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RESEARCH ARTICLE

CHARACTERIZATION OF PULP AND PAPER MILL EFFLUENT AND ITS BIOLOGICAL TREATMENT BY ISOLATED BACTERIAL CONSORTIA

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ABSTRACT

Pulp and paper mill released large amount of wastewater which affects the environment. These mills uses highly toxic chemical for pulping and bleaching processes. The intention of this research paper is to identify predominant bacteria in pulp and paper mill effluent. In addition to evaluate the degradation efficiency of bacterial isolates and combination of bacterial isolates by shake flask method. Biological treatment methods involve the utilization of bacterial consortia found to be effective in removal of colour, lignin and COD. This study indicates that bacterial consortia treatment gave a reduction of colour 55%, lignin 25% and COD 64% in 72 hrs at 37^0C incubation temperature and pH 7.

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INTRODUCTION

The Indian pulp and paper industry is over a hundred years old. In india, total number of paper mills is estimated to be around 813 with production capacity of more than 15 million tons/annum (Jain *et al*., 2015). Indian paper industries are employs water as raw material for production of different variety of paper board & newsprint (Jain *et al*., 2015). These industries are using an enormous quantity of chemical during processing and generating large quantities of waste water approximately 150-200m^3effluent/ton of paper being produced (Shanthi *et al*., 2012). The effluent generated at the pulping stage contains various compounds like dissolved lignin and its degradation products, hemicelluloses, resin acid, fatty acids, tannins and phenols (Ali and Sreekrishnan. 2001, Claxton and Houk and Hughes 1998, Lara *et al*., 1998). These organic compounds are also responsible for giving the effluent its characteristic dark brown colour and toxicity. Thus it is obligatory to treat the effluent before disposal. Various physical and chemical methods are attempted for treatment of paper mill effluent but these methods are costly and sometimes it increases BOD, COD and Colour of treated effluent (Pokhrel and Virarghavan. 2004; Ali and Sreekrishnan, 2001). Therefore biological methods involving the use of fungi, bacteria, algae and enzymes are developed because it is cost-effective and eco-friendly (Chandra and Singh 2012). Micro organisms (Fungi and bacteria) are nature s original recyclers that convert toxic organic compounds to harmless products (Raj *et al*., 2014). In treatment of paper mill effluent a variety of micro organisms including algae, fungi and bacteria are reported in which white rot fungi posses as good lignolytic activity (Souza *et al*., 2006). However, the fungal system is not stable in treatment under extreme environmental conditions as pH (4-5) is required for their growth and enzyme production. Hence, there is a need to search for biological devices that can tolerate broad pH range, to survive in extreme conditions (Sharma *et al*, 2007; Chandra *et al*., 2007). In contrast to fungi, Bacteria have been shown to have better degradation capability due to board pH (7-9) range and biochemical versality. It may play an important role in decolourization of paper mill effluent without pH adjustment. Several species of bacteria have been reported for degradation of paper effluent (Chandra *et al* 2009).

The present study is focused on developing a potential and bacterial culture capable to decolourize and detoxify high pollution load containing paper mill effluent.

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MATERIALS AND METHODS

Chemicals

All the chemicals were of analytical grade and purchased from various sources as Hi media (India) and Sigma Aldrich (USA).

Collection of sample

Samples were collected from different source as soil , sludge and effluent samples were collected from paper mill and CPPRI which is located in Saharanpur U.P. Samples were collected in sterilized polybags, bottles and stored at 4^0C.

Characterization of Effluent sample

The pH of the effluent was measured using pH meter. Chemical oxygen demand (COD), Colour and Lignin of effluent was analysed using APHA (American Public Health Association) method.

Isolation of lignolytic bacterial strain

Bacteria were Isolated from different soil sample using serial dilution method .1gm of soil sample was added to10 ml of sterile water and homogenous suspension is obtained by shaking vigorously and serially diluted with autoclaved distilled water to 10^{-4} and 10^{-5} dilutions and flooded on MSM agar plate containing (g/l) Na_2HPO_4 (2.4), K_2HPO_4 (2.0), NH_4NO_3 (0.1), $MgSO_4$ (0.01), $CaCl_2$ (0.01), D-glucose (5.0) peptone (0.5) and agar (18).The plates were supplemented with 0.075% lignin and incubated at 34^0C for 24 to 48 hrs. The isolates showing growth at MSM agar plates were purified by repeated sub culturing on nutrient agar plates (Raj *et al*., 2014).

Screening of lignolytic bacterial strain

Different bacterial strains were screened for their ability to degrade pulp and paper effluent. For screening of bacterial strains Nutrient broth (25ml) was prepared in 250 ml Erlenmeyer flasks and inoculated with isolated bacteria then the flask was incubated at 34^0C and 120 rpm for 24 hrs in shaking incubator. The isolates were taken in 50 ml centrifuge tube and centrifuged at 10,000 rpm for 15 minutes at 4^0 C. After centrifugation pellet was discarded and the supernatant was inoculated in unsterilized effluent Flasks (sample and culture). Were incubated for 24 hrs at 35^0C and 120 rpm. Sample was withdrawn for analysis of COD, Colour and Lignin. (Virendra *et al*, 2011).

