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Bacteria immobilized electrospun polycaprolactone and polylactic acid fibrous webs for remediation of textile dyes in water

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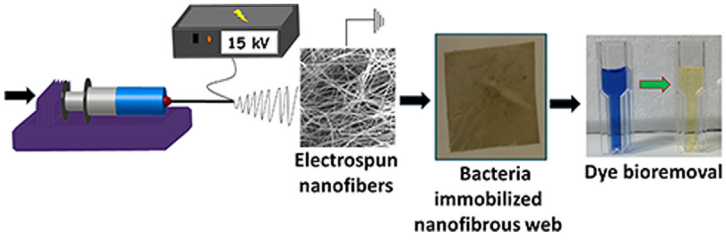
HIGHLIGHTS

- Novel bacteria immobilized biocomposites were developed for dye removal.
- Efficient textile dye removal was achieved by these biocomposites.
- Removal speeds can be improved by increasing the number of viable bacteria.
- Robust attachment of bacterial cells providing reusability of the biocomposites.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, preparation and application of novel biocomposite materials for textile dye removal which are produced by immobilization of specific bacteria onto electrospun nanofibrous webs are presented. A textile dye remediating bacterial isolate, *Clavibacter michiganensis*, was selected for bacterial immobilization, a commercial reactive textile dye, Setazol Blue BRF-X, was selected as the target contaminant, and electrospun polycaprolactone (PCL) and polylactic acid (PLA) nanofibrous polymeric webs were selected for bacterial integration. Bacterial adhesion onto nanofibrous webs was monitored by scanning electron microscopy (SEM) imaging and optical density (OD) measurements were performed for the detached bacteria. After achieving sufficient amounts of immobilized bacteria on electrospun nanofibrous webs, equivalent web samples were utilized for testing the dye removal capabilities. Both bacteria/PCL and bacteria/PLA webs have shown efficient remediation of Setazol Blue BRF-X dye within 48 h at each tested concentration (50, 100 and 200 mg/L), and their removal performances were very similar to the free-bacteria cells. The bacteria immobilized webs were then tested for five times of reuse at an initial dye concentration of 100 mg/L, and found as potentially reusable with higher bacterial immobilization and faster dye removal capacities at the end of the test. Overall, these findings suggest that electrospun nanofibrous webs are available platforms for bacterial integration and the bacteria immobilized webs can be used as starting inocula for use in remediation of textile dyes in wastewater systems.

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1. Introduction

Dyes comprise a great portion of industrial contaminants and their removal from wastewater systems is of substantial importance. Because of their diverse application areas (e.g. textile and leather industries), synthetic dyes have a great usage, whereas decontamination of them is still a major challenge and their incomplete removal from water systems can lead to environmental problems (San et al., 2014). Dyes can be removed from wastewater systems by using conventional treatment methods such as reverse osmosis (Nataraj et al., 2006), advanced oxidation process (Zhan et al., 2010) and photocatalysis (Wang et al., 2011), additionally, alternative technologies have been proposed to achieve sustainability and biofriendliness. Bioremediation is an alternative technique for removal of contaminants from wastewater systems by use of specific microorganisms, and it provides low-cost, efficient, green and sustainable remediation (Malik, 2004). Therefore, there are many attempts for bioremediation of coloring agents in the literature, and specific microorganisms have been used for this purpose (Asad et al., 2007; El-Sersy, 2007; San et al., 2014; San-Keskin et al., 2015a).

Bioremediation process can be performed with either free microorganisms or microorganism immobilized bio-integrated materials. Although free microorganisms can efficiently remove water contaminants, use of bio-integrated materials can bring some advantages over free microorganisms such as lower space and growth medium necessities, potential reusability and higher resistance to environmental extremes (Eroglu et al., 2012; Hall-Stoodley et al., 2004). Due to their versatile nature, electrospun fibrous webs have been used as carrier matrices for immobilization of specific microorganisms for decontamination of water systems (San-Keskin et al., 2015a, 2015b; Rosales et al., 2011; Sarioglu et al., 2013, 2015, 2016). Since electrospinning is a low-cost and versatile technique and it enables optimization in fiber morphology (e.g. higher surface area and porosity), electrospun fibrous webs are promising candidates for bio-integration and water filtering applications (Salalha et al., 2006).

