# Biodegradation of textile dye using consortium of bacterial strains isolated from industrial dye effluent

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# Abstract

Dyes are discharged into water through many industries such as textile industries. Disposal of these dyes into the aquatic and terrestrial environment causes serious damage. Remarkable, processes for the treatment of dye waste involve biological remediation, catalytic oxidation, filtration, and sorption process. In this study the textile industrial dye effluent was treated using biodegradation method. The degradation of dye wastewater was determined by pure bacteria and its consortium isolated from textile effluent. Three different bacterial spp. were isolated and subjected to dye decolorization assays. The isolates were identified by 16sRNA as Aeromonas aquatic AE 235, Flavobacterium ginsengiterrae DCY55, Shewanella xiamenensis S4. The percentage decolorization process was detected by UV spectrophotometer. This process was obtained at 90, 94, 95 and 98% respectively by pure of three bacterial strains B1, B2, and B3 and its consortium BC. The degradation process of dye effluent was confirmed by FTIR analysis. The physico-chemical analysis of the effluent sample was investigated before and after biodegradation treatment to emphasize the process efficiency. The results indicated that these bacterial strains have good potential for the biodegradation of dye from textile waste water.


# 1. Introduction

Dye wastewater from textile industries is one of the most complicated industrial wastewaters to treat. Textile dyes participate as the most important environment polluting agents. The textile industry is a large water consumer and makes large volumes of contaminated water. One of such example one of the most famous dyeing factories located in tenth of Ramadan city, Sharqiaa, Egypt. This textile factory which is produces millions of liters of improperly treated effluents that are released directly without giving proper treatment. Synthetic dyes freed into the environment in the form of effluents by textile, leather, food, paper and printing industries give rise to severe ecological damages. Wastewater producing from dyeing and finishing processes has an opposite impact in terms of total organic carbon, biological oxygen demand and chemical oxygen demand. The compound aromatics of dyes make them stable and complicated to be biodegraded [1]. Dyes are assorted as cationic (basic dyes); anionic (reactive dyes, direct and acid); and nonionic (disperse dyes). The chromophores in anonic and nonionic dyes essentially consist of azo groups or anthraquinone types. Anthraquinone based dyes are more recalcitrant to degradation due to their combine aromatic structures. The metal complex dyes are predominately based on chromium. Azo dyes are the most greatly used and record for 60% of the overall dye structures [2]. Effluent generated from textile dye is highly colored because of the residual dye in it which does not attach to the fabric. However it is also a well-known but generally ignored fact that these synthetic dyes are mostly toxic, carcinogenic and mutagenic. Furthermore, color of the dyestuff is harmful the aquatic environment by decreasing light penetration, gas solubility and intervention of phytoplankton photosynthesis They are also harmful to the environment because of their bonds which are resistant to breakdown with the potential for the persistence and accumulation in the environment [3].

The physical or chemical treatment processes are usually applied for treated of dye wastewaters. These involve electro-kinetic coagulation, electrochemical destruction, electro-flocculation, flocculation

combined with flotation, membrane filtration, ion-exchange, irradiation, precipitation, ozonation, and katox treatment process including the use of activated carbon and air mixtures [4]. However the developed physical and chemical methods present several drawbacks such as high cost, high generation of sludge, high-energy-requiring irradiation methods requires a lot of dissolved $O_2$, excessive use of chemicals, secondary pollution, low efficacies, and high operational cost etc. [5]. The treatment systems based on using microorganisms capable of degrading dyes have received increasing interest, Owing to their cost effectiveness, ability to produce less sludge and environment benignity. It is now recognized that various microorganisms including fungi, bacteria, yeasts and algae able to decolorize and even totally mineralize many types of dyes under specific ecological conditions. Bacteria cleave the azo bond (–N = N–) by azoreductase enzyme, results in the formation of colorless amines and subsequently simpler compounds [6]. The activity of microbial decolorization relies on the adaptability and the activity of chosen microorganisms [7]. Microbial consortia have been greatly used in cleanup of a number of pollutants in lab scale and large scale bioremediation studies. It is generally concept that the microbial consortia are more effective than pure cultures in biodegradation process. It is generally thought that microbial consortia are more effective than pure cultures in biodegradation. This is possibly because broader enzymatic capacity is achieved [8]. The objective of the present study is to treat of the industrial dye effluent sample using biodegradation technique. The current study deals with the isolation, identification and screening of bacterial species which able to decolorize textile dye effluents. Decolorization of dye by bacterial species is investigated by FTIR analysis.

