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Advances in Bioresearch

Decolorization of Reactıve Black-B textile dye using bacterial isolate NB1, NB3 and NB4 Decolorization of Reactıve Black-B textile dye using bacterial isolates

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ABSTRACT
In the present study, isolation and screening of bacteria from textile dye effluents and evaluation of their ability to decolorize reactive dyes is reported. About 157 bacterial isolates were isolated from textile effluents. Screening of dye-decolorizing bacterial isolates gave three potent isolates namely NB1, NB3, & NB4 which have shown 83, 99, and 96% reactive black b dye decolorization within 24 h. The decolorizing activity was measured every 2 h for 100 mg/l of reactive black b at 597 nm. Furthermore, the effect of carbon sources, nitrogen sources, temperature and, pH for dye-decolorization were investigated. The NB1 showed 91 % Reactive Black B dye (100 mg/l) decolorization in nutrient broth containing wheat flour (1.0 g/l), peptone (1.0 g/l) at 37 °C, pH (7.0) within 12 h. The NB3 showed 99 % reactive black b dye (100 mg/l) decolorization in nutrient broth containing wheat flour (1.0 g/l), peptone (1.0 g/l) at 37 °C, pH (9.0) within 12 h. The NB4 showed 96 % reactive black b dye (100 mg/l) decolorization in nutrient broth containing wheat flour (1.0 g/l), peptone (1.0 g/l) at 37 °C, pH (6.0) within 12 h.
Keywords: *dyes, decolorization, reactive dyes, characterization, textile effluent, toxicity.*

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INTRODUCTION

Several colored effluents that contain dyes are released from the textile, food, leather, dyestuff, and dyeing industries. The textile industry is one of the largest producers of effluents contaminated with dyes [1]. The residual dyes released from these effluents introduce different organic pollutants into natural water resources and land [2]. Approximately 80,000 tonnes of dyestuff and pigments are produced in India. It has been estimated that 10,000 different textile dyes are commercially available worldwide, and the annual production is estimated to be 7×10^5 metric tonnes; 30% of these dyes are used in excess, which is 1000 tonnes per annum [3]. During the dying process, about 2% of these dyes fail to bind to the substrate and are discharged in aqueous effluents [4]. The wastewater from textiles, when directly released into the surface water without treatment, can cause a rapid depletion of dissolved oxygen and lead to significant environmental damage [5]. When dyes are available in the water system, the sunlight penetration into deeper layers is significantly reduced, distorting photosynthetic activity, resulting in deterioration of water quality, lowering the gas solubility, and finally causing acute toxic effects on aquatic flora and fauna. Most dyes released from wastewater, including their breakdown products, are toxic, carcinogenic, or mutagenic to humans and other life forms. [6, 7]. Various physicochemical methods, such as adsorption on activated carbon, electrocoagulation, flocculation, froth flotation, ion exchange, membrane filtration, ozonation, and reverse osmosis, are used for the decolorization of dyes in wastewater. These methods are inefficient, expensive, have less applicability, and produce waste in the form of sludge, which again needs to be disposed off [8]. However, the microbial decolorization and degradation of dyes have gained considerable interest from researchers as it is inexpensive, eco-friendly, and produce less sludge [9, 10]. Our aim was to isolate bacteria with reactive black b dye decolorization abilities. Therefore, isolation and screening of

bacterial isolates were done for decolorization of reactive black b dye and the factors affecting its decolorization were investigated.

MATERIALS AND METHODS

Collection of samples and physicochemical analysis

The textile effluent samples were collected from the outlet of the dyeing unit from Ahmadabad and Ankleshwer, Gujarat, India. Sampling site conditions like pH (7.0) and temperature (30°C) were recorded. Samples were then moved to the laboratory for further studies following standard protocols and were persevered at 4°C. A physicochemical analysis of the collected samples was carried out.

Chemicals

Reactive black b was procured from the textile industry unit from Narol, Ahmedabad, Gujarat. The nutrient medium was purchased from Hi-Media, Mumbai, India. The other chemicals used in the study were of analytical grade.

