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Biodegradation and decolorization of pulp and paper mill effluent by anaerobic and aerobic microorganisms in a sequential bioreactor

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Summary

Paecilomyces sp. and *Pseudomonas syringae* pv *myricae* (CSA105) were isolated from sediment core of drainage of the pulp and paper mill industry. Fungi and bacteria were applied for treatment of pulp and paper mill effluent in a two-step and three-step fixed film sequential bioreactor containing sand and gravel at the bottom of the reactor for immobilization of microbial cells. Degradation of chlorinated phenols and formation of their metabolites were determined by high performance liquid chromatography. The microbes exhibited significant reduction in colour (88.5%), lignin (79.5%), chemical oxygen demand (87.2%) and phenol (87.7%) in two-step aerobic sequential bioreactor, and colour (87.7%), lignin (76.5%), chemical oxygen demand (83.9%) and phenol (87.2%) in three-step anaerobic-aerobic sequential bioreactor.

Introduction

Over recent decades significant quantities of organochemicals are released by industrial, agricultural, and domestic activities into the environment. Microorganisms rapidly degrade many chemicals and thereby eliminate them from the environment, others are degraded slowly, accumulate in the environment and occasionally exhibit toxicity (Alexander 1981). Pulp and paper mills are categorized as a core sector industry and are the fifth largest contributor to industrial water pollution. Approximately 155 million tons of wood pulp is produced worldwide and about 260 million is projected for the year 2010 (Bajpai 1999). Pulp and paper production has increased globally and will contribute to increase in the near future. For every tonne of paper produced, these mills generate 220–380 m^3 of highly coloured and potentially toxic wastewater (Eriksson & Kolar 1985).

The pulp and paper industry is considered to be a highly energy intensive and polluting industry. In recent times, the high cost of energy inputs and increased environmental concerns are forcing the pulp and paper industry to look for cost-effective and environmentally friendly alternatives. Application of biotechnology in the pulp and paper sector is one such alternative to achieve both energy and cleaner production. Pulp and paper mills utilize huge amount of lignocellulosic components of plants, and use chemicals during manufacturing, and are generally regarded as polluting industries because of their output of lignosulphonates, chlorophenols, chloroabietic acid and chlorohydrocarbons (Mc-leay 1979).

Although physical and chemical methods are available for treatment of pulp and paper mill effluent, they are less desirable than biological treatment because of cost-ineffectiveness and residual effects. Biological treatment is known to be effective in reducing the organic load and toxic effects of pulp and paper mill effluent. There have been several attempts to use biological methods to decontaminate effluent from Kraft mills because of their ability to degrade lignin by several microorganisms (Haggblom & Salonen 1992). Anaerobic treatment of a Kraft bleaching plant effluent was studied with focus on the removal of chlorinated organic compounds and biochemical oxygen demand (BOD). When the hydraulic retention time (HRT) was 10 h or longer, the overall removal of organically bound chlorine, measured as adsorbable organic halogens (AOX) was 50%, extractable organic chlorine (EOX) 60% at an HRT of 15 h, BOD (70%) and COD (20%) were observed (Yu & Welander 1994). The anaerobic process plays a prominent role in handling wastewater containing huge amount of sludge. Anaerobic biological

treatment can also efficiently destroy chlorophenolic compounds, mutagenicity and acute toxicity. Anaerobic treatment has become the most commonly used method for the treatment of medium and high strength effluents, with low generation of surplus sludge and cost-effectiveness. Different anaerobic technologies have been applied for the treatment of less concentrated effluents, providing good treatment efficiencies at low hydraulic retention times (Hickey *et al*. 1995). Chlorinated phenols are important chemicals because of their widespread application in agriculture, industry, commercial product formation and preservation. They are highly toxic and persist in water and soil for a longer time and adversely affect flora and fauna (Thakur *et al*. 2001). In aerobic treatment the fungi are able to remove colouring materials and lignin compounds, but they are not efficient in degrading chloro-organics. However, bacteria viz. *Pseudomonas*, *Flavobacterium*, *Xanthomonas*, *Nocardia*, *Aeromonas* and *Arthobacter* are known to utilize lignocellulosic components of the bleached plant effluent including organochlorine compounds (Vora *et al*. 1988). A mixed population of bacteria and protozoa has been utilized to degrade lignin sulphonate from paper mills. *Pseudomonas putida*, *Nocardia coralina* and *Torula* sp. have been used for degradation of lignin (Bajpai & Bajpai 1997). Therefore, in the present investigation, potential microbial strains (fungal and bacterial) isolated from pulp and paper mill effluent were examined for degradation and detoxification ability and organic load, and applied for consequent pollution abatement by the best strain in sequential bioreactor treatment.

