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RECENT DEVELOPMENTS IN BIOTECHNOLOGY RELATED TO PULP AND PAPER INDUSTRY.

V. Ramaswamy & T. Ramanathan
SPB - Projects and Consultancy Limited.
Esvin House, Perungudi
Madras - 600 096

Abstract

Bio-technology in simpler terms means exploitation of naturally occurring biological processes for industrial applications. The pulp and paper industry depends on the naturally occurring lignocellulosic material as the source of raw material. In nautre, the micro-organisms break down this abundantly available material into carbon-di-oxide and water thus maintaining the carbon cycle.

Instead of allowing the micro-organisms to convert them into carbon-di-oxide and water they can be efficiently exploited in the process and to convert conventional waste material into useful products. While industrial physicao chemical processes operate under high temperature, the biological conversion processes operate under much milder conditions thus resulting in energy savings. Most of them are efficient at room temperature and neutral pH.

Some of the process where considerable progress has been achieved include :

- Growing of genetically superior trees
- Development of disease and pest resistance
- Bio-pulping
- Bio-bleaching
- Ethanol production from wood residues
- Cultivation of mushrooms
- Waste water treatments

The paper presents data on some of the above mentioned and also results of the experiments carried out in our laboratory in the field of bio-pulping, bio-bleaching and anaerobic waste water treatment. An isolate of white rot fungus *Schizophyllum commune* which has the ability to preferentially degrade the middle lamella lignin was identified based on a systematic screening of a number of fungi. Pulping of eucalyptus wood pretreated with the fungus resulted in pulp of higher yield, lower kappa number and higher brightness.

Treatment of bagasse chemi thermo mechanical and chemi mechanical pulps with fungal enzymes resulted in 2 to 6 points gain in their brightness. Subsequent peroxide bleaching of the same clearly indicated that the fungal enzymes have modified the exposed functional groups of the lignin molecules rendering them more accessible to the bleaching agent.

A pilot plant reactor developed by us for the treatment of bagasse pulp mill waste water has been functioning steadily since last six months. The system has been quite stable and 55% reduction in the COD and 70% reduction in the BOD has been achieved.

1.0 Introduction

Bio-technology in simpler terms means exploitation of naturally occurring biological process for industrial applications. The pulp and paper industry mainly deals with lignocellulosic material as the source of raw material. The carbon cycle in the enviroment is kept up by the activity of micro-organisms on these abundant ligno-cellulosic material. They are broken down into carbon-dioxide, water and humic substances due to the microbial activity. Instead of allowing the microbes to convert them into carbon-dioxide and water, they can be efficiently exploited for the production of a number of industrially and commercially valuable products like fodder proteins, ethanol, methane, phenolics and other energy rich basic chemicals.

Micro-organisms or the enzymes produced by them can be used to convert the conventional waste material into useful products. While the industrial physico-chemical processes operate under high pressure and temperature, the biological conversion processes operate under much milder conditions thus resulting in energy savings. Most of them are efficient even at room temperature and neutral pH.

Some of the processes where the exploitation of bio-technology in paper industry has received interest are :

- Growing of genetically superior trees
- Incorporation of nitrogen fixing genes into tree crops
- Development of disease and pest resistant clones
- Improving the symbiotic relationship between the roots and mycorhiza thus enhancing their nutrient uptake
- Bio-pulping and bio-bleaching
- Separation of bark by preferential bio-pretreatment
- Ethanol production from wood residues
- Cultivation of mushrooms on non-pulpable wood and wastes.
- Waste water treatment etc.

2.0 Bio-Technology & Forestry

The issue of increased economic productivity from our commercial forests is crucial. A simple process of more intensive management of the existing stands can boost the productivity[1]. The various functions that are actively involved in the growth of trees are :

-- The tree form and structure

-- Photosynthetic efficiency

-- Avoidance of water stress

-- Cambial production in the tree

-- Respiratory mechanisms

-- Capacity to seek and acquire nutrients and water

Bio-technology basically involves the manipulation of most of these functions at the genetic level or DNA level. The phenomenal successes that have been achieved in agricultural crops like tobacco indicate that a similar possibility existes even in the tree crops. The asexual means of production of offsprings that resemble the parent is termed "clonning". Traditionaly vegetative propagation like cutting is used for clonning. The trees can be clonally propagated and put to field trials in nearly one third the time required by the conventional methods. The superior trees can be indentified only after the trees have reached maturity and propagation by cutting at this stage becomes very difficult. Tissue culture offers an alternative for clonning the superior trees. More than 200 trees have been established as callus cultures, and in 25 cases complete seedlings have been produced[2]. Besides clonning, tissue culture also offers the potential to rapidly screen and pick the desirable genotypes. Traits such as growth efficiency, photosynthetic efficiency, stree tolerance, disease resistance, frost, drought, salinity heavy metal and herbicide tolerance can be easily screened by tissue culture.

