

BACTERIAL DECOLORIZATION OF TEXTILE DYE- ORANGE 3R

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ABSTRACT:

The dye decolorizing isolates, *Bacillus* sp., *Klebsiella* sp. *Salmonella* sp. and *Pseudomonas* sp. were isolated from the textile effluent samples collected from Elampillai, Tamil Nadu. Different parameters such as various carbon source, nitrogen source, temperature, pH and inoculum size were optimized for decolorization of Orange 3R by using bacterial isolates. *Pseudomonas* sp. and *Bacillus* sp. showed maximum dye decolorization of 89% at the end of 144h under optimum condition. But the *Bacillus* sp. was found to be more efficient in dye decolorization. All parameters studied in this paper were found to be effective for all isolates. The results reported here warrant further investigation to establish the usefulness of these isolates for bioremediation and biodegradation application such as waste water treatment. High decolorization extent and facile conditions show the potential for this bacterial strain to be used in the biological treatment of dyeing mill effluents.

Keywords: *Orange 3R, Textile dye, Decolorization, Textile effluent*

[I] INTRODUCTION

Water is life but now a-days due to the advancement in industrialization, it is spoiling a lot. Many contaminants present in wastewater, such as acids, bases, toxic organic and inorganic dissolved solids, and colors. Among them, colors are considered the most undesirable and are mainly caused by dyes [1]. Dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to biodegrade [2]. The textile industry utilizes about 10000 different dyes and pigments. The worldwide annual production of dyes is over 7·10⁵ tons [3,4]. The dyestuff usage has been increased day by day because of tremendous increase of industrialization and man's urge for color [5]. Synthetic dyestuffs are used extensively in textile, paper, printing industries and dyehouses. The effluents of these industries are highly colored and the disposal of these wastes into

receiving waters causes damage to the environment. Dyes may significantly affect photosynthetic activity in aquatic life because of reduced light penetration and may also be toxic to some aquatic life due to the presence of aromatics and metals, chlorides, etc. Dyes tinctorial value is high: less than 1 ppm of the dye produces obvious coloration [6].

Removal of color from dye bearing wastewater is a complex problem because of difficulty in treating such wastewaters by conventional treatment methods [7]. Ozonation, photooxidation, electrocoagulation, adsorption, activated carbon, froth flotation, reverse osmosis, ion exchange, membrane filtration and flocculation, are applied for color removal from textile effluents [8-10]. These physico-chemical methods are less efficient, costly, of limited applicability and produce wastes, which are

difficult to dispose off. In some cases, traditional biological procedures were combined with chemical or physical treatment processes to achieve better decolorization. As a viable alternative, biological processes have received increasing interest owing to their cost, effectiveness, ability to produce less sludge and environmental benignity [11]. Biological processes have potential to convert or degrade the pollutant into water, carbon dioxide and various salts of inorganic nature. The isolation of potent species and thereby degradation is one of the interests in biological aspect of effluents treatment [5].

In recent years a number of studies have focused on some microorganisms that are able to degrade and absorb dyes from wastewater. A wide variety of microorganisms are capable of decolorization of a wide range of dyes some of them are as bacteria: *Escherichia coli* NO3 [12], *Pseudomonas luteola* [13], *Aeromonas hydrophila* [11]; fungi: *Aspergillus niger* [14], *Phanerochaete chrysosporium*, *Aspergillus terricola* [15], *P. chrysosporium* [16]; yeasts: *Saccharomyces cerevisiae*, *Candida tropicalis*, *C. lipolytica* [17]; algae: *Spirogyra* species [18], *Chlorella vulgaris* [19], *C. sorokiniana* [20], *Lemna minuscula* [21], *Scenedesmus obliquus*, *C. pyrenoidosa* and *Closterium lunula* [22]. Malachite Green (MG) is a triphenyl methane dye, which is most widely used for coloring purpose, amongst all other dyes of its category [23]. MG has properties that make it difficult to remove from aqueous solutions. If the solution containing MG discharged into receiving streams it will affect the aquatic life and cause detrimental effects in liver, gill, kidney, intestine and gonads. In humans, it may cause irritation to the gastrointestinal tract upon ingestion. Contact of MG with skin causes irritation and redness and pain. Upon contact with eye will lead to permanent injury of human eyes and laboratory animals [24].