Enrichment and isolation of dye-decolorizing bacteria

Enrichment technique was performed to isolate bacteria which are capable of dye decolorization by adding 10 ml of effluent to 90 ml of nutrient broth containing 100 mg/l reactive black b dye in 250 ml of Erlenmeyer flask and incubated at 37ºC, 100 rpm for 24 h. After 24 h, 10 ml of nutrient broth was used to inoculate another 90 ml nutrient broth containing 100 mg/l reactive black b dye and incubated under at same conditions. The isolates were obtained after appropriate dilution of above enrichment sample and plating on nutrient agar medium. Colonial characteristics were noted down and morphologically distinct colonies were isolated on nutrient agar plates.

Screening of dye-decolorizing bacteria

The isolates were screened by inoculating each bacterial isolates in 10 ml nutrient broth medium containing reactive black b (100 mg/l) and incubated at 37°C, 120 rpm for 24–48 h. Samples were withdrawn every 2 h interval from the nutrient broth and centrifuged at 3,000 rpm for 20 minutes for removal of cells. 2.0 ml of supernatant was used to measure the absorbance of reactive black b at 596 nm using a UV-Visible spectrophotometer (Thermo-Fisher, USA).

Characterization of selected bacterial isolates

Gram staining, morphological characterizations and biochemical tests were performed for selected bacterial isolates. Morphological characteristics like size, shape, and arrangement of the cells were done by Gram's staining. Colony characteristics include size, shape, margin, texture, appearance, elevation opacity, pigmentation. The biochemical tests like starch hydrolysis; gelatinase, lipase, catalase, oxidase production, carbohydrate fermentation, indole production test, methyl red test, Voges-Proskauer test, nitrate reduction, hydrogen sulphide production, and citrate utilization.

Decolorization of reactive black b dye by bacterial isolates NB1, NB3, and NB4

Decolorization was studied in Erlenmeyer flasks (250 ml) containing nutrient broth (100 ml) amended with reactive black b dye (100 mg/l). Inoculum preparation was done by inoculating each bacterial culture in 10 ml nutrient broth medium and incubated at 37°C, 120 rpm for 24–48 h. The absorbance of cells were recorded at λ_{max} = 600 nm. One percent of freshly grown NB1, NB3, & NB4 culture having 1.0 O.D were inoculated in each flask. The decolorization of reactive black b dye was examined as decrease in absorbance (A) at 596 nm using a UV-Visible spectrophotometer. Two millilitres of decolorized sample were removed and centrifuged at consistent intervals of 0, 2, 4, 6, 8, 10, and 12 h, respectively. Medium without dye and inoculum was used as blank. All experiments were carried out in triplicate, and the standard deviation was calculated. The bacterial isolates showed maximum decolorizing ability which were utilized for subsequent investigations. The decolorization capacity shown by different isolates were measured in terms of percentage decolorization and calculated as follows:

$$\% \text{ decolorization} = \frac{(\text{Initial absorbance} - \text{Final absorbance})}{\text{Initial absorbance}} \times 100$$

Optimization of culture conditions for efficient decolorization

The one variable at a time method was used to check the impact of different factors on the decolorization of reactive black b by NB1, NB3, & NB4. Effect of different dye concentrations (100–300 mg/l), pH (4–10), temperature (20–45 °C), carbon sources (1%w/v; glucose, wheat flour, glycerol, and starch), and nitrogen sources (1%w/v; ammonium sulphate, sodium nitrate, peptone, and yeast extract) were tested for dye decolorization. The data presented is mean of three replicates.

Effect of pH:
The pH of the nutrient medium containing reactive black b (100 mg/l) was set at 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 using 0.1 M HCl or NaOH. Medium was inoculated using 1.0 ml inoculum and incubated at 37°C for 24 h.

Effect of Temperature:
Decolorization of reactive black b dye by the bacterial isolates were studied at incubation temperature of 20, 25, 30, 35, and 40°C with initial reactive black b dye concentration of 100 mg/l, 1.0 ml inoculum, at pH 7.0 for 24 hr.

Effect of dye concentration:
Effect of dye concentration (100, 150, 200, 250, 300 mg/l) on the decolorization of reactive black b dye was investigated. During the study of dye concentration for dye decolorization, pH and temperature were set at 7.0 and 37°C, respectively.

Effect of carbon source:
To investigate the effect of various carbon sources on dye-decolorization, 1% (w/v) different carbon sources (glucose, wheat flour, glycerol, and starch) were supplemented in nutrient medium, respectively. 1 ml Inoculum was added in 100 ml nutrient medium containing 1% (w/v) of different carbon sources and 100 mg/l reactive black b in a 250 ml Erlenmeyer flask. The bacterial isolates NB1, NB3, & NB4 were inoculated in nutrient broth at 37°C with 120 rpm for 24 h. Samples were taken every 2 h from the cultural broth and used to measure the cell density and dye concentration.

