International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 7 Special Issue : 525-535 July 2014

Biochemistry

Bioremediation potential of *Comamonas acidovorans* MTCC 3364 for the removal of sulfonated di-azo dye Reactive Black B

Darshan Rudakiya and Kirti Pawar*

Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences, New Vallabh Vidyanagar, Anand - 388 120, Gujarat, India.

**Corresponding author: drkirtipawar@rediffmail.com*

Abstract

Azo dyes are a chief class of synthetic colorants, which are released by the majority of the textile industries. The effluents of dyes disrupt the ecosystem so removal of these dyes is major concerned by using cost-competitive and eco-friendly method. The present study was aimed to study the decolorization efficiency of the textile azo dyes by *Comamonas acidovorans* MTCC 3364 and optimize the environmental condition for maximum decolorization and degradation of Reactive Black B (RBB) dye. Optimization of various environmental parameters like pH and temperature was studied in which maximum decolorization was obtained at 37°C, pH 7.0 under static condition within 24 hours. The addition of co-substrates lactose and yeast extract increased the rate of decolorization. The bacterial strain was able to decolorize high concentration of RBB dye (1 g l^{-1}) up to 8^{th} cycle. Vanillin was added as a redox mediator which showed the highest rate of decolorization (1.062 mg $l^{-1} h^{-1}$) and thiourea was added as an inhibitor, which showed highest inhibition (0.246 mg $l^{-1} h^{-1}$). Incubation of dye with a non-growing (free) cells and dead cells resulted in removal of dye from the buffer, indicating the biosorption and adsorption mechanism. Immobilization cell studies revealed that activated immobilized cell preparations decolorized RBB dye up to 10 cycles showing remarkable operational stability. The degradation analysis of RBB was further confirmed by HPTLC and FTIR techniques.

Highlights

- Spectrum of different azo dyes was studied to evaluate the efficiency of color removal by *Comamonas acidovorans* MTCC 3364, which depicted maximum color removal of RBB.
- "One factor at a time" methodology was used to optimize the different physico-chemical parameters for RBB color removal.
- Effect of redox mediators and inhibitors revealed that maximum decolorization was obtained in the presence of vanillin whereas thiourea inhibited the decolorization.
- Free living cell and dead cell studies depicted the biosorption and adsorption mechanisms.
- Degradation analysis was done using HPTLC and FTIR techniques, in which degraded products had different R_f values suggested transformation of dye.

Keywords: Azo dye decolorization, *Comamonas acidovorans* MTCC 3364, FTIR spectroscopy, Optimization, Reactive Black B

Synthetic dyestuffs are extensively used in textile, food, leather, paper printing industries and dye houses. Azo dyes account for about one-half of all dyes produced. During manufacturing and usage an estimated 10-15% of total dyes are released into the environment (Verma and Madamwar 2002). Even at low concentrations, water soluble azo dyes can cause waste water streams to become highly colored and remain unaffected (Khalid *et al.,* 2012; Liao *et al.,* 2013). 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 (Daassi *et al.*, 2013). 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 (Khehra *et al.,* 2005; Gahlout *et al.,* 2013). Over the past few decades, major research on the biological degradation of dyes have focused on fungal and its enzymatic systems, whereas the bacterial azo dye removal processes have been less studied (Chang and Lin 2001).

Different physico-chemical treatment are used for the wastewater treatment like flocculation, coagulation, adsorption, membrane filtration, precipitation, irradiation, ozonization and Fenton's oxidation (Lodha and Choudhari 2007; Garg *et al.,* 2013). These methods are in dye removal, particularly for non-ionic dyes are expensive and add operational complexity to the process and generate large amounts of dye contaminated sludge (Saratale *et al.,* 2011). Reactive Black B is an azo dye having high molecular weight (991.82 g mol^{-1}). Degradation of RBB was studied by various physico-chemical methods (Huang *et al.,* 2009; Shih *et al.,* 2013; Perez *et al.,* 2013). Due to the inherent drawbacks of physical as well as chemical approaches to dye removal, the use of biological methods for the treatment of textile wastewaters has received attention as a more cost-effective alternative (Olukanni *et al.,* 2006; Patel *et al.,* 2012).

Different strains of *Comamonas* could degrade distinctive types of azo dyes. *Comamonas* VS-MH2 could degrade a mixture of four distinct reactive azo dyes (Pathak *et al.,* 2011). Direct Red 5B, Direct Blue GLL and Reactive Blue HERD were degraded by *Comamonas* sp. UVS (Jadhav *et al.,* 2008, 2009; 2011). *Comamonas acidovorans* has a vital role in the degradation of natural as well as complex organic compounds like 4-nitrobenzoate and cocaine (Peter *et al.,* 1992; Lister *et al.,* 1996).

Comamonas acidovorans MTCC 3364 has also been routinely reported for bioconversion of different steroids and heavy metal removal (Pawar *et al.,* 2011; Rudakiya and Pawar 2013a, b). The main purpose of study was to investigate effective decolorization of RBB dye by this strain and to study various physico-chemical parameters, co-substrate addition and repeated use of dye. The effect of salinity, redox mediators and inhibitors on decolorization of dye was studied. The preparations of free living cells, dead and immobilized cells were also evaluated for the dye decolorization. UV-Visible Spectroscopy, HPTLC and FTIR spectroscopy were used to analyze the decolorization and degradation of dye.

2. Materials and Methods

2.1 Dyestuff and chemicals

Nutrient broth, nutrient agar, L-tyrosine and vanillin were purchased from Hi-media (Mumbai, India). All other chemicals used in the study were of the highest purity available and of an analytical grade. Reactive dyes used in the study were of industrial grades and dyes were obtained from Manibhadra Enterprise, Ahmedabad, Gujarat (India). All dyes stock solutions (1g l^{-1}) were prepared in distilled water.

