RESEARCH PAPER

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Biobleaching and biopulping of bagasse fiber using co-culture of *Trichoderma viridae* and *Aspergillus glaucus* fungi

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The world's second largest population is in India and hence, provides a challenging task for the government and organizations to various issues. Chemical pulping and bleaching are used to increase the paper yield and quality; however it leads to release of pollution and hazardous to ecological health (Bajpai and Kondo, 1999).Therefore, an alternative method are sought and worked out by different scientists. The Microfungi such as *Aspergillus glaucus*, *Trichoderma viridae* were selected and their co-culture resulted in a significant performance.

Key words : Biobleaching, Biopulping, Bagasse fiber

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INTRODUCTION

Pulp and papers are manufactured from raw materials containing cellulose fibers generally wood, recycled paper and agriculture residues (Santhosh Kumar *et al.,* 2009). In developing countries about 60% of cellulose fibers originate from non-wood materials such as bagasse, cereal straw, bamboo, reeds, esparto grass, jute, flax and sisal. These fibers could be supplied from wood and non-wood plant materials (Kirkpatrick, 1991). Mostly non-wood fibers are preferred for paper making, since they are eco-friendly and more sustainable. Hence, felling of trees could be arrested. Biopulping and biobleaching are promising cost effective alternatives involving the use of microbes or their enzymes to reduce and/or replace the harmful chemical extraction of hemicelluloses and lignin without affecting the cellululose and fiber strength of paper products (Kanmani, 2009; Irfan *et al.,* 2010; Mishra and Thakur, 2010). Lignin is a multifunctional natural polymer that has the potential to be developed into a major industrial raw material for a multitude of applications. After cellulose and hemicelluloses, lignin is considered to be the most abundant natural polymer present on planet earth contains 300 billion metric tons of lignin, with an annual biosynthetic rate of production of 20 billion metric tons. This can be removed by microbial enzymes for getting higher quality paper.

Bagasse as raw material:

Bagasse is the fibrous residue left after sugarcane is crushed for extraction of juice and is therefore, a by-product of the sugar industry. Bagasse has been established as a successful raw material for the manufacture of a wide range of paper and paperboard. India is the world's largest producer of sugarcane producing about 150 million tons of sugarcane per annum. Bagasse obtained from sugar mills is known as mill wet Bagasse is approximately one-third of the total sugarcane crushed, and the yield of paper from Bagasse is about one sixth (Rao, 1989). Bagasse has low lignin content, and lends itself to easy pulp ability with high yield.

Biological pulping:

Pre-treatment of wood chips with lignolytic fungi to decrease the energy requirement for subsequent mechanical pulping and to increase the strength of the pulp produced has been the most successful approach in bleaching (Nair *et al.,* 2010). These lignolytic fungi produce lignin modifying enzymes (LME) such as,

laccases, Mn-dependent peroxidase, lignin peroxidase. Microbial enzymes are enabling new technologies for processing pulps and fibers. The potential for environmentally benign, efficient lignin removal spurred research that led to the discovery of lignin-degrading enzymes in the early 80s, and to extensive (Krik and Jeffries, 1996). The xylanase of *B. subtilis* was found efficeient in biobleaching (Manimaran and Vatsala, 2007).

RESEARCH METHODOLOGY

Microbes used for biological bleaching:

The micro fungi selected in this study for the pretreatment of sugarcane bagasse for the production of pulp and paper were *Trichoderma viridae* and *Aspergillus glaucus.*

Source:

The above represented fungi were isolated from different soil samples obtained from the residential area of Ariankuppam, Puducherry.

Isolation:

Serial dilution is the most specified technique for the isolation of the specific soil fungus. Hence, dilutions were made up to 10^{-1} to 10^{-6} and transferred to PDA plates. After incubation the single and pure colony was isolated and maintained. Then the pure culture was morphologically identified for further use of biobleaching and biopulping.

Enzyme assay:

The xylanase activity was determined according to the method of Baily *et al.* (1992). The substrate solution contained 1% birchwood xylan (sigma) xylan dissolved in 0.05M sodium phosphate buffer (pH 8.0). The reaction mixture consisted of 1.8ml of substrate solution and 0.2 ml of appropriately diluted xylan enzyme. After 5 min incubation at 50^0C, the reaction was stopped by the addition of DNS solution. The liberated reducing sugars (xylose equivalent) were estimated by the DNS method. Cellulase activity was determined as usual method. The enzyme activity was expressed in nKat. One nKat of enzyme activity is defined as the amount of enzyme that catalyses the release of 1nmol of xylose per second.

