

BIODEGRADATION OF TEXTILE DYE ANTHRAQUINONE VAT BLUE 4 BY *Pseudomonas aeruginosa*

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ABSTRACT

Uncontaminated and Vat blue 4 contaminated soil were screened for heterotrophic bacterial population and the bacterial density were found to be 19.3×10^4 and 5.5×10^4 CFU/gm respectively. The bacterial genera of dye contaminated soil was dominated by *Pseudomonas* sp. (32.5 %) followed by *Bacillus* sp. (27.5 %), *Aeromonas* sp. (15.0 %), *Micrococcus* sp. (12.5 %) and *Achromobacter* sp. (12.5 %). The optimum inoculum load, pH and temperature were found to be 5%, 7 and 35°C respectively. The initial pH of the effluent prepared using Vat Blue 4 was 8.5. The free cells of *P. aeruginosa* reduced the pH to 7.44 after 24 hours of treatment. The values of TDS, BOD₅, COD and colour removal of Vat Blue 4 were reduced up to 62.21, 89.00, 87.95, and 95.00 % respectively by *P. aeruginosa*.

Keywords: *Pseudomonas aeruginosa*, Anthraquinone Vat blue 4, Biodegradation, Decolourization, dyeing industry effluent, Textile effluent

INTRODUCTION

Rapid industrialisation and tremendous increase of human population increase the conventional solid and liquid waste and it create problems to environment and mankind. One of the most important problems is the textile dye pollution to

land and water. Generally, dyes are coloured compounds, which are capable of being fixed in fabric and are classified in accordance with their application. Synthetic dyes are extensively used in textile, paper, pharmaceutical, food and cosmetic industries [1, 2, 3]. In this connection,

decolourisation of textile dyes has caused much industrial and scientific concern. Their abatement through the process of degrading them prior to disposal deserves scientific and public concern as they are as such mostly carcinogenic or mutagenic. In recent years, the method of bioprocessing of dyes has gathered much scientific concern. Many methodologies such as membrane filtration [4], chemical coagulation [5], ion exchange [6] and ozone treatment [7] are being developed for processing textile wastewater. A review of different methodologies adopted to process these wastewaters proves that biological treatment is the best opted methodology as it does not adversely influence the water quality characteristics of the wastewater which in turn will decide the survival of variable biotic constituents.

Vat Blue 4 is one of the common dyes used in the dyeing process of silk and cotton and they appear in wastewaters of dyeing industries in Tamil Nadu. Though few studies have been carried out on the biodegradation of the reactive anthraquinone dyes, there is paucity of research on anthraquinone vat dyes. The present study focuses on the prime objective of investigating the biodegradation of anthraquinone vat blue 4 using indigenous bacteria, *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

The dye-contaminated soil samples were collected from Kallidaikurichi, of Tirunelveli district, Southern Tamil Nadu, which is well known for handloom production with flourishing dyeing units. The soil samples were collected both from uncontaminated and the dye contaminated sites in clean and sterile polythene bags. The samples were kept in icebox and then brought to the laboratory for microbiological analysis. The total heterotrophic bacterial population of the soil were analysed by the pour plate technique employing nutrient agar. Morphologically dissimilar and prominent colonies were picked and restreaked on nutrient agar to ensure purity and were identified up to the generic level [8]. The Bergey's manual of

Systematic bacteriology [9] was also referred in the identification procedures.

Different concentrations of 100 to 1000 ppm Vat Blue 4 were incorporated individually in the nutrient agar plates. 1.0 ml of the soil suspension was pour plated on the dye incorporated nutrient agar plates and incubated at 37°C for 24 to 72 hours and bacterial growth was observed. The dye degrading bacteria isolated from sample with high concentrations of dye were taken up for further studies. Four bacterial strains were selected (DS-1, DS-2, DS-3 and DS-4) based on the efficiency of the dye degradation ability. To evaluate their dye degradation efficiency these isolates were streaked on dye incorporated plates. *Pseudomonas aeruginosa*, DS 2 was able to produce clear and distinct zone of clearance of dye around the growth. This strain was selected for further investigation.

