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ORIGINAL ARTICLE

Development of a fungal consortium for the biosorption of cadmium from paddy rice field water in a bioreactor

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Abstract The largest contributor to cadmium (Cd) pollution of agricultural fields is the use of high-phosphate fertilizers. Conventional techniques for the removal of metal ions from wastewater has several limitations and, therefore, a novel technique is required. Biosorption is the removal of metals and related elements or compounds from a solution by biological materials. Numerous types of biomass have been researched for their uptake capacity, with fungal biomass appearing to be the most promising candidate. In this study, the potential of three fungi, i.e. *Gliocladium viride* AI003, *Mucor* sp. HI33 and *Aspergillus niger* AH09, as a compatible/incompatible consortium for the biosorption of cadmium from paddy water was evaluated. Seven different combinations were investigated as possible consortia. Maximum biosorption was found for the consortium of 48-h-old *Mucor* sp. HI33+72-h-old *Gliocladium viride* AI003+72-h-old *Aspergillus niger* AH09. This consortium showed the maximum percentage removal of Cd (99.98%) after 8 days of incubation and significantly reduced the biological oxygen demand (85.76%).

Keywords Biosorption · Cadmium · *Gliocladium viride* · *Mucor* sp. · *Aspergillus niger*

Introduction

Industrial, agricultural and domestic activities of humans have affected the chemical composition of natural waters, resulting in the generation of wastewater containing high levels of pollutants (Gupta et al. 2009). Anthropogenic activities often result in the release of metallic pollutants into bodies of water where the metals tend to persist indefinitely, either being transported directly to the oceans, deposited and stored in floodplains, or circulated until eventually accumulating through the food chain, thus posing a severe threat to the environment (Volesky and Holan 1995). Of particular concern are the metals released from agricultural practices. The effluent from agricultural fields contains a range of different pollutants that eventually end up in rivers and seas. Direct use of untreated water by aquatic flora and fauna has toxic effects due to contamination by heavy metals (Gutnick and Bach 2000). Exposure to cadmium (Cd) can cause a wide range of diseases, including kidney damage, cancer and bone diseases (Iqbal and Edyvean 2005). The consumption of Cd accumulated in rice causes proximal tubule damage, anaemia and a severe loss of bone minerals, resulting in fractures (Reilly 2002). Additionally, cases of *itai–itai* disease have been recognized amongst people living in Cd-contaminated areas (Waalkes 2000).

During the past two decades, a considerable amount of research has been directed towards investigating the use of microbial cell as a tool for bioremediation (Davis et al. 2003). Fungi possess a large capacity for removing a wide range and large amounts of metal ions from aqueous solutions and in many cases outperform activated carbon and ion exchange resins. Interest is currently focusing on the use of two or more different microorganisms (a "consortium") to effect biotransformations in natural environments. Mixed culture fermentations are widely used in biotechnological applications for the production of antibiotics, enzymes, fermented food and the bioconversion of domestic wastewater sludge. Strain compatibility is a key

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factor in mixed culturing and has to be developed for each application. Encouraging results have been reported by many researchers for the treatment of sludge by mixed culture (Zahangir et al. 2003; Molla et al. 2001; Friedrich et al. 1987). Therefore, the aim of the study reported here was to develop a novel and cost-effective bioremediation technique for the removal of Cd from the drainage waters of paddy rice fields by optimizing the compatible fungal consortium for biosorption of Cd.

Materials and methods

Organisms

Three locally isolated mesophilic fungal strains, i.e. *Gliocladium viride* AI003, the thermophilic strain *Mucor* sp. HI33 and *Aspergillus niger* AH09, were used in this study. The strains were isolated from the local habitat and selected after a screening test of cadmium ($CdCl_2$) tolerance to evaluate their potential as biosorbents. All cultures were maintained on 5.0% (w/v) potato dextrose agar (PDA; Oxoid, Thermo Fischer Scientific, Waltham, MA) slants.

Paddy water

Paddy water was collected from the paddy rice fields of Jislani Khurd Village (District Nankana, Pakistan) and characterized physically and chemically (Table 1). Glucose (2.0%) was added to the paddy water as a co-substrate in all experiments.

Inoculum preparation

The cultures grown on PDA agar slants were transferred into 500-mL Erlenmeyer conical flasks containing 150 mL of sterile medium supplemented with 20 g/L glucose and 10 g/L yeast extract. The amount of inoculum was kept at 4.0%. The flasks inoculated with *G. viride* AI003 and *A. niger* AH09 were incubated at 30°C and those with *Mucor* sp. HI33 at 45°C, in a rotary shaking incubator [model 3033; Gesellschaft für Labortechnik (GFL), Burgwedel, Germany]. The suspended fungal mycelia were harvested after 48 and 72 h by filtration through filter paper (Whatman, Maidstone, Kent, UK) and the biomass was used as inoculum.

