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BIOREMEDIATION OF AZO DYE AND TEXTILE EFFLUENTS USING *PSEUDOMONAS PUTIDA* MTCC 2445

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Abstract – Due to industrialization in the last century, lots of chemical including dyes are manufactured and used in various industrial process. In India, out of total export about 1/3 is from textile industries and largely accounts for aquatic and soil pollution due to the presence of the various dyes. Approximately 2500 small or medium scale or large-scale textile industries are scattered all over the country in different clusters. In the present study *Pseudomonas putida* MTCC 2445 has been selected based on its ability to degrade the textile dyes in primary screening. Strain MTCC 2445 was found to degrade Yellow HEGR dye was observed after 84 h of incubation. The maximum decolorization at concentrations of 50 mgL^{-1} was 68.7%. In direct textile industry effluent, *P. putida* was able to decolorize 25 and 50 % textile effluent concentration after 12 h of incubation time. The effect of different temperatures showed that maximum decolorization was at 37 °C after 84 h of incubation. The maximum decolorization of Yellow HEGR dye (45.8 %) and textile effluent (31.0 %) was observed at pH 8.0 after 84 h and 96 h of incubation, respectively. The effect of aeration was studied on the decolorization of dyes and effluents. It was observed that the static conditions showed better decolorization than shaking at 200 rpm. The non-living cells showed no decolorization of dyes or effluent confirming biodegradation of dyes and effluent. It was found during phytotoxicity assessment that, biodegraded textile effluent is less toxic as compared with the non-treated effluent. *P. putida* was found an appropriate strain to reduce the dye load in media broth and textile effluent under laboratory conditions.

INTRODUCTION

Our biosphere is under constant threat from continuing environmental pollution and out of all type of pollutions, industrial water pollution is quiet problematic. Increased dimensions of the industrialization and global population has intensified the problem of water pollution (Madhavi and Rao, 2003). Effluent discharge from various textile industries is the main cause of water pollution. Nearly 10,000 various dyes are produced annually, worldwide. Chemical dyes are commonly used in textile, dyeing, printing of different forms of the paper, colour photography, food formulations, cosmetic and other industrial processes. In India, textile industries play an important role as it account for around 1/3 of total export. There are approximately more than 2500 small or large textile industries are distributed all over the country. Due to increase in industries, lot of chemicals including dyes are manufactured and used in day to day life (Murthy and Naidu, 2012). Out of various activities in textile industry, chemical processing contributes about 70% towards pollution. It is well known that textile processing units utilize lots of water for different processes like sizing, desizing, scouring, bleaching, dyeing, printing, finishing and washing (Dos Santos *et al.,* 2007). In a textile industry about 40-65 L of waste water is generated per kg of cloth processed. Approximately 2 % of textile dyes are discharged directly in waste water effluent and 10% are subsequently discharged during the textile colouration process to the environment from textile and dyeing industries (Pearce *et al.,* 2003).

Wastewater discharge from textile industries are complex solution, which are highly coloured and toxic. The concentration of dye contained in the effluent is generally in the range of 10-200 mgL^{-1}. Several dyes are visible in water even at concentration as low as l mgL^{-1}, therefore the textile

wastewater is generally highly coloured. The dyes are bio recalcitrant and xenobiotic compounds. Textile effluent is characterized primarily by dyes and high concentration of BOD, COD, heavy metals, total dissolved and suspended solids, and high pH. This coloured effluent into the environment is objectionable not only for of colour, but also as many dyes from wastewater are mutagenic to life (Weisburger, 2002). The discharge of textile wastewater into surface water bodies also leads to obstruction in light infiltration and oxygen mixing into water bodies (Slokar and Le-marechal, 1998; Bae and Freeman, 2007). This effluent can sweep into the water table and pollute the ground water or where it has been discharged without appropriate treatment into water bodies. Even the minor change in pH of water body as a result of discharged effluent, can cause grave change in water composition and chemistry. Their affect on water bodies also affect photosynthetic organisms and consequently impact negatively on food chain (Asamudo *et al.,* 2005).

