Biotechnology applications in pulp and paper industry

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ABSTRACT

Biotechnology has aroused wide interest due to its diverse applications in various fields. It is likely to gain promising position in pulp and paper sector also. This review highlights the various aspects of biochemical processes used in pulp and paper industry. Forest agriculture, biopulping and bio-bleaching, pollution abatement and colour removal, production of chemicals and bio-energy generation from lignocellulosic wastes of the industry are the areas where biotechnology is likely to play a major role in coming decades. Various pretreatment methods used to enhance enzymatic and microbiological attack on lignocellulosic wastes are also examined. The micro-organism responsible for deterioration of raw materials and products are also discussed

INTRODUCTION

Recent advancements in the field of biotechnology and bio-engineering have led to their diversifying applications in various fields such as food, medicine, agriculture, manufacture of usefu lchemicals, metallurgy, pollution abatement, waste utilisation, etc. Application of biotechnology is very old in pulp and paper industry. These wastes can not only be handled but can also be usefully utilized through biochemical routes. Use of appropriate biotechnology—forest agriculture, bio-pulping, and bio-bleaching will improve productivity and lead to energy conservation. Various secondary treatment processes like oxidation pond, aerated lagoon, activated sludge, trickling filter, rotating biological surface, all the processes are biological treatment processes. Lignocellulosic materials are available in abundance and are the most easily renewable natural resource on the earth. During recent years various biotechnological processes have received much attention in manufacture of valuable commercial products such as ethanol, single cell protein, fodder yeast and bio-energy from lignocellulosic wastes. Wastes rich in lignicellulosics are generated in large quantity at various stages of paper production and utilisation.

This paper presents an overview of the biotechnology applications in pulp and paper industry for pollution abatement and colour removal; forest agriculture, bio-pulping and biobleaching, manufacture of useful chemicals and bio-energy from lignocellulosic wastes.

FOREST AGRICULTURE

Biotechnology can be helpful in boosting up the forest productivity by clonning of superior trees using tissue culture techniques; by developing trees that assimilate naturally occurring nutrients or applied fertiliser; enhancing nutrient uptake by improving the symbiotic relationship between tree roots and mycorrhizae, developing microbial fertilisers; developing trees having more resistance to diseases and pests, selecting soil bacteria to produce toxic compounds for the control of insects and pests. The growth rates of trees can be enhanced by increasing the rate of photo synthesis and/or improved breeding methods[1,2]. The other field where genetic engineering can provide valuable contribution is in altering body plan of trees[3].

BIO-PULPING

Microbial delignification of lignocellulosic materials by microorganisms and their cellulase-less mutants and

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enzymes can be highly cost effective as these require mild condition compared to conventional chemical and mechanical processes which are carried out at high temperature and pressure. Bio-pulping will help in reducing energy consumption. White rot fungus and enzymes extracted from wood-rot fungus such as *Phanerochaete chrysoporium* are able to degrade lignin as well as the other wood components[4-10]. By the action of fungi and enzymes lignin molecule is weakened and some of the carbon-carbon linkages and carbon-oxygen linkages are splitted making the lignin molecule more accessible to further treatment[2]. Pulp made from spruce wood by treating the chips with *Sporotrichum pulverulentum* showed an average saving of 23% in the energy requirement in further refining. Thus marginal reduction in lignin content by pre-biological treatment gives substantial saving in energy[11,12]. Laboratory data of chemical pulping of eucalyptus wood pretreated with *Schizophillum commune* white rot fungus by SPB-Projects and Consultancy, Madras show higher yield, brightness, breaking length, and burst factor[3].

BIO-BLEACHING

Bleaching operation can be carried out by micro-organism requiring less costly and deleterious bleaching conditions. Costly and deleterious chemicals like chlorine, chlorine dioxide, hypochlorite, hydrogen peroxide, etc. will be needed in lesser amounts. Bond cleavage by use of fungal enzymes makes the lignin more succeptible to attack by bleaching agents. Net result of bio-bleaching is increase in brightness with same level of yield, higher viscosity, reduced effluent load, less chemical requirements and less energy requirement. Some of the experiments were carried out by SPB-Projects and Consultancy Limited, Madras. It was observed that CTMP and CMP pulp from bagasse on treatment with a crude enzymes resulted in 2 to 6 unit improvement in the brightness and further improvement was anticipated by purified enzyme optimising operating conditions[3].

BIOLOGICAL TREATMENT FOR POLLUTION ABATEMENT AND COLOUR REMOVAL

Paper industry utilises wide variety of raw materials. Various process technologies used are kraft pulping, sulphite pulping, soda pulping, neutral semi-sulphite, chemical pulping, and mechanical pulping. In India large integrated paper mills which account for 60% of total production are based on kraft pulping and utilise hardwood and bamboo as main raw material. Small paper mills which account for about 40% total production are based on soda pulping and utilise agricultural residues and wastepaper. Sulphite pulping process is used by only one mill in India. Newsprint mills utilise hardwood, bamboo and bagasse as raw materials and use mechanical pulp 70-75%, from stone ground wood, refiner mechanical pulping, thermo-mechanical pulping, cold soda refiner mechanical pulping and 25-30% kraft pulp.