2.2 Microorganisms and culture conditions

The strain of *Comamonas acidovorans* MTCC 3364 was purchased from Microbial Type Culture Collections, Institute of Microbial Technology, Chandigarh, India. The pure culture was maintained on nutrient agar slants and stored at 4°C. The organism was sub-cultured every month. Decolorization studies were performed using the nutrient broth medium.

2.3 Spectrum of different reactive dye decolorization

For screening of decolorization potential of the strain towards different dyes, flasks containing 100 ml of nutrient broth were inoculated with 1 ml of previous grown culture

of the strain. Flasks were incubated on a rotary shaker at 120 rpm for overnight. Next day, 100 mg l^{-1} of different sterilized dyes were added to the respective flasks and incubated for 24 hours at 37°C in static or shaking condition. 2 ml of sample was withdrawn at the time of estimation and centrifuged for 10,000 rpm for 5 min to remove cell biomass. The absorbance of supernatant was measured for dye decolorization spectrophotometrically by monitoring the absorbance maxima of the respective dyes using a UV–visible spectrophotometer. The decolorization expressed in % of the dye decolorization was calculated as follows:

Decolorization efficiency (%) = $[(OD_0 - OD_t) / OD_0] \times 100$

Where,

OD_0 is the absorbance at the time of dye addition to the culture,

OD_t is the absorbance after time t of addition of dye to the culture

2.4 Optimization of Reactive Black B decolorization

All parameters were optimized using the single factorial methodology for decolorization of Reactive Black B. All the decolorization experiments were performed in triplicates.

2.4.1 Effect of agitation and time of incubation on dye decolorization

Cells need oxygen for enzyme activity, synthesis of new molecules and generation of new cells. Nutrient medium along with culture in various conditions like static and shaking were kept as well as 100 mg l^{-1} of dye was inoculated at the time of culture inoculation and overnight grown culture at 37°C for 24 hours. % decolorization was measured from respective flasks after 24 hours of inoculation of dye.

2.4.2 Effect of carbon and nitrogen source on dye decolorization

The organism was supplemented with additional carbon and nitrogen sources to enhance the ability of decolorization of dye. The medium was supplemented with additional different carbon sources (2 g l^{-1}) as well as nitrogen sources (2 g l^{-1}). % decolorization was measured from the respective flask after 24 hours of inoculation of dye.

2.4.3 Effect of physico-chemical parameters on dye decolorization

Physico-chemical parameters like temperature and pH are also effective parameters of cells' growth and its' activities so these parameters were optimized for RBB decolorization. 100 ml of overnight grown culture and 100 mg l^{-1} of RBB dye was incubated under static condition at the different temperature (4°C to 60°C) and pH (3 to 11). % decolorization was measured after 24 hours.

2.4.4 Effect of concentration of dye and split dose studies

Different concentrations of dye like 50 mg l^{-1} to 1000 mg l^{-1} were added in the overnight grown culture. Flasks were incubated for 24 hours at 37°C under static condition and % decolorization observed after 24 hours. A spilt dose study was also done using 100 mg l^{-1} of dye along with overnight grown culture. RBB was added repeatedly for eight consecutive cycles. % decolorization was measured after 24 hours in the respective flask.

2.4.5 Effect of medium salinity on growth and decolorization

Flasks containing overnight grown culture were supplemented with different salt concentration (1% to 5% w/v) and inoculated with 100 mg l^{-1} of dye. Control (without salt) was also taken and incubated at 37°C for 24 hours. % decolorization was measured after 24 hours.

2.4.6 Effect of redox mediators and inhibitors on dye decolorization

Redox mediators are intermediate of the metabolism which is studied for the decolorization of RBB. Different redox mediators as well as inhibitors were inoculated with culture medium and dye was inoculated after 24 hours of incubation. Percent decolorization was measured after 24 hours in the respective flask.

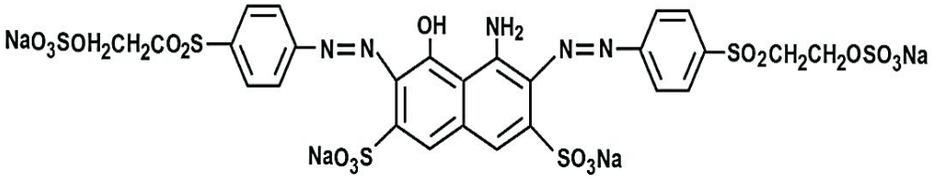


Figure 1: Chemical structure of sulfonated di-azo dye Reactive Black B

2.5 Decolorization using free living cells, dead cells and immobilized cells

Using alternative reaction media like free cells, pre-treated cells, dead cells and cells entrapped in 5% calcium alginate were prepared for decolorization studies. Free cells harvested from pre-decided volume of overnight grown culture were suspended in phosphate buffer (pH 6.85) and one set of the cells was killed by autoclaving. 100 mg l^{-1} dye was added in each set and % decolorization was measured after 24 hours. Similarly, harvested cells were entrapped in 5% calcium alginate gel and incubated with 100 mg l^{-1} Reactive Black B dye in Tris-buffer (pH 6.85), in four variables, namely, non-activated immobilized cells, overnight activated beads in peptone broth, activated beads were supplemented with lactose, and dextrose. % decolorization was measured after 24 hours. The reusability of activated beads was also tested.

2.6 Degradation analysis

Degradation analysis was monitored using HPTLC and FTIR spectroscopy. The HPTLC analysis was carried out using pre-coated silica gel 60 F254 plate (Camag, HPTLC). 8 µl of the sample was spotted on TLC plates using a micro syringe. The solvent system used was n-propanol: ethyl acetate: water (1:1:2). The dye chromatogram was observed by exposing to the ultraviolet light (254 nm) and in visible light. For FTIR analysis, the control and experimental (degraded dye product) and vanillin mediated degraded product were mixed with spectrophotometrically pure potassium bromide (KBr). The prepared pellets were fixed in sample holder and scanned by exposing to mid-infrared light region of 400 to 4,000 cm^{-1}.