The genus *Enterococcus* comprises Gram-positive, non-sporulating and facultative aerobe cocci which are tolerant to environmental extremes (Fisher and Phillips, 2009). The genus *Halomonas* comprises Gram-negative, rod shaped and halophilic bacteria that have the ability to grow at extremely salty environments (Lee et al., 2005). *Clavibacter michiganensis* is the only member of the genus *Clavibacter*, and it comprises Gram-positive and aerobic bacteria (San et al., 2012). In the present study, a dye bioremediating bacterial strain, *Clavibacter michiganensis*, was successfully immobilized onto electrospun nanofibrous webs of polycaprolactone (PCL) and polylactic acid (PLA). The PCL and PLA nanofibrous matrices are known as biocompatible and biodegradable (Bhavsar and Amiji, 2008; Jung et al., 2010) polymeric materials which would be very suitable to be used in applications of dye bioremediation. These bacteria encapsulated nanofibrous webs were tested for their removal capacities against a commercial reactive textile dye (Setazol Blue BRF-X), and the results have shown that these webs have the potential for dye remediation in water. Reusability of bacteria immobilized nanofibrous webs was also tested and the results suggest that these webs can be reused for several times while increasing the number of immobilized bacteria and reducing the time required for bioremediation. These types of bacteria-integrated electrospun nanofibrous materials could be promising candidates for potential wastewater treatment applications.

2. Materials and methods

2.1. Materials

The chemicals and reagents (polycaprolactone (PCL, Mw ~70.000–90.000, Scientific Polymer Products, Inc.), polylactic acid (PLA, NatureWorks LLC Co.), dichloromethane (DCM, ≥99% (GC), Sigma-Aldrich), N,*N*-Dimethylformamide (DMF, ≥99% (GC), Sigma-Aldrich), textile dyes (Setazol Blue BRF-X, Setazol Turquoise Blue G (Setaş, Turkey)), LB broth (Luria-Bertani, Sigma-Aldrich) and Agar (Sigma-Aldrich)) were procured and used without any purification (all of them were of high purity available and were of analytical grade). Millipore Milli-Q Ultrapure Water System was used for providing deionized water.

2.2. Procurement of the bacterial strains and 16S rRNA gene sequencing analysis

The bacterial strains utilized in this study were isolated from different resources. *Enterococcus hermanniensis* and *Clavibacter michiganensis* isolates were isolated from İvedik wastewater treatment plant (Ankara, Turkey), while *Halomonas variabilis* isolate was isolated from Salt Lake (Ankara, Turkey). The isolates were enriched in LB medium (Luria-Bertani: 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl in 1 L of distilled water) and then stock cultures were prepared. These three bacterial strains were chosen due to their capability to grow in extreme conditions and their fast growing abilities. The stock cultures were stored for short periods (at 4 °C) and fresh cultures were prepared from those samples prior to the further use.

The species identities of the isolates were determined via 16S rRNA gene sequencing analysis. DNeasy Blood & Tissue Kit (QIAGEN, Germany) was utilized for bacterial DNA isolation. PCR amplification and further sequencing were performed with the following concentrations: 1.25 U Platinum Taq polymerase, 0.2 mM dNTP, 0.4 pmol T3 (ATTAACCCTCACTAAAGGGA) and T7 (TAATACGACTCACTATAGGG) primers encompassing the entire 16S gene, 1.5 mM MgCl and 1X Taq buffer (Rijpens et al., 1998). The PCR steps were as follows: initial denaturation at 96 °C for 5 min and further 30 cycles of denaturation at 96 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 30 s and a final elongation at 72 °C for 5 min. DNA sequencing and analysis was done via ABI 3130xl Genetic Analyzer by using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The 16S rRNA gene sequences of the isolates were analyzed by NCBI's Bacterial Blast Tool (http://www.ncbi.nlm.nih.gov) for determination of the species identities.

2.3. Electrospinning of PCL and PLA nanofibrous webs

PCL and PLA nanofibers were produced by using different binary solvent systems. The homogenous electrospinning solutions were prepared by dissolving PCL and PLA in DCM/DMF (1/1 (v/v)) and DCM/DMF (7/3 (v/v)) solvent mixtures at 15% (w/v) polymer concentrations for PCL and PLA nanofibers, respectively. The electrospinning solutions were loaded in a 3 mL syringe fitted with a metallic needle of 0.4–0.6 mm inner diameter, and these were located horizontally on a syringe pump (model KDS-101, KD Scientific, USA). The metallic needle of the syringe was clamped by one of the electrode of high-voltage power supply (Spellman, SL30, USA) and the metal collector was grounded. Electrospinning parameters were adjusted as follows: feed rate of solutions = 0.5 mL/h, applied voltage = 10–15 kV, tip-to-collector

distance = 10–12 cm. The electrospinning apparatus was put in a Plexiglas box and electrospinning was performed at 24 ± 1 °C and 20% relative humidity. The deposited nanofibers/nanowebs were dried overnight at room temperature in a fume hood before use in application studies.

