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SCIENCE DIRECT®
Bioresource Technology 97 (2006) 218–223
BIORESOURCE TECHNOLOGY

# Colour removal of anaerobically treated pulp and paper mill effluent by microorganisms in two steps bioreactor

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Received 2 November 2004; received in revised form 21 February 2005; accepted 21 February 2005
Available online 25 April 2005

**Abstract**

In the present study sequential anaerobic and aerobic treatment in two steps bioreactor was performed for removal of colour in the pulp and paper mill effluent. In anaerobic treatment, colour (70%), lignin (25%), COD (42%), AOX (15%) and phenol (39%) were reduced in 15 days. The anaerobically treated effluent was separately applied in bioreactor in presence of fungal strain, *Paecilomyces* sp., and bacterial strain, *Microbrevis luteum*. Data of study indicated reduction in colour (95%), AOX (67%), lignin (86%), COD (88%) and phenol (63%) by *Paecilomyces* sp. where as *M. luteum* showed removal in colour (76%), lignin (69%), COD (75%) AOX (82%) and phenol (93%) by day third when 7 days anaerobically treated effluent was further treated by aerobic microorganisms. Change in pH of the effluent, and increase in biomass of microorganisms substantiated results of the study, which was concomitant to the treatment method.


*Keywords:* Pulp and paper mill; Effluent; Microorganisms; Bioremediation; AOX; Lignin; Treatment; Decolourisation

## 1. Introduction

Pulp and paper mill is the major industry in our country. The heavy demand for the paper helps in steady expansion of paper industries. In 1951 there were 17 paper mills in the country, producing 0.13 million tons paper per annum. The number has gone up to 406 in 2002, producing 1.9 million tons paper per annum. Pulp and paper mills are utilizing huge amount of lignocellulosic components of plants, and using chemicals during manufacturing, and generally regarded as polluting industries because of huge amount of waste material enter into the environment (Chaudhary et al., 2002; Hossain et al., 2001; Jayaramraja et al., 2001; Pokhrel and Viraraghavan, 2004).

Effluent released by pulping and bleaching are amongst the most polluter and are characterized by parameters unique to these wastes such as colour and organic halides (AOX) (Taseli, 1997; Elisa et al., 1991; Fitzsimans et al., 1989). The untreated effluents from pulp and paper mills discharged into water bodies, damages the water quality. The brown colour imparted to water due to addition of effluents is detectable over long distances. The effluents have high biological and chemical oxygen demands (BOD and COD), lignin compounds and their derivatives. The dark brown colour is due to the formation of lignin degradation products during the processing of lignocellulosics from paper and pulp manufacture. The undiluted effluents are toxic to aquatic organisms and exhibit a strong mutagenic effect. Further more some compounds in the effluents are resistant to biodegradation and can bioaccumulate in the aquatic food chain (Sundman et al., 1981; Crawford et al., 1987).

Several methods have been attempted for the removal of colour from the pulp and paper mill effluents. These can be classified into physical, chemical and biological

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doi:10.1016/j.biortech.2005.02.022

methods. Physical and chemical processes are quite expensive and remove high molecular weight chlorinated lignins, colour, toxicants, suspended solids and chemical oxygen demand. But BOD and low molecular weight compound are not removed efficiently (Singh and Singh, 2004). The biological colour removal process is particularly attractive since in addition to colour and COD it also reduces BOD and low molecular weight chlorolignins (Nagarathnamma et al., 1999; Barton et al., 1996).

Anaerobic digestion is a process frequently employed for the secondary treatment of industrial wastewater (Pearson, 1990; Welander et al., 1999). It has many potential advantages in comparison to aerobic treatment such as lower sludge production, lower chemical consumption, smaller land requirements due to smaller reactors and energy production in the form of methane. Typical COD removal data for the treatment of paper mill waste waters shows that a relatively constant removal efficiency of about 80% can be achieved and that the treated effluent has a COD concentration of about 800 mg/l.

