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Optimisation for enhanced decolourization and degradation of Reactive Red BS C.I. 111 by *Pseudomonas aeruginosa* NGKCTS

N. T. Sheth · S. R. Dave

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Abstract Soil samples collected from dye contaminated sites of Vatva, Gujarat, India were studied for the screening and isolation of organisms capable of decolourizing textile dyes. The most efficient isolate, which showed decolourization zone of 48 mm on 300 ppm Reactive Red BS (C.I.111) containing plate, was identified as *Pseudomonas aeruginosa*. Reactive Red BS (C.I.111) was used as a model dye for the study. The isolated culture exhibited 91% decolourization of 300 ppm dye within 5.5 h over a wide pH range from 5.0 to 10.5 and temperature ranging from 30 to 40°C. The culture was able to decolourize more than 91% of Reactive Red BS under static conditions in presence of either glucose, peptone or yeast extract. Addition of 300 ppm of Reactive Red BS, in each step, in ongoing dye decolourization flask, gave more than 90% decolourization within 2 h corresponding to 136 mg l^{-1} h^{-1} dye removal rate. The isolate had the ability to decolourize six different reactive dyes tested as well as the actual dye manufacturing industry's effluent. The degradation of the dye was confirmed by HPTLC.

N. T. Sheth
Microbiology Department, Gujarat Vidyapith, Sadra 382 320, India

S. R. Dave (✉)
Department of Microbiology, School of Sciences, Gujarat University, Ahmedabad 380 009, India
e-mail: shaileshrdave@yahoo.co.in

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Introduction

Synthetic dyes are extensively used in textile dyeing, paper printing, colour photography, pharmaceutical, food, cosmetics and other industries. Approximately 10,000 different dyes and pigments are used industrially and over 7×10^5 metric tonnes of synthetic dyes are produced annually worldwide (McMullan et al. 2001). During textile dyeing, the amount of dye lost in effluent is dependent upon the class of dye used, varying from only 2% loss when using basic dyes to a 50% loss when reactive dyes are used (McMullan et al. 2001). Approximately 20% of these losses enter the environment through effluents from wastewater treatment plants (Kirk and Othmer 1993). In India, an average mill discharges about 1.5 million litres of contaminated effluent per day, which leads to chronic and acute toxicity (Sandhya et al. 2005). On the other hand, certain azo dyes exhibit toxic effects towards microbial populations and can be toxic, mutagenic and/or carcinogenic to animals (Pavan et al. 2008). Also, the possible contamination of drinking water supplies is of concern because azo dyes are known to be enzymatically degraded in

the human digestive system, producing carcinogenic substances (Kirk and Othmer 1993).

Methods of effluent treatment for dyes may be classified broadly into three main categories; physical, chemical and biological. The physical and chemical methods include adsorption, flocculation, coagulation, irradiation, incineration and ultra filtration. The main disadvantages of physical and chemical methods are (1) difficulty in handling and (2) requirement of high capital costs (Chakraborty et al. 2003; Georgiou et al. 2003; Papić et al. 2000).

In recent years, some fundamental work has revealed the existence of a wide variety of microorganisms capable of decolourizing a broad range of dyes (Dave and Dave 2009; Mielgo et al. 2002; Robinson et al. 2001; Stolz 2001). *Pseudomonas luteola* is reported for decolourization of reactive azo dyes, but it was not effective under continuous shaking or completely static condition (Hu 1994, 2001). Limited data is available on decolourization of Reactive Red BS (C.I.111), which is diazo, vinyl sulphone dye. Moreover, the time required for the decolourization of the model dye as well as other tested azo dyes was less than 24 h. Therefore, the possibility of biodecolourization of Reactive Red BS (C.I.111) was exploited with the following objectives: (1) isolation and characterization of culture capable of decolourizing the model dye, (2) optimization of decolourization parameters, (3) confirmation of degradation of the dye, (4) to assess the toxicity of the degraded product, (5) to assess the ability of the culture to decolourize higher concentrations of Reactive Red BS (C.I.111) and actual dye industry waste.

