Heliyon 7 (2021) e08102

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The degradation of textile industry dyes using the effective bacterial consortium

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ARTICLE INFO

Keywords:
Azo dye
Bacteria
Biodegradation
Consortium
Decolourization
Textile effluents

ABSTRACT

The effluents from textile industries without proper treatment contains a remarkable amount of synthetic dyes which are harmful to the environment and a big challenge globally to degrade it with a eco-friendly way. Conventional methods are extremely energy-consuming, non-effective and generate a toxic sludge impacting the environment. Several microorganisms can be utilized to treat these effluents. The research deals with five bacteria isolated from textile effluent and their consortium for the biodegradation ability of Novacron dyes. The isolates were identified through the Biolog™ identification system and molecular technique. Biodegradation was confirmed by measuring optical density (OD) optimizing conditions (pH 7.0, temperature 37 °C, 10 % inoculums and 100 mg/L dye) under static condition. The isolates started decolourization at 24 h whereas, the consortium started decolourization at 18 h and exhibited a maximum after 72 h. The presence of low molecular weight protein as metabolite supported the biodegradation and non hazardous to environment. This study revealed that these bacteria might have degradation potentials, and research results will help to set up dye removal eco-friendly methods to expose the dye effluents to environment in future.

1. Introduction

The rapidly developing economy of Bangladesh mainly depends on the textile and ready-made garments industries [1]. The country's prime option of foreign exchange earnings is exporting textiles and garments products [2]. Bangladesh seized 2nd position in producing garments just after China in 2016 [2]. The discharge of polluted water from industrial sources, which causes serious environmental hazards, is an excellent threat to living beings. In Bangladesh, little or no treatment is carried out before the discharge of industrial textile effluent [3].

Water pollution, where effluents come from dye-based industries like textile industries, serves as a principal source and is one of the most concerning environmental pollution threatening our biodiversity [4, 5]. Generally, textile dyes are mostly alienated into azo, reactive, triphenylmethane, heterocyclic, polymeric compositions etc. [6, 7]. Among all the textile dyes, azo dyes are most suitable to use in the textile industry. They are very stable to light, water, heat, bleach, detergents, and perspiration due to their quality and π-conjugated azo bond individuality [8, 9]. It is projected that about 5–10 % of total dyes are released in textile waste water streams during the dyeing process that ultimately reaching the natural water bodies without treatments [10]. Most of the dyes and their converted product (aromatic amines) have a toxic effect on aquatic existence and some have still carcinogenic and mutagenic effects on human and other animals [11, 12]. Moreover, the dye can reduce light penetration, gas solubility and can interfere with phytoplankton photosynthesis that disturbs the aquatic environment [13, 14]. Besides, the discharge of untreated dye effluent into useable water resources similar to rivers and lakes altered the pH level, thus enhance BOD, COD, and TOC assessments [15]. So, treatment of effluents (dye-contamination) from the textile industries is necessary to control environmental pollution.

Furthermore, azo-dyes from textile is sometimes complicated to entirely degrade through conventional Physico-chemical strategies such as coagulation, flocculation, reverse osmosis technique, and activated carbon adsorption [16, 17]. Moreover, such traditional treatments have various disadvantages, including excess chemicals, more energy consumption, the formation of a huge quantity of sludge, and secondary pollution [18, 19]. In contrast, the degradation of azo dyes can be performed by a wide variety of microorganisms such as bacteria, fungi,

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https://doi.org/10.1016/j.heliyon.2021.e08102
Received 6 June 2021; Received in revised form 23 July 2021; Accepted 28 September 2021

yeast, and algae [15, 20, 21]. They can collapse dyes and convert them into CO_2 and H_2O. So, the mineralization and detoxification of azo dyes through biodegradation is now emerging as an effective and productive approach [22, 23, 24]. But many of these studies used single cultures which convert the azo dye into aromatic amines as degradation metabolites [25]. In addition, specificity to a particular nature of the dye, less effective to huge amount dye-containing wastewater treatment limits their extensive use [26, 27]. Alternatively, high decolourizations, as well as detoxification activity and more resistance to intense pH (acidic or alkaline), temperature (high or low) and high dye concentrations, were showed by mixed cultures [15, 26]. To focus on these issues, the objectives of the present research work are designed to form a potential culture consortium as well as pure bacterial isolate to decolourize the structurally five diverse textile azo-dyes under aerobic conditions and metabolites for its physio-chemical nature.

2. Materials and methods

2.1. Design of experiments

This research was conducted at the Industrial Microbiology Laboratory, Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dr. Qudrat-I-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh. This research involved identifying dye degrading bacteria isolated from textile effluents in our previous study, measuring decolourization potential of single and consortium bacterial isolates, determining the effect of decolourized effluent and characterization of azo reductase enzyme.

2.2. Dyes, chemicals, and culture media

Azo dyes named Novacron Ruby, Novacron Super black, Novacron Yellow, Novacron Navy, Novacron Blue DK used in this study were collected from Swiss Colors Ltd., Bangladesh. Biochemical reagents and Microbiological media (Hi-Media, India), RNase free water and TAE buffer (Thermo Fisher Scientific), PCR supermix (Invitrogen), Agarose and SDS-polyacrylamide gel (Invitrogen, Germany), Coomassie Brilliant Blue (Promega, USA) and the additional chemicals employed here from Sigma, USA.

2.3. Identification of selected bacterial strains

The isolated five bacterial stock cultures that eventually isolated from textile effluents and enriched in the nutrient broth media. After 24 h streak plate technique was done on nutrient agar media; thus, single and pure colony was picked up for identification. These bacterial strains were identified with morphological and Gram staining characteristics, Biolog™ identification system, and PCR identification system.

2.3.1. Morphological characterization

Characterization of colony morphology (colour, shape and size)of bacterial isolates were performed by streaking (sub-cultured) on nutrient agar media, and microscopic observations were carried out following 24 h of incubation [28].

