BIODEGRADATION OF REACTIVE TEXTILE DYES BY BACTERIAL ISOLATES

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ABSTRACT

Most of the textile industries in Bangladesh dispose reactive dyes in the environment without any treatment and pollute the environment severely. To obtain bacteria having remarkable ability to decolorize and degrade reactive textile dyes, 30 bacterial isolates were isolated from the effluents collected from two textile mills and two leather industries. Screening of these isolates for dye decolorization and degradation capability was performed in nutrient broth medium by using eight structurally different reactive textile dyes. Among these bacterial isolates, 12 isolates showing one or more dye decolorizing ability within 48 hours of incubation were identified. Morphological, cultural and biochemical characterization indicated two isolates as *Aeromonas*, three as *Pseudomonas*, three as *Bacillus*, two as *Serratia*, one as *Citrobacter* and one as *Morganella*. Decolorization and degradation capability of *Aeromonas, Pseudomonas* and *Bacillus* was optimized by using Novacron Super Black G, one of the eight reactive dyes used above. Physicochemical conditions for decolorization of Novacron Super Black G by *Aeromonas*, *Bacillus* and *Pseudomonas* isolates were optimized. These bacteria decolorized and grew well in a high concentration of the dye up to 500 mg /l. *Aeromonas sp., Bacillus sp.* and *Pseudomonas sp*. showed significant dye decolorization by 93, 92 and 91%, respectively at 200 mg/l of dye concentration after 96 h of incubation under optimum conditions. Biodegradation and decolorization of reactive dye was confirmed using UV-VIS Spectrophotometry and Fourier Transform Infrared Spectroscopy (FTIR). Peaks at 600 nm at different incubation times were observed and the peak of parent dye compound was completely disappeared after 96 h of incubation. This result clearly indicated that the dye had been catabolized and utilized by these bacterial isolates. High decolorization extent and facile conditions showed these bacterial isolates might be potential in the biological treatment of dyeing mill effluents.

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INTRODUCTION

Environmental pollution has become one of the major concerns of today's world. Due to rapid industrialization and urbanization, large amount of wastes are generated and discharged into the environment and causing major pollution problem. Among many pollutants, effluents from textile industries are the major source of aquatic environmental pollution. Synthetic dyes are widely used in the textile, pharmaceutical, cosmetic, paper and food industries [1]. Usually synthetic dyes have a complex aromatic molecular structures which make them more stable and more difficult to biodegradation [2]. About 10000 different dyes and pigments are used in textile industries and the worldwide production of dyes is over 7·105 tons per annum [3, 4]. For tremendous increase of industrialization and man's urge for color, the dyestuff usage has been increasing day by day [5].

Reactive dyes, including many structurally different dyes, are extensively used in the textile industries because of their wide variety of color shades, high wet fastness profiles, ease of application, brilliant colors and minimal energy consumption. Azo, anthraquinone and phthalocyanine dyes are the three most common groups of reactive dyes [6], most of which are toxic and carcinogenic [7]. Reactive dyes may significantly affect the photosynthetic activity in aquatic lives because of reduced light penetration and may also be toxic to aquatic lives due to the presence of aromatics, metals, chlorides, etc. Due to high tinctorial value of dyes, less than 1 ppm of the dye produces obvious coloration [8].

Different physical and chemical methods including adsorption, coagulation–flocculation, oxidation and electrochemical methods can be used to remove dyes from wastewater [9, 10]. However, both the physical and chemical methods have many disadvantages in application, such as high-energy costs, high-sludge production, and formation of by-products [11]. Conversely, bioprocessing can overcome these defects because it is cost saving and environmentally benign.

In recent years, a number of studies have focused on some microorganisms that are able to degrade and absorb dyes from wastewater [7, 12, 13]. A wide variety of microorganisms are capable of decolorization of a wide range of dyes. Some of them are bacteria namely *Escherichia coli* NO3 (12), *Pseudomonas luteola* [14] and *Aeromonas hydrophila* [13], some are fungi such as *Aspergillus niger* [15], *Aspergillus terricola* [16], *Phanerochaete chrysosporium* [17], some are yeasts namely *Saccharomyces cerevisiae* [18], and some are algae such as Sp*irogyra* species [19], *Chlorella vulgaris* [7, 20], *Lemna minuscula* [21], *Closterium lunula* [22]. Bacteria can degrade and even completely mineralize many reactive dyes under certain conditions [13, 23, 24, 25]. The intermediate metabolites generated during the decolorization process, such as aromatic amines, can be degraded by the hydroxylase and oxygenase produced by bacteria [1]. Some new bacterial strains capable of decolorizing a broad-spectrum of dyes have also been isolated and characterized [26] and bacterial degradation of reactive dyes is often initiated under anaerobic conditions by an enzymatic biotransformation step [27, 28].

Bangladesh has emerged as the one of the largest garment-manufacturing nations in the world and its garment sector has become the second largest sector of the country's foreign exchange earnings and 50% of its industrial work force. The textile industries use large quantity of water in its production processes and are discharged into sewers and drains [29]. The textile dyeing industries located throughout the country's major cities namely Dhaka, Gazipur, Narayanganj and Chittagong generate large amount of effluents, sewage sludge and solid waste materials everyday which are being directly discharged into the surrounding channels, agricultural fields, irrigation channels, surface water without any kind of treatment. Highly polluted and toxic waste waters finally enter into many of the rivers of

Bangladesh such as Buriganga, Turag, Shitalakkhya and Karnafuli. Textile and dyeing industrial effluents may cause alteration of the physical, chemical, and biological properties of aquatic environment by continuous change in temperature, odor, noise, turbidity etc that is harmful to public health, livestock, wildlife, fish, and other biodiversity [30]. The physicochemical and anionic parameters of the effluents of the Bangladesh were extremely higher than the standard value recommended by Department of Environment (DOE). The presence of dyes in surface and subsurface water is making them not only aesthetically objectionable but also causes many water borne diseases, viz. mucous membrane, dermatitis, perforation of nasal septum and severe irritation of respiratory tract. Textile effluents impart a minor fraction of chemical load to the environment; its integrity renders the environmental quality fairly deplorable. For this, a large number of people living near textile industries are now being threatened due to the environmental degradation [31].

In the present study reported herein, we isolated and identified *Aeromonas*, *Bacillus* and *Pseudomonas* species that decolorized reactive dyes used in the textile industries. The general characteristics, decolorization efficiency under different physicochemical parameters were investigated and the mechanism of decolorization is discussed.