Materials and methods

Dyes and media

Dyes

The dyes used for the decolourization and degradation studies were Reactive Red BS (C.I. RED 111), Reactive Magenta HB (C.I. Violet 26), Reactive Violet 5R (C.I. Reactive Violet 5), Red HE8B (C.I. Red 152), Reactive Blue (C.I. Blue 222), Reactive Black G (Mixture of C.I. Reactive Black 5 and C.I. Orange 16) and Reactive Black B (C.I. Reactive Black 5). All these dyes were procured from Ganesh Dye chemical Industry, Ahmedabad, India. Reactive Red BS (C.I. 111) was used as a model dye in the study.

Nutrient media

Pseudomonas aeruginosa NGKCTS was routinely grown at 31 ± 2°C in Nutrient Broth (NB) containing g l^{-1}: peptone, 10.0; meat extract, 3.0 and NaCl, 5.0. Medium pH was adjusted to 7.4 ± 0.2.

For medium optimization 2^3 factorial experiments were designed in NB supplemented with g l^{-1}: glucose, 10; yeast extract, 2; peptone, 10 and Reactive Red BS, 300 mg l^{-1}. The culture was preserved on Nutrient Agar (NA) slants.

For toxicity assay, Ashby's Mannitol Agar (AMA) containing g l^{-1}: mannitol, 20; K_2HPO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.2; K_2SO_4, 0.1; $CaCO_3$, 5.0; agar, 30.0; and Yeast Extract Mannitol Agar (YEMA) containing g l^{-1}: yeast extract, 1.0; mannitol, 10.0; NaCl, 0.1; $MgSO_4 \cdot 7H_2O$, 0.2; K_2HPO_4, 0.5 and agar, 30.0 were used. The chemicals and media components used in the study were of analytical grade procured from S.D. Fine Chemical and HiMedia Laboratories respectively.

Isolation, inoculum preparation and preservation of decolourizing bacteria

Dye contaminated soil samples were collected from textile processing house, Vatva, Ahmedabad, India. Soil sample of 1.0 g was added to 100 ml sterile distilled water in 250 ml Erlenmeyer flask. The inoculated flasks were kept on environmental shaker agitating at 120 rpm and 31 ± 2°C for 24 h. The obtained suspensions were diluted 100 times with sterile normal saline and 0.1 ml of this was streaked on NA plates containing 300 ppm of dye and decolourization zone was measured. Colony showing the largest zone of decolourization was transferred on another plate containing 300 ppm dye, and the procedure was repeated for five times. The selected culture was preserved on NA slant containing 300 ppm of Reactive Red BS. A cell suspension (3×10^7 cells ml^{-1}) from preserved slant was inoculated in NB containing 300 ppm of dye. This was incubated at 31 ± 2°C. On 90–95% decolourization of the added dye, 20% (v/v) actively growing culture was used as inoculum for next

cycle. The culture suspension in the decolourized broth was standardised. The cells were harvested by centrifugation at 6,000*g* for 25 min and the inoculum was standardised with respect to optical density of 0.8 at 660 nm, using UV-Visible spectrophotometer (Jasco V-530, Japan).

Identification of dye decolourizing bacteria

Isolate giving largest zone of decolourization of Reactive Red BS was selected and streaked on NA plate for further purification and the process was repeated three times. Suspension prepared from a distinctly isolated colony from the 3rd transfer, was used for the inoculation of various biochemical media. The selected isolate was transferred on BUG medium (Biolog® Inc., USA) and prepared suspension was inoculated in GN Biolog plates as per the standard protocol provided (Biolog 2001). All the media and Biolog® plates were incubated for 6–24 h at 32 ± 2°C. Results were recorded and interpreted for identification using Biolog® software (Biolog Inc., USA).

Genomic DNA was isolated from the pure culture pellet using—GeneiPure Bacterial DNA purification Kit-117290. The 16S rDNA fragment was amplified using high-fidelity PCR Polymerase. Universal 16S rDNA primers and instant ligation kit 105611 were used for the process. PCR product was cloned in pGEMT vector. 1.5 kb insert were sequenced in ABI3100 (16 capillary) sequencer.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and were in the units of the number of base substitutions per site. Codon positions included were 1st + 2nd + 3rd + noncoding. All positions containing gaps and missing data were eliminated from the dataset. There were a total of 1,464 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

Decolourization study and quantification

For the decolourization study, 10 ml of the uninoculated and inoculated broths were centrifuged at 6,000*g* for 25 min (REMI C-24 centrifuge) and the supernatants were checked for decolourization at 520 nm. Dye concentration of the decolourized broth was quantified by comparing its absorbance with the absorbance of known concentrations of Reactive Red BS and this was used to calculate the dye removal rate (mg l^{-1} h^{-1}) and expressed as percentage of decolourization (Chen et al. 1999; Kumar et al. 1997). Percent decolourization was calculated as:

$$\text{Decolourization}\ (\%\ ) = \frac{I - F}{I} \times 100$$

where I = initial absorbance and F = absorbance of decolourized medium.