## 2. Materials And Methods

# 2.1. Sample collection

Industrial wastewater sample was collected from one of the most famous dyeing factories located in tenth of Ramadan city, Sharqiaa, Egypt. The sample was withdrawn from end point of the production line containing all types of pollutants that’s may be found from such industries.

# 2.2. Physico-chemical properties analysis

The collected effluent sample has been analyzed to determine its physico-chemical parameters. The various parameters viz., Temperature, pH, suspended solids (TSS), biological oxygen demand (BODs), chemical oxygen demand (CODs), phosphate, nitrate, sulfide and color were analyzed in the laboratory by High Technological Institute, 10th of Ramadan city, Egypt.

# 2.3. Isolation of dye degrading bacterial isolates from dye effluent wastewater

Sriram and Reetha [9] described the isolation of dye degrading bacterial strains by mixed of 1 ml wastewater sample with 9 ml of sterile saline water, and then it was serially diluted up to concentration of $10^{-8}$. Then, 0.1 ml of serially diluted samples from each concentration of samples were transferred to sterile nutrient agar plates and evenly spread throughout the plates. The nutrient agar plates were

incubated at 37 °C for 24 h. After incubation, the bacterial colonies were isolated and purified from the plates. The well grown bacterial cultures used for upcoming screening technique and stored at 4 °C.

## 2.4. Screening test for dye degradation ability of bacterial isolates

The bacterial isolates were tested as individual and consortium for their ability to degrade the textile dye by incubating them on 100 ml of nutrient agar medium with 10 ml of dye effluent. The nutrient agar medium incubated at 37 °C for 24 h. After the incubation, plates were observed for clear zone. The strains that showed higher decolorizing potential were selected for upcoming experimentation. The screened bacterial culture was transferred to nutrient agar slants and maintained as stock cultures at 4 °C in a refrigerator [10].

## 2.5. Identification of selected bacterial isolates by 16sRNA sequencing

Three selected dye degrading bacterial isolates were named as B1, B2 and B3 based on their dye degrading ability, and they were identified using 16 s RNA in Sigma Scientific Services Co., Egypt. The extraction of DNA: the selected bacterial cells were inoculated on LB broth medium and incubated at 37˚C for 24 hours. The bacterial culture harvested through the enrichment medium (LB) up to $8 \times 10^8$ cells. DNA was isolated using protocol of Gene Jet genomic DNA purification Kit (Thermo) (Sigma Scientific Services Co., Egypt). To amplify the 16S rRNA genes of isolates, a polymerase chain reaction (PCR) was accomplished using (5'-AGA GTT TGA TCC TGG CTCAG-3') (5'-GGT TAC CTT ACG ACT T-3') as forward and reverse primer respectively. The GeneJET™ PCR Purification Kit used for the cleanup of PCR to the PCR product. A 45 µl of bounding buffer was added to the completed PCR mixture. This mixture was then thoroughly transferred from step 1 to the GeneJET™ purification column. Then, the mixture was centrifuged for 30–60 s at > 12000 xg, then the flow was discarded. A 100 µl wash buffer was added to the GeneJET™ purification column, centrifuged for 30–60 s, neglected the flow-through and place the purification column back into the collection tube. The mixture was centrifuged at empty GeneJET™ purification column for an additional 1 min to completely remove any residual wash buffer. The purification column was transferred to a clean 1.5 ml microcentrifuge tube. A 25 µl of elution buffer was then added to the center of the column membrane, which then centrifuged for 1 min, throw away the column and store the purified DNA at -20 °C. After that the purification of the PCR products, the DNA sequence of the positive clone was subjected to a similarity search BLAST on the NCBI website (http://www.ncbi.nlm.nih.gov) and deposited into Gen-Bank. Many relevant 16S rRNA gene sequences with validly published names were chosen as references from the Gen-Bank.

