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Short communication

Metal and dye removal using fungal consortium from mixed waste stream: Optimization and validation

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ABSTRACT

Response surface methodology (RSM) involving two-level-three factors (2^3) full factorial design of experiment (DOE) was employed to optimize the concentrations of three media components (yeast extract, urea and ammonium nitrate) for a fungal consortium comprising *Aspergillus lentulus*, *Aspergillus terreus* and *Rhizopus oryzae*. The interaction between three variables was studied and modelled for four responses: chromium, copper, dyes (mixture of Acid Blue 161 and Pigment Orange 34) removal and biomass production. The results showed that yeast extract had a significant effect on Cu(II) removal (87.6 mg L^{-1}) and biomass production while dye removal was significantly affected by the combination of nutrients. It increased from 80.28% to 97.26% as the amount of urea and ammonium nitrate was increased from 0.5 g L^{-1} to 0.75 g L^{-1}. A 50% reduction in the nutrient cost incurred for multiple pollutant removal was achieved by RSM based optimization. The results were validated by treating different industrial effluents supplemented with key media components. The utility of fungal consortium in simultaneous removal of dyes and metals from complex synthetic solution as well as industrial effluents has been demonstrated.

1. Introduction

In the recent years, extensive research has occurred on biological remediation of heavy metals and dyes (Gupta et al., 2010; Mittal et al., 2010, 2013). However, metal bioaccumulation has been largely studied under single metal or dye exposures (Kaushik and Malik, 2009; Bhatia et al., 2011; Gupta et al., 2011; Singh et al., 2012; Asgher and Bhatti, 2012; Akar et al., 2013; Mishra and Malik, 2013). Such studies do not exactly reflect the real situation in wastewaters containing metal ions and dyes together. Some investigations with metal-dye mixtures indicate that double/multiple stress may influence the bioaccumulation performance. Cu(II) uptake and reactive dye decolorization ability of growing *Candida tropicalis* was tested as a function of initial Cu(II) and dye ion concentrations, both singly and in mixture by Gonen and Aksu (2009). Poorer performance under the dual stress as compared to single exposure in metal–dye mixtures was noticed. A suitable combination of efficient strains can handle both metal and dye simultaneously without compromising the removal efficiency (Jadhav et al., 2010) as mixed cultures are better able to withstand pollutant stress due to close interactions and protections offered by the partners. Therefore, it is desirable to investigate the potential of microbial consortiums in handling mixed metal–dye waste streams. However, the nutrient demands, substrate uptake rates and pollutant affinities of the consortium members may be different from each other.

Process optimization for biological removal of hazardous contaminants has emerged as an important initiative towards reducing the process cost (Gonen and Aksu, 2009). Previous studies from our group on the nutrient optimization for Cr(VI) removal (Sharma et al., 2009) and Acid Navy Blue (ANB) removal (Kaushik and Malik, 2011) using RSM, presented interesting observations. It was revealed that the nutrient requirement is pollutant specific with yeast extract being crucial for Cr(VI) removal while urea being important for ANB removal. Hence, process optimization for multiple pollutant removal through a consortium shall be a challenging task. This would mimic the actual process performance under field conditions. However, no such study is reported in the literature. Hence, the present study attempts to optimize the process conditions (media components) for simultaneous dye and metal removal employing a fungal consortium developed earlier (Mishra, 2013). So far, this is the first reports utilizing fungal consortium for the simultaneous removal of metal and dye from synthetic medium.

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2. Experimental

Two metals (copper and chromium) as Potassium dichromate [$K_2Cr_2O_7$] and copper sulphate [$CuSO_4 \cdot 5H_2O$] salts and two industrial quality metal complex azo dyes (Acid Blue 161 and Pigment Orange 34) were used (Sup. Table 1). The stock solutions of 10 g L^{-1} were prepared for each metal and dye in double distilled water.

2.1. Microorganism and growth conditions

A previously developed (Mishra, 2013) fungal consortium was employed comprising three fungal strains viz. *Aspergillus lentulus* (FJ172995.1), *Aspergillus terreus* (KC354516.1) and *Rhizopus oryzae* (KC354517.1). *A. lentulus* was best for Cu(II) removal (76.6% in 120 h) and AB removal (96.7% in 42 h), *A. terreus* was best for Cr(VI) removal (100.0% in 96 h) whereas, *R. oryzae* was best for PO removal (98.4% in 24 h). The fungal isolates were maintained on Potato dextrose agar slants at 4 °C, individually, and freshly revived culture was used for each experiment.

