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ScienceDirect
Dyes and Pigments 74 (2007) 723–729
DYES and PIGMENTS
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Isolation, characterization and decolorization of textile dyes by a mixed bacterial consortium JW-2

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Received 27 April 2006; accepted 8 May 2006
Available online 23 June 2006

Abstract

Soil samples collected from dye contaminated sites of Jetpur, Gujarat were exploited for isolation of dye decolorizing organism. A microbial consortium JW-2 was selected based on its efficiency, showing maximum and faster decolorization of textile dyes. The consortium consisted of three isolates. Identification of isolates by 16SrRNA technique revealed that the organisms were *Paenibacillus polymyxa*, *Micrococcus luteus* and *Micrococcus* sp. The concerted metabolic activity of these isolates led to complete decolorization of Reactive Violet 5R (100 ppm) within 36 h whereas individual isolates could not show decolorization even on extended incubation. The culture exhibited good decolorization ability in the pH range from 6.5 to 8.5 and temperature from 25 to 37 °C. The consortium showed complete decolorization utilizing low amount of co-substrates like glucose (0.1% w/v) and yeast extract (0.05% w/v) and could also utilize a cheaper carbon source like starch in place of glucose as an alternative co-substrate. The consortium had the ability to decolorize nine different dyes amongst 10 tested. Potential of this consortium JW-2 to decolorize textile effluent containing a mixture of textile dyes is to be carried out using appropriate bioreactors.

Keywords: Bacterial consortium; Identification; 16SrRNA; Textile dyes; Decolorization

1. Introduction

Synthetic dyestuffs are extensively used in textile, paper printing industries and dyehouses. During manufacturing and usage, an estimated 10–15% of total dyes are released into the environment [1]. Even at low concentrations water soluble azo dyes can cause waste streams to become highly colored. In addition to color certain azo dyes and their biotransformation products have been shown to be toxic and in some cases these compounds are carcinogenic and mutagenic [2]. Microbial degradation and decolorization is an environment friendly and cost-competitive alternative to chemical decomposition processes. Many microorganisms belonging to different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolorize azo dyes [3]. Pure fungal cultures have been used to develop bioprocesses for the mineralization of azo dyes [4]. However, the long growth cycle and moderate decolorization rate limit the performance of fungal decolorization system [5]. In contrast, bacterial decolorization is normally faster, but it may require a mixed community to mineralize azo dyes through a combined metabolic mode of anaerobic–aerobic sequence [6]. Mixed culture studies may be more appropriate for decolorization of azo dyes. About 80% of color removal in an effluent sample containing mixture of azo and diazo reactive dyes was observed by using mixed bacterial culture [7]. As the catabolic activities of microorganisms in mixed consortium complement each other, obviously syntrophic interactions present in the mixed communities can lead to complete mineralization of azo dyes [6].

The main aim of this study was to investigate dye decolorizing potential of a consortium isolated from contaminated site of an industrial estate and provide biologically favorable

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doi:10.1016/j.dyepig.2006.05.005

environment required to accomplish dye decolorization in an effective manner.

2. Experimental

2.1. Materials

2.1.1. Dyes

The dyes used in the study were of industrial grade and procured from Mantung Dyestuff Industry, Vatva GIDC, Ahmedabad, India and they were Ponceau Red GR, Procion Golden Yellow HR, Reactive Navy Blue HER, Reactive Blue MR (RBMR), Reactive Black B, Reactive Violet 5R, Red HE8B, Procion Red H7B, Reactive Yellow FG, Reactive Golden Yellow. Reactive Violet 5R (RV5R) was used as a model dye for all the optimization experiments (Fig. 1).

2.1.2. Medium

All chemicals used in the study were of analytical grade. The microbial consortium, JW-2 was routinely grown at 37 °C in Bushnell and Haas broth (BHB) (in g L^{-1}: $MgSO_4$, 0.2; K_2HPO_4, 1.0; $CaCl_2$, 0.02; $FeCl_3$, 0.05; NH_4NO_3, 1.0) with or without glucose (0.1% w/v) and yeast extract (0.05% w/v) (BGY).

