Application of biotechnology for organic solid waste utilization

Dhingra H. K.*, Shivhare Preeti*, Panesar K. S.*, Bohidar P R.*, Mohindru V. K.* and Pant R **

ABSTRACT

A large amount of solid waste from pulp and paper industry is produced which not only causes environmental problems due to disposal but also remains unused. Disposal of waste on land induces a number of ecological problems. Hence waste utilization and reduction instead of disposal on land will be an environmental necessity. To date, utilization of waste for agricultural purpose and incineration has been developed and CPPRI has also done work in these areas. Pulp and paper mill wastes mainly sludge are, in general, largely cellulosic and are therefore amenable to conversion to glucose. If glucose can be produced cheaply from a low cost raw material such as paper mill sludge, many interesting avenues of producing commercially useful products will open up. With the application of biotechnology, it will be possible to convert cellulose to glucose and thus a range of products viz. food, chemicals and fuel/energy can be made from the lignocellulosic waste of pulp and paper industry. The biotechnology approach is also more environmental friendly.

This review highlights the various aspects of bioconversion of solid waste to energy in form of gas that is methane and liquid fuel that is ethanol, to protein rich food production and to highly valued organic chemicals. The process being used at pilot plant scale or lab scale has also been described. The mechanism of enzymatic hydrolysis of cellulose to glucose and the pretreatment methods used to enhance enzymatic and microbiological attacks on lignocellulosic wastes have been examined. The advantages and disadvantages of biotechnology approach and also theoretical economical aspects have been reviewed.

Introduction :

Environmental and economic characteristics require the re examination of traditional waste management practices. Land disposal of untreated solid waste can lead to sanitary and odour problems. Now, solid waste is generally recognized as both a major problem and an underutilized resource for material and energy recovery. To date, utilization of paper mill waste for agricultural purpose and incineration has been developed. In CPPRI also, scientists have done much work on making briquettes from WWT sludge, straw dust and other organic waste and also on preparation of compost from these wastes. Both these byproducts from wastes are important from Indian economic point of view. Because India is an agricultural country, so compost can be utilized as a soil conditioner/fertilizer to improve the yield of crop. Similarly, by making briquettes, we can substitute these for coal and thus can save fossil fuel.

In addition to these utilization approach,there are other promising by-products like ethanol, methane, protein rich food and organic chemicals which can be obtained from pulp and paper mills solid waste and also crucial for Indian economy. The sufficient amount of cellulose present in such wastes warrants their consideration for production of ethanol, methane and protein rich food. Crude cellulose in the form of waste may be available free except for handling

---

Central Pulp & Paper Research Institute
P.O. Box No. 174 SAHARANPUR-247001 (U. P.)

cost. In these days of increased concern for the environment, wastes may even have negative costs. The crucial in the developing technology for production of ethanol, protein rich food is optimizing the conversion of cellulose to its monomer glucose. The traditional approach to hydrolyze cellulose to glucose is acid hydrolysis. But due to poor yiled of glucose, low concentrations and high reactor cost, there is a problem of economical conversion of cellulose to glucose. The another promising approach to obtain glucose from cellulose is by biotechnology application that is by enzymatic hydrolysis of cellulose. Perhaps the most promising aspect of this process lies in the fact that it can help to reduce solid waste build up and in the meantime produce useful products.

Enzymatic hydrolysis of cellulose to glucose is both older and newer than acid hydrolysis. Older because the microbial decomposition of biomass has been an integral part of the carbon cycle since its inception. Newer because it has only been recently that research on the mechanism of microbial degradation of cellulose has provided the conceptual framework on which to base an enzymatic conversion plant. With the discovery of fungal strains of high cellulolytic activity, it became conceptually possible to consider the positive aspects of enzymatic hydrolysis of cellulose in waste disposal and the production of chemicals, energy, and food instead of merely the negative aspect viz. The decomposition of textiles and wood.

Any lignocellulosic waste can be converted into sugars such as cellobios and glucose by using cellulase enzyme. Also biological conversion process are more likely to leave the lignin fractions in a form suitable for further use while acid hydrolysis frequently results in extensive lignin degradation.

This paper presents an overview of the biotechnology application in pulp and paper industry for conversion of lignocellulosic wastes to useful products like ethanol, methanol, food and other valuable chemicals. The pretreatment methods required for effective hydrolysis of lignocellulosic waste has been also presented. The mechanism of enzymatic hydrolysis and also other points related to economic evaluation has also been presented.

Enzymatic Hydrolysis of cellulose to Glucose :

The most important aspect for the conversion of cellulosic waste of food and energy and other valuable products is economical feasible processing of cellulosic waste to reducing sugar. This would then open a wide range of potential fermentation processes based on glucose to produce useful material. The enzymatic hydrolysis of cellulose to glucose is being discussed in brief below.

A. Mechanism :

The enzymatic hydrolysis of celloulose is a complex phenomenon. The cellulase, the enzyme capable of hydrolyzing cellulose contains two components, C_1 and C_x which are responsible for hydrolysis of cellulose. There are different opinions about the specific order of their activity. Whatever the specific order of their activity, the cellulase complex contains the components enumerated below according to the favoured currentconcept of enzymatic hydrolysis[2]2.