Characteristics of the effluents depend upon the raw materials used and pulping and paper making processes. Characteristics of effluents from large integrated mills based on bamboo and hardwood, newsprint mills, agrobased and waste paper mills is given in Table—1[13-14]. Various commonly employed effluent treatment systems for paper industry are given in Fig. 1[15]. All biological treatment processes used are capable of reducing BOD/COD, toxicity, foaming tendency and dispersed turbidity, however, slight reduction in chlorinated phenols and colour is achieved. Comparison of various biological treatment processes is given in Table 2[16-20]. Each process has its own advantages and disadvantages.

The various aerobic treatment processes used are stabilisation ponds, aerated stabilisation pond and activated sludge. In the process of degradation of organic compound oxygen is being consumed by the cells present in the waste to their growth and in turn organic compounds decomposed into simple compounds viz. CO_2, SO_2, H_2O, H_2S etc. progressively.

Stabilisation Ponds

Stabilisation ponds are being used since long in paper industry and are still very common in developing countries as these are less expensive, however, land requirement is large. About 50-90% BOD reduction is obtained at loading of 4.89 to $58 \times (10)^{-5}$ kg/m²/day (22.6-26.8 kg/acre/day)[17]. Aerated stabilisation basin is an outgrowth of oxidation pond and is very common in Indian paper industry. Three basic types of aerated stabilisation ponds-aerated oxidation pond, facultative

TABLE 1—Characteristics of Effluents from Pulp and Paper Mills

Raw Materials	Integrated Pulp and Paper Mills	Newsprint Mills	Agrobased Small Paper Mills	Waste Paper based Mills
Raw Material	Bamboo, Hard wood	Salai, Bamboo Hardwood	Rice straw, Wheat straw Bagasse etc.	Waste paper
Waste water, m^3/Tonne	250-350	200	200-380	70-150
pH	6.0-9.0	7.2-7.3	6.0-8.5	6.0-8.5
Pollution Load, kg/Tonne Paper				
Suspended solids	100-150	100	90-240	50-80
BOD_5	35-50	45	85-270	10-40
COD	150-200	135	500-1100	50-90

TABLE 2—Comparison of Various Biological Treatment Processes

Treatment Method	Area requirement	Load range kg/m^3/day	H. R. T.	BOD_5 Reduction	Remarks
1	2	3	4	5	6
Stabilisation Pond	Very large	0.055-0.01	10 days or more	50-80%	Capable of buffering accidental spills of strong waste without upset, no maintenance, no mechanical aeration required, aeration takes place by natural means from air. Large Land requirement, suitable where land is inexpensive.
Aerated Lagoon	Large	0.04-0.2	5-10 days	50-90%	Capable of shock load absorption, general stability, less capital and operating cost than activated sludge. Temperature sensitivity and large land requirement than activated sludge.
Trickling Filter	Small	1-5 kg $BOD_5/m^3/d$		40-75%	Low BOD removal efficiency, not suitable for large scale volume, low sludge, large temperature drop, time to start after a stop-a week, operating cost high.
Activated Sludge	Small	1-3 kg $BOD_5/m^3/d$	2-10 hrs	70-95%	Relative compactness, heat conservation inherent operation flexibility. Inherent disadvantage sensitivity to shock loads, excess sludge, temperamental behaviour. Maintenance, installation and operating cost high compared to aerated logoon. Time to restart after a stop 3-4 weeks.
Rotating Biological System	Small			80-90%	Higher surface area requiring higher cost, suitable for small volumes.
Anaerobic	Small	10-20 kg COD/m^3/d	4-12		Lower electrical power, production of energy, rich biogas, lower nutrient demand, lower sludge, highly concentrated effluent can be treated, no noise or odour nuisance. Some of the disadvantage long start up period, diluted and cold effluents can not be easily treated.
Land Disposal	Large area	200 lb/acre/day 225 kg/ha/day		90-95%	Low cost, high purification and simplicity of operation. Some of the disadvatages are large land requirement, soil sealing odour, freezing problem in cold weather.