2.2. Screening of dye decolorizing bacterial cultures

Soil samples collected from areas around dye industries in Vatva industrial estate, Ahmedabad and washing sites of textiles near textile industrial estate of Jetpur, Gujarat were used for screening of dye decolorizing cultures by enrichment culture technique using BHM amended with dye (RV5R, 100 mg L^{-1}) as a sole source of carbon and energy or along with glucose and yeast extract.

Dye containing media (100 mL) in 250 mL Erlenmeyer flasks were inoculated with 10 mL of soil suspension (10% w/v) and incubated in orbital shaker and under static condition at 37 °C. Repeated transfers were carried out in fresh dye containing media till stable dye decolorizing cultures were obtained showing consistent growth and decolorization during successive transfer. The plates containing dye and media (BGY) were inoculated with the JW-2 culture.

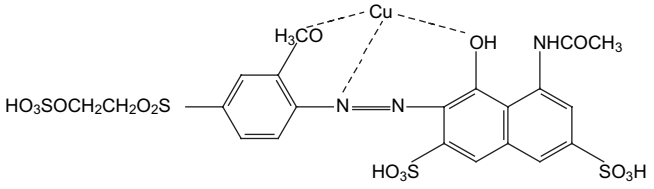

Fig. 1. Structure of Reactive Violet 5R; C.I. no. 18097; λ_{max} − 558 nm.

2.3. Identification

2.3.1. 16SrRNA-based identification

The culture that showed consistent decolorization was subjected to separation on dye containing agar plates. DNA isolation and genomic DNA extraction of the isolated cultures were done as described by Ausubel et al. [8].

2.3.2. PCR primers and amplification conditions

Universal bacterial 16SrRNA gene primers corresponding to *Escherichia coli* position 8f and 1527r were used for polymerase chain reaction (PCR) amplification of the 16SrRNA gene [9]. Purified PCR products were sequenced using internal overlapping primers [9]. Sequence was initially analyzed at NCBI server (http://www.ncbi.nlm.nih.gov/) using BLAST (blastn) tool and corresponding sequences were downloaded. Similarly matrix was prepared using Dnadist program in PHYLIP analysis package [10] using Jukes Cantor corrections. Phylogenetic tree was constructed by neighbour-joining method using the MEGA package. The sequences have been deposited at GenBank with accession numbers DQ 513325, DQ 513326, DQ 513327 for JW-21, JW-22, JW-23, respectively.

2.4. Dye decolorization studies in liquid medium

BGY medium containing constituents of BHM along with glucose (0.1% w/v) and yeast extract (0.05% w/v) amended with 100 ppm of RV5R was inoculated with bacterial culture (5% v/v) and incubated at 37 °C under static or shaking condition. Samples were withdrawn at different time intervals and analyzed for growth and decolorization.

2.5. Study of physicochemical parameters

Decolorization was studied using various carbon and nitrogen sources, at varying pH (3–11.5), dye concentrations (100–500 ppm) and temperature (20, 27, 40, 45, 50 °C).

2.6. Analysis of growth and decolorization

Growth was monitored spectrophotometrically. The cell pellet obtained upon centrifugation (6000 rpm for 15 min) of 5 mL culture was resuspended in 5 mL distilled water and its absorbance was studied at 660 nm. Decolorization was determined by measuring absorbance of culture supernatants at absorbance maxima of respective dyes.

3. Results and discussion

3.1. Screening of dye decolorizing bacterial cultures

Isolation was carried out from various sites contaminated with waste from textile and dyestuff industries. Jetpur was one of the areas explored for isolation of bacterial culture from soil samples for decolorization and degradation of textile dyes and effluent. A consortium JW-2 was isolated from the

washing sites of the Jetpur textile industrial estate by enrichment culture technique employing RV5R dye as sole source of carbon and energy. Isolation was also attempted by employing glucose and yeast extract as co-substrates since majority of reports have obligate requirements of labile carbon source for the functioning of dye decolorizing bacteria [5].