By intergrating tissue culture techniques with genetic engineering, new characters like nitrogen fixation can be incorporated into the genotypes which will enable the trees to directly fix atmospheric nitrogen for their nutrition. This will, in turn, minimise the need to add fertilizers. Researchers in several laboratories have been able to regenerate whole crops from transformed cells bearing foreign "marker" genes.

One of the other field where genetic engineering can provide valuable contribution is in altering the body plan of the trees. The "narrow crown phenotypes" illustrates one of the morphological variants. These trees grow strongly in hight and girth and the growth of branches is reduced.

3.0 Bio-Technology and Degradation of Ligno-Celluloses

3.1 Bio-Pulping

Lignin provides the structural support to trees. The single major processing effort in the pulp and paper industry is delignification. The white rot group of fungi have the inbuilt mechanism to degrade lignin in addition to the other cell wall components. Even if complete lignin degradation is not possible, considerable amounts of the complex molecule can be degraded/altered to bring about savings in the energy inputs during pulping. Spruce wood chips on treatment with an isolate of white rot fungi revealed 23% reduction in the energy inputs[3] during mechanical pulping. The lignin molecule is weakened by the action of the fungi/enzymes. Some of the carbon-carbon linkages and carbon-oxygen linkages are split. The cleaving of these bonds render the lignin molecule more accessible to subsequent action of the cook liquor.

It has been shown that the lignin in one part of the wood may be acted upon by the fungus while it is not acted upon in yet another part of the wood[4]. In our laboratory a systematic screeing of a number white rot fungi was taken up to identify isolates which act preferentially to degrade middle lamella lignin leading to defibration. An isolate of *Schizophillum commune* has been identified by us which brings about such an alteration. The results of chemical pulping of eucalyptus wood pretreated with the isolate are presented in Fig 1 & 2.

Swedish workers have clearly demonstrated that even marginal reduction in the lignin content of wood and mechanical pulps can bring about considerable savings in the energy inputs required for refining. The results are presented in Table 1 & 2.

Currently the time required after treatment is in the order of days and can be reduced to hours with the identification of the right strains and otehr treatment conditions. The treatment can be done either during the transport or storage in the yard.

TABLE 1

ENERGY DEMAND* FOR REFINING SPRUCE WOOD CHIPS**(5)

TENSILE STRENGTH (Nm/g)	BIOLOGICALLY TREATED*** PULP (kWh/t)	REFERENCE (kWh/t)
15	375	500
25	830	1000
35	1360	1860

NOTE:
* ENERGY DEMAND IN BEATING IN A PFI MILL FOLLOWING DEFIBRATION.
** TREATED WITH A CELLULASE MUTANT OF SPOROTRICHUM PULVERULENTUM
*** AFTER GRAFFIN, TAPPI, 68, 1985, 57.

TABLE 2

ENERGY CALCULATIONS FOR REFINING OF MECHANICAL PULPS* (6)

SL. NO.	DESCRIPTION	UNIT	PARAMETER
A	ENERGY REQUIRED (J/T OF PULP)	MILLION	1.37
B	ESTIMATED PULP PRODUCTION FOR THE YEAR 2000 AD (t/yr)	MILLION	7.09
C	ESTIMATED ENERGY CONSUMPTION (J/yr)	TRILLION	9.73
D	BIO-PULPING ENERGY SAVING FACTOR	--	0.23
E	ANNUAL ENERGY SAVING USING BIO-PULPING (J/yr)	TRILLION	2.23

(* A x B = C, C x D = E)

3.2 Bio-Bleaching

The conventional bleaching of the pulp in the industry is brought about by the utilisation of various chemical reagents like chlorine, hypochlorite and peroxide. These chemicals are expensive and in additon the sewered waste water from these stages causes considerable effluent pollution and mild toxicity problems. After the alkaline exraction stage, unbleached kraft hardwood pulps have been treated with Xylanases of *Escherichia coli* clone to yield pulps which are bright and have a kappa number 24% lower than the corresponding control samples[7]. In addition, the viscosity of the pulps were also higher as compared to the controls. This again is due to the modification of the lignin in the pulp rendering them more accessible to the bleaching agents.