3. Results and discussion

3.1 Spectrum of different reactive dye decolorization

Comamonas acidovorans MTCC 3364 was tested for its ability to decolorize various textile dyes. It was found that the strain could decolorize variety of different dyes within 24 hours. Structural differences in the azo dyes are known to affect decolorization as it is observed in the differences in the percent decolorization of different dyes by *Comamonas acidovorans* MTCC 3364. It could decolorize Reactive Black B and Reactive Orange 16 with maximum efficiency 90.35 ± 0.71% and 89.53 ± 0.88%, respectively (Table 1). It also decolorized with limited efficiency, Reactive Yellow 16 and Reactive Violet 1. It might be structural complexity of dyes and presence of $–NO_2$ and $–SO_3$ groups (Hu and Wu 2001) which could be accounted for the low efficiency of decolorization of the specific dye by *Comamonas acidovorans* MTCC

Table 1: Decolorization of different textile dyes by *Comamonas acidovorans* MTCC 3364

No.	Azo Dyes	λ_{max} (nm)	% Decolorization
1	Reactive Blue 160	612	65.52 ± 0.83
2	**Reactive Black B**	**598**	**90.35 ± 0.71**
3	Reactive Yellow 16	426	44.99 ± 0.35
4	Reactive Red 141	512	70.40 ± 0.32
5	Reactive Orange 16	492	89.53 ± 0.88
6	Reactive Violet 1	517	50.59 ± 0.36
7	Reactive Blue 3R	577	71.20 ± 0.38
8	Reactive Red ME6BL	545	77.72 ± 0.57
9	Reactive Orange H2R	490	68.53 ± 0.10
10	Reactive Orange 125	421	41.71 ± 0.05

3364. Since the maximum perecent decolorization was observed for Reactive Black B dye, effect of different parameters was studied for Reactive Black B dye.

3.2 Optimization of Reactive Black B decolorization

3.2.1 Effect of agitation and time of addition on dye decolorization

Azo dyes are reported to be efficiently degraded under anaerobic or limited aerobic conditions (Khehra *et al.,* 2005; Asad *et al.,* 2007; Dawkar *et al.,* 2008; Jadhav *et al.,* 2011). A combination of two parameters, RBB was effectively degraded, i.e. 89.64 ± 0.37% in a static condition and dye was inoculated after overnight grown culture. This result depicted that cells needed agitation for their growth, but cells required less oxygen for color removal of RBB. Flasks were kept in shaking condition or addition of dye and culture same time showed less decolorization, i.e. 17.88 ± 0.8% - 19.58 ± 0.89%. The comparison of results in Figure 1 indicated that aerobically grown culture of the organism was efficient in dye decolorization when the dye was added after growth and incubated under static condition, whereas the toxic effect of dye on culture growth resulting in a remarkable reduction in percent decolorization when dye was added during inoculation of the organism. The results are in accordance with the observations of other researchers with different organisms and can be attributed to competition of oxygen and dye for the reduced electron carriers under aerobic conditions as reported by Asad *et al.,* (2007). Thus, static condition was selected for further studies.

Table 2: Decolorization of Reactive Black B by *Comamonas acidovorans* **MTCC 3364 in presence of different co-substrates**

Co-substrates	% Decolorization			
	3 hours	6 hours	12 hours	24 hours
Control	34.53 ± 0.35	49.65 ± 1.04	62.45 ± 0.49	89.44 ± 1.12
Carbon sources				
Glucose	35.43 ± 0.42	49.78 ± 0.94	64.07 ± 0.43	90.60 ± 0.99
Dextrose	43.59 ± 0.38	57.88 ± 0.58	70.42 ± 0.49	93.30 ± 0.78
Lactose	48.36 ± 0.67	67.06 ± 0.83	75.93 ± 0.55	93.97 ± 0.84
Sucrose	36.03 ± 0.15	50.81 ± 0.48	63.59 ± 0.29	90.63 ± 0.73
Maltose	22.54 ± 0.47	39.48 ± 0.12	51.35 ± 0.48	84.60 ± 0.63
Mannitol	30.69 ± 0.49	34.57 ± 0.48	53.01 ± 0.94	86.97 ± 0.95
Xylose	32.59± 0.73	42.19 ± 0.82	62.45 ± 0.92	90.93 ± 1.06
Starch	38.95± 1.12	72.27 ± 0.95	62.48 ± 0.49	91.24 ± 1.23
Nitrogen Sources				
Beef extract	39.49 ± 0.58	52.53 ± 0.98	65.69 ± 0.81	93.24 ± 0.49
Yeast extract	41.63 ± 0.39	57.37 ± 0.68	67.12 ± 0.47	94.03 ± 0.92
Peptone	38.94 ± 0.57	51.77 ± 0.91	62.46 ± 0.59	92.58 ± 0.64
Urea	32.41 ± 0.48	47.52 ± 0.49	56.51 ± 0.93	84.83 ± 0.96
Ammonium sulphate	21.47 ± 0.59	39.62 ± 0.68	47.45 ± 1.28	77.42 ± 1.33
Ammonium chloride	25.33 ± 0.76	36.13 ± 0.58	46.54 ± 0.86	79.80 ± 0.36
Sodium nitrate	24.59 ± 0.82	41.38 ± 0.15	55.35 ± 0.83	82.31 ± 0.88

3.2.2 Effect of carbon and nitrogen source on dye decolorization

Decolorization efficiency of RBB increased by the addition of carbon sources in the medium, among different carbon sources maximum decolorization obtained was 93.97 ± 0.84% in a medium supplemented with lactose in the medium. The attained result specifies lactose is the most easily utilizing and help in the decolorization rather than glucose. These carbon sources were further studied with immobilized beads for the determination of decolorization. Maltose and mannitol gave less decolorization which showed minor inhibition of co-substrate in the decolorization (Table 2). Bayoumi *et al.,* (2010) also observed that *Comamonas acidovorans* TM-1 supplemented with starch, and dextrose showed highest decolorization of different dyes rather than glucose. The medium was also supplemented with organic and inorganic nitrogen sources in which yeast extract showed highest decolorization was 94.03 ± 0.92%; significantly, less decolorization efficiency was observed in inorganic nitrogen sources like ammonium sulphate, ammonium chloride and sodium nitrate showed similar efficiency of decolorization of RBB dye as shown in Table 2. Chen *et al.,* (2003) also observed that bacteria could efficiently degrade textile dyes by using yeast extract and other organic nitrogen sources.