## 2.6. Dye decolourization assay

Dye decolourization experiments were achieved in four 250 ml Erlenmeyer flasks for effluent dye sample. The each flask containing 100 ml of nutrient broth with 10 ml of dye wastewater, the pH was adjusted to 7 ± 0.2. After that, the flasks were autoclaved at 121 °C at 15 lbs pressure for 15 minutes. Then, the

autoclaved flasks were inoculated with 5 ml of bacterial inoculum of each isolates and bacterial consortium. The flasks were put in shaking incubator with 150 rpm of agitation speed and incubated at 30 ºC for 24 h. After the end of incubation period, the different culture media were filtered and centrifuged at 8000 rpm for 20 minutes. Decolourization was assessed by measuring absorbance at 510 nm of the supernatant with the help of spectrophotometer at wavelength maxima (λm) of respective dye [10]. Decolourization assay was determined in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated from the following equation,

$$\% \text{ Decolourization} = \frac{\text{Initial OD - Final OD}}{\text{Initial OD}} \times 100$$

# 2.7. Conformation of decolourization process by FT-IR analysis

Decolourized culture medium by pure and bacterial consortium was centrifuged at 8000 rpm for 20 min. The Infrared (IR) spectra of the culture supernatant (a film of each sample on KBr pellets) were obtained using a Nicolet IS-10FTIR spectrometer. The IR spectra collected within a scanning range of 500-4000$cm^{-1}$ with a resolution of 1 $cm^{-1}$ [11].

## 3. Results And Discussion

Textile dye industrial effluents are one of great sources of ecological toxicity. It not only affects the quality of drinking water but also has deleterious impact on the soil microflora and aquatic ecosystems [12, 13].

# 3.1. Isolation and molecular identification of dye degrading bacterial isolates

In the present study, three different bacterial isolates were isolated from textile dye effluent sample. The bacterial isolates were found to be motile, gram negative and rod-shaped strains. The bacterial isolates were molecular identified using 16 s RNA in Sigma Scientific Services Co., Egypt. The identified of bacterial isolate was belonging to *Aeromonas aquatic* AE 235, *Flavobacterium ginsengiterrae* DCY55, *Shewanella xiamenensis* S4 with similarity of 99, 96 and 98% respectively. El Bouraie and Salah El Din [14] reported that the *Aeromonas hydrophila* isolate can be considered as potential bioremediation for the treatment of dye industrial wastewater. Xu et al. [15] have investigated isolation of *Shewanella decolorationis* S12 for the ability of dye decolourization.

# 3.2. Screening of dye decolorizing bacterial isolates using plate technique

In the present study, three bacterial strains were screened for potential decolourization of textile dyes effluent sample was achieved by plate assay [16]. The selected bacterial strains were tested separately and consortium of all for their ability to decolorize dye wastewater sample and the results were tabulated in Table (1). The bacterial strains exhibiting strong decolourizing activity were also reported by Hassan et al. [17]. The maximum percentage decolourization was recorded by mixture of three tested strains in the plate containing dye effluent sample as zone inhibition, followed by *Shewanella xiamenensis, Flavobacterium ginsengiterrae* and *Aeromonas aquatic* after 24 h. Karthikeyan and Anbusaravanan [18] reported the isolation and screening of microorganisms able to decolourizing diverse azo dyes from wastewater treatment sites polluted with dyes. Azo-dyes were degraded by extracellular peroxidase released by *Falvobacterium* sp [19].

Cell walls consist fundamentally of polysaccharides, proteins and lipids exhibit abundant functional groups. The dyes can interact with these active groups on the cell surface in a different manner. So, the degradation efficiency of dyes by microbial biomass is due to ion-exchange mechanisms [20]. Many researchers have mentioned that a higher degree of biodegradation and mineralization of textile dye effluents can be expected when co-metabolic activities within a microbial community supplement each other. In such a 'consortium', the organisms can act synergistically on a variety of dyes and dye mixtures. One organism may be able to cause a biotransformation of the dye, which consequently renders it more accessible to another organism that otherwise is unable to attack the dye [21].

