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Full Length Article

Optimization of Factors for Enhanced Phycoremediation of Reactive Blue Azo Dye

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Abstract

Synthetic dyes are widely used in textile, leather and other dye-stuff industries. A large fraction of the dyes applied during the dyeing processes are released into wastewater. Therefore, the wastewater from dye-related industries is densely colored, with high chemical oxidation and biological oxidation demand. This wastewater must be treated prior to discharge into wastewater streams to prevent pollution of surface and groundwater, and minimizing risks to public health. The present study was undertaken for the first time in Pakistan to optimize the factors for algae possessing high potential to degrade Reactive Blue azo dye in liquid medium. Among eighty eight (88) isolates of alga, two showed high decolorization, which were used to optimize the decolorization of Reactive Blue azo dye. Both the selected algal strains showed an excellent potential to decolorize 100 mg L^{-1} Reactive Blue in seven days in the liquid broth.The optimum conditions determined for the two selected algal strains for decolorization of Reactive Blue (100 mg L^{-1}) were pH 7.0, temperature 30°C, light conditions of 16 h, when inoculum size of 1 mL/10 mL was maintained and dye solution was supplemented with 5 g L^{-1} N and 5 g L^{-1} P as co-substrates.

Key words: Phycoremediation; Algae; Wastewater; Azo dye

Introduction

Wastewater flowing out from textile industries not only adversely affects plant growth (Yousaf *et al.*, 2010) but also disturbs the aquatic ecosystem and other beneficial microorganisms (Kaur *et al.*, 2010). The effluent discharged by their textile units contains a large number of dyes which are discharged into environment without treatment. Dyes containing an azo group (-N=N-) are very commonly used colorants worldwide. Over one million tons of dyes are produced annually, of which more than 50% are azo dyes (Stolz, 2001; Pandey *et al.*, 2007; Bibi *et al.*, 2012). When these azodyes are applied to the fabric, large quantity of these dyes remain unfixed and is released directly into the wastewater during textile processing depending upon the relative low efficiencies in dyeing and finishing. About 50% of reactive dyes, 20% disperse dye and 2% of basic dyes being unfixed on fabric are released in wastewater (Tan *et al.*, 2000; Boer *et al.*, 2004). Discharge of this textile effluent into the surrounding environment has a number of implications. For instance, presence of azo dyes as contaminant in surface water leads to aesthetic problems, obstruct light penetration and oxygen transfer into water bodies, thus affecting photosynthetic activities of aquatic plants (Umbuzeiro *et al.*, 2005; Ozturkand Abdullah, 2006).

Recently, algae have been widely used in wastewater treatment as they often colonize the ponds naturally and have fast growth rates and high nutrient removal capabilities. Moreover, algae offer a low-cost and effective approach to remove contaminants in tertiary wastewater treatment, while producing potentially valuable biomass (Muñoz and Guieyssea, 2006). Textile effluents have broader pH and temperature, which is strongly dependent on the quantity of salts, dyes and other substances present therein. Though, the algae are very sensitive to the varying temperature and pH, some show the capability to perform their functions over wider ranges (Khan *et al.*, 2009; Prasad *et al.*, 2011). Consequently, there is a dire need to regulate optimum pH, temperature and other conditions for maximum efficacy of phycoremediation of azo dyes, finding out suitable P and N levels as well as optimization of cultural conditions such as inoculum size and light conditions to hasten the phycoremediation process.

Keeping in view the above discussion a series of optimization were planned with the objectives to optimize the environmental conditions for maximum phycoremediation of azo dye and find out additional use of N and P for the higher efficiency of phycoremediation.

