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EFFECT OF DIFFERENT PARAMETERS ON BIODECOLOURIZATION OF AZO REACTIVE RED RB DYE FROM TEXTILE EFFLUENT

T. Madhuri, B. Lakshmi Kalyani, P. Suvarnalatha Devi

Environmental Microbiology Laboratory, Department of Applied Microbiology, Sri Padmavati Mahila Visvavidayalam (Women's University), Tirupati-517502, Andhra Pradesh, India.

ABSTRACT

The aim of the present study is to isolate textile dye effluent degrading organisms. The synthetic textile dye effluents released into the environment, pollute soil and water ecosystem. Many of these effluents are toxic, carcinogenic and mutagenic in nature which effect aquatic and soil flora and fauna. Thus there is a need of processing these effluents before releasing into the environment. The Bacteria was isolated from soil contaminated with textile dye effluent by serial dilution followed by pour plating technique on nutrient agar medium. The ability of degradation was assessed by decolourization assay. Four isolates (RR1, RR2, RR3 and RR4) had the ability to degrade the dye effluent at different concentrations. The effect of pH, temperature, carbon and nitrogen sources and time course of decolourization was observed. The isolated RR2 and RR3 showed significant decolourization of dye at 600ppm.The ideal temperature was 370C and pH 7 and 9. Both isolates RR2 and RR3 showed optimum growth in media supplemented with sucrose and glucose as carbon source and RR2 showed good growth in ammonium sulphate and RR3 in peptone as a nitrogen source. The result concludes that the RR2 and RR3 isolates showed marked decolourization for textile dye effluent.

Key Words: Azo dye, Decolourization, Bioremediation, Red RB

INTRODUCTION

Among the textile industries, one of the most extensively used as dyes are synthetic chemicals. Approximately 10,000 different dyes and pigments are used industrially and 0.7 million tonnes of synthetic dyes produced annually, worldwide (Dawker *et al.,* 2008; Rafi *et al.,* 1990). India's dye industry produces different type of dyes and pigments. Nearly 7,00,000 tonnes of dyes are produced annually Worldwide (Zollinger, 1987). Production of dye stuffs and pigments in India is close to 80,000 tonnes. India is the second large exporter of dye stuffs and intermediates after China (Mathur *et al.,*2005). Azo dyes being the large group of synthetic dyes constitute up to 70% of all known commercial dyes produced (Carliell *et al.,* 1998). Synthetic dyes generally classified in to reactive, acidic, vat, dispersing, direct and sulphur etc. During the dyeing process, approximately 10-15% of the dyes are released in to the wastewaters, causing serious environmental and health hazards (Chen *et al.,* 2003).Disposal of these dyes into the environment causes serious threat, since they may significantly affect the photosynthetic activity of hydrophtes by reducing light penetration and also toxic to aquatic organisms due to their breakdown products (Hao *et al.,* 2000; Aksu *et al.,* 2007). Dyes may also be toxic to some aquatic life due to the presence of aromatics and metals, chlorides etc (Gupta et al., 2003). The textile finishing generates a large amount of waste water containing dyes and represents one of the largest causes of water pollution (Maulin *et al.,* 2013). Considerable attention has been given in evaluating the capability of microorganisms in decolourizing and degrading the azo dyes. Many studies on the decolourizing capability of microorganisms especially fungi and bacteria have been reported (Feng *et al.,* 2012). Various physical and chemical methods have been used for the removal of dyes from wastewater effluent (Jadhav *et al.,* 2008). However, implementation of physical and chemical methods have inherent drawbacks of being economically unfeasible, unable to completely remove the recalcitrant azo dyes and organic metabolites may cause pollution problems and involving complicated procedures (Forgaus *et al.,* 2004; Jadhav *et al.,* 2008).

Corresponding Author:
P. Suvarnalatha Devi, Department of Applied Microbiology, Sri Padmavati Mahila Visvavidayalam (Women's University), Tirupati-517502, Andhra Pradesh, India; Email: drsuvarnapallipati@gmail.com

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MATERIALS AND METHODS

Dyes and characterization

Dye – Reactive red RB was chosen for decolourization in the present study and was provided by a dyeing unit, Satravada, Chittoor District of Andhra Pradesh, India.