Microorganisms are capable of utilizing a variety of complex chemicals including dyes as their sole source of carbon but only few researchers have been successful in isolating culture capable of utilizing dyes as sole source of carbon [11]. Despite our repeated attempts we were not able to isolate the culture capable of decolorizing RV5R using it as sole source of carbon and energy. However, in presence of glucose and yeast extract as co-substrates a number of cultures capable of decolorizing RV5R were isolated from soil samples collected. But on successive transfer few cultures failed to show consistent decolorization on second and third transfer. Similar experiences have been reported previously by other researchers [12]. An isolate JW-2 showed consistent decolorization on successive transfers and upon prolonged enrichment the culture decolorized RV5R within 38 h. The consortium when subjected to further isolation three isolates JW-21, JW-22 and JW-23 were obtained on solid medium containing BGY amended with dye. Each individual culture was tested for its decolorizing ability on liquid medium in combination with other pure culture and none of the cultures showed decolorization even on extended incubation but all three isolates when mixedly inoculated in liquid medium showed complete decolorization of RV5R. Similar observations were made by Khehra et al. when a consortium HM-4 based on combinations of four selected isolates was developed which showed decolorization efficiency of 20 mg of AR-88 per litre in 20 h whereas the individual isolate took 60 h to achieve complete decolorization [3]. Senan and Abraham also developed a consortium of three organisms to degrade a mixture of dyes by co-metabolism and observed that the consortium could decolorize efficiently all the three dyes tested [13]. Nigam et al. made similar observation during their studies with PDW mixed bacterial culture consisting of two bacterial species (*Alcaligenes faecalis* and *Comamonas acidovorans*) and neither of which had capability of showing decolorization individually. Nigam et al. showed that PDW consortium exhibited decolorization and as well as growth under anaerobic condition, while under aerobic condition they only exhibited growth but not decolorization [14]. However, in our experiments no decolorization was observed under anaerobic condition.

3.2. Identification of bacterial consortium based on 16SrRNA-based identification

Identification of each bacterial culture from consortium was done on the basis of 16SrRNA gene sequence. By the use of internal primers, 1.4 kb sequence of amplified 16SrRNA gene fragment was determined. This amounts to more than 90% of the gene. The sequence of JW-21 showed 99% similarity with *Paenibacillus polymyxa* (AY302439), JW-22 and JW-23 showed 99% similarity with *Micrococcus luteus* (AJ409096) and *Micrococcus* sp. (AJ313024), respectively. Figs. 2 and 3 show the phylogenetic relationship between different members of the genus *Paenibacillus* and isolate JW-21 and the genus of *Micrococcus* and isolates JW-22 and JW-23, respectively.

3.3. Decolorization and growth of bacterial culture under static/shaking condition

Figs. 4 and 5 show the growth and decolorization profiles of mixed bacterial culture under static and shaking conditions, respectively. The consortium exhibited decolorization up to 93% within 38 h under static condition whereas under shaking condition the culture showed 24% decolorization of RV5R dye in 38 h. Under shaking condition the culture though showed faster growth, showed only lesser decolorization. The results clearly indicated that decolorization was not dependent on biomass concentration but was significantly correlated with dissolved oxygen. Maximum decolorization of 72 and 63% was observed under anaerobic and microaerophillic conditions, respectively, in case of *E. coli* [15]. Initial step in bacterial decolorization of azo dyes is azoreductase-mediated reduction of azo bond resulting in the formation of aromatic amines [16]. It is known that azoreductase driven bacterial decolorization of azo dyes is normally inhibited by the presence of oxygen primarily due to competition in oxidation of reduced electron carriers (e.g. NADH) with either oxygen or azo groups as the electron receptor [17]. Microbial growth events and bacterial decolorization events are independent as decolorization via azoreductases is repressed under aerobic conditions [6]. *Aeromonas hydrophila* was observed to be sensitive to DO level and decolorization of azo dye would not take place at

