Biodegradation and Detoxification of Congo Red by Intuitive Bacterial Strain TVU-CR4

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Environmental pollution has been renowned as one of the foremost problems of the modern world. Ever-increasing industrialization and urbanization result in the discharge of hazardous waste to the environment, which in turn creates more pollution. Environmental biotechnology is persistently intensifying its efforts in the biological treatment of colored textile effluents, which is an environmental friendly and low cost alternative to physico-chemical processes. In the present study, effluent samples were collected from various textile and dyeing industries located in and around Vellore District, Tamilnadu, India and were exploited for the screening and isolation of bacterial strains that were capable of decolorizing Congo red. Optimization of cultural conditions was carried out to maximize decolorization efficiency of the bacterial isolate TVU-CR4 towards Congo red. Decolorization efficiency was found to be optimized at 35°C, neutral pH, after 24 h of incubation. Static conditions proved to be effective in maximizing decolorization. Increase in dye concentration decreased decolorization efficiency of TVU-CR4. Among the various carbon and nitrogen sources investigated; glucose and yeast extract instigated maximum decolorization. Detoxification was confirmed by phytotoxicity assay using Macrotyloma uniflorum.

Keywords: Congo red, Detoxification, Macrotyloma uniflorum, Phytotoxicity, Textile azodye

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Introduction
William Henry Perkin in 1856, accidentally discovered the world’s first commercially successful synthetic dye and named it as ‘Mauveine’. Since then, more than 1, 00,000 new synthetic dyes have been generated (Hemapriya and Vijayanand, 2014). Synthetic dyes are colored substances that color fibers permanently, without losing its color even when exposed to sweat, sunlight, water and many chemical substances including oxidizing agents and also to microbial attack (Rai et al., 2005; Saratale et al., 2011). These dyes were used in different industries, with an annual consumption of about 0.7 million tons.
worldwide. Synthetic dyes were extensively used in many fields of up to date technology, for example in various branches of the textile industry, in leather tanning industry, in paper production, in food industries, in agricultural research, in cosmetic industry, in light harvesting arrays, and in photo-electrochemical cells (Hemapriya and Vijayanand, 2013). Of the approximately $10^9$ kg of dyestuffs estimated to be manufactured annually throughout the World, the two most widely used in the textile industry are the azo and anthraquinone groups (Križanec and Marechal, 2006; Forss, 2011). Nearly all the dyestuffs used by the textile industry are azo dyes.

Global extension of textile industry has led to an alarming expansion in the utilization of such synthetic dyestuffs, resulting in a rise in environmental pollution due to the contamination of effluents (Pandey et al., 2007; Shyamala et al., 2014). In addition to the unpleasant appearance of the textile industries wastewater, the disposal of these untreated effluent waters into natural receiving waters causes damage to the environment as they significantly affect the photosynthetic activity in aquatic life due to reduced sunlight penetration and may also be toxic to aquatic flora and fauna due to the presence of hazardous metals, chlorides etc. and the breakdown products of dyes in them (Cetin and Donmez, 2006; Fan et al., 2009; Vijayanand et al., 2017).

Environmental pollution has been renowned as one of the foremost problems of the modern world. The escalating demand for water and the deteriorating supply has made the treatment and reuse of industrial effluents as an attractive option.

Therefore treatment of industrial effluent containing aromatic compounds becomes necessary prior to their final discharge into the environment (Shyamala et al., 2014). Moreover, the frequently high volumetric rate of industrial effluent discharge in combination with increasingly stringent legislation, make the search for appropriate treatment technologies an important priority. So, necessitate of efficient and economic processes to treat these effluents have become an alarming concern. As a consequence, there has been a mounting interest in biotechnological processes.

Implementation of different physico-chemical techniques including coagulation/flocculation, membrane filtration, ultrasonic mineralization, precipitation, flotation, adsorption, ion exchange, ion pair extraction, electrolysis, advanced oxidation process (chlorination, bleaching, ozonation, Fenton’s oxidation and photo catalytic oxidation) and chemical reduction have inbuilt drawbacks of being economically unfeasible (more energy consumption and chemical uses), unable to remove the recalcitrant azo dyes and/or their organic metabolites completely, generating a significant amount of sludge that may cause secondary pollution problems (Anjaneyulu et al., 2005; Vijayanand and Hemapriya., 2013).

The microbial decolorization and degradation of synthetic dyes has been of considerable interest since it is inexpensive, eco-friendly and produces a less amount of sludge (Kalyani et al., 2009).

Thus, the present study investigates the efficacy of textile effluent adapted bacterial strain to mediate biodecolorization and biodegradation of Congo red.

