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Bioremediation of chromium complex dyes and treatment of sludge generated during the process

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ABSTRACT

Bioremediation of chromium complex dyes in synthetic solutions and actual effluent was performed using growing *Aspergillus tamarii* in batch and continuous bioreactors. Maximum removal of color (90.00 ± 0.20 and 74.00 ± 0.10%) and chromium (94.00 ± 0.10 and 77.50 ± 0.10%) was obtained from synthetic solutions (100 mg/L) of acid brown 45 and acid blue 158 dyes, respectively in batch mode. Optimization of process parameters such as initial concentration of dye, pH and time was performed using response surface methodology (RSM). Biosorption, bioaccumulation and biodegradation of the dyes during bioremediation were supported by TEM and GC-MS analyses. Maximum removal of color (64.50 ± 0.10 and 45.00 ± 0.10%) and chromium (67.00 ± 0.10 and 49.00 ± 0.10%) was obtained at 220 h HRT using solutions (100 mg/L) of acid brown 45 and acid blue 158 dyes, respectively in continuous mode. The removal of color (86.00 ± 0.10 and 65.00 ± 0.20%) and chromium (100.00 ± 0.10 and 81.00 ± 0.10%) was observed in batch and continuous modes, respectively using actual effluent. The lower removal values were obtained using desorbed and dead biomass as compared to actively growing biomass. Phytotoxicity test was conducted using seeds of *Cicer arietinum* to examine the toxic effect of dye solution before and after bioremediation. Anaerobic digestion of residues (after bioremediation of dyes) indicated maximum methane production of 41.00 ± 0.20% on 16th day of operation.

1. Introduction

Various metal complex dyes (chromium, cobalt, copper and nickel complex dyes) are widely used in textile and leather industries as coloring agents (Aksu and Balibek, 2010; Li and Guthrie, 2010). The effluents from these industries are deadly toxic to the environment, plants and animals due to the presence of synthetic dyes associated with heavy metals (Akar and Tunali, 2006; Ghosh and Das, 2014, 2015a). Many physico-chemical techniques, in spite of having several drawbacks are in use for the treatment of dye and metal contaminated effluents (Jia et al., 2002; Deng et al., 2003; Sheng et al., 2004; Banerjee and Dastidar, 2005; Malaviya and Rathore, 2007). Various micro-organisms (bacteria, algae, fungi, yeast, etc.) have been reported to remove separately different heavy metals and dyes from aqueous solutions (Sağ and Kutsal, 2000; Mehta and Gaur, 2001; Wang and Chen, 2006; Bishnoi et al., 2007; Chhabra et al., 2008; Ranjusha et al., 2010; Sinha et al., 2012). However, very little information is available on simultaneous removal of metal and dye from synthetic solution of metal complex dye using micro-organisms (Kalpana et al., 2011). The bioremediation of the pollutants by micro-organisms usually takes place via biosorption, biodegradation and bioaccumulation (Dursun et al., 2003; Yadav et al., 2012; Carro et al., 2013). The functional groups on the cell wall such as carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups are responsible for binding the pollutants on the cell surface (Ghosh et al., 2015b). Fungi have higher resistance to dyes and metals, higher surface area and higher biomass yield compared to other micro-organisms (Kalpana et al., 2011). Fungi are highly efficient for biodegradation of dyes due to the presence of various oxidoreductive enzymes (peroxidases, manganese peroxidases, lignin peroxidases and laccases) (Anastasi et al., 2010; Ghosh et al., 2015b). Also, in some conditions

Abbreviations: AAS, Atomic Absorption Spectroscopy; Chromium: Cr, Copper: Cu; COD, Chemical Oxygen Demand; CSTR, Continuous Stirred Tank Reactor; EDX, Energy Dispersive X-Ray Analysis; FTIR, Fourier Transform InfraRed Spectroscopy; GC-MS, Gas Chromatography–Mass Spectrometry; HRT, Hydraulic Retention Time; RSM, Response Surface Methodology; STP, Standard Temperature and Pressure; TEM, Transmission Electron Microscopy; TDS, Total Dissolved Solids; TIC, Total Ion Chromatogram; TSS, Total Suspended Solids; VFA, Volatile Fatty Acid.

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the matabolites formed appear to be very toxic after biodegradation of dyes (Phugare et al., 2011; Kabra et al., 2011). Therefore, in-depth studies are needed on bioremediation of metal complex dyes using fungi. Further to reduce a large number of experiments in conventional batch process and hence the overall cost, the response surface methodology can be applied for optimization of various process parameters (Jain et al., 2011). Moreover, disposal of the sludge generated during bioremediation assumes great importance as it is rich in pollutants in concentrated manner. Many researchers reported anaerobic digestion consisting of hydrolysis, acidogenesis and methanogenesis phases for solid and semi-solid organic wastes (Ali and Sreekrishnan, 2000; Lastella et al., 2002; Sreekrishnan et al., 2004; Themelis and Ulloa, 2007; Khalid et al., 2011; Gupta et al., 2015). However, there is a lack of study on anaerobic digestion of fungal residues after bioremediation of dyes and metals. Incineration is also a popular treatment technique for disposal of hazardous wastes (Beylot and Villeneuve, 2013).