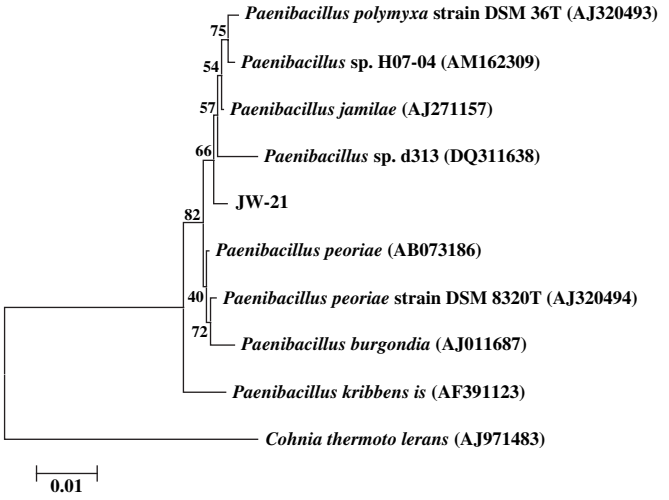

Fig. 2. Phylogenetic tree based on 16SrRNA gene sequence comparisons over 1436 bases showing the relationship between members of genus *Paenibacillus* and isolate JW-21. *Cohnia thermotolerans* has been taken as out group. The sequences have been retrieved from NCBI database and the tree has been drawn using neighbour-joining method in MEGA software. The bar represents distance values calculated in MEGA and values at nod represent percentage of 1000 bootstrap replicates. Numbers in bracket represent GenBank accession numbers.

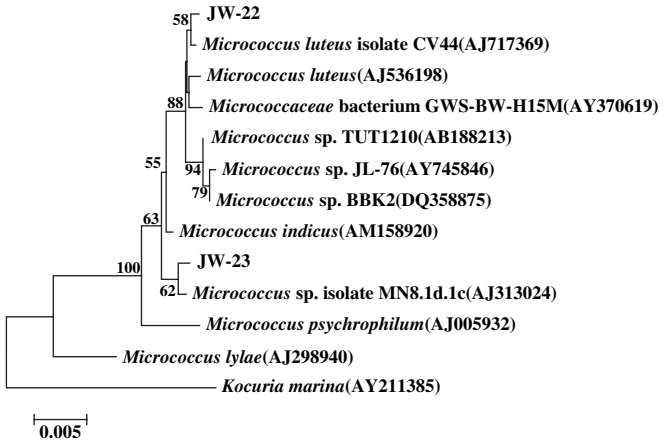

Fig. 3. Phylogenetic tree based on 16SrRNA gene sequence comparisons over 1436 bases showing the relationship between members of genus *Micrococcus* and isolate JW-22 and JW-23. *Kocuria marina* has been taken as out group. The sequences have been retrieved from NCBI database and the tree has been drawn using neighbour-joining method in MEGA software. The bar represents distance values calculated in MEGA and values at nod represent percentage of 1000 bootstrap replicates. Numbers in bracket represent GenBank accession numbers.

a DO concentration higher than 0.45 mg L^{-1} [18]. Therefore, facultative or obligate anaerobes are necessary for azo dye reduction.

3.4. Effect of medium composition

Consortium JW-2 could completely decolorize RV5R only when glucose and yeast extract were supplemented in the medium as co-substrates along with BHM medium. In absence of glucose and yeast extract, bacterial consortium showed lesser decolorization (40%) which indicates the necessity of supplementary carbon source for growth and decolorization of dyes [12]. Dyes are deficient in carbon content and biodegradation without any extra carbon source is found to be very difficult [19]. Using yeast extract as a sole carbon source in the medium decolorization efficiency of RV5R by JW-2 was found to be 25%.

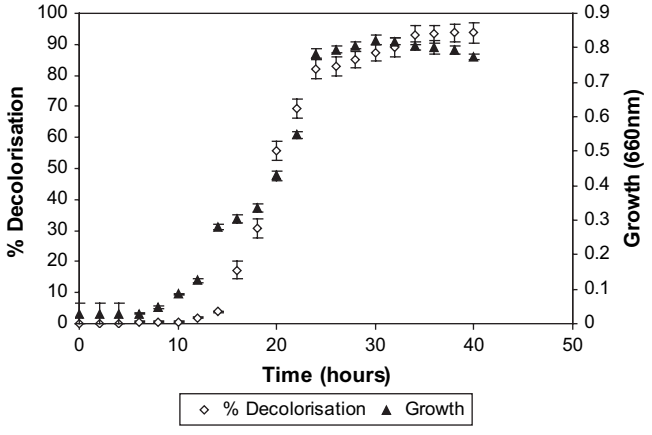

Fig. 4. Decolorization of Reactive Violet 5R by mixed bacterial consortium JW-2 under static condition at 37 °C.