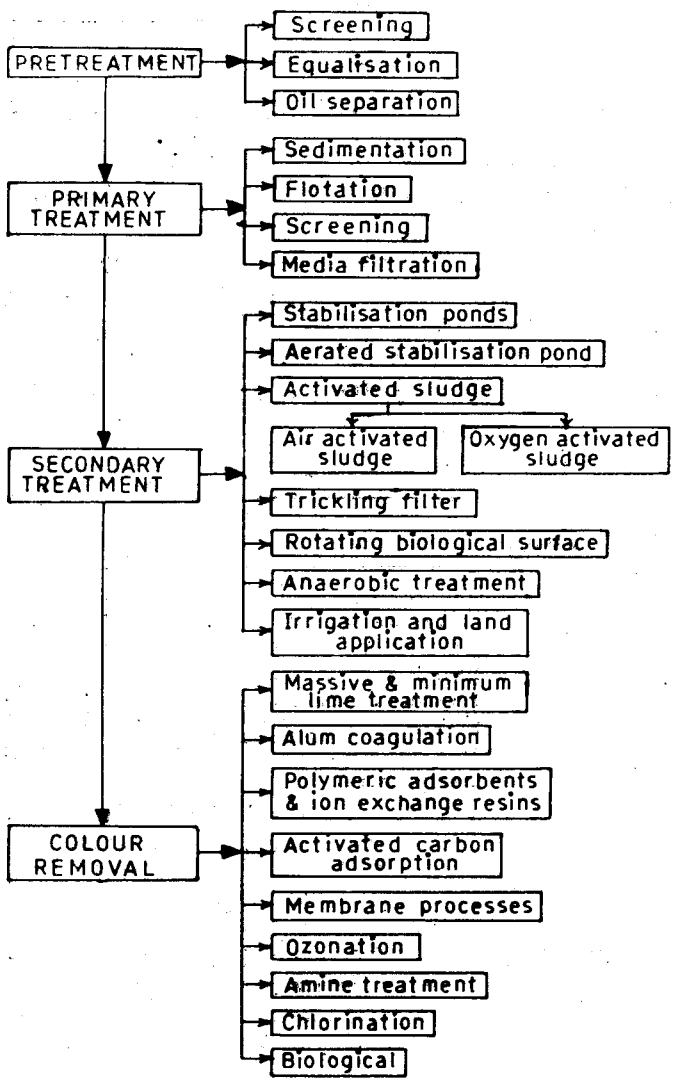

FIG.1 - EXTERNAL EFFLUENT TREATMENT METHODS IN PULP AND PAPER INDUSTRY.

aerated lagoon and completely mixed aerated lagoons are used. The efficiency of BOD removal in areated stabilisation pond is 80-95%[16]. Aerated stabilisation basin system requires about 0.5-0.6kg of oxygen per kg of BOD removed[16].

Activated Sludge Process

Activated sludge method has been very common in pulp and paper industry all over world and many effluent treatment plants based on activated sludge process are operating in India. Both surface aerations and diffused aeration technologies are used. The detention time is 3 to 8 hr. Most activated sludge units are designed to have 0.46-0.54 kg of oxygen transferred for each kg of BOD_5 removed[17]. The nutrient demand of activated sludge process is BOD_5 : N:P ratio of 100:5:1[17]. The amount of sludge generated is 0.23-0.34 kg/kg of BOD_5 removed and is difficult to dewater[16]. Activated sludge process is succeptible to upset due to shock loads of BOD or pH swings. Effectiveness of an activated sludge is dependent on the protoxoan count in the system. Various modifications which have been used in paper industries are-contact stabilisation process and extended aeration. Oxygen activated sludge process has received growing interest during recent years due to availability of pressure swing absorption technology for producing pure oxygen in small quantities at an economical cost. Oxygen activated sludge allows very high transfer rate of oxygen into the activated sludge and requires small size aeration basin, less retention time, small volume of sludge with better dewatering characteristics[16]. Cost of effluent treatment system of employing activated process in a typical Indian Paper mill based on kraft pulping and cold soda refined mechanical pulping is about Rs. 1.00/kg of BOD removed and Rs. 76.10 per tonne of paper produced[21].

Trickling Filter and Rotating Biological Disc System

Although trickling filters and rotating biological discs have been tried in paper industry, low BOD removal efficiency and higher surface area requirement for treating large volumes of effluents which are discharged from paper industry, however, have restricted their acceptability. The development of plastic media in case of trickling filters has helped to overcome some of the above mentioned deficiencies.

Land Application and Irrigation

Land application and Irrigation by pulp and paper mill effluent is very common in many part of the world and has been tried by many paper mills in India also. Land applications is also a biological treatment in broad sense and is the cheapest method of effluent treatment and is best suited to developing countries where financial constraints have restricted the growth of the industry. Various systems of land application are: (i) Irrigation—direct application of effluent, (ii) Infiltration and percolation where effluent is spread at high rate onto uncultivated land and the effluent is purified as it percolates, and (iii) Overland flow—in-

cludes the flow of effluent through natural vegetation across a gentle sloping large impervious track of land[22]. The rate of application of effluent in land disposal vary with soil condition, effluent characteristics, type of crop climatic condition and application rates. Although land application of effluent is cheap and high removal is possible, the main drawback with system is large land requirement and public resistance to some extent when used for irrigation purpose. Many paper mills in India have tried the application of effluent for irrigation purposes and have found quite suitable for irrigation of the crops as well as plantation[23-28].