MATERIALS AND METHODS

CHEMICALS

To obtain bacterial isolates with a high performance of decolorizing capability, eight structurally different textile reactive dyes namely Novacron Yellow S3R (NY-S3R), Novacron Blue SGL (NB-SGL), Novacron Ruby S3B (NR-S3B), Novacron Navy FNBN (NN-FNBN), Novacron Super Black G (NSB-G), Novacron Turquise HGN (NT-HGN), Novacron Dark Blue WR (NDB-WR), Novacron BR Blue FNG (NBRB-FNG) were collected from a textile industry located at Kalarpool, Patia, Chittagong, Bangladesh. NSB-G of these dyes was selected for detailed decolorization studies to optimize physicochemical parameters and to elucidate the mechanisms of decolorization. Maximum wavelength of NSB-G was obtained by UV-vis spectrophotometer (Shimadzu, Japan).

SAMPLE COLLECTION AND ESTIMATION OF TOTAL VIABLE BACTERIAL COUNT

Samples were collected in sterile vials from effluents and discharge sites of two textile and two leather industries located at Kalarpool, Patia and Oxygen Circle in Chittagong, Bangladesh. Samples were transported immediately to the laboratory and used in the experiment. Total viable bacterial count (TVBC) in these samples were enumerated as described previously [32, 33].

ISOLATION, SCREENING AND IDENTIFICATION OF DYE DECOLORIZING BACTERIA

From nutrient agar plates used for TVBC, 30 bacterial colonies were randomly selected for obtaining pure culture. Primary screening for decolorization capability of isolated bacteria was carried out for two days using eight reactive dyes. For screening the bacterial isolates having the dye degradation capability, one loop full of each bacterial isolate was inoculated in 10 ml sterile nutrient broth (glucose: 5 gm/l,

peptone: 5 gm/l, beef extract: 1.5 gm/l and yeast extract: 1.5 gm/l) containing any of the eight dyes in the test tubes at concentration of 200 mg/l. Isolates that decolorized at least one of these eight dyes within 48 hours were taken for further screening (up to seven days). Dye decolorizing isolates were identified on the basis of morphological, cultural and biochemical tests according to Bergey's Manual of Systematic Bacteriology [34].

MEASUREMENT OF DECOLORIZATION EXTENT

Samples (2 mL) were collected every 24 h and centrifuged at 8000 rpm for 10 min. Decolorization extent was determined by measuring the absorbance of the culture supernatant at 600 nm using a UV-visible spectrophotometer. Decolorization extent was calculated using the following equation:

$$\text{Decolorization extent (\%)} = \frac{OD1 - ODt}{OD1} \times 100$$

Where, OD_1 refers to the initial absorbance, OD_t refers to the absorbance after incubation with bacterial isolates.

EFFECTS OF DIFFERENT PHYSICOCHEMICAL PARAMETERS ON DECOLORIZATION OF NSB-G

The effects of different physicochemical parameters on dye decolorization by bacterial isolates was investigated by using NSB-G which is one of the most widely used dyes in the textile industries. From the bacterial isolates, one isolate from each of the genus *Aeromonas* (isolate no. 27), *Bacillus* (isolate no. 26) and *Pseudomonas* (isolate no. 28) were selected for this purpose. To investigate the effects of initial dye concentration, media containing 50, 100, 200, 500, and 1000 mgl $^{-1}$ dye were subjected to decolorization. The pH of the media was adjusted to 7.0 and the experiment was conducted at 37 °C. To obtain the optimum temperature for dye decolorization, investigation was done at 22, 30, 37 and 45 °C, and the initial pH of the media was 7.0. To observe the effects of different initial pH on the decolorization, media were adjusted to different pH at range of pH 5.0 to 9.0. The culture was carried out at 37 °C. The effects of different organic carbon sources on decolorization of NSB-G was investigated with supplementation of 0.5% glucose or sucrose or maltose in a medium containing 0.5% yeast extract, 0.05% $(NH_4)_2SO_4$, 0.266% KH_2HPO_4, 0.432% Na_2PO_4. In order to investigate the effects of organic nitrogen sources on decolorization of NSB-G, 5% of beef extract, peptone and yeast extract was supplemented separately to a medium containing 0.5% glucose, 0.05% $(NH_4)_2SO_4$, 0.266% KH_2PO_4, 0.432% Na_2PO_4. Experiments were performed in 20 ml glass tubes containing 15 ml broth medium without agitation.

DECOLORIZATION MANNER OF NSB-G BY AEROMONAS, BACILLUS AND PSEUDOMONAS SP.

In order to investigate the decolorization manner of NSB-G by bacterial isolates, medium containing NSB-G was inoculated either with autoclaved bacterial culture (heat treated) or untreated bacterial culture. After 24, 48 and 96 h of incubation at 37 °C, bacterial cultures were centrifuged at 8000 rpm and the supernatant was scanned from 200 nm to 800 nm using a UV-visible spectrophotometer to detect the presence of new compounds in the medium.

FOURIER TRANSFORM INFRARED SPECTROSCOPY ANALYSIS

The infrared spectra of dyes and their metabolites were recorded on KBr pellets with a SHIMADZU IR spectrometer (Model:Prestige 21). Solution of degraded products was extracted by ethyl acetate. Solvent was removed by rotary evaporator and dried in vacuum. Then Fourier Transform Infrared Spectroscopy (FTIR) spectra of pure dyes and their degraded products were recorded on KBr pellets.

STATISTICAL ANALYSIS

For statistical analysis, Student's t test was used. A *P* value of <0.05 was considered to be statistically significant. Data were presented as the means ± standard errors of the means (SEM) of at least three independent experiments, or as noted in the figure legends.

RESULTS AND DISCUSSION

BACTERIAL LOAD IN THE INDUSTRIAL EFFLUENTS

VBC is one of the important parameters for determining water quality. The total bacterial count of receiving water can provide extremely valuable statistics in monitoring various types of pollutants. The heterotrophic bacteria were counted by the spread-plate technique by using nutrient agar. Total viable bacteria found in the effluents was in the range 3.7×10^2 to 5×10^2 CFU ml^{-1} (Table 1). TVBC (2×10^6) of normal pond water was also enumerated. Data reported herein suggested that these effluents may be toxic to the growth and survival of normal microflora.

