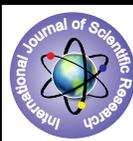


## Studies on Soil Biomass and Xylanases Producing Bacteria in Paper Mill Effluent Affected Soil



**KEYWORDS :** xylan, xylanases, paper mill effluent, xylanases producing bacteria PI introduce the abstract as blow:

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### ABSTRACT

*Paper mill effluent contains organic and inorganic toxic materials, which affects the soil physico-chemical parameters and microbial population and its diversity. Phenol is one of the toxic aromatic compounds which have deleterious effects on soil micro flora as well as on vegetation in effluent affected areas. In the present study, the physico-chemical parameters of paper mill effluents obtained from Nagaon Paper Mill (NPM), Jagiroad, Assam were analysed. All total nine strains, capable of growing in phenol containing MSM medium at a concentration range of 100 to 1000ppm, were isolated. Pure cultures of phenol-degrading microbial strains were obtained by growing on enrichment medium (Mineral salt medium) containing 500ppm. Three strains were found to be efficient among the nine strains, which is confirmed by measuring their capability of phenol degradation with respect to incubation time. This study provides scope for future study of identification of phylogenetically closely related species for phenol degradation in industrial wastes and their use in eco-friendly and more economic way, with the development of technology.*

### INTRODUCTION

In recent past land degradation has reduced the productive capacity due to intensive and mechanized management practices and industrial waste disposals. In India, paper mill industry falls under 17 identified categories of the highly polluting industries defined by the Ministry of Environment and Forests, Government of India.

The conventional bleaching of paper pulp with chlorine results in the expulsion of effluents containing chloro-aromatic compounds and chloro-lignin derivatives, and carcinogenic substances to the environment, resulting in incomplete degradation of lignin leading to disposal of brown coloured effluents. The degradation of lignin is further impaired by the re-precipitated and re-located xylan. Thus, lignin degradation can be effected by the removal of this xylan veil which makes the lignin prone to less drastic oxidatives. The hydrolysis of xylan leads to easy acceptance of oxidative chemicals for the bleaching process. Expulsion of hazardous chlorinated compounds and other chloro-lignin derivatives formed during conventional pulp production to the environment cause serious public concern. Xylan, the second most abundant polysaccharide (Christove and Prior, 1993; Ali et. al., 1999; Wong and Maringer, 1999) and a major component in plant cell wall is linked to lignin and cellulose (Puls, 1997; Connerton et. al., 1999) and its hydrolysis by xylanases eases the removal of lignin, the chromogenic precursor. Viikari et. al. (1986) were the first to demonstrate that xylanases could be useful in paper and pulp industry effecting delignification in bleaching process. Xylanases are of great importance to pulp and paper industries as the hydrolysis of xylan facilitates release of lignin from paper pulp and reduces the level of usage of chlorine as the bleaching agent (Shoham et. al., 1992). Several studies have been conducted to assess the deleterious effects of effluents from paper and pulp industries.

Cellulase-free xylanases selectively remove hemicellulose components with minimal damage to cellulose (Jurasek and Paice, 1986; Srinivasan and Rele, 1995). According to Kantelinen et. al. (1991) hydrolysis of re-precipitated and re-absorbed xylan or xylan-lignin complex, is the major action in xylanase catalysed enzymatic treatment. As a result the pulp becomes more accessible to bleaching chemicals. Potential applications of xylanases include bioconversion of lingo-cellulosic material to fermentative products (Wong et. al., 1988). A treatment with xylanases can improve the chemical extraction of lignin from pulp (Bajpai et. al., 1994). These results in significant saving of chemicals required for bleaching thereby reducing the release of toxic chlorine compounds into the environment.