Anaerobic Treatment

Anaerobic treatment has received considerable interest in pulp and paper industry and many large scale effluent treatment systems utilising anaerobic treatment are in operation in many countries. The anaerobic decomposition involves the breakdown of oaganic matter in the absence of oxygen and various steps involved are hydrolysis, acid formation and methane formation which proceed simultaneously. Various available anaerobic systems are shown in Fig. 2[19,28,29]. Power and nutrient demand and sludge production is lower and a methane rich biogas is produced. Anaerobic treatment plant employing mixed reactor is working in Pudmjee Pulp and Paper Mill Ltd. having 60 TPD capacity. The BOD and COD reduction efficiencies of about 80% and 60% respectively has been achieved with specific gas production ranging from 0.45-0.5m³/kg COD destroyed with 75% methane and 25% CO_2 content in biogas. Net saving in oil Rs. 10,000 per day and as power and chemicals correspond to Rs. 15 lakhs every month[30-32].

Effluents from the pulp and paper industry contain sulphur compounds which have an unfavourable effect on the efficiency of the process (1mg of S^{2-}=2mgCOD), the biogas quantity (max 30% loss) and the biogas quality (1 to 5% H_2S)[33]. The reaction of sulphate reducing bacteria is faster than the methane bacteria and in case enough sulphate is present, all hydrogen will be consumed by sulphate reducing bacteria and the reaction[33].

$$4\,H_2 + CO_2 \longrightarrow CH_4 + H_2 \qquad \text{(i)}$$

is displaced by

$$4\,H_2 + H_2SO_4 \longrightarrow H_2S + H_2O \qquad \text{(ii)}$$

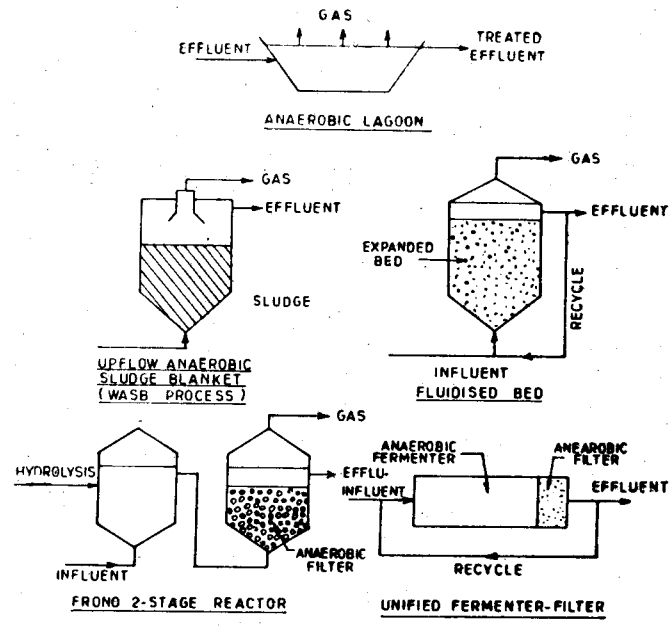

FIG.2 - ANAEROBIC TREATMENT SYSTEM.

This lowers the rate of methanegenesis. Some of the other problems are odour and corrosion. The anaerobic plants need special protection against biological corrosion. Another development of anaerobic treatment which has received attention by many paper mills abroad is high rate anaerobic-aerobic treatment which consisted of high rate anaerobic treatment and convert the organic substance into methane and C_{O_2} and is followed by aerobic treatment[28,34,35].

Biological Treatment for Removal of Colour and Chlorinated Phenols

Removal of colour from pulp and paper mill effluents has been a cause of major concern as the colour bodies are resistant to removal by conventional waste treatment methods, such as aerated lagoon and activated sludge processes, commonly used in paper industry. Lignin and its degradation products are the main contributors to the colour and toxicity of bleach plant effluent. Lignin is resistant to degradation by most of the micro-organisms and only high fungi can completely metabolise the lignin micromolecule, probably because only higher fungi can oxidatively degrade high molecular weight aromatic materials[36].

White rot fungi among various organisms have been found capable of completely degrading and metabolising lignin. The most important among the white

rot fungi that degrade lignin are *Phanerochaete chrysosporium, Polyporous versicolor* and *Sporotrichum puluerluentum*[36-41]. The degradation of lignin by white rot fungi is by and large an oxidative process and requires oxygen[41]. The fungi require an additional carbon source as co-substrate[42]. The lignolytic system is not induced by lignin but it is synthesised in response to nitrogen depletion as a result of primary growth[43]. During degradation of lignin both lignin and co-substrate are consumed without further mycelial growth.

Chlorinated phenols are major part of chlorinated organic compounds which are formed during bleaching. These chlorinated organic phenols are toxic, mutagenic and carcinogenic. Removal of these chlorinated phenols have been achieved by the biological treatment process white rot fungus by Forest Products Lab/North Carolina State University Mycelial Colour Removal process[44]. Mechanisms for degradation include methylation, oxidation and reduction[45].

As the physico chemical methods are very costly in comparison to biological treatment, there has been increasing interest in development of economically viable biological treatment with the help of white rot fungi. The biggest drawback of lignin biodegradation so far has been slowness and incompleteness of the process requiring long time.