Table 1. TVBC in water of different sources

Sample	**TVBC (CFU/ml)**
Textile Effluent	5×10^2
Textile Effluent Dumping Site	7×10^5
Leather Effluent	3.7×10^2
Leather Effluent Dumping Site	7×10^4
Pond Water	2×10^6

ISOLATION, SCREENING AND IDENTIFICATION OF DYE DECOLORIZING BACTERIA

Primary screening for dye decolorization was carried out for 48 h using eight reactive dyes and 30 bacterial isolates (Table 2).

Table 2. Screening of dye degrading bacteria following incubation at 37°C for 48 h

Dye	Bacterial isolates																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
NY-3SR	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	
NB-SGL	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	+	+	-
NR-S3B	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-
NN-FBN	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	-
NSB-G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-
NT-HGN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NDK-WR	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-
NBRB-FNG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	-

Bacterial isolates were cultured in nutrient broth supplemented with 200 mg/l dye. Here, NY-3SR, Novacron Yellow 3SR; NB-SGL, Novacron Blue-SGL; NR-S3B, Novacron Ruby; NN-FBN, Novacron Navy-FNBN; NSB-G, Novacron Super Black-G; NT-HGN, Novacron Turquise-HGN; NDK-WR, Novacron Dark Blue-WR; NBRB-FNG, Novacron BR Blue- FNG.

Fourteen isolates that decolorized at least one of these eight dyes were taken for further screening (up to seven days). Decolorization extent and number of decolorizing isolates were increased with further incubation after 48 h (data not shown).

Based on morphological, cultural and biochemical characteristics, fourteen dye decolorizing bacterial isolates were identified which were distributed to the bacterial genus of *Aeromonas, Bacillus, Citrobacter, Morganella/Providencia, Pseudomonas* and *Serattia* (Table 3).

Table 3. Morphological and biochemical characteristics of bacterial isolates

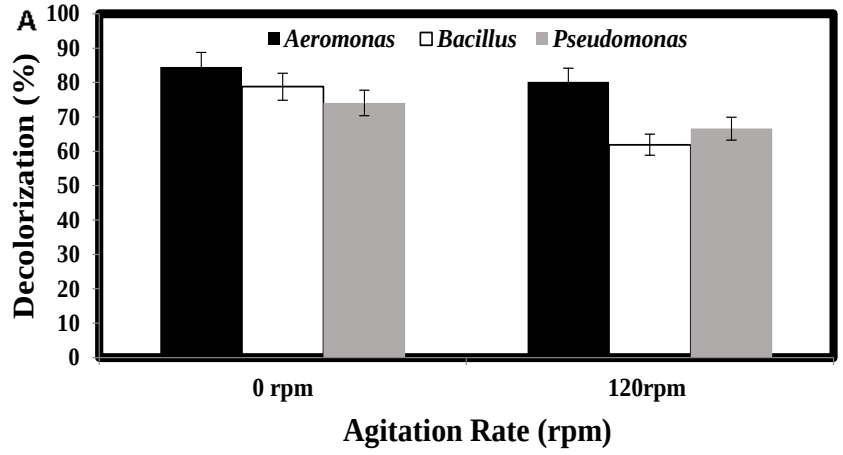
Morphological and Biochemical tests	Bacterial isolates		
	Isolate 26	Isolate 27	Isolate 28
Shape	Rod	Rod	Rod
Motility	+	+	+
Catalase production	+	+	+
Oxidase production	+	+	+
Gram staining	+	-	-
Indole test	-	-	-
Methyl red test	+	+	+
Voges-Proskauer test	-	+	-
Citrate utilization	-	-	+
Triple Sugar Iron Agar test (Slant/Batt)	K/A	A/A	K/A
Maltose fermentation	+	+	-
Lactose fermentation	-	+	+
Glucose fermentation	+	+	-
Spore test	+	+	-
Bacterial genus	+	-	-

Here, K/A, Red/Yellow; A/A, Yellow/Yellow.

Among them four isolates were identified as *Bacillus*, three as *Aeromonas* and three as *Pseudomonas*. We selected one isolate from each genus of *Aeromonas* (isolate no. 27), *Bacillus* (isolate no. 26) and *Pseudomonas* (isolate no. 28) that predominantly decolorized dyes during screening. We then performed detailed decolorization of NSB-G with these three bacterial isolates.

EFFECTS OF AGITATION AND INCUBATION PERIOD ON THE DECOLORIZATION OF NSB-G

The effects of oxygen on cell growth and dye reduction is one of the important factors [35] that need to be considered. The bacterial isolates showed good growth under agitation (data not shown) but the color removal was better in static condition (Figure 1A).



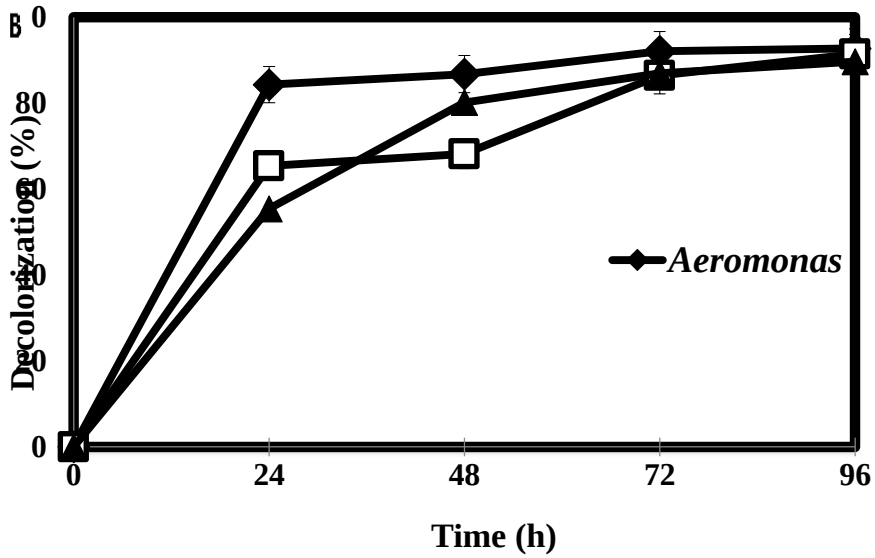


Fig. 1. Effects of agitation rate (A) and incubation period (B) on decolorization of NSB-G after 96 h of incubation at 37°C, with 200 mg/l dye, 5% inoculum and initial pH 7.0

In static condition, color removal was almost 10% more as compared to agitated condition. This result indicated that static condition was better for dye decolorization by bacteria even the growth of bacteria were less than that under agitated conditions. Similar observations were reported previously [36, 37] in which results showed that static condition was necessary for dye decolorization but the growth of culture significantly increased at shaking condition (120 rpm) and no dye decolorization was observed even after 24 h of incubation. The growth of microbial cell decreases under static condition because transfer of oxygen is limited to the broth surface and the cells are sedimented to the bottom of the flasks. Therefore, for the experiments, static conditions were adopted to investigate bacterial decolorization.

