Ann Microbiol (2014) 64:823–829
DOI 10.1007/s13213-013-0721-y

ORIGINAL ARTICLE

Potential of *Bacillus cereus* for bioremediation of pulp and paper industrial waste

Mahjabeen Saleem · Sumaira Ahmad · Mubashir Ahmad

Received: 1 February 2013 / Accepted: 17 September 2013 / Published online: 12 October 2013

Abstract Biotechnological processes have the potential to reduce environmental pollution through their application in processes aimed at resolving waste dumping problems. In this study, *Bacillus cereus* was assessed for its efficiency to decolorize pulp and paper industrial effluents. The impact of carbon source, nitrogen source, temperature, initial pH and incubation period on color reduction was also studied. The optimum pH and temperature were found to be 6.5 and 45 °C, respectively. *B. cereus* was efficient at decolorizing the effluents in the presence of glucose, xylose and starch (84, 45.5 and 66 %, respectively), but maximum color reduction (90.6 %) was obtained with 0.5 % sucrose as the carbon source in the presence of 1 % ammonium sulphate. The substantially high load of chemical oxygen demand and biological oxygen demand was decreased by about 61 and 66 %, respectively, by *B. cereus*. The molecular size distribution studies of control and bacterial-treated effluent samples revealed the degradation of high and medium molecular mass compounds. The results demonstrate the high potential of *B. cereus* as a significant candidate for color removal from pulp and paper mill effluents.

Keywords *B. cereus* · Mill effluents · Decolorization · Bioremediation

Introduction

Comprising over 70 % of the earth's surface, water is undoubtedly the most precious natural resource that exists on our planet. The pollution of water changes the quality of the subsoil to such a degree that its suitability, either for human consumption or for the support of man's natural life processes, will decrease or cease. Industries are major contributors to air and water pollution (Gannon et al. 1996). Over the past few decades the pulp and paper industry had learned to use water efficiently. Motivated by the pressure to minimize costs, increase productivity, and ensure product quality requirements, the current focus by the industry is on the optimization of existing water circuits and the development of new treatment concepts (Nagarathamma et al. 1999; Jung and Pauly 2011).

The pulp and paper industry is the sixth largest polluter of water bodies after oil, cement, leather, textile and steel industries. The dense, brown color of effluent from pulp and paper processing plants inhibits the natural process of photosynthesis in streams due to the reduced penetration of solar radiation, subsequently producing a chain of adverse effects on the aquatic ecosystem (Ali et al. 1993). The preparation of wood, the source of papermaking fiber, generates suspended solids, biological oxygen demand (BOD), dirt, grit and fibers, among others. Wastewater generated from Digesters in kraft mills, commonly referred to as "Black Liquor" (BL), contains resins, fatty acids, color, BOD content, chemical oxygen demand (COD) content, adsorbable organohalogens, volatile organic carbons (terpenes, alcohols, phenols, methanol, acetone, chloroform among others (Pokhrel and Viraraghavan 2004).

One of the specific problems associated with pulp mill effluent is the presence of high molecular weight chromophoric organic compounds which are deleterious to the general human health (Tiku et al. 2010). Tarek et al. (2011) studied different oxidation processes, such as UV, UV/H_2O_2, Fenton and photo-Fenton process, for the treatment of paper mill, while Katal and Pahlavanzadeh (2011) assessed the efficiency of the electrocoagulation technique in the treatment of paper mill wastewater by using different combinations of aluminum and iron electrodes.

Various biological treatments have been tested for their efficacy in decolorizing industrial effluents, but the results

M. Saleem (✉) · S. Ahmad · M. Ahmad
Institute of Biochemistry and Biotechnology,
University of the Punjab, Lahore 54590, Pakistan
e-mail: mahjabeensaleem1@hotmail.com

have been ambiguous, different and divergent. Many bacterial strains have been identified for the degradation of lignin, and some of these also mineralize and solubilize polymeric lignin (Zimmermann 2002). Fungi have been reported to have the capacity to remove chloro compounds with simultaneous reduction of COD, BOD and color compounds. Fungal hyphae penetrate contaminated soil, reaching not only heavy metals but also xenobiotic compounds (Leitao 2009). Chandra et al. (2011) studied the decolorization of BL by an isolated potential bacterial consortium comprising *Serratia marcescens* (GU193982), *Citrobacter* sp. (HQ873619) and *Klebsiella pneumoniae* (GU193983) by using the different nutritional as well as environmental parameters.

