

## Original Research Article

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## Bacterial Decolorization of Reactive Red: Strategic Bioremediation of Textile Dye

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### ABSTRACT

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  $\lambda_{\max}$  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 °C 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 °C 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.

#### Keywords

Reactive red, Azo dye, Decolorization, Bacteria, Optimization

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### 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 introduced 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  $7 \times 10^5$  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 dyeing 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  $36 \pm 2$  °C 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  $36 \pm 2$  °C. 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 -20°C (Roat *et al.*, 2016).

### **Inoculum preparation**

Stored master cultures were transferred on nutrient agar plate and incubated for 24 hours at 36±2 °C, 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 °C 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  $\lambda_{max}$  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 °C. The effect of temperature was studied by inoculating overnight culture and incubating in a shaker at 28°C, 32°C, 36°C and 40°C. 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 °C.

### **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.

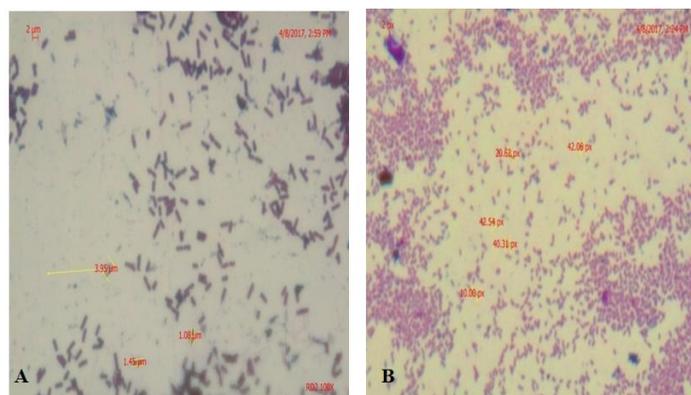
### **Decolorization of Reactive Red dye by individual isolates at different time interval**

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).

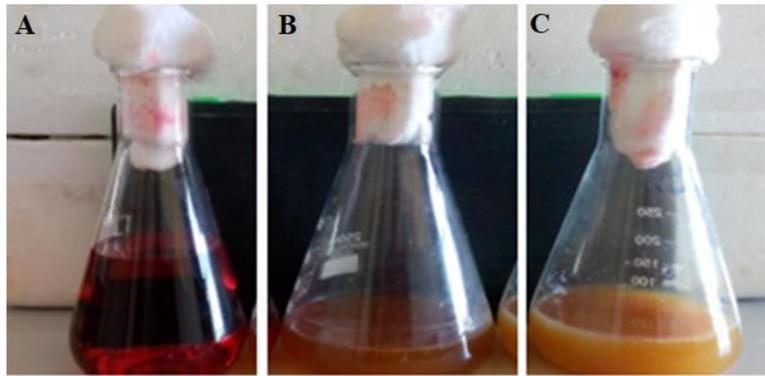
### **Reactive red dye decolorization at various concentrations**

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).

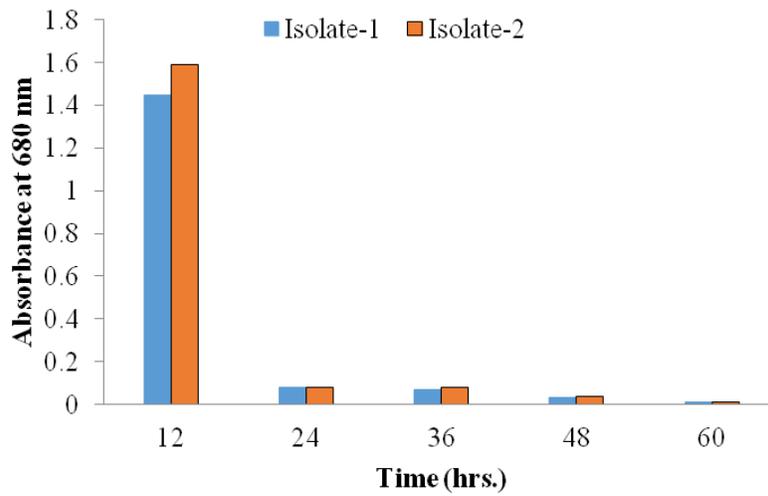
**Fig.1** Microscopic images of Gram staining reaction of (A) Isolate-1 and (B) Isolate-2