Materials and methods

Sampling site

The study was conducted on the effluent released from the Century Pulp and Paper Mill Ltd., Ghanshyamdham, Lalkuan, Nainital (Uttaranchal), situated about 7 km from the G.B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, India, on the Nainital–Bareilly Highway. This industry came into operation in June 1984. The industry uses eucalyptus wood as a raw material. The effluent was collected from inside premises near the Rayon Grade Paper Unit Laboratory and stored in refrigerator at 4 °C.

Source of aerobic microorganisms

A microbial community was isolated from the sediment core of drainage of the pulp and paper mill with regular discharge of effluent. A stable bacterial community was obtained after enrichment of microbial cells.

Chemostat culture

The bacterial community was enriched in a glass vessel, dimension 22 × 12 cm, which served as a chemostat by providing an inlet, minimal salt medium supplemented with 4-chlorosalicylic acid (10 ml/h) for the entry of fresh medium and an outlet (10 ml/h) for the removal of spent medium (Thakur 1995). Mineral salt medium had the following composition (g/l): $Na_2HPO_4{\cdot}2H_2O$, 7.8; KH_2PO4, 6.8; $MgSO_4$, 0.2; Fe $(CH_3COO)_4{\cdot}NH_4$, 0.01; Ca $(NO_3)_2{\cdot}4H_2O$, 0.05; $NaNO_3$, 0.085, and 4-CSA (2.5 mM) and trace element solution, 1 ml/l (Thakur 1995). An inlet was provided for the entry of alkaline solution to culture medium, which was maintained at pH 10. Sterile air was made to pass into the culture vessel by using an aeration pump and filter. The culture vessel kept on a magnetic stirrer was capable of maintaining the stirring at 200 rev/min and temperature at 28–30 °C.

After continuous enrichment, bacterial cells were removed and cultured on LB agar plates for structural and chemo-taxonomical analysis of the colony. The morphologically distinct isolates were identified by morphological, physiological and chemotaxonomical properties in accordance with *Bergey's Manual of Determinative Bacteriology* (Palleroni 1984). The isolates were also identified by a commercial micro-plate test (Biolog Inc, Hayward, CA) based on the utilization of 95 carbon sources (Thakur 1995). The homogeneous mixture of bacterial cells was dispensed in 96-well microplates (100 μl/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. Bacterial diversity of the stabilized consortium was enumerated on nutrient agar plates by spreading 100 μl of culture medium removed from chemostat under aseptic conditions. The colonies were further purified by streaking and restreaking alternatively on nutrient agar plates and MSM agar plates. The colonies appearing on nutrient agar plates were characterized morphologically and identified through biochemical tests and the Biolog test. All the tests were repeated three times. The fungal strain (*Paecilomyces* sp.) used in the present research was made available by Dr. I.S. Thakur, School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, and had been isolated from the sediment core of effluent drainage with regular discharge from the Century Pulp and Paper Mill Industry (Lalkuan, Uttaranchal, India).

Inoculum of fungal and bacterial strains

The fungal strain (*Paecilomyces* sp.) was enriched in potato dextrose broth for 4 days. The development of mycelium showed the growth of fungus. It was then transferred to the bioreactor. The bacterial strain (*Pseudomonas syringae* pv *myricae*, CSA 105) was grown in nutrient broth was used for treatment.

Anaerobic inoculum

Digested slurry from a biogas plant was collected from the Livestock Research Center of G.B. Pant University

of Agriculture and Technology, Pantnagar, Uttaranchal, India, situated at Nagla. This slurry was filtered with muslin cloth and active bacterial suspension was brought to laboratory for seeding material. An anaerobic bacterial consortium, which was stabilized for 3 months under anaerobic condition, was used for treatment of pulp and paper mill effluent.

Upflow anaerobic sludge blanket (UASB) reactor

For designing bench scale reactor, a plastic container (size: $25 \times 25 \times 35$ cm, volume: 20 l) was used. The reactor was provided an inlet at the bottom for up flow of effluent and an outlet at a height of 27.5 cm from bottom for discharge of treated effluent. For the collection of biogas from the reactor an outlet was provided on the top of the digester. The reactor was fed with 15 l effluent, which was inoculated with 15% (in ratio of 10% anaerobic inoculum and 5% cow dung slurry), and purging was done by liquid N_2 gas to remove O_2 from reactor. The reactor was sealed with M-seal to ensure anaerobic conditions in the reactor. An opening at the top of the reactor was used for biogas outlet, and connected to a calibrated water displacement bottle with a rubber pipe. This was done for quantifying gas production in the reactor. The reactor was left in ambient environmental conditions for stabilization of anaerobic digestion.