The absorption maximum for Vat Blue 4 was observed to occur at 650 nm. Hence the decolourisation was assayed at 650nm using a spectrophotometer. The nutrient broths (250 ml) incorporated with dye at ten different concentrations (100 to 1000 ppm) was inoculated with 24 hour old bacterial culture of *P. aeruginosa*, DS 1 at 5 % (v/v) concentration and incubated at 37°C for 48 hours. Aliquots were withdrawn from the culture broth at 12-hours intervals and centrifuged at 10,000 rpm for 10 minutes in a tabletop centrifuge and the bacterial cells were removed as pellets. The supernatant was analysed spectrophotometrically at 650 nm and the decolourisation was calculated using the following formula [10]

Percentage of decolourisation =

$$\frac{\text{Initial absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

Initial absorbance value

As a preamble to use *P. aeruginosa* for decolourisation experiments, optimization studies with regard to pH, temperature and inoculum load were also carried out.

Effluent containing vat blue 4 was collected from the dyeing unit for conducting bioremediation studies. The effluent was a mixture of anthraquinone dye, inorganic salts and water. Decolourisation of vat blue 4 by free

cells of *P. aeruginosa* was further investigated. The effluent was incorporated with vat blue 4, to get the optimum dye concentration (900 ppm). The 24 hours old culture of *P. aeruginosa* at the concentration of 5 % (containing 35×10^4 counts of DS-2) was inoculated into the effluent prepared. The physico - chemical parameters like pH, TDS, BOD₅ and COD of both the raw and treated effluents were assessed using the standard procedures [11]. The rate and extent of colour removal were analysed after 24 hours adopting the standard procedures [10].

RESULTS

Isolation and Identification of dye degrading bacteria

The values of total heterotrophic bacterial population of the uncontaminated soil and dye contaminated soil were determined by adopting pour plate technique. They were found to be 19.3×10^4 CFU/gm and 5.5×10^4 CFU/gm respectively. With the view of understanding the distribution of the heterotrophic bacteria in the study area, comparison was made between the uncontaminated and contaminated soil. Student's 't' test analysis affirmed that significant variation prevailed between the 't' tests conducted ($P < 0.001$). In the present study, a total number of forty bacterial strains were isolated and identified from the dye contaminated soil sample. The bacterial genera of dye contaminated soil were dominated by *Pseudomonas* sp., (32.5 %) followed by *Bacillus* sp., (27.5 %), *Aeromonas* sp., (15.0 %), *Micrococcus* sp., (12.5 %) and *Achromobacter* sp., (12.5 %) as shown in Table 1. Since the dominant bacterial species *P. aeruginosa* exhibited normal growth up to 1000 mg/l concentration and produced distinct zone of clearance of dye around their growth up to 1000 mg/l it was selected for further studies.

Optimization studies

The optimal amount of inoculum required for the degradation of Vat Blue 4 was determined. The inoculum load of 5 % was observed to bring about efficient decolourisation of Vat blue 4 within 24 hours (data not shown) (Fig.1). DS-2 brought about the complete decolorization of

100 to 400 mg/l of Vat Blue 4 within 12 hours. Within 60 hours of inoculation total decolorization was observed up to 900 mg/l of Vat Blue 4 (Fig.2).

The temperature optima of the efficacy of DS-2 strain in dye degradation of various concentrations such as 300, 500 and 700 mg/l were assessed in 30, 35, 40 and 45°C temperatures and the results were presented in Fig.3. The temperature optimum of DS 2 was found to be at 35°C.

