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BIODEGRADATION OF ANTHRAQUINONE DYE BY *ASPERGILLUS NIGER* SA1 IN SELF DESIGNED FLUIDIZED BED BIOREACTOR

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ABSTRACT

Biodegradation and decolorization of Drimarene blue K2-RL (anthraquinone) dye by a fungal isolate *Aspergillus niger* SA1 was studied in self designed lab scale bioreactor system with different solid supports. *Aspergillus niger* SA1, was efficiently immobilized on sand and sodium alginate beads. The immobilized cells were used in the form of fluidized bed reactor for textile dye (Drimarene blue) removal. Both the reactors were operated at room temperature and pH=5.0 in continuous flow mode with increasing concentrations of dye in simulated textile effluent. The reactors were run on fill and draw mode, with hydraulic retention times of 24-72 h. The fluidized bed bioreactor with sand as immobilizing support (FBR1) showed overall better performance as compared to fluidized bed bioreactor with sodium alginate as immobilizing support (FBR2). The average overall color, BOD and COD removal in the FBR1 system were up to 78.29, 70.81 and 83.07% respectively, with 50 ppm initial dye concentration and HRT of 24 h. While 72.19%, 86.63% and 74.74% removal of color, BOD and COD were observed, respectively, in FBR2 with the same conditions. Reductions in BOD and COD levels along with color removal proved that decolorization and biodegradation occurred simultaneously.

Key words: Drimarene blue; Biodegradation; Fluidized bed bioreactor system; Color removal, *Aspergillus niger*

INTRODUCTION

The textile industry is one of the greatest generators of liquid effluent pollutants, due to the high quantities of water used in the dyeing processes. It is estimated that approximately 280,000 tonnes of textile dyes are discharged in such industrial effluent annually worldwide (Jin *et al.,* 2007). Discharge of these colored effluents results in direct and indirect exposure of the human population to high concentrations of these dyes. The Anthraquinone group of textile dyes has a complex aromatic molecular structure which resists degradation in the environment due to their fused aromatic structure, which tends to remain color for a long time. Decolorization of these dyes has also received much attention due to their toxicity, carcinogenic and mutagenic behavior (Lu *et al*., 2008). Biological processes using microbial systems provide an alternative to the existing physical/chemical technologies (expensive and commercially unattractive) because they are more cost-effective, environment friendly, and do not produce large quantities of sludge (Kariminiaae-Hamedaani *et al*., 2007; Asad *et al*., 2007). As regulations are becoming even more stringent, there is an urgent need for technically feasible and cost-effective wastewater treatment methods in this industry (Pandey *et al*., 2007). Current researches have indicated that improving the biological treatment efficiency is the key solution to this problem due to their possibly complete mineralization of dyes at low cost (Cetin *et al*., 2006; Yang *et al*., 2009). In the present study biological removal of Drimarene blue K_2RL (anthraquinone) dye by a fungal

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isolate *Aspergillus niger* SA1 was studied in self designed lab scale bioreactor system with different solid supports. The purpose of this study was to establish a cost effective, easily maintained, high efficiency wastewater treatment technology.

MATERIALS AND METHODS

Dye and Simulated Textile Effluent (STE)

The investigated commercial dye Drimarene blue (Db) K_2RL (Anthraquinone based dye) was obtained from Kohinoor Textile Mill, Rawalpindi, Pakistan. Simulated effluent was made by adding per litre of distilled water; Acetic Acid (99.9%) =0.15 mL, $(NH_2)_2CO$= 108.0 mg, KH_2PO_4=67.0 mg, $NaHCO_3$=840.0 mg, $MgSO_4.7H_2O$=38.0 mg, $CaCl_2$=21.0 mg, $FeCl_3.6H_2O$=7.0 mg and glucose=860mg (Luangdilok and Panswad, 2000). pH of effluent was adjusted to 5 by using 0.1 M HCl and NaOH.