Bioreactor

The bioreactor designed on a laboratory scale was fabricated by using a glass column (size 5×100 cm). It has an outlet, present in the uppermost part of the reactor, and an outlet at lower part of the column. The uppermost part was provided with three openings for stirring, aeration and inoculation and of bacterial community. The stirring of the effluent was made possible by fixing a motor. To the motor, a plastic hollow tube was fixed. The terminal end of the tube was thickened and it was quite wide enough (dia., 105 cm) to be capable of swirling. In the bioreactor, oxygen was provided by passing sterile air through an air pump. The lower portion contained a layer of gravel (100 g) and sand (50 g) for immobilization of microbial cells over a plastic plate so that effluent passed through the sand substratum to the another bioreactor connected sequentially.

Two-step sequential bioreactor

The effluent collected from the pulp and paper mill was placed in plastic container on the top and was adjusted at pH 4.5 ± 0.2 for aerobic treatment by the fungus. This container was connected to the column of the bioreactor with the plastic tubes. Set I of the sequential bioreactor consisted of a column where the fungus was used for the treatment. Set II was is filled with fungus-treated effluent and the pH was adjusted at 7.0 ± 0.2 for subsequent treatment by *Pseudomonas syringae* pv *myricae* (CSA105).

Three-step sequential bioreactor

In the three step sequential bioreactor Set I was a UASB reactor where anaerobic treatment was done. Set II was filled with anaerobically treated effluent, and treated by fungus (*Paecilomyces* sp.) and set III was where the fungus-treated effluent was subsequently treated by the bacterial strain (*Pseudomonas syringae* pv *myricae*).

The effluent was supplemented with dextrose (0.1%) and urea (0.02%) as carbon and nitrogen source, respectively. During the process, the continuous supply of effluent was maintained at the rate of 20 ml/h. Samples were collected from each set of the sequential bioreactor after 1, 3, 5 and 7 days and the change in pollution load was determined.

Determination of biogas production by liquid displacement method

The biogas was determined by liquid displacement and its composition was evaluated by GLC (Raju & Ramalinghaih 1997).

Colour estimation

The colour content in the effluent was measured as described by Bajpai *et al.* (1993). In this method, sample was centrifuged at 10,000 rev/min for 30 min and the pH was adjusted to 7.6. The absorbance was measured at 465 nm and transformed into colour units.

Lignin content

Lignin in the effluent was estimated by reaction of effluent with acetic acid and sodium nitrite and ammonium hydroxide and measuring the absorbance at 430 nm (Pearl & Benson 1940).

Chemical oxygen demand (COD)

COD was determined by dichromate reflux method (APHA 1995). In this method, the sample was refluxed with potassium dichromate and sulphuric acid, and titrated with ferrous ammonium sulphate.

Phenol estimation

Total phenol in the effluent sample was measured by using the 4-aminoantipyrine colorimetric method (APHA 1995).

Analysis of chlorinated phenols and their metabolites by high performance liquid chromatography (HPLC)

The biodegradation of chlorinated phenols was determined by extraction of metabolites from effluent

samples by HPLC (Radehaus & Schmidt 1992; Thakur *et al*. 2001). In this method effluent was centrifuged at 7650 × *g* for 10 min, and chlorinated phenols and their metabolites present in supernatant were extracted with dichloromethane. For quantitative analysis, samples were separated by reverse phase HPLC with STR ODS II column (size 150 × 3.9 mm). The mobile phase was methanol and ammonium acetate buffer (0.01 M, pH 4.8) in the ratio of 70: 30 v/v, the flow rate was 1.5 ml/min and detection was at 224 nm as described earlier. Percentage utilization was estimated by measuring the peak area of metabolites.

Results

Characterization and identification of bacterial strains

After 1-month enrichment, six different type of colonies appeared on LB agar plates after stabilization of growth. These were named as CSA101, CSA 102, CSA103, CSA 104, CSA105, and CSA 106. CSA101 and CSA106 were identified as *Micrococcus luteus* and CSA102, CSA104, CAS105 were identified as *Deinococcus radiophilus, Micrococcus diversus* and *Pseudomonas syringae* pv *myricae*, respectively, but CSA 103 was not identified. Strain CSA 105 strain was found most effective for treatment, and was used for further study.