Effect of nitrogen source:
To investigate the effect of various nitrogen sources on dye-decolorization, 1% (w/v) different nitrogen sources (ammonium sulphate, sodium nitrate, peptone, and yeast extract) were supplemented in nutrient medium. 1 ml Inoculum was added in 100 ml nutrient medium containing 1% (w/v) of different nitrogen sources and 100 mg/l reactive black b in a 250 ml Erlenmeyer flask. The bacterial isolates NB1, NB3, & NB4 were inoculated in nutrient broth at 37°C with 120 rpm for 24 h. Samples were taken every 2 h from the cultural broth and used to measure the cell density and dye concentration.

RESULTS AND DISCUSSION

Collection of sample and physicochemical analysis of samples
Three effluent samples and reactive dyes were collected from the textile industries of GIDC, Vatva, Ahmadabad, Gujarat and two effluents from dye manufacturing units at GIDC, Ankleshwer, Gujarat (Figure 1 & 2; Table – 1). A physicochemical analysis of the collected samples was carried out and results were noted in Table -2.

Enrichment and isolation of dye-decolorizing bacteria
Enrichment and isolation of bacterial isolates from effluent samples were performed in nutrient broth using a serial dilution technique and incubated at 37 ºC for 24 to 48 h. In this study, total 157 bacterial isolates were obtained.

Isolation and screening of dye-decolorizing bacteria
Preliminary decolorization experiments were conducted for decolorization efficiency of 157 bacterial isolates. Based on their dye decolorization efficiency in a nutrient broth containing reactive black b dye (100 mg/l), three bacterial isolates namely NB1, NB3 and NB4 showing potential decolorization were selected for further study. The bacterial isolates NB1, NB3 and NB4 were inoculated in nutrient medium and incubated at 37°C for 12 h. Among the isolates, NB1, NB3, & NB4 have showed reactive black b dye decolorization of 94%, 99%, and 96%, respectively (Figure 3).
Another study shows that bacterial isolate *Pseudomonas* spp. (D4) was 70% decolorized the reactive black 5 dye (100mg/l) at 37°C within 24 h [11].

Characterization of selected bacterial isolates
Morphological, colonial characteristics and biochemical were performed. Morphological characteristics, Colonial characteristics and Biochemical tests for the selected NB1, NB3, & NB4 were mentioned in Table – 4, 5 and 6.

Decolorization of reactive black b dye
UV – Visible spectrophotometer was used to confirm the decolorization of reactive black b. The bacterial isolates were used to decolorize reactive black b and were pelleted through centrifugation (at 3,000 rpm for 20 min). The absence of color in the pellet of NB1, NB3 & NB4 and supernatant showed 96%, 99% and 97% dye decolorization, respectively (Figure – 4).
Other study reported that *B. amyloliquefaciens* W36 could decolorize four different dyes Coomassie brilliant blue (95.42%), Bromcresol purple (93.34%), Congo red (72.37%) and Safranin efficiently within 48–96 h [12].

Optimization of cultural conditions

Effect of pH:

The effect of pH on the dye decolorization efficiency of NB1, NB3 & NB4 was checked, over a wide range of pH 4–10. An increase in decolorization percentage from pH 6 to 9 was observed. Therefore, the optimum pH for NB1, NB3 & NB4 to decolorize reactive black b was pH 7.0, 9.0 and 6.0, respectively (figure 5). The optimum decolorization was observed for NB1 at pH 7.0, 93%, NB3 at pH 9.0, 97.3%, and NB4 at pH 6.0, 92%, after 12 h.

The reactive dyes were decolorized by bacteria with the effect of pH was studied at different pH (8, 8.5, 9, 9.5, and 10). The maximum decolorization was observed at pH 9.5, 87%, after 5 days [13].

Effect of dye concentration:

Figure 6 shows the effects of initial dye concentration on percent decolorization of reactive black b at different initial dye concentrations (100–300 mg/l) by NB1, NB3 and NB4 within 12 h (Figure 6). The increasing initial dye concentration the level of decolorization during the same time interval starts decreasing. The highest percent decolorization of 70.1, 77.9 and 72.8 were obtained at 100 mg/l whereas lowest percent decolorization of 27.0, 38.3 and 29.0 was observed at 300 mg/l by NB1, NB3 and NB4.