Microorganism and inoculum preparation

The fungal isolate *Aspergillus niger* SA1 was obtained (isolated from wastewater storage pond of local Textile Mill) from the culture collection of Microbiology Research Laboratory Quaid-i-Azam University, Islamabad. Spores were collected from a 72 h culture and suspended in 20 mL of 0.05% Tween 20 solution in sterile collection tubes. Spore suspension (5%) was used to inoculate STE Medium (100 mL) in 250mL conical flasks to a final concentration of 10^3spores/mL by using MOD-FUCH'S ROSETHAL hemocytometer (Depth 0.2mm 1/116mm^2, WEBER, England).

Immobilization of fungus

The fungal strain *Aspergillus niger* SA1 was immobilized to sand and sodium alginate beads. For the immobilization on sand, the particle size of 0.2 mm was used. The sand was washed with sterilized distilled water twice and then washed with sodium phosphate buffer. The 72 h old culture of *Aspergillus niger* SA1 was mixed with the sand and incubated at 30 ˚C incubator. Thorough mixing of the fungal mycelia and the sand particle was done each day manually for one week. Attachment of mycelia on the sand particle was checked under the microscope.

In another experiment, sodium alginate (3%) was mixed with 1mL of 5% spore suspension gently until homogenized completely. This slurry was dropped by a 60 mL syringe into 0.2 M ice cold calcium chloride solution. The spore-immobilized beads were cured in the 0.2 M $CaCl_2$ solutions for 4h to enhance their mechanical stabilities. Control beads (cell-free immobilized beads) were prepared following the above procedures except that the spores were excluded. Beads were incubated in STE on a rotary shaker at 30 °C and 120 rpm for 72 h. These freshly prepared "immobilized beads" were harvested, rinsed twice with sterilized distilled water and stored in sodium acetate buffer at 4°C. Environmental Scanning Electron Microscopic (ESEM) analysis of the immobilized and un-immobilized sand particles and sodium alginate beads was done to compare and investigate the mechanism of immobilization of fungal strains on solid support efficiently. Analysis was performed on FEI Quanta 200 ESEM at low vacuum 0.68 Torr mode by using LFD (large field detector).

Bioreactor design and operation

The reactor body consisted of plastic cylinder (3350 cm^3) with 101.6 cm height and 9 cm internal diameter. Height of the immobilized cells bed was kept upto 34.29cm. The working volume was 2000cm^3. Void volume of 1350cm^3 capacity was kept for the fluidization of the bioparticles and for the degasification of the liquid. Sterile air was supplied from the bottom of column at a flow rate of 1.5mL/min. The bioreactor main bed consisted of immobilized cells of fungi (Sand in FBR1 and Sodium Alginate beads in FBR2) and these bioparticles were fluidized in the column by air upflow. The influent port was situated at the bottom parallel to the air supplying port. The base of column consisted of sterile plastic net to prevent the entry of bio particles into the flow lines. The effluent flow line was located at the top of the column. Both the influent and the effluent flows were maintained by the peristaltic pump (manostat®).

The reactors were operated at room temperature, pH=5.0 and in the continuous flow mode. The cylinders were filled with immobilized cells (depending upon the experimental set up in the bioreactors) and STE medium containing

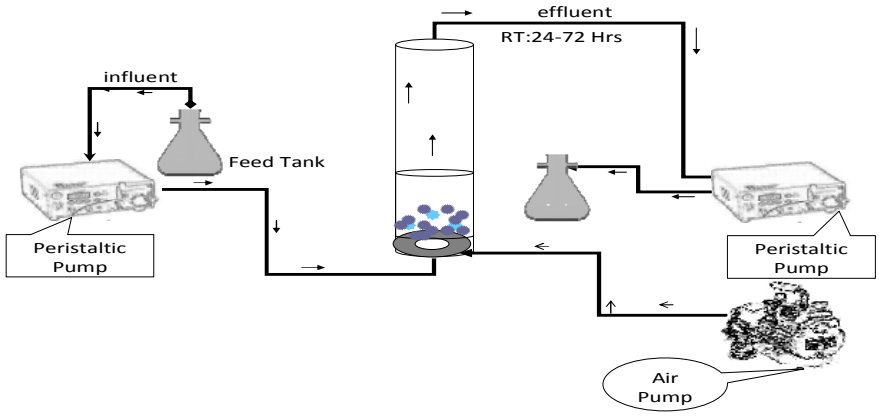


Fig. 1: Schematic diagram of the up-flow fluidized bed bioreactor with immobilized fungus