The optimal cultural conditions for maximizing biodecolorization was studied and further detoxification were investigated by phytotoxicity study using Macrotyloma uniflorum.
Materials and Methods

Sample Collection and Physico-chemical analysis

The effluent samples was collected from both textile industries and dyeing units located in and around Vellore District, Tamil Nadu, India. Samples were collected at the surface and at various depths (S1, S2 and S3) and were placed in sterile polythene bags.

Dye stuff used

Synthetic textile azo dye, Congo red used for this study was procured from a local textile dyeing unit. Stock solution was prepared by dissolving 1 g of Congo red in 100 ml distilled water. All chemicals used were of the highest purity available and of an analytical grade.

Enrichment and screening of bacterial strains decolorizing Congo red

The textile effluent samples were serially diluted and plated onto the surface of Nutrient agar medium enriched with 50 ppm of Congo red. pH was adjusted to 7.0 before autoclaving and incubated at 37 °C for 5 days. Colonies surrounded by halo (decolorized) zones were picked and streaked on Nutrient agar medium containing dyestuff. The plates were reincubated at 37°C for 3 days to confirm their abilities to decolorize Congo red. Morphologically distinct colonies of dye decolorizing bacteria were selected and re-streaked several times to obtain pure cultures.

Decolorization assay

A loopful of the selected bacterial culture was inoculated in Erlenmeyer flask containing 100 ml of nutrient broth and incubated at 150 rpm at 37°C for 24 h. Then, 1 ml of overnight broth culture of the bacterial strain was inoculated in 100 ml of nutrient broth containing 50 ppm of Congo red and re-incubated at 37°C till complete decolorization occurs.

Suitable control without any bacterial culture was also run along with experimental flasks. 1.0 ml of sample was withdrawn every 12 h and centrifuged at 10,000 rpm for 15 min. Decolorization extent was determined by measuring the absorbance of the culture supernatant at 500 nm using UV-visible spectrophotometer, according to Shyamala et al., (2014).

Decolorization efficiency (%) = Dye (i) – Dye (r) / Dye (i) X 100

Where, Dye (i) refers to the initial dye concentration, Dye (r) refers to the residual dye concentration. Decolorization experiments were performed in triplicates.

Optimization of culture conditions for biodecolorization of Congo red

Effect of incubation time, temperature, pH, agitation rates and dye concentration

Optimization of culture conditions for decolorization of Congo red was carried out by incubating the bacterial strain at different temperatures (20-50°C), different pH values of the medium (pH 4.0-10.0), different agitation speeds (0-200 rpm) and various dye concentrations (200 -1000 ppm).

Effect of carbon and nitrogen sources

The effect of various soluble carbon sources (1% w/v) (glucose, sucrose, lactose, maltose, starch) and nitrogen sources (tryptone, beef extract, peptone, yeast extract and meat extract) on dye decolorization extent of Congo red by bacterial strain TVU-CR4, was investigated.
Phytotoxicity studies

Phytotoxicity tests were performed in order to assess the toxicity of the untreated and treated dye samples. The ethyl acetate extracted products of degraded azo dyes were dried and dissolved in 5 ml sterile distilled water to make a final concentration of 100 ppm. Phytotoxicity tests were carried out on *Macrotyloma uniflorum*. 10 healthy plant seeds were treated separately with 5 ml of control dye and degraded products respectively/per day. Control sets were carried out using distilled water at the same time. Germination percentage as well as the length of plumule and radical was recorded after 7 days (Saratale *et al.*, 2009; Shyamala *et al.*, 2014).

Results and Discussion

Ever increasing demand for water and the dwindling supply has made the treatment and reuse of industrial effluents as an attractive option. Textile industries consume a considerable amount of water in their manufacturing processes. Considering both the volume and the effluent composition, the textile industry is rated as the most polluting among the industrial sectors.

Textile effluents are of global concern because they color the drains and ultimately the receiving water bodies (Olukanni *et al.*, 2006; Hemapriya and Vijayanand, 2013). In view of the need for a technically and economically satisfying treatment technology, a flurry of emerging technologies are being proposed and tested at different stages of commercialization.

Isolation and screening of bacterial strains decolorizing Congo red

Eight morphologically distinct bacterial isolates, designated as TVU-CR1 to TVU-CR8 that was capable of decolorizing Congo red were isolated from different effluent samples. Among the above mentioned isolates, TVU-CR4 isolate was found to be the efficient bacterial strain exhibiting maximum decolorization efficiency (92 %) (Table 1), which was selected for the further studies. Similarly many bacterial strains were reported to decolorize textile azo dyes (Deng *et al.*, 2008; Hemapriya *et al.*, 2010; Vijayanand *et al.*, 2017).