Standard dye solution

The dyes were dissolved in sterile distilled water to a concentration of 500 mg/10 ml.

Screening and Identification of microorganisms

Dye degrading bacteria was isolated from the textile industry effluent by serial dilution method (Madigan *et al.,* 2000). The pure isolates were maintained on nutrient agar at 4^0C for further use. These isolates were streaked on Luria bertani medium amended with dye, incubated at room temperature and observed for clear zone around the colony. Among the seven isolates four showed the decolourization activity and they were selected for further use.

Decolourization studies

Decolourization activity was expressed in terms of percentage of decolourization and was determined by monitoring the decrease in absorbance at absorption maxima (λmax 518) of respective dye. This was calculated using the following formula as described by (Sani *et al.,* 1999).

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

Effect of Physicochemical conditions on decolourization activity

Effect of pH and temperature

Various physical parameters like pH (5, 6, 7, 8 and 9) and temperatures (27°C, 37°C and 47°C) were monitored to study their effect on decolourization of reactive red RB dye.

Effect of dye concentration

Different concentrations of dye like 200, 400, 600, 800 and 1000ppm were incorporated into 250 ml Luria bertani medium, inoculated and incubated at optimum conditions.

Effect of different C and N sources

Different carbon sources like glucose, sucrose, lactose and maltose were incorporated separately into Luria bertani medium at 1% concentration level. Similarly, different sources of nitrogen like yeast extract, peptone and ammonium sulphate at 0.1% concentration were added individually.

Time course of dye decolourization

The time course of decolourization was carried out under optimum conditions are: for RR2 isolate initial dye concentration at 600ppm,pH 7, 37°C, 1% sucrose and 0.1%ammonium sulphate for RR3 isolate at initial dye concentration at 600ppm, pH 9, 37°C, 1% glucose and 0.1% peptone. Flasks were incubated up to 36 hours at their respective temperature and samples were removed at regular intervals for every 6 hours and analyzed for decolourization activity as describe above.

STATISTICAL ANALYSIS

The experiment was done in triplicate for each parameter. The results were expressed as percentage of decolourization with respect to control values and compared by two-way ANOVA and DMRT test. A difference was considered statistically significant if $p \leq 0.05$.

RESULTS

Screening of dye degrading bacteria

The total seven isolates were isolated from textile dye effluent and screened for degradation by streaking on dye amended Luria bertani medium. Among the seven isolates four showed efficient decolourization. Then the four isolates named as RR1, RR2, RR3 and RR4 respectively. Among the four degraded bacterial isolates, RR3 showed highest decolourization activity followed by RR2, RR1 and RR4 respectively.

Decolourization studies

250 ml of Luria bertani broth amended with 600ppm concentration of red RB dye was inoculated with 4 isolates individually. All the flasks were incubated in static conditions at 37^0C for 7 days. 5ml of samples was withdrawn at regular intervals and centrifuged at 10000 rpm for 10min. The supernatant was collected and the percentage of decolourization was measured at 518nm using UV-Spectrophotometer. The uninoculated dye medium supplemented with respective dye was used as blank (Jacob Thomson, 1998). Decolourization activity (%) was calculated by using the above formula and all assays were done in triplicates and the Mean value was taken for statistical analysis.

Effect of Physicochemical conditions on decolourization activity

Effect of pH and temperature

The pH exhibited varied range of effect on dye decolourization (Table 1). RR1, RR2 and RR4 isolates exhibited highest degrading capability at pH 7, whereas RR3 at pH

9, respectively. The different temperatures also showed varied range of effect on dye decolourization as shown in (Table 2). All 4 isolates exhibited highest degrading capability at 37°C respectively.

Effect of dye concentration

The different dye concentration exhibited varied range of effect on dye decolourization (Table 3). RR1, RR2, RR3 and RR4 isolates exhibited highest degrading capability at 600ppm concentration.

Effect of different C and N sources

The effect of different carbon sources exhibited varied rang of effects on dye decolourization (Table 4). RR1 and RR2 isolates showed highest degrading capability in sucrose while RR3 and RR4 showed maximum growth in glucose. The different nitrogen sources also showed effect on dye degrading of dye decolourization (Table 5). RR1and RR4 isolates exhibited highest degrading capability in yeast extract, for RR2 in ammonium sulphate and for RR3 in peptone.