# 3.3. Dye decolourization assay by bacterial strains

The bacterial strains degrade the industrial textile effluent as their energy source. Many authors have isolated several microbial strains having potential to decolorize a large number of dyes [22]. Biological decolourisation has been determined as a method to transform, degrade, or mineralize of dyes. Moreover such decolourization and degradation is an environmental friendly and cost competitive alternative to chemical decomposition processes [23].

The bacterial strains and its consortium were tested for their ability to decolorize the effluent dye textile sample. The percentage decolourization by bacterial strains was measured spectrophotometrically. The different percentage of degradation of textile dye effluents was noted in Fig. 1. The results suggested that, all the bacterial strain was able to decolorize the dye sample. The maximum biodegradation of dye was observed in culture media containing bacterial consortium at 98% followed by *Shewanella xiamenensis* at 95%, *Flavobacterium ginsengiterrae* at 94% and *Aeromonas aquatic* at 90% after 24 h of incubation time. Ogugbue and Sawidis [24] were describing the isolation and characterization of a strain of *Aeromonas hydrophila* capable of efficiently degrading triarylmethane dyes. The strain *Aeromonas hydrophila* was shown to decolorize three triarylmethane dyes tested in the range of 72 to 96% within 24 h. El Bouraie and Salah El Din [14] illustrated that the decolourization efficiency of Reactive Black 5 dye by *Aeromonas hydrophila* was obtained to be 76% at 100 mg $L^{-1}$ within 24 h.

Higher degree of biodegradation and mineralization can be expected when co metabolic activity within a microbial community complement each other. One organism may be capable of cause a

biotransformation of the dye polluted which consequently it more attainable to another organism that else is unable to attack the dye. Similar results were reported by Mahmood et al. [25] the bacterial consortium exhibited a remarkable increase in dye degradation and decolourization. The consortium decolorized range from 89 to 94% of different types of dyes within 24 h incubation.

# 3.4. Evaluation of dye decolourization

The degradation efficiency of isolated bacterial strains was determined by spectrophotometric assay. Comparison of FTIR spectrum of control textile dye effluent (before degradation) with FTIR spectrum of dye culture supernatant after decolourization clearly indicated the biodegradation of the dye by pure bacteria and its consortium strains (B1, B2, B3, BC) (see Fig. 2). The FTIR spectrum of the control dye displayed a peak at 2921.98 $cm^{-1}$ indicates the C–H stretching of alkanes while the specific peak at 1699.69 $cm^{-1}$ was attributed to the N = N stretching vibrations of the azo bonds position. The peaks at 1540.89 cm-1 for aromatic C = C stretching supported the aromatic structure of the dye. While the presence of peak at 1057.10 $cm^{-1}$ suggested stretching vibrations of primary alcohols C–OH. The FTIR spectrum of dye culture supernatant after decolourization showed diversity in the positions of peaks when compared to control dye spectrum. Absence of the peak 2921.98 $cm^{-1}$with bacterial consortium and appeared new peak at 1426.03 was due to C-H deformation of alkanes. The disappearance of peak at 1699.69 $cm^{-1}$ indicated the cleavage of dye at azo bond position in all bacterial culture that would be an essential step for color removal. On the other hand, absence of peak at 1540.89 $cm^{-1}$ with B1 and bacterial consortium suggested cleave of aromatic rings. Further, new peaks around 3800 – 3100 $cm^{-1}$ represented the amides, amines and carboxylic acid (–OH,-NH-, =C-H) of all different types of bacterial culture. The stretching vibration between –N– C = was detected at 1426–1455 $cm^{-1}$ with B1, B3 and BC. In addition, peaks at 1339.92 and 1246.75 $cm^{-1}$ were attributed to stretching vibrations of S = O and O–$NO_2$ vibration of nitrates respectively of bacterial culture B2. Meanwhile, the new peaks (668–880 $cm^{-1}$) indicated the fission of aromatic rings. From the FTIR spectra, it may be concluded that the different bacterial strains and bacterial consortium decolorizes textile dye effluent attributed to biodegradation process [26].