Optimisation of physicochemical parameters

The influence of static versus agitated condition (120 rpm), inoculum size, effect of pH, effect of dye concentration, reaction time, medium components and continuous addition of dye were studied in terms of decolourization of dye using the spectrophotometer. The effects of supplementation of glucose (0 and 10 g l^{-1}), peptone (10 and 20 g l^{-1}) and yeast extract (0 and 2 g l^{-1}) to Nutrient broth, were studied by 2^3 factorial statistical experiment (Clarke 1994).

If otherwise mentioned, experiments were performed using 20% (v/v) actively growing culture as inoculum containing 3.0×0^7 cells ml^{-1}, 300 ppm dye concentration, and 7.0 ± 0.2 pH, at 31 ± 2°C under static condition. All the experiments were performed in triplicates.

Toxicity assay

The biodegraded/decolourized products were tested for their toxic effect (Chen 2002; Ramsay and Nguyen 2002) on the agriculturally important soil bacterial flora (Gottlieb et al. 2003; Mali et al. 2000). *Bacillus cereus*, *Azotobacter* and *Rhizobium* procured from National Chemical Laboratory (NCL), Pune, India, were inoculated on Nutrient agar, Ashby's mannitol agar and Yeast extract mannitol agar respectively. Two wells of 8.0 mm diameter were made on the respective media containing plates. One well was filled with 0.1 ml of untreated dye and the other with the decolourized centrifuged broth. The plates were incubated at 28 ± 2°C for 48 h. Zone size of inhibition surrounding the well represented the index of toxicity.

Decolourization and degradation analysis of Reactive Red BS

Decolourization and degradation of the dye was analysed using UV-VS spectrophotometer (Jasco V-530, Japan). The decolourized broth was collected in microfuge tubes, centrifuged at 6,000*g* for 25 min and 15 µl of the supernatant was spotted on TLC silica gel $60F_{254}$ (200 × 200 mm) plate supplied by Merck, Germany, using a micro syringe. Mixture of isopropyl alcohol:acetone:ammonia (8:6:6, by volume) was used as the solvent system. The dye chromatogram was observed under visible and ultraviolet light (254 nm). The obtained TLC results were analysed for the degradation of dye (Mali et al. 2000). Biodegradation of Reactive Red BS 111 was further confirmed by HPTLC, Camag, Linomat 5 & Camag TLC Scanner 3 with winCATS software.

Decolourization study of other reactive dyes

The ability of *Pseudomonas aeruginosa* NGKCTS to decolourize other reactive dyes was checked in the same manner as it was performed with Reactive Red BS albeit in place of Reactive Red BS; 300 ppm of Reactive Magenta HB (C.I. Violet 26), Reactive Violet 5, Red HE8B (C.I. Red 152), Reactive Blue (C.I. Blue 222), Reactive Black G (Mixture of C.I. Reactive Black 5 and C.I. Orange 16) and Reactive Black B (C.I. Reactive Black 5) were added to nutrient broth and absorption was checked at λ_{max} of 520, 540, 565, 548, 586 and 588 nm, respectively.

Decolourization of dye manufacturing industry's effluent

The chemically treated effluent was collected from Ganesh Dye Chemicals, Ahmedabad, a dye manufacturing industry. Dye concentration was quantified by comparing it with the colour development of known concentrations of Reactive Red BS. COD (chemical oxygen demand) and pH were measured by standard procedure; 200 ml of this effluent was inoculated with 20% (v/v) developed active culture, in 500 ml Erlenmeyer flask. The system was incubated at 31 ± 2°C for decolourization study.