Amount of enzyme that liberates 16.7 nKat per ml second under assay conditions.

1 IU = 16.7 nKat

1g of pure xylan is suspended in 100 ml of 50mM sodium citrate buffer (pH 5.3) and incubated at 60^0C for 1 hour. Then the suspended mixture is stirred continuously overnight and stored at 4^0C. For long term storage, prepare aliquot and freeze at -20^0C.

Preparation of sample:

Teared the small pieces from the sample sheet to weigh a total of 3 to 4 grams. Mixed and made 3 to 4 grams (dry weight) into a pad by filtering on a buchner funnel; avoided any loss of fibres. Air dried the pad and teared into small pieces.

Kappa number of pulp:

The kappa number is the number of cubic centimeters of 0.1N potassium permanganate solution consumed by one gram of moisture free pulp under the conditions specified in this standard. The results were corrected to 50% consumption of the permanganate added.

RESULTS AND ANALYSIS

The results indicate bagasse to be one of the best substrates for both *Aspergillus glaucus* and *Trichoderma viridae* and both acted smart towards biocontrol activity which helped against contamination sensitivity. In this study *Aspergillus glaucus* showed higher xylanase enzyme activity and less lignin modifying enzyme activity

Table 1: Xylanase activity (IU/ml) and Kappa number

Culture inoculated	Xylanase activity (IU/ml)	Kappa number
Control	Nil	34.56
Aspergillus glaucus	1293.40	30.62
Trichoderma viridae	676.65	28.78
Co-culture (Aspergillus glaucus & Trichoderma viridae)	1297.76	20.73

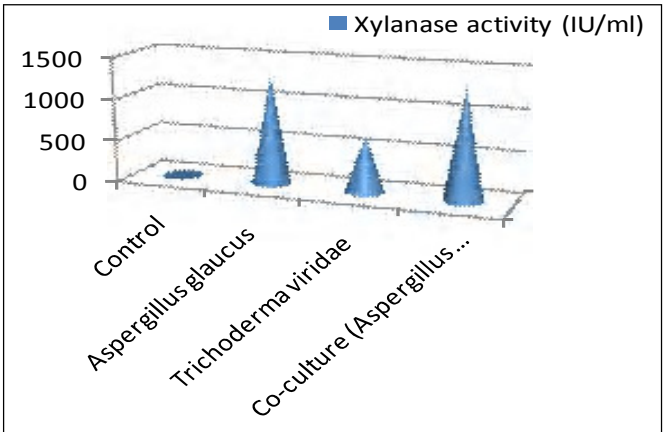

Fig. 1: Xylanase activity (IU/ml)

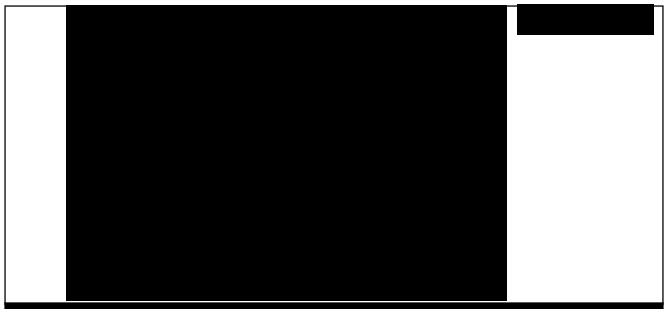

Fig. 2: Kappa number

than *Trichoderma viridae* which were clearly sorted in the Table 1. Lignin modifying enzymes were determined by means of kappa number reduction. Lower the kappa number higher the lignin modifying enzyme production (Fig. 1 and 2). Hence, an idea of using a coculture of *Aspergillus glaucus* and *Trichoderma viridae* showed valuable results in xylanase and lignin modifying enzymes production in turn considerable bleaching and pulping capacity could be seen. Even though the selected cultures are not cellulase free they are viable towards technology because of their biocontrol trait and easy availability.

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