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A STUDY ON THE COLOR REMOVAL OF DIFFERENT FORON DYES BY USING THERMOFILIC AND MESOPHILIC *BACILLUS* SPECIES

Termofilik ve Mezofilik *Bacillus* Türlerini Kullanarak Farklı Foron Boyalarının Renginin Giderimi Üzerine Bir Çalışma

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ABSTRACT

There is a significant increase in industrial activities due to the increasing population and demand. Textile and dye industries are also important groups of these industrial fields. In these fields, high amounts of dyes are used. After dyeing process, 10-15% of dyes are released into wastewaters. Existence of dyes in wastewaters is an undesired condition because they cause lots of diseases on living beings and also various problems on aquatic and terrestrial environments. Because disperse dyes do not show ionization in aquatic environments, they generally tend to bioaccumulation. Conventional biological wastewater treatment systems are not efficient for color removal of dyes. In this study, bacterial decolorization, which is an environmentally friendly and effective method, was investigated. For this aim, a thermophilic (*Bacillus firmus*) and a mesophilic bacterium (*Bacillus subtilis*) were tested in the color removal of various Foron textile dyes, a group of disperse dyes, such as Foron Brown (FB), Foron Black (FBL), Foron Turquoise (FT), Foron Violet (FV) and Foron Red (FR). The tested dyes (200 mg/L) were incubated with *Bacillus* species under the shaking condition for 24 h at various temperatures. Colors of all the dyes were efficiently removed by both bacterial species at the end of 24 h of incubation. The highest color removal rates of FB, FBL, FT, FV and FR treated with *B. firmus* were about 63, 87, 56, 77 and 74%, respectively. The maximum decolorization percentages of the same dyes incubated with *B. subtilis* were determined as 69, 80, 55, 73 and 59%, respectively.

Keywords: *Bacillus* species, color removal, foron dyes, mesophilic, thermoplilic

ÖZ

Artan nüfus ve talep nedeniyle endüstriyel faaliyetlerde önemli bir artış vardır. Tekstil ve boya endüstrileri de bu sanayi alanlarının önemli gruplarıdır. Bu alanlarda, yüksek miktarlarda boya kullanılmaktadır. Boyama işleminden sonra, boyaların %10-15' i atıksulara salınır. Atık sularda boyaların varlığı istenmeyen bir durumdur. Çünkü canlılar üzerinde birçok hastalığa ve ayrıca sucul ve karasal çevrelerde çeşitli problemlere neden olurlar. Dispers boyalar sucul ortamlarda iyonlaşma göstermediğinden genellikle biyolojik birikim eğilimindedir. Geleneksel biyolojik atık su arıtım sistemleri boyaların renginin giderimi için etkin değildir. Bu çalışmada çevre dostu ve etkili bir yöntem olan bakteriyel renk giderimi araştırılmıştır. Bu amaçla, bir termofilik (*Bacillus firmus*) ve bir mezofilik bakteri (*Bacillus subtilis*) Foron Kahverengi (FK), Foron Siyah (FS), Foron Turkuaz (FT), Foron Mor (FM) ve Foron Kırmızı (FKI) gibi dispers boyaların bir grubu olan çeşitli Foron tekstil boyalarının gideriminde test edildi. Test edilen boyalar (200 mg/L) çeşitli sıcaklıklarda 24 saat çalkalamalı koşul altında *Bacillus* türleriyle inkübe edildi. Tüm boyaların renkleri 24 saat inkübasyon sonunda, her iki bakteriyel tür ile etkin bir şekilde giderildi. *B. firmus* ile muamele edilen FK, FS, FT, FM ve FKI' nın en yüksek renk giderim oranları sırasıyla yaklaşık %63, 87, 56, 77 ve 74 idi. *B. subtilis* ile inkübe edilen aynı boyaların en yüksek renk giderim yüzdeleri sırasıyla %69, 80, 55, 73 ve 59 olarak belirlendi.