2.2. Optimization study

RSM involving three variables using two-level-three factors (2^3) full factorial design of experiment (DOE) was employed to optimize conditions for maximum metal and dye removal using fungal consortium. The interaction between three variables; Yeast extract, urea and ammonium nitrate was studied and modelled for four responses: chromium, copper, dyes (mixture of Acid Blue 161 and Pigment Orange 34) removal and biomass production. Variables and their range (Sup. Table 2) were determined on the basis of preliminary experiments (Mishra and Malik, 2012; Mishra, 2013). The following culturing medium was used for optimization study: glucose 10.0 g L^{-1}, $MgSO_4.7H_2O$ 0.1 g L^{-1}, K_2HPO_4 0.5 g L^{-1}, NaCl 1.0 g L^{-1}, pH 6.5 ± 0.2. Yeast extract was varied from 1.0 g L^{-1} to 3.0 L^{-1} whereas, urea and ammonium nitrate were varied from 0.5 g L^{-1} to 1.0 g L^{-1}. To evaluate the pure error, 29 experiments were carried with three centre point in randomized order. The media was autoclaved for 20 min at 121 °C and inoculated with 1.0% (v/v) spore suspension of all the three fungi (*A. lentulus*, *A. terreus* and *R. oryzae*) and incubated in an orbital shaker at 30 °C and 150 rpm for 120 h. Initial concentrations of metals and dyes mixture were taken as 100 mg L^{-1} dye mixture (50 mg L^{-1} each of Acid Blue 161 and Pigment Orange 34) and 100 mg L^{-1} metal mixture (50 mg L^{-1} each of Chromium (VI) and Copper). At the end of the experiment, four responses were measured. Residual Cr(VI) concentration in the medium was determined spectrophotometrically (PerkinElmer *Lambda 35*) at 540 nm using diphenyl carbazide (DPC) method. Total chromium and copper were quantified by atomic absorption spectrophotometer (PerkinElmer *AAnalyst200*) using standard protocols (APHA, 1989). Dye concentrations were measured spectrophotometrically at the absorbance maxima of each dye while dry cell weight of the generated biomass was measured gravimetrically (Kaushik and Malik, 2011). The statistical software package JMP® (SAS Institute) was used for regression analysis of experimental data and for plotting response surface. Validation was done by conducting experiment using optimized media and versatility was checked by varying relative concentration of metals (25–75 mg L^{-1}) and increasing the same to 150 mg L^{-1}.

2.3. Metals and dye removal from industrial effluents by consortium

Effluents collected from handmade paper unit and textile units, Sanganer (Rajasthan) and Electroplating cluster, Okhla (Delhi) were characterized for pH, TDS, TSS, COD and metals concentration (APHA, 1989). The effluents (unsterilized and sterilized) were supplemented with optimized nutrient media and inoculated with fungal spores (1.0%, v/v). Samples were withdrawn after 48 h for dye removal analysis and after 120 h for metal removal analysis.

3. Results and discussion

3.1. Process optimization for metals and dyes removal from mixed waste stream

3.1.1. Effect of nutrient sources on biomass production, metals and dyes removal

Preliminary studies revealed that yeast extract (YE) cannot be totally replaced from growth media during the metal bioremediation (Sharma et al., 2009; Mishra and Malik, 2012; Sharma et al., 2011). However, among the low cost alternates, urea (UR) and ammonium nitrate (AN) were found to be suitable sources for partial replacement of yeast extract (Kaushik and Malik, 2011; Mishra, 2013). Therefore, a combination of YE, UR and AN was optimized through full factorial RSM and influence on biomass production and pollutant removal efficiency was evaluated (Table 1). The responses were fitted with quadratic model equation (1)–(3) and expressed in terms of coded factors (Sup. Table 3–5). The DOE statistical data analysis was conducted for all the three responses [Biomass, Dye removal and Cu(II) removal] except Cr(VI) removal as 100% Cr(VI) removal was obtained in all sets of experiment. The ANOVA results for all the responses (Sup. Table 3–5) indicates that the quadratic model can be effectively uses to navigate the design space. High coefficient of determination values (R^2 = 0.9626–0.9885) showed that the equation was highly reliable. The above results show that the experimental values were significantly in agreement with the calculated values and also suggested that the models (Eqs. (1)–(3)) were satisfactory and accurate (Sup. Table 3).

Leverage plots show the effect of variables and their interaction on three responses. Effect of all the possible combinations of UR, AN and YE were significant for biomass production (p-value < 0.05) by fungal consortium in dye and metal mixture (Sup. Table 3). Based on the previous results (Mishra, 2013), maximum 95% dye removal, 100% Cr(VI) removal and 87% Cu(II) removal was envisaged. Since higher biomass production beyond desirable contaminant removal results in higher sludge production, no specific value was set for this parameter. The prediction profiler is a tool that can be used to select the optimal input concentration that would result in the best tradeoff between the three responses. As evident from this plot (Sup. Fig. 1), beyond 2.0 g L^{-1} YE, although Cu(II) removal increased considerably, dye removal remained almost same. Hence, for optimal metal and dye removal, 2.0 g L^{-1} YE should be used.

To study the interaction between all three variables, three dimensional response curves were plotted (Fig. 1). The results depicted in contour plots and surface plots were in accordance to that obtained with prediction profiler. Biomass production was significantly affected by individual variables as well as all the possible combinations of the variables. The process of dye removal was also significantly affected by the nutrients. Leverage plot for dye removal (Sup. Table 4) clearly depicted the similar effect of UR and YE followed by AN on dye removal from metal and dye mixture. It increased from 80.28% to 97.26% as the amount of UR and AN was increased from 0.5 g L^{-1} to 0.75 g L^{-1} and YE was increased from 1.0 g L^{-1} to 2.0 g L^{-1}. It is evident from the rising ridge of the response surface curve along the axis for UR and YE. In case of Cu(II) removal, YE plays significant role over other individual variable. Although UR and AN alone could not affect Cu(II) removal considerably, yet in combination with YE, significant amount of Cu(II) removal was achieved. When the concentration of YE was kept at 1.0 g L^{-1} and minimal amount of nitrogen supplements, UR