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Bioremediation and Detoxification of Azo dye containing effluent by *Bacillus pumilus* SRS83

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Abstract

Present study illustrates the effectual decolourization and degradation of the textile industry effluent using B. pumilus sp. SRS83, originally isolated from dye contaminated soil. The dye decolourization and degradation activity of the culture was enhanced by optimization. Addition of optimized concentration of carbon, nitrogen and phosphorus to the effluent facilitated complete decolourization of textile industry effluent within 24 h, at pH 7 and at a temperatureof 32±2 °C. Addition of starch (10 g/L), sodium nirate *(4 g/L) and potassium phosphates (0.2 g/L) were found optimum for complete decolourization of BHM supplemented with 10% of the effluent by B. pumilus SRS83 in 24 h. The organism showed a 87.18% and 84.91% reduction in COD and ADMI values, respectively of the as it used effluent having an initial COD and ADMI value 5632 and 2674 respectively, after 48 h of treatment.*

Keywords: *Bacillus pumilus*; Acid Black 210, decolourization, bioremediation, effluent.

Introduction

Coloured industrial effluent is the chief indicator of water pollution and the discharge of highly coloured synthetic dyes is aesthetically unpleasing and additionally leads to substantial detrimental effects upon release into water bodies[1,2] .Treatments of wastewater, containing synthetic textile dyes, through physicochemical techniques have proved to be practically ineffective because of the chemical stability of these pollutants[3].

Biodegradation through microorganisms or enzymes, for the remediation of synthetic dye wastewater, is a promising approach because of its cost effectiveness, efficacy and eco-friendly nature in comparison to physicochemical treatment methods. Additionally, biological methods bring out complete mineralization of organic pollutants into non-toxic products[1, 4,5].

Reportedly, pure culture of *Bacillus* sp. has been in use for decolourization of azo dyes. On the contrary, no reports are available in the literature on the use of pure culture of *Bacillus pumilus* for treatment of actual textile industry effluent.

Material and Methods

Chemicals and media: Nutrient broth, nutrient agar, Bushnell-Hass broth were procured from Hi Media Laboratories, India and Glucose, starch, sodium nitrate ($NaNO_3$), ammonium nitrate (NH_4NO_3), ammonium sulphate $(NH_4)_2SO_4$, sodium dihydrogen orthophosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were procured from Loba Chemie Limited, India. Sucrose, lactose, urea, potassium nitrate, potassium dihydrogen orthophosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Merck specialities, India. All the chemicals used during the study were of analytical grade.

Dyes and effluent: Effluents of the dyeing industries were collected from the textile industries and local dyeing plants, utilizing azo dyes, located near Indore (M.P.) and Ahmedabad (Gujarat), India. The collected effluent samples were transported to the laboratory and filtered through filter paper (Whatman no-1) to remove large suspended particles and stored at 4°C temperature, until processing to minimize biotic and abiotic changes in the collected samples and avoid contamination by non-indigenous microorganisms.

Inoculum development using AB210 dye for decolourization study: From the preserved slants, a loop full of culture of *B. pumilus* SRS83, was inoculated in 100 mL nutrient broth and incubated on a shaker (150 rpm) at 32±2 °C temperature for 18 h. If otherwise mentioned, 10% (v/v) of the actively growing log phase culture, having 2 x 10^8 cells/mL, was used as inoculum or AB210 dye decolourization studies.

Dye decolourization experiments were carried out in 250 mL Erlenmeyer flask containing 100 mL nutrient broth supplemented with 100 mg/L AB210 dye and 10% inoculum having 2 x 10^8cells/mL. All the flasks were incubated at 32±2 °C, (pH 7.0) and under static condition. For liquid medium, at regular time intervals, an aliquot (3 ml) of the medium was withdrawn and centrifuged at 10,000 g for l0 min at 4°C min. The absorbance of culture supernatant was measured, at dye λ_{max}=605 nm, in order to determine decolourization. If otherwise mentioned, all the experiments were performed in triplicates. The percentage decolourization was calculated by using the following formulation:

$$\text{Percentage of decolourization} = \frac{A_t - A_0}{A_0} \text{ X } 100$$

Where, A_t is the absorbance of the dye before decolorization, at the maximum absorption wavelength and A_0 is the absorbance after decolourization, at the same wavelength. All the decolourization experiments were performed in triplicates and abiotic controls (without microorganisms) were always included[6].