2.3.2. Biolog™ identification

Designed for species-level identification of dye degrading bacteria, Biolog™ identification (BIOLOG™, USA) MicroStation was employed base on the exploitation of 71 carbon sources and 23 chemical sensitivity assays in GEN-III microplates [29]. The isolates were cultured on universal growth (BUG) media. The newly cultured inoculum of the bacterial colony was added to the inoculating fluid-A (pre-warmed at 37 °C for 30 min) for obtaining the desired turbidity (90–98) % T for Protocols-A of GEN-III Microbial ID 20 assay techniques. The cell suspension was then transferred into the multichannel pipette reservoir. The tips of the multichannel Repeating Pipettor were loaded by drawing up the cell suspension from the reservoir. All 96 wells were inoculated with accurately 100 µl of the bacterial suspension. Subsequently, the microplates were incubating for 18–24 h at 37 °C. After incubations, the microplate was placed into the Micro Station Reader to achieve bacterial ID, and the result was given by comparing with the database using the software program MicroLog 4.20.05 (BIOLOG™, USA) [30].

2.3.3. DNA extraction and PCR amplification of dye degrading bacteria

The heat-thaw method was employed for DNA extraction of five bacterial isolates [31]. Isolates were grown in Nutrient Broth and after 24 h, 1 mL broth cultures was in use into Eppendorf and centrifuged at 10000 rpm for 5 min. Subsequently, the pellets were collected, and 100 µl RNase free water was added, thus mixed thoroughly by vortexing. The Eppendorf tubes were boiled at 100 °C in a water bath for 10 min and instantly placed in ice for another 10 min; then, the tube was centrifuged at 10000 rpm for 5 min. The supernatant was collected and stored at -20 °C that contains bacterial DNA. For PCR amplification, the reactions were done in a 30 µl mixture compiled of 22.5 µl PCR Super Mix, 1.2 µl of each forward and reverse primer (Table 1), DNA template 2 µl and 3.1 µl RNase free water 35 cycles using a thermal cycler (BioRad). Amplification states were 94 °C for 1 min, 65 °C (for primers Ps/Ps-rev), 55 °C (for primers F27/R1492) for 1 min, 72 °C for 1 min and final extension was carried out at 72 °C for 5 min. After amplification, PCR products were examined with 1.5 % agarose gel in 1X TAE buffer by electrophoresis (Compact XS/S, Biometra) and DNA bands were visualized with ethidium bromide under UV transilluminator (Biometra).

2.4. Secondary screening of previously identified bacterial strains

Stock cultures of five bacterial strains, which decolourized azo dye, were enriched in the nutrient broth media. All the pure colonies from the streak plate method were separately inoculated into 10 ml of nutrient broth medium. After 24 h, nutrient broth with individual dyes with a concentration of 100 mg/L was inoculated with selected microorganisms and incubated at 37 °C for 48 h. The colour change was noted at regular intervals. These isolates were purified by frequent streaking on nutrient agar and stored at -20 °C in Eppendorf with 40 % glycerol.

2.5. Optimization of the decolourization process

To optimize the decolourization conditions, the tests were conducted in two different incubation conditions: static (no shaking) and shaking. The test of pH, temperature, inoculum volume and concentration of dye was conducted accordingly [35].

2.5.1. Effect of pH and temperature on dye decolourization

To study the effect of pH and temperature on the decolourization capacity of the selected strains, the broth medium pH was adjusted to 5, 7 and 9 incubating at various temperatures (25 °C, 30 °C, 37 °C and 44 °C) in the static situation and decolourization was determined by measuring OD.

2.5.2. Effect of inoculums volume and concentration of dye on decolourization

The effect of initial dye concentration and the inoculum volume on the rate of decolourization was evaluated with different concentrations of

Table 1. The primer sequences used for PCR amplification to identify dye degrading bacteria.

Ps Ps-rev	5′-GGTCTGAGAGGATGATCAGT-3′ 5′-TTAGCTCCACCTCGCGGC-3′	65 °C	*Pseudomonas spp*	[32]
F27 R1492	5′-AGAGTTTGATCCTGGCTCAG-3′ 5′- GGTTACCTTGTTACGACTT-3′	55 °C	*Bacillus spp*	[33]
Efm1 Efm2	5′-TKCAGCAATTGAGAAATAC-3′ 5′-CTTCTTTTATTTCTCCTGTA-3′	52 °C	*Enterococcus faecium*	[34]

dyes (100–300 mg/L) and volume of inoculums (2 %, 5 %, 10 %, 15 % and 20 %) and incubated for 72 h. The decolourized medium's aliquot was seized after 72 h and centrifuged at 10,000 rpm for 10 min to split the bacterial cell mass. The supernatants were utilized to determine the decolourization at the absorbance of 590 nm.

2.5.3. Effect of carbon and nitrogen source on dye decolorization

The effect of different carbon and nitrogen source on decolorization capacity of the consortium 2 were investigated with NB medium containing 100 mg/L of mix dye. Different carbon sources like glucose, sucrose, and mannose were added individually to the culture medium at a concentration of 1 g/L. Similarly different Nitrogen sources (ammonium sulfate, peptone, yeast extract and beef extract) were added to the medium individually and in combination with carbon source at a concentration of 0.5 g/L. The medium was inoculated with 5% v/v of 24 h old cultures incubated in static condition for 72 h.

2.6. Decolourization activities

Fresh bacterial culture of 1 mL was inoculated in 10 ml nutrient broth containing 100 ppm of dye and incubated at 37 °C under the static aerobic condition, and pH 7.0 was maintained through the whole process. The nutrient broth (without bacterial culture) supplemented with respective dyes was used as a control. The culture suspensions were centrifuged at 10,000 rpm for 10 min at 4 °C for the elimination of biomass. Decolourization levels were determined by measuring the absorbance of the culture supernatant at 600 nm with a UV-visible spectrophotometer [36]. The percentage of decolourization was calculated by using the formula reported earlier.

% Decolourization = (Initial OD – Final OD)/Initial OD x 100

Table 2. Microorganism identification with BioLog™.