50 mg/L of dye concentration was fed into the reactor. The hydraulic retention time was 24 h for the 50mg/L of dye loading rate and 72 h for higher dye loads (100, 150, 200, 300 and 500 mg/L). All the reactors were run on fill, react, settle and draw mode. The effluent was drawn every time before the reactors were fed with the higher concentrations of dye (100, 150, 200, 300 and 500 mg/L) each time. Samples were drawn throughout all experimental set up for the estimation of different parameters (color, BOD and COD). All samples were filtered though Whattman paper no.1 and centrifuged at 5000 rpm for 10 minutes. The pellet was discarded and clear solution was used to analyze the biological removal of the dye in the bioreactor.

Analytical methods

Decolorization of dye was analyzed spectrophotometerically, monitoring decrease in absorption spectrum at λ=620nm (λmax for Drimarene blue K_2RL) using the AGILENT UV visible spectrophotometer. Chemical oxygen Demand (COD) analyses of the untreated and treated samples from bioreactors were performed by using closed reflex, colorimetric method, APHA 5220D standard method (APHA, 1995). BOD analysis was performed by using 5-Day BOD (APHA 5210 B standard method) Test (APHA, 1995).

RESULTS

Immobilization of fungus

Comparison of ESEM of the immobilized and un-immobilized sand particles, showed the efficient attachment of fungal strain on solid support (Fig. 2). The fungal mycelia covered the entire sand particle through attachment mechanism. Visual observations showed very little free cell mass, hence almost no cell leakage. The surface of sand particle due to its porosity was completely covered by fungal mycelia.

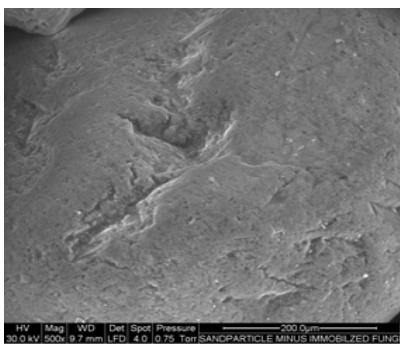
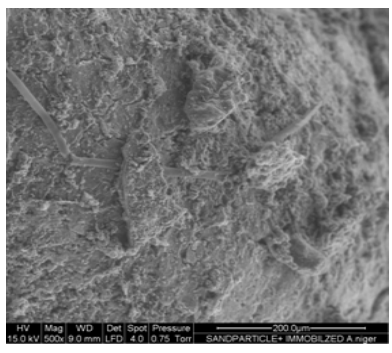
(A)   (B)

Fig. 2: Electron micrograph of Sand Particle free, (A) and with immobilized fungus (B)

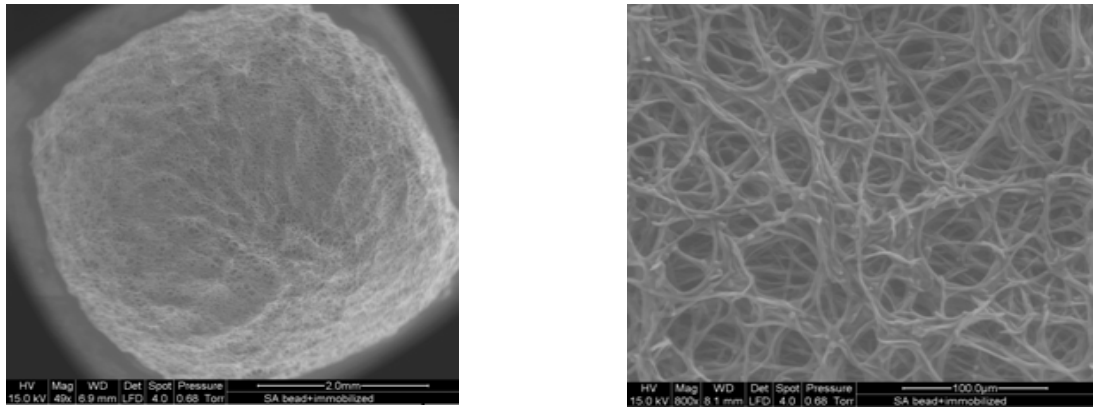


Fig. 3: Electron micrograph of cell free immobilized sodium alginate bead, (A) and entrapped mycelia of *Aspergillus niger* SA1in sodium alginate bead (B)