Optimization of culture conditions for maximizing decolorizing ability of TVU-CR4

Effect of incubation time

Results of the present study revealed that the dye decolorizing ability of the isolate was dependent on the bacterial growth. The dye decolorization process started after 4 h and reached its maximum level within 24 h and thereafter started to decline, due to the depletion of nutrients and accumulation of toxic metabolites, which inhibit the multiplication of the bacterial isolate (Fig 1). Similarly, Decolorization of Evan’s Blue by E.coli strain AKI-2 was achieved after 24 h of incubation (Aswinkumar *et al.*, 2017). In contrast, decolorization of Methyl orange by Bacillus sp. strain TVU-M4 was achieved after 32 h of incubation (Shyamala *et al.*, 2014).

Effect of temperature

Color removal efficiency of the bacterial strain TVUBK-04 increased with increase in incubation temperature, reaching highest levels between 30-40°C, with optimum being
35°C after 24 h of incubation, indicating that the decolorization process was directly proportional to the increase in incubation temperature. Decolorization activity was significantly suppressed at temperatures more than 40°C, which might be due to the loss of cell viability or denaturation of the enzymes responsible for the decolorization at elevated temperatures (Cetin and Donmez, 2006; Carvalho et al., 2008). Interestingly, the decolorization percentage was found to be reduced at temperatures below 30 °C.

**Effect of pH**

Dye decolorization efficiency of the bacterial strain TVU-CR4 was detected over a broad range of pH (5.0-9.0), with optimum decolorization being exhibited at neutral pH (7.0). pH tolerance of the decolorizing bacteria is quite important because the reactive azo dyes bind to cotton fibers by the addition or substitution mechanisms under alkaline conditions (Aksu et al., 2007). However, incubation at both acidic and alkaline pH slightly reduced the dye decolorization efficiency of the bacterial strain TVU-CR4 (Fig 3). Pure cultures of Proteus vulgaris NCIM-2027 and Micrococcus glutamicus NCIM-2168 showed maximum decolorization efficiency at neutral pH (7.0) (Saratale et al., 2009). In contrast, optimal pH values for the decolorization of Reactive Red RB by a microbial consortium and Acid orange by a halophilic bacterial consortium was found to be 8.0 (Cetin and Donmez, 2006; Vijayanand et al., 2017).

**Table.1** Bacterial Strains Decolorizing Congo red under Aerobic Conditions

| Sl. No | Isolates   | Sample Collection Site | Time taken for Maximum Decolorization | Decolorization Efficiency |
|--------|------------|------------------------|----------------------------------------|---------------------------|
| 1      | TVU-CR1    | S1                     | 60 h                                   | 64 %                      |
| 2      | TVU-CR2    | S3                     | 48 h                                   | 60 %                      |
| 3      | TVU-CR3    | S1                     | 60 h                                   | 74 %                      |
| 4      | TVU-CR4    | S2                     | 24 h                                   | 92 %                      |
| 5      | TVU-CR5    | S3                     | 60 h                                   | 71 %                      |
| 6      | TVU-CR6    | S2                     | 36 h                                   | 79 %                      |
| 7      | TVU-CR7    | S1                     | 72 h                                   | 81 %                      |
| 8      | TVU-CR8    | S3                     | 36 h                                   | 69 %                      |

Note: The isolates were considered for the table that showed above 50% decolorization ability

**Table.2** Effect of Carbon Sources on Decolorization Efficiency of TVU-CR4

| S.No  | Carbon Source (gl⁻¹) | Decolorization % |
|-------|----------------------|------------------|
| 1.    | Glucose              | 92 ± 0.02        |
| 2.    | Sucrose              | 89 ± 0.04        |
| 3.    | Lactose              | 84 ± 0.05        |
| 4.    | Maltose              | 78 ± 0.04        |
| 5.    | Starch               | 74 ± 0.02        |
**Table 3** Effect of Nitrogen Sources on Decolorization Efficiency of TVU-CR4

| S.No | Nitrogen Source (g l⁻¹) | Decolorization % |
|------|--------------------------|------------------|
| 1.   | Peptone                  | 76 ± 0.03        |
| 2.   | Yeast Extract            | 92 ± 0.04        |
| 3.   | Tryptone                 | 82 ± 0.02        |
| 4.   | Beef Extract             | 88 ± 0.06        |