The major objective of our investigation was to evaluate the potential of *Bacillus cereus* to reduce the harmful impacts of pulp mill effluent which contains BOD and COD content and to remediate the colored pulp and paper mill discharge water.

Materials and methods

Effluent sample collection

The dark-colored effluents released from a local paper and pulp mill located in the suburbs of Lahore city were used in this study. The paper and pulp mill uses various agricultural residues, mostly rice straw and bagasse, as raw materials to manufacture paper. The pulping process includes aggressive chemical treatment (high caustic loadings and chlorine-containing chemicals) of raw materials for pulp and paper production and, therefore, the effluent discharge is dark brown in color and has a high COD content due to high chlorine charges. The effluent samples were collected from wastewater discharged at the drain site of the mill in pre-sterilized Erlenmeyer flasks and preserved at 4 °C until use.

Bacterial strain

The bacterial strain used in this study was identified as *Bacillus cereus* based on biochemical tests and 16S rRNA gene sequencing (Saleem et al. 2012). Cells of *B. cereus* were inoculated into 250-ml Erlenmeyer flasks each containing 20 ml of culture medium supplemented with 0.5 % cellobiose as the carbon source and cultivated at 55 °C up to an absorbance of 0.6 at 600 nm. A sample (2 %) of this inoculum was used to inoculate fermentation medium, and the potential of these cells for decolorization of paper and pulp industrial effluent was evaluated.

Decolorization assay

The decolorization experiments were carried out in 250-ml Erlenmeyer flasks. The pH of the effluent sample solutions was adjusted to 7.0 with 1 M NaOH and the solutions then sterilized. Each flask containing an effluent sample was inoculated with 1 ml of *B. cereus* culture and incubated in a Gallenkamp orbital incubator shaker (Weiss Technik, Loughborough,Leicestershire, UK) at 150 rpm. Effluent samples were withdrawn aseptically from the flasks at regular time intervals and centrifuged at 16,800 *g* for 15 min. They were then investigated for color removal by measuring the absorbance of the supernatant at 630 nm with an UV-spectrophotometer (UV-1700 Pharmaspec; Shimadzu, Kyoto, Japan). Experiments were performed in triplicate, and the average of two experiments was taken as the final value for the supernatant. The controls were run under the same conditions but without inoculum. Percentage decolorization was calculated using the formula: decolorization (%)=$[(A_I-A_d)/A_I]\times 100$ where, A_I is the initial absorbance and A_d is the absorbance of the decolorized medium.

Optimization of decolorization process parameters

The decolorization conditions were optimized by changing one independent variable at a time while keeping the other variables constant. The influence of time on percentage decolorization of effluent was observed by incubating the effluent samples with *B. cereus* bacterial cells for different time intervals. For this study, we added 50 ml of effluent sample to a 250-ml Erlenmeyer flask and incubated this with 1 ml of bacterial culture in a Gallenkamp incubator shaker (Weiss Technik) with agitation at 150 rpm. Effluent samples were removed at regular time intervals and the percentage decolorization determined.

The effect of assay temperature on color removal was measured by incubating 50 ml of effluent samples with 1 ml *B. cereus* cells at different temperatures ranging from 30 to 55 °C. A pH range of 5.5–8.0 was used to determine the optimum pH for maximum decolorization under the optimized assay conditions. The pH of the solution was adjusted to 5.5–6.5 with HCl and to 7.0–8.0 with NaOH.

The effect of carbon sources, such as glucose, sucrose, starch and xylose, on effluent decolorization was examined as co-substrates. In these experiments, 2 % of *B. cereus* inoculum was inoculated into 50 ml of sterilized pulp and paper mill effluent sample in 250-ml Erlenmeyer flasks supplemented individually with 0.5 % of the specified carbon source and 1 % ammonium sulfate as the nitrogen source. The paper mill effluent samples without inoculum were used as control.

Gel permeation chromatography

The molecular weight distribution analysis of the treated and untreated paper mill effluents was studied by fractioning the effluent sample through a Sephadex G-75 column (Sigma Chemical, St. Louis, MO) pre-equilibrated with 0.05 N LiOH–NaCl. A 2 ml sample of effluent was subjected to gel filtration column, and fractions were eluted with a solution of 0.05 N LiOH–NaCl. A total of 48 fractions (0.5 ml each) were collected at a flow rate of 0.5 ml/min. Sample elution was monitored by measuring the absorbance at 280 nm with a UV-spectrophotometer (Shimadzu).

