Int.J.Curr.Microbiol.App.Sci (2018) *7*(9): xx-xx

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 09 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.709.xx

Bacterial Decolorization of Reactive Red: Strategic Bioremediation of Textile Dye

Sagarkumar Joshi[1*] and Nidhi Saxena[2]

[1]Department of Microbiology, School of Science, RK University, Rajkot-360020, Gujarat, India
[2]Department of Microbiology, Gyanyagna College of Science and Management, Rajkot - 360005, Gujarat, India

**Corresponding author*

Keywords

Reactive red, Azo dye, Decolorization, Bacteria, Optimization

Article Info

Accepted:
xx August 2018
Available Online:
xx September 2018

A B S T R A C T

The textile dye industries consume a substantial amount of water and produce extensive amount of waste which is contaminated by dyes like reactive dyes, azo dyes, many types of aerosols and much more non-degradable waste materials. The toxic effects of dyestuff and other organic compounds from modern effluents are harsh on human beings and also for regular habitat. Currently, most of the available dyes are aromatic and heterocyclic compounds with complex functional groups that can be converted into aromatic amines which are proved to be carcinogenic. In this research work, bacterial isolates which are proficient to decolorize the commercial dye - Reactive Red were isolated from the soil samples collected from adjacent territories of the textile industry located in Rajkot, India. The Reactive Red dye decolorization was analyzed using UV-visible spectrophotometric analysis at λ_{max} 680 nm. Optimization studies indicated that isolate-1 was found to be Gram positive rod that showed 93.59% decolorization at 60 hours with 250 mg/L Reactive Red dye concentration at 36 ºC with pH 5.5. Whereas, isolate-2 which was Gram negative bacteria exhibited 91.55% decolorization at 60 hours with 250 mg/L dye concentration at 36 ºC with pH 6.0. Both the isolates showed highest dye decolorization with sucrose as carbon source. As indicated in the present study, bacterial isolates were potential decolorizer of Reactive Red dye, which can be further exploited for commercial applications towards treatment of industrial effluent contaminated with hazardous dyes.

Introduction

Many colored effluents that contain dyes are released from food, leather, textile, dyestuff, and dyeing industries. The textile industry largely produces effluents contaminated with dyes (Marimuthu *et al.,* 2013). Different organic pollutants in the natural water resources and land are introduces by the effluents contained residual dyes (Carmen *et al.,* 2012). Approximately 80,000 - 90,000 tons of dyestuff and pigments are produced in India (Marimuthu *et al.,* 2013). It has been found that approximately 10,000 different textile dyes are commercially available worldwide and annual production is estimated to be 7×10^5 metric tons (Robinson *et al.,* 2001; Soloman *et al.,* 2009; Baban *et al.,*

2010). About 2% of dyes fail to bind to the substrate and are discharged in aqueous effluents during the dying process (Ndasi *et al.,* 2011). Azo dyes are the most preferably used dyes in the industrial sector (Murty *et al.,* 2012). They contain one or more azo groups which can resist the breakdown of dyes and accumulate in the environment at high levels with high degree of persistence (Saranraj *et al.,* 2010; Agarwal *et al.,* 2012). When dyes are present in the water system, the sunlight penetration is reduced into deeper layers which disturbs photosynthetic activity resulting in reduction of water quality, gas solubility and causes acute toxic effects on aquatic flora and fauna. Most of the dyes and their breakdown products released from wastewater are toxic, carcinogenic and mutagenic to humans and other life forms (Suteu *et al.,* 2009; Zaharia *et al.,* 2009). Various physicochemical methods are used for decolorization of dyes in wastewater, such as adsorption on activated carbon, electro-coagulation, flocculation, ion exchange, membrane filtration, ozonation and reverse osmosis but those are inefficient, expensive, have less applicability and produce wastes in the form of sludge, which again needs to be disposed off (Ogugbue *et al.,* 2011). Similarly, agro-wastes have been exploited for effective dye removal by the mechanism of biosorption (Luikham *et al.,* 2011). However, the microbial decolorization and degradation of azo dyes is inexpensive, eco-friendly process, and produces less amount of sludge (Carvalho *et al.,* 2008; Parshetti *et al.,* 2006). It has been found that many organisms are such as obligate anaerobes (e.g., *Bacteroides* spp., *Eubacterium* spp., *Clostridium* spp.), facultative anaerobes (e.g., *Proteus vulgaris*, *Streptococcus faecalis*), aerobes (e.g., *Bacillus* spp., *Sphingomonas* spp.), fungi (e.g., *Phanerochaete chrysosporium*, *Aspergillus* spp.), several yeasts and actinomycetes are used for decolorization of dyes (Dieckhues *et al.,* 1960; Adamson *et al.,* 1965; Scheline *et al.,* 1970; Dubin *et al.,* 1975; Wuhrmann *et al.,* 1980; Rafii *et al.,* 1990; Bragger *et al.,* 1997; Mehta *et al.,* 2012; Shah *et al.,* 2013; Dharajiya *et al.,* 2015; Dharajiya *et al.,* 2016). This study was carried out for the decolorization of Reactive Red dye by bacteria isolated from soil samples nearby the area of dye industry. The study also includes optimization for decolorization of Reactive Red dye by the bacterial isolates.