Among the various pH tested (4, 5, 6, 7, 8, 9 and 10) DS-2 had the maximum decolorization at pH 7 for all the three concentrations such as 300, 500 and 700 mg/l (Fig.4) tested. With DS-2 strain at neutral pH, 100 % of decolourisation was achieved after 12 hours especially at the concentration of 300 and 500 ppm. In the case of 700 ppm total decolourisation could be observed after 24 hours.

Biodegradation and Decolourisation studies

Table 2 depict the ability of biodegradation and decolourisation of anthraquinone dye by the free cells of *P. aeruginosa*. The initial pH of the effluent prepared using Vat Blue 4 was 8.5. The free cells of *P. aeruginosa* reduced the pH to 7.44 after 24 hours of treatment. The values of TDS, BOD₅, COD and colour removal of Vat Blue 4 were reduced up to 62.21, 89.00, 87.95, and 95.00 % respectively by *P. aeruginosa*.

DISCUSSION

The bacterial cultures meant for bio bleaching were isolated from the dye contaminated soil near the dyeing industries. *Pseudomonas* sp. was found to be the predominant genera constituting 32.5 % and it was followed by *Bacillus* spp (27.5%), *Aeromonas* sp. (15.0%) and *Micrococcus* sp. (12.5.0%) and 12.5% of *Achromobacter* sp. It deserves mention here the samples of the present study were collected from an area contaminated by anthraquinone dyes. Mariappan *et al.* [12] reported that the heterotrophic bacterial count in sediments ranged from 13.2×10^7 to 32.0×10^7 CFU/gm of soil. When compared to the present study, higher number of total viable bacterial count (8×10^6 CFU/ml) was reported for the azo dye contaminated water of river Bhadar [13 -15]

Jadhav *et al.* [16] have reported isolation of organisms adapted to high dye concentration from sites near textile industries complex. The appreciable amount of THB recorded in the soil ecosystem by the above authors could be attributed to continued exposure of the heterotrophic microbial population to the dye enriched wastewater. Perhaps, viable isolates may thrive and multiply to sustain the decomposition of organic matter in the bottom of the soil even in the presence of high dye containing overlying water. A trend similar to the present study in the generic composition especially with reference to *Pseudomonas* sp., (30.0 %), *Bacillus* sp., (23.3 %), *Micrococcus* sp., (13.3 %) and *Achromobacter* sp., (13.3 %) was reported by Sudhakar *et al.* [17]. Six bacterial strains with commendable capability of degrading textile dyes were isolated from sludge and lake mud samples by Chen *et al.* [3]. They selected *Aeromonas hydrophila* for further research because of its comparatively higher efficiency in colour removal. Three species of *Pseudomonas* and one species each of *Bacillus* and *Escherichia* were isolated by Mariappan *et al.* [12] from the azo dye contaminated soils collected from Nagercoil and Rajapalayam of Tamil Nadu. Prevalence of dye resistant microorganisms in dye-contaminated soil was reported by Knapp and Newby [18]. It is well known that soil is predominantly inhabited by the gram negative *Pseudomonas* sp. and by virtue of the presence of a wide variety of extra chromosomal genetic material it survive environmental hostilities; *Pseudomonas* sp BSP-3 and BSP-4 have been recorded to produce distinct zone of clearance of azo dye Black-E in concentrations up to 300 ppm [12].

The necessity of optimization experiments especially in dye degradation was formerly reported by Chen *et al.* [19]. In the present investigation the maximum inoculation attempted was 5. Further enhancement of the dose of the bacteria especially as immobilized cells was observed to low the pH up to 6.9 and since the pH below this is not suitable for the survival of fishes. Hence the levels beyond 5 % inoculum were never attempted in the present