3.2.3 Effect of physico-chemical parameters on dye decolorization

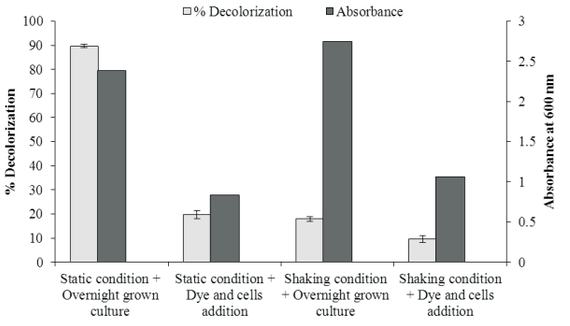


Figure 2(a): Effect of mode of incubation (shaking/static) and time of dye addition (after overnight grown culture/dye and cells addition same time) on decolorization of Reactive Black B

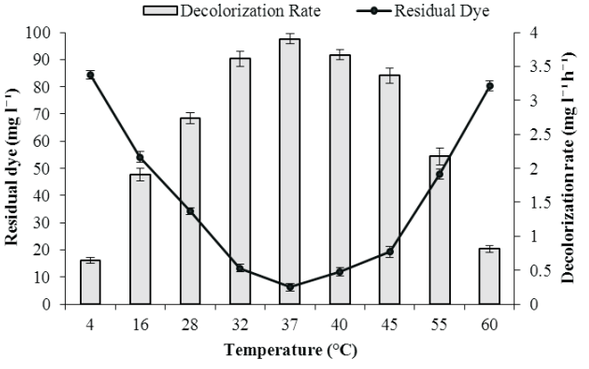


Figure 2(b): Effect of temperature (°C) on decolorization of Reactive Black B

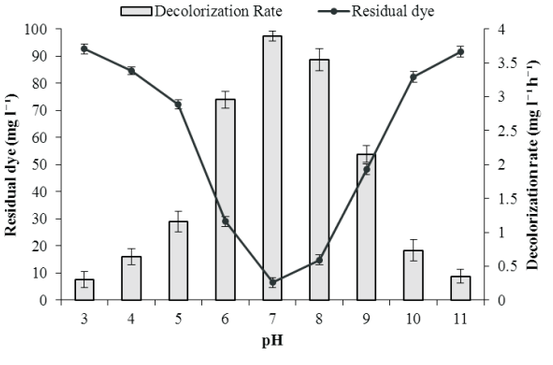


Figure 2 (c): Effect of pH on decolorization of Reactive Black B

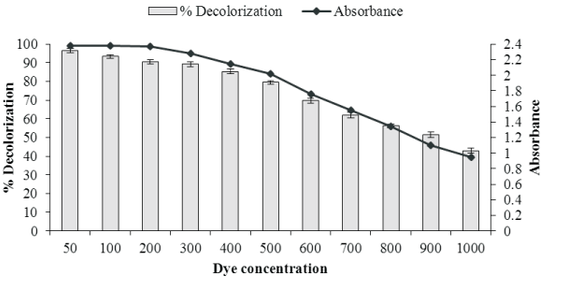


Figure 2(d): Effect of increasing concentration of Reactive Black B on decolorization by Comamonas acidovorans MTCC 3364

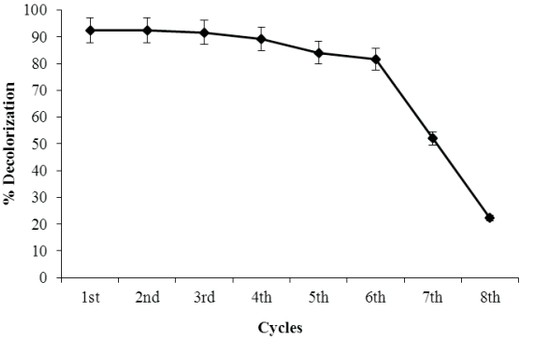


Figure 2(e): Effect of split dose addition on decolorization of Reactive Black B

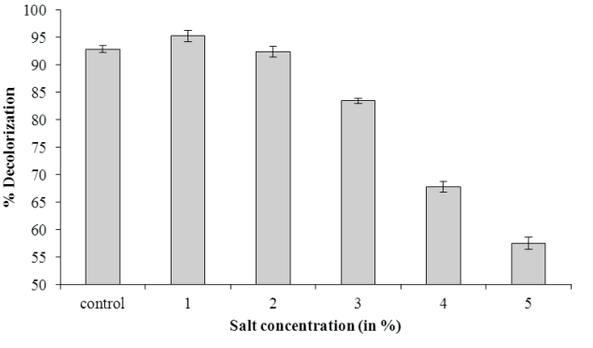


Figure 3(a): Effect of salt concentration on decolorization of Reactive Black B

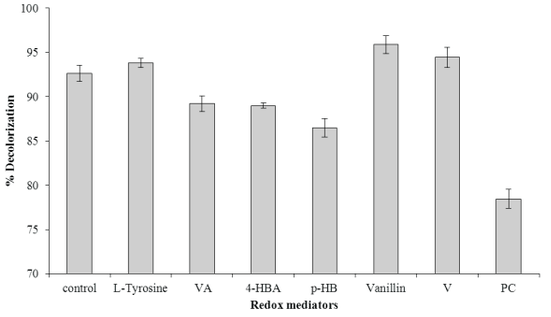


Figure 3(b): Effect of redox mediators on decolorization of Reactive Black B by Comamonas acidovorans MTCC 3364 VA: veratryl alcohol, 4-HBA: 4-hydroxy benzoic acid, p-HB: p-hydroxy benzaldehyde, V: veratryaldehyde, PC: phenol crystals.

