



Original Research Article

Optimization of culture condition for enhanced decolorization of Reactive Orange 16 by *Comamonas acidovorans* MTCC 3364

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ABSTRACT

Keywords

Comamonas acidovorans MTCC 3364; Reactive Orange 16; Bioremediation; Azo dye decolorization.

Many synthetic azo dyes and their metabolites are toxic, carcinogenic, and mutagenic so removal of azo dyes using cost-effective and eco-friendly method is major aspect. *Comamonas acidovorans* MTCC 3364 has been routinely reported for different steroid bioconversion and heavy metal removal. The main purpose of this study is to check the decolorization efficiency of *Comamonas acidovorans* MTCC 3364 for different dyes and to optimize the condition which gives maximum decolorization of Reactive Orange 16 dye. The effect of various physicochemical parameters including condition, carbon and nitrogen sources, temperature, pH and dye concentration were studied. The % decolorization of dye was determined by UV-Visible spectroscopy. This bacterial strain efficiently decolorizes Reactive Orange 16 at 37°C, pH 6.85 within 24 hours giving 99.03 ± 0.5 % dye decolorization under optimum environmental conditions.

Introduction

The growth of the worldwide textile industry in the years since then had seen a commensurate increase in the use of such synthetic dyes, and this has been accompanied by a rise in pollution due to wastewater contaminated with dyestuff (Pandey *et al.*, 2007). Many synthetic azo dyes and their metabolites are toxic, carcinogenic, and mutagenic (Myslak and Bolt, 1998). Moreover, numerous reports indicate that textile dyes and effluents have toxic effects on the germination rates and biomass of several plant species which have important ecological functions, such

as providing a habitat for wildlife, protecting soil from erosion and providing the organic matter that is so significant to soil fertility (Ghodake *et al.*, 2009a; Kapustka and Reporter, 1993).

A number of biotechnological approaches have attracted interest with regard to tackling azo dye pollution in an eco-efficient manner, mainly with the use of bacteria and often in combination with physicochemical processes. Microbial or enzymatic decolorization and degradation is an eco-friendly cost-competitive

alternative to chemical decomposition process that could help reduce water consumption compared to physic-chemical treatment methods (Rai *et al.*, 2005; Verma and Madamwar, 2003). 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 is belonging from Comamonadaceae family of the β -proteobacteria. It 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 been routinely reported for bioconversion of different steroids like progesterone, testosterone and cholesterol (Pawar *et al.*, 2011; Rudakiya and Pawar, 2013a) and heavy metals like chromium and mercury removal and tolerance (Rudakiya and Pawar, 2013b). The main purpose of study was to investigate efficacy dye decolorization of Reactive Orange 16 dye by this strain and to study various physicochemical parameters like condition, carbon and nitrogen sources, temperature, pH, salt (NaCl) concentration, metal ions concentration was evaluated by using UV-Visible Spectroscopy.

Materials and Methods

Dyestuff and chemicals

Nutrient broth, nutrient agar and other media were purchased from Hi-media Pvt Ltd. Metals and chemicals were purchased from standard chemical suppliers. All chemicals were highly pure and of an

analytical grades. All dyes were obtained from Manibhadra Enterprise, Ahmedabad.

Micro-organisms and culture conditions

Different strains of *Comamonas acidovorans* were purchased from Microbial Type Culture Collections, Institute of Microbial Technology, Chandigarh, India but *Comamonas acidovorans* MTCC 3364 was used for study. The pure culture was maintained on nutrient agar slants and stored at 4°C. The organism was sub-cultured every month.

Screening of dye decolourization

Flasks containing 20 ml of nutrient broth (13 gm/lit) were autoclaved at 121°C for 15 min, cooled and inoculated with 100 μ l of previous grown culture of *Comamonas acidovorans* MTCC 3364 in aseptic condition. Flasks were incubated overnight on a rotary shaker at 120 rpm. Next day 200 μ l of sterilized dye (10,000 ppm in the water) was added to the flasks and incubated for 24 hours at 37°C in static condition. Reactive Violet 1, Reactive Blue 3R, Reactive Black B, Reactive Red 141, Reactive Orange 16, Reactive Blue 160, Reactive Yellow 16 was used for screening of dye decolourization. 2 ml of sample was withdrawn at the time of dye addition and after 24 hours of dye addition and centrifuged for 10,000 rpm for 5 min. Dye decolourization was determined spectrophotometrically by monitoring the absorbance of samples at λ max of the respective dyes using a UV-Visible spectrophotometer. The decolourization expressed in % of the dye decolourization was calculated as follows:

$$\% \text{ Decolourization} = \frac{OD_0 - OD_t}{OD_0} \times 100$$

Where,

OD_0 is the absorbance value of the initial dye concentration,

OD_t is the absorbance value of the dye concentration in sample at time t.