Aqua Culture Treatment

Treatment of wastewater using aquatic weeds like water hyacinth and contail has also received considerable interest as they have been found effective in reducing concentration of organics, mineral, and various heavy metal like cadmium, chromium, mercury, silver, etc. Pilot plant trial conducted by Pulp and Paper Research Institute, Jaykaypur, Orisa showed that reduction of about 70-80% in COD, suspended solid 80% and colour to a great extent was possible[29,46].

PROBLEMS CREATING MICRO-ORGANISMS IN PULP AND PAPER INDUSTRY

Decay of the wood and bamboo by micro-organism growth of deposit called slime through pulp and paper mill system, growth of the micro-organism on finished products are the areas where cellulases and homicellulases and sulphate reducing micro-organisms cause degradation of wood, bamboo, paper, equipments and are responsible for losses of millions of rupees all over the world. These micro-organisms affect the efficiency and econom yof the mills by reducing yield of pulp from moth eating of wood and bamboo, causing corrosion due to slime in various areas and producing odor on finished product and spoiling the quality of paper. Moth-eating of bamboo and wood results in lower yield, nonuniform cooking' higher specks and lower strength. Production of hydrogen sulfide by sulphate reducing bacteria is the primary cause of biologically induced corrosion and is very common in paper industry. This is a pitting type of corrosion which occur when there are local accumulation of slime and fibre deposits that allow development of anaerobic state necessary for the growth of sulphate reducing bacteria[17]. Problems associated with slime deposit are loss of production due to frequent washups, wire and felt cleaning, more frequent felt repair, more breaks, decreased equipment performance, increased cost of heat, chemicals, filler, fiber and water and increase in cost of finished product. Appreciable losses from microbiological deposits is reported in pulp and paper industry[17]. The principal wood rotting fungi are *Merculuis lacrymans, Coniophora cerebella, Poria vailianti, Paxilluis panuoides* and *Lentinus lepideus*[47].

The organisms involved in slime are primarily spore forming and nonspore forming, filament fungi of the mold type and yeast like organisms[17]. The principal elements of the environment influencing biodeterioration are pH, oxidation-reduction potential, air temperature and humidity. Many bacteria and fungi produce the enzymes cellulase and cellobiase, and hemicellulase which degrade cellulose. The rate of slime build up depends upon type of pulp, the efficiency of pulp washing, temperature and pH changes, aeration, nature of make up water, and the nature of system[48]. The organisms causing coatings deterioration include fungi and bacteria, mosses, terminates, etc.[47]. Metal, stone, bride, concrete and plaster materials also attacked by micro-organism. Biotechnology and genetic engineering can be helpful in developing suitable technology for reducing the losses.

BIOTECHNOLOGY IN UTILISATION OF LIGNOCELLULOSIC WASTE

Wood was primarily used as source of paper making fibre, timber and cellulose for rayon. However, there is growing interest all over world in wood, forest residues, crop residues; and lignocellulosic wastes from paper industry as source of chemicals, energy and even food, Various cellulosic waste materials like bark, saw dust, chipper house dust, centricleaner rejects, waste paper, spent liquor from sulphite and alkaline pulping and clarifier sludge of paper mill can be utilised as source of various useful chemicals like ethanol, butanol, furfural, acetic acid, formic acid, vanillin, single cell protein and fodder, fuel oil and bio-energy, activated carbon, directly as mulch and soil conditioner. Due to economic reasons, commercial application of various processes of manufacture of chemicals and as energy source has been restricted earlier as it is more expensive source of energy than coal and petroleum.

Chemical analysis and polysaccharide composition of various fibres raw materials is given in Table 3[49-54]. Product profile of lignocellulosic waste material is given in Fig. 3.