This study aims to investigate the potential of bacterial cultures for decolorization of effluent containing a textile dye, Orange 3R dye decolorization by bacterial cultures with respect to various nutritional sources (carbon and nitrogen), environmental parameters (temperature, pH, inoculum size) was optimized.

[II] MATERIALS AND METHODS

2.1. Chemicals and media

Textile dye, Orange 3R (Figure 1) and dye effluents were collected from a dyeing industry located at Elampillai, Salem (TN). All microbiological media and medium ingredients were purchased from HiMedia Laboratories (Mumbai, MH, India).

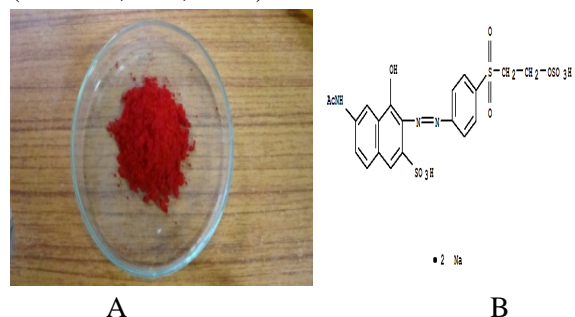


Fig. 1 Orange 3R dye sample (A) and its chemical structure (B)

2.2. Isolation, screening and identification of dye degrading bacteria

The dye decolorizing bacteria were isolated from textile dye effluent by serial dilution and plating appropriate dilutions on modified Zhou and Zimmermann (ZZ) agar medium containing (g/L), 5-yeast extract, 5-glucose, 0.5-(NH₄)₂SO₄, 2.66-KH₂HPO₄, 4.32-Na₂HPO₄, 100 mg-Dye (Orange 3R), 20-agar (pH 7.0). All the isolated cultures were studied by inoculating them in effluent basal medium containing (g/L of dye containing effluent), 5-yeast extract, 5-glucose, 0.5-(NH₄)₂SO₄, 2.66-KH₂HPO₄, 4.32-Na₂HPO₄ (pH 7.0). The inoculated medium was incubated at 30°C for six days under shaking

culture conditions After seven days decolorization effect was seen visually. The isolates showing more decolorization of the Orange 3R were selected for further studies. Dye degrading isolates were identified on the basis of morphological and biochemical tests according to Bergey's Manual of Systematic Bacteriology [25].

2.3. Dye decolorization evaluation

The dye decolorizing bacteria were screened using modified method of Arun Prasad and Bhaskara Rao [26]. Decolorization activity was performed in 100 ml of ZZ medium containing 0.02g of Orange 3R and 10% (v/v) inoculum of each isolate used separately. Uninoculated dye medium served as control. Inoculated medium and control was incubated at 30° C for six days under shake culture condition. About 2 ml samples were withdrawn aseptically and centrifuged at 8,000 rpm for 15 minutes. The clear supernatant was used for measuring absorption at 600 nm using UV-Vis spectrophotometer (Shimadzu, Japan). The percent decolorization of effluent was determined by using the formula:

$$D = [A_0 - A_1] / A_0 \times 100$$

where, D, decolorization in %; A_0 , initial absorbance; A_1 , final absorbance

2.4. Dye decolorization optimization

Decolorization of orange 3R textile dye by all four isolates was optimized with respect to the effect of 1%, carbon sources (glucose, sucrose, mannitol), 0.25%, nitrogen sources (beef extract, peptone, yeast extract), temperature (4, 27, 37°C), pH (5-9), and inoculum (2-10%). Initial experiments were carried out with 10%, (v/v) inoculum of each selected isolate in ZZ medium and ZZ medium without culture was served as control. All the flasks were incubated at 30°C under shaking conditions (150 rpm) for six days.

After six days the samples were withdrawn and analysed for percent decolorization.