1. C_1 is an enzime whose action is still open to question, with some still considering it a special enzime with the peculiar ability to act on highly crystalline substrates, while the majority now consider C_1 an exoglucanase and cellobiohydrolase which acts only after endo—enzymes have acted.
2. C_x is a group of endo–β 1, 4–glucanases which randomly cleave the cellulose chain preferentially at internal linkages rather than at the terminal linkages which are favoured by the exoglucanases. As many as six of these endoglucanases have been isolated from a single organism.
3. β-Glucosidase, more specifically cellobiase, which acts on the β–dimer of glucose, cellobiose. β-glucosidases and exo--β--1, 4--glucanases act in common on glucose oligomers from cellobiose to cellohexaose, with the small oligomers being most repidly hydrolyzed by the β–glucosidase and the larger ones by the exo--glucanases.

The kinetics of enzymatic hydrolysis involving as it does the complex system of cellulase components is understandably complex too. An important factor is the degree of order or crystallinity of the substrate. Another is its lignin content. The more accessible regions are preferentially hydrolyzed at a faster rate than the highly ordered regions. Whether or not the cellulase enzymes have any access to the cellulose at all and the extent of that access, can determine not only the rate of hydrolysis but in the case of highly lignified cellulose can completely prevent hydrolysis. The rate of hydrolysis decreases with increases in degree of crystallinity and lignification.

B. Production of Cellulases :

The future application of enzymatic hydrolysis of cellulose to glucose on a commercial scale will depend to a great extent on the availability of high activity cellulase at low cost. Considerable effort has been and is being expanded on the improvement of the technology of cellulase production.

Although cellulases are produced by insects, molluscs, protozoa, bacteria, and thousands of fungi, only the organism in the last category seem to be suitable for large scale production of cellulase. Insects and molluscs grow too slowly and isolation of their cellulase is scarcely feasible. Protozoa and bacteria are difficult to grow, and the cellulase must be extracted from association with the cells. However, the cellulolytic fungi grow rapidly on simple media and secrete their cellulases into the medium so the enzymes can be separated easily by filtration.

Of the thousands of fungi which have been studied for their ability to degrade cellulose and the hundreds which have been found to prodcue cell-free enzymes that can be used to hydrolyze cellulose, one species, Trichoderma viride, stands out for the ability of its extracellular enzyme to hydrolyze crystalline cellulose and for the storage stability ot these enzyme preparation.

C. Saccharification of Cellulose :

Although the complete hydrolysis of cellulose to glucose by enzymes is both conceptually and experimentlly feasible, many obstacles still remain to its routine practice on a commercial scale. The factors that influence the rate and extent of cellulose hydrolysis include the enzyme concentration, initial concentration of enzyme, the susceptibility of the substrate to hydrolysis as determined by its degree of crystallinity and lignin content, inhibition of the cellulase components by the hydrolysis products, and inactivation of the enzymes during the reaction.

Out of these the most important factor governing the rate and extent of hydrolysis is the susceptibility of the substrate to the enzyme system used, Although amorphous cellulose is hydrolyzed rapidly, the crystalline portion of the cellulose are hydrolyzed at much slower rate. The presence of lignin can completely inhibit access of the enzymes to the cellulose. Since most of cellulosic waste is more or less delignified, this factor has important implications for the ultimate utility of enzymatic hydrolyis.

In combination the above factors lead to a rapid decrease in hydrolysis rate with time. After initial rapid hydrolysis of amorphous cellulose, the residual becomes increasingly crystalline. Inhibition by the reaction products and enzyme inactivation are both more severe for the cellulase components which act on crystalline cellulose so hydrolysis of the crystalline residue becomes even slower.

Enzyme recovery after hydrolysis would be very important for any commercial process. Enzyme in solution could be recovered by adsorption on fresh cellulose, but if the enzyme was partially inactivated, fresh enzyme would have to be added as well. Enzyme adsorbed on solid hydrolysis residues can also be recycled, but its activity remains uncertain. Indications are that a large fraction of the enzyme activity is retained at lower temperatures, so some degree of recycle should be possible.

Although enzymatic hydrolysis of cellulose can provide 100% yield of glucose, reaction is much slower than acid hyrolysis, requiring days rather than hours or minutes for completion. The reactor capacity must be much greater than in acid hydrolysis for the given production rate, but on the other hand the severe corrosion problems and expensive corrosion resistance materials associated with acid hyrolysis are avoided. However, recycle of the more expensive enzyme catalyst is more critical than in acid hydrolysis.

Enzymatic hydrolysis is severely restricted by substrate susceptibility and can be completely prevented by the presence of lignin. It is mandatory that an inexpensive pretreatment to improve enzyme accessibility be developed in order for enzymatic hydrolysis to reach its potential importance.

Pretreatment for Cellulosic Break Down

As discussed, there are mainly two physical barriers to facile cellulose hydrolysis. Cellulose crystallinity impedes hydrolysis by either dilute acid or enzymatic hydrolysis. And the presence of lignin can completely prevent enzymatic hydrolysis. The goal of removing one or both of these barriers has led to considerable research effort which has been reviewed from both the theoretical and practical perspective. The various approaches taken may be conveniently grouped in to two categories-chemica and physical[1-6,19,25].

Chemical Treatment :

Swelling of cellulosic material with alkaline reagent is one way to increase hydrolysis rate of native cellulose. Treating the cellulose material with sodium hydroxide of different concentration increases hydrolysis rate to various extent depending upon the type of material. Another long standing approach to upgrade the feeding value of lignocellulosic material involves treatment with aqueous or gaseous ammonia.

Ammonia exerts a strong swelling action on cellulosic materials and can effect a phase change in the crystal structure from cellulose I to cellulose III. As an added benefit, the ammonia treatment would provide some nitrogen increasing its food value. Steaming of cellulosic waste is yet another approach to increase their digestibility.