Time course study for decolorization of NSB-G by the three bacterial isolates revealed that decolorization was almost saturated (~90%) after 72 h of incubation. However, decolorization of NSB-G by *Aeromonas* increased sharply to achieve more than 84 % of the total decolorization after 24 h. On the other hand, decolorization of NSB-G by *Bacillus* and *Pseudomonas* isolates after 24 h was only about 65% and 55%, respectively. *Pseudomonas* was reported to decolorize 54% of dye from effluent of a dyeing industry [38].

EFFECTS OF INITIAL DYE CONCENTRATION ON THE DECOLORIZATION OF NSB-G

Initial dye concentration has strong inhibitory effects on dye decolorization and degradation [39, 40]. Decolorzation activity of *Aeromonas* sp., *Bacillus* sp. and *Pseudomonas* sp. was studied using different initial concentrations of NSB-G ranging from 50 to 1000 mgl^{-1} (Fig. 2).

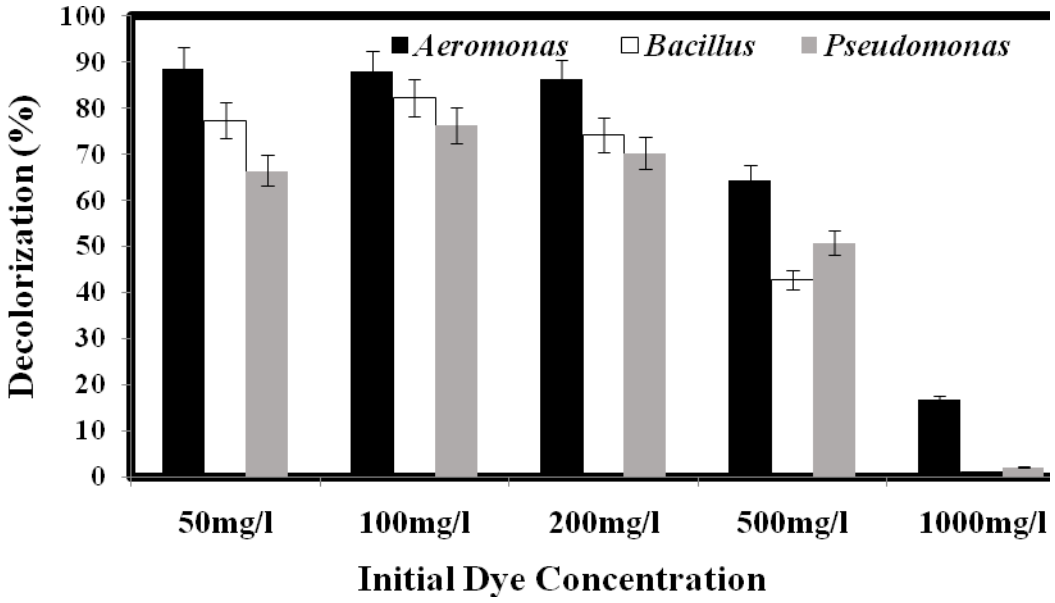
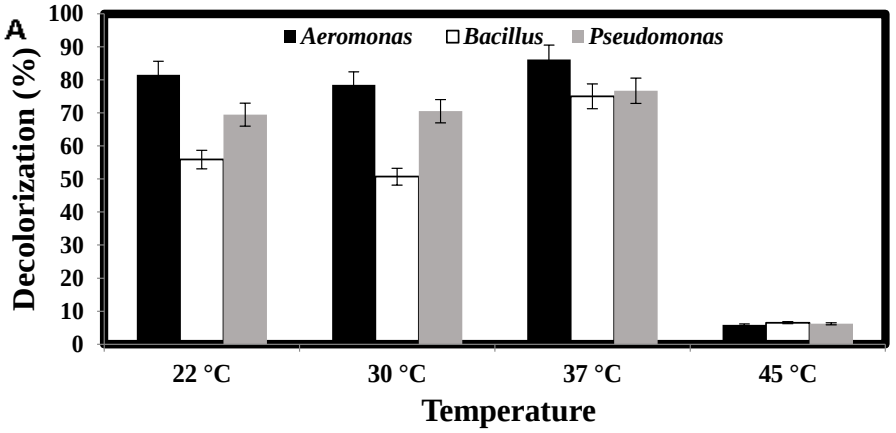


Fig. 2. Effects of initial dye concentrations on decolorization of NSB-G after 96 h of incubation at 37°C with 5% of inoculum and initial pH 7.0.

Intensity of decolorization was measured after 96 h of incubation. It was observed that the decolorization decreased with an increase in dye concentration. However, decolorization of NSB-G by the three bacterial isolates was nearly same up to 200 mgl^{-1} of dye concentration. Maximum decolorization achieved by *Aeromonas, Bacillus* and *Pseudomonas* isolate was approximately 88, 82 and 76%, respectively with 100 mgl^{-1} dye concentration. Decolorization activity decreases with dye concentration more than 200 mgl^{-1}. Decolorization was significantly decreased with 500 mgl^{-1} of dye concentration, and at 1000 mgl^{-1} of initial dye concentration, decolorization was strongly inhibited.

EFFECTS OF TEMPERATURE AND PH ON THE DECOLORIZATION OF NSB-G

The optimum temperature for maximum dye decolorization by *Aeromonas, Bacillus* and *Pseudomonas* isolates was 37 °C although an appreciable decolorization occurred at 22-30 °C. This optimum temperature for dye decolorization was in agreement with *Pseudomonas* sp. to decolorize malachite green, fast geeen, brilliant green, congo red and methylene blue [41]. Fig. 3A depicted that decolorization levels by *Aeromonas, Bacillus* and *Pseudomonas* isolates at 30 °C were 91, 66 and 93%, respectively.