Anahtar kelimeler: *Bacillus* türleri, renk giderimi, foron boyaları, mezofilik, termofilik

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INTRODUCTION

Due to the increase in industrial activities, new chemicals, most of which are organic macromolecules, are emerging in the environment. Dyes, an important group of these macromolecules, are widely used in many industrial fields like leather, textile, paint, paper, food, cosmetics, medicine, toys and plastics (Chen, Wu, Liou & Hwang, 2003; Mohana, Shrivastava, Divecha & Madamwar, 2008; Rauf & Ashraf, 2012; Shah, Dave & Rao, 2012). More than $7x10^5$ metric tons of synthetic dyes are produced worldwide every year and used in various fields (Colak, Ata & Olgun, 2009). Because of the disperse dyes, a group of synthetic dyes can be applied to many synthetic fibers, the utilization of these dyes in the textile industry has increased after the discovery of synthetic fibers (Neamtu, Yediler, Siminiceanu, Macoveanu & Kettrup, 2004). But, some disperse dyes can cause allergic reactions like eczema or contact dermatitis (Hatch & Maibach, 1995). However, these dyes do not ionize in aquatic environments and tend to bioaccumulation. Due to its chemical stability and low biological degradation, the treatment of wastewater containing these dyes by conventional biological wastewater treatment systems is not efficient (Banat, Nigam, Singh & Marchant 1996; Aksu, 2005).

There has been a great increase in wastewater production because of the rapidly increasing urbanization and industrialization in the last few decades. Especially, the high consumption of water-based on production in the textile industry significantly increases the formation of wastewater (Garg, Garg & Mukherji, 2020). The wastewaters discharged from textile and dye industries have high BOD, COD, color, pH, temperature and metal constituents. However, because the chemical structures of the dyes in the wastewater are quite complex, these wastewaters are very resistant to degradation (Shah et al., 2012). Most of the varied synthetic dyes in these wastewaters are toxic and carcinogenic (Wang et al., 2009; Franciscon et al., 2010; Yuan, Chen, Cao & Hong, 2020). After the release of textile wastewaters into the aquatic ecosystems, the penetration of sunlight into water is significantly reduced, and as a result, photosynthetic activity and also gas solubility are decreased (Maqbool et al., 2016). In this case, water quality also reduces due to the decreased dissolved oxygen amount. In addition, dyes entering the aquatic ecosystem cause acute toxic effects on flora and fauna in the water, occurring serious environmental problems worldwide (Saratale, Saratale, Chang & Govindwar, 2011; Yuan et al., 2020). However, the wastewaters containing textile dyes adversely affect human health as well as other living things (Khan & Malik, 2018). Accordingly, various bacteria and other organisms used in environmental biotechnology applications can provide suitable solutions for the treatment of textile dye wastewater, and thus contribute to a healthy life (Lellis, Fávaro-Polonio, Pamphile & Polonio, 2019).

The purpose of this work is comparatively investigation of the decolorization of Foron group dyes which are less tested than the other dyes in the literature by using mesophilic and thermophilic bacteria.

MATERIALS AND METHODS

Microorganisms

In this study, two different *Bacillus* strains (*Bacillus firmus* (Accession number KJ434782), a thermophilic bacterium, and *Bacillus subtilis* ATCC 6633, a mesophilic bacterium) were tested for decolorization of various Foron dyes. To produce their stock cultures, these bacteria were transferred to the plates containing fresh Nutrient Agar (NA) every 3-4 weeks and then, B. firmus and B. subtilis were incubated at 50 ºC and 30 ºC for 24 h, respectively.

Foron Dyes

Several disperse dyes as Foron Brown (FB), Foron Black (FBL), Foron Turquoise (FT), Foron Violet (FV) and Foron Red (FR) were used in the studies and they were added to Nutrient Broth (NB) media at a final concentration of 200 mg/L.

Preparation of Bacterial Cultures

The samples were taken from the stock solid bacterial cultures and then transferred to sterile 50 mL of NB media. In order to obtain the liquid bacterial cultures to be used in the experiments, B. firmus and B. subtilis were incubated at 50 ºC and 30 ºC for 24 h under agitated conditions (150 rpm), respectively.

Decolorization Studies

The maximum light absorbed wavelengths of FB, FBL, FT, FV and FR textile dyes were determined via the spectrophotometric scans, as 521 nm, 591 nm, 751 nm, 565 nm and 611 nm, respectively. After preliminary preparations, B. firmus and B. subtilis liquid cultures were inoculated into NB media containing FB, FBL, FT, FV and FR at final concentrations of 200 mg/L. B. firmus was incubated separately at 40, 45, 50 and 55 ºC, and B. subtilis, a mesophilic bacterium, was incubated at 25, 30, 35 and 40 ºC to determine the highest color removal of the 5 dyes tested. After 24 h of the incubation, the color removal activities of both bacteria were determined by spectrophotometric (Shimadzu-UV-1601, UV/Visible) scans. All experiments were performed in 3 replicates and the decolorization percentages were calculated with SPSS 15.0 package program.