Effects of C, N, P sources on effluent decolourization: The effect of different nutritional parameters such as carbohydrate sources such as; starch, glucose, lactose and sucrose), inorganic nitrogen sources such as; ammonium sulphate, ammonium chloride, sodium nitrate, ammonium nitrate and urea) and inorganic phosphorus sources (sodium phosphate and potassium phosphates) were evaluated so as to find the most suitable carbon, nitrogen and phosphorus source. Each carbohydrate and nitrogen source was individually added to 250 mL flask containing 100 mL BHM so as achieve a final concentration of 1 % w/v while the phosphates were added similarly at a concentration of 0.1% w/v. Thereafter, to optimize the concentration of best carbon, nitrogen and phosphorus source they were studied in the range of 0.2-1.0%, 0.2-1.0% and 0.01-0.1% w/v, respectively for the treatment of diluted effluents.

Unless otherwise stated, all the experiments were performed in triplicates in 100 mL flask filled with 100 mL BHM (pH 7.0) as growth medium supplemented with 10% v/v effluent. 10% v/v of culture having 2 x 10^8 actively growing cells/mL was used as inoculum. All the flasks were incubated at 32±2 °C under static condition.

Undiluted textile dye effluent decolourization: The nutritional and cultural conditions, as optimized above, were used to study the decolourization of textile industry effluents. Decolourization of actual textile effluent was carried out in the 100 mL Erlenmeyer flask filled with 100 mL of the unsterilized textile industry effluent supplemented with optimized C, N, and P sources. The control and experimental effluent flasks were incubated under optimized cultural conditions of pH 8.0, temperature 32±2ºC for 48 h and static culture conditions.

The true color level independent of hue was measured using the American Dye Manufacturers Institute (ADMI 3WL) tristimulus filter method[7]. In order to observe reduction in ADMI value in the test flask, an aliquot of 3 mL were withdrawn at an interval of 24 h and percent transmittance, at 590 nm, 540 nm, and 438 nm wavelengths, before and after treatment were measured[8]. ADMI removal percent (%) is the ratio between the ADMI removal value at any contact time and the initial and ADMI value and is calculated suggested by Agrawal et al.[9].

$$\text{ADMI removal } (\%) = \frac{(\text{Initial ADMI}_{(t0)} - \text{Final ADMI}_{(t)})}{\text{Initial ADMI}_{(t0)}} \text{ x } 100$$

The COD value of textile effluent, before and after its degradation, was determined by the standard method of APHA[7].

Results and Discussion

Effluent decolourization: Studies on biotreatment of textile industry effluent by *B. pumilus* SRS83 were conducted in order to demonstrate the applicability of the culture for the remediation of industrial waste containing various azo dyes and for degradation of mixtures of such dyes in the effluent. As the effluents from textile industry have high COD therefore to enhance the decolourization and degradation of the effluent studies on optimization of nutrient supplements were also conducted.
Results of inoculum development need to be added.

Effects of varying carbon sources on decolourization of effluent

In order to optimize the nutrient composition for enhanced decolourization, COD and BOD reduction of textile effluent by using *B. pumilus* SRS83 effect of additional carbon, inorganic nitrogen and phosphorus sources was studied at static conditions as no considerable decolourization performance was observed under nutrient limited conditions.

The type of carbon and nitrogen sources used in the Bushnell Haas medium strongly affected the biodegradation activity of the isolate. As depicted in figure-1, in the Bushnell Haas Medium (control), only 8.3%, decolourization of the effluent was observed over 24 h with *B. pumilus*sp. SRS83. In order to enhance decolourization in the control medium, the medium was supplemented with extra carbon, nitrogen and phosphorus sources. Maximum (72.11%) dye decolourization by *B. pumilus* sp. SRS83 within 24 h was observed with metabolizing starch in comparison to that with glucose (69.42%), lactose (62.18%) and sucrose (66.56%).

The low decolourization extent, at C-source concentration lower than optimum, could be attributed to insufficient level of utilizable C-source so as to meet the growth requirement for colour removal by*B. pumilus* SRS83. Starch is a readily metabolizable carbon source for*B. pumilus sp.*and it acts as a reducing agent for dyes and also promotes bacterial growth and metabolism, thereby facilitating dye decolourization.

Effects of varying N and P sources on effluent decolourization

Reports have shown that decolourization of synthetic textile dye effluent was best in carbon as well as nitrogen-sufficient medium along with a twofold increase in biomass[10].

Among the inorganic nitrogen sources, sodium nitrate effected dye decolourization of 87.21% within 24 h by *B. pumilus* sp. SRS83 (figure-2). Other nitrogen sources, on the basis of dye decolourization, may be arranged in the following decreasing order of dye decolourization: ammonium nitrate (81.43%),

ammonium sulphate (82.12%) > urea (76.13%) > potassium nitrate (78.43%).

Similarly, combination of potassium dihydrogen phosphate dipotassium hydrogen phosphate showed enhanced (89.31%) dye decolourization as compared to sodium dihydrogen phosphate and disodium hydrogen phosphate showed 80.40% decolourization of effluent by *B. pumilus* sp. SRS83 and therefore, was selected as the best source of phosphate for effluent decolourization (figure-3).

