

Sequential anaerobic and aerobic treatment of pulp and paper mill effluent in pilot scale bioreactor

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Abstract: In the present study sequential anaerobic and aerobic treatment in two step bioreactor was performed for removal of colour in the pulp and paper mill effluent. In anaerobic treatment, colour 50%, lignin 62%, COD 29%, absorbable organic halides (AOX) 25% and phenol 29% were reduced in eight 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 80%, AOX 74%, lignin 81%, COD 93% and phenol 76 per cent by *Paecilomyces* sp. where as *Microbrevis luteum* showed removal in colour 59%, lignin 71%, COD 86%, AOX 84% and phenol 88% 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 microorganism's substantiated results of the study, which was concomitant to the treatment method.

Key words: Pulp and paper mill effluent, Micro organisms, Bioremediation, AOX, Lignin, Decolourisation

Introduction

Pulp and paper mill is a major industrial sector utilizing a huge amount of lignocellulose materials and water during the manufacturing process, and release chlorinated lignosulphonic acids, chlorinated resin acids, chlorinated phenols and chlorinated hydrocarbon in the effluent. The highly toxic and recalcitrant compounds, dibenzo-p-dioxin and dibenzofuran, are formed unintentionally in the effluent of pulp and paper mill. The generation of waste water and characteristics of pulp and paper mill effluent depends upon the type of manufacturing process adopted and the extent of reuse of water employed in plant. Effluent of kraft mill is highly polluted, and characterized by parameters unique to this waste such as color, absorbable organic halides (AOX) and related organic compound (Elisa *et al.*, 1991; Fitzsimans and Eriksson, 1989). The alkaline extraction stage of bleach plant effluent is the major source of colour and is mainly due to lignin and derivatives of lignin. Lignin wastewater is discharged from the pulping, bleaching and chemical recovery section. Lignin is a heterogenous, three dimensional polymer, composed of oxyphenylpropane units. The high chlorine content of bleached plant react with lignin and its derivatives and form highly toxic and recalcitrant compounds that are responsible for high biological and chemical oxygen demand. Trichlorophenol, trichloroguaiacol, dichlorophenol, dichloroguaiacol and pentachlorophenol are major contaminants formed in the effluent of pulp and paper mill. (Chaudhary *et al.*, 2002; Hossain *et al.*, 2001; Jayaramraja *et al.*, 2001; Pokhrel and Viraraghavan, 2004).

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 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 (Nagarthamma *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⁻¹.

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Chen and Horan (1998) have reported the use of a two stage anaerobic aerobic approach to remove COD and sulphate from the wastewater generated in 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 removal 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.* (2005) 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.

Materials and Methods

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.

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 of mineral salt medium (MSM) containing (l⁻¹): Na₂HPO₄ · 2H₂O 7.8 g, KH₂PO₄ 6.8 g, MgSO₄ 0.02 g, NH₄Fe(CH₃COO)₃ 0.01 g, Ca(NO₃)₂ · 4H₂O 0.05 g, NaNO₃. Trace element solution 0.085 g as described by Pfenning and Lippert (1966), 1 ml, and 4 chlorosalicylic acid (0.1 g l⁻¹). Hydrogen ion concentration was maintained between 7.0 to 7.5 throughout the course of enrichment. Microorganism served as inoculums in the chemostat was isolated from the sediment core of the effluent. The chemo stat culture was run in 2 liter glass vessel (effective volume 1 liter) provided by stirring, 250 rev/min; temperature 25°C; pH 10-12; an air flow of 500 ml min⁻¹ and medium flow rate of 10 ml/hr. 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 hr 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. A₅₉₀ was determined after 7 and 24 hr on a microtitre plate reader. The isolates were identified using the Microlog software. The tests were repeated five times.