Xylanases of bacterial origin, have been reported with either high pH or temperature optima, both being optimal character-

istics in pulp and paper industry application (Subramaniyan et. al., 1997). The problems related to processing of paper manufacture and other related chemical treatments for disposed effluents necessitate search for appropriate solutions to reduce or detoxify the toxic or organic components present in the effluent in a more economic and eco-friendly way. There is a need to isolate, identify and conserve microorganisms which are able to reduce the harmful impacts to the soil and the environment through biotechnology. The objectives of the present study are to isolate and screen the potent microorganisms present in paper mill effluent affected soil, which produce higher levels of endo xylanases, and their identification, which may assist in future research for effective and efficient treatment of paper mill effluent and similar types of waste.

### Sampling and Screening of microorganisms

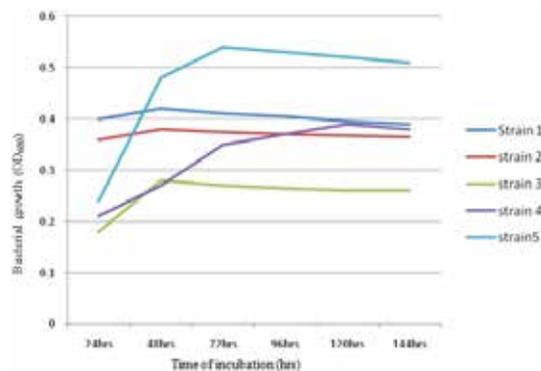
The soil samples were collected from the fields where paper mill effluent is being used for irrigation since last 20 years. Serial dilution was done for the collected soil samples in sterile saline water, upto ten -fold dilution and suspensions of 0.1ml each sample were spread onto Oat spelts xylan agar plates, sterilised at 121° C, 15 lb/inch<sub>2</sub> pressure for 20 minutes.

Isolation of alkaline xylanases producing microorganisms has been carried out by enrichment technique with xylan as a sole source of carbon in the growth media, and emphasis was given for selection of high xylanase-producing bacteria. Initial media for isolation studies contained (g/L): Wheat bran 5.0, Peptone 5.0, NaCl 5.0, Yeast extract 3.0, Agar 20.0, Media pH 7 and pH 9.5. The pH of the media was adjusted using 1% Na<sub>2</sub>CO<sub>3</sub> and 1 N HCl. The cultures isolated were spread on xylan agar plates containing 0.5% xylan instead of wheat bran as the carbon source while keeping all the other components of media the same. After six days of incubation, colonies that showed areas of clear zones with a minimum radius of 1 cm were selected for further screening in liquid medium where oat spelts xylan was the main carbon source.

The selected isolates were cultured in xylan liquid medium XLM (Horikoshi basal medium II) with xylan as the main carbon source (Horikoshi, 1991 a; Nakamura et. al., 1993a). The composition of modified Horikoshi basal medium II(g/L) : Xylan 5.0, Peptone 5.0, Yeast extract 5.0, K<sub>2</sub>HPO<sub>4</sub> 1.0, MgSO<sub>4</sub> 7 H<sub>2</sub>O 0.2, pH 7 or 9.5

The selected isolates were cultured in 100 ml of this medium in 250 ml Erlenmeyer's flasks under shaking condition at 120rpm at ambient temperature for 120 hours. Samples were drawn at every 24 hours interval, and used for measurement of optical density (OD) for bacterial population at 600nm wavelength.

**Fig.1: Bacterial growth profile as OD at 600nm against time of incubation**



**Production of xylanases**

The culture was grown for 48h in the XLM as above this was used to inoculate six 100 ml flasks each containing 15ml of XLM medium and 5g oat spelt xylan. The cultures were incubated at 28±2°C. Samples were removed at 24hours interval over a period of 6 days for analysis. The broth culture of each sample was centrifuged at 10,000 rpm for 15minutes at 4°C to separate the cells and the cell free supernatant was used as the extracellular crude enzyme preparation.