2.5. Time course of dye decolorization

The time course of decolorization was carried out under optimum conditions obtained from above studies and the optimum conditions are: for *Bacillus* sp. (1% sucrose, 1% peptone, pH 8, 27°C and 4% inoculum), for *Klebsiella* sp. (1% sucrose, 1% beef extract, pH 7, 27°C and 6% inoculum), for *Salmonella* sp. (1% sucrose, 1% beef extract, pH 8, 37°C and 10% inoculum) and for *Pseudomonas* sp. (1% sucrose, 1% beef extract, pH 6, 4°C and 10% inoculum). Flasks were incubated up to 144h at their respective temperature and samples were removed after every 24 h and analyzed for decolorization activity as described above.

2.6. Statistical Analysis

All analysis was conducted in triplicate and results presented here are the mean of triplicate \pm standard deviations (SD)

[III] RESULTS AND DISCUSSION

3.1. Isolation, screening and identification of dye degrading bacteria

The sample was collected from textile dye industry Elampillai, Salem District Tamilnadu. Textile industries have shown as increase in installed spindle age, yarn production and output of fabric and garments. It is very clear that these industries play a positive role in the Indian economical reformation [27]. Textile dye effluent sample were collected from the disposal site of effluent for screening and isolation of dye degrading bacteria. Since chances of getting microbes having the ability to decolorize the dye effluent is very high. The textile and dyeing industry are one of the industries, which contribute to the soil and water pollution. There are about 700 dyeing and bleaching units located in and around Tiruppur and Coimbatore in Tamil

Nadu state. The consume substantial volumes of water and chemicals such as bleaching liquids, soda ash, caustic soda, sulfuric acid and sodium peroxide [28]. Large amount of dye containing effluents are discharged into water bodies by these industries carrying pollution problem. This pollution problem is a topic of great public and government concern today, forced by legislation. The industrial units are now looking forward to

cost effective solutions for reduction of pollution loads to meet the regulatory requirements.

The bacterial cultures were identified by microscopic, biochemical characters were identified observations as shown in Table 1 and identified as *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp., *Pseudomonas* sp.

Test	S1	S2	S3	S4
Gram's nature	+	-	-	-
Shape	Rod	Rod	Rod	Rod
Motility	Motile	Non-Motile	Motile	Motile
Glucose fermentation	² A	² AG	AG	A
Sucrose fermentation	A	AG	-	-
Lactose fermentation	A	AG	-	-
Maltose fermentation	A	AG	AG	A
Mannitol fermentation	A	AG	AG	-
¹ TSI	³ A/A, ⁴ H ₂ S+	A/A, ⁵ G+, H ₂ S-	AG, H ₂ S+	A, H ₂ S-
Indole production	⁵ -	-	-	-
Methyl red	-	-	+	-
Voges-Prausker	-	+	-	-
Citrate utilization	⁶ +	+	+	+
Catalase	+	+	+	+
Oxidase	-	-	-	+
Isolate identification	<i>Bacillus</i> sp.	<i>Klebsiella</i> sp.	<i>Salmonella</i> sp.	<i>Pseudomonas</i> sp.

¹Triple Sugar Iron, ²Acid, ³Acid-Gas, ⁴Hydrogen sulphide, ⁵Negative, ⁶Positive

Table 1. Identification of dye decolorizing bacteria from effluent

3.2. Dye decolorization optimization

Three carbons sources such as glucose, sucrose and mannitol were used (Fig. 2). The range of activity on decolorization of orange 3R with glucose was 81.83% 56.83% 83.33% and 75.76% with *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp., and *Pseudomonas* sp., respectively. An organism, *Salmonella* sp. was found to be the most effective decolorizer. The range of activity on decolorization of orange 3R with sucrose was 87.80%, 72.36%, 86.18% and 80.49% with *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp. and

Pseudomonas sp., respectively. Bacterium *Bacillus* sp. was found to be the most effective decolorizer. The range of activity on decolorization of orange 3R with mannitol was 87.09%, 64.04%, 86.84% and 73.68%, with *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp. and *Pseudomonas* sp. and *Salmonella* sp., respectively. Also *Salmonella* sp. was found to be the most effective decolorizer. Wang et al. [29] reported a *Citrobacter* sp. decolorized by 96.2% of reactive red 180 dye with 4g/L of glucose as carbon source.