In our laboratory studies on similar lines have been initiated and bagasse CTMP and CMP pulps on treatment with a crude enzyme extract resulted in 2 to 6 unit improvement in the brightness. There is still scope to improve their performance by purfication of the enzyme and identification of the optimal treatment conditions like temperature and pH.

Bio-catalysts can act and accelerate the rate of the reaction upto 14 orders of magnitude[6]. In additon, they offer control over the products formed and thus the formation of undesirable side product can be controlled.

In fomenters where the enzymes cellulase has been used, it has been demonstrated that 50-90% of the enzymes are free in the solution and can be directly reused again[8].

4.0 Bio-Technology in Ethanol Production

The traditional method of ethanol production is by the fermentation of sugar solutions using yeasts. The formation of ethanol from the spent sulphite liquor has been an interesting possibility which has been demonstrated. These gain more importance in view of the shortage of fossil fuel availability.

Ligno-cellulosic wastes on prehydrolysis yield 70-80% sugars. These can be fermented by the conventional fermentation and distillatic techniques which are well known. The hydrolysis can be brought about by treatment with steam, a utility which is already available in the industry or by the action of cellulases.

Apparently the production of cellulase consumes nearly 60% of the total costs involved and consequently the economic viability of the process would largely depend on the reuse of the enzyme. After the saccharification of the ligno-cellulosic material, the enzyme must be either automatically released into the solution or must be in a state to be desorbed easily and cheaply. A simple elution with phosphate buffer yielded 90% recovery of the enzyme.

The industrial production of ethanol from ligno-celluloses apparently depends on the refinement in the pretreatment, hydrolysis and the fermentation steps of the process.

5.0 Waste Water Treatment

Pulp mills contribute substantially to the pollutants in the waste water. Although most of the pulp mill liquor is recycled, the small portions of the liquor that is not recovered and the inevitable spill overs still contribute nearly a third of the pollution load from the mill. Although a number of methods like coagulation, reverse osmosis absorption, ion exchange and others are known to have been tried, none of them have been commercially viable due to the high cost of operation/investment.

The industry already depends largely on the microbial technology to treat its waste waters. Oxidation ponds, lagoons, UNOX reactors, activated sludge system and others are all unit operations making use of mixed populations of microbes to destroy or detoxify wastes. The ligno-cellulosic wastes in the form of suspended solids largely contribute to the pollution loads. A number of noval methods have been proposed for the conversion of these wastes into fodder proteins. Some of the micro-organisms, fungi in particular, which have been used include *Candida utilis, Paecilomyces varioti, Sporotrichum pulverulentum, Chaetomium cellulolyticum* and others[9]. In the production of ultra-high-yield pulps like CTMP and TMP, a number of monomeric and oligomeric components are discharged into the waste water and these are excellent substrates for the growth of fungi.

5.1 Treatment of White Water

A flow sheet of a closed white water system for a newsprint mill is presented in Fig. 3[9]. Build up of organic material in the system is prevented by the incorporation of a fermenter with white rot fungi. The fermenter functions as a "kidney" and depletes the circulating water of dissolved material. The fungus grows as pellets and thus can be easily separated from the system by simple filtration. The fungal mycelium can be either used as fodder protein or could also be incorporated into the paper without altering the paper quality. The mycelial component of the paper can be as high as 1.5% of the basis weight of the paper.

5.2 Colour Removal

An example of the potential of bio-technology is in decolourizing the paper mill effluent. Lignin and its derivatives are the major contributors of the colour. The chromophore containing the compounds in the extraction stage of the pulp bleaching are predominantly polymeric chlorine containing fragments of lignin. Fungi have been known to break down the chromophores into low molecular weight colourless soluble/volatile products[10]. In our laboratory, we have been able to obtain upto 60% reduction in colour in the pulp mill effluents using white rot fungi[11].

5.3 Anaerobic Treatment

Till recently only aerobic systems were widely used for the cleaning of waste waters. The anaerobic treatment systems have, nevertheless, been receiving increased attention and a number of full-scale applications are now in existence in many parts of the world. In this process, the bacteria degrade the complex organic substances into bio-gas and sludge. 60% reduction in the COD and nearly 95% reduction in the BOD have been reported. Nearly 80% of the organic wastes are converted into methane and the balance is incorporated in the bio-mass. Some of the inherent advantages of the system are :

-- Lower energy demand for the bio-reactor
-- Lower nutrient requirements
-- Lower rate of sludge production
-- Production of bio-gas

In certain cases, the gain from the bio-gas production has been found to be higher than the operating costs thus making them a superior alternative. In our laboratory based on the preliminary bench scale studies a pilot plant reactor 0.8 m^3 capacity with a retention time of 3 days has been developed. Bagasse handling waste water is being treated and 55% reduction in the COD and upto 70% reduction in the BOD has been recorded[12]. The system has been in operation for over two months and the performance has been quite stable.