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Materials and Methods

From water samples collected from different sources, 88 different algal strains were isolated through enrichment technique. Based on their efficiency to remove color of the dye from agar medium, 20 algal isolates were selected. These algal isolates were further screened under the liquid broth medium containing 100 mg L^{-1} of Reactive Blue azo dye, from which two highly efficient algal isolates (CKW1 and PKS33) were selected to optimize the decolorization of Reactive Blue azo dye (Waqas, 2014). The isolates were identified as fresh water green algae belonging to genus *Spirogyra sp.* (CKW1) and *Cladophora sp.* (PKS33) under light microscopy as explained by Huynh and Serediak. (2006); Bellinger and Sigee (2010), where CKW1 exhibited the un-branched slippery/silky filamentous characteristics and PKS33 was with green branched filamentous distinctiveness (Waqas, 2014). The specific characteristics found for algal strains under light microscopy were assessed and compared with Wehr and Sheath (2002) to find the respective algal genera. CKW1 fit in the characters of genus *Spirogyra* while PKS33 found to be from *Cladophora.* The obtained results were also confirmed on online algal database called "Micrographia" available at http://www.micrographia.com/index.html.

Preparation of Inocula

Liquid broth was prepared by following the Modified MA medium (Ichimura, 1979). The pH of the broth was adjusted to 7 by using the 0.1 N HCl or 0.1 N NaOH. A 100 mL of broth in 250 mL conical flask were inoculated with the respective isolates. Inoculated flasks were incubated under illuminating conditions for a period of 10 days at 30°C. After incubation, optical density of 0.6±0.01 was achieved at 600 nm wavelength, using colorimeter to maintain a uniform cell density.

Optimization of Environmental Factors

Various factors were optimized to achieve the highest decolorization rate of Reactive Blue azo dye by the selected isolates of algae. All the experiments were conducted in triplicate.

Substrate (azo dye) concentration: Five levels (50, 100, 200, 400 and 600 mg L^{-1}) of Reactive Blue azo dye were used to determine the best concentration for maximum decolorization. The inoculum was added to the medium (10 mL) at inoculum: broth ratio of 1:50 and incubated at a temperature of 30°C for 7 days. Decolorization was determined by taking 1.5 mL aliquots from different test tubes and centrifuging the solutions at 10,000 rpm for 10 min to remove the cells. The absorbance of the supernatants was measured at the λmax 597 nm (for Reactive Blue) by using Spectrophotometer Model T-60 PG Instruments (Split-beam Spectrophotometer). Uninoculated blank were run to check the abiotic decolorization. Each dye level was replicated thrice.

pH: Effect of different pH ranging from 5 to 9 on the decolorization efficiency of the selected algal isolates was examined. Modified MA medium enriched with Reactive Blue @ 100 mg L^{-1} was used. The above mentioned procedure in substrate concentrationsection was repeated except changing the pH of the growth medium.

Temperature: Decolorization of Reactive Blue by the selected algal isolates was studied at different temperatures including 20, 25, 30, 35 and 40°C by using the procedure given in substrate concentration section. Modified MA medium was enriched with Reactive Blue @ 100 mg L^{-1} while pH of the medium was maintained at 7.

Inoculum size: The next optimizing condition was inoculum size. The inocula were applied at the rate of 400, 600, 800, 1000 and 2000 µL. The modified MA medium spiked with Reactive Blue @ 100 mg L^{-1} was used. pH of the medium was maintained at 7 and incubated at 30°C for 7 days.

Light conditions: Algal decolorization was then optimized with respect to illumines light conditions. A light condition of 12, 14, 16, 18 and 20 h per day was maintained. The modified MA medium spiked with Reactive Blue @ 100 mg L^{-1} was used. The pH of the medium was maintained at 7 and incubated at 30°C for 7 days.

Effect ofnitrogen (N) and phosphorus (P): In order to assess the effect of additional N and P levels on algal decolorization of azo dye, ammonium nitrate (for N) and tri-calcium phosphate (for P) were used as sources @ 1, 2, 3, 4, 5 and 6 g L^{-1}. The MA medium spiked with Reactive Blue @ 100 mg L^{-1} was used. pH of the medium was maintained at 7 and incubated at 30°C for 7 days.