The bacterial isolate *B. amyloliquefaciens* W36 was cultured in EMSM medium supplemented with 0.1% (w/v) Congo red dye decolorization 72.37% at different rotation speed (100– 250 rpm) for 96 h [14].

Effect of Temperature:

A range of temperatures from 20 to 45°C was investigated to study the impact of temperature on dye decolorization. Optimal temperatures for decolorizing reactive black b dye were 35 (83.2 %), 40 (94.5%), and 35 °C (80.8%) using NB1, NB3, and NB4, respectively (figure -7).

Another study reported, the bacterial isolate *B. albus DD1* was decolorization of RB5 was found to be at a temperature of 40°C with 73%. Also, variation in the temperature largely affected the biodegradation activities of microorganisms. [15].

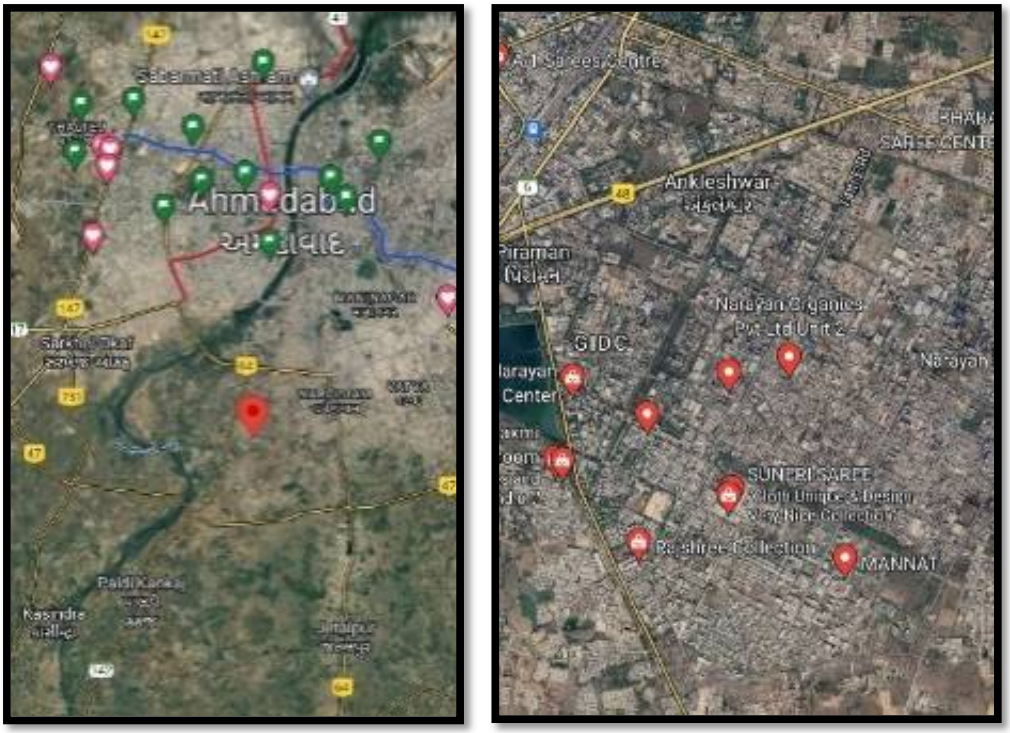
Effect of carbon source:

The effect of carbon sources was studied using wheat flour, glucose, starch, and glycerol. The efficacy of NB1, NB3 and NB4 to decolorize reactive black b dye in presence of additional carbon (1 gm %) sources was tested. The maximum percentage decolorization was observed with wheat flour (49.0, 73.4 and 55.0 %), while glucose, starch and glycerol showed a moderate decolorization value (Figure 8).

A similar observation has reported the range of activity on the decolorization of Orange 3R with 1% sucrose as a carbon source by *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp., and *Pseudomonas* sp., showing decolorization of 87.80%, 72.36%, 86.18%, and 80.49%, respectively, with the bacterium *Bacillus* sp. as the most effective decolorizer with sucrose as a carbon source [16].