Treatment of pulp and paper mill effluent in a two-step sequential bioreactor

The effluent, which was used for treatment had the following characteristics: pH 7.80, colour 5205.5 cu, lignin 6380.56 mg/l, COD 5280 mg/l and total phenol 54 mg/l. In the case of aerobic treatment, the colour was reduced 72.9, 87.3 and 57.0%, after days 1,2 and 7, respectively. Maximum colour was reduced after day 2 by the fungus (*Paecilomyces* sp.), however, after day 7, reduction in colour was less due to formation of other compounds in the bioreactor (not identified as yet). Lignin (64.2%), COD (45.0%) and total phenols (25.0%) were also reduced after day 1 treatment and 51.6, 54.4 and 68.5%, respectively, after day 7 (Figure 1a).

Fungus (*Paecilomyces* sp.)-treated effluent (day 2) was subsequently treated by bacteria (*Pseudomonas syringae* pv *myricae)* in the bioreactor and showed further changes in colour, lignin COD and total phenols. Colour (4.5%) and lignin (6.7%) were found to increase initially after day 1, however, colour and lignin were reduced to 40.9 and 35.6%, respectively, after day 7. It was observed that COD (58.3%) and total phenols (74.0%) were reduced after day 7 (Figure 1b).

Treatment of pulp and paper mill effluent in a three-step sequential bioreactor

In the three-step sequential bioreactor (batch scale) pulp and paper mill effluent was analyzed after treatment by

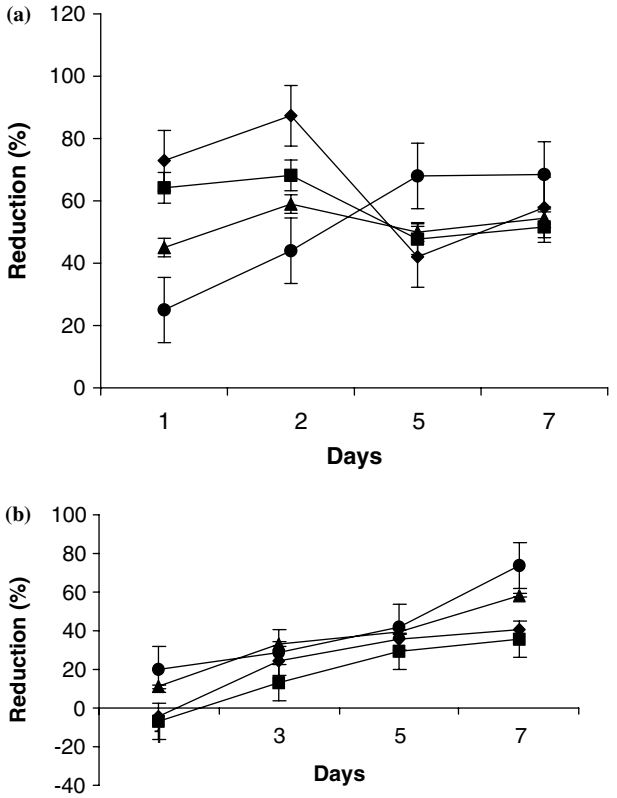

Figure 1. (a) Reduction in various parameters in pulp and paper mill treated effluent by fungus F3 (*Paecilomyces* sp.) in the two-step sequential bioreactor. (Colour indicated as ◆-◆, lignin ■-■, COD ▲-▲ and total phenols, ●-●). (b) Reduction in various pollution parameters in fungus (*Paecilomyces* sp.) treated (2d) effluent subsequently treated by aerobic bacteria (*Pseudomonas syringae* pv *myricae*) in the two-step sequential bioreactor. (Colour indicated as ◆-◆, lignin ■-■, COD ▲-▲ and total phenols ●-●).

anaerobic bacteria, subsequently treated with aerobic fungus and then aerobic bacteria (2 l reactors). Samples collected after days 0, 1, 3, 5 and 7 were analyzed to determine the reduction of pollution load in the effluent. Colour and lignin were decreased 41.5 and 35.2%, respectively after day 3, and 59.0 and 51.4%, respectively, after day 7. COD and total phenol were also reduced to 45.8 and 57.8% respectively after day 7 in effluent of the UASB reactor (Figure 2a). The pH of the effluent after day 3 was 5.03.The biogas production was observed during the run of experiment. Under controlled laboratory conditions (32 ± 2 °C) production of biogas began on day 3 after startup of the experiment. The biogas production initially started with an average of 210 ml/l/day and then gradually decreased up to day 15. After introducing the fresh effluent into the reactor the biogas production further increased. In the GLC profile of the biogas the main constituents (methane and carbon dioxide) of the biogas were observed at 0.23 and 0.65RT, respectively.