Electron micrograph (ESEM) of the immobilized sodium alginate beads showed the complete internal entrapment of fungal mycelia (Fig. 3). Surface and internal structural changes of the bead and increased porosity was also observed. A marked difference was observed in the size and color of cell free and the immobilized mycelia beads after 72 h of incubation, due to complete entrapment of fungal mycelia inside the beads. Visual observation showed the presence of free cell mass, indicating cell leakage. The irregular surface of the control beads (cell free beads) was completely distinct from the regular and loose surface of the immobilized beads. Visual observation in terms of biomass released and amount of biomass attached, proved the attachment an excellent technique as compared to entrapment. Very few free fungal mycelia, with almost no cell leakage indicated sand a better choice as a support material than sodium alginate beads.

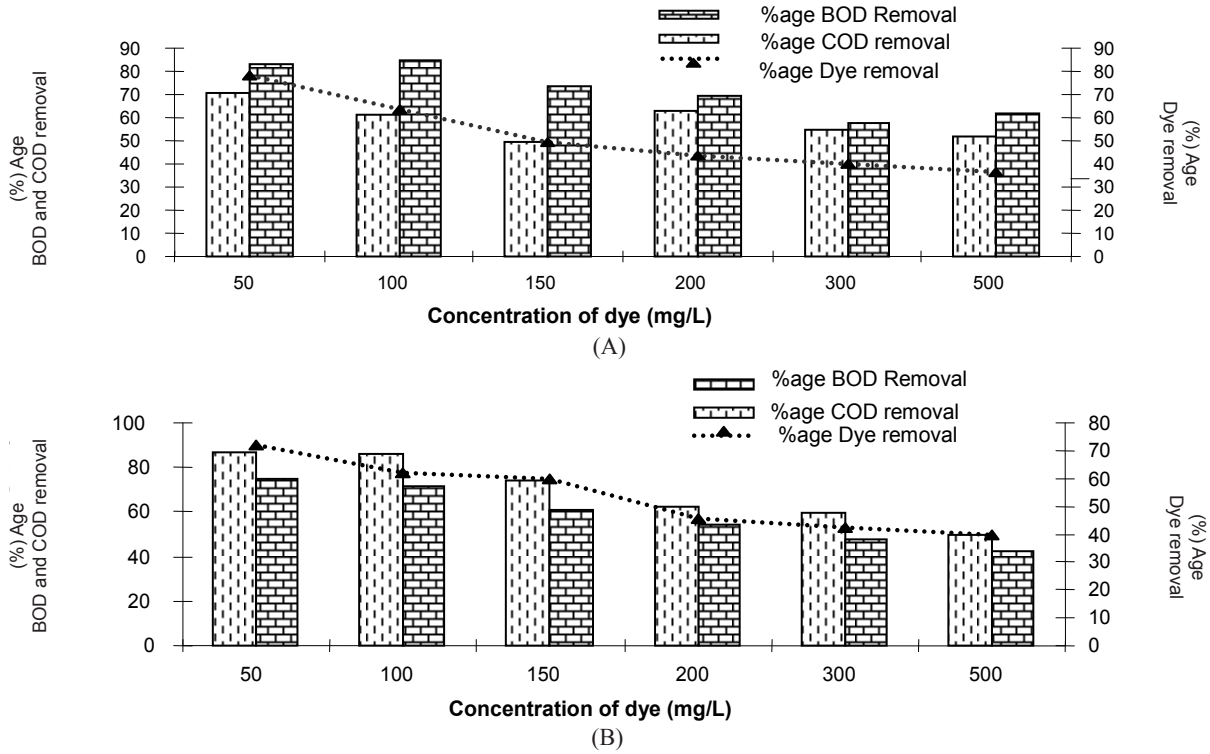


Fig. 4: Fluidized bed bioreactor treatments; A: (FBR1), B: (FBR2), showing reductions in dye, COD and BOD by immobilized fungal mycelia