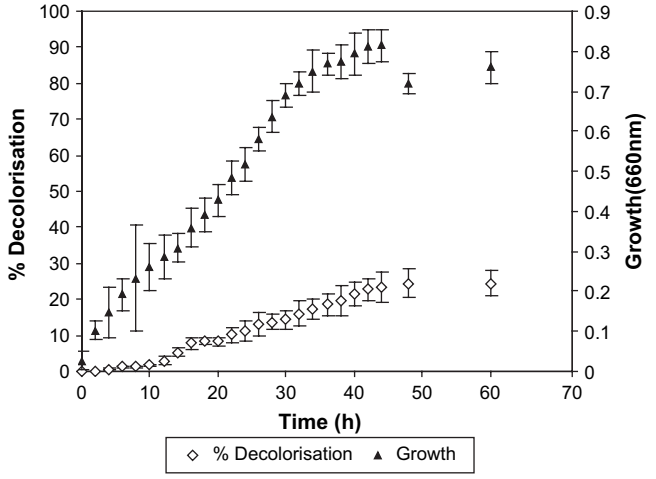

Fig. 5. Decolorization of RV5R by mixed bacterial consortium JW-2 under shaking condition at 37 °C.

The ability to decolorize RV5R in presence of other carbon sources was tested to obtain efficient and faster decolorization (Table 1). Peptone and lactose were found to be poor substrates allowing only 40 and 34% decolorization of RV5R, respectively. Sodium acetate, maltose and fructose showed 65, 77 and 89% decolorization of RV5R, respectively. However, glucose, starch and fructose served as better co-substrates showing 94, 93 and 92% of decolorization, respectively.

Glucose, showing maximal decolorization as co-substrate was tested at different concentrations (0.5–2.5 g L^{-1}). It was observed that increase in concentration of glucose from 0.5 to 1 g L^{-1} showed increase in decolorization from 25 to 93%, respectively, and with further increase in the concentration (1–2.5 g L^{-1}), decolorization was observed about 90%. Hence 1 g L^{-1} of glucose can be considered optimum for decolorization of RV5R. Decolorization of azo dye by *Pseudomonas aeruginosa* was dependent on carbohydrate metabolism [20]. It has been reported that decolorization of certain textile dyes like Orange II, AO8 and AR-88 by *Sphingomonas* sp. strain ICX was observed only in the presence of carbohydrate [21]. The metabolism of glucose resulting in production of reduced nucleotides (NADH, FADH) leads to enhanced decolorization efficiency [3].

Moreover, the ability of the culture to decolorize RV5R in the presence of different nitrogen sources other than yeast

Table 1
Effect of different carbon sources on decolorization of RV5R by bacterial consortium JW-2

Carbon sources	% Decolorization
Glucose	94 (±1.84)
Starch	93 (±1.82)
Fructose	92.4 (±1.31)
Maltose	77.9 (±1.734)
Lactose	31.6 (±1.64)
Sodium acetate	40.3 (±3.11)
Peptone	50.9 (±3.068)
BHM	32.1 (±2.82)

extract was also studied. Ammonium nitrate, ammonium chloride, potassium nitrate, peptone and urea showed poor decolorization ability (39–59%) (Table 2). Only yeast extract served as better nitrogen source and allowed 94% RV5R decolorization. Hu showed that yeast extract is a better nitrogen source for the decolorization of azo dye [22]. The decolorizing efficiency of bacterial culture tested against different yeast extract concentrations showed that concentration of yeast extract at 0.5 g L^{-1} exhibited maximum decolorization of 93% and further increase in yeast extract concentration (1–2 g L^{-1}) showed lower decolorization. *Klebsiella pneumoniae* RS-13 degraded Methyl Red with equal efficiency at glucose concentration from 0.5 to 5 g L^{-1} [23]. Growth and azo dye decolorization efficiency of *Pseudomonas luteola* was directly related to the concentration of yeast extract with 94% decolorization efficiency at 0.3% (v/v) [16]. *A. hydrophila* exhibited effective decolorization of RED RBN in presence of yeast extract resulting in 90% color removal within 2 days. The metabolism of yeast extract is considered essential to the regeneration of NADH that acts as electron donor for the reduction of azo bonds [18].