Results and discussion

Screening and identification

Morphologically distinct five bacterial cultures were isolated from soil samples that were heavily contaminated with reactive dyes. Only three of these isolates exhibited decolourization zone of 7, 19 and 24 mm. All the five isolates were resistant to 300 ppm of Reactive Red BS, suggesting natural adaptation of these isolates, as they were from dye-contaminated samples. Similar findings have been reported by Khehra et al. (2005). The isolate with 24 mm decolourization zone was selected for further study. On the 5th transfer, the selected culture showed decolourization zone of 48 mm. The two fold increase in the activity could be due to selection of most efficient strain from the isolate and improvement in the activity due to adaptation. The isolate is a Gram-negative motile rod, occurring singly. On the dye containing nutrient agar plates, the colonies were translucent and oblong in shape with a long axis parallel to the line of inoculum. On the basis of biochemical characterization and Biolog® GN plate tests (Biolog 2001); it was identified as *Pseudomonas aeruginosa*. The identity of this isolate was confirmed by 16S rRNA genes sequence analysis of 1,464 base pairs and it was designated as *Pseudomonas aeruginosa* strain NGKCTS. The sequence is deposited in GenBank with accession no. FJ556919. Phylogenetic analysis of *Pseudomonas aeruginosa* strain NGKCTS using MEGA4 software can be seen from Fig. 1. Nearest homolog genus-species was found to be *Pseudomonas aeruginosa* strain Y2P3 (Accession No. EU221381.1).

Static versus agitated

During the first five transfers, in NB, the actively growing inoculum required 10 h for decolourization. Thereafter, the culture further acclimatised to the dye concentration in the liquid medium and required only 6 h for 91.1 ± 3.0% decolourization of 300 ppm of Reactive Red BS under static condition. No considerable decolourization was observed when the flasks were incubated in agitated condition (data not shown), suggesting that a facultative anaerobic condition was favourable for the decolourization, which could be due to involvement of azo reductase for

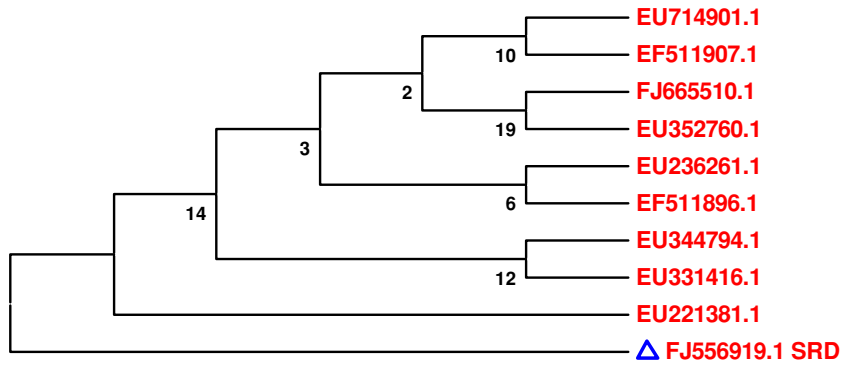

Fig. 1 Phylogenetic analysis of *Pseudomonas aeruginosa* strain NGKCTS (16S ribosomal RNA gene, partial sequence) using MEGA4 software

decolourization of azo dyes. Similar finding was reported by Sandhya et al. (2008). Chang and Lin (2000) have reported that the decolourization of azo dyes by azo reductase is normally inhibited by the presence of oxygen primarily due to competition in the oxidation of reduced electron carriers like NADH with either oxygen or azo groups as electron receptor.

Effect of inoculum size on dye decolourization

The percentage decolourizations obtained with different inoculum size were 91.1 ± 3.0%. As can be seen from Fig. 2, up to 25% (v/v) inoculum size, there was considerable difference in the time required for 91.1 ± 3.0% decolourization. Decolourization rate of 47.53 mg l^{-1} h^{-1} was observed at 20% (v/v) inoculum concentration when the dry weight of the biomass was 0.31 g l^{-1}, thus, dye removal rate comes out equivalent to 153 mg dye g $cell^{-1}$ h^{-1}, which was 8.42 times higher than that reported by Moosvi et al. (2005). Guo et al. (2008) have also reported the maximum azo acid dye removal rate of 5.3 mg dye g $cell^{-1}$ h^{-1}. Beyond 25% up to inoculum size of 50% (v/v) inoculum volume, the time required was not decreased considerably, which indicates that 25% (v/v) was the optimum inoculum size, with a dye removal rate of 57.53 mg l^{-1} h^{-1}. Further rise in inoculum size showed no beneficial effects. Similar observations have been recorded by Moosvi et al. (2005).