Effect of static and shaking condition

Flasks containing 20 ml of culture and 100 ppm of Reactive Orange 16 were incubated at static condition and shaking (120 rpm) at 37°C for overnight incubation. Next day dye decolorization observed at 492 nm and % decolorization was measured.

Effect of temperature and pH

Flasks containing 20 ml of culture and 100 ppm of Reactive Orange 16 were incubated under static condition at different temperature (4°C, 20°C, 28°C, 37°C, 42°C and 55°C). Another set of flasks containing 20 ml of culture and 100 ppm of Reactive Orange 16 was incubated at the static condition at different pH (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12). In both sets % decolorization was studied after 24 hours.

Effect of Carbon and Nitrogen sources

Dyes are deficient in carbon contents and biodegradation without any extra carbon and nitrogen source was very difficult, and to evaluate the need of a co-substrate as an electron donor different co-substrate like sorbitol, sucrose, xylose, glucose, mannitol, lactose and maltose were added to sterilize experimental test tube with 20ml nutrient broth with 1% peptone, 100 mg/l dye concentration and inoculum. The

concentration (1%) of each substrate was maintained constant and all test tubes were incubated at 37°C under static condition for 24 hours to observe. Similarly another set of nitrogen sources were also kept for study in which ammonium nitrate, ammonium persulphate, ammonium sulphate, ammonium chloride, beef extract, meat extract and urea at 1.0% concentration were added in sterilized experimental test tube with 20ml nutrient broth with 1% peptone, 100 mg/l dye concentration and inoculum. % decolorization was measured after 24 hours of incubation.

Effect of salt concentration (NaCl)

Flask containing 20 ml of nutrient broth with different concentration of salt (1% - 10%) was inoculated along with 100 µl of *Comamonas acidovorans* MTCC 3364 culture. All flasks were incubated at 37°C at rotary shaker (120 rpm). Blank (without salt) was also included. Next day dye added 100 ppm and incubated at the static condition at 37°C. % decolorization was measured after 24 hours.

Effect of different metal ions

Effect of metal ion on decolorization performance was studied by inoculating pre grown culture into experimental test tubes containing 20 ml nutrient broth supplemented with 1% peptone, and 100mg/l dye concentration and different metal ion like manganese, zinc, copper, chloride, magnesium, cadmium, lead, ferrous sulphate and cobalt at 0.1% and were incubated at 37°C under static condition for 24 hours. % decolorization was measured after 24 hours.

Results and Discussion

Spectrum of different Reactive dyes decolorization

Comamonas acidovorans MTCC 3364 was tested for its ability to decolorize various textile dyes. It was found that the strain was able to 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 % decolorization of different dyes by *Comamonas acidovorans* MTCC 3364. It decolorizes Reactive Black B and Reactive Orange 16 with maximum efficiency $90.35 \pm 0.5\%$ and $89.53 \pm 0.5\%$, respectively (Table 1). It also decolorizes with limited efficiency, Reactive Yellow 16 ($4.99 \pm 0.5\%$). Since the maximum % decolorization was observed for Reactive Orange 16 dye and Reactive Black B, effect of different parameters was studied for Reactive Orange 16 dye.

Effect of static and shaking condition

The effect of static and shaking environment on decolorization of dye were tested by incubating the cultures, containing dyes 100 ppm. The process of decolorization was monitored for 24 hours under shaking and static condition. 63% decolorization of dye was achieved with $48\mu\text{g/l}$ of biomass under static condition, but under the shaking condition, only 18% decolorization was obtained with $69\mu\text{g/l}$ of biomass (Figure 1). Decolorization was achieved under static condition while under shaking flask condition less than half decolorization was achieved. This indicates the decolorization process is inhibited in the presence of oxygen.