**Table 4** Phytotoxicity Study of Congo red and its Degradation Products on *M. uniflorum*

| S.No | Parameters Studied | Tap water | Congo red | Treated sample |
|------|--------------------|-----------|-----------|----------------|
| 1.   | Germination %      | 100       | 60        | 100            |
| 2.   | Plumule (cm)       | 22 ± 0.7  | 12 ± 0.4  | 21 ± 0.5       |
| 3.   | Radical (cm)       | 8 ± 0.6   | 5 ± 0.5   | 7.5 ± 0.4      |

**Fig. 1** Effect of incubation time on the decolorization of Congo red by TVU-CR4

**Fig. 2** Effect of temperature on the decolorization of Congo red by TVU-CR4
Effect of dye concentration

The influence of different dye concentrations (200 - 1000 ppm) were studied on decolorization ability of the bacterial strain TVU-CR4. The result shown in Fig 4 has revealed that the decolorization rate gradually decreased with the increase in initial dye concentration. As the dye concentration increased in the culture medium, a decline in

Fig.3 Effect of pH on the decolorization of Congo red by TVU-CR4

![EFFECT OF PH](image)

Fig.4 Effect of agitation speed on the decolorization of Congo red by TVU-CR4

![EFFECT OF AGITATION](image)

Fig.5 Effect of dye concentration on the decolorization of Congo red by TVU-CR4

![EFFECT OF DYE CONCENTRATION](image)
color removal was attained. At high concentration (1000 ppm), decolorization ability of the bacterial strain was greatly suppressed by Congo red. Similar result was reported by Hemapriya et al., (2010). Reduction in the color removal efficiency might be attributed due to the toxicity of the dye to bacterial cells through inhibition of nucleic acid synthesis.

**Effect of agitation**

Aeration is an important parameter that influence the color removal efficiency of microbial cells. The dye decolorization ability of the bacterial strain TVU-CR4 was found to be greatly decreased with increases in agitation speeds. At 200 rpm, the decolorization ability of the bacterial consortium was greatly inhibited (Fig 5). Static conditions proved to be effective in maximizing decolorization percentage of the consortium. Azo dye decolorization by bacterial species is often initiated by enzymatic reduction mediated by azoreductase (Zimmermann et al., 1982). According to Chang and Lin (2000), azoreductase driven bacterial decolorization is normally inhibited in the presence of O$_2$ primarily due to the competition in the oxidation of the reduced group as the electron receptor.

**Effect of carbon sources**

In a preliminary study, various soluble carbon sources were used to replace the original carbon source in the growth medium. Decolorization of Congo red was not constitutive; different levels of decolorization were found with different carbon sources. Among the various carbon sources tested, Glucose was found to be maximizing the decolorization efficiency of TVU-CR4 (Table 2). According to Khehra et al., (2005), the metabolism of glucose results in the production of reduced metabolites (NADH, FADH), which in turn leads to the enhanced decolorization capability.

**Effect of nitrogen sources**

The effect of various organic nitrogen sources (peptone, yeast extract, tryptone and beef extract) were investigated after 24 h of incubation. Among them, yeast extract was found to be the superior source in maximizing decolorizing ability of the consortium (Table 3). The metabolism of yeast extract is considered to be essential for the regeneration of NADH that acts as electron donor for the reduction of azo bonds (Carliell et al., 1995). Similar results were reported by Deng et al., (2008) and Vijayanand et al., (2017).

**Phytotoxicity study**

Phytotoxicity tests were performed in order to assess the toxicity of the untreated and treated Congo red dye samples. *Macrotyloma uniflorum* seeds treated with tap water showed 100% germination, the mean plumule length of 22±0.7 cm and the mean radical length of 8±0.6 cm. *M. uniflorum* seeds treated with control sample (untreated dye) showed 60% germination, the mean plumule length of 12±0.4 cm and the mean radical length of 5±0.5 cm. Interestingly, *M. uniflorum* seeds treated with test sample (treated dye) showed 100% germination, the mean plumule length of 21±0.5 cm and the mean radical length of 7.5±0.4 cm (Table 4). The above mentioned results clearly demonstrated the detoxification of Congo red by TVU-CR4. Similar results were recorded by many researchers (Parshetti et al., 2006; Saratale et al., 2009; Shyamala et al., 2014).

In conclusion, the concerted activities of TVU-CR4 were able to decolorize and degrade the textile azo dye Congo red with significant decolorization rate and required
less incubation time under static condition. The idealized decolorization occurred at temperature of 35°C and pH 7. The decolorization was robust even under high concentration of Congo red. External carbon and nitrogen sources have enhanced the decolorization rate. The foregoing results suggest the potential of utilizing TVU-CR4 to degrade textile effluent containing synthetic textile dyes via; appropriate bioreactor operation.

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