As lignin being the major road block to widespread utilization of the carbohydrate content of lignocellulosic material, the delignification would appear to provide a straight forward solution to enhance hydrolysis. There are various ways of delignification namely cooking with sodium hydroxide and sodium sulphide, treatment with ammonia bisulphite, sulphur dioxide etc. However, it has been reported that complete delignification is not essential and growth of enzyme on lignin removal varies with material.

But it is important to note that waste compound in the form of sludges or rejects have already received substantial pretreatment to render its carbohydrate constituents susceptible to acid enzymatic or microbiological conversion. Thus this waste material represents a tremendous potential for conversion to useful products particularly as sources dietary energy for ruminants and research in this area is in progress.

Table 1 and 2 present compositional and in vitro digestibility data for some typical pulp mill and combined pulp and paper mill residues.

TABLE—1

Composition and in vitro Digestion of Pulp mill Residues

Type of residue	Lignin	Carbohydrate	Ash	Digestibility
Groundwood fines				
Aspen	21	73	1	37
Southern Pine	31	59	1	0
Spruce	31	60	1	0
Screen Rejects				
Aspen Sulfite	19	77	2	66
Mixed hardwood Sulfite	24	65	14	54
Kraft	25	74	9	44
Chemical pulp fines				
Mixed hardwood, kraft, bleached	<1	109	1	95
Aspen Sulfite (parenchyma cells)	20	73	2	73
Southern Pine, kraft, (unbleached)	28	68	4	46

TABLE—2

Composition and in vitro Digestion of Combined Pulp and Paper mill Sludges

Type of residue	Lignin	Carbohydrate	Ash	Digestibility
Groundwood mill				
Mixed species + mixed chemical pulps	50	41	38	24
Southern pine + mixed hardwood kraft	24	60	15	19
Semichemical Pulp Mill				
Aspen	20	71	2	57
Aspen + mixed				

hardwood	55	29	13	6
Chemical pulp mill				
Deinked waste paper, tissue	23	71	22	72
Milk carton stock	28	67	25	65
Mixed chemical pulps, tissue	17	76	13	60
Aspen and spruce sulfite	45	46	45	35

Physical Pretreatment :

Some have emphasized heavily on the chemical pretreatments; however some have stressed more towards physical methods.

The reason for physical treatment has been stressed due to its twofold results.

1. Enhanced enzymatic susceptibility (increased surface area and decreased crystallinity).
2. Increased bulk density (increased slurry concentration in the hydrolysis reactor).

The subdivision of cellulosic waste to very small particle size yields a product remarkably susceptible to hydrolytic bacterial or enzymatic attack. Vibrating ball milling or hammer milling provides an efficient means of size reduction. It should be noted that particle size is not the only contributing factor when considering a physical or milling treatment. The action of the mill, the milling history (i.e. time, temperature profile) all contributes to the change in crystallinity or change in susceptibility. This is shown in figure 1.

The technique of irradiating with gamma rays or by high velocity electron improves the digestibility or enzymatic attack with lower degree of polymerization, lower crystallinity and higher moisture absorption capacity. However, due to high costs this technique would appear to have little commercial interest. Another promising radiative pretreatment for inducing deep seated structural attraction is that of photodegradation. The process includes exposer of polysaccharides to high intensity ultraviolet light (3650°A) in the presence of dilute sodium nitrite which functions as a photosensitizer. It has been reported that a 24 hour irradiation pretreatment gave upto four fold increase in the rate of biodegradation of a variety of cellulosic substrate[19]. Other physical methods include application of high or low temperature or by application of pressure[25]. In high temperature applications substrates are heated for three hours at 200°C in non polar liquid

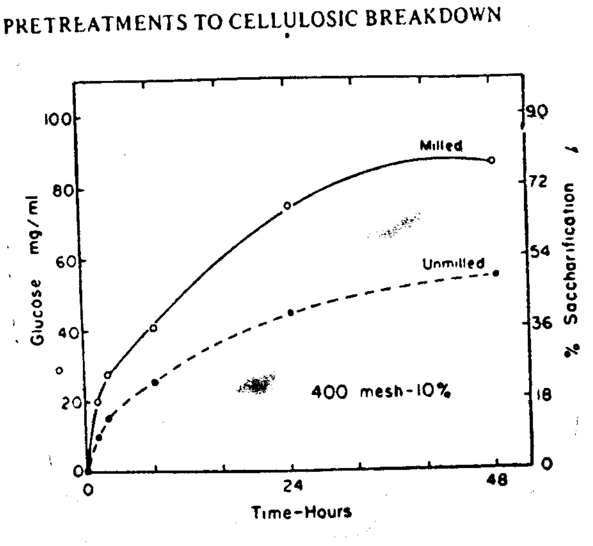


FIGURE 1: EFFECT OF MILLING ON THE SUSCEPTIBILITY OF SOLKA FLOC SW40 FRACTION PASSING 400 MESH BUT NOT PASSING 500 MESH, BALLED MILL FRACTION PASSING 400 MESH BUT NOT PASSING 500 MESH

such as kerosene or in dry air or nitrogen, and in low temperature treatment cellulosic materials are freezed. But in all these high energy is required and comparatively few advantages are obtained.

The pulp and paper mill WWT sludge may not require extensive physical pretreatment because it mostly contains fines and thus already it possess fine structure.

Protein Products for Food and Feed :

Cellulose can be used to produce food or feed calories or proteins [7–12]. Because when weighing process alternatives, yield or resource utilization is a prime consideration and yield is high in case of conversion to SCP (45—55%) or to sweet syrup (90-100%). So, to have the greatest impact on the human food chain, it is obvious that a cellulosic material should be converted to a sweet food syrup or a single cell protein.

