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Streptomyces Consortium for Enhanced Biodegrdation of Azo Blue Dye

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A *Streptomyces* consortium was developed and investigated for biodegradation of reactive sulfonated di azo dye (Reactive Blue 222). The biodegradation of azo blue dye by the consortium was further assesses with different ratios of inoculum of two different *Streptomyces* potential isolates. The optimization of process conditions was further carried out with respect to the dye concentrations, pH and temperature for the potential *Streptomyces* consortium. The *Streptomyces* consortia of 2:1 ratio was prove to enhance the biodegradation of azo blue dye to 89.39%. Dye concentration of 300 mg/L, pH of 7.0 and temperature of 35 °C showed the maximum degradation of 88.33 %, 89.06 % and 89.47 % respectively.

Keywords: Consortium, Streptomyces, Optimization, Azo blue, Biodegradation**.**

In most of the instances decolorization of dyes with pure culture was proved to be impractical, as the isolated culture would be dye specific and their application in large scale wastewater treatment plants with a variety of contaminant dyes was not feasible (Vijaraghavan, 2008). For environmental remediation, microbial consortia are found to be efficient (Senan *et al*, 2004). Mixed cultures and co cultures differ in their performance than from microbial monocultures. Interspecific interactions of microbes were known to be very important for their metabolic cooperation among the mixed cultures (Seneviratne *et al*., 2008). Microbial consortium systems can provide advantages over individual cultures as they involve the combined and inductive effects of various enzymes which can work synergistically. The complexity of microbial consortium enables them to act on a variety of pollutants (Watanabe and Baker, 2000).

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Though most of the research works on dye degradation have been carried out using fungal and bacterial consortia but the work pertained to indigenously isolated *Streptomyces* synergism for biodegradation and detoxification of azo dye is missing. Keeping this view as well as to overcome the problems of partial degradation, long reaction time and formation of toxic metabolites, a *Streptomyces* consortium was developed and investigated for biodegradation of reactive sulfonated di azo dye – reactive blue 222. Degradation of reactive sulphonated di azo dye – reactive blue 222 which was known to be recalcitrant, was investigated at different ratios of *Streptomyces* consortia, pH and Temperature.

According to Slater and Lovatt (1984), it is a commonly accepted observation that due to the concerted activity of a multimember consortium, often the biodegradation rate of a compound is faster in nature. Watnable (2000) stated that, the complexicity of microbial consortium enables them to degrade variety of pollutants. This phenomenon of degrading the variety of pollutant is generally due to syntrophic interactions among

the mixed communities which lead to complete mineralization of azo dyes (Chang *et al.,* 2004; Fude *et al*., 1994; Khehra *et al.,* 2005; Asgher *et al*., 2007). Degradation by consortia of microorganisms / mixed culture naturally enhances the process of biodegradation since, individual strains attack the dye molecule at different positions or they may utilize the decomposed products produced by one strain which will be further decomposed by another strain (Mohana et *al*., 2008; Coughlin *et al.,* 1997; Schliephake *et al*., (2000). The efficacy of decomposition considerably depends on the chemical character of the synthetic dye and biodegradation capacity of the microbial consortium (Chang *et al*., 2001). No data were available on the biodegradation of reactive blue 222 by *Streptomyces* consortium but limited data were found on biotreatment of C.I. Acid Red 119 (AR-119) acidic diazo dye and that to with an initial dye concentration of only 40 mg/ L by consortium (Khehra *et al.,* 2005).

MATERIALS AND METHODS

Formulation of consortia for effective dye degradation

The previously isolated two potential isolates of *Streptomyces* DJP15 and *Streptomyces* DJP27 were assessed in combination for effective degradation of azo blue dye. Effect of inoculums size in different combinations was mainly focused for effective dye degradation, as per the method of Senan and Abraham (2004) and Khadijah *et al*., (2009).

