Isolation and screening of a bacterial strain for the degradation and decolourization of pulp and paper mill wastewater

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Abstract

Pulp and paper mill effluent contains organic, inorganic and coloring compounds which have high pH, biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), and phenols along with lignin. When untreated or poorly treated pulp and paper mill effluents discharged into the environment, it causes adverse effects on aquatic as well as terrestrial environments. Due to the heavy pollution load of pulp and paper mill industries, the removal of pollutants present in the effluent is very necessary for environmental safety. Thus, in this study deals with the bioremediation of pulp and paper mill effluent by the bacterial strain PLP 2 was isolated from contaminated site of pulp and paper mill industry. The isolated bacterial strain PLP 2 was effectively reduced colour (69%) and lignin (44%) after 144 h of treatment at 35°C, pH 7.5 and 120 rpm in presence of glucose (1.0%) and peptone (0.5%) as additional carbon and nitrogen source. Therefore, this study showed that the isolated bacterial strain can be useful for the effective bioremediation of pulp and paper mill effluent.

Key Words: Pulp and paper mill effluent, Bioremediation, Pollutants, Lignin

Introduction

The pulp and paper industry uses large amounts of fresh water and different types of chemicals during manufacturing process of paper making and generates large quantities of recalcitrant toxic compounds and coloured effluents which have an adverse effect on the environment (Subramanium. 1976; Saraswathi and Saseetharan 2010). Pulping stages produced 40-45% toxic effluent and these effluents are heavily loaded

with organic matter i.e. Chlorolignins chlorinated phenols, lignosulphonic acids, various types of surfactants, biocides and plasticizers (Ali and Sreekrishnan 2001; Lacorte et al., 2003). According to the Ministry of Environment and Forest, Government of India, the pulp and paper industrial sector is in the "Red Category" list of 17 industries having a high polluting potential due to its pollution menace and it is compulsory for pulp and paper mills to obey the appropriate standards set by Central Pollution Control Board (CPCB 2001). The wastewater produces from pulp and paper mills are characterized by high colour, BOD, COD, toxic chlorinated compounds, resin acids, suspended solids, tannins and sulphur compounds along with lignin (Pokhrel and Viraraghavan, 2004). Some highly toxic pollutants like dibenzo-p-dioxins and dibenzofurans are inadvertently produced during pulp and paper manufacturing process (Takada et al., 1996). Because of high pollution load and coloring substances, pulp and paper mill effluents cause severe aquatic and soil pollution. In aquatic system, it blocks the photosynthesis and also decreases the level of dissolve oxygen (DO) which adversely affects fauna and flora and causes toxicity to aquatic water bodies (Sponza. 2003; Lattore et al., 2007) while in contaminated soil it showed accumulation of toxic pollutants and metals. Furthermore, these pollutants are also reported as mutagenic, clastogenic, carcinogenic and endocrinic (Jenkins et al., 2003; Savant et al., 2006).

Various physicochemical methods (i.e. adsorption, filtration, sedimentation, coagulation, ozonation, oxidation etc.) or combination of these methods are reported but these methods are high energy intensive, very high cost and sometimes it increases COD, BOD of the treated effluent and produces large amount of secondary sludge (Ramos et al., 2009). Afterward, it is very important to develop a cost effective, environmental friendly and efficient method to remediate the pulp and paper mill effluent. Biological methods including fungi, bacteria and algae are reported, in which white rot fungus are widely used (Chuphal et al., 2005; Jaganathan., 2009). If the pH is high and oxygen level is limited the fungal system cannot be used as is generally common in pulp and paper mill effluent but the bacterial system can work under such conditions. Chandra et al. (2007) reported *Bacillus* sp. for kraft lignin degradation. Moreover, various strains such as *Bacillus megaterium, Azotobacter*, *Serratia marcescens*, *Brevibacillus agri* etc can also degrade lignin (Morii et al. 1995; Hooda et al. 2015). The lignin degradation rate of bacteria is low as

compared to fungal strains so there is a need to search capable lignin degrading bacteria strains. Therefore, the aim of the present study to isolate a bacterial strain with potential lignin degrading and colour decolourization efficiency for the treatment of pulp and paper mill effluent