Materials and Methods

Dyes and chemicals

The textile dyes (azo dye compounds), namely Reactive Red, was procured from the Ranjit dyeing and printing industry, Rajkot, Gujarat, India. Nutrient agar media and all other chemicals used were of analytical grade and purchased from HiMedia, India.

Bacterial isolation and culture conditions

The bacteria were isolated from soil sample which was collected from nearby area of Ranjit dyeing and printing industry, Rajkot, Gujarat, India. From the collected composite soil sample 1% w/v of soil sample was aseptically inoculated in nutrient broth containing Reactive Red dye 250 mg/L in a 250 mL Erlenmeyer flask. The bacteria were enriched in Nutrient broth medium amended with 250 mg/L of Reactive Red dye (Pokharia *et al.,* 2013; Roat *et al.,* 2016). After 24 hours of incubation at 36 ± 2 ºC and at aerobic condition dilution tubes were prepared from the enriched culture. From each of the dilution tubes, 0.1 mL sample was inoculated on the nutrient agar plate containing Reactive Red dye (250 mg/L) using spread plate technique, followed by incubation for 24 hours at 36 ± 2 ºC. Isolates were screened for ability to decolorize the dye and highest zone of decolorization producing two colonies were selected for further experiments. The selected

isolates were then purified by streaking on nutrient agar added with 250 mg/L of the Reactive Red dye and the single colony pure cultures were stored in 15% glycerol at -20ºC (Roat *et al.*, 2016).

Inoculum preparation

Stored master cultures were transferred on nutrient agar plate and incubated for 24 hours at 36±2 ºC, and observed for purity of the culture. A well isolated colony was taken from the plate and inoculated in 50 mL nutrient broth and incubated on a shaker at 180 rpm and 36 ± 2 ºC temperature for 24 hours followed by standardization to 0.5 McFarland turbidity for all further experiments.

Morphological and biochemical identification of bacterial isolates

Bacterial isolates decolorizing the dye were characterized on the basis of their morphology and biochemical tests (Roat *et al.*, 2016). Gram's staining used for morphological characterization and according to their Gram's reaction biochemical tests were carried out, such as, sugar fermentation, IMViC, catalase, nitrate reduction, hydrogen sulfide production and motility.

Analytical techniques

Nutrient broth supplemented with Reactive Red dye was used as a control. A volume of 10% v/v of pre-cultured bacterium was added to 50 mL of Nutrient broth medium added with different concentrations (50, 100, 150, 200, 250 and 300 mg/L) of Reactive Red dye. The bio-decolorization of Reactive red by both the isolates was observed for 60 hours. In order to monitor the decolorization process, the samples were withdrawn at 12 hours interval, centrifuged at 10,000 rpm for 15 min and filtered through syringe filter (PVDF, Millipore, Inc.); and optical density was measured using UV/Vis spectrophotometer at the corresponding λ_{max} of the dye (680 nm) and was compared with the uninoculated control. The color removal efficiency of the bacteria was determined by following formula (Lade *et al.*, 2015).

$$\text{Decolorization (\%)} = \frac{(\text{Initial absorbance} - \text{final absorbance})}{\text{Initial absorbance}} \times 100$$