# 3.5. Physico-chemical properties of textile dye after biodegradation

The industrial wastewater sample which collected from located studied. This industry discharges the pinky color effluent with dye and toxic compounds into the open environment. The physico-chemical characteristics of collected sample and after biotreatment by consortium of three bacterial strains were listed in Table (2) with standard methods of USA, 1995 and according to the allowable limit of Ministerial Resolution no. 44, 2000. The data in Table 2 was illustrated that temperature recorded in wastewater and treated samples at 25 °C which lower than the permissible limit. The pH value was recorded at 8.2 and 7.3 before and after biotreatment respectively. The pH was alkaline in nature and samples have pH within the permissible limit also reported Manikandan et al. [27]. The electrical conductivity was reduced in

biotreatment sample at 800 µ.s. Electrical conductivity is commonly used as a measure of salinity of waste water. The value of total dissolved solid was obtained from dye wastewater sample 7400 mg/l which reduced into 500 mg/l in biotreated sample. High concentration of dissolved solids affects the density of water and influences solubility of gases in water (like oxygen) and osmoregulation of freshwater organisms [28]. Dos Santos et al. [29] illustrated that the presence of dyes in textile effluent contributes to high levels of suspended solids (SS), high chemical oxygen demand (COD) and biological oxygen demand (BOD). The total suspended solids were recorded at 20 mg/l in biotreated sample which lower than the dye wastewater sample. The bacterial consortium was reduced the chemical oxygen demand at 250 mg/l its effect on the quality of freshwater and subsequently cause harm to aquatic life [28]. The biological oxygen demand was recorded at 4000 mg/l which reduced into 200 mg/l with bacterial consortium, the COD and BOD which above the recommended global level [30]. The phosphate, nitrate and sulfide contains were recorded lower than the permissible limit in all different sample. The Phosphate and nitrate are major nutrients needed by living microorganisms for their physiological processes in ecosystem. However, they are considered as pollutants if their concentration is more than recommended limit.

Table 1
Screening of different bacterial strains for dye degradation by plate assay

| Code of microorganisms | Name of bacterial strains | Zone information (in mm) |
|---|---|---|
| B1 | *Aeromonas aquatic* AE 235 | 2.2 |
| B2 | *Flavobacterium ginsengiterrae* DCY55 | 2.4 |
| B3 | *Shewanella xiamenensis*t S4 | 2.5 |
| BC | Bacterial consortium from three strains | 3.0 |

Table 2
Physico-chemical properties of textile dye effluent sample and after treatment by bacterial consortium

| Experiments analysis | Dye effluent sample | Dye sample after biological treated | Allowable limit |
|---|---|---|---|
| Temperature $^{o}C$ | 25 | 25 | 43 |
| pH | 8.2 | 7.3 | 6-9.5 |
| Suspended soiled (mg/l) | 111 | 20 | 800 |
| T.D.S. (mg/l) | 7400 | 500 | 2000 |
| B.O.Ds | 4000 | 200 | 600 |
| C.O.Ds | 9000 | 250 | 1100 |
| Conductivity (µ.s) | 12500 | 800 | 4000 |
| Phosphate (mg/l) | 2.64 | 0.5 | 25 |
| Nitrate (mg/l) | 52 | 20 | 100 |
| Sulfide (mg/l) | 1.48 | 0.4 | 10 |
| Oil& Grease | 20 | 3 | 100 |
| Color | Pinky | Colorless | Colorless |

# 4. Conclusions

Wastewaters from textile industries demonstrate a threat to the environment, as major amount of chemically several dyes are utilized. The present study concludes that the degradation of dye wastewater was determined by pure bacteria and its consortium. All isolated bacterial strains capable of degrade the textile dye while the use of microbial consortium was very efficient than individual culture in dye decolorization process. This attributed that microbial consortium of isolates may have an efficient enzymatic system for the cleavage of parent dye. The biodegradation process of dye was confirmed by FT-IR analysis. Biodegradation gives an advantage for treatment of textile dye effluents and use of bacterial consortium is sufficient for treatment of the industrial wastewater of polluted area.