Effect of temperature

Microbial growth is temperature specific and decolorization is catalyzed by the enzyme, are affected by incubation temperature. Variation in temperature decreases or increases the enzyme activity. At 37°C , the maximum decolorization of dyes (74.04) within 24 hours was obtained and at a 20°C incubation temperature, minimum decolorization (8.27) was achieved. Beyond 37°C , dye decolorization decreased. Thus, the dye decolorizing activity of culture was found to increase with increase in incubation temperature (Figure 2) from 10° to 37°C with maximum activity attained at 37°C . The percentage removal of dye decreases with further increase in temperature up to 55°C . Similar kind of observation was noted in case of *Pseudomonas* sp. For decolorization of Malachite Green, Fast Green, Congo Red and Methylene Blue (Mali *et al.* 2000)

Effect of pH

Ionic strength (pH) of the medium is of prime importance for almost all physiological processes. Optimal degradation or synthesis occurs at a certain pH value. This experiment was designed to evaluate the influence of pH on the decolorization of various textile dyes. Bacterial cultures generally exhibit maximum decolorization at pH value near 7.0. Decolorization rate increases with an increase in pH from 5 to 7 but further increase in pH toward the alkaline side lead to decrease in % decolorization, still it showed decolorization at pH 8 (57.29 %) but then after decolorization rate decrease at pH 9. The maximum decolorization (58.15%) was obtained at pH 6.85 within 24 hours (figure 3).

Table.1 Decolorization of different reactive dyes by *Comamonas acidovorans* MTCC 3364

No.	Dye	λ_{\max} (nm)	% Decolorization
1	Reactive Blue 160	612	65.52
2	Reactive Black B	598	90.35
3	Reactive Yellow 16	426	04.99
4	Reactive Red 141	512	70.40
5	Reactive Orange 16	492	89.53
6	Reactive Violet 1	517	10.59
7	Reactive Blue 3R	577	12.20

Figure.1 Effect of culture condition on decolorization of Reactive Orange 16 dye

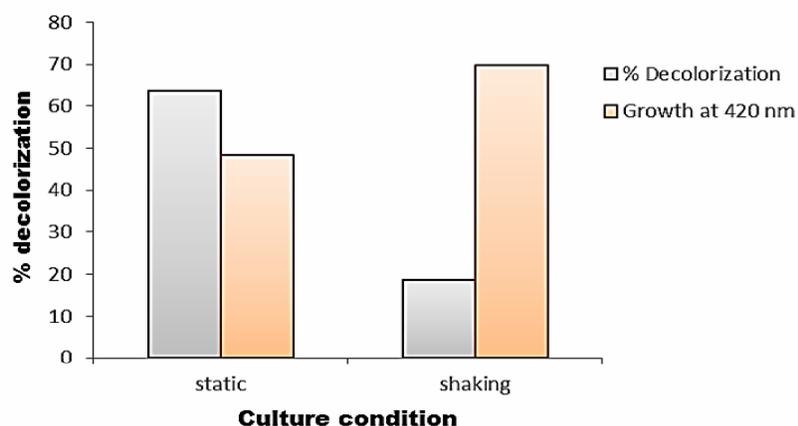
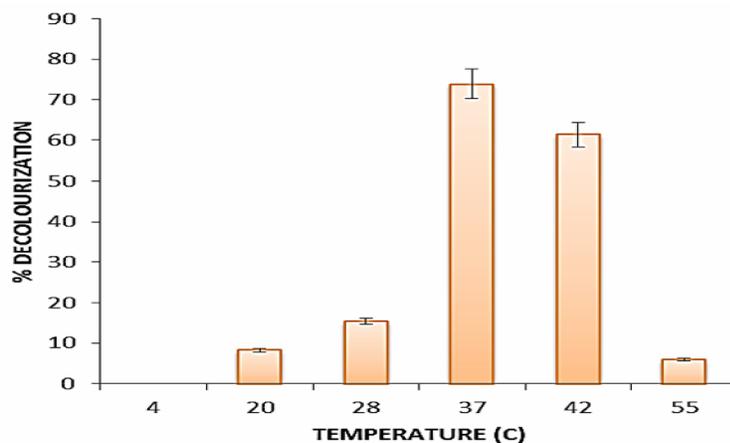


Figure.2 Effect of Temperature on Decolorization of Reactive Orange 16 dye



Bacterial species *K.Pneumonia RS-13* could decolorized Methyl Red at pH 6-8 while *Acetobacterliquefaciens S-1* completely decolorized the same dye at pH 6.5 (Wong and Yuen,1996).