Effect of pH and temperature on the decolorization

In order to study the effect of pH and temperature, the sterilized Nutrient broth was amended with 250 mg/L of Reactive Red dye. The medium was maintained at different pH *viz*., 5.0, 5.5, 6.0 and 6.5. A 10% v/v overnight culture was inoculated in the flasks and incubated in a shaker at 36 ± 2 ºC. The effect of temperature was studied by inoculating overnight culture and incubating in a shaker at 28ºC, 32ºC, 36ºC and 40ºC. The medium was maintained at pH 6.0. The measurement of decolorization of the total dye concentration was performed at an interval of 12 hours up to 60 hours (Lalnunhlimi and Veenagayathri, 2016).

Effect of carbon sources on the decolorization of dye

The effect of carbon sources was studied using various compounds, such as fructose, glucose, lactose and sucrose, at a concentration of 1% and they were added individually as a supplement to Nutrient broth for the decolorization of Reactive Red. A 10% v/v of the overnight grown culture was inoculated in the flasks and incubated in a shaker at 36 ± 2 ºC.

Results and Discussion

Reactive dyes are widely used in many industries. These reactive dyes are degraded

by a wide range of microorganisms. Aerobic and anaerobic bacteria from different environments have the ability to reduce reactive dyes into genotoxic compounds. The objective of this study was to isolate bacteria that can be used for the removal of Reactive Red dye from textile wastes.

Isolation and screening of Reactive Red dye decolorizing bacteria

The initial enrichment of the bacterial isolates for the Reactive Red dye degradation indicated two bacterial strains designated as isolate-1 and isolate-2 to be efficient. The screening experiments for color removal were carried out under acidic pH and aerobic conditions. Selection of the isolates was carried out by considering the highest zone of decolorization on nutrient agar plate containing 250 mg/L of Reactive Red dye.

Morphological and biochemical characterization of bacterial isolates

Two potent isolates of bacteria which can decolorize the Reactive Red were isolate-1 and isolate-2 which were Gram positive rod and Gram negative short rod, respectively (Fig.1). On culture plate isolate-1 showed opaque, white, large, concave, non-pigment forming and rough colony while isolate-2 shows opaque, off-white, small, pinpointed, smooth, non-pigment forming colony. Other biochemical characters are shown in Table.1.

Decolorization of Reactive Red dye by individual isolates at different time interval

Individual bacterial isolates were analyzed for the decolorization of reactive red at 250 mg/L (Fig. 2). Isolate-1 showed maximum decolorization of 93.59% and isolate-2 showed maximum decolorization of 91.55% for Reactive red dye under optimum conditions (Fig. 3).

Reactive red dye decolorization at various concentrations

The ability of the isolated bacteria to decolorize the dye Reactive Red at various concentrations (100, 150, 200, 250, and 300 mg/L) was investigated. The rate of decolorization increased with increase in initial dye concentration from 100 to 250 mg/L, whereas decolorization decreased at 300 mg/L are shown in Fig.4. This study was conducted under acidic conditions. The optimum concentration for efficient dye decolorization was found to be 250 mg/L for Reactive Red, where 92.11% and 90.31% of the dyes were decolorized by isolate-1 and isolate-2, respectively (Fig.4).

Fig.1 Microscopic images of Gram staining reaction of (A) Isolate-1 and (B) Isolate-2

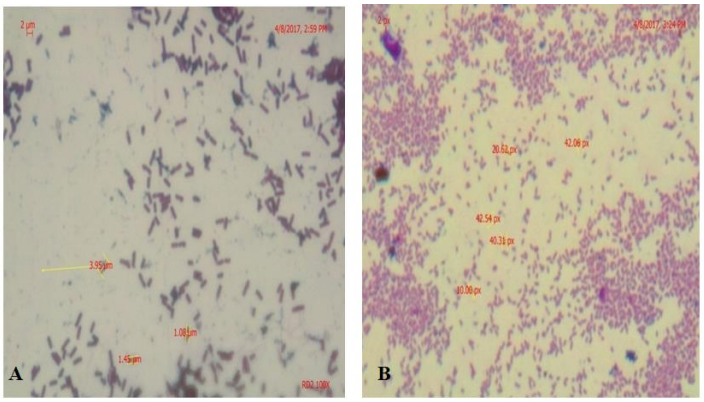

Fig.2 Dye decolorization by two indigenous isolates. (A) Control; (B) Isolate-1; (C) Isolate-2