Anaerobically treated (day 3) effluent was further treated by the aerobic fungus in the 2-l bioreactor. Colour, lignin, COD and total phenols were reduced up to 59.1, 53.3, 55.2 and 39.8%, respectively, after day 2 treatment by fungus (Figure 2b). Aerobic fungus F3

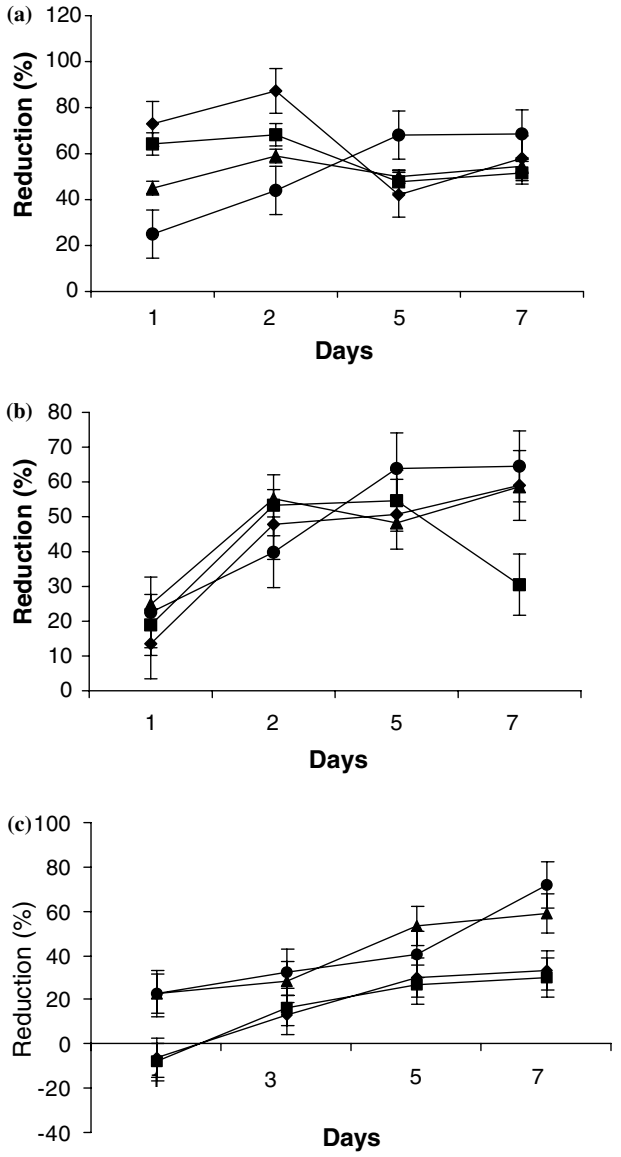

Figure 2. (a) Reduction in various pollution parameters in pulp and paper mill effluent treated by anaerobic microorganism in the three-step sequential bioreactor (Colour indicated as ♦-♦, lignin ■-■, COD ▲-▲ and total phenols ●-●). (b) Reduction in various pollution parameters in anaerobically treated effluent (3d) subsequently treated by fungus (*Paecilomyces* sp.) in three-step sequential bioreactor. Colour indicated as ♦-♦, lignin ■-■, COD ▲-▲ and total phenols ●-●. (c) Reduction various pollution parameters by subsequent treatment of anaerobic and aerobic fungus (*Paecilomyces* sp.) treated effluent (3d) by aerobic bacteria (*Pseudomonas syringae* pv *myricae)* in the three-step sequential bioreactor. (Colour indicated as ♦-♦, lignin ■-■, COD ▲-▲ and total phenols ●-●)