Effect of nitrogen source:

The efficiency of NB1, NB3 and NB4 to decolorize reactive black b dye in presence of additional nitrogen (1%) sources was tested in order to obtain efficient and faster decolorization. The NB1, NB3 and NB4 showed maximum percentage dye decolorization of 54.0, 77.0 and 66.0 % with yeast extract were (Figure 9). *Bacillus amyloliquefaciens* W36 was investigated with different nitrogen source for Congo red dye decolorization and efficiency of (NH4)2SO4, NaNO3 were achieved at 72.80% or 65.97%, respectively [12].



Textile industrial site **Dye & chemical manufacturing site**

Figure 1: Sites for sample collection

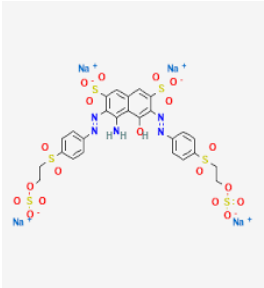
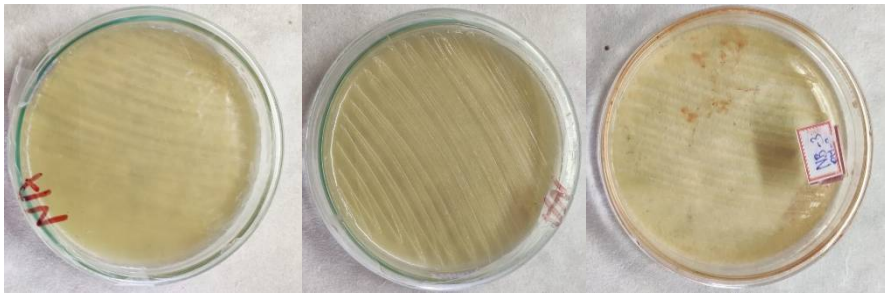
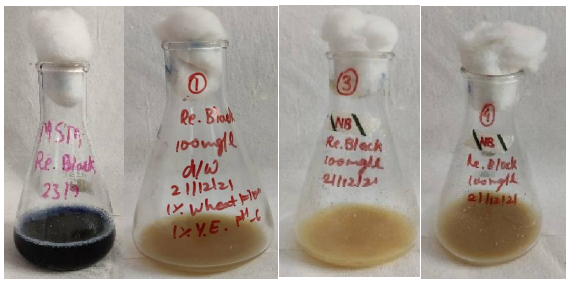