Table 1 Physical and chemical analysis of paddy rice water

Characteristics	Value
Cadmium (mg/L)	80.21±0.01
pH	6.51±0.02
Electrical conductivity $\times 10^5$ at 25°C	557±1.00
Total cations (meq/L)	7.60±0.05
Total anions(meq/L)	8.30±0.02
Na_2CO_3 (meq/L)	2.99±0.01
Total dissolved solid particles	5.82±0.09
Sodium adsorption ratio (ppm)	541±1.00

Each value is the mean of three replicates; the standard error (SE) of the mean is also indicated

Designing of consortia

The following different consortia of *G. viride, Mucor* sp. and *A. niger* (subsequently denoted as G, M and A, respectively) were tested as a biosorbent in a fermentor in the order presented below.

1. (G+A): *G. viride* and *A. niger* (0.25 g/L each of 72-h-old cultures);
2. (48 M+A): *Mucor* sp. (0.25 g/L of 48-h-old culture) and 0.25 g/L of 72-h-old culture of *A. niger*;
3. (48A+G): *A. niger* (0.25 g/L of 48-h-old culture) and *G. viride* (0.25 g/L of 72-h-old culture);
4. (48 M+G): *Mucor* sp. (0.25 g/L of 48-h-old culture) and*G. viride* (0.25 g/L of 72-h-old culture);
5. (48 G+A): *G. viride* (0.25 g/L of 48-h-old culture) and 0.25 g/L of 72-h-old culture of *A. niger*;
6. (48 M+G+A): *Mucor* sp. (0.17 g/L of 48-h-old culture) and 0.17 g/L each of 72-h-old culture of *G. viride* and *A. niger*;
7. (48 G+M+A): *G. viride* (0.17 g/L of 48-h-old culture) and 0.17 g/L each of 72-h-old culture of *Mucor* sp. and *A. niger*.

Biosorption studies and procedures

Biosorption studies were carried out in a 1.5-L glass fermentor (model MBF-500ME; Tokyo Rikakikai Co. (Eyela), Tokyo, Japan) with a 600 mL working volume, i.e. 600 mL of paddy rice water with a known concentration of Cd supplemented with 2.0% glucose as co-substrate was transferred to the fermentor and inoculated with a 4.0% biomass of the different consortia . The fermentor was allowed to run at 30°C while the aeration and agitation rates were maintained at 1.4 L/L/min (v/v/m) and 200 rpm respectively throughout the 8-day experimental period. Samples were withdrawn at 2-day intervals and the performance of the compatible/incompatible mixed fungal culture examined by inoculating the sample onto a PDA plate and incubating the plate at 30°C for 8 days. The growth pattern of the mixed fungal strains was observed visually on the PDA plate, and the fungal mycelium and fruiting bodies were studied microscopically on slide cultures (model E-100; Nikon, Tokyo, Japan).

Analysis of Cd and biological oxygen demand

Biomass was removed from the effluent after centrifugation at 10,000 *g* for 5 min and the supernatant used for analytical purposes. Biological oxygen demand (BOD) was measured by standard methods described in the American Public Health Association (APHA) manual (APHA, AWWA, WPCF 1995), and residual Cd was detected with a atomic absorption spectrophotometer (M Series 08260033; Thermo Electron Corp, Altrincham, UK).

Results and discussion

Among the seven combinations of fungal strains tested, our results indicate that the 48 M+G+A, 48 G+M+A, G+A and 48 G+A combinations were compatible, but that the degree of compatibility varied among the mixed experimental designs in terms of Cd adsorption efficiency, biomass production and BOD reduction. The other three combinations, namely, 48 M+G, 48A+G and 48 M+A, were observed to be incompatible. Microscopic examination of the slide cultures revealed that in incompatible consortia, one of the cultures was completely replaced by another culture. Similar results were also recorded on PDA plates inoculated by mixed inoculums from the 8-day fermentation vessel. The maximum biosorption of Cd, i.e. 99.98% removal of Cd ions, was obtained with fungal consortium of 48 M+G+A (Fig. 1). Removal of Cd by the other compatible combinations of fungal strains, namely, 48 G +M+A, G+A and 48 G+A, was 99.11, 97.31 and 96.03%, respectively. The removal of Cd by the incompatible consortia of 48 M+G, 48A+G and 48 M+A was 75.53, 64.68 and 81.88%, respectively. Almost linear sorption rates were observed over the 8-day period of fermentation. Strain compatibility was found to be the determining factor for successful mixed culture processes and that the combination of incompatible fungi greatly affected Cd biosorption. Similar results have been reported by Molla et al. (2001). Zahangir et al. (2003) reported that among six combinations of four fungi, only the compatible mixed culture of *Phanerochaete chrysosporium* and *Aspergillus niger* (P/A) showed the maximum potential for the treatment of sludge.