(*Paecilomyces* sp.) reduced the maximum colour and lignin after day 2 treatment, therefore, the fungus-treated (day 2) effluent was subsequently treated by aerobic bacteria (*Pseudomonas syringae* pv *myricae*) and it was observed that colour and lignin were increased to 5.9 and 8.1% on day 1, possibly due to the process of polymerization of compounds in the bioreactor by bacterial treatment. The significant findings emerged from the study that decrease in colour, lignin, COD and phenol after day 1 reached a maximum on day 7. Data of this study indicated reduction in colour (32.9%), lignin (29.9%). COD (58.7%) and total phenols (71.5%) after day 7 by CSA 105 (Figure 2c). In the comparative treatment study in the two- and three-step sequential bioreactors, it was observed that colour (81.6%), lignin (68.2%), COD (68.4%) and total phenols (51%) were reduced by fungus F3 after day 2, and colour (88.5%), lignin (79.5%), COD (87.2%) and total phenols (78.7%) were observed after day 7 in the effluent treated by the aerobic fungus and subsequently treated by bacteria in two-step aerobic sequential bioreactor. In the three-step sequential bioreactor colour (43.74%), lignin (40.15%), COD (17.23%) and total phenols (47.9%) were observed in anaerobically treated effluent, however, colour (87.7%), lignin (76.5%), COD (83.9%) and total phenols (87.2%) were reduced after day 7 treatment by anaerobically treated effluent subsequently treated by fungus and bacteria. In totality, microbes exhibited significant reduction in colour (88.5%), lignin (79.5%), chemical oxygen demand (87.2%) and phenol (87.7%) in the two-step aerobic sequential bioreactor, and colour (87.7%), lignin (76.5%), chemical oxygen demand (83.9%) and phenol (87.2%) in the three-step anaerobic-aerobic sequential bioreactor.

The metabolites extracted from pulp and paper mill effluent were analyzed for chlorophenols by HPLC. The results indicated the presence of pentachlorophenol (PCP), trichlorophenol (TCP), dichlorophenol (DCP), and 4-chlorophenol (MCP), which were determined by the retention time of the individual metabolites. The metabolites extracted from untreated and treated effluent suggested that PCP (85%), TCP (100%), DCP (97%) and MCP (100%) were degraded in aerobic treatment (Figure 3a and b), however in anaerobic–aerobic treatment, PCP (80%), TCP (95%), DCP (97%), MCP (100%) and phenols (93%) were reduced (Figure 3c).

Discussion

In recent years, environmental concerns coupled with stringent governmental regulation have prompted research on the environmental compatibility of industrial effluent. Great emphasis is being laid on improvements in a variety of in-mill processes and equipment changes for reducing the pollution load of wastewaters. Environmental laws have been legislated for installation of primary and secondary effluent treatment plants in the mills. The effluent discharged from the Century Pulp and Paper Mill carries a high BOD and COD load due to the presence of organochlorine compounds generated during the pulping and bleaching processes. These organic compounds in the effluents are degradation products of lignin. Among these, tri-, tetra-, and pentachlorophenol, chlorinated catechols, chlorinated guaicols and dioxins are of particular importance, as they are known to be highly recalcitrant, and responsible for colour, mutagenicity, carcinogenicity and toxicity of the effluent (Abbasi 1985).