COD measurement

The effluent samples treated with bacterial cells and the controls were evaluated for COD. The pH of 50 ml of effluent samples in Erlenmeyer flasks was adjusted to 6.0–8.0. The flasks were incubated with 1 ml of *B. cereus* culture for 24 and 48 h under the optimized temperature in a Gallenkamp orbital shaker. The COD of the effluent samples was measured by using Lovibond COD reactor model ET 125 Sc (Tintometer GmbH, Dortmund, Germany).

BOD measurement

The biologically treated effluent samples were evaluated for BOD using untreated effluent sample as the control. In this assay, 50 ml of effluent was inoculated with 1 ml of bacterial culture and incubated under optimized conditions of pH and temperature for 108 h in a Gallenkamp orbital shaker. The BOD of the effluent sample was measured using a Complex Lovibond BOD Oxidirect measuring system).

Results and discussion

Effect of physicochemical factors

The medium composition greatly influenced bacterial cell growth. A comparable impact of medium on growth was observed by Lodato et al. (2007) when these authors cultivated *Pseudomonas* sp. in different growth media during dye conversion experiments. The maximum percentage of decolorization was observed when the microorganism attained the maximum conversion of substrate carbon, thereby demonstrating a relationship between metabolic activity of the microorganism and its ability to complete the decolorization process. The boosting effect of *B. cereus* treatment on pulp mill effluent was evaluated by using various process constraints, such as incubation time, temperature, pH and different substrates. This bacterial strain was chosen on the basis of a number of characteristics, including short generation time, relatively fast growth rate, ability to grow at a higher temperature, pH and its prospective industrial applications.

Effect of incubation time on decolorization

Microorganisms propagate at a specific rate and in a distinctive way. The extent of the lag phase and generation period, respectively, and overall cell biomass production vary in different microbes, and these have a profound influence on the specific characteristics of the microbes. The effect of incubation time on the removal of chromophoric material from the dark-colored effluent of the paper and pulp mill was observed by incubating the samples with bacterial cells for different time intervals (Fig. 1). The bacterial cell growth was monitored by absorbance measurement using the UV/visible light spectrophotometer (UV-2450; Shimadzu). The decolorization of colored mill wastewater by *B. cereus* seems to be closely associated with the microbial growth. Similar results of bacterial growth-mediated decolorization of industrial effluents have also been reported by Galai et al. (2010).

The bacterium *B. cereus* exhibited a maximum impact on color reduction after 24 h of incubation at 40 °C and shaking at 150 rpm and a 2 % (v/v) starter culture. This result shows that *B. cereus* could be a promising candidate for the bioremediation of paper and pulp mill effluents. In one study involving the four different bacterial strains, Mishra and Thakur (2010) reported that incubation with one alkalotolerant *Bacillus* isolate (LP1) resulted in a 25 % decrease in the color of the pulp and paper mill wastewater after 48 h. In another study on a pulp and paper mill effluent, Oliveira et al. (2009) observed a 42.3 % reduction in color by *Paenibacillus* sp. and a 41.87 % reduction in color by *Bacillus pumilus* after 48 h treatment at pH 9.0. Fungal strains isolated from soil and the marine environment are particularly interesting for their potential to decolorize pulp and paper mill black-colored discharge. Saritha et al. (2010) reported a final reduction of decolorization of 78.6 % following incubation of flasks containing effluent samples with fungal cultures at 27 °C for 21 days; however, a major reduction was monitored within 5 days.

Effect of temperature on decolorization

The applications of thermostable and active microorganisms in the paper and pulp industry have been progressively increasing since specific microorganisms can play a valuable role in

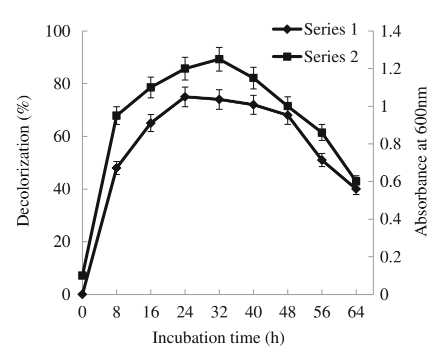

Fig. 1 Effect of time course on cell growth and effluent decolorization by *Bacillus cereus.* Color reduction was measured at intervals of 8 h. The data is representative of the mean of three experiments. *Series 1* Cell growth, *Series 2* decolorization

lowering environmental pollution by reducing the content of harmful chlorinated organic compounds in effluents.