The reduction in BOD and biomass production is shown in Fig. 2. BOD removal was significantly influenced by the fungal combinations (consortium). The results indicate that a maximum reduction of BOD (85.76%) occurred in the compatible mixed culture of 48 M+G+A after 8 days of fermentation, followed by the consortium of 48 G+M+A (75.63%), G+A (66.13%) and 48 G+A (62.1%). BOD reduction was directly related to the compatibility, loss of pollutant and biomass formed during the experiment. The maximum biomass (18 mg/L) was produced by the fungal consortium of 48 M+G+A, followed by 48 G+M+A, G+A and 48 G+A in decreasing order. Our results are in complete agreement with those of other researchers. Castillo et al. (1994) and Gutierrez-Correa et al. (1999) reported higher biomass production by mixed culture in solid state fermentation, and Friedrich et al. (1987) found that the mixed culture of *Aspergillus awamori* and *Trichoderma reesei* reduced the BOD by 70% in the treatment of apple distillery waste.

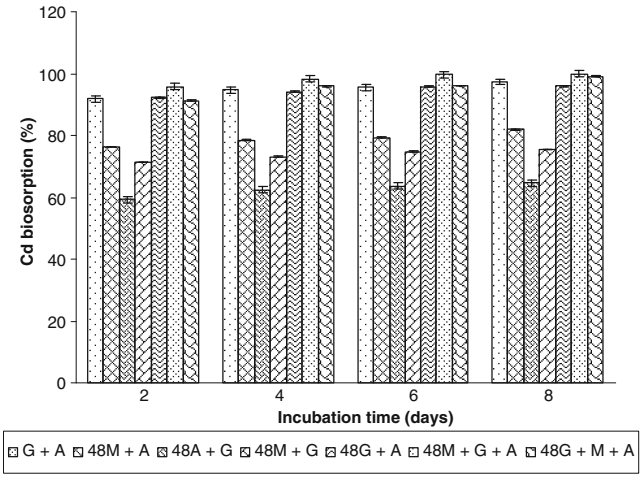

Fig. 1 Biosorption of cadmium (*Cd*) with different fungal consortia. Data are presented as the mean; *error bars* show the standard deviation (SD) among three replicates. Biosorption conditions: pH 6.5, temperature 30°C, inoculum size 4.0%, agitation speed 122 rpm, Cd concentration 80.21 mg/L. For the definition of culture consortia, see list in text (Designing of consortia)

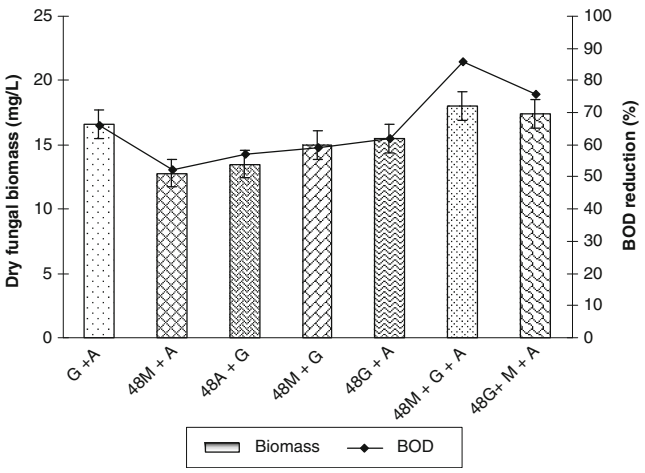

Fig. 2 Plot of fungal biomass formed (dry weight, mg/L) and reduction in biological oxygen demand (*BOD*; %) after 8 days of culture with different fungal consortia. Data are presented as the mean; *error bars* show the SD among three replicates. Biosorption conditions are as given in the caption to Fig. 1

Conclusion

The results of our study indicate that *Gliocladium viride* AI003, *Mucor* sp. HI33 and *Aspergillus niger* AH09 show potential compatibility properties for the biosorption of Cd from paddy rice field water. More studies are needed to optimize all of the environmental conditions for maximum biosorption and to examine regeneration of the biosorbent and immobilization of biomass.

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