Time course of dye decolourization

The present study reveals the high decolourization of textile dye effluent by four isolates with optimization of conditions at 84%, 90%, 94% and 80% of decolourization of dye at 36 hours.

DISCUSSION

Screening of dye degrading bacteria

The process of degradation of textile dyes by employing microorganisms particularly bacteria were also carried out to reduce environmental pollution (Srividhya *et al.,* 2012). The present study was carried out to examine the degradation of dye by isolating the bacteria from textile dye effluents. Among the seven isolates, only four isolates showed positive results for dye decolourization, as indicated by the change and disappearance of colour of the dye from the dye-containing media of the petri plates. A zone of different decolourization around the bacterial colony which might be due to the production of extracellular enzymes by the bacteria during the biodegradation of tested dye (Joshni *et al*., 2011; Ajay kumar pandey *et al.,* 2012). RR3 and RR2 isolates exhibited maximum decolourization when compared to RR1 and RR4.

Effect of Physicochemical conditions on decolourization activity

Effect of pH and temperature

The pH has a maximum effect on the efficiency of dye decolourization and the optimal pH for colour removable is between 6.0 and 10.0 for most of the dyes (Chen *et al.,* 1999). From the above data it can be inferred that RR3 (91%) at pH 9.0 is the ideal for its activity and is more efficient in decolourizing azo dye, similarly pH plays a great influence in decolourization of Reactive Red 2 dye. These findings are closely similar with alkaline pH for *Bacillus* species at pH 9.0 showed decolourization percentage was 64.34% by (EI-Sersy *et al.,* 2007). The results revealed that RR3 is more efficient in decolourizing azo dye at 37°C is the ideal temperature for its activity. Our results similar to that (Ponaj *et al.,* 2011) were reported as the range of activity on decolourization of orange 3R with *Bacillus sps at* 37°C was 78.57%. Centin *et al.* (2006) reported that decolourizing activity was suppressed at 45°C, might be due the loss of cell viability or deactivation of the enzymes responsible for decolourization at higher temperature.

Effect of dye concentration

Maximum dye degradation was observed at 600ppm concentration for four isolates. However, when the dye concentration was high, the isolates showed less capability. Similar results were also mentioned by (Khalid *et al*., 2012).Dye concentration can influence the efficiency of microbial decolourization through a combination of factors including the toxicity imposed by dye at higher concentration (Sahasrabudhe *et al.,* 2011).

Effect of different C and N sources

RR3 and RR4 exhibited maximum growth in glucose. Dyes being deficient in Carbon sources the biodegradation of dyes without any extra Carbon sources is very difficult (Senan *et al.,* 2004). The reason for low decolourization at sucrose, fructose and maltose might be that this carbon sources could not meet the good growth requirements for the bacterial isolate. Wang *et al.,* (2009) reported a *Citrobacter sps,* decolourized by 96.2% of reactive red 180 dye with 4g/l of glucose as carbon source. Bacterial utilization of azo dyes as a source of carbon and energy has been reported by (Yatome *et al.,* 1993; Dykes *et al.,* 1994).RR2 showed maximum in ammonium sulphate and RR3 in peptone as a nitrogen source. From the above data it was revealed that RR3 is more efficient in decolourizing azo dye with peptone is the ideal nitrogen source for its activity .The similar results was observed by (Chen *et al.,*1999;Liu *et al.,* 2006). The presence of peptone regenerates NADH and this acts as an electron donor for the azo dye reduction. In addition, peptone significantly enhances the strain's activity of azo dye decomposition and colour removal rate was increased with rise in peptone concentration (40 g l-1). The nitrogen sources were less efficient than carbon source availed by microorganism (Ola *et al.,* 2010).