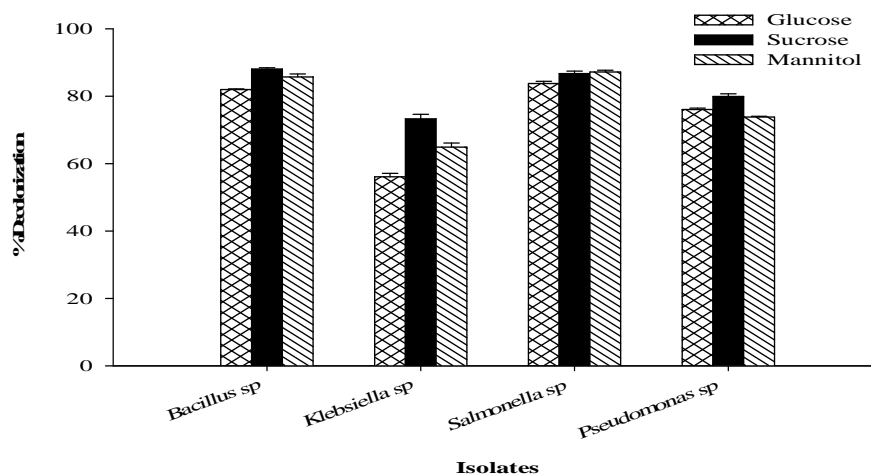


Fig. 2. Effect of different carbon sources on decolorization of Orange 3R dye by bacterial isolates (144h, 30°C, 150 rpm, error bars represents the standard deviation (\pm) mean of triplicate analysis)

Three nitrogen sources such as beef extract, peptone and yeast extract were used (Fig.3). The range of activity on decolorization of orange 3R with beef extract was 84.67%, 67.33%, 70.67%, 87.33% with *Bacillus sp.*, *Klebsiella sp.*, *Salmonella sp.*, and *Pseudomonas sp.*, respectively. *Pseudomonas sp.* was found to be the most effective decolorizer. The range of activity on decolorization of orange 3R with peptone was 85.29%, 64.71%, 42.16% and 78.43% with *Bacillus sp.*, *Klebsiella sp.*, *Salmonella sp.* and *Pseudomonas sp.*, respectively. *Bacillus sp.* was found to be the most effective decolorizer. The range of activity on decolorization of orange 3R with yeast extract was 82.81%, 70.17%, 53.91% and 81.25% with *Bacillus sp.*, *Klebsiella sp.*, *Salmonella sp.*, and *Pseudomonas sp.*, respectively. A bacterium *Bacillus sp.* was found to be the most effective decolorizer. Mathew and Madamwar [30] reported use of 0.1% yeast extract for decolorization of ranocid fast blue dye but it was for bacterial consortium.

Three different temperatures such as 4, 27 and 37°C were used (Fig. 4). The range of activity on decolorization of orange 3R with 4°C was 78.57%, 46.83%, 41.27% and 73.81% with *Bacillus sp.*, *Klebsiella sp.*, *Salmonella sp.* and *Pseudomonas sp.*, respectively. *Bacillus sp.* was found to be the most effective decolorizer. The range of activity on decolorization of orange 3R with 27°C was 79.36%, 52.38%, 42.06% and 73.02% with *Bacillus sp.*, *Klebsiella sp.*, *Salmonella sp.* and *Pseudomonas sp.*, respectively. A bacterium *Bacillus sp.* was found to be the most effective decolorizer. The range of activity on decolorization of orange 3R with 37°C was 78.57%, 44.44%, 48.41%, 72.22% with *Bacillus sp.*, *Klebsiella sp.*, *Salmonella sp.* and *Pseudomonas sp.*, respectively. *Bacillus sp.* was found to be the most effective decolorizer. Overall decolorization efficiency was not temperature dependent but a report showed a suppressed decolourizing activity at 45°C, this might be due to the loss of cell viability or deactivation of the enzymes responsible for decolourization at higher temperature [31].