3.5. Effect of pH and temperature on decolorization

Maximum decolorization of about 90% was observed in a wide range of pH from 6.5 to 8.0 (Fig. 6). Rate of decolorization decreased at lower pH (3–6) and at higher pH (9.0–11.0). Rate of decolorization (2.0–2.5 mg L^{-1} h^{-1}) increased from pH 6.5 to 7.5. *K. pneumoniae* RS-13 was found to completely degrade Methyl Red at pH 6.0–8.0 while *Acetobacter liquefaciens* S-1 completely degraded Methyl Red at pH 6.5 [23]. Mali et al. found that pH between 6.0 and 8.0 was optimum for decolorization of triphenylmethane and azo dyes by *Pseudomonas* sp. *E. coli* and *P. luteola* both exhibited best decolorization rate at pH 7.0 with constant decolorization rates up to pH 9.5 [24]. The strain GM3 of *Pseudomonas* sp. showed its highest activity in narrow pH range of 7–8 and the activity dropped by about 50% when pH was deviated from the slightly alkaline level by 0.5 units [25].

Fig. 7 shows that rate of decolorization of bacterial consortium was the highest at 37 °C showing maximum decolorization of about 93%. Rate of decolorization decreased with decrease in temperature to 20 °C showing 50% decolorization. The culture showed decolorizing ability of about 75% when temperature increased to 45 °C but rate of decolorization

Table 2
Effect of different nitrogen sources on decolorization of RV5R by bacterial consortium JW-2

Nitrogen sources	% Decolorization
Ammonium nitrate	45 (±2.94)
Ammonium chloride	32.9 (±1.95)
Potassium nitrate	31.1 (±1.87)
Peptone	33.7 (±2.92)
Urea	47.4 (±3.047)
Yeast extract	94 (±2.62)

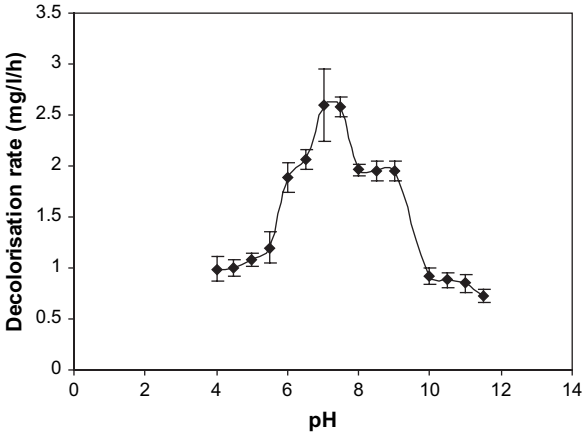

Fig. 6. Effect of pH on decolorization of RV5R by bacterial consortium JW-2.

reduced when temperature was further increased to 50 °C showing decolorization of 50%. This fact implies that the local temperature in the microenvironment of the soil samples has a very significant effect on decolorization activity. Wong and Yuen reported that *K. pneumoniae* RS-1 and *A. liquefaciens* S-1 are mesophiles and they could grow and decolorize *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) under varied temperature (23, 30, 37 °C) [26].

3.6. Effect of initial dye concentration

Rate of decolorization increased proportionately with increase in initial dye concentration up to 100 ppm (Fig. 8). Further increase in dye concentration resulted in slight reduction in decolorization rates. Lower decolorization rate at high dyestuff concentration was reported [27] and was predicted to be due to the inhibitory effects of high dyestuff concentration. Similar observations have been recorded earlier for decolorization of triphenylmethane dyes by *Kurthia* sp. [28].

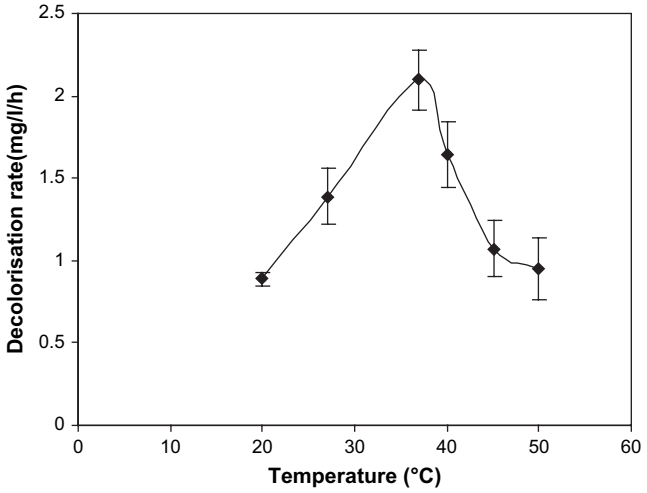

Fig. 7. Effect of temperature on decolorization of RV5R by bacterial consortium JW-2.