6.0 Utilisation of By-Products

Yet another field where bio-technology can be beneficially employed is with respect to the bio-conversion of the various lignin breakdown products. Compounds such as vanillin, dimethyl sulphonates, dimethyl sulphoxide, furfural, methyl mercaptan etc. can be produced by controlled hydrolysis.

The second largest agricultural export from Japan is the mushroom *Lentinus edodus*. Studies revealed that the growth of the mushroom can be increased ten fold by growing them in a mixture of oak wood and its bark.

7.0 Conclusion

Bio-technology has a number of potential application in the pulp and paper industry. The tantallising possibilities include the raising of superior trees, bio-pulping and bleaching, waste water treatment and others. Great strides are being made in the incorporation of some of these recent findings into existing facilities and it will not be long before these become part of the accepted processes. India being a tropical country has a wide array of fungal species which are probably a lot more virulent than some of the temperate species of which most of the early fundamental studies have been carried out. Concerted efforts to boost the research activities in some of the above mentioned areas are bound to yield valuable results.

8.0 Acknowledgements

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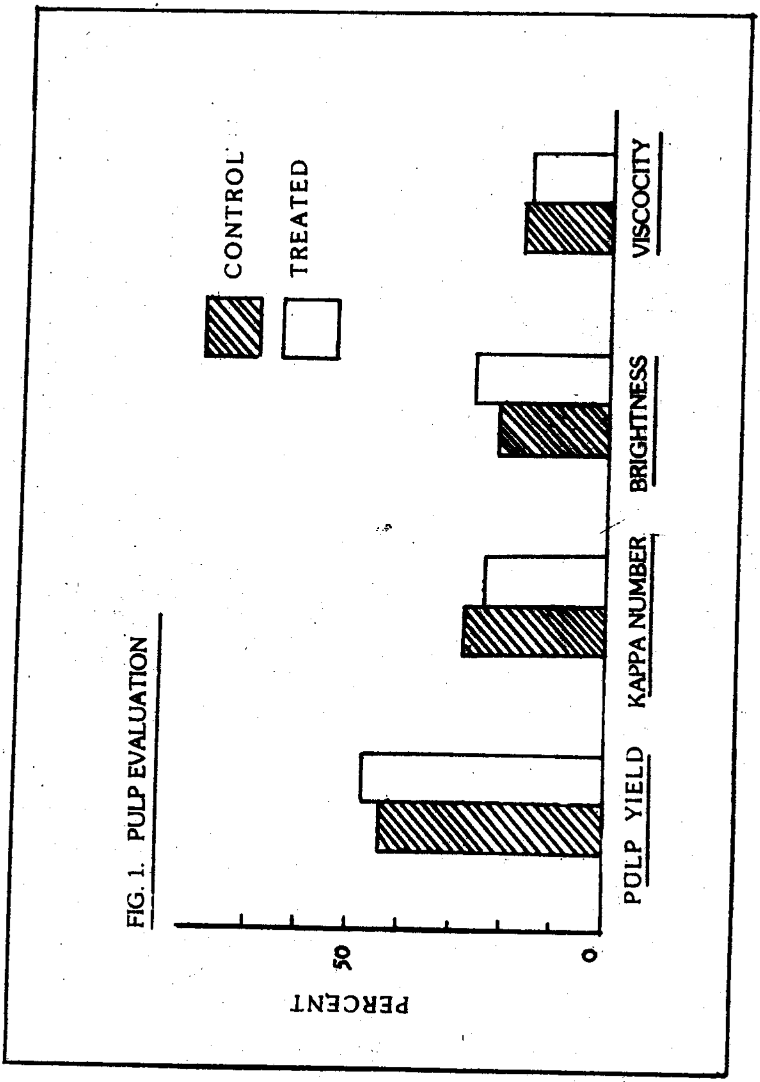