Figure 2: Chemical structure of reactive black b dye



NB1 **NB3** **NB4**

Figure 3: Cultural characteristics of NB1, NB3 and NB4



Control NB1 NB3 NB4

Figure 4: Reactive black b dye decolorization

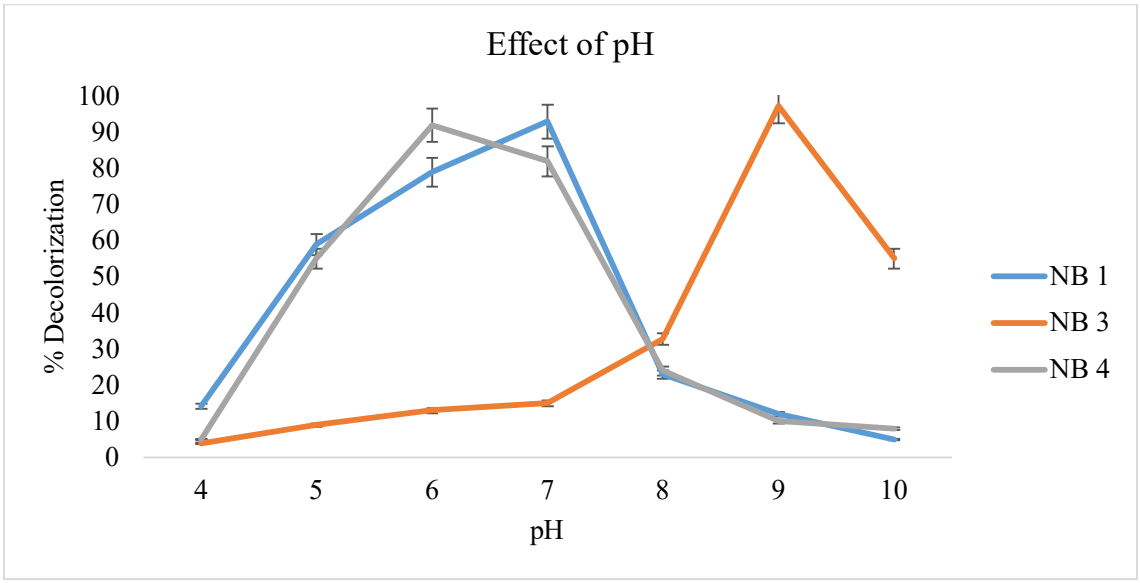


Figure 5: Effect of pH on reactive black b decolorization

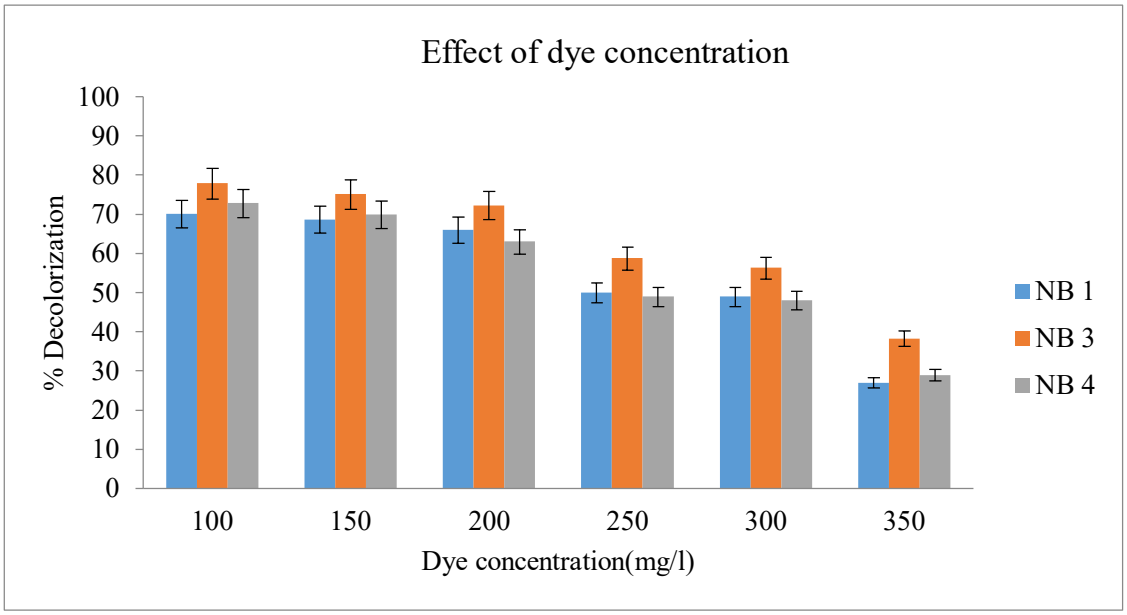


Figure 6: Effect of dye concentration on decolorization

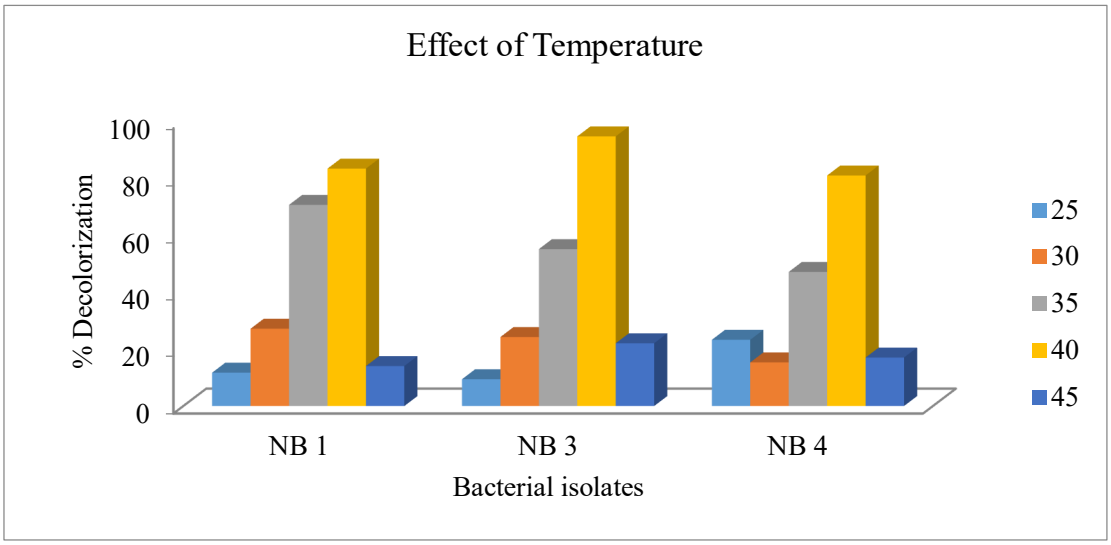


Figure 7: Effect of temperature on reactive black b decolorization