Cellulosic waste after delignification through a mild, fermentation can be used to feed animals. The research work is going on to explore the use of nitrogen fixing bacteria in conjunction with microorganisms that decompose cellulose. The attractive feature of such an animal feed would be the saving of energy from not having to add ammonia to microorganism growing on cellulose. However it is believed that protein demand is increasing and will continue to increase in the feasible future. So much emphasis is being given to protein food like SCP and microprotein from cellulosic waste material.

The use of microorganism in the preparation of protein food has a history. The products concerned are cheese and fermented soyabean product. They are examples in which microbial activity is used to alter substantially the nature of proteinaceous raw material to give a food product which may be stored for a longer period (cheese) or which is in more acceptable form (soyabean curd) So these are example of protein modification by microorganism. The more sophisticated application of biotechnology is in growth and harvesting of microbial mass which then becomes the protein food products e.g. single cell protein (SCP) and mycoprotein.

Single Cell Protein :

The nutritional value of microbial material can be quite high with respect to many important factors; not the least is protein which is in large proportion in the cell dry weight of most species. The use of microbial protein to contribute to the world protein supply is being considered. The route for its use may be either indirect, as a protein component of animal feed stuffs, reducing requirement for such material such as soyabean meal and fish meals or as a material for direct inclusions in the human diet.

The protein obtained through microbial activity is termed as "Single Cell Protein". Its production depends upon the large scale growth of suitable microorganisms and their subsequent harvesting and processing to a food product.

In the university of Waterloo, trials at pilot plant scale has been run to produce SCP by utilizing ETP sludge from paper mill and other cellulosic waste material like saw dust, straw dust etc[9]. They are using a fungus called Chaetominum Cellulolyticum for converting Cellulosic waste material to protein food namely SCP food. This food contained about 45% protein like soyameal but is lower in carbohydrates content and higher in fats, has more appropriate amino acid profile which makes it more acceptable as a food— and its high vitamin content while soyameal has almost no vitamins. This food can be used for pigs, chickens and cattle and even for human beings. This fermentation process converts one ton of wastes into half a ton of protein. The fungus will live and work over a temperature range for 25—45° C.

Mycoprotein

Mycoprotein is a food product which consists basically of fungal mycelium. The organism used is a strain of 'Fusarium graminearum' which was isolated originally from a sample of soil and the process and product are the result of an extensive program of experimentation, dovelopment and testing. Mycoprotein is produces at pilot plant scale by continuous fermentation, using glucose as substrate, with other nutrients and ammonia and ammonium salt as the nitrogen source[7]. Following the fermentation stage, the culture is subjected to heat treatment to reduce the ribonuclic acid content and the mycelium is then separated by vacuum filtrating. This product can be used even for human consumption.

In comparison to animal proteins, the production of Myco Protein shows advantageous features.

In addition to the growth rate advantage common to all SCP products the conversion of substrate to protein shows a much greater efficiency than conversion of feed by farm animals. This is shown by the data in Table 3 and it should be remembered that animal feeds need to contain a proportion of protein, possibly as much as 15—20% depending on the species and the method of husbandry. The main nutritional characteristics of myco protein are also shown in table 4 which also gives a comparison with beef.

TABLE 3

Myco Protein and Animals.
Conversion rates in protein formation

	Starting material	Product	
		Protein	Total
Cow	1 kg feed	14g	68 g beef
Pig	1 kg feed	41g	200 g pork
Chicken	1 kg feed	49g	240 g meat
Fusarium graminearum	1 kg carbohydrate +inorganic nitrogen	136g	1080 g wet cell mass

TABLE 4

Typical composition of Myco Protein compared to beef

	% content (dry weight basis)	
Component	Mycoprotein	Raw lean Beefstack
Protein	47	68
Fat	14	30
Dietry fibre	25	Trace
Carbohydrate	10	0
Ash	3	2
RNA (rubonuclic acid)	1	Trace

Fuel Alcohol production from waste :

An alternative to cellulosic waste conversion to glucose or SCP or biogas is the production of alcohol [13-16,26]. Alcohol is another key process intermediate in the cellulose conversion scheme since alcohol can be used for food and chemical production or for energy synthesis. With respect to energy synthesis alcohol can be converted into ATP i.e. biological energy. (see figure 2). Alcohol can be burnt directly in internal combustion engine. Indeed *alcohol is* a non polluting antiknocking fuel.

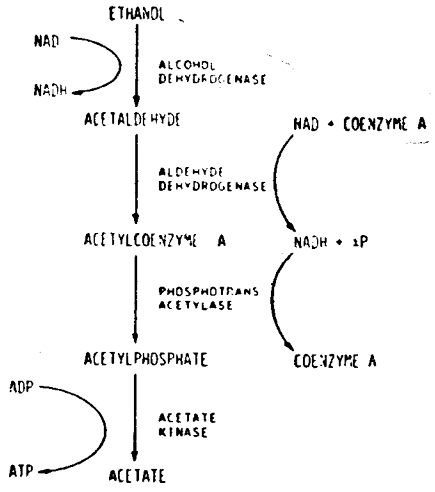


FIGURE 2 : SCHEME FOR CONVERTING ALCOHOL INTO ATP

Let us have a brief introduction to the energetic of cellulosic waste utilization. The one alternative is to burn the cellulosic waste material to make heat and then to generate power, the another way of its utilization as mentioned is hydrolysis of cellulose to glucose followed by conversion of glucose to other valuable products such as alcohol.