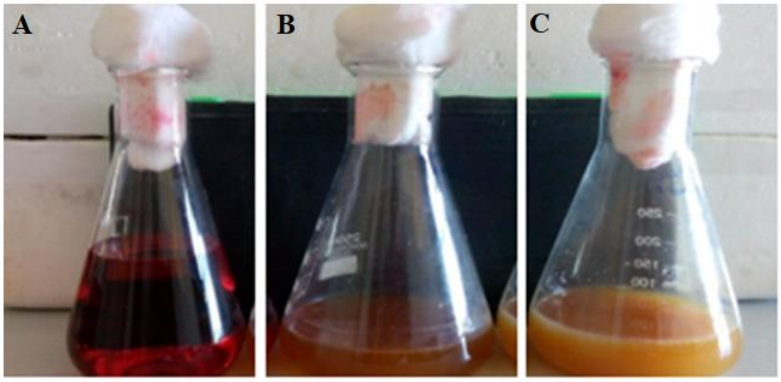

Fig.3 Decolorization of Reactive Red dye by isolate-1 and isolate-2 at different time interval

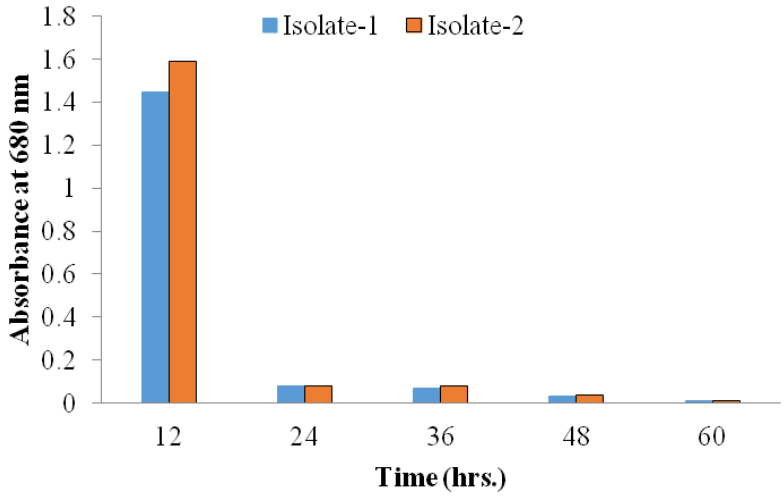

Fig.4 Decolorization of Reactive Red dye by (A) isolate-1 and (B) isolate-2, at different dye concentrations

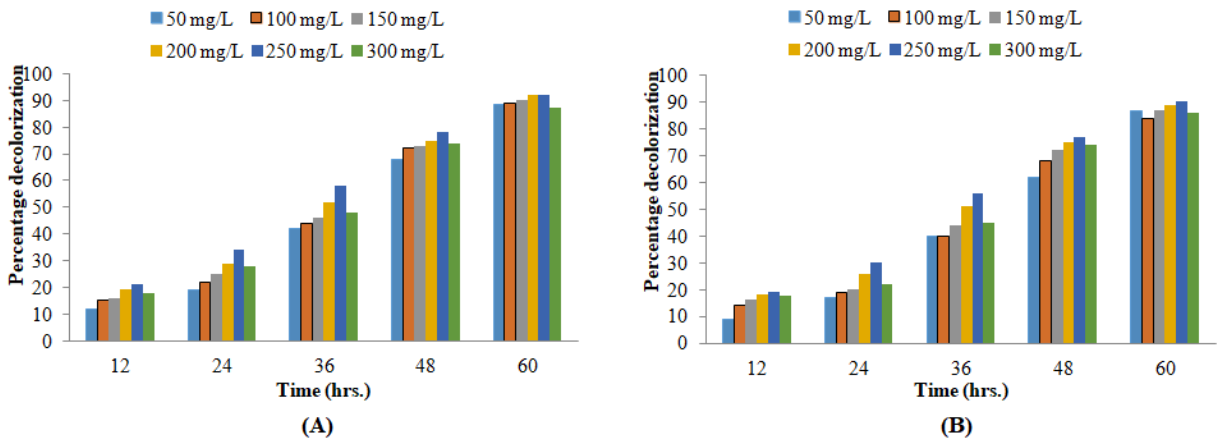

Fig.5 Decolorization of Reactive Red dye at different pH by (A) isolate-1 and (B) isolate-2