Statistical Analysis

Data were analyzed performing ANOVA and significant differences in treatment means were compared according to least significance difference test (LSD) at $P \leq 0.05$ (Steel *et al.*, 1997).

Results

Two algal strains, *Spirogyra sp.* (CKW1) and *Cladophora sp.* (PKS33) were investigated for the optimization of various incubation/environmental conditions in order to achieve the maximum decolorization rate of Reactive Blue azo dye.

Substrate (Azo Dye) Concentration

The substrate (azo dye) concentration significantly affected the decolorization potential of algae (Fig. 1). Decolorization potential of both strains to decolorize Reactive Blue azo dye was almost similar at substrate concentration of 50 mg L^{-1} and 100 mg L^{-1}. Thereafter, the amount of decolorization decreased with an increase in substrate concentration.

Strain CKW1 showed the highest decolorization at 50 and 100 mg L^{-1} dye concentration was observed after 7 days. The amount of decolorization by strain PKS33 was comparable with that of CKW1 at all tested concentration of dye. However, when dye concentration was more than 100 mg L^{-1}, strain PKS33 showed slightly better decolorization of the dye than strain CKW1. Based on these results, 100 mg L^{-1} dye concentration was used for subsequent studies.

pH: Selected strains of algae were able to decolorize the dye over a wide range of pH (Fig. 2). Maximum decolorization by the both tested strains was recorded at pH between 7 and 8. In case of PKS33 (strain isolated from salt range), a rapid increase in decolorization was observed as the pH increased from 6 to 7. However, a relative decrease in decolorization was found when pH was increased from 8 to 9. Overall, dye decolorization was 64% at pH 5, 67% at pH 6, 83% at pH 7, 80% at pH 8 and 77% at pH 9. Decolorization of the dye by CKW1 (strain isolated from textile wastewater) was 74% at pH 5, 83% at pH 6, 86% at pH 7, 88% at pH 8 and 78% at pH 9. In general, pH 7-8 favored both tested strains to decolorize the dye and pH 7 was found to be optimal and used for subsequent studies.

Temperature: An increase in the temperature from 25 to 30°C had a positive impact on the decolorization of Reactive Blue (Fig. 3). Decolorization of the dye Reactive Blue was found optimal at temperature 30°C as CKW1 and PKS33 were able to decolorize the dye by 85 and 80%, respectively. However, decolorization rate in both strains dropped gradually as the temperature increased to 35 or 40°C. Overall, a similar trend was observed in case of temperature regarding the decolorization potential of both selected algal strains.

Light conditions: There was an increase in the decolorization of azo dye by both strains with an increase in day light conditions (Fig. 4). Optimal day light period to decolorize Reactive Blue azo dye for both tested strains was 16 h. However, decolorization rate in both strains remained constant as the day light period increased from 16 h. In general, a similar trend was observed in case of day light period regarding the decolorization potential of both algal strains. However, strain CKW1 performed marginally better at all tested levels in terms of decolorization of azo dye. A day light conditions of 16 h was selected for subsequent studies.

Inoculum size: Fig. 5 illustrates an increase in the decolorization of azo dye by both the strains with the increase in inocula size from 400 to 1000 µL. Optimal inoculum size to decolorize Reactive Blue azo dye for both tested strains was 1 mL per10 mL substrate. Decolorization rate in both strains was almost same when inoculum size increased from 1 mL per10 mLsubstrate.

Effect of nitrogen (N): In order to assess the effects of additional nitrogen source on algal decolorization, ammonium nitrate was used as nitrogen source. A gradual increase in the additional N source increased decolorization of azo dye by both the strains (Fig. 6).