FIG. 1. PULP EVALUATION

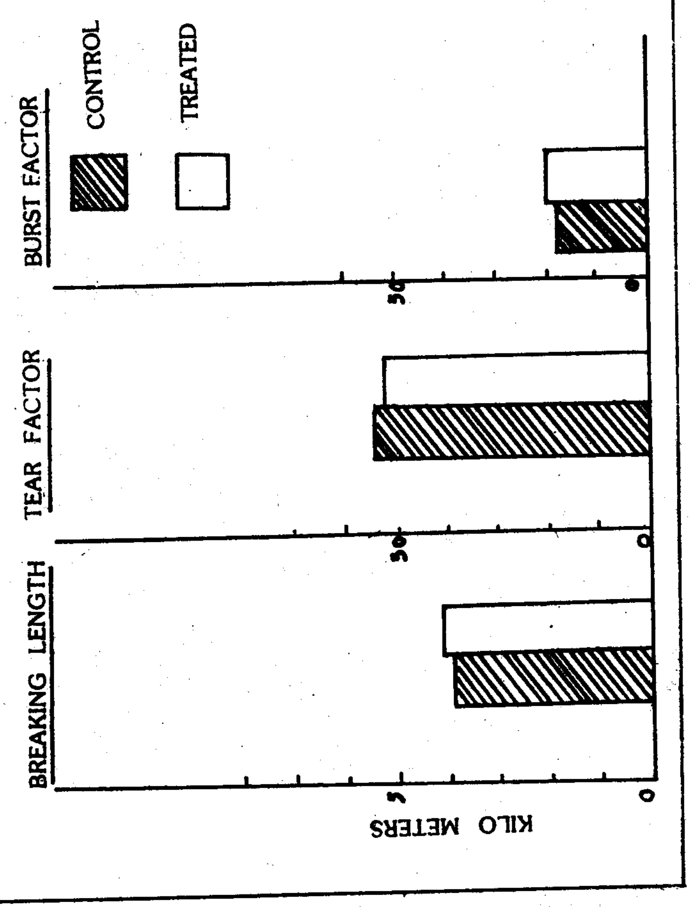
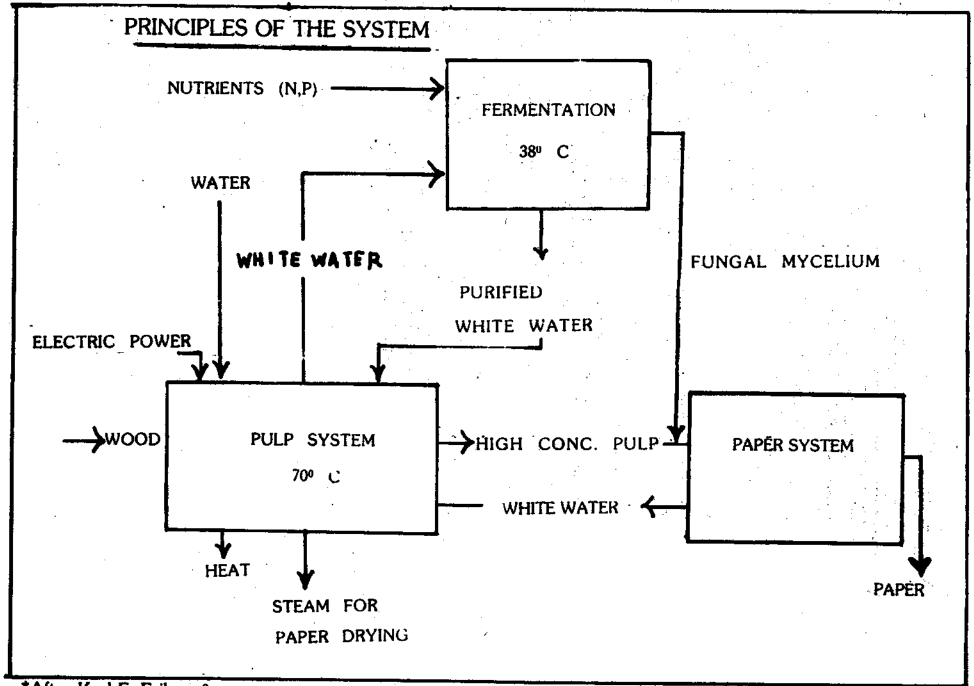


FIG. 2. PHYSICAL STRENGTH PROPERTIES

FIG. 3. FLOW SHEET OF A CLOSED WHITE WATER SYSTEM OF A NEWSPRINT PULP & PAPER SYSTEM*



*After Karl E. Erikson[9]