After World War II, people across the world became conscious towards the polluting environment. U.K and U.S.A. are the earliest countries to make legislation towards clean environment. Control and prevention of the industrial waste discharge is presently one of the major areas of scientific activity and planning. Rapid industrialization result in the discharge of large amount of waste to the environment, which in turn creates more pollution. Thus the current study was focused on the decolourization of textile dyes and bioremediation of textile effluent using *Pseudomonas putida*.

MATERIALS AND METHODS

Cultures and reagents

The culture was procured from Microbial Type Culture Collection Centre (MTCC), India. The textile dye was a kind gift from a local textile industry (Haryana), India. The textile effluent was obtained from Effluent Treatment Plant (ETP) of local textile industry, where effluent was stored for chemical treatment before disposal.

Decolourization of textile dyes and effluent

The decolourization of textile dye, Yellow HEGR, at various concentrations (10-200 mgL^{-1}) in mineral broth medium was studied. The decolourization of textile effluent at various concentrations (25-100 %, v/v) was also studied. Decolourization of dye media was recorded as the decline in absorbance of the cell free extract at their particular peak maxima (*i.e.* Yellow HEGR – 400 nm and Textile effluent – 555 nm). The amount of decolourization was observed spectrophotometrically using UV/VIS Spectrophotometer. Decolourization extent was articulated in terms of % decolourization (Sani and Banerjee, 1999).

$$\text{Decolourization (\%)} = \frac{\text{Absorbance (Initial)}-\text{Absorbance (Initial)}}{\text{Absorbance (Initial)}} \times 100$$

Optimization of process for textile dyes and effluent decolourization

The culture condition for decolourization of textile dye was optimized. The decolourization progression was optimized by using the one variable one-time strategy; varying one factor at a time by maintaining the formerly optimized conditions.

The effect of different temperatures on decolourization was observed. The mineral dye broth media comprising dye and effluent (Yellow HEGR: 200 $mgL^{-1}$and textile effluent: 100%) was inoculated 1% inoculum and incubated at different temperatures *i.e.,* 15 °C, 25 °C, 37 °C and 45 °C. The% decolourization was studied by measuring absorbance spectrophotometrically, after every 12 h interval for 4 days.

Microorganisms has a pH range within which growth and activity is possible and usually has a well-defined optimum pH. The optimal pH for the textile dyes and effluent decolourization was considered at different pH values, *i.e.* 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0. The decolourization was measured for 4 days at regular intervals of 12 h.

The effect of shaking and non-shaking cultural conditions on decolourization activity was studied under optimum pH and temperature conditions. Further, to confirm that the decolourization of textile dyes was either due to biodegradation (biological activity) or adsorption (non-biological activity) experiment was performed using living and non-living bacterial isolates.

Phytotoxicity studies

Phytotoxicity studies were carried on *Vigna radiata* seeds. The Petri dish method was used to study the seed germination (Sharma and Kumar, 2002; Rehman *et al.,* 2009). The seeds were surface sterilized (0.1% $HgCl_2$) and washed. Thereafter, the

effect of 25-100%, v/v textile effluent on seeds was detected. Further, 100 % textile effluent was used to study phytotoxicity. Sterilized petri plates with filter paper, these seeds were spread, treated with raw and treated textile effluent and incubated at 25 ± 2 °C. The extent of seed germination in percentage was observed every 24 h and the emergence of 2 mm length radical was considered as standard for germination. Textile effluent (3 mL) was further added to the petri dish after 7 days and seedlings were measured. Further, root and shoot lengths of 15 days old seedlings were measured. The plant growth parameter such as (shoot length, root length, wet shoot weight, dry shoot weight, wet root weight, dry rootweight and vigor index) were noted in 15th day old seedlings. Various seed germination constraints were studied; % germination, germination speed, peak value and emergence index.

RESULTS AND DISCUSSION

Decolorization of Yellow HEGR dye and textile effluent

The decolorization of different various dyes was studied at concentrations 50, 100, 150 and 200 mgL^{-1}. With increase in incubation period decolourization increased up to 96 or 108 hrs and then it slightly decreased and become nearly constant.