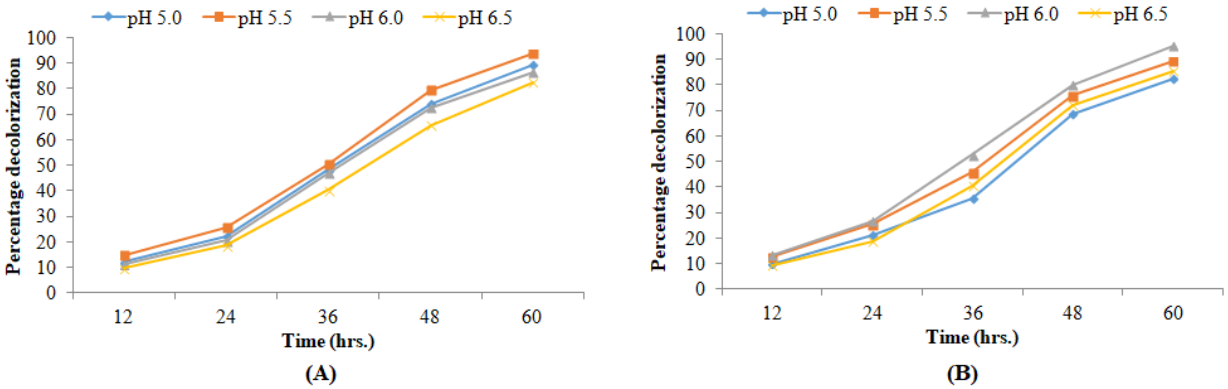

Fig.6 Decolorization of Reactive Red dye at different temperatures by (A) isolate-1 and (B) isolate-2

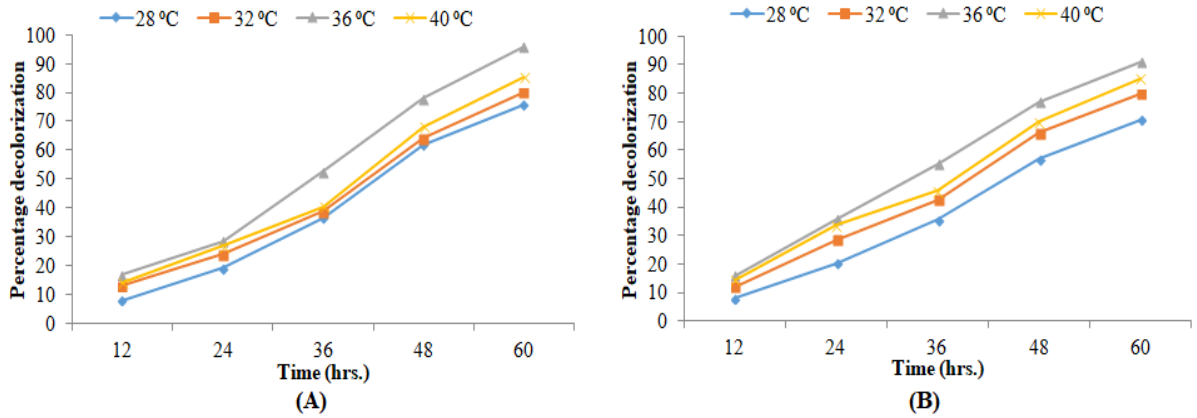

Fig.7 Decolorization of Reactive red dye with different carbon source by (A) isolate-1 and (B) isolate-2