Effect of Carbon sources

The strain was able to grow on all tested carbon sources, but its decolorization activity was observed to be influenced by the type of nutrient Carbon source (Figure 4). The decolorization activity obtained after 24hours by the static cultures growing on, sorbitol, mannitol, sucrose, lactose, glucose, maltose and xylose as carbon source (1%). The isolated culture exhibited maximum decolorization (87.16%) of Reactive Orange 16 within 24 hours when lactose was supplemented in the medium. In the absence of co-substrate bacterial culture was unable to decolorize the dye which indicated that the availability of supplementary carbon source seems to be necessary for the growth and decolorization of dye. In the presence of nutrient broth xylose, maltose, glucose and sucrose, dye completely degraded in 24hours. In Cibacron Red P4B *B.cereus* grew best with NH_4NO_3 /Glucose and NH_4NO_3 /Sucrose combination (81%) showed by Ola *et al.* (2010) and in absence of glucose only 20% decolorization obtained reported by Boonykamol *et al.* (2009).

Effect of Nitrogen sources

The potential of *Comamonas acidovorans* MTCC 3364 to decolorize Reactive Orange 16 with different nitrogen source is shown in figure 5. The effect of various nitrogen sources ammonium nitrate, ammonium persulfate, ammonium sulphate, ammonium chloride, beef extract, meat extract and urea were studied

at a concentration of 0.1%, Meat extract was found to be the best nitrogen source supporting 100% decolorization after 24hours. Next to this, ammonium sulphate, beef extract and ammonium chloride were found to be suitable nitrogen sources which showed 99%, 98% and 97 % decolorization respectively. In the presence of inorganic nitrogen sources tested, decolorization achieved was 20-60 %. Sarswati and Balakumar 2009; Ola *et al* 2010 had also reported best growth and decolorization of Cibacron Black PSG (75%) by *B.cereus* in yeast extract/lactose combination.

Effect of salt concentration (NaCl)

Dye manufacturing industries use large amount of salt in the manufacturing process, high salt concentration mainly causes osmotic imbalance thus, it is necessary to check the sustainability of the bacterial isolate under high salt environment. For this study, different concentrations of salt (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10%) were used. The bacterial isolate showed efficient decolorization and cell growth up to 6% (w/v) after that salt concentration decline was observed due to high salinity which leads to the low percentage of dye decolorization. Figure 6 shows that maximum decolorization (80.10%) and cell growth was observed at 1% salt concentration. Boonyakamol *et al.*, (2009) described the effect of decolorization was inhibited by high salt concentration at 1-1.5% concentration.

Effect of different metal ions

The effects of metals like ferrous sulphate, zinc sulphate, lead, magnesium sulphate, manganese chloride, copper sulphate, cobalt chloride, cadmium chloride, and

Figure.4 Effect of carbon source on decolorization of Reactive Orange 16 dye

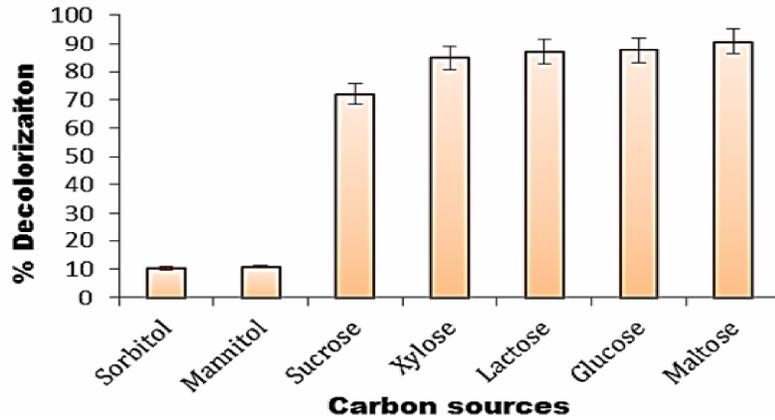
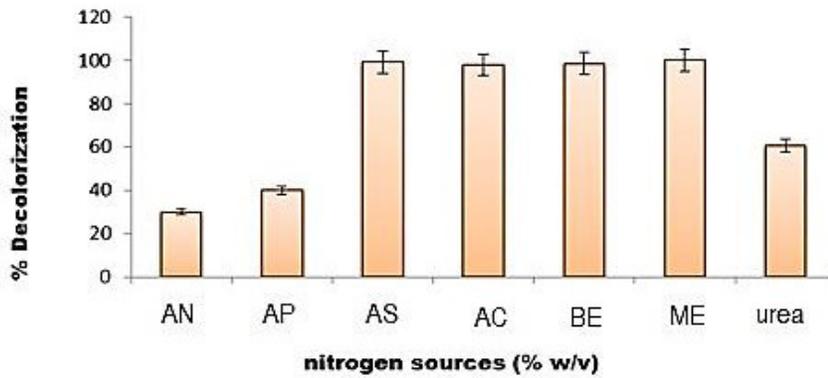


Figure.5 Effect of nitrogen source on decolorization of Reactive Orange 16 dye



AN: Ammonium Nitrate, AP: Ammonium Persulfate, AS: Ammonium Sulphate, AC: Ammonium Chloride, BE: Beef Extract, ME: Meat Extract.