Yellow HEGR dye

The decolourization of Yellow HEGR dye by *P. putida* after 12 h of incubation showed 26.6, 22.5, 11.6 and 2.15 % decolourization at 50, 100, 150 and 200 mgL^{-1} concentration of dye respectively (Figure 1a). The maximum decolourization of Yellow HEGR dye was observed after 84 h of incubation (Figure 1a). The maximum decolourization at concentrations of 50, 100, 150 and 200 mgL^{-1} was 68.7%, 65.7%, 49%, 34.2%, respectively (Figure 1a). Previously, Jirasripongpun *et al.* (2007) isolated four strains of bacteria (*Enterobacter* sp., *Serratia* sp., *Yersinia* sp. and *Erwinia* sp.) from soil of waste disposal locations of textile processing unit with their ability to degrade C.I. Reactive Red 195. Seesuriyachan *et al.* (2007) isolated *Lactobacillus casei* 1500 having ability to degrade various azo dyes on MRS (II) agar plates from soil of dairy wastewater treatment plant.

Decolourization of textile effluent

Outcome of numerous concentrations of textile effluent (25-100 % v/v) on decolorization was considered (Figure 1b). After 12 h of incubation, *P. putida* was able to decolorize 25 and 50 % textile effluent concentration. However, no decolorization was observed at 75 and 100 % textile effluent concentration after 12 h of incubation. The maximum decolorization of 44.8 %, 39.5% and 30.6% was observed at 25, 50, and 75 % textile effluent concentration after 96 h of incubation (Figure 1b). Even at 100 % textile effluent concentration, *P. putida* decolourized 16.6 % of effluent after 96 h of incubation (Figure 1b). Earlier, Prasad and Rao (2010) isolated 30 bacterial strains from textile effluent samples by serial dilution technique on Luria bertani agar media for decolourization of textile dyes.

Optimization of process conditions for decolourization

The effect of different temperatures showed that maximum decolourization was at 37 °C after 84 h of incubation (Figure 2a). The maximum decolourization of Yellow HEGR was 34.1%, (Figure 2a). The textile effluent was decolourized maximally after 96 h of incubation at 37 °C with a decolourization of 16.7 %. However, no decolourization was observed at 15 °C (Figure 2b). Vijaykumar *et al*. (2007) observed similar results with bacterium *Kerstersia* sp. strain VKY1 decolourized naphthalene containing azo dyes at temperature 38 °C under aerobic conditions. Meehan *et al*. (2001) reported optimum temperature of 37 °C for decolourization of azo dye Remazol Black B by bacteria *Paenibacillus azoreducens* sp. nov. Pourbabaee *et al*. (2005) reported decolourization of azo dye methyl orange by newly discovered *Bacillus* sp. strain PS at 37 °C within 2 days. Adedayo *et al.* (2004) observed optimal colour removal of azo dye methyl red at 30-40 °C. Khalid *et al*. (2008) found that optimum temperature for decolourization of azo dyes *Shewanella putrefaciens*AS96 was 35 °C. They also observed decreased in decolourization by change in temperature on both sides, *i.e*. less than or greater than 35 °C.

The effect of different pH was studied on decolourization of different dyes and effluent at their optimum temperatures (Figure 2c). The maximum decolourization of Yellow HEGR dye (45.8 %) and textile effluent (31.0 %) was observed at pH 8.0 after 84 h and 96 h of incubation, respectively (Figure 2d). Comparable results were also described by Dawkar *et al.* (2008) that an isolate *Bacillus* sp.