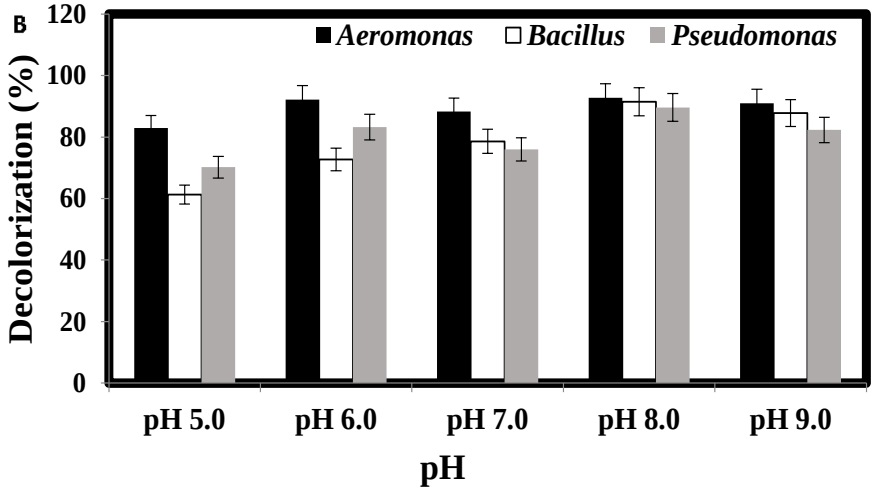


Fig. 3. Effects of temperature (A) and pH (B) on decolorization of NSB-G after 96 h of incubation with 200 mg/l dye and 5% inoculum. The initial pH in (A) and the temperature in (B) was 7.0 and 37 °C, respectively.

Decolorization extent was significantly suppressed at 45°C, which might be due to the loss of cell viability or deactivation of the enzymes responsible for decolorization [(42)]. This result indicated that temperature 30°C to 37°C may play better role in decolorization activity of *Aeromonas, Bacillus and Pseudomonas* isolates.

The effects of initial pH on the dye decolorization by the three bacterial isolates was investigated (Fig. 3B). It was found that the maximum level of dye decolorization by *Aeromonas, Bacillus* and *Pseudomonas* isolates was observed at alkaline pH with peak at pH 8.0. However, significant levels of decolorization by the three bacterial isolates were observed at pH 5.0- 7.0 although the lowest level of decolorization was noticed at pH 5.0. However, the dcolorization level by *Aeromonas* at pH 5.0 - 7.0 was significantly higher compared to that of *Bacillus* and *Pseudomonas* isolates. This result indicated that these isolates could decolorize reactive dyes in a relatively wide range of pH, make them suitable for practical bio-treatment of dyeing mill effluents. The pH tolerance of decolorizing bacteria is quite important because reactive azo dyes bind to cotton fibers by addition or substitution mechanisms under alkaline condition [(43)].

EFFECTS OF ORGANIC CARBON AND NITROGEN SOURCES ON THE DECOLORIZATION OF NSB-G

To investigate the effects of carbon and nitrogen sources on the decolorization efficiency, the medium was supplemented with different organic carbon and nitrogen sources (Fig 4). Maximum decolorization by *Aeromonas* (~70%), *Bacillus* (~65%) and *Pseudomonas* (~70%) was obtained with glucose as a carbon source (Fig. 4A).

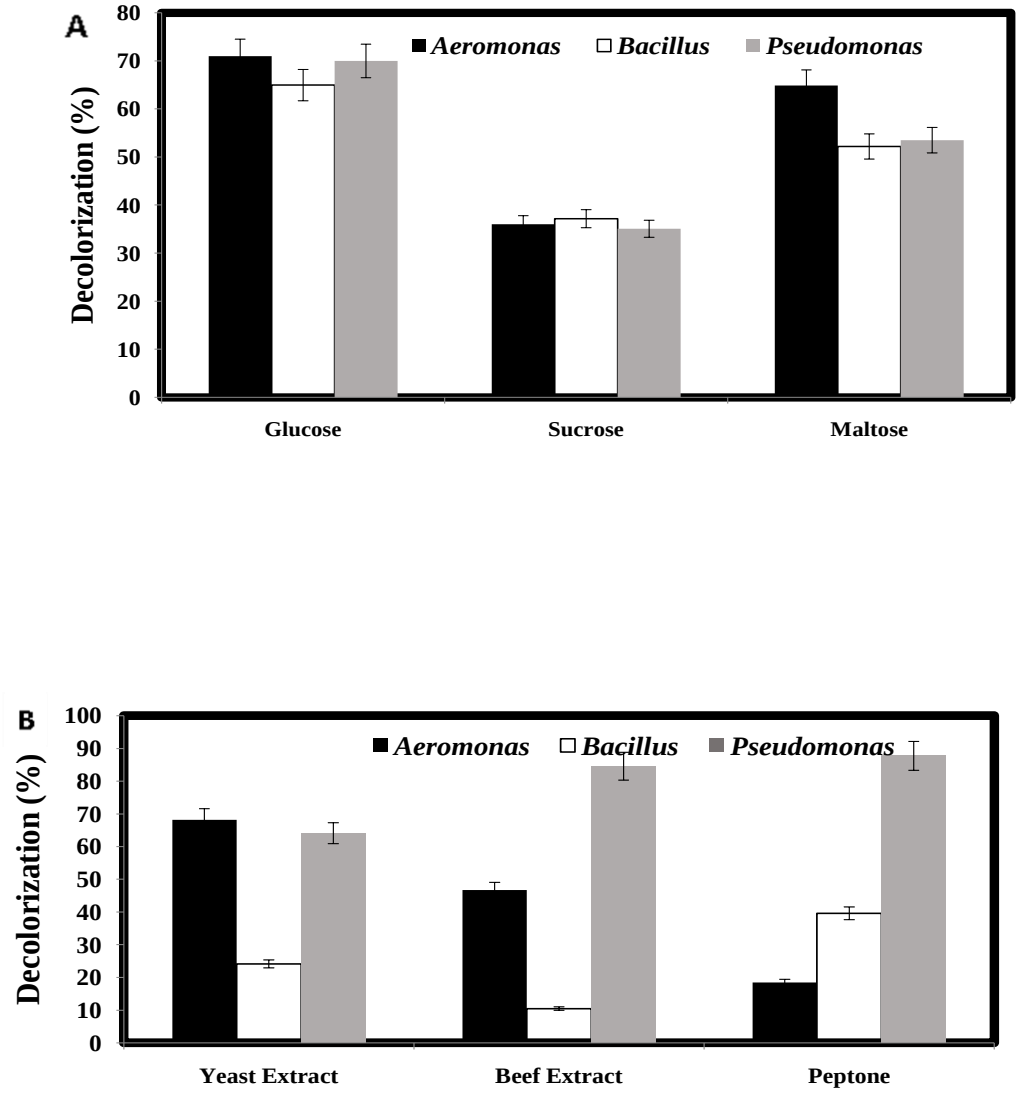


Fig. 4. Effects of different organic carbon (A) and nitrogen (B) sources on decolorization of NSB-G after 96 h of incubation at 37°C and pH 7.0 with 200 mg/l dye and 5% inoculum.