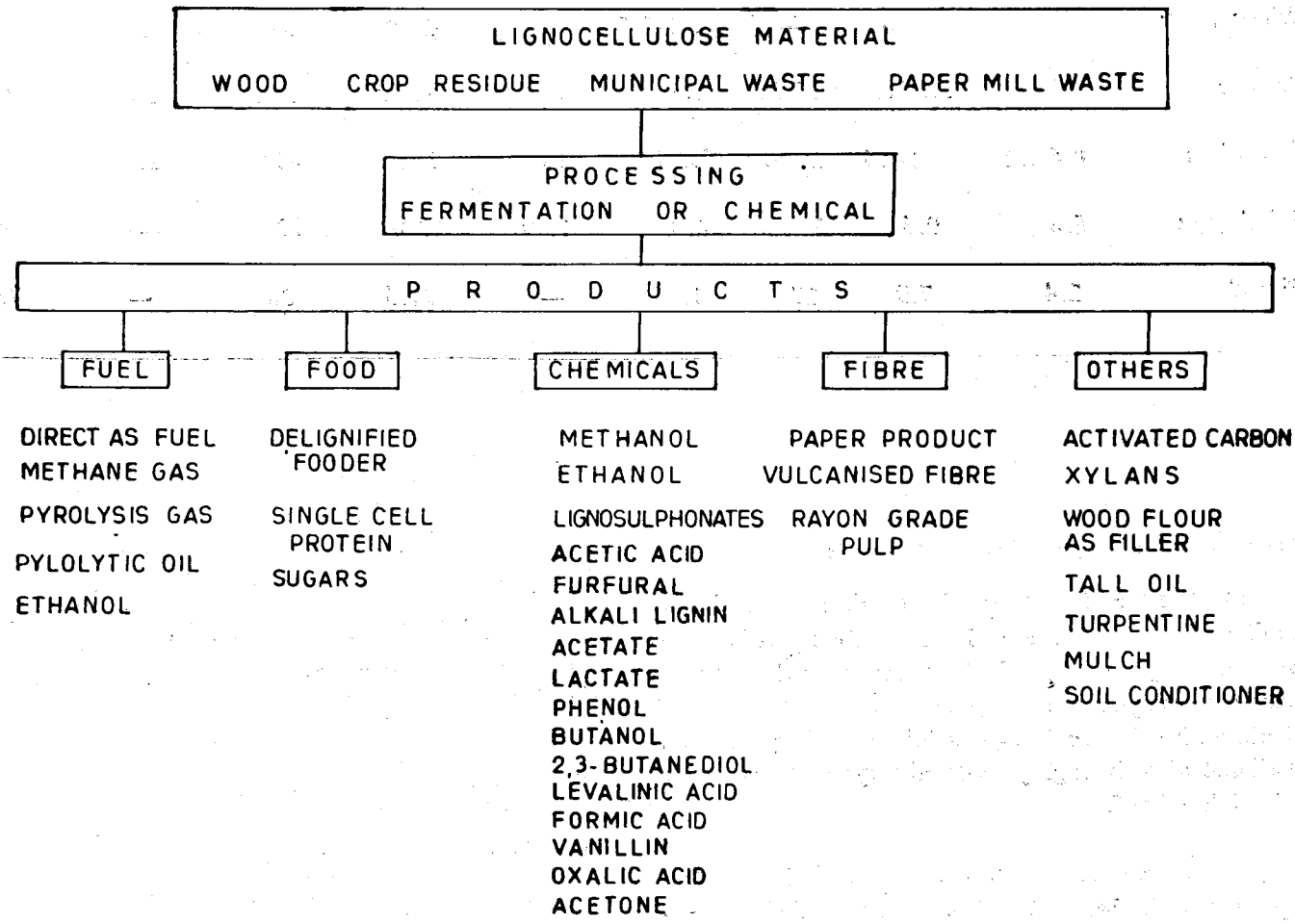

FIG. 3 - PRODUCT CHART OF LIGNOCELLULOSIC MATERIALS.

TABLE 3—Proximate Analysis of Various Raw Materials

Raw Material	Ash %	Solubilities			Pento-sans	Lignin	α-cellu-lose	Hemi-cellu-lose	Holo-celulose
		Hot water	1% NaOH	Alcohol benzene					
Bamboo	2-3	5-9	15-20	1-3	18-21	15-30	40-49	19-21	70-80
Softwood	0.2-0.5	3-5	10-20	2-4	8-9	23-33	55-60	10-15	72-80
Hardwood	0.3-0.5	5-6	10-20	2-4	18-20	16-25	45-50	20-25	75-88
Rice straw	8-16	10-15	40-48	3.8-6	24-30	10-22	26-28	24	60-74
Wheat straw	8-10	8-12	—	3.2-5.5	23-28	21.0	32	21	—
Bagasse	1.8	3.5-3.8	27-36	1.2	27-30	16-22	30-35	28	72
Jute sticks	0.6-1.2	1-1.5	25-30	1.2-1.8	18	18-21	41	25	76
Sabai grass	6.0	9.5	38-90	4.0	23.9	22.0	33	—	66.4
Kenaf	2.4	7.9	27	1.86	—	21.5	38	—	72

Pretreatment of Lignocellulosic Materials

Most of the lignocellulosic materials are resistant to direct fermentation and the rate of enzymatic conversion of lignocellulose is slow because of the physical barrier to enzymatic attack due to close association of lignin with cellulose in lignocellulose of the plant, cell wall and also the highly ordered crystalline structure of cellulose itself.

Various pretreatment processes used can be classified into physical, chemical, and biological or their combination (Fig. 4). Ball milling or hammer milling is most popular physical method for increasing cellulose digestibility. However, it is a more energy intensive

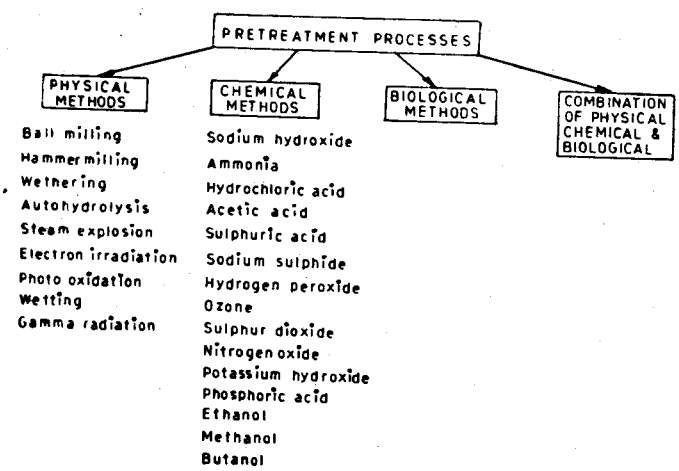

FIG. 4 - PRETREATMENT PROCESSES TO INCREASE MICROBIAL DIGESTIBILITY.