Figure.6 Effect of salt on decolorization of Reactive Orange 16 dye

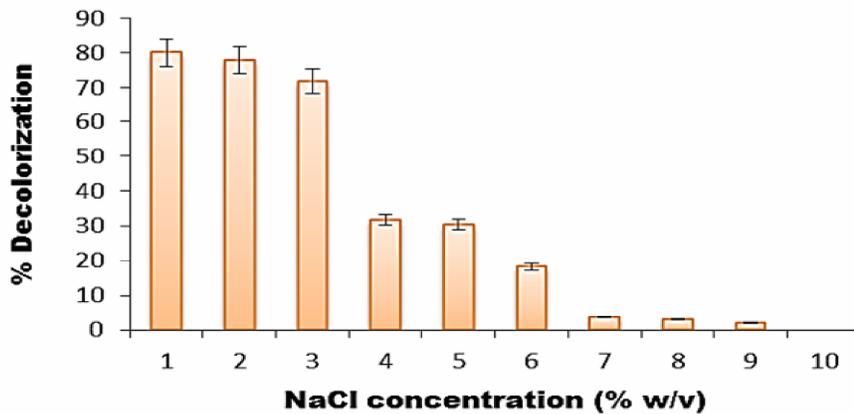
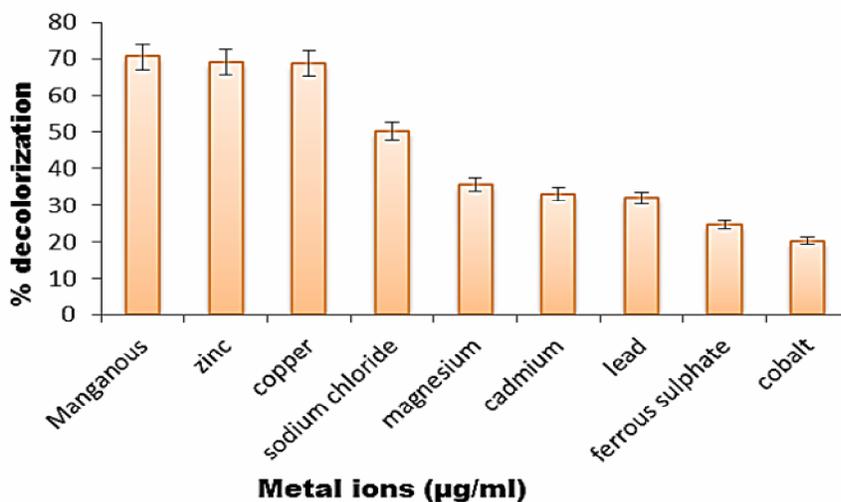


Figure.7 Effect of metal ion on decolorization of Reactive Orange 16 dye

sodium chloride were tested on decolorization of Reactive Orange 16 dye by the static cultures of organism. Results showed that at 0.05 g/l concentrations of metals, the maximum (70.52%) and minimum (20.25%) decolorization was obtained in the presence of manganoussulphate and cobalt chloride respectively. The decolorization achieved in the presence of other metals was, zinc sulphate (69%), copper sulphate (68.72%), sodium chloride (50.21%), magnesium sulphate (35.54%), cadmium chloride (33%), lead (32.1%) and ferrous sulphate (24%) as shown in Figure 7. In some cases, for e.g. cadmium chloride, lead, ferrous sulphate, and manganese sulphate, as the concentration of metal increases the decolorization activity decreases. Among the metal studied cobalt was found to be most inhibitory, but still it shows 20% degradation in 24 hours.

The overall study concluded that physicochemical parameter has significant influence on Reactive Orange 16 dye removal efficiency. *Comamonas*

acidovorans MTCC 3364 has shown a significant change in percentage of dye decolourization with optimized various physicochemical parameters, including temperature, pH, culture condition, co-substrate, nitrogen source, salt concentration and metal ions. Isolate has shown maximum decolourization of Reactive Orange 16 at 37°C, pH 6.85 using lactose best electron donor and yeast extract as a nitrogen source under static condition. Thus, further studies are required with this isolate to explore its ability for actual industrial applications like waste water treatment.