Two step bioreactor: The effluent collected from the pulp and paper mill was placed in 100 liter plastic container; In which 10% anaerobic inoculum (from cow dung) was added and purging was done by liquid N₂ gas to remove O₂ from the container. This container is connected to the column (bioreactor) with the plastic tubes having set I of the sequential bioreactor consists of a column where fungus was used for the treatment. Set I of glass bioreactor consisted 50 liter effluent transferred from anaerobic container. Effluent of set I bioreactor was inoculated with *Paecilomyces* sp. fungus strain at the rate of 10% (w/v) of the effluent and supplemented with carbon (sucrose 0.2%) and nitrogen (urea 0.1%) as carbon and nitrogen source. This column was connected with another reactor below in sequential way (set II) where effluent from set I was further treated with bacterial strain *Microbrevis luteum* (10% inoculum). During this process continuous supply of effluent was maintained. The sample was collected after 0, 1, 3 and 7 days from anaerobic tank, set I and set II respectively and change in pollution load was determined.

Aerobic bioreactor: Sequential aerobic bioreactor was made by fabrication of glass vessel size 100 liter, which was filled up to 10 cm, layer by gravel as solid support for immobilization of microbial cells. The column was equipped with stirring and aeration facilities and was connected with another vessel size 100 liter in a sequential way. *Paecilomyces* sp (F₃) fungal strain was applied for the treatment of pulp and paper mill effluent in first set and bacterial strain, *Microbrevis luteum* of the microbial community from the chemostat in second set bioreactor. The effluent was supplemented with sucrose 0.2% and urea 0.1%. The samples were collected after on day 1, 3, 7 and 15 from the set I and set II, respectively and change in pollution load was determined.

Analysis:

pH: The pH of the effluent is measured with the help of using electronic pH meter. The electrode is immersed into the sample solution, stirred gently and after few seconds the reading on the pH meter scale is displayed, which is noted.

Phenol and chemical oxygen demand: Effluent after treatment was analyzed for phenol, chemical oxygen demand as described in APHA (1995). Total phenol in the effluent sample was measured by using 4 amino antipyrine colorimetric method. COD was determined by a dichromate reflux method. In this method, the sample is refluxed with potassium dichromate and sulphuric acid and titrated with ferrous ammonium sulphate.

Colour: The colour content in the effluent was measured as described by Bajpai *et al.* (1994). In this method sample was centrifuged at 10,000 rpm for 30 min and pH was adjusted to 7.6. Absorbance was measured at 465 nm and was transformed into colour unit. Lignin was measured according to the method of Pearl and Benson (1990).

Lignin: The lignin of the effluent was estimated using the method of Pearl and Benson (1990). In this method, the sample was centrifuged at 10,000 rpm for 30 min to remove all the suspended matter. The pH of the supernatant was then adjusted to 7.6 with 2 M NaOH. The sample (50 ml) was mixed with 1 ml CH₃COOH 10% and 1 ml NaNO₂ 10%. After 15 min, 2 ml of NH₄OH was added. The mixture was left for 5 min and absorbance was measured at 430 nm. For blank, 1 ml CH₃COOH 10% was added in 50 ml distilled water and 2 ml NH₄OH. After 15 min, 1 ml of NaNO₂ 10% was added. After 5 min, OD was taken at 430 nm. The absorbance value was transformed into lignin content ppm.

Absorbable organic halogen: Absorbable organic halogen was analyzed by IDC multi X-2000 AOX Analyzer. Hundred milliliters samples were drawn from the each stage of the experiment conducted with fungus and bacterium at an interval of 0, 1, 3, 7 and 15 day's interval. Similarly the same amount of the sample was drawn from the sequential treatment consisting of three sets, *viz.* anaerobic treatment (set I), treatment with F₃ (set II) and treatment with bacteria (set III) for the same period. The pH of these samples was adjusted to 2 with the help of HNO₃ 15%. Initially the constituent was concentrated by adsorption onto activated carbon. In the case of AOX, interfering inorganic substances must be removed from the loaded carbon by rinsing with NaNO₃ solution. In order to convert the halogenated hydrocarbons (HHC) into an analyzable state, the content was combusted in an oxygen stream at about 950°C, whereby hydrogen halide, CO₂ and water were formed. After drying of the pyrolytic gases, the halide is determined by microcolometry. The silver ions needed for halide precipitation were generated electrolytically at a silver anode. After quantitative conversion of the halide, the concentration of silver ions in the electrolyte increases. This moment is the end point of titration, which

can be recognized by means of a polarized pair of indicator electrodes. The halide quantity was computed by means of Faraday's law from the amount of charge consumed until analytic conversion was complete.