# Declarations

### Availability of data and materials

Not applicable

### Competing interests

The authors declare that they have no competing interests

### Authors' contributions

**H.S. El-Sheshtawy: (**Corresponding author) performed the biological treatment section, analyzed, interpreted the data in the manuscript and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

**M. Abdelkreem:** Collected the wastewater sample and determined the physic-chemical characterization after and before biological treatment.


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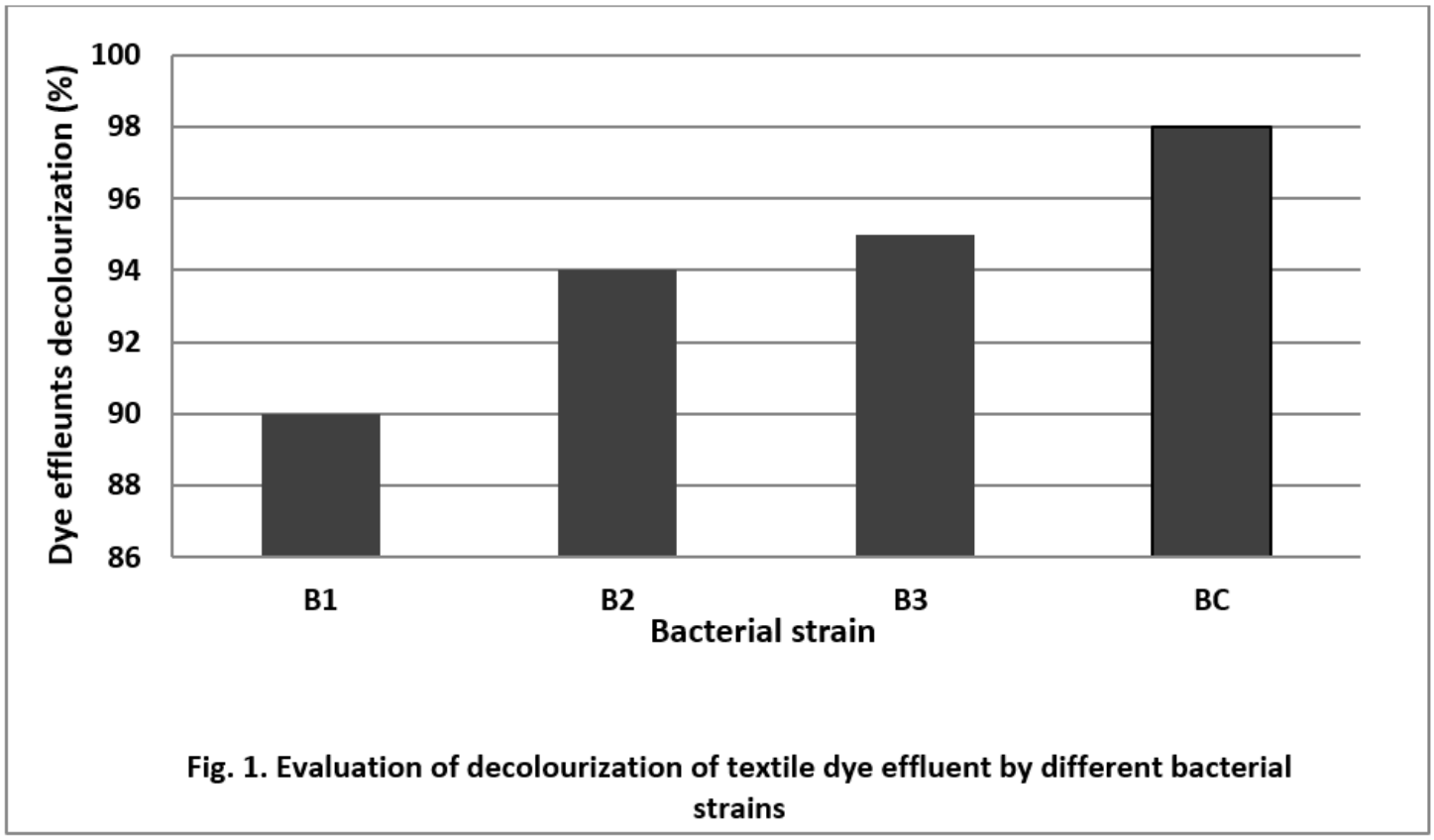
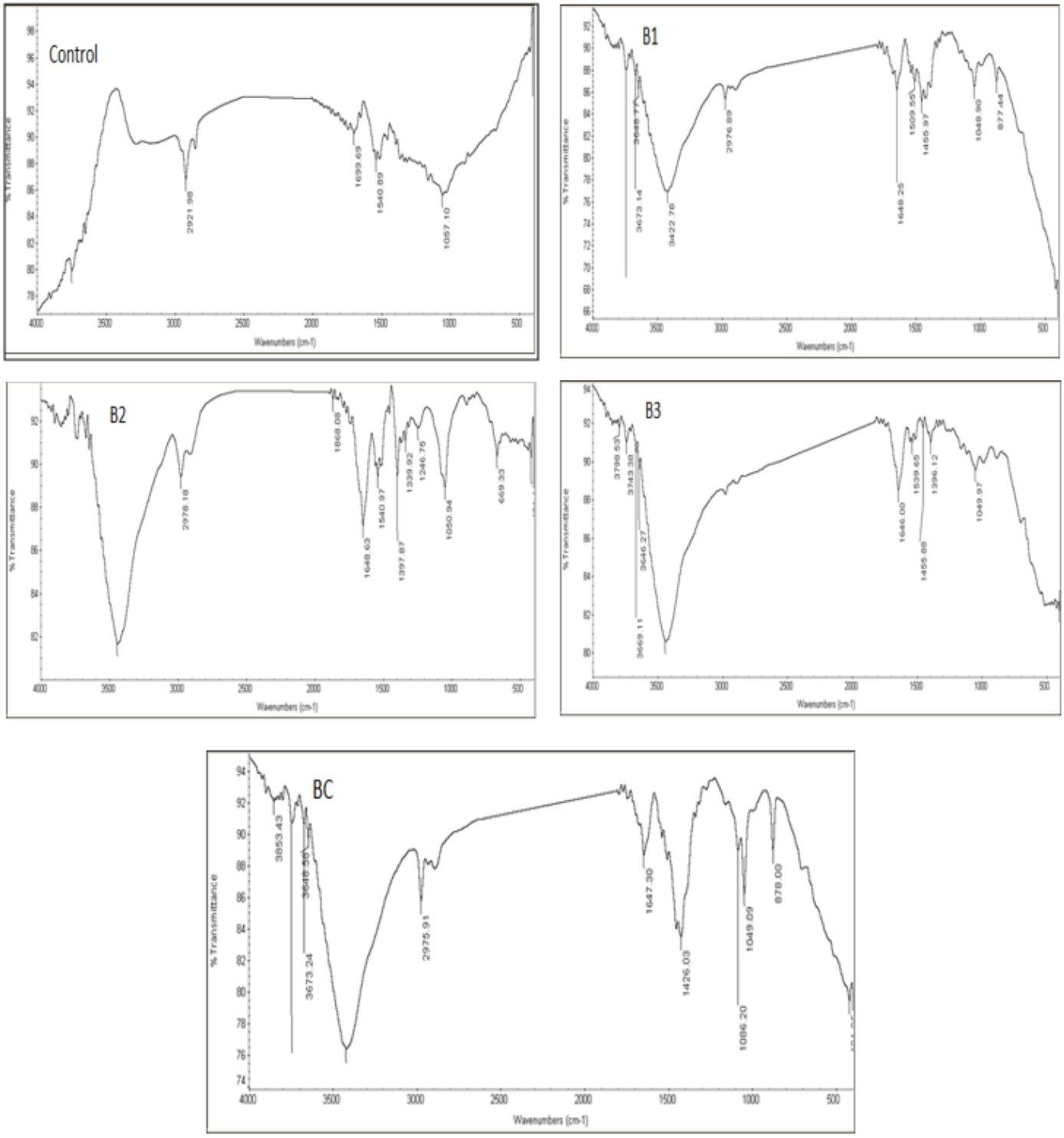
# Figures




## Figure 1

Evaluation of decolourization of textile dye effluent by different bacterial strains




## Figure 2

Infrared spectrum analysis of decolorized culture media by pure and bacterial consortium