The decolorization ability of these isolates were decreased in the absence of the carbon source (data not shown here). This suggests that the dye molecule could not serve as a sole source of carbon. Glucose was reported as best carbon source for maximum decolorization (85 %) of Remazol brilliant blue by *Polyporus* sp. S133 [(44)].

About 68 % decolorization of NSB-G was observed by *Aeromonas* in the presence of yeast extract as the nitrogen source. However, peptone was the best nitrogen source for the decolorization of NSB-G by *Bacillus* and *Pseudomonas*. In the absence of the organic nitrogen source, the decolorization ability of these isolates were increased (data not shown). It might be due to the use of dye molecule as the sole nitrogen source. Ponraj et al. [45] reported peptone as the most effective nitrogen source for the decolorization of Orange 3R dye by *Bacillus* sp. Similar results were also reported by *Enterococcus faecalis* strain YZ66 for decolourization of reactive yellow 145 [46].

DECOLORIZATION OF VARIOUS TEXTILE DYES

Out of seven structurally different reactive dyes with the concentration of 100 mgl^{-1}, six dyes were efficiently decolorized by *Aeromonas and Pseudomonas* sp. after 48 h (Fig. 5). A maximum decolorization extent of approximately 95 and 86% were recorded for *Aeromonas* and *Pseudomonas,* respectively when Novacron Ruby S3B was used. When the other five dyes except Novacron Turquise HGN were used, *Aeromonas* and *Pseudomonas* decolorized ~ 68 - 87% and ~ 56 - 74%, respectively. On the other hand, *Bacillus* sp. decolorized four dyes where maximum decolorization (~64%) was obtained with NSB-G. The variation in the decolorization of different reactive dyes might be attributable to the structural diversity of the dyes [47]. It is believed that anthraquinone dyes are more recalcitrant than azo dyes [48].

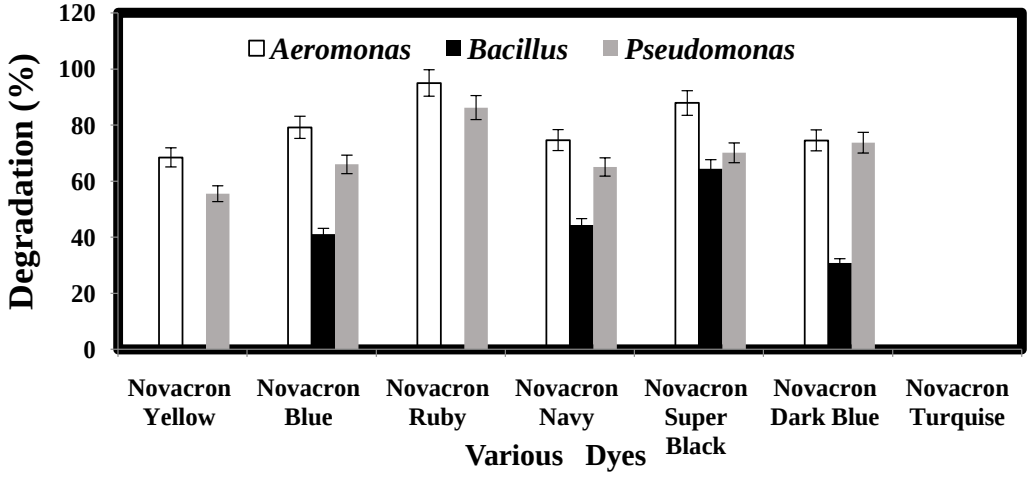
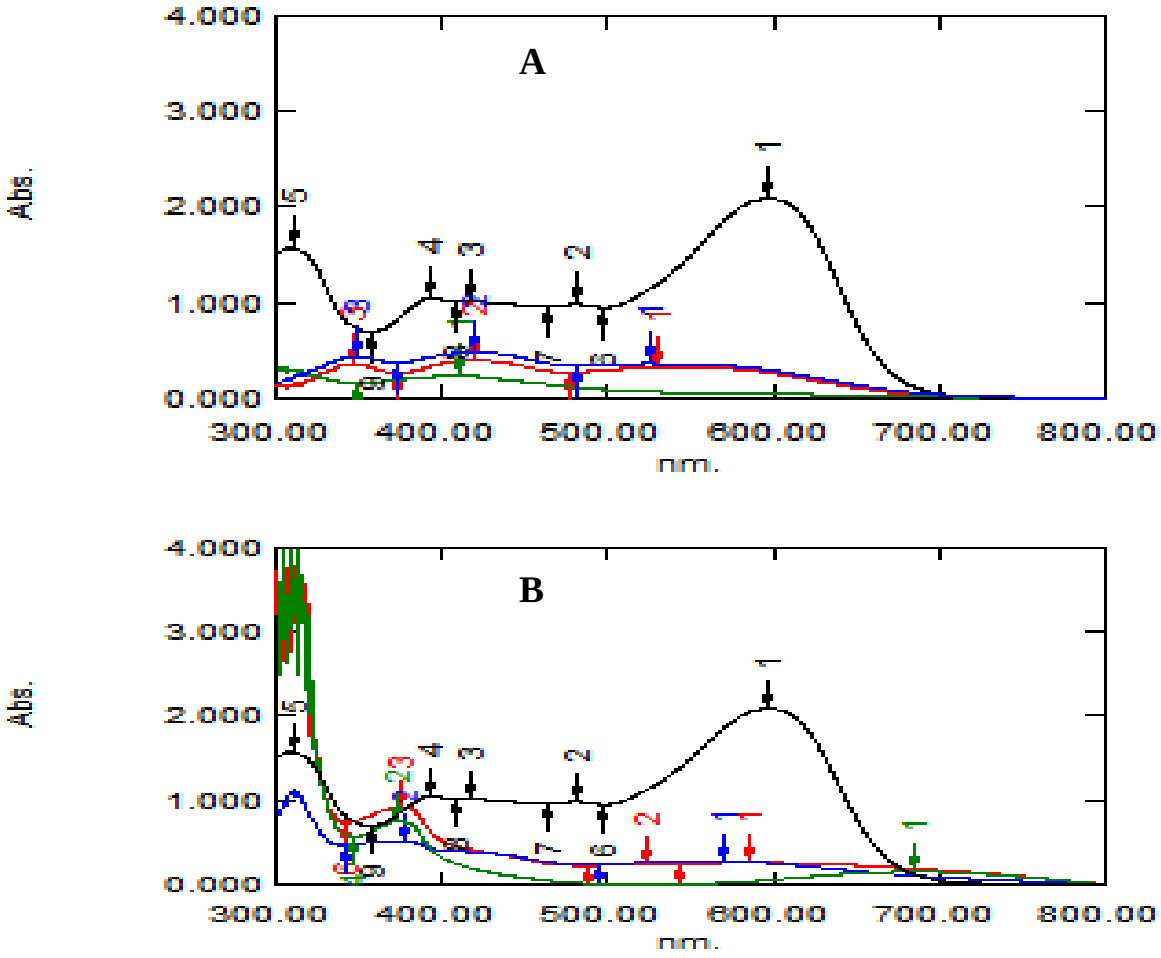