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References

- Boonyakamol, A., Imai, T., Chairattananokorn, P., Higuchi, T., Sekine, M., Ukita, M, 2009. Reactive Blue 4 decolorization under mesophilic and thermophilic anaerobic treatments. Appl. Biochem. Biotechnol. 152:405-417.
- Ghodake, G. S., A. A. Telke, J. P. Jadhav, and S. P. Govindwar, 2009a. Potential of Brssicajunceain Order to Treat Textile Effluent Contaminated Sites. Int. J. Phytoreme, 11, 1.
- Jadhav, U., Dawkar, V., Ghodake, G., Govindwar, S., 2008. Biodegradation of Direct Red 5B, a textile dye by newly isolated *Comamonas* sp. UVS. J.f Hazard.Mat. 158:507-516.
- Jadhav, U., Dawkar, V., Telke, A., 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., Govindwar, S.P. 2011. Effect of Metals on Decolorization of Reactive Blue HERD by *Comamonas* sp. UVS. Water. Air. Soil. Pollut. 216:621-631.
- Kapustka, L. A. and M. Reporter, 1993. Terrestrial Primary Producers, Handbook of Ecotoxicology, p. 278, P. Calow, Ed., Blackwell Scientific Publications, Oxford.
- Lister, D.L., Sproule, R.F., Britt, A.J., Lowe, C.R., Bruce, N.C., 1996. Degradation of Cocaine by a Mixed Culture of *Pseudomonas fluorescens* MBER and *Comamonas acidovorans* MBLF. Appl. Environ. Microbiol. 62:94-99.
- Myslak, Z. W. and H. M. Bolt, 1998. "Occupational Exposure to Azo Dyes and Risk of Bladder Cancer," Zbl. Arbeitsmed. 38:310.
- Ola, I. O., Akintokun, A. K., Akpan, I., Omomowo, I. O., and Areo, V. O., 2010. Aerobic decolorization of two reactive azo dyes under varying carbon and nitrogen source by *Bacillus cereus*. Afr. J. Biotechnol. 9 (5):672-677.
- Pandey, A., P. Singh, and L. Iyengar, 2007. Bacterial Decolorization and Degradation of AzoDyes. Int. Biodeter. Biodegrad., 59: 73 (2007).
- Pathak, H., Patel, S., Rathod, M., 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. Bioresour. Technol. 102:10391-10400.
- Pawar, K., Lad, N., 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 Res. Sci. Technol. 3:01-04.
- Peter, G., Bont, J., 1992. Degradation of 4-nitrobenzoate via 4-hydroxylaminobenzoate and 3,4-dihydroxybenzoate in *Comamonas acidovorans* NBA-10. Achiev. Microbiol. 158: 381-386.
- Rai, H., M. Bhattacharya, J. Singh, T. K. Bansal, P. Vats, and U. C. Banerjee, 2005. Removal of Dyes from the Effluent of Textile and Dyestuff Manufacturing Industry: A Review of Emerging Techniques with Reference to Biological Treatment. Crit. Rev. Environ. Sci. Technol., 35:219.
- Rudakiya, Darshan M., Pawar, Kirti, 2013a. Bioconversion of progesterone using aqueous two phase system by

- Comamonas acidovorans* MTCC 3364. Inter. J. Biotechnol. 111:211-214.
- Rudakiya, Darshan M., Pawar, Kirti, 2013b. Evaluation of remediation in heavy metal tolerance and removal by *Comamonas acidovorans* MTCC 3364. IOSR J. Environ. Sci. Toxicol. Food Technol. 5(5):26-32.
- Saraswathi, K., & Balakumar, S., 2009. Biodecolorization of azo dye (Pigmented Red 208) using *Bacillus firmus* and *Bacillus laterosporus*. J. Biosci. Technol. Vol 1 (1), 1-7.
- Verma, P. and D. Madamwar, 2003. Decolorization of Synthetic Dyes by a Newly Isolated Strain of *Serratia marcescens*, World J. Microbiol. Biotechnol., 19:615.
- Wong, P. K., Yuen, P. Y., 1998. Decolorization and Biodegradation of N, N'-dimethyl p-Phenylenediamine by *Klebsiella pneumoniae* RS-13 and *Acetobacter liquefaciens* S-1. J. Appl. Microbiol., 85:79-87.