Strain	PROB (%)	SIM	DIST	ID
C-2	0.674	0.674	4.612	*Bacillus pumilus/safensis*
C-4	0.524	0.524	6.775	*Bacillus thuringiensis*
C-6	0.646	0.646	5.066	*Enterococcus faecium*
CS-34	0.986	0.776	2.958	*Pseudomonas aeruginosa* (Lab1)
CS-37	0.753	0.753	3.593	*Pseudomonas aeruginosa* (Lab2)

2.6.1. Effect of single culture on individual and mix dye

After 24 h pure culture of all five bacterial inoculums was inoculated to the nutrient broth with each of the five different azo dyes with a concentration of 100 mg/L. These were incubated at 37 °C, and the decolourization was determined by measuring OD at regular intervals.

Nutrient broth with mixed dye (Novacron Ruby, Novacron super Black, Novacron Blue DK, Novacron Navy, Novacron Yellow) was taken to different tubes and inoculated with all five bacterial inoculums to each tube. These were incubated at 37 °C, and the biodegradation was determined by measuring OD at regular intervals [18].

2.6.2. Effect of bacterial consortia on individual and mix dye

Based on the effect of pure inoculums on each of the single dyes, 4 different consortiums were used.

Consortia were:

Consortium-1: *Pseudomonas aeruginosa* (Lab 1), *Bacillus pumilus*.

Consortium-2*: Pseudomonas aeruginosa* (Lab 1), *Enterococcus faecium*.

Consortium-3: *Pseudomonas aeruginosa* (lLab 2), *Bacillus thuringiensis, Enterococcus faecium*.

Consortium-4: *Bacillus thuringiensis*, *Bacillus pumilus*, *Enterococcus faecium*.

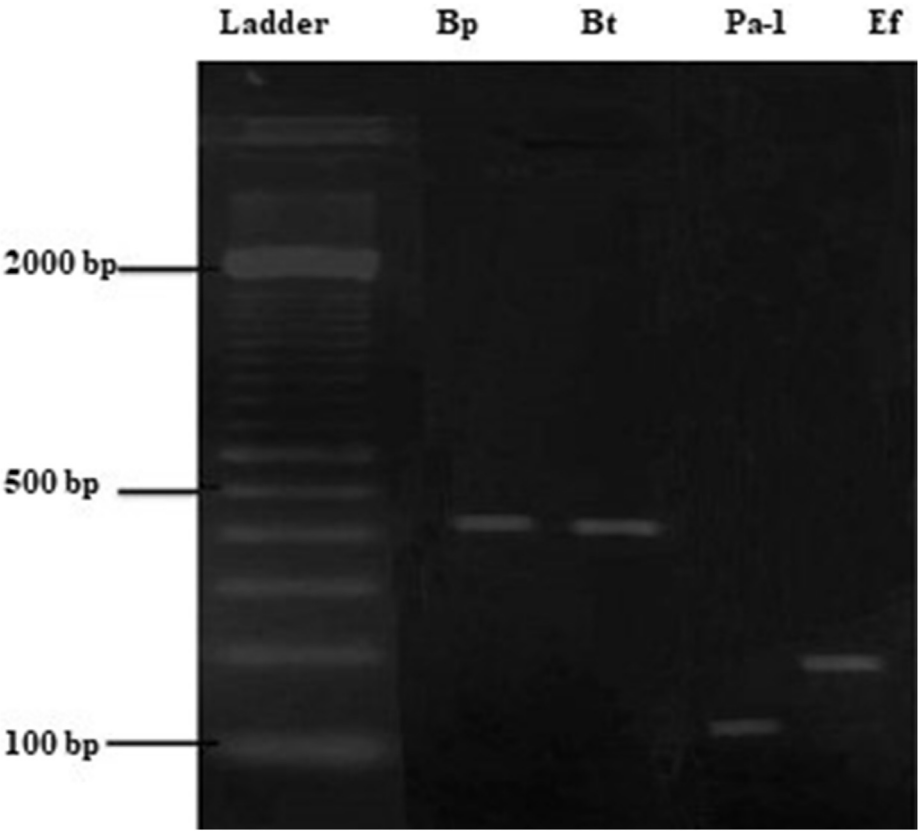

Figure 1. Gel electrophoresis with 1.5 % agarose.