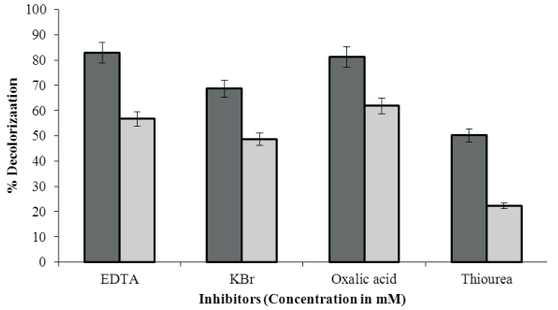


Figure 3(c): Effect of inhibitors on decolorization of Reactive Black B (■ 1mM, - 2.5mM of inhibitors)

Liao *et al.,* (2013) reported temperature as low as 25°C for decolorization of Reactive Black B by *Bacillus cereus*. Figure 3(a) depicted the effect of temperature on RBB decolorization by *Comamonas acidovorans* MTCC 3364. Cell activity was highest shown at 37°C, which gave 93.77 ± 1.43% decolorization. *Comamonas acidovorans* MTCC 3364 showed optimum activity at 37°C for RBB decolorization. This temperature is suitable for industries, and waste-water treatment plants can easily maintain this temperature. Increase in temperature of incubation by 5 to 10°C did not result into remarkable fall in the % decolorization of the dye indicating its suitability for industrial applications. The pH of the culture medium is critical to the growth and metabolic activity of cells. *Comamonas acidovorans* MTCC 3364 showed the highest activity at pH 7.0, which was 93.46 ± 1.87% decolorization of RBB (Figure 3(b)). It also showed efficient decolorization on slightly alkaline condition pH 8.0. Acidic pH showed the drastic decrease in % decolorization. The result shows the influence on growth and hence on decolorization efficiency. Similar effect of pH has been reported by Liao *et al.,* (2013) on RBB decolorization by *Bacillus cereus* strain HJ-1 where pH 8.0 was found to be optimum for dye decolorization.

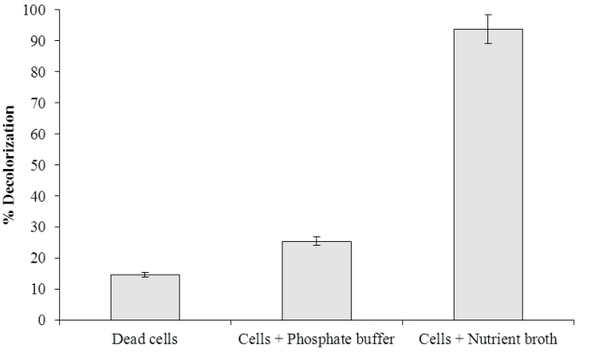


Figure 4(a): Decolorization of Reactive Black B using free cells of Comamonas acidovorans MTCC 3364

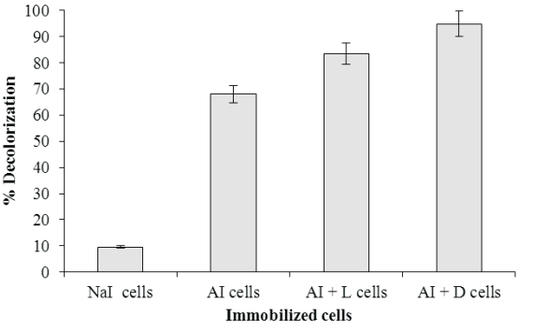
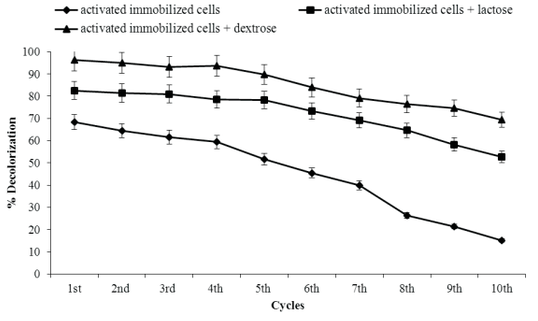


Figure 4(b): Effect of immobilized cells of Comamonas acidovorans MTCC 3364 on decolorization of Reactive Black B (NaI cells: Non-activated Immobilized cells, AI cells: Activated Immobilized cells, AI + L cells: Activated Immobilized cells + Lactose and AI + D cells: Activated Immobilized cells + Dextrose)

3.2.4 Effect of concentration of dye and split dose studies

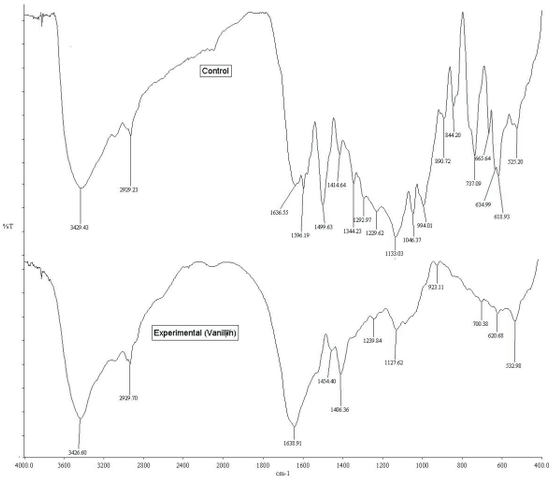
Different initial dye concentration was selected in the range from 50 mg l^{-1} to 1 g l^{-1} in which *Comamonas acidovorans* MTCC 3364 decolorized dye from 96.57