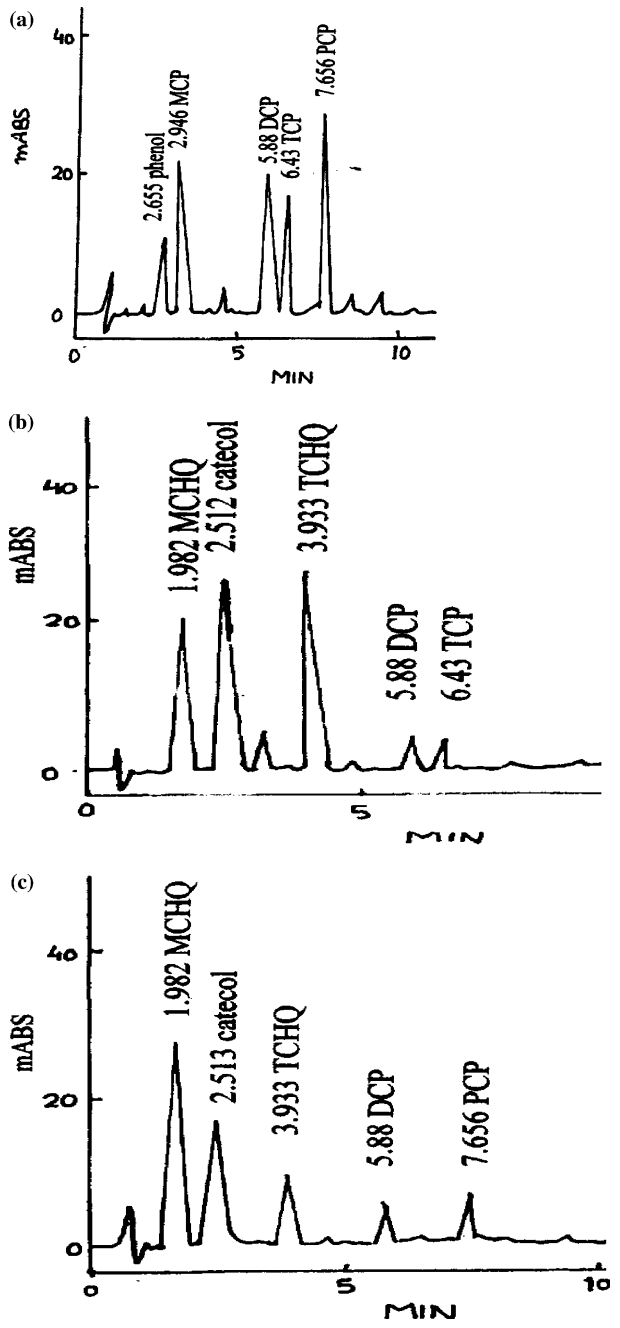

Figure 3. (a) HPLC profile of chlorinated phenol present in the untreated pulp and paper mill effluent (PCP, pentachlorophenol; TCP, trichlorophenol; DCP, dichlorophenol; and MCP, 4-chlorophenol). (b) HPLC profile of chlorinated phenol degradation and metabolites formed in the two-step sequential bioreactor by aerobic microorganisms (PCP, pentachlorophenol; TCP, trichlorophenol; DCP, dichlorophenol; MCP, 4-chlorophenol; MCHQ, chlorohydroquinone and TCHQ, tetrachlorohydroquinone). (c) HPLC profile of chlorinated phenol metabolites formed in the three-step sequential bioreactor by anaerobic and aerobic microorganisms. (PCP, pentachlorophenol; TCP, trichlorophenol; DCP, dichlorophenol; MCP, 4-chlorophenol; MCHQ, chlorohydroquinone and TCHQ, tetrachlorohydroquinone).

Thakur (1995) enriched a bacterial community in mineral salt medium containing 4-chlorosalicyclic acid in the chemostat. The growth of the population was stabilized after 150 days, because cells were physically adapted to the 4-CSA due to nutritional interaction between the members of bacterial community. Thakur *et al.* (2001) studied the application of bacterial strains for utilization of chlorinated phenols because of its genetic potentiality to degrade chlorinated benzoate, salicylate and phenoxyacetate. From the data obtained it was predicted that aerobic treatment is more successful for treatment of pulp and paper mill effluent than anaerobic treatment. Significant reduction in colour, lignin, COD and total phenols were observed. In aerobic treatment the fungus is able to remove organic chlorine from chlorolignins and to attack high and low molecular mass chlorolignins (Bajpai & Bajpai 1994).

It was observed that the three-step sequential bioreactor treatments by anaerobic and aerobic (fungus and aerobic bacteria) microorganisms showed a reduction in pollution load. In the anaerobic treatment, biogas is produced which can be utilized for energy generation. But aerobic treatment (aerobic fungus + aerobic bacteria) was more significant than anaerobic–aerobic treatment (anaerobic + aerobic fungus + aerobic bacteria).

HPLC analysis suggested that chlorophenols (pentachlorophenol, trichlorophenol, dichlorophenol, and 4-chlorophenol) were degraded to their metabolites chlorohydroquinone (MCHQ), tetrachlorohydroquinone (TCHQ), catechol. Available data of earlier studies indicate that chlorophenols are mineralized to chlorine-free end products (da Homa *et al.* 1987). The peaks of different chlorinated phenols that were observed at day 0 treatment decreased on days 1 and 3, and were converted into different metabolites like chlorohydroquinone, tetrachlorohydroquinone and catechol. These metabolite peaks were slowly reduced but had not completely disappeared on day 7. This suggests that *Paecilomyces* sp. and *Pseudomonas* sp. were able to degrade the chlorinated compounds present in the effluent but not completely eliminates the metabolites. Metabolites were extracted and analyzed by HPLC indicated complete utilization of contaminants; however, some other metabolites were detected after bacterial treatment. Apajalathi *et al.* (1986) isolated *Rhodococcus chlorophenolicus*, which degraded PCP to the corresponding tetra- or trichlo-hydroquinone and 1,2,4,-trihydroxybenzene.

On the basis of the above results, it could be concluded that environmental contaminations may be viewed as an ecosystem malaise, while bioremediation can be regarded as a kind of environmental medicine. It is possible that the existing fungal and bacterial isolates can be applied for pilot and scale-up process level for reduction of pollution parameters in pulp and paper mill effluent.