investigation. Sudhakar *et al.* [12] has reported such pattern of relationship among the concentration of azo dye (100 ppm), inoculum load (3.0 %) and time of exposure (36 hours) in their experiments with azo dye Black-E employing the native bacterium *Pseudomonas* BSP-4. Red RBN was decolourised (95.0%) by *Proteus mirabilis* within twenty hours at a dye concentration of 1.0 g/l [19]. Knapp and Newby [18] achieved maximum degradation of azo dyes using bacterial strains at neutral pH. Zimmermann *et al.* [20] also employed neutral pH for the degradation of azo dye using *Pseudomonas* KF 46. *Proteus mirabilis* has been observed to accomplish maximum colour removal at the optimal pH ranging from 6.5 to 7.5 [19]. Chang *et al.* [21] proved that *Pseudomonas luteola* caused rapid decolourisation of an azo dye Reactive Red 22 at pH ranging from 7 to 9. Grigsby *et al.* [22] also reported an enhanced degradation of azo dyes at 37°C by native micro flora. *Proteus mirabilis* displayed a good colour removal at an optimum temperature ranging from 30 to 35°C [19]. Chang *et al.* [21] registered an optimum decolourisation of Reactive Red 22 at 37°C by *P. luteola*. Ability of *Pseudomonas aeruginosa* CR-25 to decolorize and degrade reactive dye Remazol Black B (RBB) was studied under static and shaking conditions by Joe *et al.* [23]. They obtained a 96% dye decolorization at 50mg/L dye concentration; however, it reduced to 60% when dye concentration increased to 300mg/L. The strain had best decolorization at 37°C and pH 7 under static conditions.

Among the several bacterial strains *P. aeruginosa* is found to be potential strains with synergistic mode of bio bleaching the coloured effluents. About 60 to 70 % of colour removal has been observed and is associated with 2 to 3 fold decrease in BOD₅ and COD as reported by Ashoka *et al.* [24]. Sani and Banerjee [10] effected the decolourisation of synthetic effluents of azo dye by growing cells of *Kurthia* sp and in case of Magenta, nine-hour-old broth of *Kurthia* sp. was used for decolourisation. They also recorded a COD reduction to the tune of 90.00 %. The free cells of *P. aeruginosa*

brought about 100 % decolourisation of Orange I (40 hours) and Orange II (72 hours) at concentrations ranging from 50 to 100 ppm [25]. Sarnaik and Kanekar [26] reported that *P. alkaligenes* and *P. mendocina* degraded 4 di and tri phenyl methane up to 78 % in 15 hour at a concentration which is similar to that of the real effluent. Methyl Red (100 mg/l) has been reported to be completely degraded in seven days by *Aeromonas liquefaciens* [27]. Hu [28] investigated the aerobic decolourisation of Red G, Remazol Black B, RP₂B and V₂RP (0.1 % w/v concentration) by free cells of *P. luteola* and reported that the dyes were degraded at the rate of 76, 55, 23 and 51 % respectively within 120 hours. The free cells of methanogenic culture have been observed to effect 90.10 and 95 % of colour removal of the anthraquinone dyes, RB 4 and RB 19 [29]. Fontenot *et al.*, [30] achieved an incomplete degradation i.e., 78% colour removal of anthraquinone dye RB 4 in both amended and unamended cultures at 35⁰ C. *Pseudomonas* sp. has been observed to bring about a COD removal of 84 % in Congo red and 85 % in Direct Black within five days [31]. In simulated wastewater the values of BOD removal efficiency recorded under aerobic condition, static and shaking condition and aeration-static-aeration have been recorded to be 59.37, 87.50 and 90.62% respectively [32]. Under anaerobic treatment condition along with the usage of a fluidized bed reactor Sen and Demirer [33] recorded 82.00% reduction of COD, 94.50 % of BOD₅ and 59.00 % reduction of colour. Under aerobic conditions with a bacterial consortium Buitron *et al.* [34] recorded 99 % colour removal and 73 % mineralization of the azo dye Acid red 151 at the concentration of 50 mg/l. Biodecolourisation ability of *B. thuringiensis* was also checked for 100 ppm of C.I. Acid brown 14, C.I. Acid black 210, C.I. Acid violet 90 and C.I. Acid yellow 42 dyes. It brought about 90–98% decolourisation of all the dyes in 24 h; COD and TDS was reduced from 246 to as low as 34 ppm and 668 to 187 mg/l within 24 hours due to biological treatment [16]. Direct Red 5B concentration decolorized was 1100 mg/l in nutrient broth within 125 hours