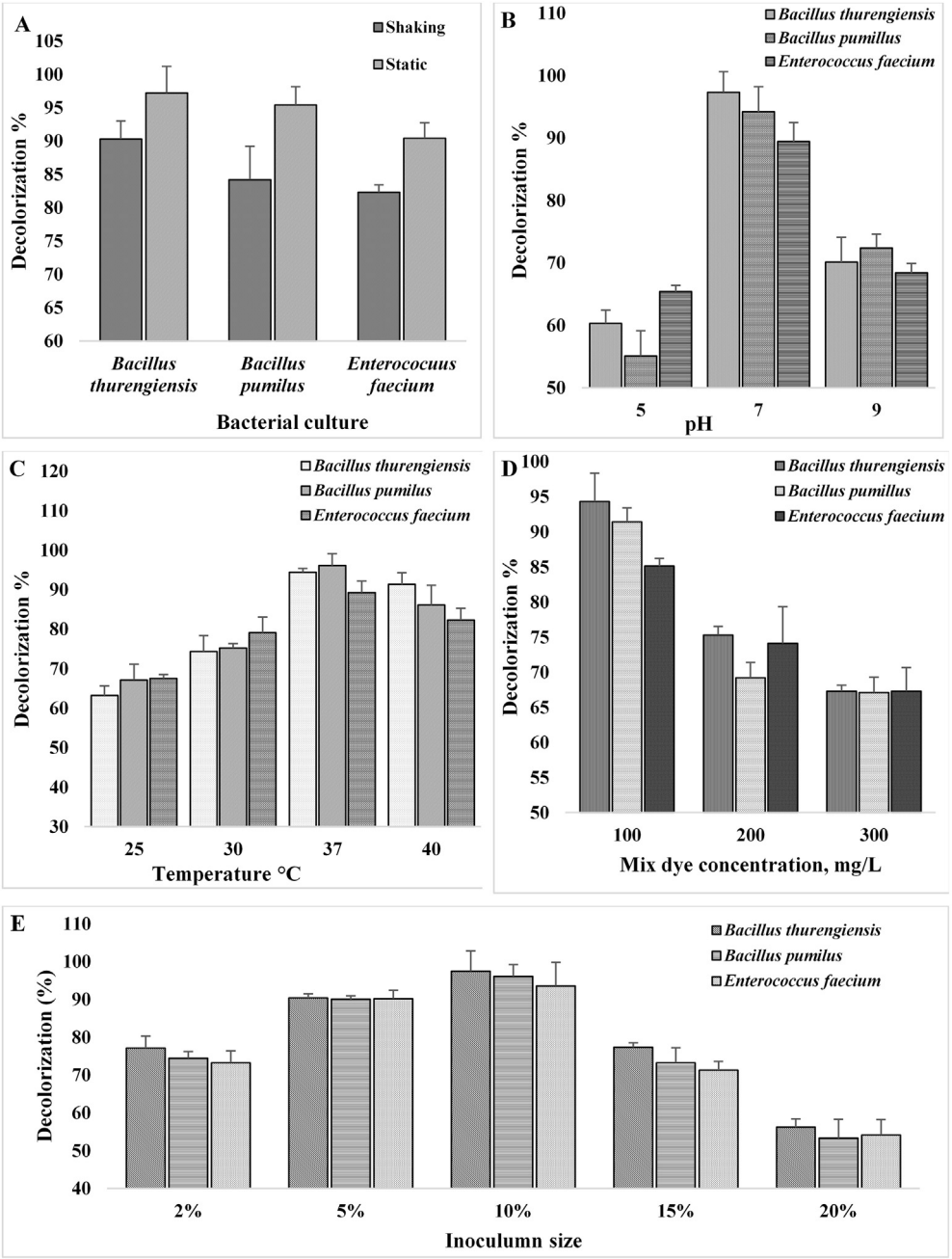

Figure 2. Optimization of decolorization process (A) incubation condition, (B) effect of pH, (C) temperature, (D) dye concentration and (E) iInoculumn size with mix dye.

Each of the consortiums was added to the medium containing single dye incubated at 37 °C decolourization was checked at different time intervals. Mix sterile dye (100 mg/L) containing nutrient medium was inoculated with another type of consortium (4 classes) and incubated at 37 °C. The biodegradation was determined by measuring OD at regular intervals.

2.7. Partial analysis of bacterial metabolites

By the acetone precipitation method [37], the decolourized broths with particular bacterial cells were centrifuged at 10,000 rpm for 10 min at 4 °C, and the supernatant was collected. Ice cold (−20 °C) acetone was put into three times (150 µl) the sample amount (50 µl) to the Eppendorf tube. After mixing properly and incubating at -20 °C for overnight, centrifugation was carried out at 13,000–15,000 rpm for 30 min at 4 °C. The supernatant was disposed of, and the acetone was allocated to evaporate at room temperature for 30 min. Subsequently, 200 µl of SDS buffer was added to the protein pellet. To find out the protein profiles extracted throughout the Acetone precipitation method, SDS-PAGE analysis (Bio-Rad, USA) was performed with 5 % stacking and 10 % separating gel at 20 mA followed by Coomassie blue staining. After the gel run, it was stained and destained in 0.1 % Coomassie Brilliant blue and 10 % Acetic acid for approximately 2–4 h at 100 rpm, respectively.

2.8. Effect of decolourized metabolites on beneficial bacteria

The decolourized products of dyes were investigated for their toxic consequence on agriculturally significant soil bacterial flora. *Bacillus*

cereus was inoculated into Mueller-Hinton agar. Three wells were prepared and filled with every decolourized broth. Plates were incubated at 37 °C for 24 h. A zone of inhibition surrounds the well-represented index of toxicity. An untreated dye medium was used as a control.

2.9. Statistical analysis

All data were statistically analyzed by one-way analysis of variance (ANOVA), and the difference between the means of samples was evaluated by the least significant difference at a probability level of 0.05. The results were stated as the mean ± standard error of three independent experiments.

3. Result and discussion

3.1. Morphological feature of isolated bacteria

The isolates that confirmed enhanced decolourization potential were grown on a Nutrient agar plate. Among these five (5) isolates, the isolates C-2 and C-4 were large with irregular margin. The isolates CS-34 and CS-37 were small with irregular margin, followed by the isolate C-6 was pinhead in size and have around the margin. Three (3) isolates, specifically C-2, C-4 and C-6, were gram-positive and the rest two (2) isolates (CS-34 and CS-37) were gram-negative. All the isolates were rod-shaped, except the isolate C-6 was cocci.

3.2. Identification of bacteria using BIOLOG™ system

All strains were identified correctly up to the species level with the BIOLOG™ system (Table 2). C-6, C-2, C-4, CS-34 and CS-37 isolates were identified as *Enterococcus faecium, Bacillus pumilus/safensis, B. thuringiensis, Pseudomonas aeruginosa* (Lab1) and *Pseudomonas aeruginosa* (Lab2).

3.3. Molecular identification of amplified DNA genus products

Genus-specific, universal, and species-specific primers (Table 1) were used in this study to identify the dye degrading bacterial isolates. After running through in 1.5 % agarose (Invitrogen) gel with 100 bp DNA ladder (Invitrogen), the PCR products were under UV transilluminator (Biometra). Two bands of 460 bp amplicon [33] were found in lane-Bp and lane Bt for isolate C-2 and C-4 as for isolate CS-34 one band of 120 bp amplicon [32] was observed in lane-Pa-1 and one band of 190 bp amplicon [34] was found in lane-Ef for isolate C-6. Three isolates (C-2, C-4, and CS-34) were confirmed at the genus level, and isolate C-6 was confirmed at the species level (Figure 1). No band was found in negative control without a DNA template, which suggests that the PCR products correspond to the DNA template. After electrophoresis was done in 1.5 % agarose gel visualization of the gel under UV transilluminator. Bp, Bt, Pa-1, and Ef indicate isolate C-2, C-4, Pa-1 one, and CS-34 which sequentially showed two 460 bp size bands, one 120 bp size band, and one 190 bp size band. All four isolates were confirmed as *Bacillus* sp, *Pseudomonas* sp and *Enterococcus faecium*.