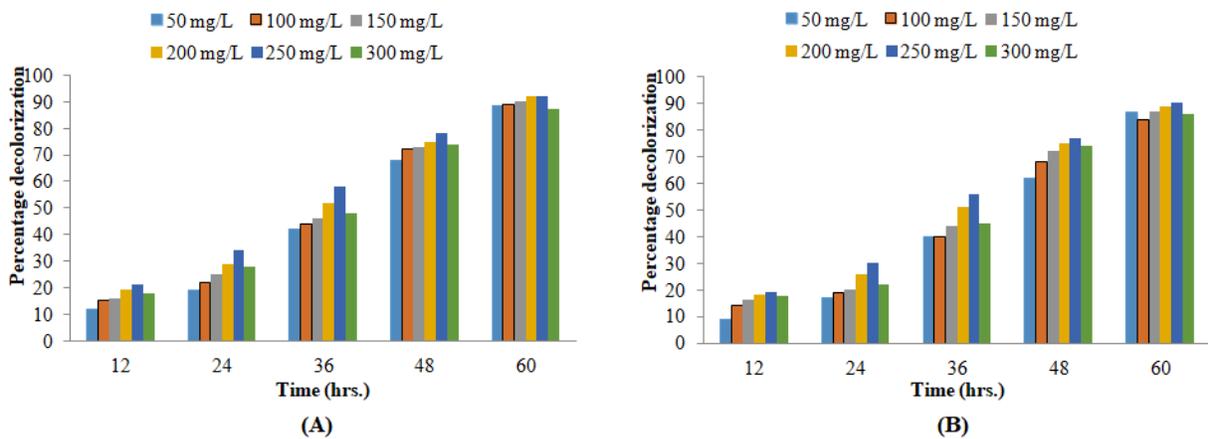
**Fig.2** Dye decolorization by two indigenous isolates. (A) Control; (B) Isolate-1; (C) Isolate-2



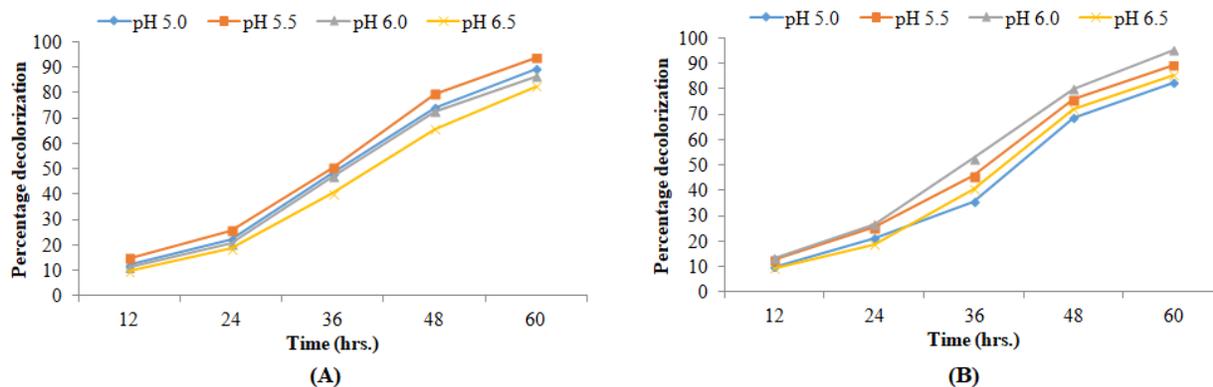
**Fig.3** Decolorization of Reactive Red dye by isolate-1 and isolate-2 at different time interval