Effect of pH on dye decolourization

The Hydrogen ion concentration showed profound effect on the biological activities of the organism (Fig. 3). The optimum pH range for decolourization

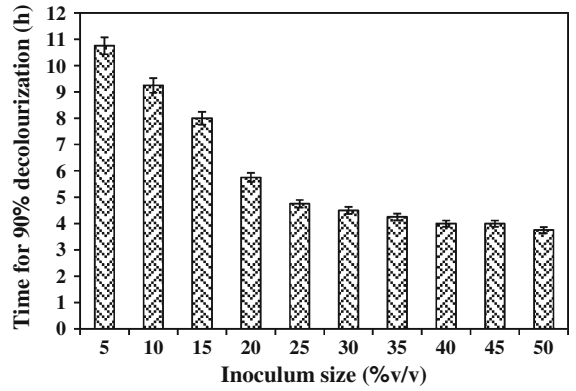

Fig. 2 Effect of inoculum size [(5–50% v/v) containing: 3.0×10^7 cells ml^{-1}], on decolourization of 300 ppm of Reactive Red BS C.I. 111 by *Pseudomonas aeruginosa* NGKCTS. pH: 7; 31 ± 2°C; static condition. Results are means of triplicate experiments (SD is indicated with *error bars*)

was observed between 6.0 and 7.5 wherein decolourization was achieved in less than 6 h. The highest decolourization rate was observed at pH 7.0. Bhatt et al. (2005) also have found that pH 7.0 was optimum for the decolourization of diazo-dye Reactive Blue 172 by *Pseudomonas aeruginosa* NBAR12. Thus, all the remaining experiments were performed at pH 7.0.

Effect of dye concentration

The reactive dye molecule is a complex structure and about 12% of the synthetic textile dyes are lost in wastewater streams. Varying amounts of these dyes are present in the effluents. Keeping this point in mind, the influence of dye concentration on the decolourization ability of the organism was investigated. It can be observed from Fig. 4 that the time

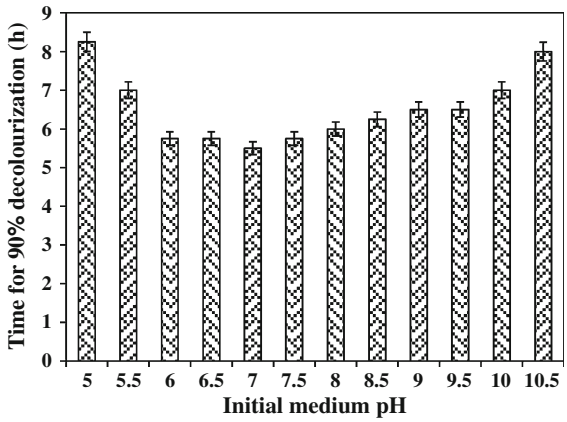

Fig. 3 Influence of initial medium pH (5–10.5), on decolourization of 300 ppm of Reactive Red BS C.I. 111 by *Pseudomonas aeruginosa* NGKCTS. Inoculum size [(20% v/v) containing: 3.0×10^7 cells ml^{-1}]; 31 ± 2°C; static condition. Results are means of triplicate experiments (SD is indicated with *error bars*)

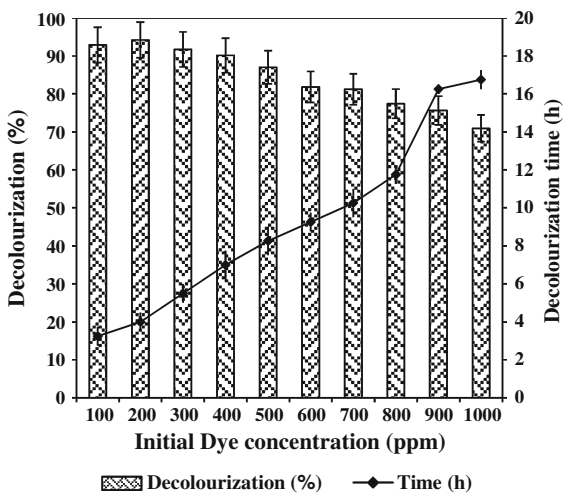

Fig. 4 Effect of Initial dye concentration (100–1,000 ppm), on decolourization of Reactive Red BS C.I. 111 by *Pseudomonas aeruginosa* NGKCTS. Inoculum size [(20% v/v) containing: 3.0×10^7 cells ml^{-1}]; 31 ± 2°C; static condition; pH: 7. Results are means of triplicate experiments (SD is indicated with *error bars*)

required for decolourization of the dye was directly proportional to the concentration of dye in the system. The highest decolourization rate of 55.45 mg l^{-1} h^{-1} was obtained at 700 ppm dye concentration with 81.2% decolourization. Dye concentration of 800 ppm and above showed gradual inhibition of decolourization ability of the organisms.