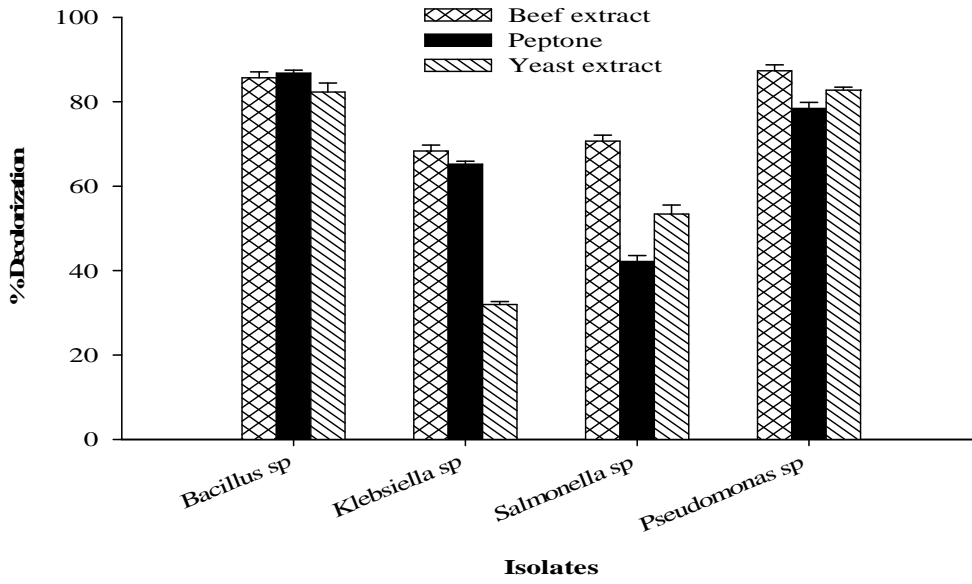


Fig. 3. Effect of different nitrogen sources on decolorization of Orange 3R dye by bacterial isolates (144h, 30°C, 150 rpm, error bars represents the standard deviation (\pm) mean of triplicate analysis)

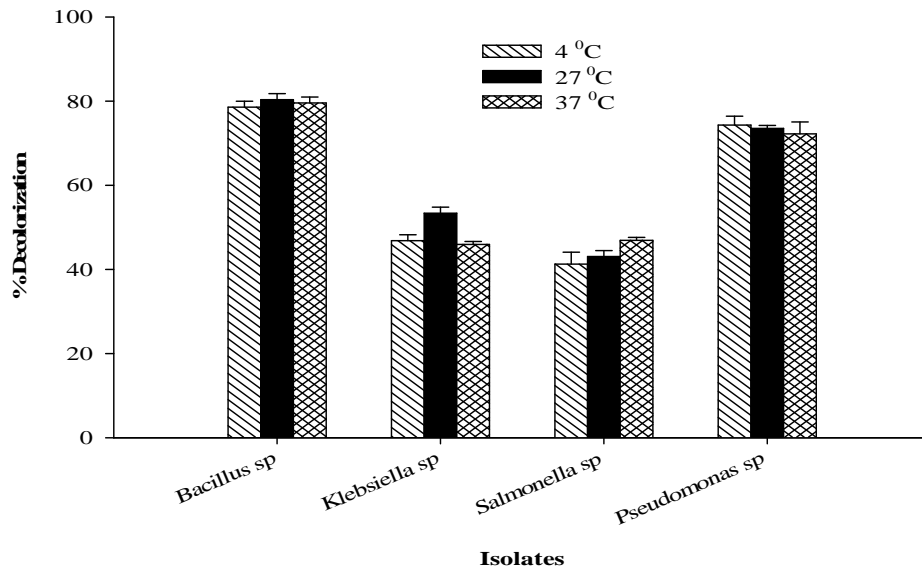


Fig. 4 Effect of different temperatures on decolorization of Orange 3R dye by bacterial isolates (144h, 30°C, 150 rpm, error bars represents the standard deviation (\pm) mean of triplicate analysis)