A brief energetic comparisons is being given in the following para :

The photosynthetic equation, and its reverse, combustion or respiration, can be represented by :

$$CO_2+H_2O \underset{\text{Combustion}}{\overset{\text{Photosynthesis}}{\longleftrightarrow}} O_2+\underset{1/6\ \text{glucose}}{(CH_2O)} \qquad \Delta G=114.4\text{kcal}$$

The equation as shown is for glucose formation, or combustion, but the energetics for cellulose are not much different. The splitting of water by electrolysis and the reverse process of combustion are given by :

$$2H_2O \underset{\text{Combustion}}{\overset{\text{Electrolysis}}{\longleftrightarrow}} 2H_2+O_2 \qquad \Delta G=+113.4\ \text{kcal}$$

Thus

$$2H_2+CO_2 \longleftrightarrow [CH_2O]+H_2O \qquad \Delta G—O$$

In other words, 1/6 mole of glucose (CH_2O) is energetically equivalent, in terms of combustion, to 2 moles of H_2 gas. When glucose is converted to CO_2 and ethanol by fermentation, only a small part of the potential energy is lost:

$$\underset{\text{1/2 Glucose}}{(CH_2O)_3} \longrightarrow C_2H_5OH + CO_2 \qquad \Delta G = -26.4\,\text{kcal}$$

Thus for the combustion of ethanol with O_2

$$C_2H_5OH + 3O_2 \longrightarrow 3H_2O + 2CO_2 \qquad \Delta G = -316.0\,\text{kcal}$$

or —105.6 kcal per carbon atom of original glucose, compared with -114 4 kcal for the combustion of glucose. The energy loss is therefore only 8.8kcal per carbon atom. In living yeast cells, of course, this is partly conserved in the formation of 2 mol of ATP per mol of glucose fermented and this is what keeps the yeast going.

Since so little energy is lost when cellulose is converted into ethanol, and since the yield of ethanol can be very high this route represents a very efficient conversion of cellulose to a clean liquid fuel.

There are two biological processing options for conversion to ethanol which have become focal points;

1. The application of fungal extracellulose cellulases to produce fermentable sugars and then ethanol.
2. A direct fermentation to ethanol and other chemicals using thermophilic anaerobes.

1. Extracelluler Cellulases :

These have been applied for converting municipal/pulp mill (digester rejects, primary sludges and digester fibers) solid wastes to ethanol. In a pilot scale operation at University of Arkansas 1 ton/day of solid waste comprising of 55% cellulose, 300 Lit/day of 95% (v/v) ethanol has been produced. Hydrolysis is accomplished with a mutant strain of Trichoderma reesei. The crude T. reesei culture was used in the hydrolysis step with no purification or concentration steps. The sugars were fermented by yeast in the same fermenter vessel. Higher rate of hydrolysis has been claimed by use of a simultaneous saccharification and fermentation (SSF) step The addition of yeast to the saccharification initially prevents any built up of glucose that would cause feed back inhibition of the cellulases. Cellobiose does not create a problem (which usually) in the SSF step because the enzymes produced by T. reesei in University of Arkansas process contain ample B—glucosidase to convert all the cellobiose to glucose making supplemental enzyme unnecessary. A brief process flow diagram is shown in figure 3 and detail is available elsewhere[13].

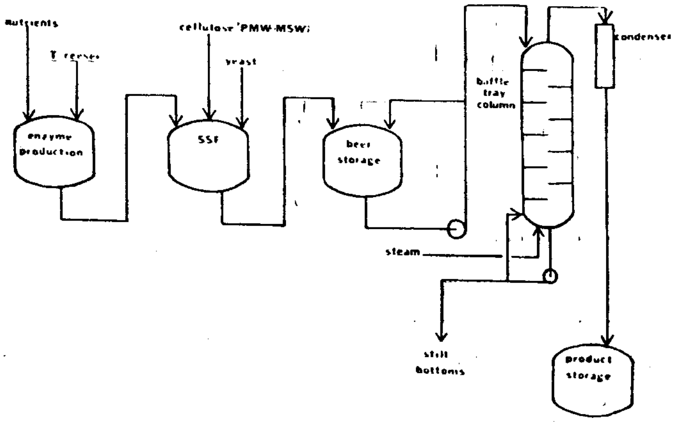


FIGURE 3: PROCESS FLOW DIAGRAM OF PILOT PLANT FOR ETHANOL PRODCTION

The other pilot process called Natick Process is based on enzymatic hydrolysis of a cellulosic material such as urban waste, wheat straw and popular to yield a sugar solution i e. subsequently ferment to ethanol. The hydrolysis step is separate from the fermentation step. The reported glucose concentration after hydrolysis is in the range of 100–120 g/1. This yields 40 to 50 g/1 of ethanol when fermented. The SRI Willke process is based on the enzymatic hydrolysis of cellulosic material like newsprint and agricultural wastes such as corn stover. This process also incorporates a proposed enzymatic recycle step.

2. Direct fermentation of Cellulosic Materials :

There is a growing interest in the application of the thermophilic microorganisms to industrial processes. A feature of the microorganisms being investigated for ethanol production is their heterofermentye capability. Clostridium thermocellum converts cellulose to acetic and lactic acids as well as to ethanol. C. thermohydrosulfuricum metabolizes to ethanol and a broad rage of carbon sources including pentose and starch. Genetic manuplation of metabolic pathways is required to direct electron flow towards the reduced biochemical product dictated by economic and market consideration. There could be an application for site directed mutagenesis such as the use of insertion sequences to shut off gene activity. A candidate would be lactate dehydrogenase in C. thermocellum, e.g. if ethanol were the chosen product. Implanting ferredioxin NAD and NADP reductases in C. thermicellum might be attempted as a way to direct electron flow towards ethanol production.