RESULTS AND DISCUSSION

There are limited numbers of studies in the literature on the decolorization of Foron dyes. Moreover, most of these studies were performed by using white rot fungi. In a study, Sadaf and Bhatti tested the mixture biomass of *Ganoderma lucidum* and *Coriolus versicolor* for decolorization of Foron Turquoise SBNL and the highest decolorization value was detected as about 17% at 30 ºC (Sadaf & Bhatti, 2011). In another work, the color removal efficiency of Foron Brill Red SRG1200 by *Pleurotus ostreatus* liquid culture was investigated and the decolorization rate was determined as 16% on the 3rd day of the incubation while it was 75% on the 10th day (Kowsalya, 2014). Hassan was studied the decolorization of 4 different disperse dyes such as Foron Turquoise SBLN-200, Foron Blue RDGLN, Foron Red RDRBLS and Foron Yellow SE4G using various white rot fungi (Pleurotus ostreatus IBL-02, *Phanerochaete chrysosporium* IBL-03, Coriolus versicolor IBL-04, Ganoderma lucidum IBL-05, and *Schizophyllum commune* IBL-06). It was stated that all of the white rot fungi except *Phanerochaete chrysosporium* IBL-03 were decolorized the tested Foron dyes at rather low rates (less than 16%) in the 24th hour but the color removal rates gradually increased in the further incubation times (Hassan, 2009).

In this study, a mesophilic and a thermophilic bacteria were used for decolorization of Foron group dyes which are less tested than the other dyes in the literature. Five different Foron dyes at high concentration (200 ppm) were incubated at 25-40 ºC with the mesophilic bacterium B. subtilis and at 40-55 ºC with the thermophilic bacterium *B.* firmus for 24 h.

B. firmus, a thermophilic bacterium, at the end of a short incubation period of 24 h, has significantly removed the colors of all Foron group dyes tested at all temperatures studied (Figure 1).