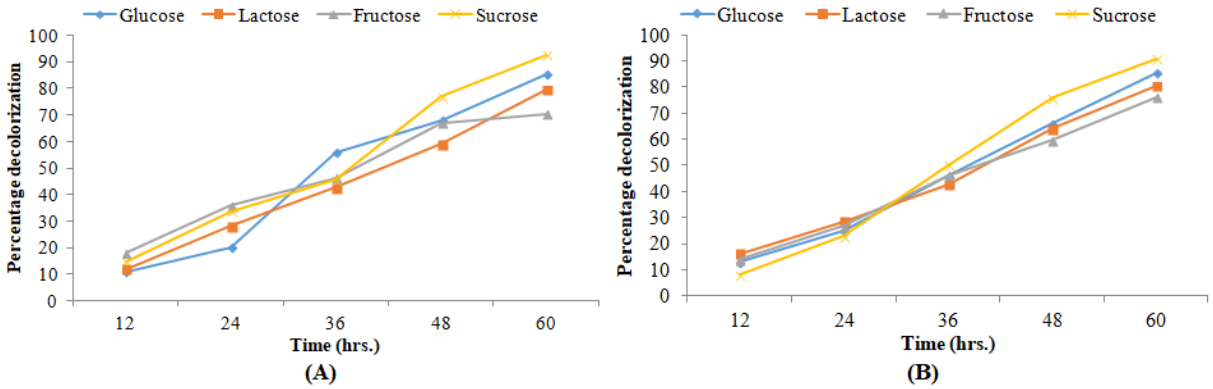

Table.1 Biochemical characteristics of isolates

S. N.	Biochemical test	Isolate-1	Isolate-2
1.	**Sugar Fermentation**		
	Lactose	Variable	Acid only
	Glucose	Acid only	Acid and Gas
	Sucrose	Acid only	Acid
	Mannitol	Acid only	Acid
2.	**IMViC test**		
	Indole test	–	–
	Methyl red test	–	+
	Voges-proskauer test	+	–
	Citrate utilization test	+	+
3.	**Catalase test**	+	+
4.	**Nitrate reduction test**	+	+
5.	**Hydrogen disulfide**	–	+
6.	**Motility**	+	+
– : Negative; +: Positive			

The maximum decolorization was observed at dye concentration of 200 mg/L in the past study (Lalnunhlimi and Krishnaswamy, 2016). Hence, the bacterial isolates used in the present study can tolerate dye concentration up to 250 mg/L and can efficiently decolorize the Reactive Red dye.

However, more than 250 mg/L dye could be little toxic to the cells as the rate of decolorization was reduced beyond 250 mg/L.

Effect of pH

The effect of pH was studied at different pH (5.0, 5.5, 6.0 and 6.5) with both bacterial isolates. All the pH allowed growth of the bacteria. The maximum decolorization was observed at pH 5.5, which was 93.59% by isolate-1 and at pH 6.0, which was 95.2% by isolate-2 at the end of the 60 hours (Fig.5). The pH tolerance of decolorizing bacteria is quite important because reactive azo dyes are bound to cotton fibers by addition or substitution mechanisms under acidic conditions and high temperatures (Lalnunhlimi and Veenagayathri, 2016). In one of the research, it has been indicated that bacteria (*Microbacterium* sp.) can efficiently decolorize azo dye at slight acidic pH (5.0) (Roat *et al.*, 2016).

Effect of temperature

The effect of temperature was analyzed at 28 ºC, 32 ºC, 36 ºC and 40 ºC. The temperature 36 ºC enhanced the growth of the bacteria and showed maximum decolorization of dye that was 93.95% with isolate-1 and 91.55% with isolate-2 by the end of the 60 hours (Fig. 6). Similarly, 36 ºC was found as an optimum temperature for the azo dye decolorization by bacterial cell (Lalnunhlimi and Krishnaswamy, 2016).

So, most of the bacteria isolated and used as a dye decolorizer are having optimum temperature around 37 ºC. It is important to note that, the bacterial isolates having optimum decolorization temperature as 37 ºC can be used in the in-situ remediation of the dye contaminated sites.