3.4. Optimizing the decolourization process of azo dye

Figure 2 depicted the change in the extent of dye decolorization in response to varying incubation condition, pH, temperature, dye concentration and inoculums size. The best decolorization exhibited with static incubation at pH 7 (Figure 2A and B). The results showed that the decolorization rate increased with increment of temperature from 25-37

Figure 3. Decolorization of Azo dye by single isolates and consortium. (A) Decolorization of Novacron Navy by single isolates, (B) Decolorization of Novacron Ruby by single isolates, (C) Decolorization of Novacron Black by single isolates, (D) Decolorization of Novacron Blue dk by single isolates, (E) Decolorization of Novacron Yellow by single isolates, (F) Decolorization of different Azo dye by consortium.

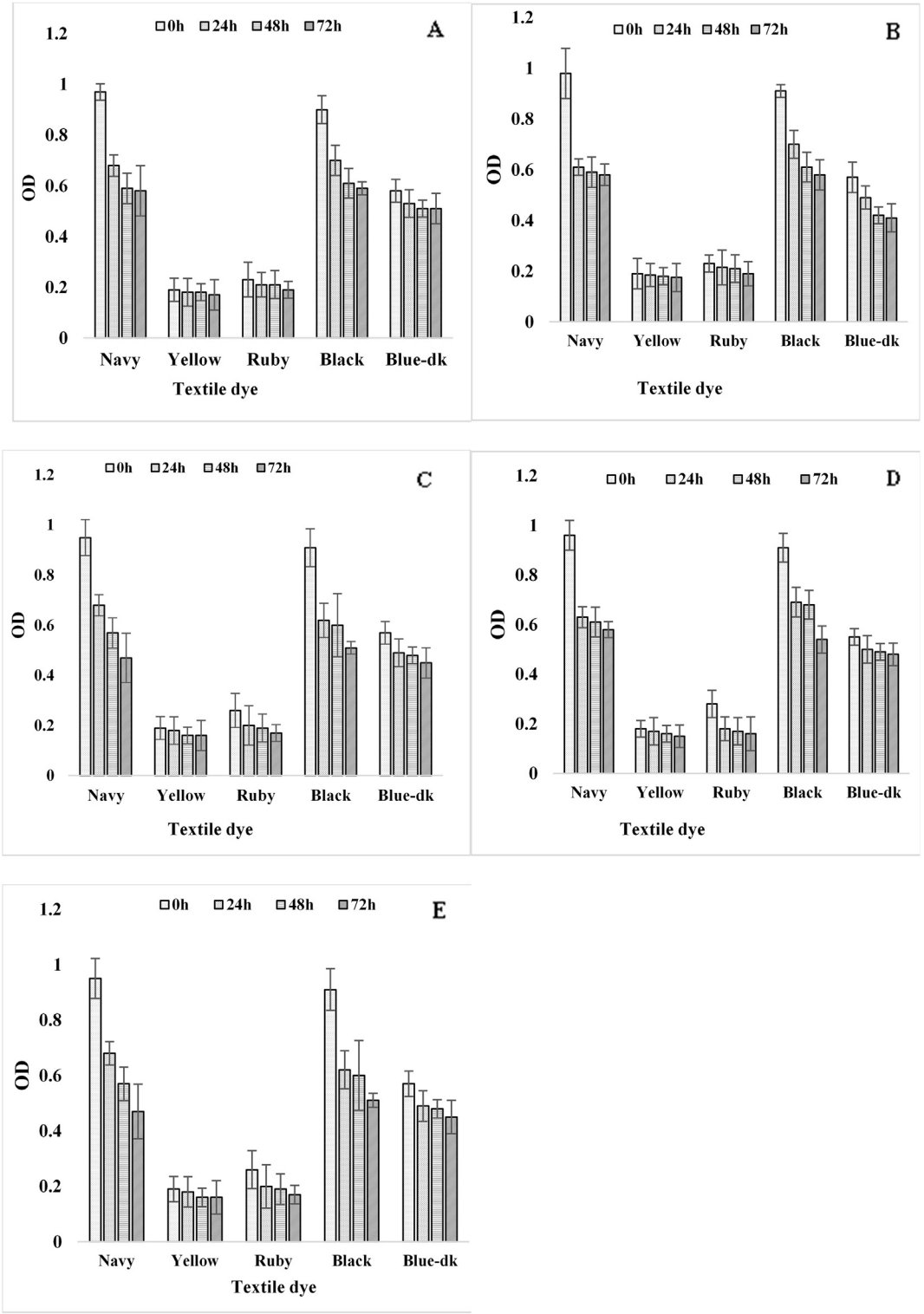

Figure 4. Biodegradation of single dye. (A) *P. aeruginosa* (Lab 1), (B) *P. aeruginosa* (Lab 2), (C) *B. pumilus*, (D) *B. thuringiensis* and (E) *E. faecium.*

°C. At 44 °C, the decolorization ability was sharply reduced. Highest decolorization of 98.2 % was noticed at 37 °C (Figure 2C). The study clearly showed that due to the increment of dye concentration, decolorization efficiency was decreased (Figure 2D). After 72 h of incubation at 37 °C in static condition, 77.1 % decolorization was achieved in 2 % (v/v) of bacterial inoculums (Figure 2E). Decolorization of dyes was optimum at pH 7 and efficiency changed with different pH. However, the decolorization rate decreases under extremely alkaline pH and under acidic pH. Some previous study indicated that the present study results were similar with their finding [35, 38]. The same study noted that temperature beyond 37 °C had an adverse effect for both growth and decolorization. This could be owing to a greater production of enzymes and maximal growth conditions of the bacterial culture for its dye decolonization ability. Decolorizing activity was significantly suppressed at 44 °C, which might be due to the loss of cell viability or deactivation of the enzymes responsible for decolorization at 44 °C [35, 38].