Each 1mL spore suspension of 3 days old cultures of both potential isolates of *Streptomyces*: (*Streptomyces* DJP15 and *Streptomyces* DJP27) were inoculated to 100 mL conical flask containing 50 mL of starch casein broth (pH 7.0) and incubated at 35 °C for 5 days to develop the consortia. Three different ratios (v/v) of consortia were formulated by inoculating both isolates of *Streptomyces* DJP15 and *Streptomyces* DJP27 at different ratios of inoculum size, namely1:1%, 2:1 % and 1:2% respectively. Azo blue dye at the concentration of 300 mg/L were added individually to the inoculated flask and incubated at 35 °C for 3 days. Samples of 5 mL culture broth were drawn at every 6 h and percent decolourisation was determined as mentioned earlier.

Optimization of conditions for effective dye degradation by consortia

Optimization of important conditions such as dye concentration, pH and temperature for effective dye degradation by the consortia of potential isolates of *Streptomyces* for azo blue dye was carried out as per the protocol prescribed by Dave and Dave (2009). Percent degradation of dye was determined by spectrophotometric method (Prachi and Anushree, 2009). 2:1 and 1:2 ratios of Streptomyces consortia were optimized for the degradation of azo blue dye. Effect of one parameter at a time, keeping others constant (as mentioned earlier) was followed.

Optimization of dye concentration

The maximum dye degradation under static state by the potential consortia of *Streptomyces* at different concentrations of dye was assessed following broth culture method as mentioned earlier. The azo blue dye was examined at the concentrations of 300, 350, 400, 450 and 500 mg/L. The percent dye degradation was calculated as mentioned earlier.

Optimization of pH

Various levels of pH were optimized for effective dye degradation by the potential consortia of *Streptomyces*, 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. Percent dye degradation was calculated in different pH, at every 6 h of incubation, up to 3 days, as mentioned earlier.

Optimization of temperature

Different ranges of temperatures were optimized for effective dye degradation by potential consortia of *Streptomyces*, following broth culture method as mentioned above. The effect of temperature on maximum dye degradation was examined by keeping the inoculated broth at 25, 30, 35, 40 and 45 °C. The percent dye degradation by the test isolates at different ranges of temperatures was calculated, at every 6 h, as mentioned earlier.

RESULTS

Our study demonstrates the efficient degradation of azo blue (Reactive Blue 222) by specific consortium of *Streptomyces*, developed

by mixing two indigenously isolated strains of *Streptomyces* at different ratios. The three different ratios (1: 1, 2:1 and 1:2) of consortium were developed by mixing two indigenously isolated strains of *Streptomyces*; *Streptomyces* DJP15 and *Streptomyces* DJP27 and particularly tested for their ability to check the enhanced degradation of blue dye.

The *Streptomyces* consortia of 1:1, 2:1 and 1: 2 ratios showed 69.69%, 89.39% and 73.23 % of azo blue degradation respectively (Fig. 1). All the three ratios of consortia showed their maximum percent degradation in less time of 36 h than the pure potential culture *Streptomyces* DJP15 which took 48 h of time for its maximum degradation. Among three ratios of consortia, least percent degradation (69.69 %) was exhibited by the consortia of ratio 1:1 at 36 h. The ratio 2:1 of the *Streptomyces* consortia proved to be the best one showing highest percent degradation (89.39 %) in short time of 36 whereas the consortia of ratio 1:2 showed 73.23% degradation keeping its place between the best and the least degraders.

It was found from the study that, consortia with a ratio 2:1 are potential degrader for blue and orange dyes. It was noted that, in the biodegradation of blue dye, in all the ratios of consortia at every interval of 6 h incubation