Effect of carbon sources

To examine the influence of carbon sources on the decolorization of Reactive Red dye (250 mg/L), carbon sources such as glucose, lactose, sucrose and fructose were supplemented in the media. It was found that sucrose could enhance the growth of the bacteria more effectively than other carbon sources (Fig. 7). The decolorization of Reactive Red dye reached a maximum of 92.67% with sucrose as a carbon source followed by glucose, lactose and fructose which showed 85.25%, 70.29% and 79.56% of decolorization, respectively with isolate-1 and decolorization of Reactive Red dye reached a maximum of 90.85% with sucrose as a carbon source followed by glucose, fructose and lactose which showed 85.25%, 76.25 and 80.25% of decolorization, respectively with isolate-2 (Fig. 7). It is important finding as the bacterial isolates utilized simple form of carbon sources like glucose and fructose for the reproduction and maintenance of the cells. After acclimatization at the higher concentration of dye, the isolates used more complex carbon sources like sucrose for efficient dye decolorization. This will improve the efficiency of the bacterial isolates to utilize more complex molecules such as azo dyes which lead to the improvement of the decolorization efficiency. Similar results were found by Lalnunhlimi and Krishnaswamy, (2016) as they reported sucrose as an optimum carbon source for the decolorization of dyes.

Present study showed that enriched bacterial strains isolate-1 and isolate-2 can efficiently decolorize Reactive Red dye up to 93.59% and 91.55%, respectively in 60 hours. The bacterial isolate-1 and isolate-2 shows maximum decolorization ability of Reactive Red dye at pH 5.5 and pH 6.0, respectively. The physical parameters such as pH, temperature and carbon sources play an important role in enhancing of the decolorization efficiency. Future work on the identification of isolates, evaluation of the mechanism for decolorization and metabolic pathway present in the bacterial isolates can be helpful in enhancing the decolorization of azo dyes.

Acknowledgement

Authors acknowledge the School of Pharmacy, RK University for the research facilities towards efficient execution of the experiments.

References

Adamson RH, Dixon RL, Francis FL, Rall DP (1965) Decolorization of industrial effluents – available methods and emerging technologies – a review. Proceedings of the National Academy of Sciences of the United States of America 54:1386-1391.

Agarwal T, Singh R (2012) Bioremedial potentials of a moderately halophilic soil bacterium. Journal of Pharmaceutical and Biomedical Sciences 19(19):1-6.

Baban A, Yediler A, Ciliz NK (2010) Integrated water management and CP implementation for wool and textile blend processes. CLEAN–Soil, Air, Water 38(1):84-90.

Bragger JL, Lloyd AW, Soozandehfar SH, Bloomfield SF, Marriott C, Martin GP (1997) Investigations into the azo reducing activity of a common colonic microorganism. International Journal of Pharmaceutics 157(1):61-71.

Carmen Z, Daniela S (2012) Textile organic dyes–characteristics, polluting effects and separation/elimination procedures from industrial effluents–a critical overview. Inorganic pollutants ten years

after the Stockholm convention-environmental and analytical update 2012:55-86.

Carvalho MC, Pereira C, Goncalves IC, Pinheiro HM, Santos AR, Lopes A, Ferra MI (2008) Assessment of the biodegradability of a monosulfonated azo dye and aromatic amines. International Biodeterioration & Biodegradation 62(2):96-103.

Dharajiya D, Shah M, Bajpai B (2015) Biosorption of Acid Black 52, an azo dye from aqueous solution using pre-treated biomass of *Aspergillus fumigatus* A23. Pollution Research 36(4): 667-676.

Dharajiya D, Shah M, Bajpai B (2016) Decolorization of simulated textile effluent by *Phanerochaete chrysosporium* and *Aspergillus fumigatus* A23. Nature Environment and Pollution Technology 15(3): 825-832.

Dieckhues B (1960) Research on reductive splitting of azo dyes by bacteria. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 1. Abt. Medizinisch-hygienische Bakteriologie, Virusforschung und Parasitologie. Originale 180:244-249.

Dubin P, Wright KL (1975) Reduction of azo food dyes in cultures of *Proteus vulgaris*. Xenobiotics 5(9):563-571.

Lade H, Kadam A, Paul D, Govindwar S (2015) Biodegradation and detoxification of textile azo dyes by bacterial consortium under sequential microaerophilic/aerobic processes. EXCLI Journal 14:158-174.