In our study, we used a temperature range of 30–55 °C to determine the influence of temperature on the percentage reduction in decolorization. Effluent samples were inoculated with *B. cereus* and incubated in a Gallenkamp incubator shaker at 150 rpm for 24 h. The maximum color removal occurred at 45 °C, but further increases in incubation temperature had no significant effect on color reduction (Fig. 2). Another strain of *B. cereus* has been reported by Galai et al. (2010) for color removal efficiency, with percentage decolorization increasing with increasing incubation temperature up to 40 °C but with maximum activity attained at 27 °C after 72 h. In another study, *Bacillus* sp. strain LP1 reached maximum percentage decolorization at 35 °C after a 48-h incubation at 200 rpm in the presence of 2.5 % sucrose and 10 % BL (Mishra and Thakur 2010).

In terms of industrial applications, it is convenient to be able to carry out biotechnological processes at high temperatures due to the reduction of contamination risks, amplified rate of the reaction, reduced viscosity and high percentage of yield (Krahe et al. 1996). It has also been reported that growth rate, biomass yield and the reaction mechanism are significantly influenced by the temperature (Blaga et al. 2008). In this context, this bacterial culture tested here seems to be a suitable strain for the bioremediation of pulp and paper mill effluent.

Effect of pH on decolorization

A pH range of 5.5–8.0 was used to determine the optimum pH for maximum percentage decolorization of mill effluent by *B. cereus* (Fig. 3). The results established that the organism is proficient for decolorizing effluent samples over a pH range of 6.0–7.0; however, maximal color discharge from the effluent samples occurred at pH 6.5. A new *B. cereus* strain isolated by Giwa et al. (2012) from contaminated food exhibited a maximum decolorization rate at pH 7.0, while a pH optima range of 3.5–4.0 was reported by Mehna et al. (1995) and Prasad and Joyce (1991) for decolorization of effluents by *Trametes versicolor* and *Trichoderma* sp., respectively. In contrast, Sahoo and Gupta (2005) reported an optimum pH of 8.0 for high-efficiency effluent decolorization by *Aspergillus fumigatus*. In a different study, the treatment of pulp and paper mill effluent by *B. pumilus* and *Paenibacillus* sp. enhanced color reduction at pH 9.0 as compared to treatment at pH 7.0 and pH 11.0 (Oliveira et al. 2009).

Effect of different cosubstrates on decolorization

Bacillus cereus was employed for the assessment of its potential to decolorize effluents in the presence of various substrates present at an initial concentration of 0.5 % under the optimized conditions of temperature and pH. After 48 h, we observed a slight decrease (8.5±0.3 %) in effluent color even in the absence of a carbon source (control). After a 48-h incubation in the presence of 1 % ammonium sulfate as the nitrogen source, supplementation of the effluent with xylose reduced the color by 45.5 %, whereas the color reduction was 66.0 and 84.0y when starch and glucose, respectively, were used as cosubstrates. However, under similar conditions, maximum increase in color removal (91.6 %) was observed with 0.5 % sucrose, thereby establishing its affirmative impact on color discharge (Fig. 4). With respect to the nitrogen source, these results are in contrast to those reported by Sahoo and Gupta (2005) who found that no additional nitrogen source is required for the effective decolorization of pulp and paper mill effluent. However, Mehna et al. (1995) reported the improved decolorization efficiency of *Trametes versicolor* when ammonium nitrate was added to the pulp and paper mill effluent samples at a concentration of 1.75 g/l. Jadhav et al. (2008) also reported decolorization by a microbial consortium of *Galactomyces geotrichum* and *Bacillus* sp. using various nitrogenous compounds. In terms of supplementation of the effluent with various substrates, Mishra and Thakur (2010) observed maximum color removal in the presence of 2.5 % sucrose at pH 8 and