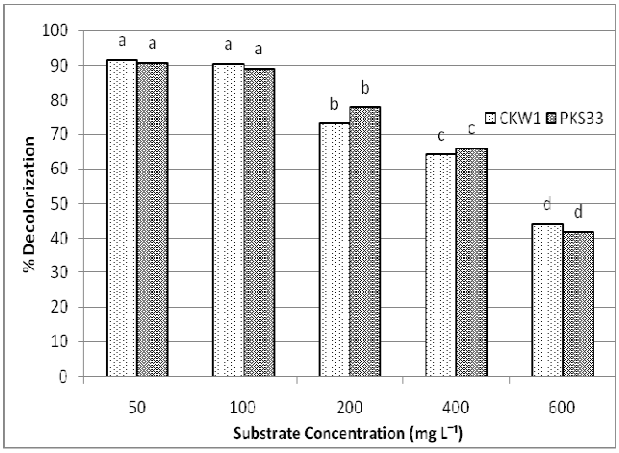

Fig. 1: Effect of substrate concentration on decolorization of Reactive Blue dye by the selected algal strains. Different letters at top of the bars indicate significant difference according to LSD (3.41) at $P \leq 0.05$

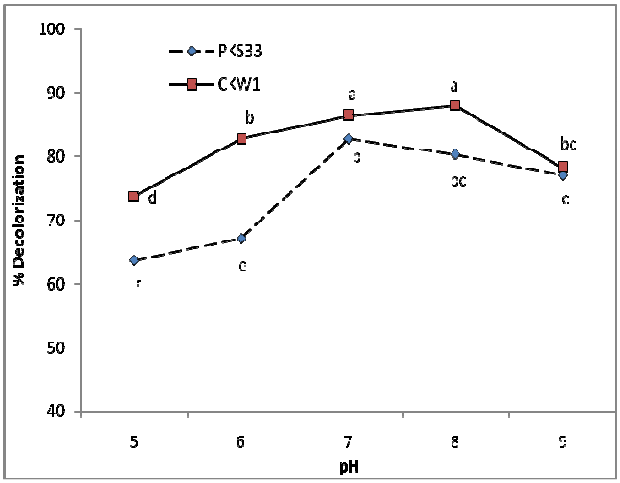

Fig. 2: Effect of pH on decolorization of Reactive Blue dye by the selected algal strains. Different letters indicate significant difference according to LSD (3.12) at $P \leq 0.05$

Maximum decolorization of 95 and 93% was observed with CKW1 and PKS33, respectively at N concentration 5 g L^{-1}. In general, both strain showed similar decolorization percentage when N concentration was increased from 5 to 6 g L^{-1}. The strain CKW1 performed marginally better than PKS33 at all tested levels.

Effect of phosphorus (P): The data summarized in Fig. 7 showed that addition of P in the form of tri-calcium phosphate, affected the decolorization of Reactive Blue azo dye by selected algal isolates. The results were similar to that obtained incase of N. A gradual increase in the decolorization of dye by both the strains with the increase in additional P source was found. Maximum decolorization of up to 90 and 88% was observed with CKW1 and PKS33, respectively at P concentration 5 g L^{-1}. Both strain showed a

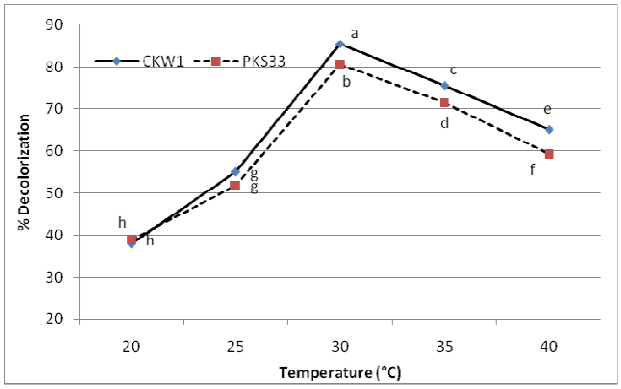

Fig. 3: Effect of temperature on decolorization of Reactive Blue by the selected algal strains. Different letters indicate significant difference according to LSD (3.97) at P ≤ 0.05