± 1.26% to 43.02 ± 1.40%, respectively (Figure 4(a)). Overnight grown culture could tolerate the high dose of Reactive Black B, and culture could degrade RBB up to 500 mg l^{-1} efficiently. However, the presence of high initial concentration of Reactive Black B showed some substrate inhibition supporting the observations of Dawkar *et al.,* (2008). Initial high concentration of the dye was found to be inhibitory for efficient decolorization of dye. Hence a split dose addition of RBB was attempted for eight cycles (100 mg l^{-1} in each dose) in which *Comamonas acidovorans* MTCC 3364 decolorized efficiently within 24 hours, and it decolorized above 90% for three consecutive cycles and above 80% for the rest of three cycles (Figure 4(b)). Because of the higher decolorization in the split dose studies, this organism primarily can apply in the continuous waste-water treatment, which comes from industries. The results are in accordance with the reports by Jadav *et al.,* (2011) where the similar inhibitory effect of initial dye concentration has been reported and split dose addition recommended.



3.2.5 Effect of medium salinity on growth and decolorization

Industrial effluents containing dyes are known to have high toxicity. Hence it was decided to study the effect of elevated NaCl concentration (Figure 5) which was found to affect RBB decolorization in which addition of high salt concentration reduced the decolorization of RBB dye by the organism. 1% of salt concentration showed 95.28 ± 1.04% RBB decolorization. This result data depicted that organism needed some amount of salt to decolorize dye, but high concentration of salt acted like inhibitor to cells' growth as well as dye decolorization. Decolorization of textile azo dyes by halophilic and halotolerant bacteria has been reported by Asad *et al.,* (2007) where it has been reported that dye degradation occurred in the presence of up to 20 % NaCl with dye tolerance up to 10,000 mg l^{-1}. The results of present study highlight the potential of *Comamonas acidovorans* MTCC 3364 for dye decolorization in presence of salt.



3.2.6 Effect of redox mediators and inhibitors on dye decolorization

Various redox mediators have been reported to improve dye decolorization (Samet et al., 2011), of which vanillin was reported to be the most suitable mediator for dye decolorization and cost effectiveness. Different mediators were tested for RBB decolorization by the selected strain and were found to improve decolorization of the dye. L-tyrosine, veratryaldehyde and vanillin showed higher decolorization of RBB dye in 24 hours. Vanillin containing nutrient broth media gave 95.88 ± 0.99% result within 24 hours, which is maximum % decolorization of Reactive Black B dye (Figure 6(a)) and also affected the degradation of RBB dye, which was observed in FTIR analysis. L-tyrosine and veratryaldehyde observed 93.80 ± 0.53% and 94.43 ± 1.12%, which showed higher activity rather than control. Inhibitors inhibit the dye decolorization process by interfering biochemical pathway of cells and also inhibit the growth of the cells. Increased amount of inhibitors directly decreased degradation of RBB. Dawkar et al., (2005) observed that EDTA and NaBr were inhibitors for Bacillus sp. UVS, and it significantly decreased in dye decolorization. It is well evident to

figure 6(b) that all inhibitors gave 30 to 35% of inhibition in which thiourea gave maximum inhibition, which was a 68% reduction of the normal extent of decolorization. 2.5 mM of KBr and thiourea showed only 48.70 ± 0.51% and 22.27 ± 0.54% respectively.

3.3 Decolorization using free, pre-treated and immobilized cells

In order to test the reusability and the importance of metabolic activity of cells for dye decolorization, the cells of the cells were harvested from overnight grown culture and incubated with the dye in the buffered medium. Free cells did not show acceptable decolorization of Reactive Black B dye, which means that cells required a minimal amount of nutrients for effective decolorization (Figure 7). Dead cells also showed 14.60 ± 0.33% of decolorization, which highlights the role of RBB adsorption in contributing towards decolorization of azo dye by *Comamonas acidovorans* MTCC 3364. Cells of *Comamonas acidovorans* MTCC 3364 was immobilized in Calcium alginate gel and when applied directly for Reactive Black B decolorization, showed very low decolorization efficiency. Cell obtained in the beads could degrade and decolorize only 9.54 ± 0.56% of dye. Beads had to be activated in peptone broth overnight to get a remarkable improvement of 68 ± 0.89% RBB decolorization. Addition of different carbon sources like lactose and dextrose showed 82-93% decolorization (Figure 8(a)). This showed that cells needed a metabolic function for Reactive Black B decolorization. These beads were reused for ten times and each time RBB decolorization was 90.53 ± 0.87% and 95.88 ± 0.57% by using dextrose and lactose respectively as shown in figure 8(b). It is a significant application that we can use immobilized cells and recycle it with efficient dye decolorization and degradation.

3.4 Degradation analysis

In the present study decolorization of Reactive Black B was due to the biodegradation and is not only visible decolorization. Spectrophotometric analysis of RBB showed a maximum absorbance at 598 nm and decrease in absorbance of samples withdrawn after decolorization using *Comamonas acidovorans* MTCC 3364. If the removal of dye is attributed to the process of biodegradation, a major visible-light absorbance peak would be disappeared (Chen *et al.,* 2003; Saratale *et al.,* 2009). The result obtained indicates that color removal may be largely due to biodegradation. HPTLC was done for the control and degraded sample (vanillin mediated). Control sample showed a single band at its R_f value is 0.84 whereas the degraded sample showed three bands at its R_f value is 0.76, 0.59 and 0.43. Degraded products formed new bands and such type of transformation also occurred. The FTIR spectrum of control dye compared with vanillin mediated degraded product as shown in Figure 8. Brominated and iodo compounds showed a strong peak in dye, which is at 690-500 cm^{-1} region. Degraded product showed the weak peak of halogenated compounds, which indicate that removal of halogenated compounds from Reactive Black B dye. Present of a normal alkane with asymmetrical stretching of methylene groups in Reactive Black B dye and also in their products. Reactive Black B dye showed from 1636.55-994.01 cm^{-1} in which dye contains –C=C- stretching vibration of conjugated and unconjugated alkenes, mainly it contained vinyl group and C-O, C=N or phenolic C–O vibrations. In case of control dye peak around 1499.63 cm^{-1}, assigned to (N=N) azo-bond vibration, peaks around 1596 cm^{-1} and 1414 cm^{-1} may be characteristics of C=C aromatic skeletal vibration and azo linkages (–N=N–) on aromatic structures, bands at region 2,960–2,850 cm^{-1} originate from CH_3 asymmetric, CH_3 symmetric vibrations and CH_2 asymmetric stretching vibration. Degraded products of RBB dye showed weak stretching vibration in azo groups and aromatic compound, which mainly indicate that degradation of azo dye might be occurred. Disappearance of the classical azo bond peak indicates efficient degradation of the dye rendering it less toxic for release in the environment. These data strongly revealed that degradation of RBB dye has occurred.