Fig. 5 Decolorization of various textile dyes after 48 h of incubation at 37° C and pH 7.0 with 5% inoculum and 100 mg/l dye.

BACTERIAL DECOLORIZATION MANNER OF NSB-G

There are different mechanisms of dye decolorization. It may be by bacterial adsorption [49] or degradation [50]. Dyes are only adsorbed onto the surface of bacterial cells in the case of adsorption, whereas new compounds are produced when dyes are degraded by bacterial enzymes. In adsorption, examination of the absorption spectrum reveals that the intensity of all peaks decreases approximately in proportion without any new peak. If the dye removal is attributed to biodegradation, either the major

visible light absorbance peak will completely disappear or a new peak will appear [51]. In the cultures with addition of heat-killed bacterial cells, around 20% decolorization was recorded after 96 h incubation, which might be due to the adsorption by dead bacterial cells, and this was also confirmed by the presence of colored cell pellets. In the culture where cells were not killed, approximately 86, 74 and 70% decolorization were achieved by *Aeromonas, Bacillus* and *Pseudomonas* isolates, respectively after 96 h and the cell pellets were not pigmented. Meanwhile, a UV–vis spectral scan (200–800 nm) of the supernatant after 96 h decolorization showed that the maximum absorbance wavelength in visible spectra shifted from 600 nm to 410nm, 378 nm and 373 nm by *Aeromonas, Bacillus* and *Pseudomonas* isolates respectively (Fig. 6). The absorbance peak at UV spectra did not disappear in the end of decolorization, which indicated that NSB-G was not completely mineralized while some new metabolites were formed in the culture.



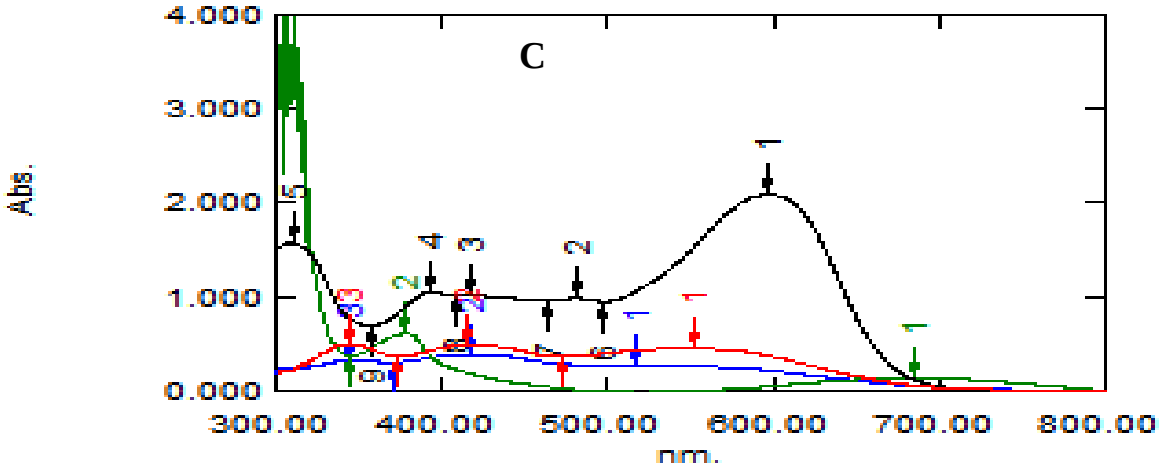
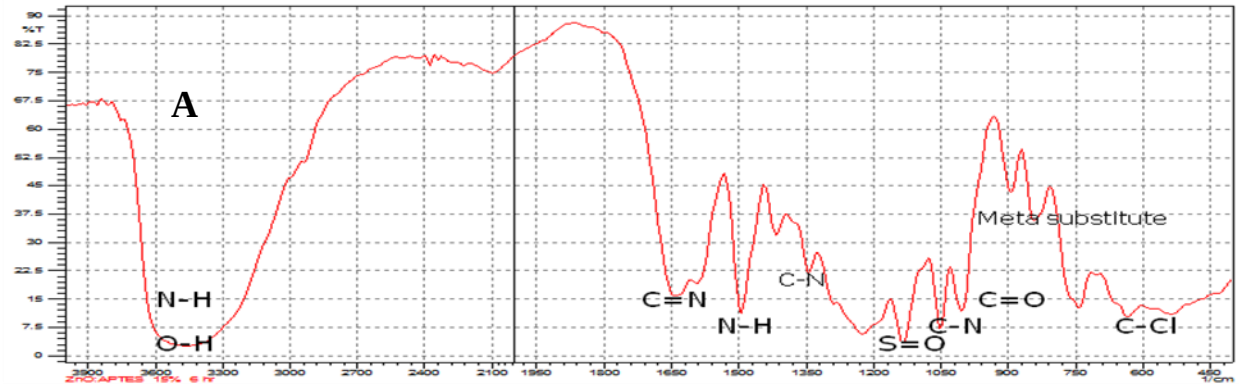


Fig. 6. Variation in the UV-vis spectra of NSB -G before and after decolorization by *Aeromonas* (A), *Bacillus* (B) and *Pseudomonas* sp (C). (Black line, 0 h which indicated before decolorization; blue line, 24 h; red line, 48 h; green line, 96 h).