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References

Abassi, A. 1985 Occurrence, toxicity and treatment of lignin in pulp and paper effluents. State of art. *IE(I) Journal of Environment* **65**, 1–8.

Alexander, M. 1981 Biodegradation: Problems of molecular recalcitrance and microbial fallibility. *Advances in Applied Microbiology* **7**, 35–80.

Apajalathi, J.H., Karpahoja, P. & Salkinoja, M.S. 1986 Degradation of polychlorinated phenols by *Rhodococcus chlorophenolicus*. *Applied Microbiology and Biotechnology* **25**, 62–67.

APHA, 1995 *Standard methods for the examination of water and waste water*. 19th ed. Washington, DC: American Public Health Association. ISBN 0875532233.

Bajpai P.K., 1999 *Proc. of Interaction meeting on Biotechnological Application in the Pulp and Paper Industry*. Central Pulp and Paper Research Institute, Saharanpur. 1999. pp. 411.

Bajpai, P. & Bajpai, P.K. 1997 Reduction of organochlorine compounds in bleach plant effluent. *Advances in Biochemical Engineering and Biotechnology* **57**, 213–259.

Bajpai, P. & Bajpai, P. K. 1994 Mini Review: Biological colour removal of pulp and paper mill wastewater. *Journal of Biotechnology* **33**, 211–220.

Bajpai, P., Mehna, A. & Bajpai, P.K. 1993 Decolorization of Kraft Bleach Plant Effluent with the white rot fungus *Trametes versicolor*. *Process Biochemistry* **28**, 377–384.

da Homa, M.F., Haddad, A.I. & Abd-El-Bary, M.F. 1987 Treatment of phenolic wastes in an aerated submerged fixed-film (ASFF) bioreactor. *Journal of Biotechnology* **5**, 279–292.

Eriksson, K.E. & Kolar, K.C. 1985 Microbial degradation of chlorolignins. *Environmental Science and Technology* **19**, 1086–1089.

Haggblom, M.M. & Salonen, M.S. 1992 Biodegradability of chlorinated organic compounds in pulp bleaching effluents. *Water Science and Technology* **24**, 161–170.

Hickey, R.F., Jones, W.W. & Veiga, M.C. 1995 The start-up operation, monitoring and control of high-rate anaerobic treatment system. *Water Science and Technology* **24**, 207–255.

Mc-leay, D.J. 1979 Influence of dilution water on the toxicity of kraft pulp and paper mill effluent including mechanism of effect. *Water Research* **13**, 221–236.

Palleroni, N.J., 1984 Pseudomonas. In *Bergey's Manual of Systematic Bacteriology*, eds, Krieg, N.R. & Holt, J.G. pp 141–198. Baltimore: Williams & Wilkins. ISBN 0683041088.

Pearl, I.A. & Benson, H.K. 1940 The determination of lignin in sulphide pulping liquor. *Paper Trade Journal* **111**, 35.

Radehans, P. & Schmidt, S. K. 1992 Characterization of noval *Pseudomonas* sp. that mineralizes high concentration of PCP. *Applied and Environmental Microbiology* **58**, 2879–2885.

Raju, K. & Ramalinghaih, S. 1997 Methane production from orange waste. *Indian Journal of Environmental Health* **30**, 20–22.

Thakur, I.S. 1995 Structural and functional characterization of a stable, 4-cholrosalicylic-acid-degrading bacteria community in a chemostat. *World Journal of Microbiology and Biotechnology* **11**, 643–645.

Thakur, I.S., Verma, P. & Upadhyaya, K. 2001 Involvement of plasmid in degradation of pentachlorophenol by *Pseudomonas* sp. from a chemostat. *Biochemical and Biophysics Research Communications* **286**, 109–113.

Vora, K.A., Singh, C. & Modi, V.V. 1988 Degradation of 2-fluorobenzoate by a Pseudomonad. *Current Microbiology* **17**, 249–254.

Wieser, M., Eberspacher, J., Vogler, B. & Lingens, F. 1994 Metabolism of 4-chlorophenol by *Azotobacter* sp. GP1: Structure of the meta cleavage product of 4-chlorocatechol. *FEMS Microbiology Letters* **116**, 73–78.

Yu, P. & Welander, T. 1994 Anaerobic treatment of kraft bleaching plant effluent. *Applied and Microbiology and Biotechnology* **40**, 806–811.