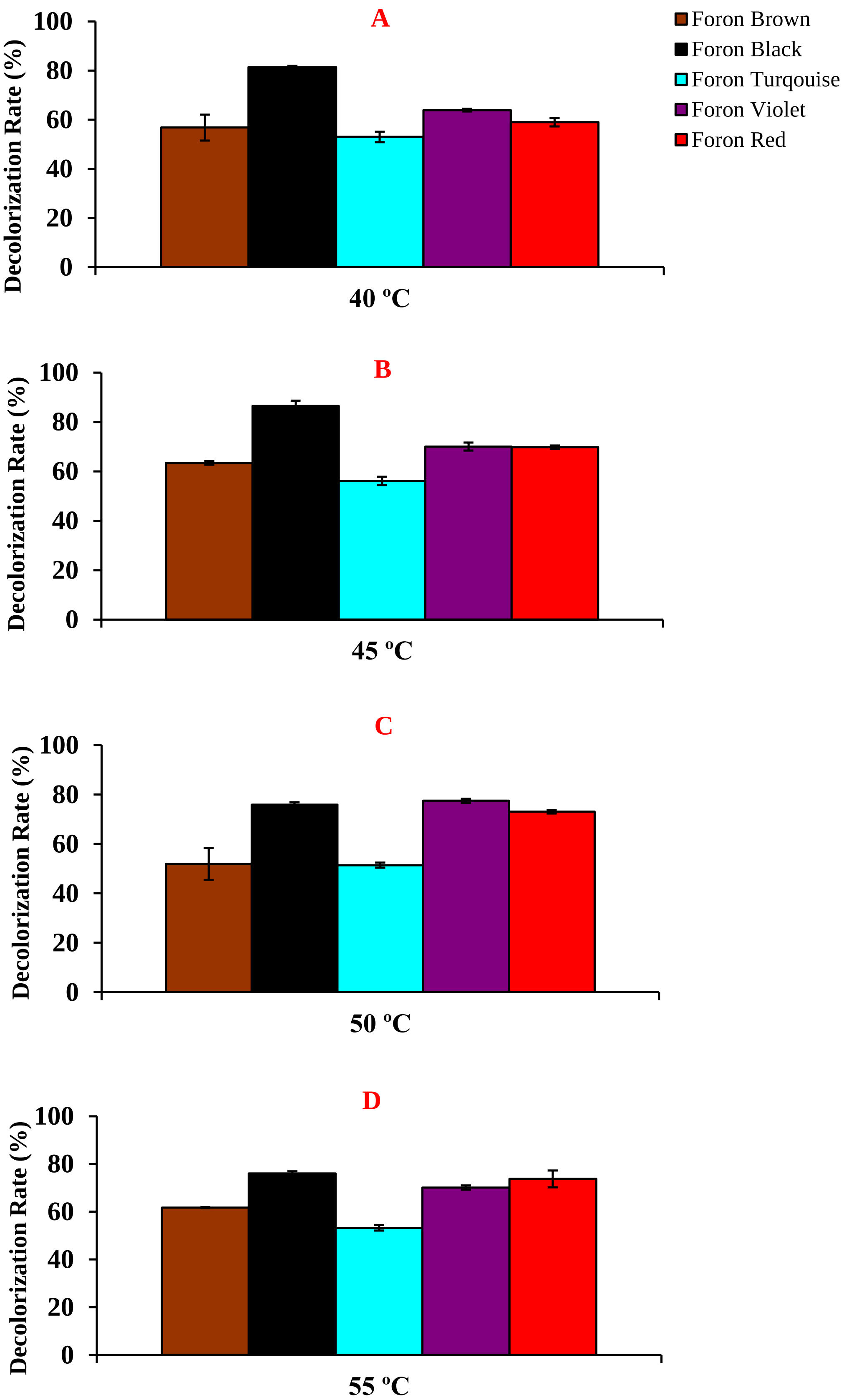

Figure 1. The color removal percents of Foron group dyes as a result of 24 h incubation with B. firmus at 40 ºC (A), 45 ºC (B), 50 ºC (C), 55 ºC (D).

The highest decolorization rates of FB, FBL and FT dyes obtained after 24 h incubation at 45 ºC by B. firmus were detected as 63%, 87% and 56%, respectively. On the other hand, the maximum decolorization rates of FV and FR dyes at the end of incubation by *B.* firmus were detected at 50 ºC. The photographs and spectrophotometric scans of color removal of the dyes tested at 45 ºC and 50 ºC by B. firmus are shown in Figure 2 and Figure 3, respectively. The disperse dye decolorization ability of Bacillus firmus was also investigated by Arora et al. Accordingly, Dianix CC and Dianix S group disperse dyes at 50 mg/L were incubated with Bacillus firmus under 100 rpm at 35 ºC. Among the Dianix CC dyes, the maximum decolorizations were determined at 13th day and 24th day for Yellow CC and Royal Blue CC as about 87% and 67%, respectively. The highest decolorization rates among the Dianix S dyes were measured as about 40% and 26% on the 4th day for Navy S-2G and Rubine S-2G, respectively (Arora, Saini & Singh, 2011).

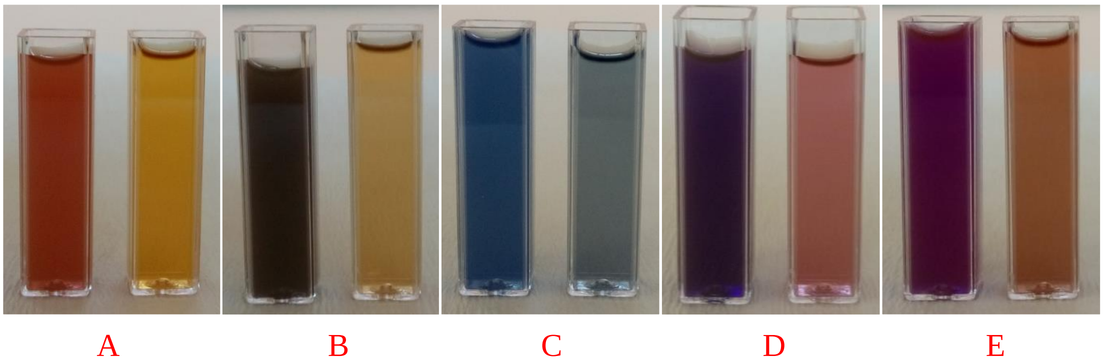

Figure 2. The photographs of color removals compared to the controls that resulted after the incubation of FB (A), FBL (B), FT (C), FV (D) and FR (E) dyes with B. firmus.