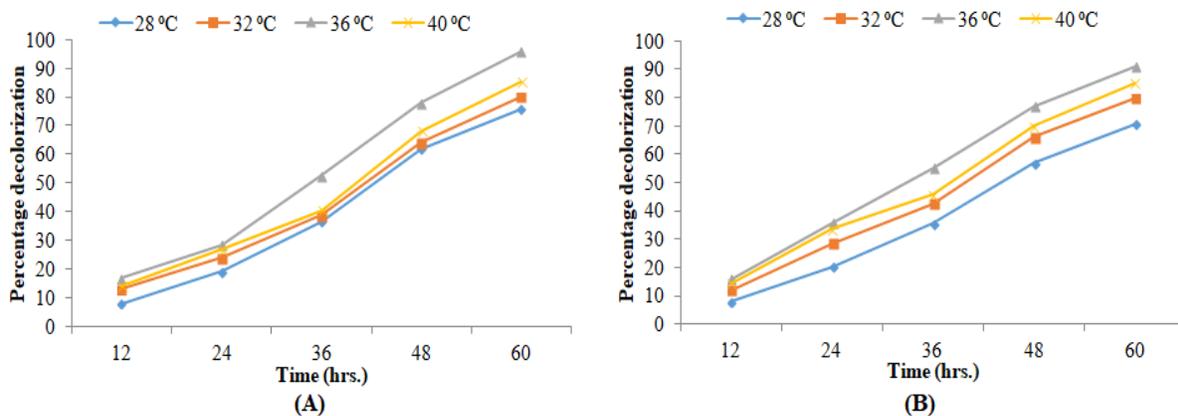
**Fig.4** Decolorization of Reactive Red dye by (A) isolate-1 and (B) isolate-2, at different dye concentrations



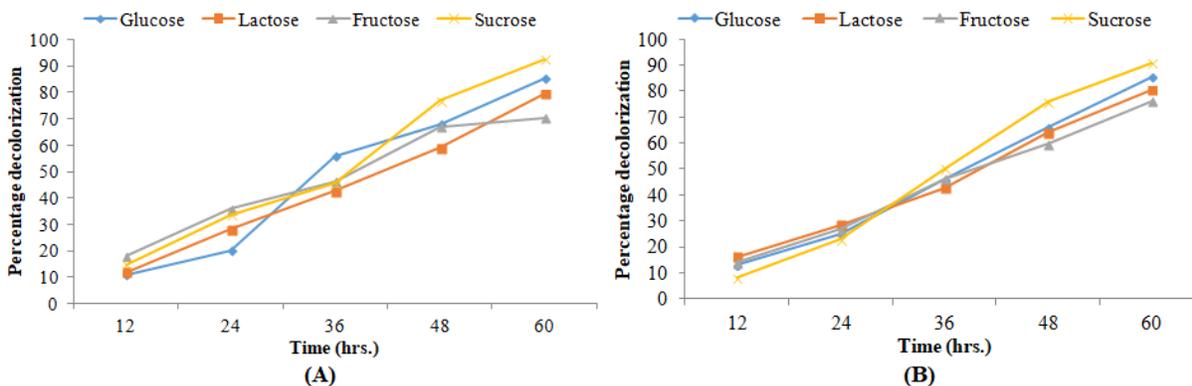
**Fig.5** Decolorization of Reactive Red dye at different pH by (A) isolate-1 and (B) isolate-2



**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    |
|---------------------------|-------------------------------|-----------|--------------|
| <b>1.</b>                 | <b>Sugar Fermentation</b>     |           |              |
|                           | Lactose                       | Variable  | Acid only    |
|                           | Glucose                       | Acid only | Acid and Gas |
|                           | Sucrose                       | Acid only | Acid only    |
|                           | Mannitol                      | Acid only | Acid only    |
| <b>2.</b>                 | <b>IMViC test</b>             |           |              |
|                           | Indole test                   | –         | –            |
|                           | Methyl red test               | –         | +            |
|                           | Voges-proskauer test          | +         | –            |
|                           | Citrate utilization test      | +         | +            |
| <b>3.</b>                 | <b>Catalase test</b>          | +         | +            |
| <b>4.</b>                 | <b>Nitrate reduction test</b> | +         | +            |
| <b>5.</b>                 | <b>Hydrogen disulfide</b>     | –         | +            |
| <b>6.</b>                 | <b>Motility</b>               | +         | +            |
| – : 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 °C, 32 °C, 36 °C and 40 °C. The temperature 36 °C 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 °C 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 °C. It is important to note that, the bacterial isolates having optimum decolorization temperature as 37 °C 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.

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