2. Materials and Methods

2.1. Sample collection

Sludge and effluent samples were collected from Century Pulp Paper Mill Lalkuan, Uttarakhand, India on the Nainital-Bareilly highway. Sludge of wastewater treatment plant is a rich source of bacterial community. The sludge samples were collected in presterilised test tubes using a sterile spatula and the effluent samples were collected in plastic container (5L). These samples were transported to the laboratory in iceboxes and stored at 4℃ for further studies.

2.2. Chemicals

The reagents used in this study were of analytical grade. The kraft lignin was purchased from Sigma Aldrich (USA). All other reagents including glucose, peptone, agar and salts for bacterial culture and degradation studies were purchased from Hi-Media (Mumbai, India).

2.2. Physico-chemical analysis of pulp and paper mill wastewater

The physico-chemical analysis of pulp and paper mill effluent was done according to the standard methods for the examination of water and wastewaters (APHA, 2012). The collected pulp and paper mill effluent was analyzed for pH, temperature, biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), Total nitrogen, chloride, phosphate and sulphate (APHA, 2012). Colour and lignin present in pulp and paper mill effluent were measured using CPPA (1974) and Pearl and Benson (1940) methods.

2.3. Isolation and Screening of lignin degrading bacterial strains

For the isolation of potential lignin degrading bacterial strain, one gram of sludge sample collected from contaminated site was serially diluted and spread on lignin amended mineral salt medium (composition (g/l): Na_2HPO_4, 2.4; KH_2PO_4, 2; NH_4NO_3, 0.1; $MgSO_4$, 0.01; $CaCl_2$, 0.01; D-glucose, 1%; Peptone, 0.5%; Agar, 15) agar plates. After 48 hr of incubation period, colonies with different morphology were selected

and purified by repeated plate streak method on lignin amended mineral salt medium (L-MSM) agar plates. Further, the isolated bacterial strains were screened on the basis of their tolerance limit with increased concentration of lignin (200-1500 mg/L) on MSM agar plates. The secondary screening was done on the basis of lignin and colour removal capability in MSM-broth amended with synthetic lignin (100 ppm, w/v) and assessed at the regular interval of 24, 72, 96, 120 and 144 h with respect to control.

2.4. Morphological and Biochemical Characterization of Bacterial Isolates

The morphological and biochemical characterization of isolated lignin degrading bacterial strain was characterized morphological and biochemically as per the methods described in Cowan and Steel's Manual for Identification of Medical Bacteria (Barrow and Feltham 1993).

2.5. Degradation of pulp and paper mill effluent pollutants by axenic Culture

The degradation experiments were carried out in triplicate in 250 mL Erlenmeyer flasks containing 95 ml of autoclaved pulp and paper mill effluent. 5% (v/v) of activated overnight grown bacterial suspension of was aseptically transferred to 250 mL flasks containing 95 mL autoclaved pulp and paper mill effluent. The inoculated flasks were incubated at 32°C under shaking condition of 140 rpm for 144 h. Flasks were monitored at regular time intervals by withdrawing samples for bacterial growth, reduction in colour and lignin concentration. A separate set of uninoculated flask was taken in parallel as control (Raj et al., 2014).