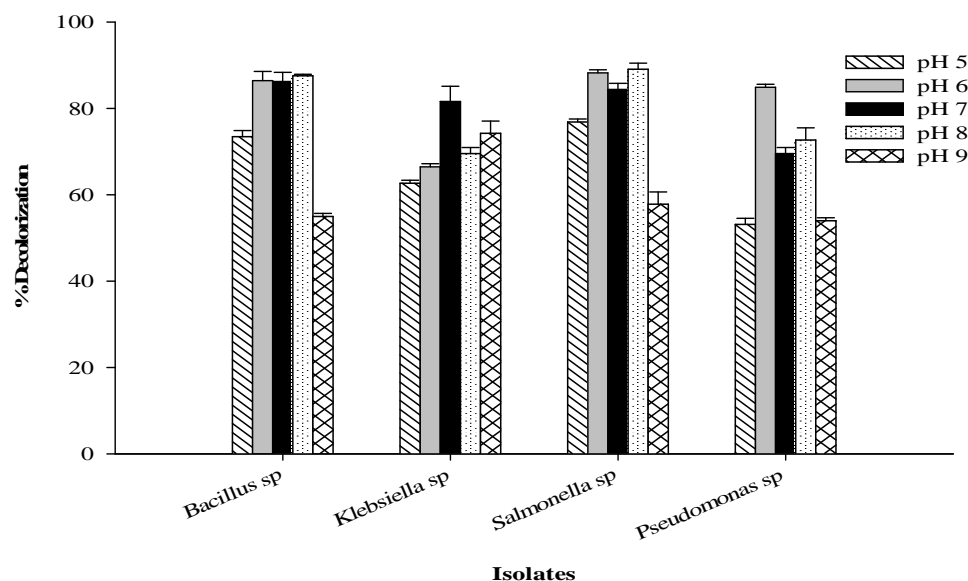


Fig. 5. Effect of different pH on decolorization of Orange 3R dye by bacterial isolates (144h, 30°C, 150 rpm, error bars represents the standard deviation (\pm) mean of triplicate analysis)

Pseudomonas sp. shows the highest decolorization (89.06% and 86.72%) at pH 6 and pH 8. *Bacillus sp.* shows the highest decolorization of 86.72% at pH 7 while, *Klebsiella sp.* showed the highest decolorization at pH 9 (Fig. 5). The optimum pH was found to be between pH 6 to 8 for maximum removal of dye. The pH has a major effect on the efficiency of dye decolorization, and the optimal pH for color removal is often between 6.0 and 10.0 for most of the dyes [32]. The pH tolerance of decolorizing bacteria is quite important because reactive azo dyes bind to cotton fibers by addition or substitution mechanisms under alkaline conditions and at high temperatures [33].

The 2%, 4%, 6%, 8% and 10% of inoculums are used for the four isolates to degrade the textile dye effluent (Fig. 6). Decolorization activity of *Bacillus sp.* has high (86.72%) in 4% of inoculums. Decolorization activity of *Klebsiella sp.* has high (67.19%) in 6% of inoculums. Decolorization activity of *Salmonella sp.* has high (53.91%) in 6% of inoculums.

Pseudomonas sp. have highly decolorize (50%) with 10% of inoculums. Kumar et al. [34] used a mixed culture for decolorization of reactive azo-dye and reported 98% decolorization at 10% inoculum size.

3.3. Time course of dye decolorization

The present study report the high decolorization of textile dye effluent by bacteria with optimization of conditions. Specific conditions used for maximum decolorization effect (Fig. 7). *Bacillus sp.* and *Pseudomonas sp.* were produced similar level (89%) of decolorization of Orange 3R at 144h followed by 80 and 76% by *Salmonella sp.* and *Klebsiella sp.*, respectively. In literature *Pseudomonas aeruginosa* decolorized Orange 3R dye by 93.06% [35]. Jothimani and Prabhakaran [36] has reported 59% dye removal from a dyeing industry effluent using *pseudomonas* after 4 days of inoculation and with *Bacillus sp.* Different dyes may be used, this attributed to differences in dye structures as has also been reported earlier for other microbial cultures [37].