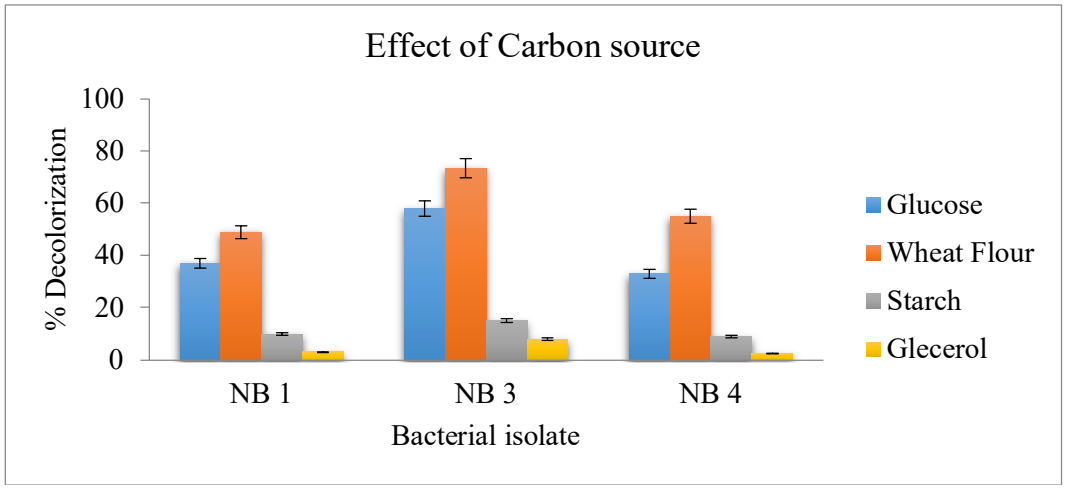


Figure 8: Effect of carbon source on reactive black b decolorization

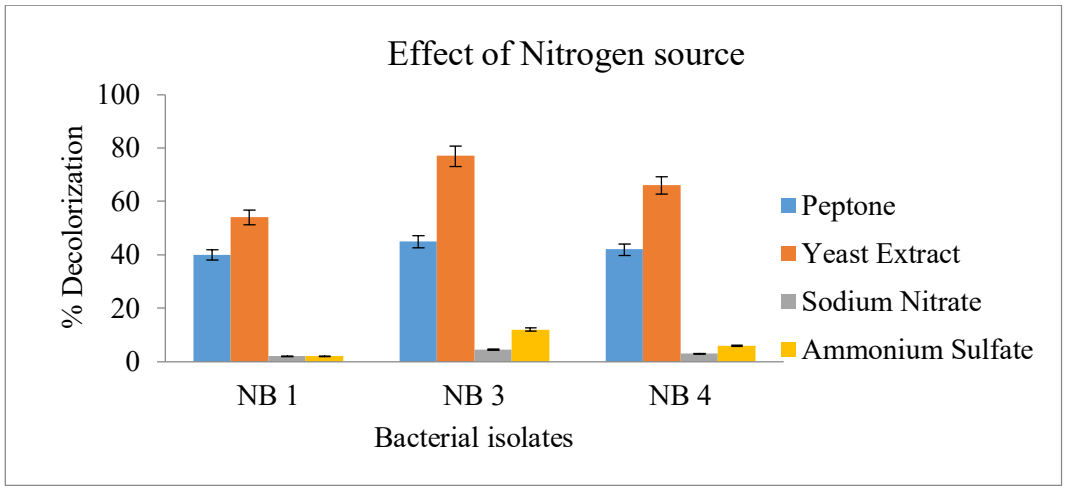


Figure 9: Effect of nitrogen source on reactive black b decolorization

Table – 1 – Site for sample collection

Site for sampling :	22.963128, 72.556681	**pH**	**temp**	**color**
Ahmadabad	collection tank	7	34	greyish
	aeration tank	7	33	black
	final tank	8	34	greenish
Ankleshwer	21.621284, 73.025169			
	collection tank	7	34	greenish
	final tank	8	34	Light green