3. Results and Discussion

3.1. Physico-chemical characteristics of pulp and paper mill effluent

The physico-chemical characteristics of pulp and paper mill wastewater were as follows pH: 8.3 colour: 1132 Co-Pt (Cobalt-Platinum), COD: 825, BOD: 647, sulphate: 2.8, lignin: 433 respectively as shown in Table 1. The pulp and paper industries are one of the biggest consumer of natural resources (water and wood) and energy and discharged various types of toxic pollutants to the environment (Chandra and Singh, 2012). The high pH and sulphate value of pulp and paper mill wastewater was due to the presence of sodium sulphide and sodium hydroxide which is used in pulping process (Chandra et al., 2011). Lignin and its derivatives cause high COD and colour in pulping effluent. Phenols are harmful to both fauna and flora even at very low concentration. 1.0 µg/ml concentration of phenol inhibits the photosynthesis of diatoms and blue algae and if the range between100-400 µg/ml causes complete inhibition of photosynthesis (Kostyeav,

1973). Metal present in the wastewater might be due to the accumulation of these metals by plants which are used as raw material as well as from various chemicals used during manufacturing process.

3.2. Isolation and Screening of Bacterial Strains

Initially, seven morphologically distinct bacterial strains were isolated from pulp and paper sludge samples collected from the outlet of pulp and paper industry. The isolated bacterial strains were purified by the repeated plate streak method on L-MSM agar plates. In primary screening the isolated bacterial strains were grown on L-MSM agar plates with increasing concentration of lignin (100-1200mg/L). Out of seven three bacterial strains (PLP 1, PLP 2 and PLP 3) were selected that were able to grow at highest concentration of lignin i.e. 1200 mg/L. In secondary screening all these bacterial strains were grown in lignin (100 mg/L) amended MSM broth. Out of three only one bacterial strain (PLP 2) reduced colour and lignin content more efficiently and this bacterium was selected for further studies.

3.2 Biochemical characteristics of isolated bacterial strain

The isolated bacterial strain PLP 13 was identified as gram positive, rod shaped and motile. Moreover, this bacterium showed positive for starch and casein hydrolysis test while negative for indole, citrate utilization and H_2S production test. The various morphological and biochemical tests are shown in **Table 2**.

3.3 Degradation and decolourization study of pulp and paper mill wastewater

For biodegradation studies additional mineral salts like glucose (1.0%) and peptone (0.5%) were added in the effluent and conducted for 144 h. The bacterial growth and reduction in lignin and content concentration during the treatment process is shown in Fig.1 (a and b). The growth of the bacterium was good initially and maintained upto 120 h, thereafter it declined. After 24 h significant reduction in colour and lignin was observed. These results indicated that this bacterium used glucose and peptone initially and after that it utilized glucose and peptone along with lignin which resulted decrease in lignin and colour concentration of the effluent. The initial colour and lignin content present in effluent was 1132 CU and 433 mg/L, respectively, but after bacterial treatment it reduced upto (69%) and (44%) respectively. Similar work has also been reported by many authors (Raj et al., 2014; Singhal and Thakur, 2009). Gupta et al. (2001) found that the treatment of pulp and paper mill effluent using *Aeromonas formicans* showed colour (85%), lignin (80%) and COD (70%) after 8 days of incubation. Removal of colour (61%), lignin (53%), BOD (82%),

COD (78%) and phenol (77%) by Bacillus sp. within 6 days of treatment (Raj et al., 2007). Raj et al. (2014) found that *Paenibacillus sp*. remove 68% colour, 54% lignin, 83% BOD, 78% COD and 86% phenol after 6 days of incubation at 34±1℃ and 120 rpm. Zainith et al., (2019) isolated manganese peroxidase producing *Bacillus aryabhattai* strain and found that it effectively reduces colour 67% and lignin 54% within 144 h of incubation time.

4. Conclusion

Lignin and colour present in pulp and paper mill effluent are considered as major environmental pollutants due to their toxic effects on environment as well as human health. The lignin degrading bacterial strain was isolated from pulp and paper mill effluent contaminated site. The isolated bacterial strain PLP 13 was found effective degradation and decolourization of pulp and paper mill effluent. The maximal removal efficiencies of colour (69%) and lignin (44%) were observed within 144 h of incubation period. Therefore, this study concludes that this bacterium is capable for the effective degradation and decolourization of pulp and paper mill effluent.