process[55-61]. The technique of irradiating wood or straw by gamma rays or by high velocity electron improves digestibility of these materials with lower degree of polymerisation, lower crystallinity and higher moisture absorption capacity[62,63]. Steam explosion process is conducted with high pressure and temperature followed by rapid depressurisation[64,66]. Sodium hydroxide treatment results in destruction of lignin structure and hydeative swelling of the cellulose and decrease in cellulose crystallinity[67-69]. Sulphur dioxide gas treatment of cellulose for increasing digestibility is most promising technique as SO_2 is less expensive than Sodium hydroxide[55,59]. Pretreatment of lignocellulose with hydrogen peroxide in presence of manganese compounds has been found to be beneficial[70]. A new type of attrition bioreactor for conversion of waste cellulose has been developed bo Rya and Lee[71] which perform pretreatment and hydrolysis at the same time. Pretreatment of hardwood biomass with supercritical ammonia dramatically enhance the succeptibility of substrate to enzymatic hydrolysis[72,73]. Other chemicals which have been tried for pretreatment are sulphuric acid[74-76], hydrogen chloride gas[77], phosphoric acid[76], ethylenedimamine[77], hydrogen fluoride[78], and ethanol and butenol (in organo-solvo processes)[79].

Ethanol from Lignocellulosic Biomass

Ethanol can be produced from lignocellulosic materials including agricultural residues, wood and forest residues and biochemical processing of biomass to ethanol has great potential as the biochemical processes are almost of 100% efficiency[80-84]. Lignocellulose is a complex structure of three major components-cellulose, hemicellulose and lignin and these must be processed separately to achieve high efficiency. Flow diagram for conversion of lignocellulose to ethanol is shown in Fig. 5[80-84]. Cellulose is hydrolysed by the acid and enzymatic process to glucose which is further fermentated to ethanol. The xylose separated during fermentation can be fermented to ethanol and can be combined with ethanol produced from glucose fermentation.

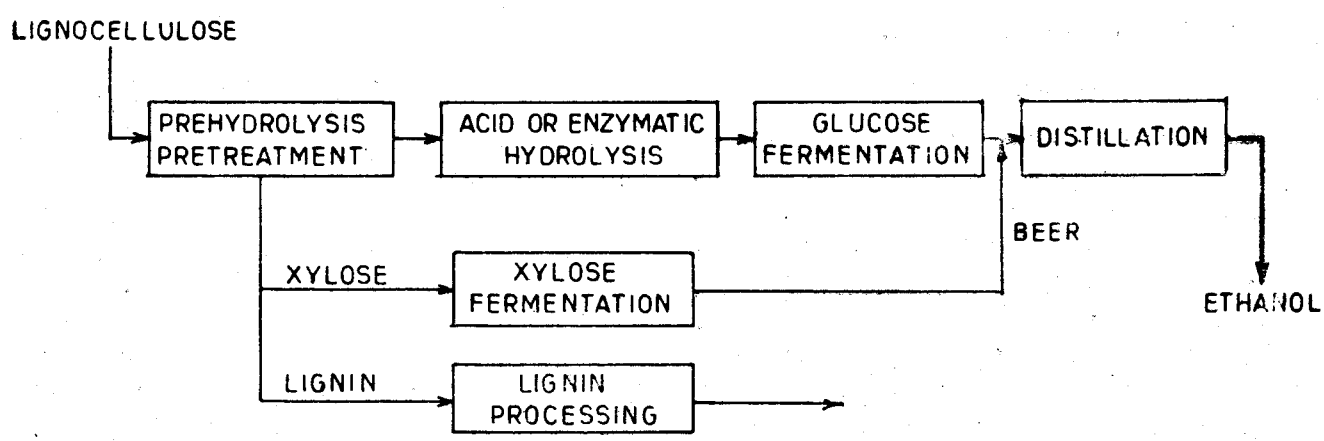

FIG.5 - CONVERSION OF LIGNOCELLULOSE TO ETHANOL

Enzymatic hydrolysis processes are attractive because of very high yield and production of sugar that are relatively clean and free of either products or degradation products[30]. The various step involved are pretreatment, enzyme production, hydrolysis and fermentation. The hydrolysis and fermentation can be carried out separately by separate hydrolysis fermentation (SHF) process or by simultaneous saccharification and fermentation process (SSF). The SSF processes have advantage over the SHF process as they reduce end product inhibition by sugars increasing the yield and product concentration and reducing the amount of enzyme required. Xylose fermentation which accounts for 30-60% of the total sugars in the biomass can be carried by bacteria, fungi, yeast or enzyme-yeast system[80-84].