Size of inoculum: The percent change in the size of inoculum was measured in the culture filtrate. In this process culture filtrate was placed in preweighed centrifuge tubes and centrifuged at 10,000 rpm for 30 min. After discarding the supernatant, the centrifuged tube was kept for drying at 80°C overnight in an oven. Then the centrifuged tube with the cell mass was weighed. The samples for different analysis subjected to removal of colour and lignin was used for the changes in biomass represented the cell growth of the fungal and bacterial cells. The biomass measurement was made for each and every sample in triplicate.

Results and Discussion

Eight fungal strains (F₁, F₂, F₃, F₄, F₅, F₆, F₇ and F₈) were isolated on potato dextrose agar plate by serial dilution method, and bacterial community developed in the chemo stat. The isolates were applied in batch culture for analysis of pollutants of pulp and paper mill effluent after supplementation of 0.2 and 0.1% glucose and urea. It was observed that F₃ identified as *Paecilomyces* sp. and *Microbrevis luteum* by Biolog test method showed maximum potential to remove colour and other parameters. So these microbial strains were applied in laboratory scale testing of 2 liter sequential bioreactor.

The biotreatability study of fungal and bacterial strain for the treatment of pulp and paper mill effluent was performed in anaerobic and aerobic culture conditions. Data recorded on this aspect clearly indicated that the reduction of pollution load by fungal and bacterial strain was more pronounced in immobilized cells in glass column compared to anaerobic set of bioreactor (Fig.1 and 2). The parameters used as indicators of pollution load are COD, lignin, colour, AOX, phenol, and pH. Sequential treatment was conducted with anaerobic and aerobic microorganisms. In set I pulp and paper mill effluent was treated by anaerobic microorganisms. After anaerobic treatment, data indicated significant reduction in colour 50%, lignin 62%, COD 29%, AOX 25% and phenol 29% in 8 days incubation (Fig. 1). Slight increase in biomass and pH (from pH 8 to 6.6) were also observed during treatment but biomass increase was much lower in anaerobic treatment than aerobic treatment. Aerobic treatment with fungal strain *Paecilomyces* sp, showed significant removal in colour 80%, lignin 81%, COD 93%, AOX 76% and phenol 74% after 7 days treatment, while bacterial (*Microbrevis luteum*) treatment indicate significant reduction in colour 60%, lignin 70%, COD 80%, AOX 84% and phenol 88% after 7 days treatment (Fig. 2). The rate of reduction of COD in all treatments was higher during initial days (*i.e.* 1, 3, days); with a decline at later stage (Fig. 1 and 2). Significant increase in biomass 27% and change