2.4. Dye bioremoval experiments

LB broth was utilized as the bacterial growth medium for dye bioremoval experiments. The pH was kept at 7.0. Initially, three different bacterial isolates (*Enterococcus hermanniensis*, *Clavibacter michiganensis* and *Halomonas variabilis*) were tested as free bacterial cells against two different commercial reactive textile dyes (Setazol Blue BRF-X, Setazol Turquoise Blue G) to determine the dye with the higher biodegradability and the bacterial isolate with the highest bioremoval capacity. The remaining dye concentrations were determined by spectrophotometric measurements and it was found that, Setazol Blue BRF-X gives specific absorbance at 609 nm and Setazol Turquoise Blue G gives specific absorbance at 626 nm. For this test, all initial dye concentrations were kept constant at 50 mg/L and all bacterial isolates were incubated at 30 °C for 48 h. Since *Clavibacter michiganensis* isolate has shown the highest bioremoval performance among three different isolates especially against Setazol Blue BRF-X in this test (Fig. S1), further dye bioremoval studies were performed by *Clavibacter michiganensis* isolate against Setazol Blue BRF-X dye. Equivalent samples of pristine PCL and PLA webs with equal weights (w/v ratio of 0.5 mg/mL) were prepared for bacterial immobilization and consecutive application studies. These webs were added directly to *Clavibacter michiganensis* cells containing (~10^7-10^8 cfu/mL) LB broth for bacterial attachment. Bacterial incubation was ended after 7 days and bacterial immobilization was monitored by SEM (Scanning Electron Microscopy) analysis. The bacterial immobilization was also checked by detachment of the immobilized bacterial cells from equivalent samples and further OD_{600} measurements. The detachment protocol includes sequential vortexing and sonication at cold temperatures (to preserve bacterial cell viability) (Sarioglu et al., 2015). For testing the bioremoval capabilities of bacteria/PCL and bacteria/PLA webs, three different (50, 100, 200 mg/L) initial dye concentrations were selected and bacteria immobilized web samples were added to dye containing LB media as inoculants. The bacteria immobilized web samples were incubated for 48 h at 150 rpm and 30 °C. Free bacteria cells and pristine web samples were also tested for their dye removal capabilities, as positive and negative controls, respectively. Liquid samples were collected periodically and then centrifuged at 8000 rpm for 5 min, and the supernatant fractions were used for spectrophotometric measurement of the dye, to avoid optical density interference from bacterial cells. All tests were done in triplicate.

The removal capacities (Q_{eq}) of free bacteria cells, and bacteria immobilized web samples were calculated by Eq. (1) (1):

$$Q_{eq}(mg/g) = \left(C_0 - C_f\right) V/M \quad (1)$$

where C_0 is the initial dye concentration (mg/L), C_f is the final dye concentration (mg/L), V is the volume of the solution (L) and M is the total bacterial biomass (g) at equilibrium (Buchko et al., 1999).

2.5. Scanning electron microscopy (SEM)

Millimeter-length PCL and PLA webs were prepared for SEM analysis to evaluate bacterial immobilization on nanofibrous webs. The sample fixation was done as previously mentioned (Sarioglu et al., 2015). In brief, bacteria immobilized web samples were washed several times with PBS buffer and incubated overnight in 2.5% glutaraldehyde solution (prepared in PBS buffer) for sample fixation. Then the web samples were washed again with PBS buffer and the samples were dehydrated by immersion in a series of EtOH solutions (30%–96%). At the end, samples were coated with 5 nm Au–Pd for SEM imaging (Quanta 200 FEG SEM, FEI Instruments, USA). While the fixation protocol was not applied for pristine PCL and PLA webs, they were also coated with 5 nm Au–Pd prior to SEM imaging.

2.6. Adsorption isotherms and kinetics studies

Adsorption isotherm coefficients and their estimated values were determined upon three isotherm models (Langmuir, Freundlich, and Toth) using the isotherm parameter fitting software IsoFit (Wagner et al., 2005). The reactions orders were evaluated upon the R^2 values of zero, first, second and third order plots of free bacteria, bacteria/PCL and bacteria/PLA samples.