[17]. Removal of COD was 94% at static condition [17]. *Commomonas* UVS showed 100% decolorization of Direct Red 5B (DR5B) dye at 40°C and pH 6.5. Joe *et al.* [24] observed that the initial COD of the medium consisting of 100mg l⁻¹ RBB was 2000 mg l⁻¹. After the treatment with *P. aeruginosa* CR-25 for 24h, the COD was only marginally reduced to 1962mg l⁻¹ with 90% dye decolorization. After 24hours, the decolorized sample was subjected to shake flask conditions and COD was measured at 30, 36, 42 and 48 hours. The COD values were correspondingly reduced to 1490, 1080, 422 and 340mg l⁻¹ at 30, 36, 42 and 48 hours respectively. There was regular reduction in COD after every 6 hours under shake flask conditions and finally it was reduced to 340mg l⁻¹. Efficient decolorization of textile industry effluent containing disperse dye Scarlet RR was achieved by a developed bacterial-yeast consortium BL-GG within 48 hours with 68 and 74% reduction in BOD and COD values respectively, while the same consortium decolorized the solution containing single dye Scarlet RR (50 mg l⁻¹) within 18 hours (pH 9.0, 40 °C, static condition) with 98% reduction in color [35].

Most of the research works conducted so far with textile dyes were mainly concerned with BOD, COD and colour removal. They have aimed at the determination of optimum pH for the bacterial strains to efficiently degrade the textile dyes. However, there is brevity of information in these studies regarding the fate of reduction of TDS and pH. The permissible pH limit for the discharge of textile industry effluent is to the tune of pH 9 [29]. It warrants mention here that during the process of biodegradation this pH limit was never observed to surpass the stipulated hyper pH condition.

CONCLUSION

The present study on biodegradation of anthraquinone vat dyes is first of its sort and provides first hand information on the degradation of anthraquinone vat dyes which are commonly used in the dye industry in South India especially Tamil Nadu. Moreover, it

deserves mention here that most of the dyeing industry effluents pollute many of the aquifers, which supply fresh water to the public living in and around the dyeing industrial units. Absence of perennial rivers in Tamil Nadu enhances this problem, as the wastewater cannot be diluted to non-hazardous levels. Hence biological methods need to be developed in such areas to check the extent of pollution of these dye wastes and their impact on the wastewater receiving aquatic ecosystems.

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Tables and Figures:

Table 1: Percentage prevalence of different bacterial genera of the dye contaminated soil

S. No	Bacterial genera	Total no. of isolates	No. of strains identified	Percentage prevalence
1	<i>Pseudomonas</i> sp	40	13	32.5 %
2	<i>Bacillus</i> sp	40	11	27.5 %
3	<i>Aeromonas</i> sp	40	6	15.0 %
4	<i>Micrococcus</i> sp	40	5	12.5 %
5	<i>Achromobacter</i> sp	40	5	12.5 %

Table 2. Decolourisation of effluent of anthraquinone dye Vat Blue 4 by free cells of *P. aeruginosa* (DS 2)

Parameters	Control	Treated with <i>P. Aeruginosa</i> (Mean Value)	Extent of reduction (%)
pH	8.5	7.44	87.52
TDS*	3215	2000	62.21 %
BOD ₅ *	3670	3250	89.00 %
COD*	8254	7260	87.95%
Color (optical density value)	1.95	0.10	0.10 (95.00%)

- **In the case of TDS, BOD₅ and COD the quantities removed after treatment are furnished**



Fig.1. Decolourisation of Vat Blue 4 by DS-2