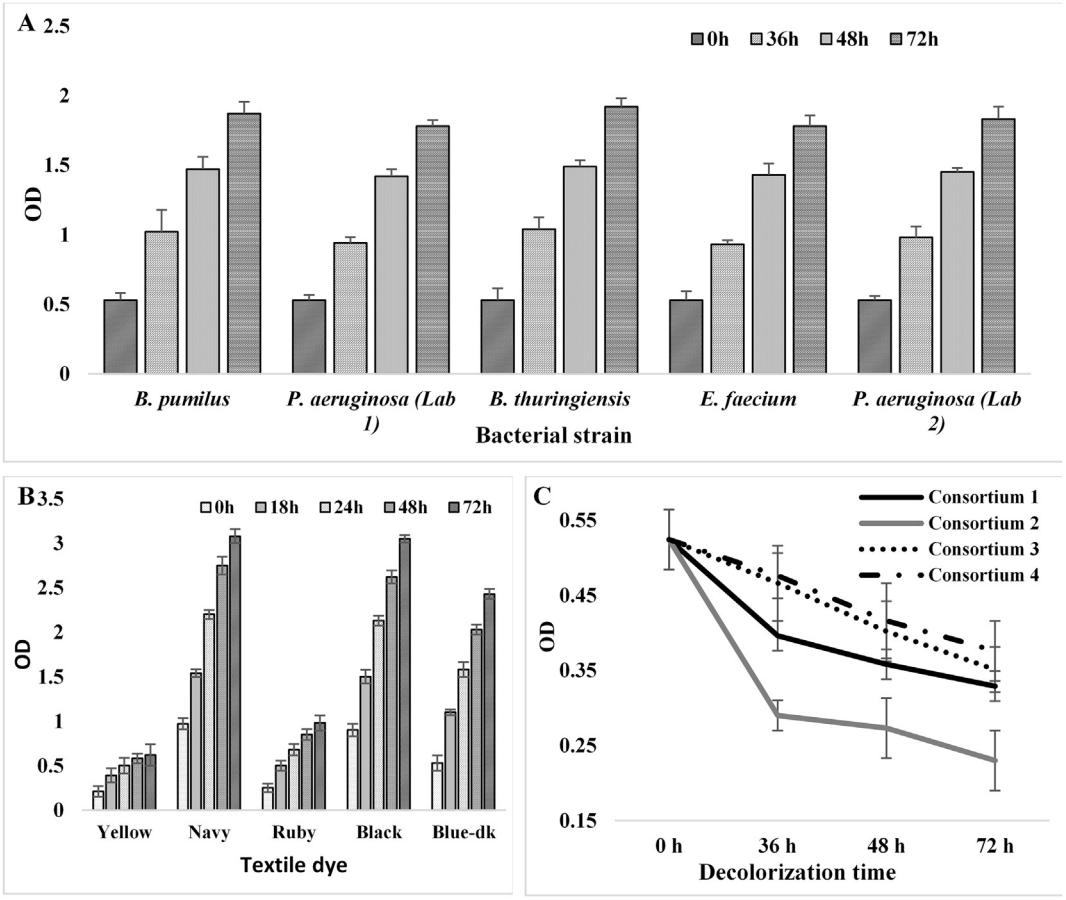

Figure 5. Decolourization activites of individual dye with single isolates (A), with consortium 2 (B) and mix dye with all consortium (C).

With an increment of inoculums size, the extent of decolorization also increased and 97.4 % decolorization was achieved in 10 % (v/v) of inoculums. A further increase in volume of inoculums resulted in decreased decolorization efficiency of the bacteria (Figure 2E). Similar studies have been reported [39, 40] where a decrease in the efficiency of decolorization was observed with increase in initial dye concentration. With subsequent increase in dye concentration toxic effect of dye and its metabolites became dominant, leading to inhibition in decolorization. The decrease in the efficiency of color removal with increase in concentration of dye can be due to toxic effect of dye and inadequate amount of biomass to uptake this higher concentration of dye and the ability of the enzyme to recognize the substrate efficiently at the very low concentrations [35, 41]. With the increase of inoculums size gradually decolorization efficiency also increased up to certain limit. Further increasing causes decreased decolorization. Bhatt *et al* [42] reported the similar result early depletion of nutrient occurs as the inoculums volume increased and hence biological process of decolorization involving microorganisms require an optimum amount of microbial cells.

There was no change in the colour decolorization within 08 h, possibly indicating the lag phase for adaptation to the environment. After 24 h, decolourization was visualized. Novacron black and Novacron blue dk dye take additional time compared to other dyes. This phenomenon

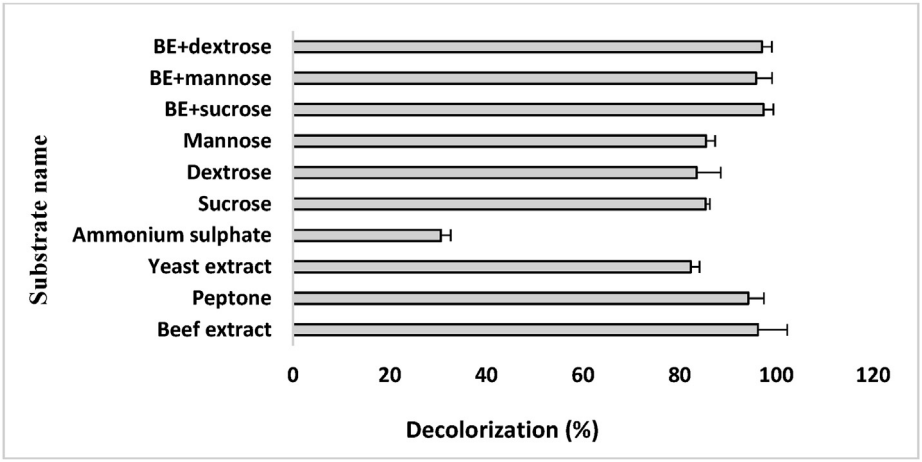

Figure 6. Effect of various carbon and nitrogen source on dye decolorization with consortium 2.