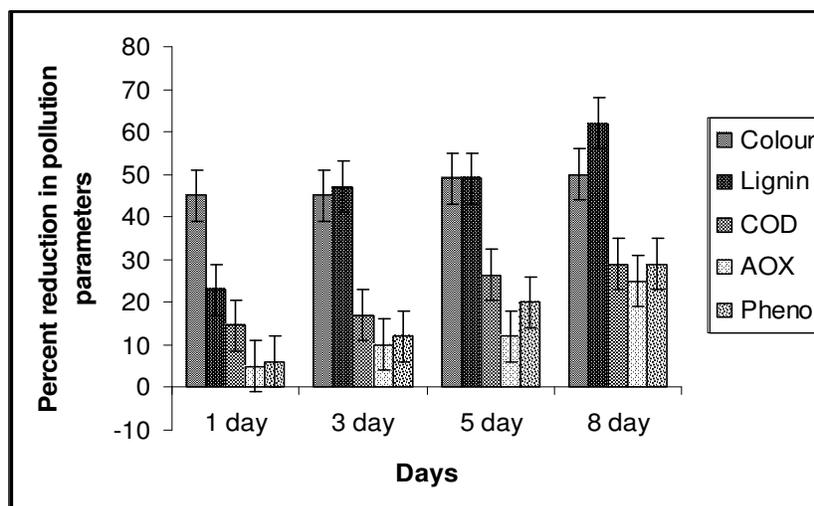


Fig. 1: Changes in AOX (mg l^{-1}), colour (CFU), lignin (mg l^{-1}), COD (mg l^{-1}) and phenol (mg l^{-1}) in anaerobic treatment of pulp and paper mill effluent in two step bioreactor 100 liter during different time intervals. Values are mean of three replicates \pm S.E. Significant at 0.05 level

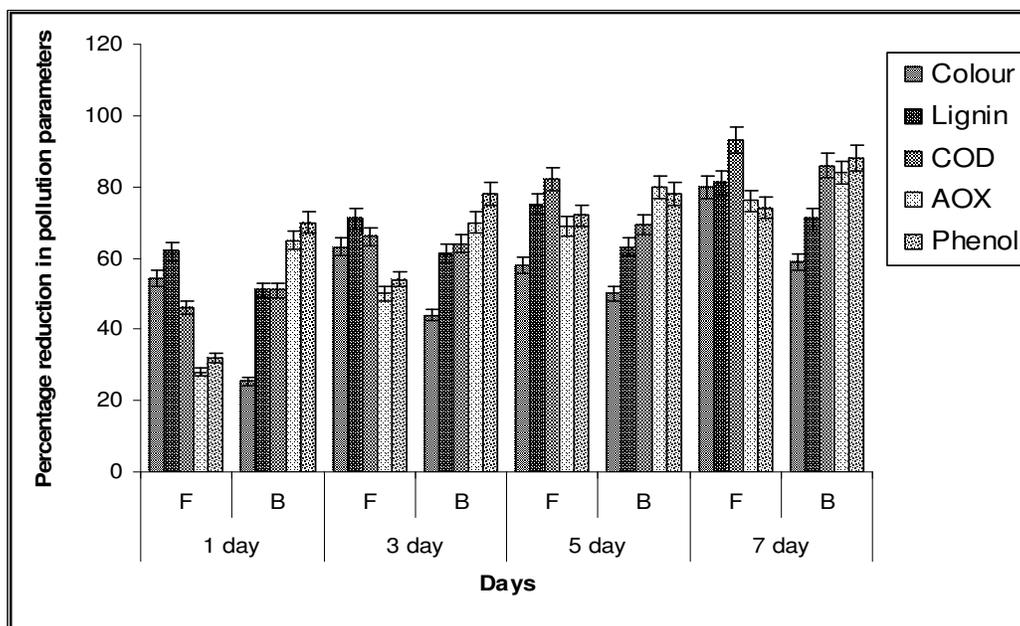


Fig. 2: Changes in AOX (mg l^{-1}), colour (CFU), lignin (mg l^{-1}), COD (mg l^{-1}) and phenol (mg l^{-1}) in aerobic treatment of pulp and paper mill effluent by fungus F (*Paecilomyces* sp.) and bacteria B (*Microbrevis luteum*) during different days anaerobically treated effluent in two step bioreactor 100 liter during different time intervals. Values are mean of three replicates \pm S.E. Significant at 0.05 level

in pH (from pH 8.0 to 5.0) was also observed with fungal and bacterial treatment.

Eight fungal strains isolated from pulp and paper mill effluent, the decolorization potency of *Paecilomyces* sp was higher on day 1 followed by *Phoma* sp and *Paecilomyces varioti*, (Thakur, 2004). Therefore, in present study *Paecilomyces* sp was selected for treatment of pulp and paper mill effluent in 2 liter sequential anaerobic and aerobic bioreactor. Results of anaerobic treatment showed that all the pollution parameters were decreasing slowly during different time intervals. No increase in

biomass was observed during the anaerobic treatment. This suggests that anaerobic treatment resulted in slow growth of anaerobic microorganisms and less sludge production. Slight change in pH indicated the slow degradation of toxic compounds present in the effluent. Slow AOX reduction in anaerobic treatment indicated the elimination of chlorinated compounds from the effluent. The anaerobic treated effluent was separately applied in bioreactor in presence of fungi and bacteria in step second and third of bioreactor for further elimination of pollution parameters. Data of the study indicated that degradation of pollution parameters was comparatively fast in aerobic treatment

then anaerobic treatment. It may be due to unique capability of aerobic microorganisms to secrete enzymes that efficiently degraded colour causing compounds and chlorinated phenols from pulp and paper mill effluent (Livernoche *et al.*, 1983; Pokhrel and Viraraghavan, 2004).