**Xylanases assay**

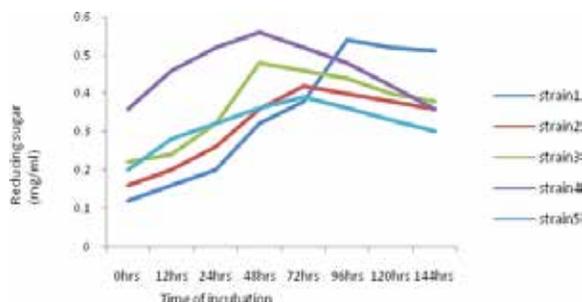
Endo-xylanases (1,4-B-D-xylan xylanohydrolase EC 3.2.1.8.) was assayed by the method of Bailey et. al. (1992) with some modifications using 0.5% oat spelt xylan. Enzyme blanks were prepared adding the DNS reagent prior to the enzyme addition so that only the reducing sugars present in the enzyme preparations would be determined. The reagent blank was prepared in the same manner but 200µl phosphate buffer (0.2 M pH 7) was used instead of enzyme. The reaction was terminated by adding 3ml of dinitrosalicylic acid reagent by keeping in boiling water bath at 100°C for 5 minutes. The concentration of reducing sugars released was estimated against the absorbance readings of xylose standard at 540 nm. The stock solution for xylose standard was prepared in 10µmole/ml concentration and appropriate dilutions were used as the standard. One unit of endo-xylanase activity was defined as µmoles of xylose liberated per minute per ml of enzyme preparation.

**Table 1: Soluble Protein, Reducing sugar and Xylanase Activity of cell free culture supernatant at the time of maximum enzyme production**

Strain no.	Period of maximum enzyme production (time of incubation) in hours	Xylanases activity of cell free culture supernatant at the time of maximum enzyme production			
		Soluble Protein mg/ml	Reducing sugar µl/ml	Xylanases Activity IU /ml	
				pH 7.0	pH 9.0
1	120	2.87	542±0.9	21.2	50.8
2	96	2.6	411±0.5	31.8	56.2
3	72	2.7	423±0.5	14.5	24.6
4	72	3.61	554±1.1	82.3	100.84
5	96	2.44	372±0.86	62.3	92.5

**Fig.2: Reducing sugar content in culture of different strains**

**at different time of incubation**



**Identification of bacteria**

The selected microorganisms were identified following Bergey’s Manual of Systematic Bacteriology. Microbiological tests were done on fermentation mode, detection of spores, motility test for the vegetative bacteria, Gram staining etc. which helped in classification of the isolates to respective genera. The biochemical tests were carried out using the procedure given in standard microbiological text (Collins et. al., 1989) and for identifying the bacterial isolates Bergey’s Manual of Systematic Bacteriology was followed.

**Result and Discussion**

Most of the bacteria strains displayed maximum xylanases production at the post-exponential or stationary phase of growth (Samain et. al., 1992; Nakamura et. al., 1993a; Fenandez-Espinar et. al., 1992). Broth culture initiated with different strains, at pH 7.0 and 9.0, lead to variation in xylanase activity (Table 1). Among the twenty strains isolated, only five showed comparatively high potential for xylanases production. The strains which showed clear zone on the xylan agar plates were selected and analysed for xylanase activity. It was observed that strain 4 resulted in the highest xylanase activities of 82.3 IU/ml and 100.84 IU/ml at the reaction pH of 7 and 9.2 respectively (Table 1).

Reducing sugar levels showed steep increase in the early hours of growth which was presumed to be due to the xylanases present in considerable amounts in the inoculums causing the hydrolysis of xylan in the medium except strain 1 (Fig.2).

Morphological and biochemical tests for the selected bacterial isolates have led to the conclusion that Strain1 to strain5 were identified as *Bacillus cereus*, *B. pumilus*, *Alkaligenes sp.* *Arthrobacter sp.* and *Enterobacter sp.* respectively. Among these five strains, *Arthrobacter sp.* was found to be most promising for exploration as xylanase producer. There is enormous scope for technological development, genetic analysis and molecular manipulations for more efficient and improved strains for making bleaching process more effective bioremediation of paper mill effluent and other related industrial wastes.

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