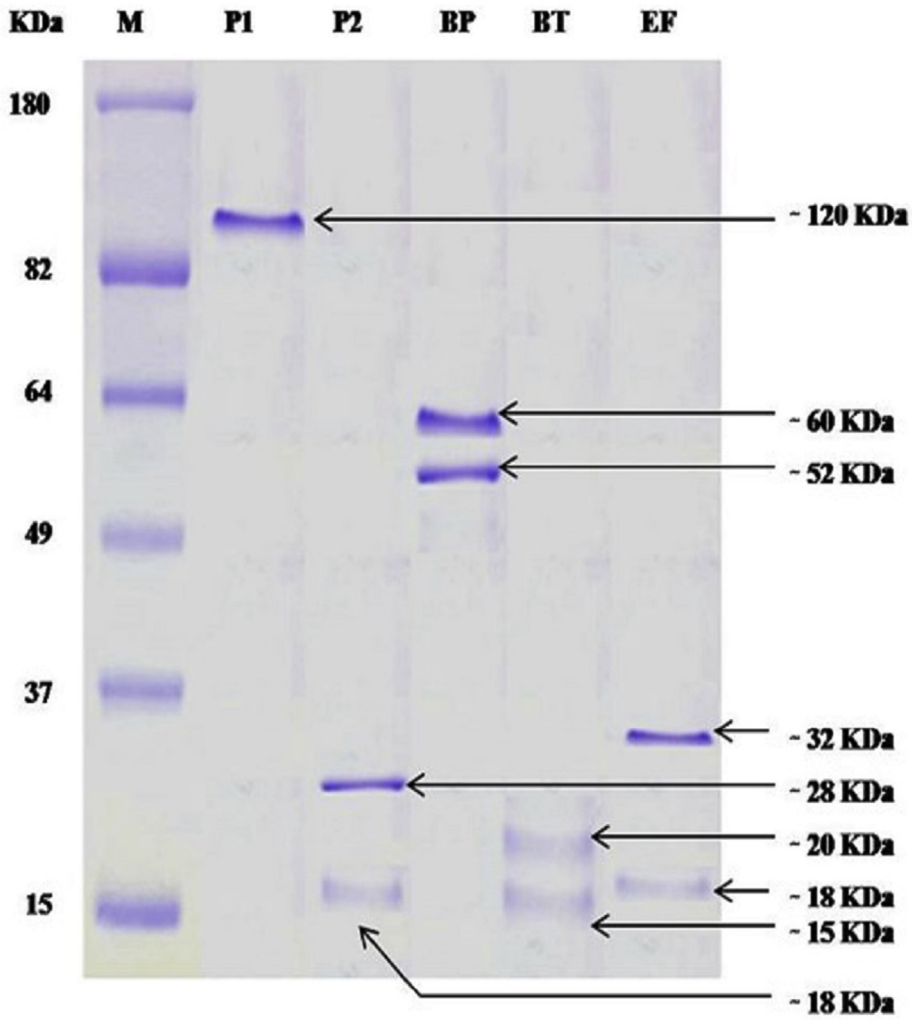

Figure 7. SDS-PAGE analysis of the bacterial enzyme. M: Marker, P1–*P. aeruginosa* (Lab 1), P2–*P. aeruginosa* (Lab 2), BP-*B. pumilus,* BT-*B. thuringiensis,* EF-*E. faecium.*

occurs owing to the complex structure and nature of textile dyes [43, 44]. Decolourization of Azo dye by all five single isolates and consortium was studied and visualized in Figure 3.

3.5. Effect of single isolate on single and mixed dye

All bacterial strains named *E. faecium, B. pumilus, B. thuringiensis* and *P. aeruginosa* were able to decolourize all five dyes: Novacron Ruby, Novacron Black Novacron Blue dk, Novacron Navy, and Novacron Yellow (Figure 4). The decolourization or biodegradation was authenticated by the magnitude of OD at 600 mn. Decolourization extent by pure bacterial inoculum to single as well as mix dye was various. Among all five isolates, *E. faecium* and two strains of *P. aeruginosa* showed a better effect on single as well as mixed dye. The effect of single isolates on a single dye was better than an effect on mixed dye [7, 45]. As evident, after 48 h, very little biodegradation of dyes was observed. This may be

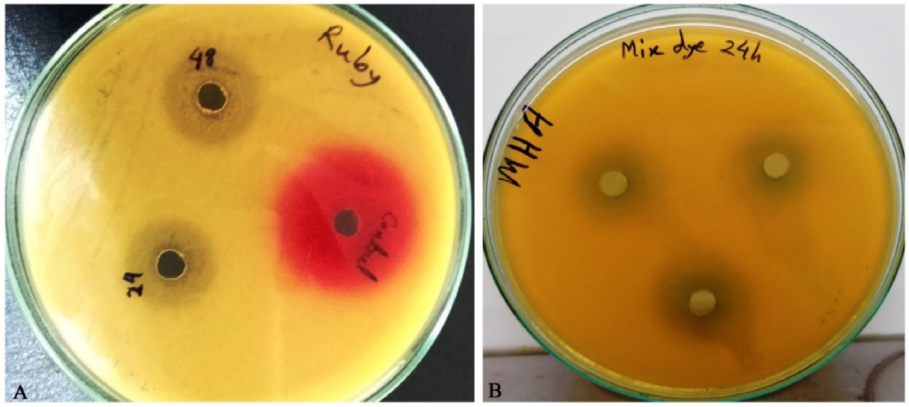

Figure 8. Toxic effect of treated and untreated Ruby (A) and treated Mix dye (B) on the beneficial bacterium.

caused by the culture approaching the death stage that reduced the enzymatic activity since maximum decolourization of the single dye was perceived after approximately 72 h. Similar studies have been reported in various literature [12, 46], whereas the rate of decolourization increased with the increasing time, and after a certain period, it showed no further decolourization [47, 48]. Fascinatingly, among the five isolates, *E. faecium* and two strains of *P. aeruginosa* showed maximum decolourization of the single dye after 72 h.