Cost of enzyme production is an important parameter affecting the economy of the bioconversion process from lignocellulose to ethanol and it is very essential that in the saccharification process high sugar yield per enzyme is obtained. As much as 60% total processing expenses have been attributed to the cost of enzyme production[85]. Factors affecting yield are pretreatment, inhibition of enzyme action by heat or the degradation products, enzyme and substrate concentration, absorption of cellulase onto cellulose, speed of enzyme action and degree of agitation.

Single Cell Protein from Cellulosic Solid Wastes

Various solid wastes like primary clarifier sludge, tertiary centricleaner rejects, waste paper from municipal garbage, waste newsprint which have been traditionally disposed of as land fill or incineration can be used for the production of single cell protein by using both mesophilic and thermophilic micro-organism[86-92]. Upgrading of the six different primary clarifier sludge and a tertiary centricleaner rejects into protein rich animal feed using the cellulolytic fungus *Chaetomium cellulolyticum* was reported by Pamment et al.[86]. In the preliminary production upto 28% crude protein content of product has been oatained at specific growth rate of upto 0.12 hr^{-1} on direct utilisation of waste[86]. Studies were carried out by David for converting newsprint and other waste paper to a high animal feed supplement[92]. Production of microbial protein from pulp mill fines using *Themomonosporce fussa* a thermophilic actinomycete has been reported by Crawforb et al.[87]. The protein was found to have good nutrient quality and contains no strongly toxic material. Fermentation of waste mechanical fibres from a newsprint mill by white rot fungus *Sporotrichum pulveruten tum* to protein have been also reported[88].

Utilisation of Spent Sulphite Liquor

Spent sulphite liquor contains many useful chemicals. Spent sulphite liquor has about 90% organic material. The composition of organic material is generally 50-60% ligno-sulfonates, 20-25% monosaccharides, 5-10% palysaccharides and 10-15% low molecular weight acids[93]. The fermentation of sulphite spent liquor yields valuable commercial products such as ethanol, single cell protein, and fodder yeast. Large scale fermentation of the sugars of the sulphite spent liquor is now practised by many European sulphite mills in order to reduce stream pollution and to obtain valuable products especially ethanol. Pulp yield and alcohol yield are inversely related since the amount of sugar dissolved during pulping is reduced substantially as the pulp increases, this is the reason why spent sulphite liquor from dissolving pulp production is considered more attractive source for fermentation and hexose which can be fermented to alcohol increase by about 40% and total carbohydrate which can be used as protein fermentation increases by 45%[94]. Baker's yeast *Saccharomyces cerevisive Hansen* is used for fermentation[95]. In a process used by Ontario Paper Co. at Thorold an interesting combination involving fermentation of spent liquor to alcohol, production of vanilline from residue and destruction of residual organics for regeneration of inorganic chemicals is practised[96].

A process based on filamentous fungi growing on spent sulphite liquor has been commercialised in Finland for production of single cell protein (Pekilo protein). The product contains an excellent balance of amino acids lysine and methionine. A reduction of 50% in BOD is achieved[97].

Bio-energy Generation from Domestic Refuse and Sludge

The muncipal solid waste contain high percentage of waste paper especially fron domestic refuse (it may

be as high as 30-40% in wastern country and represents an abundant source of renewable cellulosic biomass for the potential production of biogas. Due to increasing costs of conventional methods of disposal such as dumping and incineration, the anaerobic digestion of domestic refuse could provide attractive means of reducing disposal costs through simultaneous reduction of waste volume and recovery of methane as an energy source. The overall reaction rate of biomethane production from domestic refuse is slow and requires long residence time and low loading rate, which is probably due to low cellulolytic activities and slow specific growth rates of the organism involved[98]. Rumen micro-organisms which have high cellulolytic activity have considerable potential. Rumen micro-organism and colonised polyurethane foam as a support has been found effective for anaerobic degradation of paper mill sludge[98,99].

CONCLUSIONS

Biotechnology application has great role to play in coming years for utilisation of lignocellulosic waste materials which are abundant source of cellulosic biomass. Some of the areas where biotechnology application are to be given immense importance are-Forest agriculture, utilisation of bark, fines, primary clarifier sludge, tertiary centricleaner rejects and waste paper from municipal garbage for manufacture of chemicals and single cell protein and bioenergy, in bio-pulping and bio-bleaching in secondary wastewater treatment and colour removal, bio-energy generation through anaerobic treatment of wastewater from kraft pulp mill and small paper mills. Although much work has been done in this field in developed countries, however, in India it is yet to receive due considerations. This field needs indepth study under Indian conditions to exploit the possibility of utilisation of lignocellulosic wastes and commercial availability of process technology for development of enzyme. The knowledge of genetic engineering and biochemical engineering can be very well utilised for development of biological inhibitors for the reduction of losses caused by various micro-organism in the raw material as well finished production and biological corrosion occurred in various section of the industry.

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