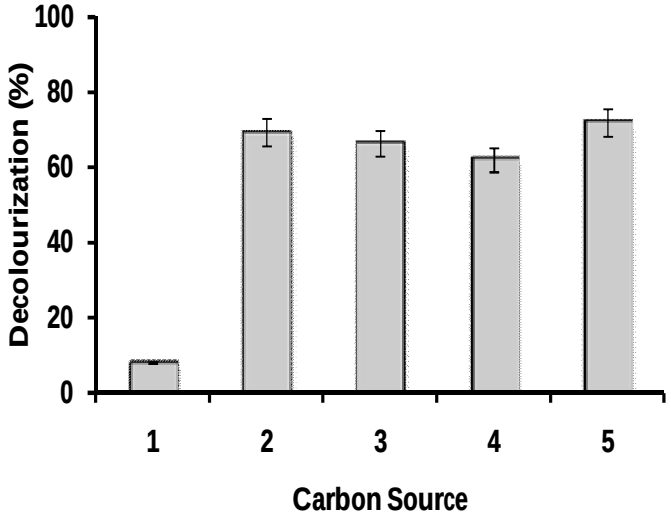

Figure-1
Effect of different carbon sources on effluent decolourization (1-BHM/Control, 2-Glucose, 3-Sucrose, 4-Lactose, 5-Starch)

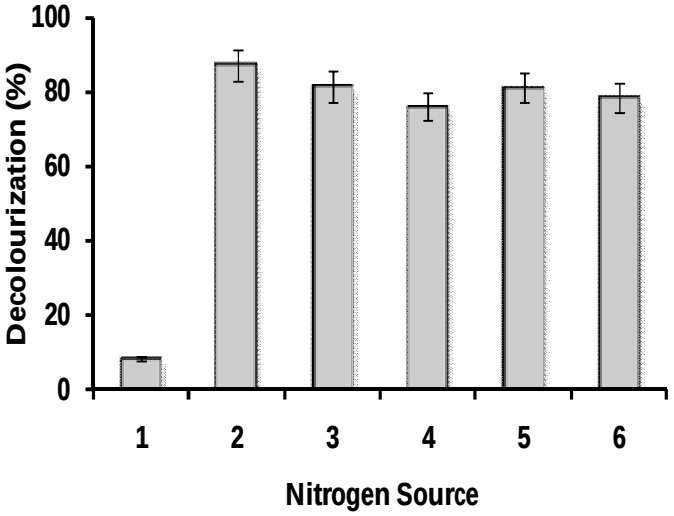

Figure-2
Effect of addition of different nitrogen sources on effluent decolourization (1-BHM, 2-$NaNO_3$, 3-NH_4NO_3, 4-Urea, 5-$(NH_4)_2SO_4$, 6-KNO_3)

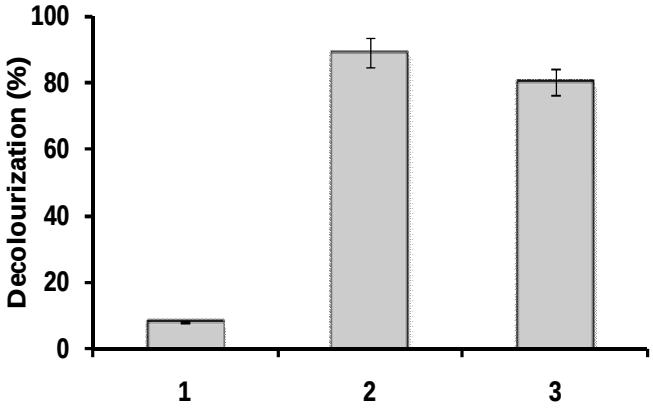

Figure-3
Effect of addition of different phosphorus sources on effluent decolourization (1-BHM, 2-Na_2HPO_4+NaH_2PO_4, 3-K_2HPO_4+KH_2PO_4)

Optimization of carbon, nitrogen and phosphorus content: Often, biodegradation of pollutants is observed to be restricted by the availability of nitrogen. The organism may have insufficient nitrogen source at lower concentration of nitrogen source or may influence the change in pH at higher nitrogen content. Thus, both low and high nitrogen source concentrations have an adverse effect on dye decolourization.

The maximum decolourization of 90.93% for the effluent was noticed at 10 g/L concentration of starch concentration for *B. pumilus* sp.SRS83as shown in figure-4. The percentage decolourization of dye for different nitrogen source concentrations is shown in the figure-5. Similarly, the maximum decolourization of 94.33% for the effluent was noticed at 4 g/L concentration of sodium nitrate. In order that during effluent decolourization by B. pumilis SRS83, the system not being deficient in available organic material and nitrogen, carbon, nitrogen and phosphorus sources were added. As shown in Fig. 6, it was observed that maximum decolourization of dye occurred at a phosphorus content of 0.2 g/L (95.8%), below which the organisms have inadequate amount of phosphorous to support its growth and moreover, high concentration of phosphorous does not support the growth. Thus, the involvement of small amount of phosphorus enhanced dye biodegradation as proved by Velan et al.[11].