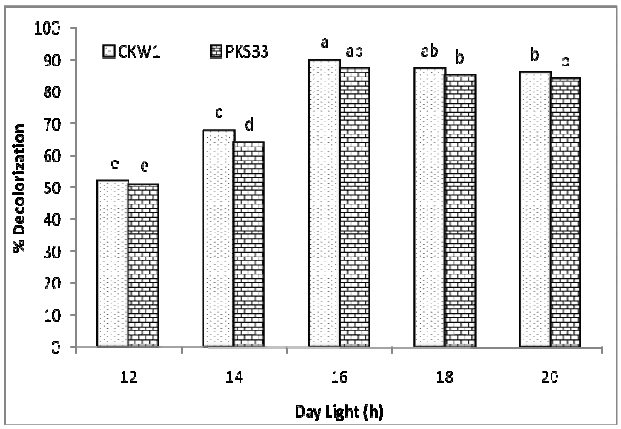

Fig. 4: Effect of day light conditions on decolorization of Reactive Blue dye by the selected algal strains. Different letters at top of the bars indicate significant difference according to LSD (3.231) at P ≤ 0.05

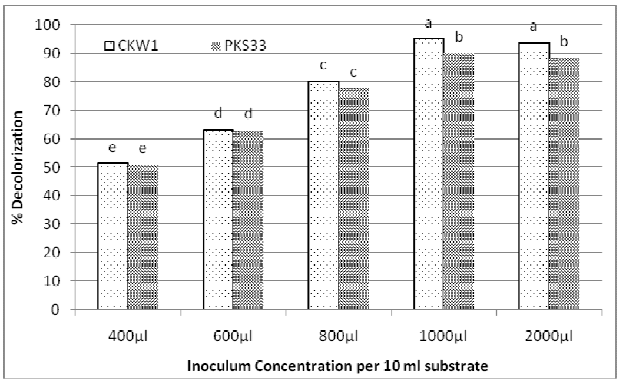

Fig. 5: Effect of inoculums size on decolorization of Reactive Blue dye by the selected algal strains. Different letters at top of the bars indicate significant difference according to LSD (3.42) at P ≤ 0.05

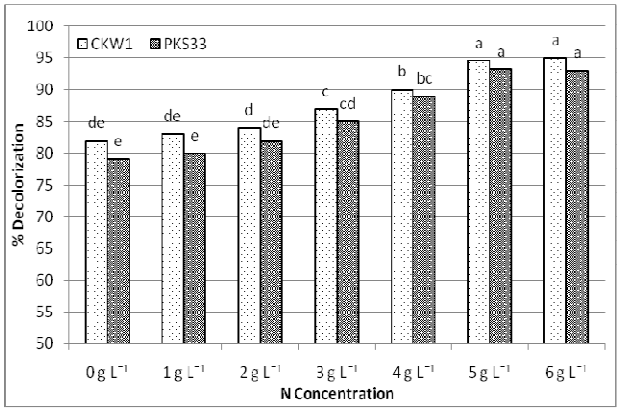

Fig. 6: Effect of additional N source on decolorization of Reactive Blue dye by the selected algal strains. Different letters at top of the bars indicate significant difference according to LSD (2.97) at P ≤ 0.05

slight decrease in decolorization of dye when N concentration was increased from 5 to 6g L^{-1}. Overall, the strain CKW1 performed marginally better than PKS33 at all the applied levels of additional P source.

Discussion

In this study, it was observed that the decolorization of Reactive Blue dye was concentration-dependent. Maximum decolorization of the dye by both algal strains was observed at 100 mg dye L^{-1} liquid medium. Decolorization rate of both strains reduced when the concentration of dyes increased above 100 mg L^{-1} liquid medium. High substrate (dye) concentrations are probably toxic to algae, inhibiting degradation of the dye. Previous investigations showed that the dye concentration can affect the rate of biodegradation of dyes and the optimum dye level could also vary from species to species. In general, high color removal efficiencies have been observed at medium dye concentrations (Rajaguru *et al*., 2000; Kapdan and Oztekin, 2003; Sponza and Isik, 2005; Khalid *et al.,* 2008a, b). Furthermore, some azo dyes contain one or more sulphonic-acid groups on aromatic rings, which can act as a deterrent to inhibit the growth of microorganisms (Chen *et al*., 2003). Another reason of the toxicity at higher concentration could be the presence of heavy metals (metal-complex dyes) and/or the presence of non-hydrolyzed reactive groups in case of reactive dyes (Sponza and Isik, 2005). The addition of nitrogen and phosphorus in the liquid medium supported the biodegradation reaction. It is very likely that both nutrients improve biomass production of algae (Kassim, 2002; Aslan and Kapdan, 2006), resulting in greater removal of dyes by algae.