Time course of dye decolourization

The time course of decolourization of reactive red RB dye under optimum conditions was 36 hours for RR2 90%,

whereas 94% of decolourization was observed in RR3 isolate within 36 hours showed (Table 6 and 7).The outcomes of this experiment indicated that RR2 and RR3 showed the decolourization process to a better extent than RR1 and RR4. However, both the species of bacteria can be inferred as good agents for the degradation of azo dye. Jothimani *et al.,* (1998) has reported 59% dye removal from a dyeing industry effluent using *pseudomonas sps.*

CONCLUSION

The present study concludes that dye degrading microorganisms RR2 and RR3from an effluent contaminated site of textile dyeing industry have potential of decolourization. This observation has established that the bacteria's are adaptive in nature and can degrade the dye contaminants. Thus, it is concluded that the bacterial isolates can be used as a good microbial source for textile waste water treatment.

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Table 1: Effect of pH on decolourization of reactive red RB dye

pH	Control	RR1	RR2	RR3	RR4
6	0.532±0.000	0.187±0.002	0.168±0.001	0.162±0.001	0.275±0.001
7	0.427±0.000	0.118±0.002	0.093±0.001	0.095±0.001	0.155±0.001
8	1.323±0.000	0.464±0.002	0.387±0.001	0.246±0.001	0.473±0.002
9	1.089±0.000	0.396±0.001	0.248±0.001	0.097±0.001	0.424±0.001

Values are the means of triplicates ± SD.

Table 2: Effect of temperature on decolourization of reactive red RB dye

Temperature	control	RR1	RR2	RR3	RR4
27°C	1.149±0.000	0.835±0.003	0.671±0.006	0.505±0.003	0.875±0.002
37°C	1.213±0.000	0.574±0.002	0.150±0.001	0.087±0.002	0.623±0.002
47°C	1.118±0.000	0.684±0.002	0.568±0.004	0.366±0.001	0.724±0.002

Values are the means of triplicates ± SD.

Table 3: Effect of dye concentration on decolourization of reactive redRB dye

Dye conc	control	RR1	RR2	RR3	RR4
200 ppm	1.126±0.000	0.294±0.001	0.277±0.001	0.102±0.001	0.397±0.001
400 ppm	0.236±0.000	0.054±0.001	0.037±0.001	0.024±0.001	0.174±0.001
600 ppm	0.538±0.000	0.064±0.001	0.058±0.001	0.048±0.001	0.095±0.002
800 ppm	0.425±0.000	0.071±0.001	0.059±0.001	0.051±0.001	0.092±0.001
1000 ppm	1.068±0.000	0.188±0.002	0.164±0.001	0.143±0.002	0.193±0.001

Values are the means of triplicates ± SD.

Table 4: Effect of carbon sources on decolourization of reactive redRB dye

'C' source	control	RR1	RR2	RR3	RR4
Glucose	1.553±0.000	0.424±0.004	0.396±0.001	0.122±0.002	0.494±0.001
Sucrose	1.265±0.000	0.325±0.005	0.214±0.001	0.101±0.001	0.441±0.002
Lactose	1.048±0.000	0.315±0.005	0.213±0.001	0.111±0.001	0.408±0.002
Maltose	1.596±0.000	0.568±0.001	0.337±0.001	0.296±0.001	0.984±0.002

Values are the means of triplicates ± SD.

Table 5: Effect of nitrogen sources on decolourization of reactive redRB dye

'N' sources	control	RR1	RR2	RR3	RR4
Yeast extract	1.043±0.000	0.206±0.004	0.147±0.003	0.084±0.002	0.253±0.003
Peptone	1.047±0.000	0.237±0.002	0.133±0.004	0.076±0.004	0.295±0.005
NH_4 SO_3	1.059±0.000	0.320±0.001	0.121±0.002	0.101±0.001	0.362±0.001

Values are the means of triplicates ± SD.

Table 6: Time course on decolourization of reactive red RB dye (RR2)

Time	Final	% decolourization
0	1.225	0
6	0.748	38
12	0.573	53
18	0.436	64
24	0.302	75
30	0.178	85
36	0.113	90

Table 7: Time course on decolourization of reactive redRB dye (RR3)

Time	Final	% decolourization
0	1.225	0
6	0.682	44
12	0.545	56
18	0.375	69
24	0.258	78
30	0.123	89
36	0.068	94

Figure 1: Decolourization assay