Effluent Treatment: The textile industry effluents usually have allow BOD/COD ratio (0.26) and therefore the contents that could not be easily degraded. Hence stabilization of the textile industry effluent was carried out using efficient *B. pumilus* sp. SRS83 under above optimized nutritional and cultural conditions. A 87.18% and 84.91% reduction in COD and ADMI values, respectively of the effluent having an initial COD and ADMI value 5632 and 2674 respectively was noticed was noticed after 48 h upon treatment by *B. pumilus* SRS83 suggesting its efficiency in industrial applications.

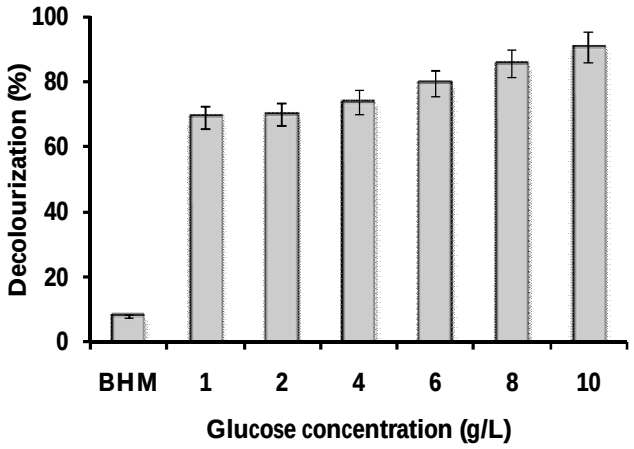

Figure-4
Effect of varying starch concentration on effluent decolourization

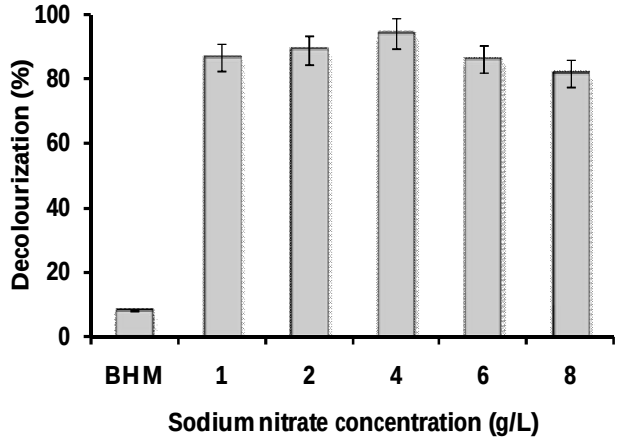

Figure-5
Effect of varying concentrations of sodium nitrate on effluent decolourization

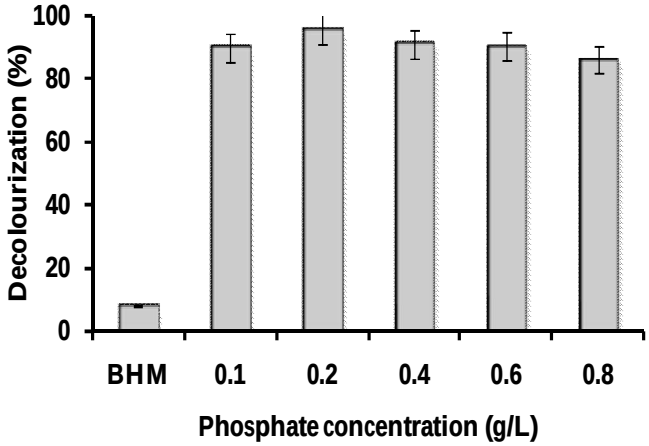

Figure-6
Effect of varying phosphate concentration on effluent decolourization

Conclusion

The obtained results have established the dye degrading efficiency of *B. pumilus* SRS83. The capacity of the strain *B. pumilus* SRS83 to tolerate, decolourize and degrade azo dyes at varying saltandinoculum concentrations gives it an advantage for treatment of textile industry wastewater. The developed strain proved to be effective for colour removal from diluted as well as actual industrial dye waste upon supplementation of simple substrates like sodium nirate, starch and potassium phosphates with significant reduction in ADMI and COD values. The organism showed as much as 87.18% and 84.91% reduction in COD and ADMI values, respectively of the effluent having an initial COD and ADMI value 5632 and 2674 respectively after 48 h of treatment by *B. pumilus* SRS83. Thus, *B. pumilus* SRS83 proved to be highly promising and can be successfully employed for the application in the treatment of dyeing wastewater and in bioremediation of recalcitrant AB210 and other azo dyes.

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