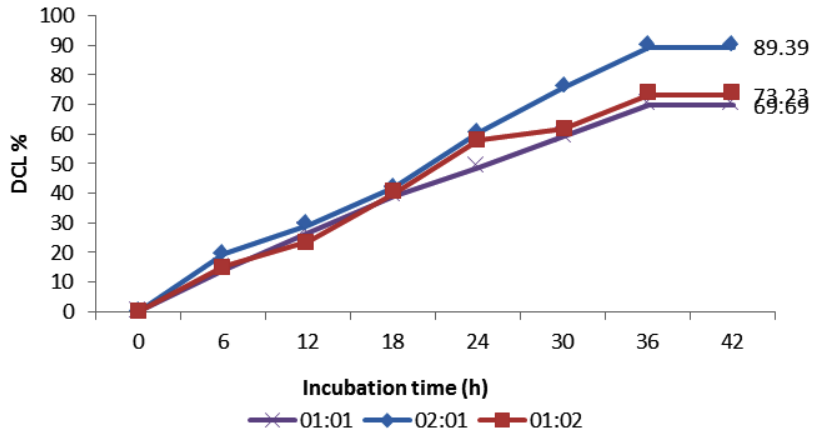

Fig. 1. Degradation of azo blue by different ratios of *Streptomyces* consortia

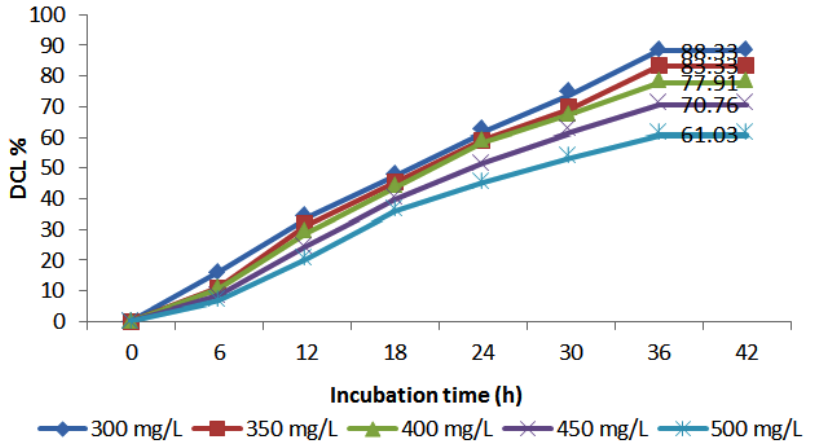

Fig. 2. Degradation of azo blue by potential consortium at different dye concentration

time, the percent degradation gradually increased indicating the degradation was synchronously related to growth of the isolates in the consortia.

Both isolates of *Streptomyces* were successfully existed as mixed cultures / co cultures at all the three different ratios of consortium in starch casein broth. The three ratios of consortia (1: 1, 2:1 and 1:2) of *Streptomyces* DJP15 and DJP27 showed varied degree of percent decolorization for azo blue. Significant reduction in the time (12 h) for final degradation of blue dye by all three consortia was noted.

Optimization of conditions for effective dye degradation by consortia

Optimization of dye concentration

Figure 2 shows the effect of dye concentration on the degradation of azo blue by potential 2:1 ratio of Streptomyces consortium. It was observed that, at concentration of 300, 350, 400, 450 and 500 mg/L of reactive blue 222; 89.50 %, 81.81 %, 74.40 %, 66.07 % and 59.33 % degradation was observed. Increase in the concentration of dye, decreased the degradation efficiency of the potential consortia. Moreover,