3.6. Effect of culture consortia on single and mixed dye

Based on the effect of single isolates, four different types of the consortium were developed (Figure 5). Biodegradation by the dissimilar consortium was different, and consortiums having dual isolates were superior to the consortium of three isolates due to the wider enzymatic capability is attained, and the development of toxic intermediary metabolites is counteracted by the selection of these dead-end products formed mainly by co-metabolism processes [47, 49]. The consortium started decolourization to single dye approximately 18 h after incubation and exhibit maximum decolourization after approximately 72 h. Similar studies have been reported in various kinds of literature [12, 50]. But consortium started decolourization to mix dye approximately 24 h after incubation. The effectiveness of the decolourization method depends on the survival, adaptability, and activities of enzymes formed by microorganisms present in the mixed cultures [4, 51].

After 16 h, there was no significant change in the dye. Interestingly, after around 36 h, some significant changes occurred, and decolourization was determined by the measurement of OD at 600 nm. Among the different types of the consortium, a higher rate of decolourization was obtained by consortium 2, which contains two isolates. The biodegradation capacity of the consortium was determined by measuring optical density and percentage decolorization presented in Table 3.

The efficacy of bacterial consortium 2 to decolorize mix dye in presence of additional carbon (1 %) and nitrogen sources (0.5 %) was tested in order to obtain efficient and faster decolorization (Figure 6). The maximum percentage of decolorization was observed with beef extract (96.16 %), while peptone and yeast extract showed a moderate decolorization value of 94.21 % and 82.27 % respectively (Figure 6). Negligible decolorization was observed for $(NH_4)_2SO_4$ as supplements of nitrogen source. Effect of carbon source was evaluated with and without beef extract as a nitrogen source. Presence of sucrose, dextrose and mannose as carbon source without beef extract showed 85.34, 83.55 and 85.43 %, respectively. On addition of beef extract along with sucrose, dextrose and mannose, the decolorization percentage was increased to 97.34, 95.87 and 97.03%, respectively. This indicates that presence of nitrogen source in media has a significant effect on the rate of decolorization, where as the presence of different carbon sources seems to be less effective to promote the decolorization of mix dye by the bacterial consortium. Similar results were obtained [35, 41] researchers observed the effect of nitrogen sources to supersede the effect of carbon sources under shaking conditions. Dye being a complex carbon ring is less preferred than a supplementary carbon source by the organisms. Hence the dye degradation potential of the organism decreases in presence of supplementary carbon source.

Table 3. Decolourization consequence of consortiums.

Consortium No.	16 h	24 h	42 h	60 h
1	64.5 ± 1.24%	73.1 ± 1.14 %	78.3 ± 0.71%	86.4 ± 3.13%
2	58 ± 4.03%	68.2 ± 3.07 %	76.3 ± 4.01%	89.2 ± 4.09%
3	70.2 ± 0.96%	78.4 ± 1.11 %	89 ± 3.0%	96.3 ± 4.12%
4	59.4 ± 3.38%	66.3 ± 0.92 %	77.2 ± 1.78%	83 ± 1.07%

3.7. Partial analysis of bacterial metabolites

The protein (enzyme) of bacterial isolates were determined by SDS-PAGE (10 % gel), thus evaluated through the standard protein marker (Figure 7). Electrophoretic patterns of the enzyme from five isolates illustrated diverse banding sizes. Bacterial enzymes on SDS-PAGE with a molecular weight of approximately 120 kDa, 60 kDa, 52 kDa, 28 kDa, 20 kDa, 18 kDa, 15 kDa have also been reported [52].

3.8. Effect of the decolourized product on beneficial bacteria

The inhibition zone was scrutinized around the wells which contain decolourized metabolites (treated dyes) after removing the bacterial cell using a centrifuge. The treated dyes as well as untreated dyes, were harmful to beneficial bacteria since all showed a zone of inhibition on the Mueller-Hinton agar plate. The toxic consequence of untreated dyes and elimination of their toxicity after biological treatment has been described by [18]. The result suggested that Azo dyes were decolourized or degraded by these isolates, but complete mineralization has not occurred. Surprisingly, the zone of inhibition was observed for both treated dyes as well as untreated dyes (Figure 8).

4. Conclusion

Rapid industrialization has commenced varieties of wastewaters, contaminated with diverse toxic constituents (dye) causing in deterioration of the environment and imbalanced ecosystem. This situation provided the impetus to the search for new source to treatment of textile dye. The result suggested a great prospect for bacteria to be used remediating pollutants (dye) from textile effluents. Among five isolates *E. faecium* and two strains of *P. aeruginosa* showed maximum decolorization at 37 °C under static aerobic conditions. With a significant decolorization rate, the consortium was able to degrade Novacron Ruby, Novacron Black, Novacron Blue dk, Novacron Navy, and Novacron Yellow and required less incubation time compared with individual strains. SDS-PAGE exploration of the isolates exhibited a broad range of bands on Electrophoretic pattern. To observe these harmful effects of treated dyes revealed that the degraded products were nontoxic to agriculturally valuable bacteria. The above results suggest that *E. faecium* and two strains of *P. aeruginosa* as well as developed consortium might be used as a promising for practical applications in decolorizing and simultaneously minimizing the toxicity of textile dyes. The metabolites formed after the decolorization of dye need to be analyzed, thus they can be used as excellent bio-agents for the biodegradation of textile dyes.

Declarations

Author contribution statement

Sadia Afrin, Hasibur Rahman Shuvo, Banjir Sultana: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Faridul Islam, Ahmed Abu Rus'd, Shamima Begum: Conceived and designed the experiments.

Md Nur Hossain: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Funding statement

This work was supported by the R & D project of the Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research, Dhaka.

Data availability statement

Data included in article/supp. material/referenced in article.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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