Thus the production of ethanol from cellulosic waste by biotechnology application is technically feasible and has been proven. Alcohol produced from all such materials is equal to synthetic alcohol in quality and performance. However, still there are many constraints and still further research work is required to make it economically viable at plant scale.

Enzymatic Enhancement of the Bioconversion of a Cellulose to Methane :

Much of the current interest in bioconversion technologies has been focussed on the conversion of cellulosic material to readily usable fuel products and one is production of methane. Anaerobic digestion of solid waste offers a promising alternative both as a waste treatment process and as a means of energy (methane) production. The mode of interest from an anaerobic digestion is twofold.

1. Production of methane that is a source of energy.
2. Utilization of the digester effluent either as a direct animal feed supplement or as a basal medium for single cell protein production.

The anaerobic digestion technology is by no means a new technology. It has been known and used in many countries for many years. Now the work is being directed towards enhancement of the yield of the process in which the cellulosic material is first hydrolyzed to glucose monomers and is the biologically converted to the final product. Since the efficiencies of the biological conversion process is highly dependent on the feed stock presented to the organisms, great interest has been focussed on improving the yield of readily metabolized simple sugars from various hydrolysis techniques. There is a great potential to enhance the bioconversion of cellulose waste to methane by enzymatic hydrolysis.

In one of the study, enzymatic hydrolysis was carried out to cellulosic waste (MSW, paper mill sludge) that had already been degraded partially by anaerobic digestion[17]. This process provided the advantage of rendering the cellulosic fraction of cellulosic waste more amenable to hydrolysis because it had already been subjected to mechanical and thermal stresses in the digester and much of the biodegradable associated material had already been metabolized. After enzymatic hydrolysis, the cellulosic waste was introduced to a second anaerobic digester to further degrade the material to biogas. Figure 4 is a diagrammatic presentation of the laboratory scale enzyme treatment and anaerobic digester process used for MSW and paper mill sludge. Enzyme used in this work was obtained from the fungus Trichodermareesci.

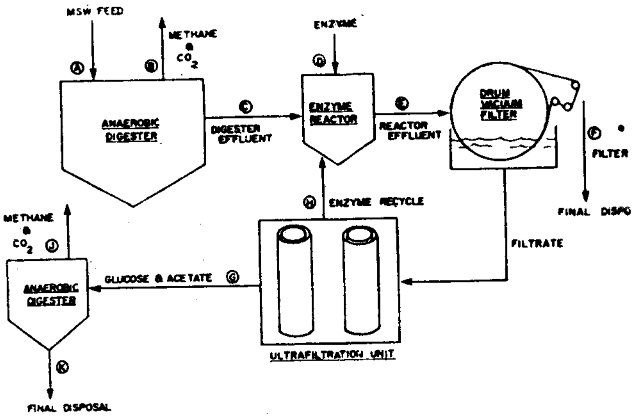


FIGURE 4 Laboratory - scale process flow diagram of Biogas generation through enzymatic hydrolysis

During the 5% MSW TS study, the digester converted 17% of the total solids and 16% of the cellulosic TS to biogas. On the average, 0.11 lit (0.17g) of biogas was produced per gram of inputs solids 95 volume % of which was methane. Thus incorporation of Enzymatic hydrolysis step in the process line system gives gain in overall conversion of solid to biogas. An additional 0.13 lit (0.14 g) of biogas was produced per gram of input solids, 58 volume % of which was methane. As a result of enzymatic hydrolysis process, 115% more biogas, 153% more methane and 82% more total solids conversion were observed than with digester I alone. In addition, approximately 100% more of the total cellulosic solids were converted to methane.

However, the effect of glucose inhibition and the relationship between enzyme recovery and extent of cellulose hydrolysis both indicate the need for further study of enzymatic hydrolysis process. If the rate of removal of glucose could be enhanced hydrolysis should proceed to completion accompanied by an increase in the overall recovery of enzyme. This would result in a significant increase in the efficiency of the process, and ultimately to a much greater destruction of MSW wastes.

Preliminary studies on paper mill waste have indicated that the application of enzymatic hydrolysis to these substrates is quite promising for the overall increase in bioconvertibility with appropriate levels of enzyme applications, both solids destruction and methane production should be greatly enhanced.

Thus it has demonstrated that feasibility of enzymatic hydrolysis as a means of optimizing the anaerobic digestion of various waste is possible.