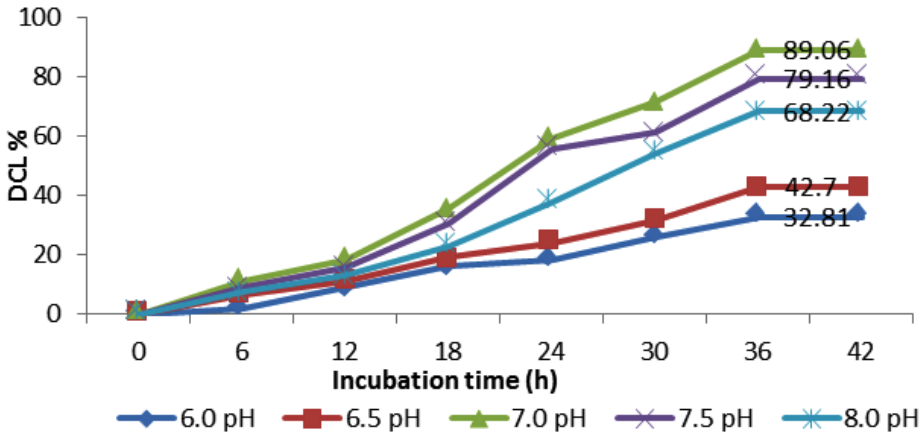

Fig. 3. Degradation of azo blue by potential consortium at different pH

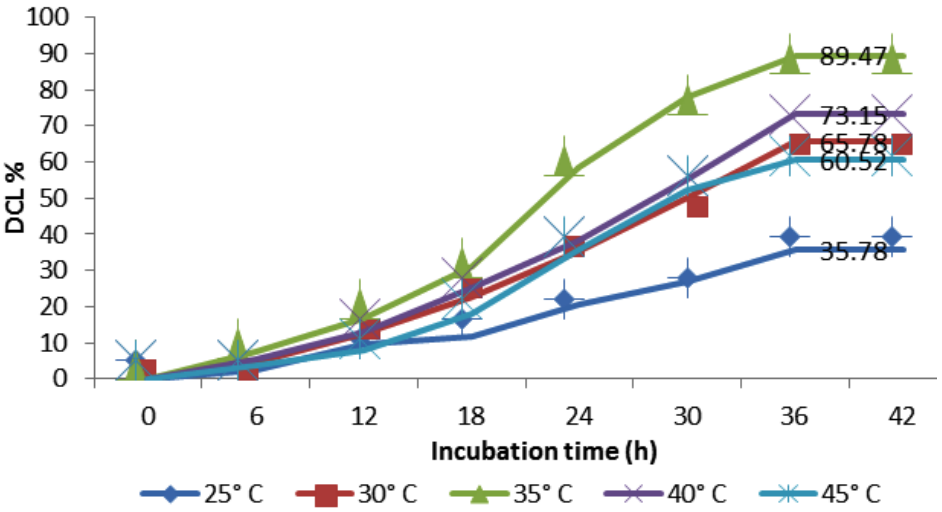

Fig. 4. Degradation of azo blue by potential consortium at different temperature

the consortia ratio showed considerable percent degradation (59.33 %) at high concentration (500 mg/L), was an significant nature of the consortia proving its tolerance property and ability to degrade more than 50 % at higher concentration of dye which was naturally lethal and toxic to most of the organisms. By this data and results it can be postulated that "Higher the concentration of the dye, lesser the degradation efficiency of the organisms".

Effect of pH on the degradation of azo blue by consortium of *Streptomyces* at 2:1 ratio was represented in Figure 3. 32.81 %, 42.70%, 89.06 %, 79.16 % and 68.22 % of azo blue degradation was recorded respectively over a pH range of 6.0, 6.5, 7.0, 7.5 and 8.0 at an incubation time of 36 h. Poor degradation activities of 32.81 % and 42.70 % were observed at acidic pH range of 6.0 and 6.5 respectively. Moderate percent degradation (79.16 %) was at pH 7.5 and good degradation (68.22 %) was recorded at alkaline pH 8.0; whereas the maximum degradation (89.06 %) was recorded at neutral pH for the consortia of 2:1 ratio.

Figure 4 represents the effect of temperature on the degradation of azo blue by *Streptomyces* consortium at 2:1 ratio. Consortia exhibited moderate percent degradation over the temperature ranges of 30 °C – 40 °C; highest being 89.47 % at 35 °C. Beyond the 40 °C the degradation efficiency of the consortium decreased which was 60.52 % at 45 °C. Lowest percent degradation (35.78 %) was noticed at low temperature 25 °C. At 30 °C and 40 °C the percent degradation recorded were 65.78 % and 73.15 % respectively.