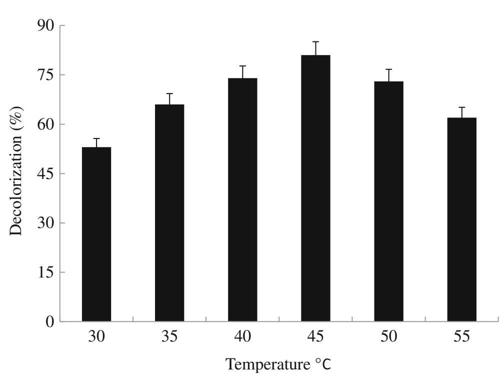

Fig. 2 Effect of temperature on decolorization of pulp and paper mill effluent by *B. cereus* after 24 h of incubation. Data are the mean of three experiments

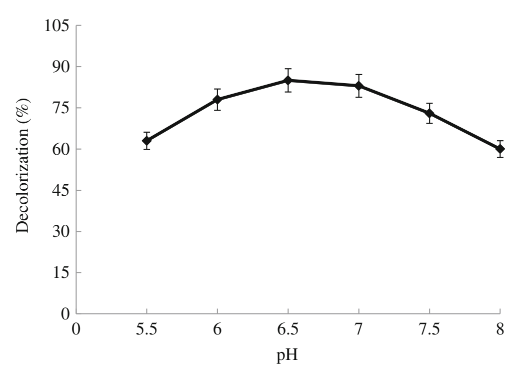

Fig. 3 Decolorization of pulp and paper mill effluent by *B. cereus* at different pH at 45 °C in a Gallenkamp orbital incubator shaker at 150 rpm after 24 h of incubation. Data are the mean of three experiments

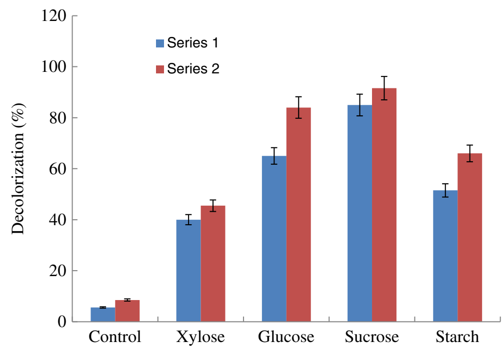

Fig. 4 Effect of different substrates on the decolorization of effluent by *B. cereus.* Data are the mean of three replicates with standard deviations (SD) and are the mean of three experiments. *Series 1* After 24 h, *Series 2* after 48 h

Table 1 Reduction in chemical oxygen demand in pulp and paper mill effluents after treatment with *Bacillus cereus* at different pH

Effluent pH	Treatment time (h)	COD value (mg/ml)	Reduction (%)
6.0	24	832±1.88	52.26
	48	771±1.70	55.76
6.5	24	680 ±1.99	61.25
	48	750 ±2.1	57.26
7.0	24	880 ±2.3	50.11
	48	894 ±2.6	49.31
8.0	24	898 ±1.8	49.23
	48	930±2.4	47.42

COD, Chemical oxygen demand

35 °C after a 48-h incubation. After optimization of various process parameters, these authors achieved a twofold increase in color and lignin removal, from 25 to 69 % and from 28 to 53 %, respectively. In another study, a mixed culture of *Bacillus* sp. and *Serratia marcescens* was found to have the potential to degrade pentachlorophenol and decolorize pulp and paper mill effluent when the samples were supplemented with 1 % glucose and 0.5 % peptone (w/v) and incubated for 168 h at 30±1 °C, pH 8.0±0.2 at 120 rpm (Singh et al. 2008).

Hence, we suggest that *B. cereus* could be an efficient strain for the decolorization of pulp and paper industry effluents and might be a practical alternative for the treatment of pulp and paper mill wastewater.

Gel permeation chromatography

The studies on molecular size distribution highlight the changes that occur in high molecular weight colored compounds present in mill effluents after their biological treatment. The pattern of molecular weight distribution of the compounds produced in *B. cereus*-treated samples and the control was deduced after running them through a Sephadex G-75 column. The elution profile at 280 nm showed a substantial decline in high and average molecular weight compounds in the biologically treated pulp and paper mill effluent samples as compared to the controls (Fig. 5). Breakdown and depolymerization of high molecular weight compounds in biologically treated effluent was assessed by the presence of small-sized organic compounds (≤ 1,000 Da) in the sample. These results are in accordance with those reported by Oliveira et al. (2009) who found that molecular weight distribution peaks area from treated effluents were reduced by nearly 60 and 70 % following incubation with *B. pumilus* and *Paenibacillus* sp., respectively, thereby confirming that the compounds present in the pulp and paper mill effluent were depolymerized during the treatment. Taseli (2008) also confirmed the ability of *Penicillium camemberti* to remove low molecular weight compounds from acidic and alkaline composite effluent, especially under shaking conditions.