Color, COD and BOD removal in bioreactors

The comparison of the UV-vis spectra of the input and output of the bioreactor treatments showed spectral shifts towards the UV region. The peaks appearing at 610 nm (λmax for Db K_2RL) in the input of the bioreactor disappeared in the output of the bioreactor, suggesting the reductions of the dye by the activity of the immobilized fungal mycelia. Additionally, almost <1% dye was found adsorbed on the support materials in abiotic controls, suggesting that the dye decolorization observed in case of all bioreactor treatments was caused by the activity of the fungal agents. Concentration of the dye in the input of the bioreactor affected the BOD and COD removal efficiency in a similar manner as dye removal efficiency i.e. increasing concentrations from 50 (at 24 h HRT) to 100, 150, 200, 300 and 500 mg/L (at 72 h HRT) decreased removal performances. Immobilized *Aspergillus niger* with bioreactor system (FBR1) removed 78.29% dye, 70.81% BOD and a higher COD reduction of 83.07% was observed, when the reactor was operated with 50 mg/L dye for 1 day (24 h HRT). In FBR1 the COD reduction was quite higher up to the last day of the reactor operation with 500 mg/L dye, while the BOD reduction was 52.03% with 36.28% dye removal (Fig. 4).

DISCUSSION

Immobilization of *Aspergillus niger* on two different solid supports showed two different mechanisms i.e. attachment (on sand particle) and entrapment (on sodium alginate beads). These two different mechanisms possessed certain advantages and disadvantages at the same time in both the systems. Sand particles proved to be good support in terms of the amount of solid biomass attached and also the less amount of cell free leakage. A similar type of results were reported in previous finding that revealed the formation of biofilm by the components of bacterial consortium on seashell pieces (Khehra *et al*., 2006). Sharma *et al* (2004) utilized the pieces (7–10 mm) of refractory bricks as immobilization support because of their high porosity and inertness. SEMs of an uninoculated brick piece showed the porous surface of the support material, which can provide a large surface area for bacterial growth and attachment. Immobilization with alginate beads (entrapment mechanism) is also preferred over other materials and offers the advantages of biodegradability, hydrophilicity, presence of carboxylic groups, natural origin, low density, mechanical stability and stability over an experimental pH range of 3.0–8.0 (Arica *et al*. 2001). Also, in the present study, the regularity in surface structure indicated the uniform distribution of the fungal mycelia inside the beads. Sodium alginate beads collected after 4-5 h of the treatment process was of dark blue color due to initial biosorption. Beads were also collected at 24 h and 72 h (depending upon the HRT of the treatment, in turn depending upon the dye loading rate) showed almost white color. This may also show that the Drimarene blue K_2RL decolorization that occurred during the first 5 h was due to biosorption of dye inside the cells and that the cells started biodegradation of the dyes after 4-5 h, which carried out further decolorization of the whole dye in the STE. Thus, the results clearly showed that a high percentage of the decolorization of this dye is mainly due to microbial biotransformation and not only due to biosorption.

In both FBR1 and FBR2 system, the immobilized fungal mycelia played an important role in removing COD, BOD and dye, obviously improving biodegradability of dye for further treatment. The rates of decolorization are higher and require less retention time as compared to the previous reports (Parshetti *et al*. 2007).

The dye, BOD and COD removal capability in both the reactor systems proved *Aspergillus niger* as the main biodecolorizing and biodegradation agent. For biological treatment of simulated textile effluent with Drimarene blue dye, the fluidized bed bioreactor with sand (FBR1) as immobilizing support was found to have better overall performance, easy maintenance and cost effectiveness than the fluidized bed bioreactor with sodium alginate beads (FBR2). The data obtained from the study will be used for up-scaling the process to an insitu bioremediation of textile wastewater.

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