DISCUSSIONS

All the three of *Streptomyces* consortia developed in the present study exhibited their maximum final degradation at 36 h for the dye failing in their way to perform complete degradation whereas; the complete decolorisation of Acid Brilliant Scarlet GR was reported by Tan *et al.,* (2012 a, b). Similar finding were also reported in the investigations of Buitro´n *et al.,* (2004) and Davies *et al.,* (2006), who investigated only one dye. Tan *et al.,* (2012 a, b) also reported the further degradation of most of the aromatic intermediates by the consortium. Enhanced decolorization and degradation of azo dye Rubine GFL (50 mg/L within 30 h) using defined consortium GG-BL of *Galactomyces geotrichum* MTCC 1360 yeast and *Brevibacillus laterosporus* MTCC 2298 bacterium was reported by Waghmode *et al.,* (2012) whereas, the individual cultures fails to completely decolorize the dye. Decolorization of Navy blue HE2R by developed consortium-PA of *A. ochraceus* NCIM-1146 fungi and *Pseudomonas Sp* SUK1 bacterium was reported (Kadam *et al.,* 2011). The degradation efficiency of the consortium may be due to the synergetic actions of oxidoreductases (Gou *et al.,* 2009; Telke *et al.,* 2009b).

A striking observation was made with respect to the *Streptomyces* consortia of ratio 2:1 which exhibited an enhanced degradation of 11.50%, showing its pronounced capacity to degrade the blue dye with a maximum percent degradation of 89.39 % at 36 h at a concentration of 300 mg/L.

Pearce *et al.,* (2003) reported that dye concentration could affect the efficiency of microbial decolorization through a combination of factors including the toxicity from higher dye concentrations and the accumulation of toxic decolorization intermediates.

Decolorization of azo dyes and simulated dye bath waste water using microbial consortium at 1 to 5 % inoculum size was reported by Dafale *et al*., (2008).

Synchronized action of microorganisms for the biodegradation of textile dye as been reported (Moosvi *et al*, 2005, Chen *et al*, 2006, Bafana *et al*, 2007, Yang *et al.,* 2009, Patil *et al*., 2010, Phugare *et al*., 2011).

Our finding were in agreement with the reports of Nigam *et al*., (1996) and Sharma *et al*., (2004) who mentioned that, the higher degree of biodegradation and mineralization can be expected when metabolic activities of mixed cultures within a microbial community complement each other. They also stressed that the advantages of mixed cultures are apparent as some microbial consortia can collectively carry out biodegradation that cannot be achieved by pure culture.

Waghmode *et al*. (2012) reported the decolorization and biodegradation at 150-200 mgL^{-1} Rubin GEL concentration by microbial consortium GG-BL. In our study all the three ratios

of consortia showed better degradation of blue dye. It was also observed that, the consortia have ability to degrade higher concentration of dye as reported by Ayed *et al.* (2010).

It is important to study the effect of pH on decolorization process, as transport of dye molecule into the cell is pH dependent and thought to be rate limiting step for decolorization of dyes (Lourenco *et al.,* 2000). The potential consortia exhibited maximum degradation at pH 7.0 which was in accordance with the findings of Phugare *et al*., (2011) who studied the bacterial consortia SDS with maximum decolorization and degradation efficiency at pH 7.0. Optimum pH for the degradation activity for both the consortia were found to be in the narrow range of 7.0 to 8.0 pH as reported by Chaube *et al*., (2010). Kumar *et al.,* (2009) recorded 96 % and 50% degradation of Remazol Black B at 7.0 pH and 5.0 pH respectively using pH sensitive consortium.

It was noted that, the ability of the consortia to show considerable degradation activity even at high temperature of 45°C. Maximum decolorization at 37°C by bacterial consortium was reported by Saratale *et al.,* (2010).

Our finding were in agreement with the reports of Phugare *et al.*, (2011 a) who found that, the bacterial consortium of *P. aeruginosa* BCH and *Providencia Sp* SDS had a potential to produce all the four dye decolorizing enzymes and gave a biodegradation advantage compared to the single bacterial strains.

CONCLUSIONS

In the bioremediation of xenobiotic anthropogens, application of mixed microbial consortium offers an advantage of complemented catabolic versatility. With a better understanding, this synergistic activity of *Streptomyces* consortia would be further exploited for the degradation of wide range of reactive azo dyes.

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