Table – 2 - Physicochemical analysis of samples

Sampling Point	Sample 1 - Collection Tank	Sample 2 - Aeration Tank	Sample 3 - Final outlet	Sample 4 - collection tank	Sample 5 - final tank
pH	4.79	6.9	6.96	<1.0	7.7
Conductivity (µs)	8930	8800	3824	5934	145
COD (mg/l)	2984	3065	424	2420	84
BOD (mg/l)	1110	1397	218	52726	1986
TDS (mg/l)	6000	5720	2316	132	40
Chloride (mg/l)	1887	1848	696	BDL	0.432
Sulphate (mg/l)	458.64	360.36	462.28	2937.2	68.15
Phenol (mg/l)	0.552	0.568	0.107	27.6	2.7
Ammonical nitrogen (mg/l)	14	12.264	6.048	22.55	3.331
Total hardness (mg/l)	415	425	447	7.003	BDL
Hardness ca+ +(mg/l)	158	206	189	BDL	BDL
Oil and grees	84.4	255.2	2.8	16.99	1.261
TOC (ppm)	186.8	448	539.6	0.119	0.129
Heavy metals					
Fe	0.256	16.13	8.672	22.55	3.331
Zn	BDL	0.994	0.466	7.003	BDL
T-Cr	BDL	0.122	BDL	BDL	BDL
Cu	0.046	0.967	0.409	16.99	1.261
Ni	0.091	0.273	0.192	0.119	0.129
Pb	BDL	BDL	BDL	8.760	BDL

Table – 3 - Reactive black b dye decolorization using selected bacterial isolates:

% Decolorization	Time (h)	NB 1	NB 3	NB 4
	2h	25%	39%	36%
	4h	55%	62%	57%
	6h	69%	74%	66%
	8h	73%	82%	79%
	10h	88%	89%	86%
	12h	97%	99%	96%

Table – 4 - Morphological characteristics of selected bacterial isolates

Gram staining	NB 1	NB 3	NB 4
Gram's reaction	Negative	Positive	Negative
Size	Medium	Small	Small
Shape	Short Rod	Short rod	Rod
Arrangements	In singly, pair and chain	In singly, pair and chain	In singly and chain

Table – 5 – Colonial characteristics of selected bacterial isolates

characteristics	NB 1	NB 3	NB 4
Size	Small	Small	Small
Shape	Circular	Round	Round
Margin	Entire	Entire	Irregular
Elevation	Convex	Flat	irregular
Texture	Dry	Creamy	Dry
Opacity	Translucent	Opaque	Translucent
Pigmentation	Grayish white	Yellow	Light Green

Table – 6 - Biochemical test of selected bacterial isolates

Gram staining	NB 1	NB 3	NB 4
Catalase test	Positive	Positive	Positive
Citrate Utilization	Positive	Negative	Negative
Gelatin Hydrolysis	Negative	Positive	Negative
H_2S Production	Negative	Positive	Negative
MR (Methyl Red) test	Negative	Negative	Positive
Urease test	Positive	Negative	Negative
VP (Voges Proskauer)	Positive	Negative	Negative
Glucose fermentation	Positive	Positive	Negative
Lactose fermentation	Positive	Negative	Negative
Maltose fermentation	Positive	Negative	Negative
Mannitol fermentation	Positive	Negative	Positive
Sucrose fermentation	Positive	Positive	Negative
Xylose fermentation	Positive	Negative	Negative

CONCLUSION

In this study bacterial isolates NB1, NB3 and NB4 were selected for the decolorization studies based on their higher potentials to decolorize reactive black b (100 mg/l). Maximum decolorization was shown by NB1 for reactive black b dyes (97%) within 24h, followed by NB3 (99%), NB4 (96%), respectively during screening. At optimal conditions, up to 97 %, 99 % and 96 % of the reactive black b dye (100 mg/l) decolorized by the bacterial isolates NB1, NB3 and NB4 respectively within 12 h. The decolorization efficiency of reactive black b dye was improved with yeast extract and wheat flour, which were used as nitrogen and carbon sources, respectively. Furthermore, the dye degradation properties of NB 1, NB 3, & NB 4 can be further used as an essential tool for the bioremediation of various dyes in textile effluents.

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Competing Interests: The authors have declared that no competing interest exists.

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