2.7. Reusability test

Reusability of bacteria/PCL and bacteria/PLA web samples were tested for bioremoval of Setazol Blue BRF-X dye. Prior to each cycle, the web samples were washed gently with Tris-HCl buffer to remove unattached bacteria. The experiments were performed at an initial dye concentration of 100 mg/L with the parameters: incubation at 150 rpm and 30 °C for 48 or 24 h. The remaining dye concentrations were measured at the beginning and at the end of each run, and the percentile removal of dye was calculated. All tests were done in triplicate.

3. Results and discussion

3.1. Identification and preliminary characterization of the bacterial isolates

The bacterial cultures were isolated from different resources where they were growing in extreme conditions, therefore they were assumed as potential candidates for bioremediation of water contaminants. 16S rRNA gene sequencing analysis was applied on these strains and the species identities of the isolates were determined to be *Enterococcus hermanniensis*, *Clavibacter michiganensis* and *Halomonas variabilis*, and they were deposited in NCBI's GenBank with the accession numbers of GU907677, GQ466171 and KX351792.1, respectively. These three isolates were then tested for their potential textile dye bioremediation capability. At the end of this test, all of the isolates have shown higher bioremoval performance against Setazol Blue BRF-X dye, while only 25–40% of removal was observed for Setazol Turquoise Blue G dye (Fig. S1). Dyes used in industry have different structural forms including acidic, basic, disperse, azo, diazo, reactive, metal-complex and anthraquinone based dyes (Binupriya et al., 2010). Although reactive dyes in this group are very efficient for dyeing fabrics, removal of them from effluent is highly difficult due to their high solubility in water and poor biodegradability (Muthu, 2017). Both of Setazol Blue BRF-X and Setazol Turquoise Blue G dyes are reactive dyes. Here, our preliminary studies have shown that the bioremoval performance of the three isolates we used against Setazol Turquoise Blue G was not very satisfying. When compared with Setazol Blue BRF-X, the lower biodegradability of Setazol Turquoise Blue G is probably due to the higher numbers of cyclic groups and multiple bonds in the dye structure, as shown in a previous study for Reactive Turquoise Blue 15 (AT15) dye (Osugi et al., 2003). In addition, among three different isolates, *Clavibacter michiganensis* isolate has shown the highest bioremoval performances against

both Setazol Blue BRF-X and Setazol Turquoise Blue G dyes. Therefore, Setazol Blue BRF-X dye was selected as the target contaminant and *Clavibacter michiganensis* isolate was selected as the remediating organism for further textile dye bioremediation studies.

3.2. Bacterial immobilization on PCL and PLA webs

Two biodegradable and biocompatible polymers (Bhavsar and Amiji, 2008; Jung et al., 2010), PCL and PLA were selected for producing electrospun fibrous webs. These webs were then utilized as carrier matrices for bacterial integration. The electrospinning process and subsequent bacterial adhesion are summarized in Fig. 1. Bacterial immobilization was achieved by natural adhesion process in which, bacterial cells attach on the surface via physical and chemical interactions after the initial contact, then they start to colonize throughout the surface after stabilizing their location, and finally forming biofilm structures (Hori and Matsumoto, 2010). The morphologies of pristine PCL and PLA nanofibers along with their bacteria immobilized versions are shown in Fig. 2. The average fiber diameters of PCL and PLA webs were measured as around 1.3 and 0.85 μm, respectively. As shown in Fig. 2c and d, strong bacterial adhesion and biofilm structures were observed, hence 7 days of incubation was found as adequate for each sample to initiate bioremediation studies. In addition to SEM imaging, bacterial immobilization was followed by a protocol in which immobilized bacterial cells were detached from equivalent web samples and then OD_{600} measurements were applied on these detached cells to determine the approximate number of the immobilized bacteria (where $OD_{600} = 0.1$ corresponds to ~10^8 cfu/mL). The results of this experiment will be mentioned in a following section. After ending the bacterial immobilization process at day 7, equivalent samples of bacteria/PCL and bacteria/PLA webs (w/v ratio of 0.5 mg/mL) were collected to initiate dye removal experiments.

3.3. Dye removal capabilities of bacteria/PCL and bacteria/PLA webs

Bacteria/PCL and bacteria/PLA webs were tested for their dye removal capabilities at different initial dye concentrations (50, 100 and 200 mg/L). