JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2017. Vol. **11**(4), p. 1757-1765

Optimization of Process Conditions for Effective Degradation of Azo Blue Dye by *Streptomyces* DJP15

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http://dx.doi.org/10.22207/JPAM.11.4.14

(Received: 20 October 2017; accepted: 30 November 2017)

The present study was carried out to optimize the degradation process of textile azo blue dye by the potential strain *Streptomyces* DJP15 isolated from dye contaminated soil in and around Palakad Textile Industry, Palakad District, Kerala state, India. The decolourizing activity of the potential isolate *Streptomyces* DJP15 was measured spectrophotomterically at every 6 h over a period of 54 h in *starch* casein broth amended with 50 mg/L of the test dye, azo blue. It was noticed that, there was a decrease in the optical density (OD) indicating the degradation of the test dye by the potential isolate *Streptomyces* DJP15. Different incubation conditions like shake condition, static condition, dye concentration, pH and temperature were used in the present study to investigate their effect on the rate of decolorisation. The potential isolate *Streptomyces* DJP15 exhibited significant decolourisation activity at 48 h of incubation for all the degradation condition studied. The conditions optimum found for degradation of the azo blue dye by the potential isolate *Streptomyces* DJP15. The highest degradations were noticed at static conditions, 50 mg/L of dye concentration, 3% v/v inoculum concentration, 7 pH and 35 °C temperature respectively. The results of the present study confirms that the isolate *Streptomyces* DJP15 was effective in degrading the textile dye azo blue under optimized conditions.

Keywords: *Streptomyces*, textile dye, azo blue, optimization, biodecolourisation.

Azo dye is the largest and most versatile class of synthetic dyes widely used in the textile industries which accounts for more than half of the annually produced synthetic dyes. Azo dyes are classified as monoazo dyes (e.g., acid orange 52, reactive yellow 201, disperse blue 399), diazo dyes (reactive brown 1, brown 2, acid black 1, amido black), trisazo dyes (direct blue 78, direct black 19) and poly azo dyes (direct red 80) depending on the number of azo groups. On the basis of application, azo dyes are classified as reactive, disperse, direct, cationic, anionic and metalized azo dyes[1]. Amongst the azo dyes, reactive dyes the only textile colourants designed to bind covalently with cellulosic fibers and are extensively used in textile industry**.** Reactive dyes are highly water soluble due to high degree of sulphonation and non degradable in typical aerobic conditions found in conventional biological treatment systems[2]. Sulfonated azo dyes characterized by the presence of a -SO_3H- group are commonly found in industrial effluents. Most of the azo dyes are stable to light, temperature, and highly resistant to degradation[3]. Persistence of the azo dye is mainly due to sulfo and azo groups which do not occur naturally making the dyes xenobiotic and recalcitrant to oxidative degradation[4, 5]. The dyes without an appropriate treatment can persist in the environment for extensive periods of time and are deleterious not only for the photosynthetic processes of the aquatic plants but also for all the living organisms since the degradation of these can lead to carcinogenic substances[6]. These compounds

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tend to bioaccumulate in the environment, and have allergenic, carcinogenic, mutagenic and teratogenic properties for humans. Release of dyes into the aquatic system reduces the dissolved oxygen content, which ultimately causes the death and putrefaction of aquatic fauna[7]. In recent years, bioremediation has been considered as effective, specific, less energy intensive and environmentally benign process, since it results in partial or complete bioconversion of pollutants to stable nontoxic end products[8]. Microbial bioremediation process involves the improvement of natural degradation capacity of the microorganism[9]. Biodegradation using microorganisms is gaining importance as it is cost effective, environmental friendly technique producing less sludge[10] and complete degradation would lead to non toxic end products [11, 12,13]. Many microorganisms belonging to different taxonomic group of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolourize azo dyes[14,15]. Environmental factors are known to play a crucial role affecting the decolorization activity of microorganisms[16]. The physicochemical parameters may affect the stability of enzyme system involved in dye degradation, resulting in decreased performance in decolorization activity at extreme pH and temperature, which may affect the viability of strain[17]. Parameters such as various carbon source, nitrogen source, dye concentration, aeration, temperature, pH, incubation period, and inoculum size influence the decolorization efficiency of the bacteria[18,19]. The present investigation is an effort to optimize the biodegradation process of azo blue dye by previously isolated potential isolate *Streptomyces* DJP15.

MATERIALS AND METHODS

Decolurisation Experiments

The previously isolated potential strain of *Streptomyces* DJP15[20] was grown and maintained on enrichment media[21] amended with 50 mg/L of azo blue dye at a temperature of 37 °C under agitation at 180 rpm. Decolurisation experiment were carried out in 50 mL of starch casein broth the medium (soluble starch 10.0g, K_2HPO_4 2.0 g, KNO_3 2.0g, NaCl 2.0 g, Casein 0.3 g, $MgSO_4$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4$ 0.01g, Distilled H_2O 1000 mL, pH 7.0) amended with 50 mg/L of the test dye. The efficiency of degradation percentage of the azo blue dye by *Streptomyces* DJP15 was studied with respect to the varying effects of shake condition, static condition, dye concentration, inoculum size, pH and temperature for optimization of the degradation process. All experiments were done in triplicates.

Analytical methods for dye decolourisation studies

Aliquots (5 mL) of the culture media were withdrawn at time intervals of 6 h over 54 h and centrifuged at 7000 rpm for 15 min. Decolourisation was quantitatively analyzed by measuring the absorbance of the supernatant using a UV–visible spectrophotometer (Spectronic® GENESYS TM 2 PC; at maximum wavelength, λmax, of 620 nm for azo blue dye. The decolourisation rate was calculated using the equation[22].

$$\text{Dye Decolourisation percentage} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Optimization of process conditions for effective dye degradation

Optimization of important conditions such as shake condition, static condition, dye concentration, inoculums size, pH and temperature for effective dye degradation by the potential isolates of *Streptomyces* was carried out[23]. The potential *Streptomyces* DJP15 strain was examined for the degradation of azo blue dye. Effect of one parameter at a time, keeping others constant was followed. Influence of shake condition and static conditions on the maximum degradation of dye, at empirical conditions was carried out primarily before the examination of other mentioned conditions.

Influence of shake and static conditions

50 mL of starch casein broth was added into 100 mL Erlenmeyer conical flask and sterilized. 1 mL of azo blue dye, at the concentration of 50 mg/L was added to the broth independently. 1 mL of 3 days old cultures of test isolate *Streptomyces* DJP15 was inoculated to the broth and incubated at 35 °C for 3 days, under shake condition (on shakers at 180 rpm) as well as static conditions. 5 mL of the incubated broth was drawn at every 6 h and centrifuged at 7000 rpm for 15 min. Absorbance of the supernatant was recorded using UV vis spectrophotometer at 620 nm for azo blue dye. The percent degradation of the dye was calculated as mentioned earlier.

Optimization of dye concentration

The maximum dye degradation under static state by the potential isolate *Streptomyces* DJP15at different concentrations of dye was assessed following broth culture method as mentioned earlier. Degradation of azo blue dye was examined at the concentrations of 50, 100, 150, 200, 250 and 300 mg/L. The percent dye degradation was calculated as mentioned earlier.

Optimization of inoculum size

Inoculum size was optimized for effective dye degradation by the test isolate *Streptomyces* DJP15, following broth culture method as mentioned above. Inoculum size of 3 days old test isolate at 1, 2, 3, 4 and 5 % (v/v) were assessed for maximum dye degradation.

Optimization of pH

Various levels of pH were optimized for effective dye degradation by the test isolate *Streptomyces* DJP15, following broth culture method as mentioned above. pH 6.0, 6.5, 7.0, 7.5 and 8.0 of the medium were adjusted using dilute acidic and alkaline solution of hydrochloric acid and sodium hydroxide respectively.

Optimization of temperature

Various ranges of temperatures were optimized for effective dye degradation by the test isolate *Streptomyces* DJP15, following broth culture method as mentioned above. The effect of temperature on the maximum dye degradation was examined by keeping the inoculated broth at 25, 30, 35, 40 and 45°C respectively. The percent dye degradation by the test isolate at different ranges of temperatures was calculated as mentioned earlier.

RESULTS

Optimization of process conditions for effective dye degradation

In the present study, an attempt was made to optimize the degradation of azo blue dye by the potential *Streptomyces* strain DJP15. Effect of various process parameters like shake condition, static condition, dye concentrations, inoculum size, pH and temperature were studied. The efficiency of *Streptomyces* DJP15 isolate was evaluated for the degradation of azo blue dye. The effect of shake condition, still condition, dye concentration, inoculum size, pH and temperature was studied with an aim to determine the optimal conditions required for degradation of the azo blue dye in starch casein broth.

Influence of static and shake conditions

The percent degradation of azo blue dye by potential isolate *Streptomyces* DJP15 at shaking and static conditions were as shown in the Figure 1. The strain *Streptomyces* DJP15 showed maximum degradation of 65.26% for azo blue dye under continuous shaking conditions at 48 h of incubation time. Under still condition, a sudden increase in the percent degradation of 12.63 % by *Streptomyces* DJP15 for blue dye was observed. The isolate *Streptomyces* DJP15 exhibited 77.89 % of maximum degradation at a incubation time of 48 h under static conditions. It was found that the isolate *Streptomyces* DJP15 showed more percent degradation under static conditions than shaking conditions. These results showed that the isolate *Streptomyces* DJP15 was more effective and potential in degrading azo blue dye under still conditions than shaking conditions.

To the best of our knowledge, it is the first report on degradation of sulfonated reactive di azo textile dyes (azo blue) by *Streptomyces* strains.

Optimization of dye concentration

Figure 2 shows the effect of initial concentration of dye ranging from 50–300 mg/L. The percent degradation of azo blue dye after 48 h of incubation by *Streptomyces* DJP15 was found to be 76.66, 71.25, 68.42, 64.78, 61.53 and 45 % at initial dye concentrations of 50, 100, 150, 200, 250 and 300 mg/L respectively. It was further noted that the degradation of the dye was concentration dependent. It was clear from the observation that, percent degradation of dye increased with an increase in time, irrespective of initial dye concentration. Further, percent degradation of dye decreased with an increase in dye concentration. i. e. lower the concentration higher the degradation efficiency and vice-versa. In our study, the diazo dye, reactive blue 222 (azo blue) degraded up to 76.66 % at 48 h of incubation with an initial dye concentration of 50 mg/L by the isolate *Streptomyces DJP15*.

Optimization of inoculum size

Effect of inoculum size (1 - 5% v/v) with time on degradation of azo blue dye was represented in the Figure 3. The result in Figure 3 depicts that, at every dose of inoculum, dye degradation increased with time during 6 to 48 h

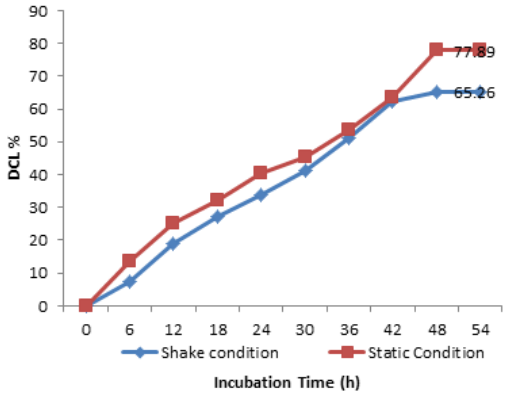

Fig. 1. Degradation of azo blue by potential isolate Streptomyces DJP15 at shake and static condiotns

incubation. After 48 h, the percent degradation of azo blue dye was found to be 76.03, 77.68, 80.99, 81.81 and 81.81 % at inoculum sizes of 1, 2, 3, 4, and 5 % v/v respectively. When the inoculum size was increased up to 3.0 % (v/v), the extent of degradation increased to 80.99% at 48 h of incubation. No drastic or considerable increase or decrease in the percent degradation was observed when the inoculum size was increased to 4.0 and 5.0 % (v/v).The maximum dye degradation (80.99 %) was attained at 3.0 % (v/v) inoculum at 48 h. Therefore, 3.0 % (v/v) dose of *Streptomyces* DJP15 inoculum was selected as optimum for the degradation of azo blue dye.

Optimization of pH

Effect of pH (6.0 – 8.0) on the degradation of azo blue dye by *Streptomyces* DJP15 was shown

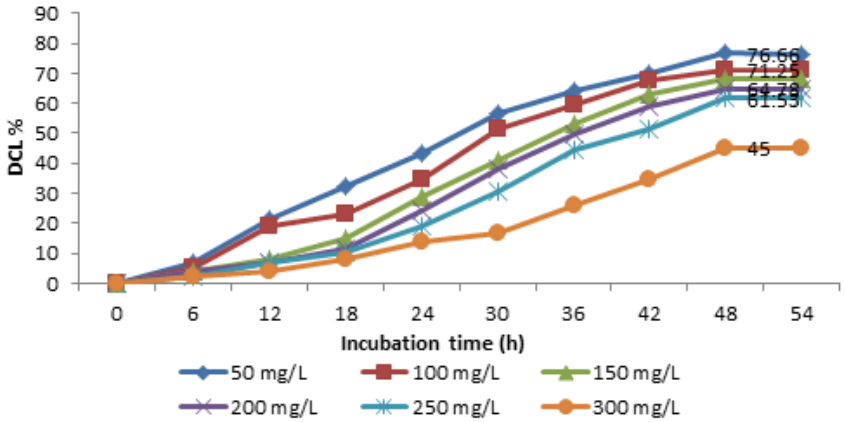

Fig. 2. Degradation of azo blue by Streptomyces DJP15 at different dye concentrations

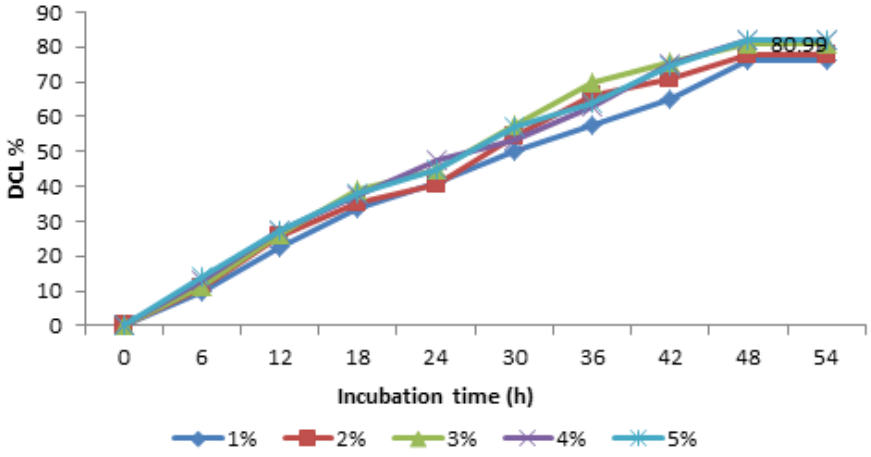

Fig. 3. Degradation of azo blueby Streptomyces DJP15 at different inoculum size

in Figure 4. In our study, it was noticed that, an increase in pH from 6 to 7 enhanced the rate of degradation significantly. However, degradation rate was the highest between 7-7.5 pH.Highest degree of degradation occurred at optimum pH 7.0 at 48 h of incubation. The results further revealed that, any deviation in the pH from optimum, decreased the extent of dye degradation. From the Figure 4 it was clearly noted that, the percent degradation of azo blue dye increased with increase in time irrespective of pH. The maximum percent degradation (76.31 %) of dye was found at pH 7 after 48 h of incubation period. Good percent degradation (72.10 %) was observed at pH 7.5. Further, increase in pH from 7.5 to 8.0, decreased the percent degradation of azo blue to 67.89 %. Least percent degradation (57.36 %) was recorded at pH 6.0. 62.63 % degradation was noticed at pH 6.5. It was clearly understood that, degradation was lower in acidic pH than alkaline pH.

Optimization of temperature

Figure 5 shows degradation of azo blue dye by *Streptomyces* DJP15 with time at different temperatures (25, 30, 35, 40 and 45°C). The percentage degradation of azo blue dye at 25, 30, 35 40 and 45 °C was found to be 57.89, 74.21, 79.47, 68.94 and 64.73 % respectively. It was clear that, percent degradation of dye increased with an increase in temperature from 25 to 35 °C. The percentage removal of dye was decreased with further increase in temperature up to 45 °C. Degradation activity was significantly suppressed at 25 °C than other temperatures, which might be due to the loss of cell viability or deactivation of the enzymes responsible for degradation at 25 °C (Cetin 2006).Further, increase in the temperature resulted in the decrease in the percent degradation. This may be due to the that at higher temperatures, thermal deactivation to the enzyme responsible for degradation may occur.

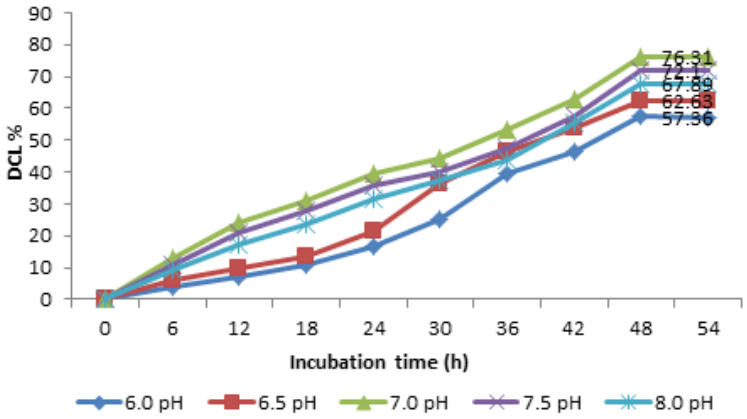

Fig. 4. Degradation of azo blue by Streptomyces DJP15 at different pH

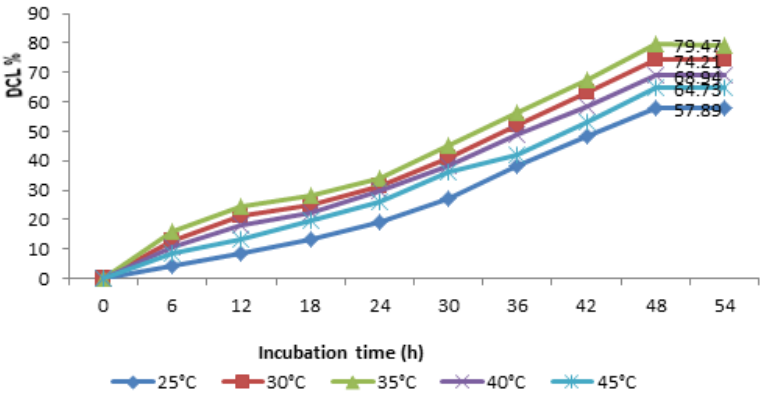

Fig. 5. Degradation of azo blue by Streptomyces DJP15 at different temperature

Extended period of incubation further to 6 h (54 h), decreased the percent degradation may be due to the decline phase of the isolate growth curve in all the conditions subjected for the study.

DISCUSSIONS

Microbes posses more than one mechanism for dye degradation[24]. Decolorisation of dye was enhanced by static condition as previously reported by the researchers[25]. Other researchers also reported that, static conditions were suitable for dye degradation process[26, 27, 28]. Generally stationary culture condition dominates over shake culture condition[29, 30, 31]. The present study supports that still / static condition is suitable for dye degradation process as other researchers reported[32, 33]. The more efficient decolorisation of similar structurally complex dyes under shaking condition was reported[34]. Azo dye degradation of 20% in shake culture and more than 95% in still culture by *Proteus mirabilis* was reported[35]. The initial biodegradation of azo dye occurs under anoxic condition leading to reductive cleavage of azo bond which causes decolorization of the dye. During shaking condition, presence of oxygen leads to deprive the azoreductase required for azo bond cleavage. In the present study degradation was noticed both in shaking and static conditions by both the isolates but effective degradation was recorded only under static condition than shaking condition. The competition between azo dyes and oxygen for reduced electron carriers under aerobic condition was the reason for decreased decolorization at shaking condition[32]. This reveals that the enzyme azoreductase involved in the initial step of azo bond reduction must be an oxygen insensitive[9, 36]. The dye concentration can influence the efficiency of microbial decolourization through a combination of factors including the toxicity imposed by dye at higher concentration [16, 37] and the dye degradation efficiency depends on the initial dye concentration [38]. The degradation of 80% of the synthetic dyes by *Pseudomonas sp* at 50 mg/L concentration in more than 7 days was reported[39]. The 80% decolorisation of navy blue 3G at 50mg/L by *Brevibacillus laterosporus* MTCC2298 within 48 h under static condition was also reported[40]. It was noted that beyond certain size of inoculum there was no proportionate increase in degradation with further increase in size of inoculum[41]. Rate of terasil black effluent decolorization enhanced with increase in inoculum size of *B. cereus* from 2.5 to 10 %; however, further increase of inoculum up to 20 % did not cause any change in the intensity of color[42]. The pH has a major effect on the efficiency of dye decolorization, and the optimal pH for color removal is often between 6.0 and 10.0 for most of the dyes[35]. The effect of pH in degradation of the dye may be due to the transport of dye molecules across the cell membrane, which is considered as a rate limiting step for dye decolorization[43]. The high decolourization of Reactive Black 5 by *Enterobacter* EC3 at pH 7 was reported[29]. The maximum decolourization of Reactive Red 195 by *Georgenia* sp at pH 7 was also observed[44]. *E. coli* and *P. luteola* both exhibited best decolorization rate at pH 7.0[45]. Our findings also in accordance with these reports, where maximum dye degradation was noticed at pH 7.0. Inhibition of *Klebsiella pneumoniae RS-1*, and *Alcaligens liquefaciens* S-1 biodecolourisation activity at 45°C was reported[45]. Optimum temperature of 37 °C was observed for the decolorization of acid orange 10 and disperse blue 79 by *Bacillus fusiformis kmk 5*[46]. The optimum temperature of 30-40°Cas for decolorization of crystal violet by *Shewanella Sp NT0Vl* was observed[47]. The decrease in the decolorization activity at higher temperature can be attributed to the loss of cell viability or to denaturation of the azoreductase enzyme[48]. The pH and temperature exert major effect on the efficiency of dye decolorization and that optimal conditions vary between pH 7.0–10.0 and 30– 40 °C, respectively[18, 26, 35].

CONCLUSIONS

The isolate *Streptomyces* DJP15 found to be very effective and potential in degrading the textile dye azo blue. The significant and striking observation of the resistance to higher levels of azo blue dye toxicity by the strain *Streptomyces* DJP15 enables their use for in situ bioremediation because it indicates the ability of strain to withstand shock loads of dye during the bioremediation process. The results of incubation temperature showed no deactivation of the degradation ability of the isolates up to 45°C which indicates the thermo tolerance ability of the isolates. Therefore, the

isolate *Streptomyces* DJP15 could be useful for on-field process in a country like India, where temperatures reaches to above 40°C in some parts of the country during summer season. However, there is a need for further investigation to understand the enzymes and other mechanisms involved in the degradation of the azo blue dye by the isolate *Streptomyces* DJP15 in order to harness its property for bioremediating the dye contaminated habitats for clean environment and clean nature for all life.

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