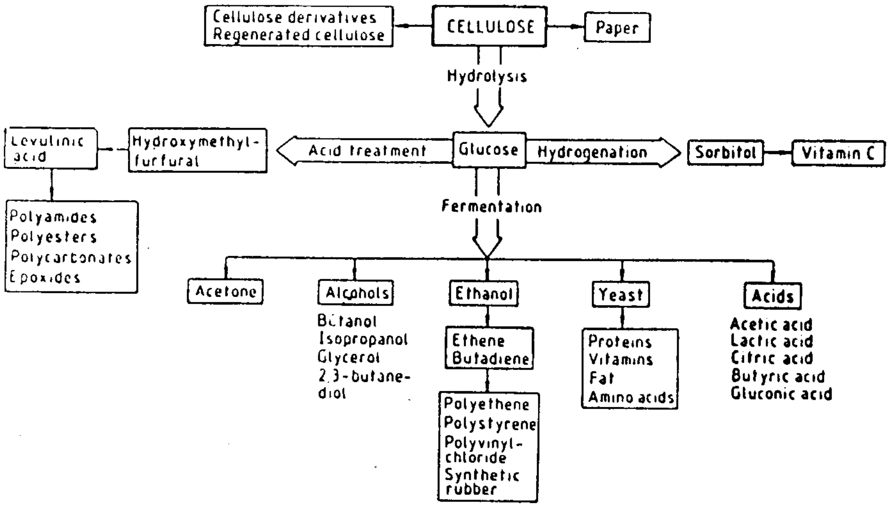
Chemicals From Cellulosic wastes

Chemicals here refers to nonpolymeric organic compounds of commerical importance. At present almost all organic chemicals and the synthetic organic polymeric material derived form them are obtained from petroleum and natural gas. This dependence is so great that the term petrochemical has become synonymous with the chemical industry. However many of these chemical can also be produced from cellulosic waste by biotechnological application. After converting cellulosic waste to glucose many interesting avenues of producing commercially useful chemicals will open up. Some of the possibilities are shown in figure 5 and detail is available elsewhere[22]. These are the most obvious one, but chemical ingenuity will undoubtedly develop many new schemes for the chemical utilization of glucose if it should become an inexpensive intermediate in cellulosic waste conversion by biotechnology application.

Economic Considerations

Economic factors in the assessment of cellulosic substance as chemical and energy resources are many and complex. No substrate nor conversion process can be singled out as significantly advantageous.

If glucose is to be the end product, then it will probably have to complete with corn syrup. If SCP is to be the end product, then in order for the SCP process to be economical, volumetric productivities of 2—4 g/L production must be achieved and it should be economically competent with soya meal and fish meal. If alcohol is to be the end product, then an intermediate product stream of glucose and other sugars must be obtained at a price that is in case of obtaining glucose form present production.



Chemical products derivable from cellulose

Figure 5

In a cellulose processing system using fungal celluloses, the cost of enzyme production plus hydrolysis cost is more than 50% of the overall cost of production. Research work in genetics and physiology is in progress to develop hyper producing strains. This is an area where gene amplification must be tried. If pushed by genetic manuplation to the physiological limit of protein production and excretion, such an improved fungal strain could have a pronounced effect on lowering the cost of production of ethanol. This remains to be determined.

Some of the points which will determine the future use of utilization of organic solid waste for production of valuable products by biotechnological application are discussed below.

Yield

When weighing process alternatives, yield or resource utilization is a prime consideration. Only resource availability will not solve the problem, it is also important that how much final product is obtainable from resource. In case of SCP conversion the yield is about 45 to 55%, in case of only glucose it is about 80% and while for ethanol conversion, yield is more than 60% thus this data indicate that there is enough resource utilization in these conversions in comparable to other products.

Raw Material Cost

Raw material costs are important economic factors in most manufacturing operations, they are critical in conversion of cellulosic material to fuel or other chemicals. The heterogeneous composition makes low yield of products inevitable when expressed as a per centage of total cellulose material. Reduction in product weight is also to be expected in going from cellulosic material with its high oxygen content to chemicals such as ethylene with no oxygen as well as from loss of CO_2 during fermentation. The maximum possible yield of ethylene from glucose would be only 31% and even of ethanol only 51%. With such yield limitations, the raw material costs must be low.

The cellulosic materials like biomass, paper pulps are costly and in this respect waste cellulosic sludge from paper making WWT is a potential raw material in biotechnology application, because it is unusable residues that must be disposed of and thus is having mostly negative cost. However, in all probabilities the minimum value that will come to be placed on cellulosic sludge waste suitable for conversion will be determined by its value as an alternative source of fuel by direct combustion or for other utilization purposes (board, compost etc.). Again, pretreatment by physical and chemical means as discussed earlier for enzymatic hydrolysis will increase the cost, but in case of cellulose sludge, this cost will be very less as it has already gone through pretreatment in paper making process.

Capital Investment :

It has been reported that capital investment costs are generally greater particularly in conversion to chemicals in comparison to conventional petrochemical processing. The reason for this is that there is difficulty in handling and storing solids compared to liquids. Estimates for ethanol plant investment for enzymatic hydrolysis vary widely, but are equal to or greater than acid hydrolysis cost.

Although the relative magnitude of capital investment requirements is discouraging in enzymatic hydrolysis, one bright spot is that the individual plant size can be much smaller for conversion. This initial plant size and thus investment considered to be necessary for economy of scale would be much low in case of conversion of cellulosic raw material to chemicals or other use by biotechnology application. This would be a great advantage.

Cost and Availability of Fossil Fuels/Protein Products :

These are the most important consideration effecting the ultimate utilization of cellulosic waste to ethanol, SCP production. Petroleum based products, (e g gasoline for motor fuel) will continue to command a high price in India and also there availability will also be scarce. Same will be the case with other fossil fuels (e.g. coal). In these circumstances, ethanol or biogas production from cellulosic waste material may represent an overlooked opportunity. Similarly, due to large population, availability of protein rich food will be scarce and also these products will cost high and their demand will increase. So SCP production will certainly be advantageous in case of low coast conversion.

Political, Social and Environmental Considerations

Classical laissez – faire economics based on supply, demand and profitabiltity are not necessarily the most important factors in determining a change in a resource based, as has been pointed out by Berg in his analysis of the history of the switch from wood to coal, and then to oil and gas as primary energy resource. Although he emphasized process technology advances as having been more important than the relative prices of resouree, a similar role may be assigned to political, social and environmental decisions.

In present times, it is also possible that governments incentives could influence decisions on the resources based to be used for the production of fuel, chemical and other products. Mechanisms such as subsidies, price supports, favourable tax treatment, price ceilings, import duties etc, can completely reverse an unfavourable venture analysis based on market place economics alone. **Instead of buying petroleum from abroad with scarce foreign exchange, a country may like to achieve self sufficiency.** The increased use of cellulosic waste material will also have great impact on environment. So these all points can favour utilization of cellulosic waste by biotechnological applications of fuels, chemicals and SCP.