Chemical oxygen demand

Decreases in both the usage of physical methods and the consumption of hazardous chemicals for paper and pulp effluent treatment are the major objectives of industrial organizations in

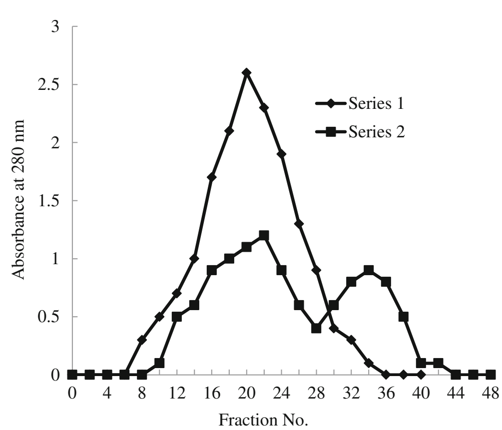

Fig. 5 Sephadex G-75 elution profile at 280 nm obtained with untreated and *B. cereus*-treated effluent. *Series 1* Control, *Series 2* After *B. cereus* treatment

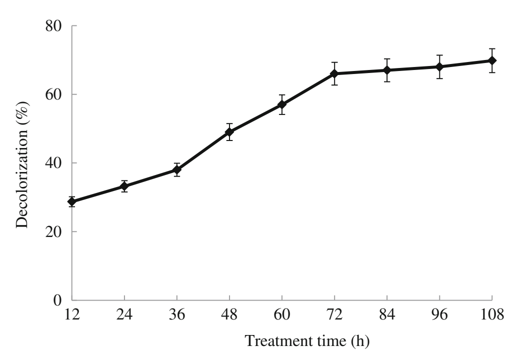

Fig. 6 Reduction of biological oxygen demand (BOD) in pulp and paper mill effluent after treatment with *B. cereus.* Data are the mean of three experiments

their attempts to lessen energy consumption and protect the environment form contamination and toxic wastes. Considerable attention is being paid to the development of new technologies and improvements in current methods for the treatment of pulp and paper mill effluents, particularly the usage of biological methods. Microorganisms are predominantly important for color removal as well as for reducing the BOD and COD of the industrial effluents (Bajpai and Bajpai 1994).

Table 1 presents the COD pattern in the untreated and *B. cereus*-treated effluent samples from the pulp and paper plant. The COD values in the untreated effluent samples were found to be 1,743, 1,755, 1,764 and 1,769 mg/ml, at pH 6.0, 6.5, 7.0 and 8.0, respectively. No significant change in the percentage reduction of COD [52.26 to 55.76 % (pH 6.0); 61.25 to 57.26 % (pH 6.5); 50.11 to 49.31 % (pH 7.0); 49.23 to 47.42 % (pH 8.0)] was observed after 24–48 h of incubation. A slight increment in COD reduction was detected with increasing pH up to 6.0, and a considerable decrease in COD reduction (61.25 to 49.23 %) was observed when the pH increased from 6.5 to 8.0 after 24 h. In comparison, after a 10-day treatment of pulp and paper mill effluent by *Pleurotus sajor-caju*, Ragunathan and Swaminathan (2004) reported a COD reduction of 61.3 % (1302.0 mg/dl). Selvam et al. (2002) reported comparable results, namely, a reduction in COD to 1,984 mg/l, corresponding to 59.3 %, by *Fomes lividus* on the tenth day. A substantial reduction (89.4 %) and (83 %) in the COD of paper mill effluent samples after fungal and bacterial consortium treatment was reported by Saritha et al. (2010) and Chandra et al. (2011), respectively. In contrast, a COD drop of only 20.46 and 19.11 % was reported for *B. pumilus*- and *Paenibacillus*-treated effluents, respectively, by Oliveira et al. (2009) at pH 7.0 after 48 h.