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Table.1 Physico-chemical characteristics of pulp and paper mill wastewater

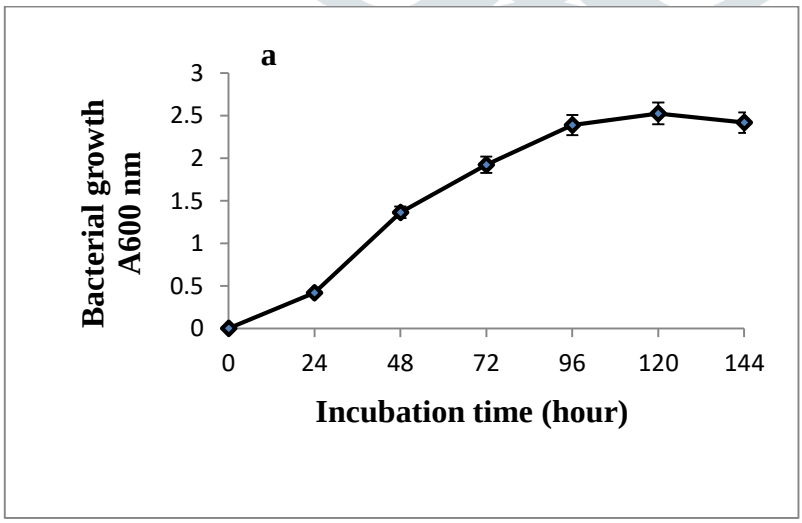
S.No	Parameters	Calculated Value
1.	pH	8.3
2.	BOD (mg/L)	647
3.	COD (mg/L)	825
4.	Lignin (mg/L)	433
5.	Colour (Co-Pt)	1132
6.	TDS (mg/L)	742
7.	Nitrate (mg/L)	54
8.	Phosphate (mg/L)	8.2
9.	Sulphate (mg/L)	813
10.	Total phenol (mg/L)	37
11.	**Heavy metals (mg/L)**	

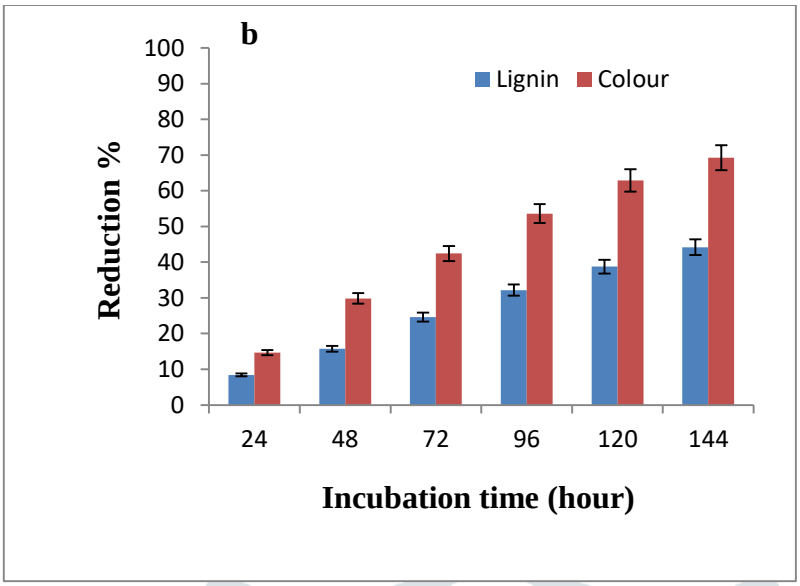
i.	Cu	0.06
ii.	Zn	7.67
iii.	Fe	9.21

Table.2 Morphological and biochemical characteristics of isolated bacterial strain

Characteristics	Bacterial strain (PLP 2)
Gram stain	Gram +ve
Shape	Rod
Colour	Light yellow
Margin	Even
Motility	Motile
Catalase test	+ve
Indole test	-ve
Casein hydrolysis	+ve
Hydrogen sulfide test	-ve
Starch hydrolysis	+ve

Fig.1 Bacterial growth (a), reduction in lignin and colour (b) by isolated bacterial strain PLP 2





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