In the present study, simultaneous removal of color and chromium from synthetic solutions of chromium complex dyes (acid brown 45 and acid blue 158) and actual textile effluent contaminated with chromium complex dyes was studied using actively growing *Aspergillus tamarii* in batch and continuous modes of operations. Optimization of parameters such as initial concentration of dye, pH and time was conducted using RSM. Phytotoxicity test was performed to determine the toxic effect of dye solution (before and after bioremediation) on the growth of *Cicer arietinum*. Desorption study on dye-contaminated biomass was carried out using different elutants and then the desorbed biomass was mechanically destructed. Biosorption studies were also performed using desorbed and dead biomass of *Aspergillus tamarii*. The sludge generated after bioremediation of synthetic solutions of dyes and effluent was anaerobically digested and finally incinerated with an aim to ensure safe disposal of the sludge.

2. Materials and methods

2.1. Dyes and effluent

Acid brown 45, acid blue 158 and actual effluent were collected from local textile industry, Delhi National Capital Region, India. Both the dyes were water soluble at normal room temperature. The chemical structure of acid blue 158 is available (PubChem, NCBI website), whereas the chemical structure of acid brown 45 is not reported anywhere. Therefore, an attempt was made in this study to determine the structural groups, chromium and copper contents of the dyes through FTIR and AAS analyses, respectively. The effluent was dark black in color with a pH value of 3.8. The concentrations of chromium and copper in the effluent were also analyzed by AAS. The TDS and TSS of the effluent were determined by gravimetric method.

2.2. Micro-organism and culture conditions

The fungus *Aspergillus tamarii* was isolated previously in the laboratory from the sludge of a textile industry and was characterized through ribotyping of 18S rRNA gene sequencing. The maximum growth of the organism was found at pH 5.0 and was reported earlier to remove different chromium complex dyes such as acid orange 86, acid orange 80 and acid black 52 (Ghosh et al., 2014; Ghosh et al., 2016a; 2016b). The chemical composition of the growth media was as follows: Glucose: 10 g/L; K_2HPO_4: 0.5 g/L; NaCl: 1 g/L; $MgSO_4$: 0.1 g/L; NH_4NO_3: 0.5 g/L and Yeast extract: 5 g/L (Ghosh et al., 2014). The growth media (100 mL) contained in 250 mL flask was inoculated with the strain after autoclaving and then incubated aerobically at 27 °C and 110 rpm. The required quantities of the dyes were added separately to the media to prepare solutions of different initial concentrations of the dyes for bioremediation studies. The pH of the growth media was adjusted by 0.1 (N) HCl or 0.1 (N) NaOH solutions. Analytical grade chemicals were used for the experiments.

2.3. Batch bioremediation

2.3.1. Growing cells

In the present study, batch experiments were performed at different initial dye concentrations (50–2000 mg/L) and at pH 5.0 to determine the tolerance level of the fungi. The strain used in bioremediation experiment was not acclimatized in the presence of dye. During growth, removal of color and chromium, specific removal of dye and chromium and biomass concentration were determined in samples withdrawn at pre-determined time intervals and centrifuged at 4000 rpm for 10 min (min). The bioremediation was monitored for 50 h. The bioremediation of dye was further studied through GC-MS, EDX and TEM analyses. Further, batch bioremediation studies were extended to include industrial effluent contaminated with metal complex dyes in batch bioreactors at pH 5.0 up to 50 h. The COD was determined to find out the total oxidizable contents present in the dye solution and effluent. The growth of plant is highly sensitive to toxic substances. To check whether toxic components are present in the solution after bioremediation of dyes, the phytotoxicity study was conducted using *Cicer arietinum* seeds which were germinated in tap water as control (Kalpana et al., 2011; Almeida and Corso, 2014). After bioremediation the dye solution was separated from fungal biomass and tested for phytotoxicity. Ten seeds of *Cicer arietinum* were grown separately in dye solution (100 mg/L) before and after bioremediation to compare the length of root and shoot and the dry weight of the germinated seeds.

2.3.2. Desorbed and dead biomass

Biomass after bioremediation of chromium complex dyes (100 mg/L solution) was dried at 60 °C for 12 h. Desorption of the biomass (1 g) was performed up to 2 h under shaking condition using double distilled water and acidic elutants. After desorption biomass was washed, mechanically crushed and tested for chromium. An investigation was made to examine the reuse potential of desorbed biomass for removal of color and chromium from synthetic solutions of chromium complex dyes. Further, fungal biomass was initially grown in the growth media in absence of any dye. The biomass was harvested, dried at 60 °C for 12 h and grounded with the help of a mortar and pestle, sieved to size fraction between 0.5 and 1.0 mm and stored in air-tight bottles for adsorption experiments. For comparative study desorbed biomass and biomass under non-living condition, 1 g each were used for removal of color from 100 mg/L synthetic solutions of dyes in shaker up to 4 h.

2.4. Bioremediation in continuous mode

The continuous stirred tank reactor (CSTR) (5 L volume with 2 L working volume) was connected to a fresh media holding tank through a peristaltic pump (Model no-D4X-01, AcuFlow, Design Innova, India) at the inlet and a tank for collecting the liquid at the outlet. The temperature of CSTR was maintained at 27–30 °C by heating externally through a heating tape joined with a temperature control module. The CSTR was equipped with an aerator at the bottom. The CSTR was initially operated in batch mode using non-sterile growth media containing acid brown 45 and acid blue 158 dyes (in separate reactors) till exponential phase of growth of *Aspergillus tamarii* was reached. Then the operation was started in

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