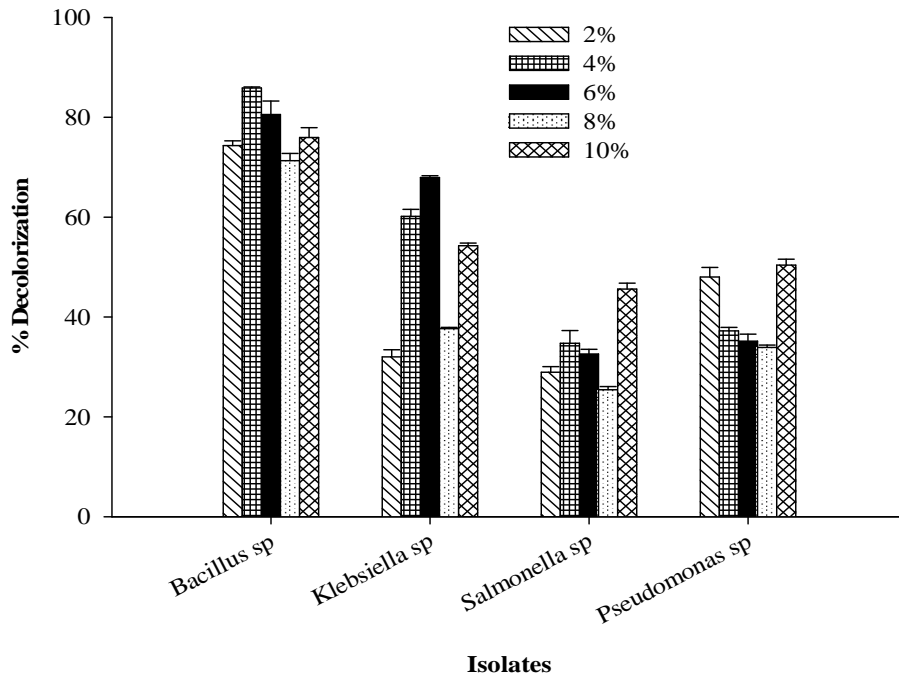


Fig. 6. Effect of different inoculum size on decolorization of Orange 3R dye by bacterial isolates (144h, 30°C, 150 rpm, error bars represents the standard deviation (\pm) mean of triplicate analysis)

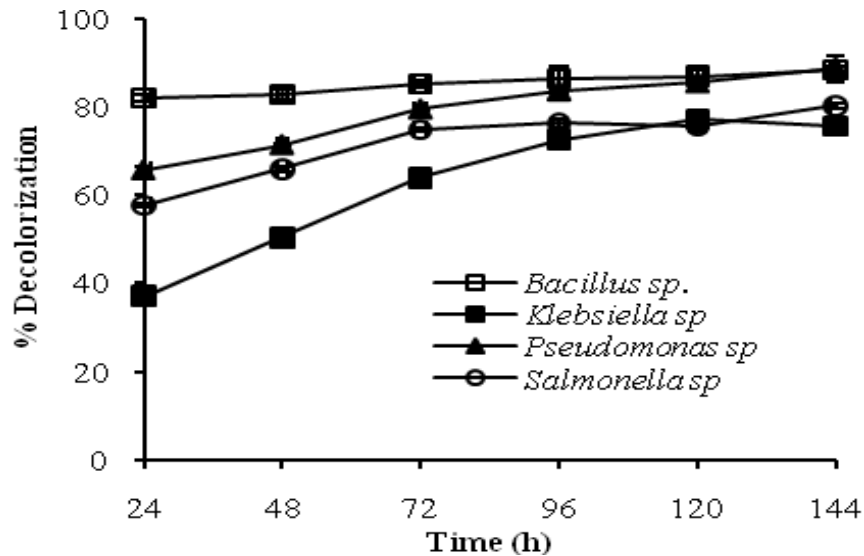


Fig. 7. Time course of decolorization of Orange 3R dye by bacterial isolates under optimum conditions (150 rpm, error bars represents the standard deviation (\pm) mean of triplicate analysis)

[V] CONCLUSION

The textile dye (Orange 3R) is degradable under aerobic conditions with a concerted effort of bacteria isolated from textile dye effluent. Nutrients (carbon and nitrogen

sources) and physical parameters (pH, temperature and inoculum size) had significant effect on dye decolorization. *Pseudomonas sp.* showed highest decolorization of Orange 3R dye effectively during optimization and more interesting *Pseudomonas sp.* showed consistent

decolorization of textile dye (Orange 3R) throughout the study.

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