A significant application of the COD parameter is to determine the quantity of organic pollutants in effluents. As such, our results showing a significant drop in COD in contaminated paper and pulp mill discharge samples demonstrate an effective degradation of such pollutants by *B. cereus*, further indicating the potential of this microbe for the bioremediation of pulp and paper mill effluents.

Biological oxygen demand

Pulp and paper mill effluent can described by its different characteristics, such as BOD (40,000–50,000 mg/l), COD (200,000 mg/l), dark-brown color, temperature, highly alkaline pH, etc. It is estimated that one tonne of paper generates 150 m^3 of effluent that is highly lethal in nature (Pokhrel and Viraraghavan 2004). Hence, an inclusive management approach is needed to avoid the harmful impacts of pulp and paper mill effluents. The prerequisite for the industrial use of biotechnology is the existence of organism with the proficiency to degrade various mill effluents. We therefore evaluated the ability of *B. cereus* for BOD using untreated and treated effluent samples. The BOD experiment was carried out under the optimized conditions of temperature (45 °C) and pH (6.5), employing the untreated sample as a control. The BOD exhibited moderate rates up to 36 h of incubation, but thereafter we noted rapid and effective degradation rates to 72 h at which time point the BOD reduction was 66 % (Fig. 6). After 72 h of digestion, the decrease in BOD was not significant and became almost constant. Singh et al. (2008) demonstrated the potential of a mixed culture of two bacterial strains (*Bacillus* sp. and *Serratia marcescens*) to reduce the high load of BOD (81.25) in pulp and paper mill wastewater, and Belsare and Prasad (1988) reported a BOD reduction of 46.6 % with *Schizophyllum commune* using effluent from a bagasse-based paper mill. Interestingly, Saritha et al. (2010) reported a BOD reduction of 65.3, 51.3, 100 and 100 % in effluent treated with *Penicillium chrysogenum*, *Trametes hirsuta* and two unidentified fungal isolates MF and SF, respectively, but only after 17 days.

BOD is the amount of dissolved oxygen used by the organisms to break down organic complexes in waste and is frequently used to measure the amount of pollutants in wastewater. Our results show the proficiency of *B. cereus* for effective BOD reduction in pulp and paper mill effluents.

Public opinion and government regulations on ecological pollution have forced the paper industry to develop novel techniques to reduce the different contaminants in effluents emanating from the bleaching plants of the paper and pulp mills. One such approach is the use of biological methods which permit the development of more sustainable and ecofriendly procedures capable of reducing the use of toxic compounds and subsequently minimizing them in the pulp and paper mill effluents. The number of industries in Pakistan has grown rapidly in recent years, and most factories and mills do not have any appropriate treatment strategies. The presence of various pollutants in the effluents discharging from the bleaching plants can cause extreme damage to the habitat adjoining such mills. The *B. cereus* strain studied here has the ability to endure, decolorize and reduce toxic and recalcitrant compounds present at high concentration in pulp and paper mill effluents, thereby providing a safe mode of dumping in the surroundings.

References

Ali F, Sarma TC, Saikia CN (1993) Pulp and paper from certain fast-growing plant species. Bioresour Technol 45:65–67

Bajpai P, Bajpai PK (1994) Biological color removal of pulp and paper mill waste waters. J Biotechnol 33:211–220

Belsare DK, Prasad DY (1988) Decolorization of effluent from the bagasse-based pulp mills by white-rot fungus, *Schizophyllum commune*. Appl Microbiol Biotechnol 28:301–304

Blaga A, Avramova T, Stefanova L, Mutafov S (2008) Temperature effect on bacterial azo bond reduction kinetics: an arrhenius plot analysis. Biodegradation 19:387–393

Chandra R, Abhishek A, Sankhwar M (2011) Bacterial decolorization and detoxification of black liquor from rayon grade pulp manufacturing