Biodegradation of NSB-G by bacterial isolates was confirmed by IR spectroscopic analysis (Fig. 7). Peaks in the control dye spectrum represented the stretching vibrations of S=O at 1134 cm^{-1} and asymmetric stretching at 1051 cm^{-1} for C–N. The stretching vibrations of C–O showed a band at 1004 cm^{-1}. C–N stretching at 1342 cm^{-1} represented nature of aromatic amine group present in parent dye compound. The stretching at 1494 cm^{-1} and 3452 cm^{-1} represented the presence of free NH group in parent dye structure. The stretching vibrations of C=N showed a band at 1639 cm^{-1}. The stretching vibrations of C-Cl showed a band at 632 cm^{-1}. The FTIR spectrum of 48 h extracted metabolites showed significant change in positions of peaks when compared to those of the parent (Fig.7). Comparison of FTIR spectrum of control dye with extracted metabolites clearly indicated the biodegradation of the parent dye compound was occurred by *Aeromonas, Bacillus* and *Pseudomonas*.



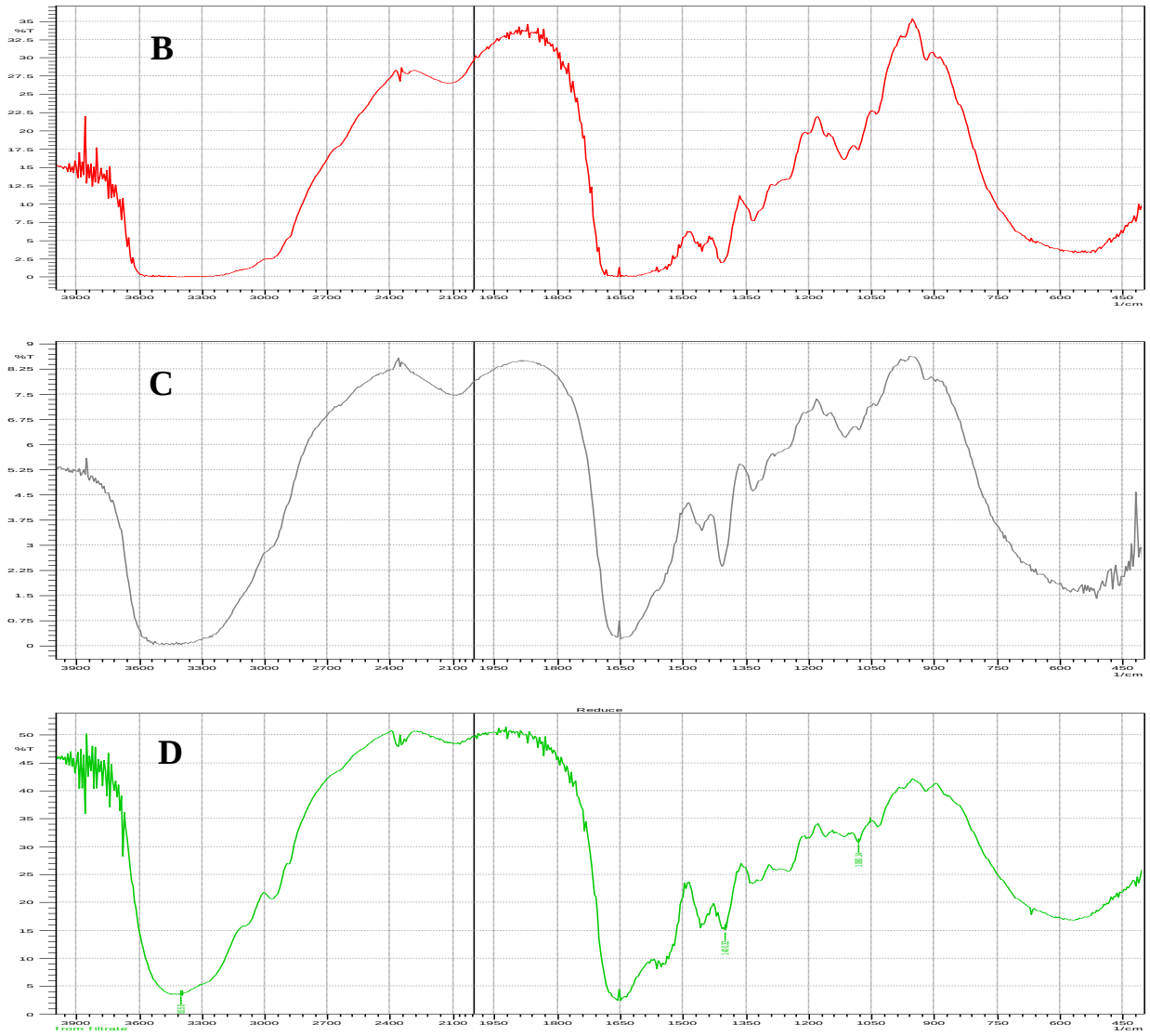


Fig. 7. Biodegradation analysis of NSB-G by infrared spectrophotometer after 48 h of incubation (A, Parent Dye; B, degradation by *Aeromonas*; C, degradation by *Bacillus* and D, degradation by *Pseudomonas.*

CONCLUSIONS

In this study, decolorizing bacterial isolates were isolated from effluents and discharge sites of textile and leather industries. Fourteen isolates showed single or multiple dye decolorizing ability. Physicochemical parameters for decolorization of NSB-G by *Aeromonas, Bacillus* and *Pseudomonas* isolates were optimized. *Aeromonas, Bacillus* and *Pseudomonas* sp. showed decolorizing activity through degradation mechanism rather than adsorption. With high degradative and decolorizing activity against various reactive dyes commonly used in the textile industries, it may be proposed that *Aeromonas,*

Bacillus and Pseudomonas isolates have potential practical application in the biotransformation of various dye effluents.

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