Formulation of Consortia

The paper mill effluent is complex as it contains organic or inorganic toxic compounds as lignin, phenolics, cellulose and hemicelluloses which is difficult to degraded by single bacterial strain. It harms the soil physicochemical properties, ecosystem and microbial population. So there is a need for the formulation of effective bacterial consortia which can degrade the effluent in minimum time period. The bacterial strains were selected on the basis of growth on nutrient agar media containing lignin respectively (Virendra *et al*., 2011).

Screening of formulated consortia

Different bacterial strains were screened for their ability to degrade pulp and paper effluent. For screening Nutrient broth (25ml) was prepared in 250 ml Erlenmeyer flasks and inoculated with isolated bacteria then incubated the flask at 34^0C and 120 rpm for 24 hrs in shaking incubator. The isolates were taken in 50 ml centrifuge tube centrifuged at 10,000 rpm for 15 minutes at 4^0C. After centrifugation pellet was discarded and the supernatant was inoculated in 90 ml of unsterilized effluent (sample and culture). Flasks were incubated for 24 hrs at 35^0C and 120 rpm. Samples were withdrawn for analysis of COD, Colour, Lignin was estimated (Virendra *et al*., 2011).

Optimization of Parameter

The pulp and paper mill effluent is deficient in nitrogen as well as phosphorous constituent, so nitrogen and phosphorous were supplemented during wastewater treatment. Various parameters as incubation time varies from 24 to 96hrs, inoculum density ranges from 5% to15%, temperature 30 to 45^0C and pH 4-9 were optimized in order to get efficient reduction. After the optimization of selected parameters, the experiments were repeated in order to check the reproducibility among the results with isolated bacterial strains.

Bacterial growth

Bacterial biomass/turbidity was measured at 620 nm by the UV-Vis spectrophotometer.

Estimation of colour of the effluent

Colour was measured at 465nm by UV-Vis spectrophotometer. The sample was centrifuged at10, 000 rpm for10 minutes to remove all the suspended particle .After centrifugation, pH of the supernatant was adjusted to 7-7.6 by the help of acid/base by pH meter. Later the sample was used to take absorbance at 465nm.

Estimation of lignin of the effluent

The lignin content present in effluent was measured according APHA (American Public Health Association).The sample was centrifuged at10, 000 rpm for 10 minutes to remove the turbidity. After centrifugation pH of the supernatant was adjusted to neutral. Sample was used to take absorbance at280 nm against Blank.

Chemical oxygen demand (COD)

COD was measured by the open reflux methods according to APHA (American Public Health Association). In this method sample was digesting with potassium dichromate and sulphuric acid for 2 hrs and titrated with ferrous ammonium sulphate. (Chandra *et al*., 2008).

RESULTS

The pulp and paper mill face problem of the large amount of waste water. It affects the ecosystem and environment. The

treatment of pulp and paper mill effluent has been studied using a number of bacterial strains. In the present study, different bacterial strain was isolated from soil, wood based mill effluent containing lignin and chemicals and many other compounds. The selected bacterial consortia were found to be potential for the degradation of effluent generated from pulp and paper mills.

Characteristics of Effluent

Effluent sample was collected from paper mill which located at Saharanpur U.P India. COD of paper mill effluent varies from 800 to 1200mg/l. Colour of the effluent sample varies from 600-800 PCU and Lignin varies from 100-200g/l.

Isolation of Bacteria

The bacterial strains were isolated from soil sample collected near the paper mill. 50 bacterial strains were showing growth on MSM plates from the soil sample. The isolated bacterial colonies were diverse in their morphologies ranging from small size to large size, flat to irregular whitish to florescent smooth to wrinkled periphery. 26 bacteria were screened out of 50 isolates, showing reduction in all the pollutants in which 4 bacteria were showing more reduction and were selected for formulation of consortia.

Screening of formulated consortia

Screened bacterial strains showing maximum reduction in COD, colour and lignin were selected for the formulation of consortium that composed of 2 bacteria. Three consortia were selected and tested for COD, colour and lignin of effluent.

Table 1. Screening of formulated consortia

Bacterial Consortia	COD Reduction (%)	Colour Reduction (%)	Lignin Reduction (%)
BR6+BR7	50	45	15
BR1+BR7	35	32	10
BR2+BR5	20	15	7

As shown in table 1, maximum reduction in COD 50%, lignin reduction 15%and colour reduction 45% was obtained by bacterium consortium BR6+BR7 .So it was selected for further study.

Optimization of parameter

The various parameters like incubation time, inoculum density, pH and incubation temperature were optimized.