Concept of sequential treatment is very important because in this case both anaerobic and aerobic fungi and bacteria can be applied for treatment of effluent in different stages. Fungi has better capability to remove colour from the effluent, however, bacteria is more potent for degradation of aromatic compounds (Thakur, 2004). In addition, two or more microorganisms can be tried sequentially, one member of which may carry out the initial catabolic reactions and another may complete the rest of the metabolic pathway to mineralize the organic compounds completely. Such consortia have been developed for mineralisation of bicyclic aromatics such as chlorinated biphenyls, chlorinated dibenzofurans and naphthalene sulfonates (Pokhrel and Viraraghavan, 2004; Thakur, 2004).

Colour removal by fungal strain (*Paecilomyces* sp) and bacterial strain (*Microbrevis luteum*) was faster in comparison to anaerobic microorganisms in sequential bioreactor. This might be due to action of microbial strains immobilized in the soil on chlorolignin compounds resulting in its mineralization. Chlorinated compounds present in the effluent were degraded into their metabolites due to action of microbial strains. (Singh and Thakur, 2004).

A significant reduction in colour, lignin, COD and phenol during the experimental study in the treatment process can be considered as the result of mineralization of chlorinated compounds in the effluent and also due to the activity to aromatic ring oxidation enzymes. Effluent showed decrease in pH (acidic) during aerobic treatment due to conversion of complex organic compounds in simple inorganic acids. Increase in biomass during aerobic treatment suggested fast growth of microorganisms. Available data of earlier studies indicated that chlorinated phenols are mineralized to chlorine free end products (Homada *et al.*, 1987).

Knackmuss and Hellwig (1978) reported the cooxidation of chlorinated phenols by *Pseudomonas* sp strain B13 when the bacterium was grown in continuous culture of 4-chlorophenol. The bacteria were able to degrade all monochlorophenols and dichlorophenol isomers except 2, 4 dichlorophenols. Similarly in the result *Microbrevis luteum* was able to utilize different chlorinated phenols. Amy *et al.* (1985) tested the ability of several 2,4-dichlorophenoxy acetic acid degrading bacteria to degrade chlorinated phenols similar in structure. Anaerobic biological treatment can also efficiently destroy chlorophenolic compounds, mutagenicity and acute toxicity. The Enso fenox process was capable of removing 64-94% of the chlorophenol load and toxicity, mutagenicity and chloroform in the bleaching effluent (Smith, 1994). The decolourization was decreased to 75% when the retention time was shortened to 0.85 days.

Results of the present study indicated that sequential anaerobic and aerobic treatment is more efficient in removal of colour and chlorinated compounds, because anaerobic microorganisms were able to remove highly chlorinated substances more efficiently than aerobic microorganisms. Aerobic microorganisms removed the last remaining chlorine atom. The combined treatments typically removed 82% of the AOX, COD and chlorinated phenolics and completely eliminated chlorate. Ek and Eriksson (1987) also showed similar results and proposed a process based on ultra filtration UF methods and anaerobic and aerobic biological treatments. The UF was used to separate the high molecular mass material, which is relatively resistant to biological degradation. Lafond and Ferguson (1991) reported that anaerobic treatment in an upflow hybrid reactor removed 17-40% of AOX.

Present study concluded that sequential anaerobic and aerobic treatment helps in efficient removal of colour causing compounds and chlorinated phenol and also suggested further investigation in direction of purification and characterization of enzymes microorganisms involved in degradation of colour causing compounds.

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