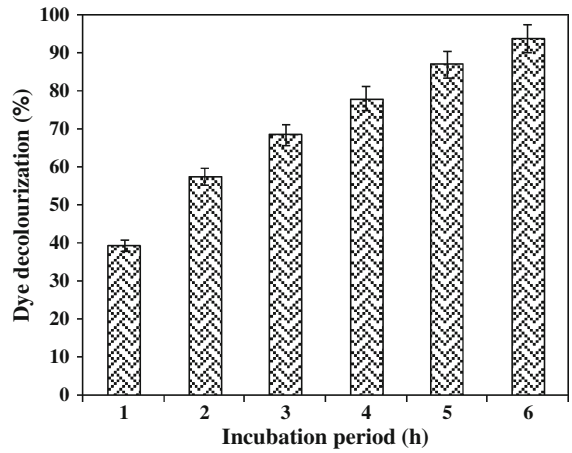

Fig. 5 Time bound decolourization of 300 ppm of Reactive Red BS C.I. 111 by *Pseudomonas aeruginosa* NGKCTS. Inoculum size [(20% v/v) containing: 3.0×10^7 cells ml^{-1}]; 31 ± 2°C; static condition; pH: 7. Results are means of triplicate experiments (SD is indicated with *error bars*)

Decolourization with function of incubation time

Gradual decrease in decolourization rate of the dye was observed with increased time (Fig. 5). In the first hour, 39.36% decolourization was obtained whereas, during 2nd to 6th h of incubation 18, 11, 10, 9 and 7% decolourization was achieved per hour, respectively. This could be due to the actively growing culture used for the study, thus no lag phase was observed and decolourization started immediately after inoculation. Thereafter, observed decrease in decolourization rate with time could be due to the decreased amount of the available dye in the system. This finding shows the need of investigating the influence of fed batch process for the dye decolourization.

Effect of continuous dye addition

The dye industry wastes are continuously discharged from the dyeing and textile industry, therefore the consequence of continuous dye addition in ongoing experiment was investigated and results are shown in Table 1. Up to the sixth addition the time required for about 90% decolourization of 300 ppm Reactive Red BS gradually decreased and it reached from 5.5 h to as low as 2 h, which is 12 times faster than the rate reported by Moosvi et al. (2005) for Reactive violet 5 even with dye amount as low as 100 ppm. The highest dye decolourization rate obtained in this

study was as high as 136 mg l^{-1} h^{-1}. The enhancement in the activity could be due to the generation of increased biomass in on going flask with addition of each new instalment of dye. After sixth addition the time required for decolourization increased and on tenth addition it reached to 6 h with decolourization rate of 30 mg l^{-1} h^{-1}. The observed increase in the decolourization rate up to sixth addition was due to increase in the microbial mass in the system, which increased from 6.0×10^7 to 2×10^9 cells ml^{-1} in the system. The decreased decolourization rate after sixth addition of the dye could be due to accumulation of the metabolites as the experiment was continued in a fed batch manner. When the results of dye addition in one instalment (Fig. 4) and dye addition in multiple fractions (Table 1) were compared, decolourization of 900 ppm of dye added in one lot required 16.25 h but when dye was added in fractions of 300 ppm, 91.1 ± 2.0% decolourization was achieved in 12.75 h. This indicates that dye addition in fraction in continuously ongoing decolourization process is more efficient. This finding suggests that continuous decolourization process with fixed biofilm could be developed to get more benefit.