Lalnunhlimi S, Krishnaswamy V (2016) Decolorization of azo dyes (Direct Blue 151 and Direct Red 31) by moderately alkaliphilic bacterial consortium. Brazilian Journal of Microbiology 47(1):39-46.

Luikham S, Malve S, Gawali P, Ghosh S (2018) A novel strategy towards agro-waste mediated dye biosorption for water treatment. World Journal of Pharmaceutical Research 7(4): 197-208.

Marimuthu T, Rajendran S, Manivannan M (2013) A review on bacterial degradation of textile dyes. Journal of Chemistry and Chemical Sciences 3(3):201-212.

Mehta P (2012) Treating textile effluents by coagulation–flocculation method using different dosing compositions. Advances in Applied Science Research 3:2514-2517.

Murty SD, Patel SD, Soni R, Bhatt N (2012) Isolation and identification of bacterial culture for azo dye degrading capability. International Journal of Research in Chemistry and Environment 2:69-79.

Ndasi NP, Augustin M, Bosco TJ (2011) Biodecolourisation of textile dyes by local microbial consortia isolated from dye polluted soils in ngaoundere (Cameroon). International Journal of Environmental Sciences 1(7):1403-1419.

Ogugbue CJ, Sawidis T (2011) Bioremediation and detoxification of synthetic wastewater containing triarylmethane dyes by *Aeromonas hydrophila* isolated from industrial effluent. Biotechnology research international 2011.

Parshetti G, Kalme S, Saratale G, Govindwar S (2006) Biodegradation of Malachite Green by *Kocuria rosea* MTCC 1532. Acta Chimica Slovenica 53(4):492-498.

Pokharia A, Ahluwalia SS (2013) Isolation and screening of dye decolorizing bacterial isolates from contaminated sites. Textiles and Light Industrial Science and Technology 2(2):54-61.

Rafii FA, Franklin WI, Cerniglia CE (1990) Azoreductase activity of anaerobic bacteria isolated from human intestinal

microflora. Applied and Environmental Microbiology 56(7):2146-51.

Roat C, Kadam A, Patel T, Dave S (2016) Biodegradation of diazo dye, reactive blue 160 by Isolate *Microbacterium* sp B12 Mutant: Identification of intermediates by LC-MS. International Journal of Current Microbiology and Applied Sciences 5(3):534-47.

Robinson T, McMullan G, Marchant R, Nigam P (2001) Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresource Technology 77(3):247-55.

Saranraj P, Sumathi V, Reetha D, Stella D (2010) Decolourization and degradation of direct azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. Journal of Ecobiotechnology 2(7):7-11.

Scheline RR, Nygaard RT, Longberg B (1970) Enzymatic reduction of the azo dye, acid yellow, by extracts of *Streptococcus faecalis* isolated from rat intestine. Food and Cosmetics Toxicology 8(1):55-58.

Shah MK, Darshan TD, Bajpai B (2013) A comparative study on decolourization of industrial dyes and real textile wastewater by white rot and non-white rot fungi. Asian Journal of Water, Environment and Pollution 10(4): 77-87.

Soloman PA, Basha CA, Velan M, Ramamurthi V, Koteeswaran K, Balasubramanian N (2009) Electrochemical degradation of Remazol Black B dye effluent. CLEAN–Soil, Air, Water 37(11):889-900.

Suteu D, Zaharia C, Bilba D, Muresan R, Popescu A, Muresan A (2009) Decolorization waste waters from the textile industry-physical methods, chemical methods. Industria Textila 60(5):254-63.

Wuhrmann K, Mechsner KL, Kappeler TH (1980) Investigation on rate—Determining factors in the microbial reduction of azo dyes. European Journal of Applied Microbiology and Biotechnology 9(4):325-38.

Zaharia C, Suteu D, Muresan A, Muresan R, Popescu A (2009) Textile wastewater treatment by homogenous oxidation with hydrogen peroxide. Environmental Engineering and Management Journal 8(6):1359-69.

How to cite this article:

Sagarkumar Joshi and Nidhi Saxena. 2018. Bacterial Decolorization of Reactive Red: Strategic Bioremediation of Tex-tile Dye. *Int.J.Curr.Microbiol.App.Sci.* 7(09): xx-xx.
doi: https://doi.org/10.20546/ijcmas.2018.709.xx