical values should be encouraged. Lignin can be recovered as a separate entity in most of the processes which are being studied, but is merely thought of as a plant boiler fuel. The recovery of high grade chemical values would substantially improve ethanol process economics.

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