Conclusion :

There is no question but that organic or cellulosic wastes could be put to a number of uses by biotechnology application, thus mitigating the effect of anticipated increase in the shortage and continuous increase in prices of fossil source raw materials. These uses could include energy production or the production of material, proteins or essential chemicals.

Economics, of course, is an overriding consideration. Economics will determine weather cellulosic waste should be collected and transported in the first place and the degree to which it can be managed and manupilated prior to utilization.

Characteristics or compositional requirements of the cellulosic waste as dictated by the particular use to which the waste is to be put or by the process by which conversion to some useful product is to be mediated. For instance, if glucose is to be produced as an intermediate or final products via enzymatic conversion (cellulase), which is substrate specific, then a relatively pure substrate with regard to cellulose content in required. Protein production is considerably less restricted but still nicely served by a highly cellulosic substrate, on the other hand substrate used as an energy feed stock need not be either purified or restricted to cellulosic composition, but could conceivably include other organic materials of either natural or manufactured origin. The method of substrate conversion as applied to energy feed stock, however, would dictate substrate composition. Biological conversion methods would be restricted to the use of easily biodegradable substrates.

The ultimate utility of any cellulosic waste, of course, depends upon its availability; more precisely the quantity that can be collected economically for consumption, whether it be in the form of primary substrate, or residues resulting from other consumptive processes. Since the usage contemplated for cellulose have arisen from but recent recognition of new needs, there has not yet been time to develop substrate sources solely for these needs. Consequently one of the most readily available sources of cellulose at present time are residues, or wastes.

Thus biotechnology application, particularly using waste can revolutionize industry and everyday life. Some analysts believe that its future economics and social impact will be deeper and more widespread. Unfortunately, biotechnology. being still in process of early development, does not posses a shape and easily defined form and much research work is needed in this field.

References :

1. Millet M.A., Baker A.J. and Satter L.D. Biotechnol and Bioeng. Symp. No. 5, 193–219 (1975).
2. Marchessault R.H., Coulmbe S, Hanal T. and Morikawa H. Pulp and paper Canada Trans. 6, TR 52 (1980).
3. Cowling B.W. Biotechnol and Bioeng. Symp. No. 5, 163—181 (1975).
4. Gaden Jr E.L. Biotechnol and Bioeng. Symp. No. 5, 161—162 (1975).
5. Pritchard G.I. etal J. Animal Sci 42,215 (1962).

6. Nystrom J. Biotechnol and Bioeng. Symp. No. 5, 221—224 (1975).

7. Beech G A., Melvin M.A. and Taggart J., 'Food, Drink and Biotechnology' in 'Biotechnology Principles and Applications'—Higgins I.J., Best D.J. and Jones J. Eds Blackwell Sci 1985.

8. Humphrey A.E. Biotechnol and Bioeng. Symp. No. 5, 49—65 (1975).

9. Turnbull Andy, Pulp and Paper Canada 83:5 (1982).

10. Crawford D.L. and McCoy E., Biotechnol and Bioeng. Symp. 13, 77 (1971).

11. Ericson, K.E. and Larsson K., Biotechnol and Bioeng. 17, 327 (1975).

12. Harkin J.M., Grawferd, D.M. and McCoy, E., Tappi 57, 131 (1974).

13 Blotkamp, P. J , Becker, D. K., and Emert, G. H. ' Pilot scale conversion of sellulose to ethanol", in 'Fuels from Biomass and wastes' Klass, D.L. and Emert, G,H Eds., Ann ArbourMich , 1981.

14. Wyn, C E , Spinder K, Gohmann, K. and Lastick S. M , Biotechnol and Bioeng. Symp. No. 17, 218 (1986)

15. Wright J. D , Power A J., Douglas, L. J. Biotechnol and Bioeng. Symp. No. 17, 285 (1986).

16. Edwards, V. H., Biotechnol and Bioeng Symp. No. 5, 321–338 (1975).

17. Higgins, G. M. Billock L. D. and Swartzbaugh, J. T., "Enzymatic Enhancement of the Bioconversion of Celloulose to Methane" in 'Fuels from Biomass and wastes' Klass, D. L. and Emert, G. H. Eds., Ann Arbour Mich., 1981.

18. Wise, D.L., Sadek, S.E.,Kispert, R. G , Anderson L. C. and Walker, D. H., Biotechnol and Bioeng. Symp. No. 17, 285–301 (1975).

19. Rogers C. J., Coleman E., Spino D. F., Scarpino P. V., and Purcell T. C., Envir. Sci. Technol., 6 (8), 715 (1972).

20. Brandt, D., Biotechnol and Bioeng. Symp, No. 5, 275--277 (1975).

21. Kennedy, J. F., Philips, G. O. Wedlock D. J. and Williams, b. A., "Cellulose and its Derivatives : Chemisrt, Bichemisrty and Applications", JOhn Wiley and Sons (1985).

22. Goldstein I. S. 'Chemicals From Cellulose' in "Organic Chemicals from Biomass", Goldstein, Z. S., CRC Press Inc., Florida, 1983.

23. Murray Moo Yong, "Comprehensive Biotechnology" Vol. 4, Pergomon Press, New York, 1985.

24. Barnard G. W., Economic Analysis Of Liquid Fuel Options' 'in Bioconversion System' Wise D. L. CRC,

25. Millet M. A. and Goedken Y. L , Tappi, 48 (6), 367 (1965).

26. Bassham J. A. Biotechnol and Bioeng. Symp. No 5, 9 (1975).