Chen and Horan (1998) have reported the use of a two stage anaerobic aerobic approach to remove COD and sulphate from the wastewater generated an integrated news print mill. COD and sulphur removals were 66% and 73% respectively. In general, anaerobic digestion is carried out at mesophilic temperatures, 35–37 °C. However the use of the thermophilic temperature range is worth considering (Rintala et al., 1991; Fujian et al., 2001) as it will give faster reaction rates and higher gas production rate. Welander et al. (1999) obtained high removals of chemical oxygen demand (COD) using anaerobic followed by aerobic biological treatment at 55 °C. Although anaerobic treatment showed a better operating economy, it was more sensitive to inhibitory compounds and suggested that recovery time after upsets may be long. Dias et al. (2004) showed that the treatment of foul condensates from Kraft pulp mills at high temperatures using a membrane bioreactor was shown to be technically feasible and it has good potential for industrial application.

The present study aims to assist the removal of colour and other pollution parameters of pulp and paper mill effluent by anaerobic treatment that was subsequently treated by fungus (*Paecilomyces* sp.) and bacterial strain (*Microbrevis luteum*) separately in two steps bioreactor.

## 2. Methods

### 2.1. Collection of sample

The combined effluent samples of pulp and paper mill were collected from the Century Pulp and Paper Mill, Lalkuan, Uttaranchal, India. The effluent was collected from inside and outside premises near Rayon Grade Paper Unit Laboratory. The effluent collected in clean plastic containers were brought to the laboratory and immediately stored in refrigerator of 4 °C until used for further analysis.

### 2.2. Isolation and identification of microorganisms

Fungal strains were isolated from sediment sludge of the pulp and paper mill out side premises and eight morphologically distinct fungal isolates were obtained on potato dextrose agar plates. The fungi were identified based on microscopic and morphological structures as colour, texture, mycelium and spore formation and attachment into the filaments. Bacterial strains were developed in the chemostat by continuous enrichment containing mineral salt medium (MSM) containing ($l^{-1}$): $Na_2HPO_4 \cdot 2H_2O$, 7.8 g; $KH_2PO_4$, 6.8 g; $MgSO_4$, 0.02 g; $NH_4Fe(CH_3COO)_3$, 0.01 g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.05 g; $NaNO_3$, 0.085 g; trace element solution as described by Pfenning and Lippert (1966), 1 ml, and 4 chlorosalicylic acid (0.1 g/l). pH was maintained between 7.0 and 7.5 throughout the course of enrichment. Microorganism served as inoculums in the chemostat was isolated from the sediment core of the effluent. The chemostat culture was run in 2 l glass vessel (effective volume 1 l) provided by stirring, 250 rev/min; temperature 25 °C; pH 10–12; an air flow of 500 ml/min and medium flow rate of 10 ml/h. Samples of the culture were collected under aseptic conditions. The growth of the bacterial community was determined by colony forming unit (cfu) by serial dilution. The microbial cells appeared on the nutrient agar plate were characterized depending upon morphology of colonies based on diameter, colour, opacity, form, elevation, margin smoothness, texture and spreading nature. The different colonies appeared on nutrient agar plates, were streaked on another nutrient agar plates. The process was repeated three times to ensure the purity of each isolate. The morphologically distinct isolates were identified by morphological, physiological and chemotaxonomical properties in accordance with Bergy's Manual of Determinative Bacteriology (Collin and Lyne, 1989; Palleroni, 1984). The bacterial isolates were also identified by a commercial microplate test (Biolog, Incorporated, Hayward, CA) based on the utilization of 95 carbon sources (Thakur, 2004). In this method isolates grown at 24 h were removed from the petriplates and diluted with saline. The homogeneous mixture of bacterial cells was dispensed in 96-well microplates (100 µl per well) and incubated at 30 °C. $A_{590}$ was determined after 7 and 24 h on a microtitre plate reader. The isolates were identified using the Microlog software. The tests were repeated five times.