paper industry and detection of their metabolic products. Bioresour Technol 102:6429–6436
Galai S, Limam F, Marzouki MN (2010) Decolorization of an industrial effluent by free and immobilized cells of *Stenotrophomonas maltophilia* AAP56. Implementation of efficient down flow column reactor. W J Microbiol Biotechnol 26:1341–1347
Gannon RW, Osmond DL, Humenik FJ, Gale JA, Spooner J (1996) Goal-oriented agricultural water quality legislation. Water Resour Bull 32: 437–450
Giwa A, Giwa FJ, Ifu BJ (2012) Microbial decolourization of an anthraquinone dye C.I. reactive blue 19 using *Bacillus cereus*. Am Chem Sci J 2(2):60–68
Jadhav SU, Jadhav MU, Kagalkar AN, Govindwar SP (2008) Decolorization of Brilliant Blue G dye mediated by degradation of the microbial consortium of *Galactomyces geotrichum* and *Bacillus* sp. J Chin Inst Chem Eng 39:563–570
Jung H, Pauly D (2011) Water in the pulp and paper industry. Treatise Water Sci 4:667–683
Katal R, Pahlavanzadeh H (2011) Influence of different combinations of aluminum and iron electrode on electrocoagulation efficiency: application to the treatment of paper mill waste water. Desalination 265:199–205
Krahe M, Antranikian G, Markl H (1996) Fermentation of extremophilic microorganisms. FEMS Microbiol Rev 18:271–285
Leitao AL (2009) Potential of *Penicillium* species in the bioremediation field. Int J Environ Res Public Health 6:1393–1417
Lodato A, Alfieri F, Olivieri G, Di Donato A, Marzocchella A, Salatino P (2007). Azo-dye conversion by means of Pseudomonas sp. OX1. Enzyme Microb Technol 41:646–652
Mehna A, Bajpai P, Bajpai PK (1995) Studies on decolorization of effluent from a small pulp mill utilizing agriresidues with *Trametes versicolor*. Enzyme Microb Technol 17:18–22
Mishra M, Thakur IS (2010) Isolation and characterization of alkalotolerant bacteria and optimization of process parameters for decolorization and detoxification of pulp and paper mill effluent by Taguchi approach. Biodegradation 21:967–978
Nagarathamma R, Bajpai P, Bajpai PK (1999) Studies on decolorization, degradation and detoxification of chlorinated lignin effluents by *Ceriporiopsis subvermispora*. Process Biochem 34:939–948
Oliveira PL, Duarte MCT, Ponezi AN, Durrant LR (2009) Use of *Bacillus pumilus* CBMAI 0008 and *Paenibacillus* sp. CBMAI 868 for color removal from paper mill effluent. Braz J Microbiol 40:818–826
Prasad DY, Joyce TW (1991) Color removal from kraft bleach plant effluent by *Trichoderma* sp. Taappi 74:165–169
Pokhrel D, Viraraghavan T (2004) Treatment of pulp and paper mill wastewater. Sci Total Environ 333:37–58
Ragunathan R, Swaminathan K (2004) Biological treatment of a pulp and paper industry effluent by *Pleurotus* spp. World J Microbiol Biotechnol 20:389–393
Sahoo DK, Gupta R (2005) Evaluation of ligninolytic microorganisms for efficient decolorization of a small pulp and paper mill effluent. Process Biochem 40:1573–1578
Saleem M, Rehman A, Yasmin R, Munir B (2012) Biochemical analysis and investigation on the prospective applications of alkaline protease from a *Bacillus cereus* strain. Mol Biol Rep 39: 6399–6408
Saritha V, Maruthia YA, Mukkanti K (2010) Potential of bioremediation of industrial effluents. Bioresources 5:8–22
Selvam K, Swaminathan K, Song MH, Chae KS (2002) Biological treatment of a pulp and paper industry effluent by *Fomes lividus* and *Trametes versicolor*. World Microbiol Biotechnol 18:523–526
Singh S, Chandra R, Patel DK, Reddy MMK, Rai V (2008) Investigation of the biotransformation of pentachlorophenol and pulp paper mill effluent decolorisation by the bacterial strains in a mixed culture. Bioresour Technol 99:5703–5709
Tarek SJ, Montaser YG, Ibrahim ES, Eglal RS, Rabab AN (2011) Comparative study among different photochemical oxidation processes to enhance the biodegradability of paper mill waste water. J Hazard Mater 185:353–358
Taseli BK (2008) Fungal treatment of hemp-based pulp and paper mill wastes. Afr J Biotechnol 7:286–289
Tiku DK, Kumar A, Chaturvedi R, Makhijani SD, Manoharan A, Kumar R (2010) Holistic bioremediation of pulp mill effluents using autochthonous bacteria. Int Biodeterior Biodegrad 649:173–183
Zimmermann W (2002) Degradation of lignin by bacteria. J Biotechnol 13:119–130