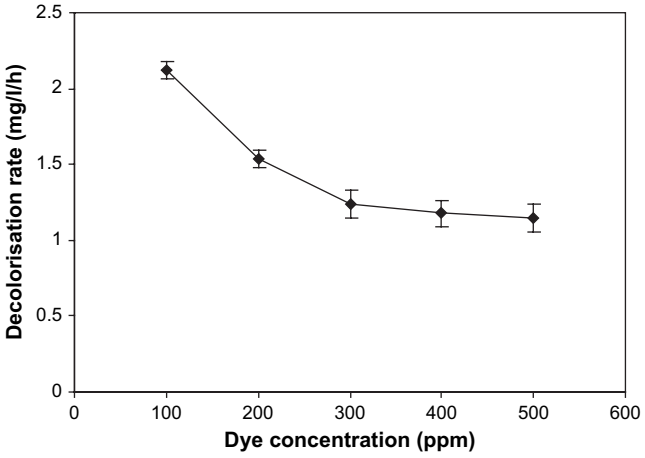

Fig. 8. Effect of dye concentration on decolorization of RV5R by bacterial consortium JW-2.

3.7. Spectrum of dyes decolorized by bacterial consortium

Industrial effluent consists of a mixture of various dyes. Ability of our consortium to decolorize different dyes was studied (Table 3). There was rapid decolorization observed for all dyes used in the study within 24 h. Reactive Navy Blue HER, Reactive Violet 5R, Red HE8B, Procion Red H7B, Reactive Yellow FG, Reactive Black B showed decolorization in the range of 89–92%. Procion Golden Yellow, Reactive Golden Yellow showed decolorization in the range of 60–65%. While RBMR showed only 5% decolorization. This may be attributed to the structural differences [29]. The decrease in decolorization efficiency might be due to the toxic effect of dyes or the blockage of the active sites of azoreductase enzymes by dye molecule with different structures [3]. Low decolorization may also be attributed to the presence of sulphonic groups [30].

4. Conclusion

The consortium containing bacterial cultures *P. polymyxa*, *M. luteus* and *Micrococcus* sp. exhibited good decolorization ability in a mixed form. These bacterial species have not been reported so far to our knowledge for dye decolorization. The culture exhibited good decolorization ability at pH from 6.5 to 8.5 and temperature from 25 to 37 °C, which are normal operation parameters for conventional wastewater treatment systems. The bacterial consortium was able to grow and decolorize dyes under static conditions. Agitated cultures, though exhibited good growth, showed poor decolorization. The mixed culture seems to have potential application in treatment of dye bearing wastewaters as it exhibited efficient decolorizing ability for nine out of 10 dyes tested. The ability of culture to utilize cheap co-substrate such as starch for dye decolorization gives it an advantage for treatment of textile industry wastewaters. However, potential of culture needs to be demonstrated for its application in treatment of real dye bearing wastewaters using appropriate bioreactors.

Table 3
Decolorization of spectrum of dyes by bacterial consortium JW-2

Sr. No.	Dyes	% Decolorization
1	Ponceau Red GR	90 (±1.825)
2	Procion Golden Yellow HR	69.2 (±3.01)
3	Reactive Navy Blue HER	90 (±1.824)
4	Reactive Blue MR	5 (±0.91)
5	Reactive Black B	88.5 (±1.825)
6	Reactive Violet 5R	92 (±2.08)
7	Red HE8B	87.2 (±0.91)
8	Procion Red H7B	93.3 (±2.94)
9	Reactive Yellow FG	88.8 (±1.97)
10	Reactive Golden Yellow R	66.3 (±1.82)

Acknowledgement

The work was supported by Department of Biotechnology, New Delhi, India. I would thank Rashesh Parikh and Dr Yogesh Shouche, NCCS, Pune for the sequencing of cultures.

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