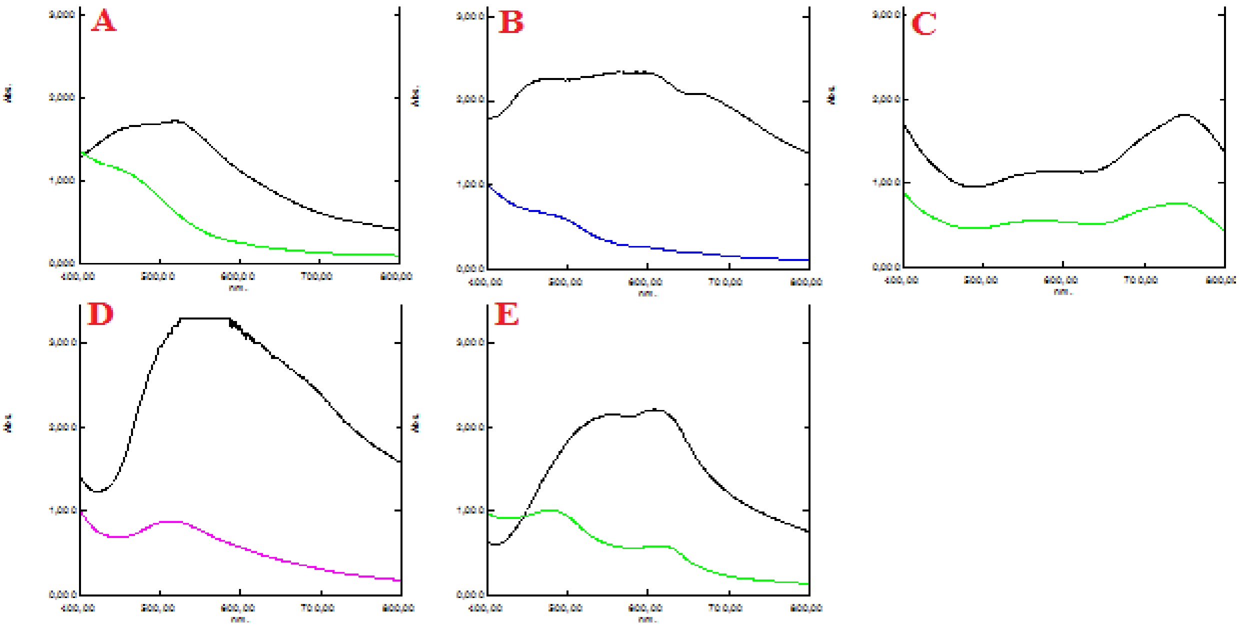

Figure 3. The spectrophotometric scans of the color removals obtained after the incubation of FB (A), FBL (B), FT (C), FV (D) and FR (E) dyes with the thermophilic bacterium B. firmus.

Similarly, these five different Foron group textile dyes were highly decolorized by the mesophilic bacterium B. subtilis as a result of 24 h of incubation in all temperatures investigated (Table 1).

Table 1. The color removal percents of Foron group dyes as a result of 24 h of incubation with B. subtilis at 25 ºC, 30 ºC, 35 ºC and 40 ºC.

Temperature (°C)	Percents of Color Removal (%) of Foron Group Dyes				
	FB	FBL	FT	FV	FR
25	62	76	52	69	51
30	66	77	53	70	52
35	69	80	55	73	56
40	65	77	49	73	59

After the incubation with the mesophilic bacterium, the maximum color removal rates of FB (69%), FBL (80%) and FT (55%) dyes were determined at 35 ºC. The highest percentages of color removal for FV and FR dyes were obtained as 73% and 59%, respectively, as a result of incubations by this bacterium at 40 ºC. The highest color removal results with B. subtilis at 35 ºC and 40 ºC were shown at the photographs (Figure 4) and spectrophotometric scans (Figure 5). In a study performed by using Bacillus subtilis, Sharma et al. reported the maximum decolorization rate of Disperse Yellow 211 (100 mg/L) as 80% at 32.5 ºC (Sharma, Singh & Dilbaghi et al., 2009).