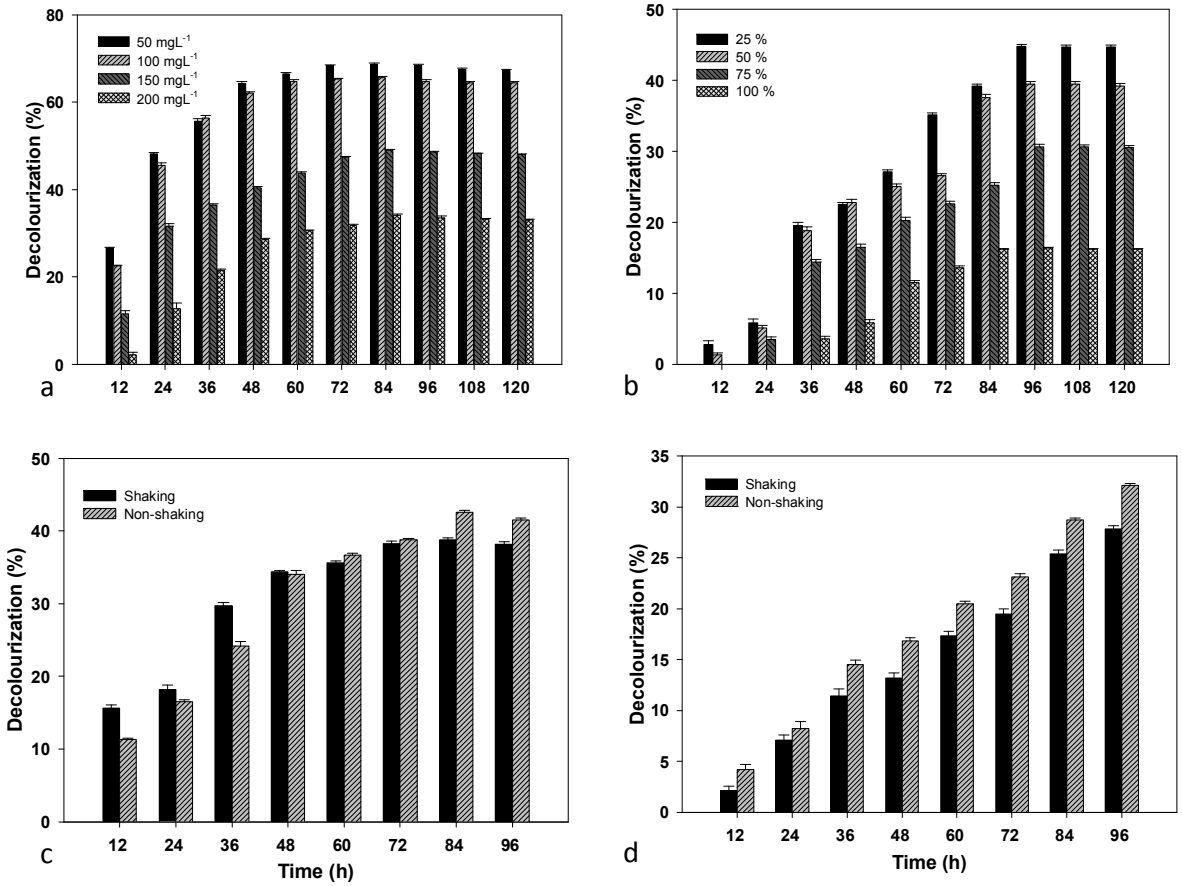

Fig. 1a. Decolorization of Yellow HEGR dye at different concentrations.
b. Decolorization of textile effluent at different concentrations.
c. Effect of agitating and non-agitating conditions on decolorization of Yellow HEGR dye.
d.Effect of agitating and non-agitating conditions on decolorization of textile effluent.

VUS and was able to degrade (100%) dye Brown 3REL at alkaline pH (6.5-12) within 8 hrs. Telke *et al*. (2009) reported 88% decolourization of sulfonated azo dye C.I. Reactive Orange 16 by *Bacillus* sp. ADR at optimum pH (7-8). Jirasripongpun *et al.* (2007) observed that decolourization of Reactive Red 195 was highest at pH 7.0 by *Enterobacter* sp. Liu *et al*. (2006) reported decolourization of azo dyes by bacteria *Rhodopsudomonas palustris* at optimum pH of 8.0. Meehan *et al*. (2001) observed optimum pH of 7.0 for decolourization of azo dye Remazol Black B by bacteria *Paenibacillus azoreducens* sp. nov. Khalid *et al*. (2008) found that pH 7-8 was optimum for decolourization of azo dye (Disperse Orange 3) by *Shewanellaputrefaciens* AS96.

The effect of aeration was studied on the decolourization of dyes and effluents. It was observed that the static conditions showed better decolourization than shaking at 200 rpm (Figure 1c & d). Kalyani *et al*. (2008) found that *Pseudomonas* sp. SUK1 decolourized Red BLI (50 mgL^{-1}) 99.28% within 1 hr under static condition. Mabrouk and Yusef, (2008) observed that decolourization of Fast Red was efficient in static compared to shaked cultures by *Bacillus subtilis* HM. Jirasripongpun *et al.* (2007) detected that decolourization of Reactive Red 195 by *Enterobacter* sp. in shaking/static conditions and found that shaking at 150 rpm did not result in any different decolourization from static conditions.

To rule out the possibility of dyes and effluent decolourization by adsorption (non-biological activity), decolourization was assessed using living and non-living (autoclaved) cells. The non-living cells showed no decolourization of dyes or effluent confirming biodegradation of dyes and effluent. Sirianuntapiboon and Prasertsong (2008) reported similar findings. These findings confirm that the decolourization activity is due to bacterial metabolism and not due to physical adsorption.

Phytotoxicity studies

The consequence of various concentrations of raw effluent (25-100 %) affected the germination of *Vigna radiata* seeds with noteworthy change in the rate of seed germination. The lower concentrations, 25% and 50 %, showed 22.2% and 15.5 % germination within 24 h, respectively (Table 1). However, higher concentrations of 75% and 100% showed lethal effect on seeds within 24 h (Table 1). The maximum germination was after 72 h of incubation. The toxicity analysis of treated effluent was completed at 100% non-diluted effluent. The various germination speed, emergence index, vigor index and peak value etc. are shown in (Table 2). Raw effluent showed 30.3% seed germination and 3.03 germination speed while seed treated with bioremediated effluent caused in % germination and germination speed of 70.9% and 5.16,

Table 1. Germination percentage at different conc. of textile effluent.

Time Period(hrs)	% Germination			
	Textile effluent conc.			
	25%	50%	75%	100%
24	22.2±0.56	15.5±0.62	0	0
48	45.5±0.53	44.3±0.81	20.2±0.48	11.1±0.53
72	70.2±0.38	57.2±0.45	20.5±0.39	13.3±0.45