Media optimization by 2^3 factorial experiments

It was observed that for 92.0 ± 1.0% decolourization the required reaction time ranged between 4.25 h to as high as 8.0 h. The presence of glucose slightly reduced the decolourization ability and the required time period was increased from 5.75 to 7.0 h. Supplementation of nitrogen sources like peptone and yeast extract increased the decolourization ability and time period was reduced from 5.75 h to as low as 4.25 h. When the concentration of glucose, peptone and yeast extract was increased from 0 to 10, 10 to 20 and 0 to 2 g/l, the reaction time was as high as 8.0 h. The shortest reaction time was observed in presence of 20 g/l peptone and 2.0 g/l yeast extract in the medium. For the shortest decolourization time, absence of glucose and presence of peptone and yeast extract are needed. Chen et al. (2003) have also reported that presence of glucose inhibited decolourization activity in six bacterial strains isolated from sludge samples and mud lakes, capable of degrading textile dyes. The reduction in the decolourization ability of *Pseudomonas aeruginosa* NGKCTS in presence of glucose could be due to the preferential use of glucose by the organism as compared to Reactive Red BS.

Table 1 Effect of continuous dye addition into ongoing flask system

Number of dye addition	Total added dye in medium (ppm)	Time (h)	Decolourization (%)
1	300	5.5	91.1 ± 1.0
2	600	4.0	91.1 ± 3.0
3	900	3.25	91.1 ± 2.0
4	1,200	3.0	91.1 ± 1.5
5	1,500	2.75	91.1 ± 1.2
6	1,800	2.0	91.1 ± 2.5
7	2,100	3.0	91.1 ± 3.0
8	2,400	3.25	91.1 ± 2.5
9	2,700	3.25	91.1 ± 1.5
10	3,000	6.0	60.3 ± 3.0

300 ppm of dye was added to the same broth at each step on decolourization

Toxicity assay

No zone of inhibition observed in untreated as well as treated dyes, indicated that the biodegraded or decolourized product was non-toxic to the tested beneficial bacterial flora of the soil. The toxic effect of untreated Malachite green, Brilliant green, Fast green, Methylene blue and Congo red and removal of their toxicity after biological treatment has been reported by Mali et al. (2000).

Decolourization and degradation analysis of Reactive Red BS

Spectrophotometric analysis (at 520 nm) of inoculated medium containing Reactive Red BS showed a decrease in the peak as compared to the uninoculated medium. Evidence of dye removal can be observed with the absorbance at λ_{max} being virtually zero in the medium inoculated with the culture (Fig. 6). Moreover, the peak of the inoculated medium shifted towards the UV region as reported by McMullan et al. (2001).

The degradation of Reactive Red BS 111 was checked on TLC and no spot was observed in visible light whereas spots with different R_f values were observed as compared to untreated dye in UV light

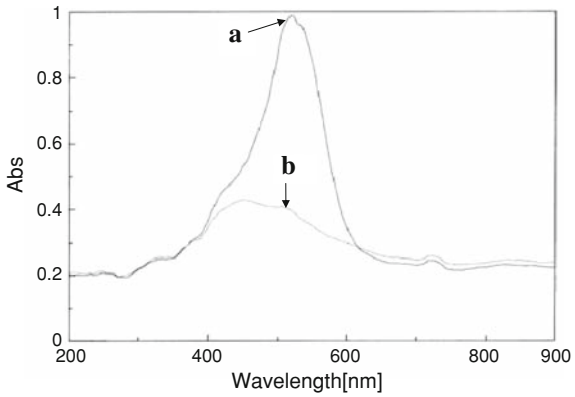

Fig. 6 Spectrophotometric analysis of decolourization of 300 ppm of Reactive Red BS C.I. 111 in nutrient medium with/without *Pseudomonas aeruginosa* NGKCTS, using UV–VS spectrophotometer (Jasco V-530, Japan)

(data not shown). The degradation was further checked by HPTLC analysis, which can be seen from the data (Table 2). When area occupied by all the peaks were considered, peaks 6, 5 and 1 were responsible for major area corresponding to 52.4, 17.2 and 14.2% of total area of the peaks. When biodegradation was considered; 100,100, 97 and 88% reduction was observed in area of peak numbers 4, 7, 6 and 5, respectively, as compared to untreated dye. On the other hand, there was some increase in area of peak number 1 and 8; these peaks mainly correspond to components of NB, which was used as a nutrient medium. Moreover, the development of peak 2 in treated dye was a new development, but it corresponds to only 5.56% of the total area, occupied by all the peaks. This indicates the degradation of major dye components.