4. Conclusion

Comamonas acidovorans MTCC 3364 showed an ability to decolorize different reactive azo dyes. It showed a higher ability to decolorize and degrade Reactive Black B dye. The dye decolorizing efficiency was further enhanced by optimizing physico-chemical parameters and showed highest degradation at 37°C, pH 6.85 within 24 hours. Vanillin as a redox mediator showed the positive effect on efficiency of dye decolorization as well

as degradation and thiourea showed highest inhibition of decolorization. Free living and dead cells indicated that cells needed minimal amount of metabolite and dead cells depicted biosorption and adsorption mechanism. Immobilized cells give efficient decolorization so it can be used for waste-water treatment. FTIR analysis showed that the degradation of Reactive Black B is better in vanillin mediated degradation. These promising results suggest the applicability of *Comamonas acidovorans* MTCC 3364, which can be utilized in the decolorization of wastewater effluents containing dyes in optimum conditions.

Acknowledgement

The authors acknowledge Charutar Vidya Mandal, Vallabh Vidyanagar, Anand for providing the infrastructure, laboratory, chemicals, computational and other necessary facilities for the successful completion of this work. Authors also thank to MTCC for providing the cultures used for the study and SICART, Vallabh Vidyanagar for extending analytical facilities for this study.

References

Asad, S., Amoozegar, M., Pourbabaee, A., Sarbolouki, M., and Dastgheib, S. 2007. Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. *Bioresource Technology* **98:** 2082–2088.

Bayoumi, M., Bahobil, L., and Sakawey, E. 2010. Biodecolorization and Biodegradation of Azo dyes by Some Bacterial isolates. *Journal of Applied Environmental and Biological Sciences* **1:** 1- 25.

Chang, S.J., and Lin, Y.C. 2001. Decolorization kinetics of recombinant *Escherichia coli* strain harbouring azo dye decolorization determinants for *Rhodococcus* sp. *Biotechnology Letters* **23:** 631–636.

Chen, K.C., Jane, Y.W., Liou, D.J. and Hwang, S.C.J. 2003. Decolorization of the textile dyes by newly isolated bacterial strains. *Journal of Biotechnology* **101:** 57–68.

Cheng-Gang, N., Ying, W., Xue-Gang, Z., Guang-Ming, Z., Da-Wei, H., Min R. and Xiang-Wei, L. 2012. Decolorization of an azo dye Orange G in microbial fuel cells using Fe (II)-EDTA catalyzed persulfate. *Bioresource Technology* **126:** 101–106.

Daassi, D., Zouari-Mechichi, H., Frikha, F., Martinez, M.J., Nasri, M., and Mechichi, T. 2013. Decolorization of the azo dye Acid Orange 51 by laccase produced in solid culture of a newly isolated *Trametes trogii* strain. *3 Biotech* **3:**115–125.

Dawkar, V., Jadhav, U., Jadhav, S. and Govindwar, S. 2008. Biodegradation of disperse textile dye Brown 3REL by Bacillus sp. VUS. *Journal of Applied Microbiology* **105:** 14–24.

Dawkar, V., Jadhav, U., Telke, A. and Govindwar, S. 2009. Peroxidase from *Bacillus* sp. VUS and its role in the decolorization of textile dyes. *Biotechnology and Bioprocess Engineering* **14(3):** 361-368.

Gahlout, M., Gupte, S., and Gupte, A. 2013. Optimization of culture condition for enhanced decolorization and degradation of azo dye reactive violet 1 with concomitant production of ligninolytic enzymes by *Ganoderma cupreum* AG-1. *3 Biotech* **3:** 143-152.

Garg, S.K., and Tripathi, M. 2013. Process parameters for decolorization and biodegradation of Orange II (Acid Orange 7) in dye- simulated minimal salt medium and subsequent textile effluent treatment by *Bacillus cereus* (MTCC 9777) RMLAU1. *Environmental Monitoring and Assessment* **185:** 8909-8923.

Hu, T.L. and Wu, S.C. 2001. Assessment of the effect of azo dye RP2B on the growth of nitrogen fixing cyanobacterium-*Anabena* sp. Bioresource Technology, 77: 3–95.

Huang, C.P., Huang, Y.F., Cheng, H.P. and Huang, Y.H. 2009. Kinetic study of an immobilized iron oxide for catalytic degradation of azo dye Reactive Black B with catalytic decomposition of hydrogen peroxide. Catalysis Communications, 10: 561–566.

Jadhav, U., Dawkar, V., Ghodake, G. and Govindwar, S. 2008. Biodegradation of Direct Red 5B, a textile dye by newly isolated *Comamonas* **sp.** UVS. *Journal of Hazardous Materials* **158:** 507–516.

Jadhav, U., Dawkar, V., Telke, A. and Govindwar, S. 2009. Decolorization of Direct Blue GLL with enhanced lignin peroxidase enzyme production in *Comamonas* sp. UVS. *Journal of Chemical Technology and Biotechnology* **84:** 126–132.