Effect of incubation time

The experiment was also performed in order to optimized the incubation time for 24-96 hrs. The maximum reduction in all pollutants as COD reduction 55%, lignin reduction20% and colour reduction 50% was estimated after 72 hrs of incubation time and after that it did not favour more reduction in pollutants of effluent. Bindhya and Vijiyan (2015) reported reduction in colour 18%, lignin 25% and COD 36% after 120 hrs of incubation time.

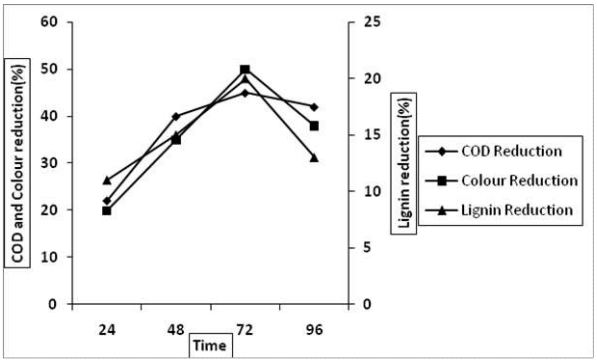

Fig. 1. Effect of incubation time on pollutant of effluent

The results in our study is better than Bindhya and Vijiyan study in terms of less incubation time as it takes less time for reduction of pollutants.

Effect of inoculum density

According to table 3 it is shown that maximum reduction in all the pollutant as COD reduction 58%, lignin reduction 23% and colour reduction 53% was obtained with inoculum density 10% by bacterium consortium BR6+BR7.

Table 2. Effect of inoculum density on pollutant of effluent

Inoculum density	COD Reduction (%)	Colour Reduction (%)	Lignin Reduction (%)
5%	43	25	15
10%	58	53	23
15%	38	20	13

Effect of pH

The pH of the medium is one of the most critical parameter that affects the growth of bacterial strains. Optimization was carried out with this consortium for getting more reduction in all the pollutants. It was resulted that bacterial consortia (BR6+ BR7) has shown maximum reduction in COD 60%, lignin25%and Colour 55% at pH 7 where as there is less reduction was obtained at pH 9. Our study is correlated to Raj *et al*., 2007 & Jacob *et al*., 2014 who reported maximum reduction in pollutant at pH 7. Eaton *et al*., 1982 also supported our study that shown up to 40% reduction in COD at pH 7.

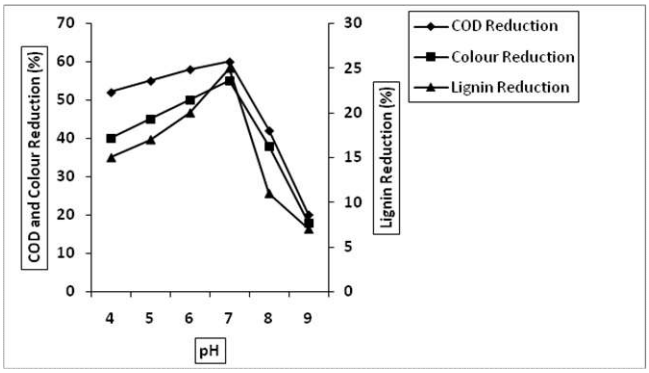

Fig.2. Effect of pH on pollutant of effluent

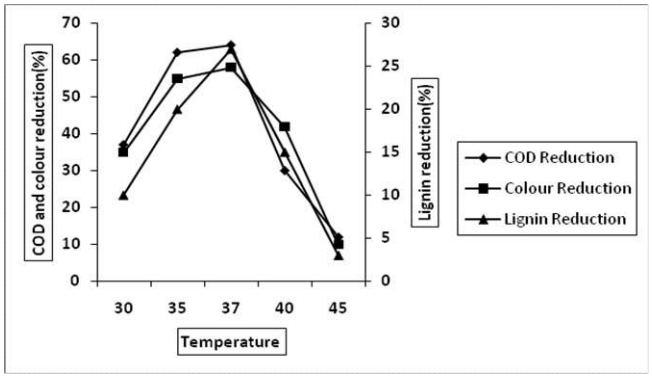

Fig.3. Effect of temperature on pollutant of effluent

Effect of incubation temperature

The effluent was treated at different temperature using bacterial consortia. It is resulted that bacterial strains have shown maximum reduction COD 64%, lignin 25% and colour 55% at 37^0C and very less reduction was found at 43^0C. Jacob *et al*., 2014 reported reduction up to 60.3% in COD and colour reduction up to 20.3 % at 35 to 37^0C.

Conclusion

This present study concluded that the isolated bacterial strains have an ability to reduce the COD, colour and lignin of the paper mill effluent. The mixed culture might be useful for degradation and decolourization of pulp paper mill effluent. These bacterial strains shows reduction of COD 64% Colour 58% and lignin 27% after 72 hrs of incubation time at 37^0C. This should help the paper mills in achieving the stipulated norms as specified by the statutory bodies. The reduction efficiency achieved is relatively higher than that reported by earlier values.

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