Spectrum of reactive dyes decolourization

Pseudomonas aeruginosa NGKCTS was also found to be efficient in removing 85–95% of colour from Reactive Magenta HB (C.I. Violet 26), Reactive Violet 5, Red HE8B (C.I. Red 152), Reactive Blue (C.I. Blue 222), Reactive Black G (Mixture of C.I. Reactive Black 5 and C.I. Orange 16) and Reactive Black B (C.I. Reactive Black 5), even at 300 ppm concentration, in less than 24 h (Table 3). Among the dyes studied under the experimental conditions Reactive Blue (C.I. Blue 222) was found to be the most resistant to the decolourization activity of the isolate. The obtained results show the decolourization ability of the isolate over a broad range of reactive dyes, which could be exploited for the treatment of waste containing multiple dyes simultaneously.

Decolourization of dye manufacturing industry's effluent

The main aim of the entire work was to develop a biological process for the treatment of the industrial effluent. The wastewater from dye manufacturing industry with 10,000 ppm of Reactive Red BS 111 showed 86% decolourization and 76% reduction in the COD in 72 h of contact time (Table 4). The most interesting point is no nutrient addition was needed for the treatment of the industrial waste. The left out

Table 2 HPTLC analysis of the treated and untreated Reactive Red BS C.I. 111 by *Pseudomonas aeruginosa* NGKCTS (winCATS planar Chromatography Manager)

Peak	Positive control			Negative control			Test		
	Rf	Area	% of total area	Rf	Area	% of total area	Rf	Area	% of total area
1	0.13	2670.4	14.19	0.12	1667.2	62.27	0.11	3639.8	62.38
2	0.31	194.1	1.03	–	–	–	0.35	324.4	5.56
3	0.39	507.1	2.69	–	–	–	0.44	462.3	7.92
4	0.51	1614.1	8.58	–	–	–	–	–	–
5	0.61	3221.3	17.38	0.67	125.0	4.67	0.61	401.2	6.88
6	0.71	9832.7	52.28	0.71	275.1	10.28	0.70	229.6	3.93
7	0.81	372.8	1.98	–	–	–	–	–	–
8	0.95	361.5	1.87	0.94	610.0	22.78	0.93	777.5	13.33

Positive control: nutrient broth + 500 ppm dye (uninoculated); negative control: nutrient broth; test: decolourized broth (inoculated) (Solvent system isopropanol:acetone:ammonia; 8:6:6, by volume)

Table 3 Decolourization spectrum of reactive dyes by *Pseudomonas aeruginosa* NGKCTS

No.	Dye (C.I. number)	λ_{max} (nm)	Incubation time (h)	Chemical nature	Decolourization (%)
1	Reactive Red HE8B (C.I. Red 152)	520	7.00	Diazo	92.75
2	Reactive Magenta HB (C.I. Violet 26)	540	4.75	Monoazo	93.30
3	Reactive Blue (C.I. Blue 222)	565	23.50	Diazo, vinyl sulphone	84.95
4	Reactive Violet 5R (C.I. Reactive Violet 5)	548	4.50	Monoazo, vinyl sulphone	95.63
5	Reactive Black G (C.I. Reactive Black 5 + C.I. Orange 16)	586	11.00	Diazo, vinyl sulphone	85.21
6	Reactive Black B (C.I. Reactive Black 5)	588	10.25	Diazo, vinyl sulphone	88.55

The concentration of respective dye in each of the broth was 300 ppm

Table 4 Characteristics of dye manufacturing industry's effluent

Waste	Characteristics of the waste		
	pH	COD (mg l^{-1})	Dye concentration (mg l^{-1})
Before treatment	7.43	2,746	10,080
After treatment	7.76	640	1,390

nutrient carried along with inoculum was found to be sufficient for the decolourization activity. This finding could make the process economically viable.

Conclusions

- The optimum conditions for *Pseudomonas aeruginosa* NGKCTS to degrade Reactive Red BS (C.I.111) were pH 7.0, inoculum size 25% (v/v) and at static condition.
- Presence of glucose showed negative effects on decolourization.
- Fed batch process was found to be better and decolourization time was reduced to as short as 2.0 h.
- Organisms were able to decolourize other reactive dyes tested and actual industrial effluent.
- Obtained highest decolourization rate was 12 times higher than the highest rate reported. The increased bio-decolourization ability could be due to the selection of efficient strain and optimization of the parameters.

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