Jadhav, U.U., Dawkar, V.V., Kagalkar, A.N. and Govindwar, S.P. 2011. Effect of Metals on Decolorization of Reactive Blue HERD by *Comamonas sp*. UVS. *Water Air Soil Pollution* **216:** 621–631.

Khalid, A., Kausar, F., Arshad, M., Mahmood T., and Ahmed, I. 2012. Accelerated decolorization of reactive azo dyes under saline conditions by bacteria isolated from Arabian seawater sediment. *Applied Microbiology and Biotechnology* **96 (6):** 1599-1606.

Khehra, M.S., Saini, H.S., Sharma, D.K., Chadha, B.S., and Chimmi, S.S. 2005. Decolorization of various azo dyes by bacterial consortium. *Dyes and Pigments* **67:** 55-61.

Kodam, I., Soojhawon, P.D., Lokhande, K.R., Gawai, 2005. Microbial decolorization of reactive azo dyes under aerobic conditions. *World Journal of Microbiology & Biotechnology* **21:** 367–370.

Liao, C.S., Hung, C.H. and Chao, S.L. 2013. Decolorization of azo dye reactive black B by *Bacillus cereus* strain HJ-1. *Chemosphere* **90:** 2109–2114.

Lister, D.L., Sproule, R.F., Britt, A.J., Lowe, C.R., and Bruce, N.C. 1996. Degradation of Cocaine by a Mixed Culture of *Pseudomonas fluorescens* MBER and *Comamonas acidovorans* MBLF. *Applied and Environmental Microbiology* **62:** 94–99.

Lodha, B. and Choudhari, S., 2007. Optimization of Fenton-biological treatment scheme for the treatment of aqueous dye solution. *Journal of Hazardous Materials* **148:** 459–466.

Olukanni, O.D., Osuntoki, A.A., and Gbenle, G.O. 2006. Textile effluent biodegradation potentials of textile effluent-adapted and non-adapted bacteria. *African Journal of Biotechnology* **5:** 1980–1984.

Patel, Y., Mehta, C., and Gupte, A. 2012. Assessment of biological decolorization and degradation of sulfonated di-azo dye Acid Maroon V by isolated bacterial consortium EDPA. *International Biodeterioration and Biodegradation* **75:** 187-193.

Pathak, H., Patel, S., Rathod, M. and Chauhan, K. 2011. In vitro studies on degradation of synthetic dye mixture by *Comamonas* sp. VS-MH2 and evaluation of its efficacy using simulated microcosm. *Bioresource Technology* **102:** 10391–10400.

Pawar, K., Lad, N., and Pawar, S.P. 2011. Simultaneous Spectroscopic estimation of 11-β, 17-α, 21-trihydroxy-4-pregnene-3, 20-dione, 21-o-succinate and its 1(2)-Dehydrogenated Product during Bioconversion by *Comamonas acidovorans* MTCC 3364. *Recent Research in Science and Technology* **3:** 01-04.

Perez, A., Poznyak, T ., and Chairez, I. 2013.Effect of additives on ozone-based decomposition of Reactive Black 5 and Direct Red 28 dyes. *Water Environment Research* **85(4):** 291-300.

Peter, G., and Bont, J. 1992. Degradation of 4-nitrobenzoate via 4-hydroxylaminobenzoate and 3,4-dihydroxybenzoate in *Comamonas acidovorans* NBA-10. *Achieves in Microbiology* **158:** 381-386.

Rudakiya, D., and Pawar, K. 2013a. Bioconversion of progesterone using aqueous two phase system by *Comamonas acidovorans* MTCC 3364. *International Journal of Biotechnology* **111:** 211-214.

Rudakiya, D., and Pawar, K. 2013b. Evaluation of remediation in heavy metal tolerance and removal by *Comamonas acidovorans* MTCC 3364. *IOSR Journal of Environmental Science, Toxicology and Food Technology* **5(5):** 26-32.

Samet, Ş., Gedikli, S., Aytar, P., Güngörmedi, G., Çabuk, A., Hür, E., Ünal, A. and Kolankaya, N. 2011. Decolorization Potential of Some Reactive Dyes with Crude Laccase and Laccase-Mediated System. *Applied Biochemistry and Biotechnology* **163:** 346-361.

Saratale, R.G., Saratale, G.D., Chang, J.S. and Govindwar, S.P. 2009. Decolorization and biodegradation of textile dye Navy blue HER by *Trichosporon beigelii* NCIM-3326. *Journal of Hazardous Materials* **166:** 1421–1428.

Saratale, R.G., Saratale, G.D., Chang, J.S., and Govindwar, S.P. 2011. Bacterial decolorization and degradation of azo dyes: A review. *Journal of the Taiwan Institute of Chemical Engineers* **42:** 138–157.

Shih, Y., Ho, C.H., and Huang, Y. 2013. Photo-Fenton oxidation of azo dye Reactive Black B using an immobilized iron oxide as heterogeneous catalyst. *Water Environment Research* **85(4):** 340-345.

Verma, P., and Madamwar. D. 2002. Comparative study of transformation of azo dye by different white rot fungi. *Indian Journal of Biotechnology* **1:** 31-36.

Waghmode, T.R., Kurade, M.B., Kabra, A.N. and Govindwar, S.P. 2012. Degradation of Remazol Red dye by *Galactomyces geotrichum* MTCC 1360 leading to increased iron uptake in *Sorghum vulgare* and *Phaseolus mungo* from Soil. *Biotechnology and Bioprocess Engineering* **7:** 000-012.

Wang, H., Qiang, S., Zheng, X.W., Tian, Y., Xiong. X.J. and Zheng,T.L. 2009. Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by *Citrobacter* sp. CK3. *International Biodeterioration and Biodegradation* **63:** 395–399.