An increase in pH from 5 to 7caused significant increase in the rate of dye decolorization by algae, however decolorization rate was highest at pH ranging from 7 to 8. The pH 7-8 was proved to be the best pH for both the selected isolates for maximum decolorization. Most likely

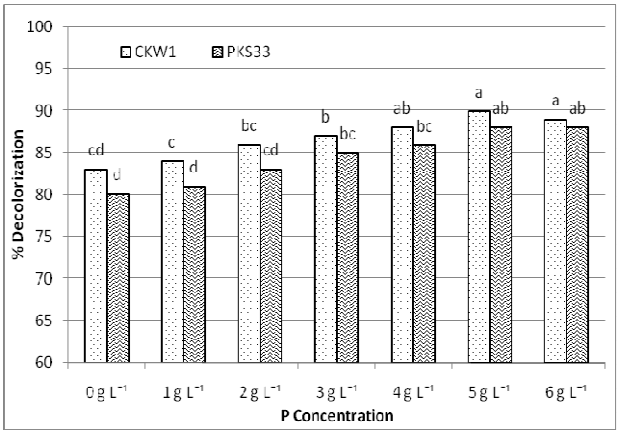

Fig. 7: Effect of additional P source on decolorization of Reactive Blue dye by the selected algal strains. Different letters at top of the bars indicate significant difference according to LSD (3.01) at $P \leq 0.05$

pH affects the enzymatic activity involved in decolorization of dye in addition to cellular growth of algae. Prasad *et al.* (2011) reported that biological treatment can effectively decolorize azo dyes over a wide range of pH (6–9). However, optimum pH for growth and decolorization was found to be 8 because maximum decolorization (90%) was recorded at this pH. Maximum dye decolorization by the selected algal strains was observed at 30°C and further increase in temperature beyond 30°C inhibited decolorization of Reactive Blue. High temperature probably caused thermal deactivation of algal enzyme(s) responsible for decolorization of azo dyes. Previously, Guo*et al*. (2008) reported that 28 to 35°C may be an optimal temperature for the decolorization of dyes.

Light is one of the basic requirements for algae to carry out the autotrophic activities. In addition to that algae also need a light/dark regime for efficient photosynthesis (Cheirsilp and Torpee, 2012), in our results that is why algae used 16 h light period per 24 h to be the most effective in decolorization of azo dye. These findings are well in line with the results of Al-Qasmi *et al*. (2012), which explains the light-dark regime for efficient microalgae growth. Related results have also been reported by Cheirsilp and Torpee (2012); Khoeyi *et al.* (2011). Similarly, the effect of inoculum size of phycoremediation is evident from results that higher the inoculum size more will be the decolorization of azo dye. This might be due to the fact that larger inoculum size helps algae in obtaining higher algal densities and biomass (Lopez-Elias *et al.*, 2008) which could bring in more decolorization efficiencies.

Conclusion

The selected algal strains were able to decolorize synthetic azo dye effectively in liquid medium at a dye concentration of 100 mg L^{-1}, pH 7, and 16 h of day light with 1000 µL inoculum size at 30°C. Phycoremediation can be studied further, to efficiently decolorize azo dyes present in real textile wastewater. This implies that the use of such algal isolates in biological treatment system could be helpful in reducing the threat posed by these pollutants in textile industry effluent.

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