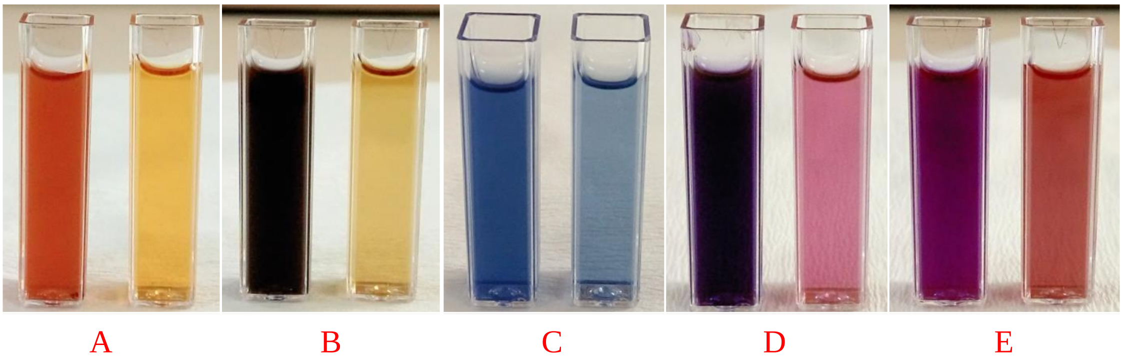

Figure 4. The photographs of color removal compared to the controls that resulted after the incubation of FB (A), FBL (B), FT (C), FV (D) and FR (E) dyes with B. subtilis.

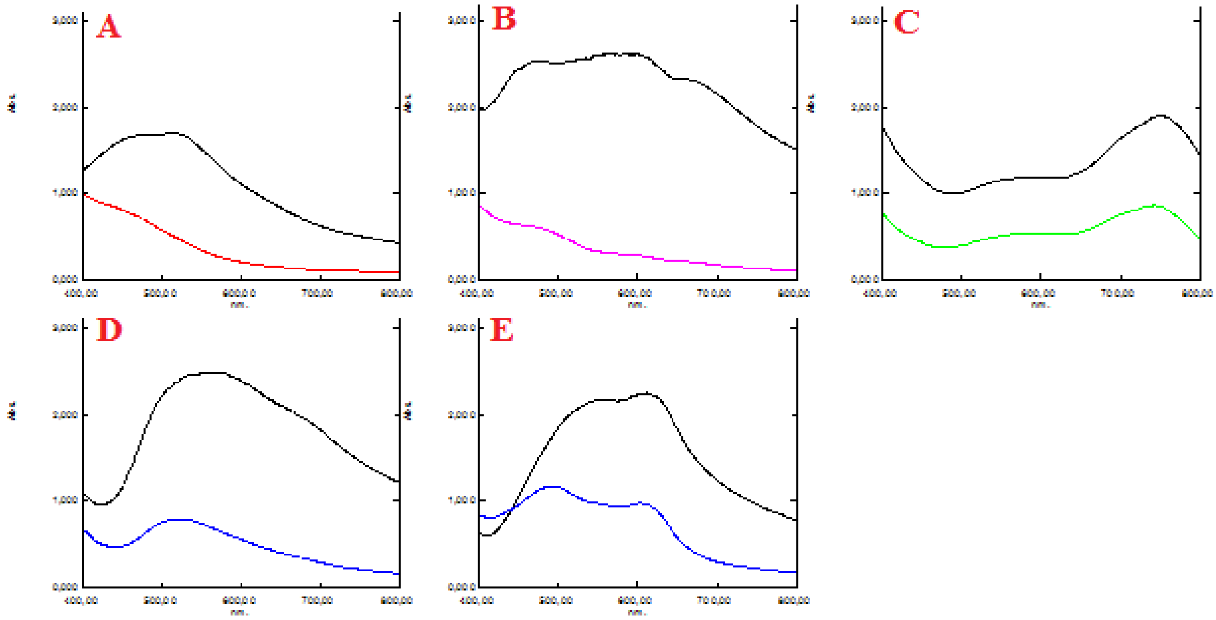

Figure 5. The spectrophotometric scans of the color removals obtained after the incubation of FB (A), FBL (B), FT (C), FV (D) and FR (E) dyes with B. subtilis.

CONCLUSION

As a result of intensive use of disperse dyes, the formation of toxic aromatic amines and insufficiencies in the treatment of wastewater containing these dyes cause environmental concern. In order to preserve the health of living beings and reduce the environmental pollution, it is very important to remove the colors of textile and dye industry wastewaters with powerful and environmentally friendly treatment processes and then discharge them into the receiving environments. According to the data of the present work, various disperse dyes such as Foron dyes can be effectively decolorized with mesophilic and thermophilic bacteria under different conditions. This study had shown that, in simple, economical and short-term working conditions, the dark colors of many different dyes could be effectively removed both at high and low temperatures with the bacteria that had different characteristics. Accordingly; the microbial decolorization method is a cost-effective and efficient way and has not the disadvantages of physicochemical and conventional biological treatment systems.

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