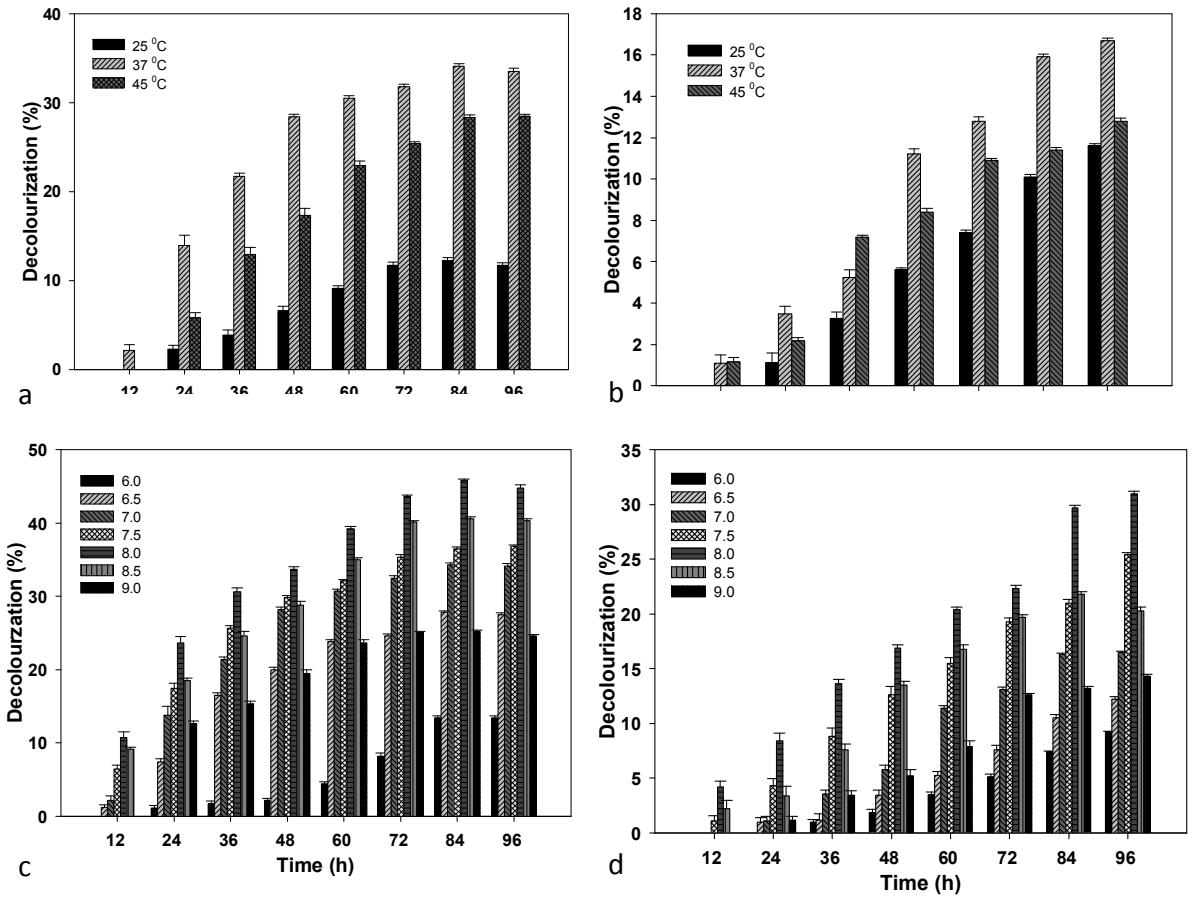

Fig. 2. Optimization of dye and textile effluent decolourization by *Pseudomonas putida*.
a. Consequence of temperature on decolourization of Yellow HEGR dye.
b. Consequenceof temperature on decolourization of textile effluent.
c. Consequence of pH on decolourization of Yellow HEGR dye.
d. Consequence of pH on decolourization oftextile effluent.

Table 2. Different Parameters studied for germination treated with raw and bioremediated effluent.

Sr. No.	Parameter(s)	Raw Effluent	Textile effluent treated with *P. putida*
1.	% Germination	30.3± 0.67	70.9± 0.61
2.	Germination Speed	3.03± 0.51	5.16± 0.44
3.	Emergency Index	2.0± 0.40	7.10± 0.51
4.	Peak Value	3.03± 0.35	5.16± 0.29
5.	Vigor Index	0.582± 0.42	274.5± 0.46
6.	Shoot Length (cm/seed)	0.067± 0.32	3.110± 0.44
7.	Root length (cm/seed)	0.160± 0.54	3.13± 0.45
8.	Wet Root Weight (mg/seed)	2.5± 0.38	40.50± 0.50
9.	Wet shoot weight (mg/seed)	18.0± 0.47	76.43± 0.56
10.	Dry Root weight (mg/seed)	0.503± 0.31	1.94± 0.33
11.	Dry shoot weight (mg/seed)	5.01± 0.44	7.84± 0.46

respectively (Table 2). The emergency index, peak value and vigor index was (2.0, 3.03, 0.582); (7.10, 5.16, 274.5) for controland *P. putida*, respectively (Table 2). Seedling length was significantly stimulated by bioremediated effluent, while raw effluent showed no significant effect of germination. The shoot length and root length were also improved in seeds treated with *P. putida* (3.110 and 3.13) as compared to control (0.067 and 0.160). Wet root weight (40.50), wet shoot weight (76.43), dry root weight (1.94) and dry shoot weight was (7.84) also higher for seeds treated with *P. putida* (Table 2). Hence, this resulted that effluent treated with *P. putida* was better for seed germination as compare to raw effluent. Mohammad and Khan (1985) that the germination of Kidney bean (*Phaseolus aureus*) and lady's finger (*Abelmoschus esculentus*) seeds were affected adversely when 75% and 100% concentrations of textile effluent were used, while no effect upto 50% concentration of textile effluent was noticed.

CONCLUSION

In the present study, a dye decolourization strain *P. putida* MTCC 2445 was selected and subjected for further analysis. The application of *P. putida* decolourizes the recalcitrant textile dye such as, Yellow HEGR. We concluded that *P. putida had* great potential as biocatalyst in view of its activity and stability at alkaline pH, temperature, rate of agitation and involvement of non-adsorption mechanism well as the ability to decolourize dye. The nontoxic behaviour of the treated effluent was found during the phytotoxicity assessment. A pilot-scale bioremediation study will be conducted with this valuable strain for actual industrial applications.

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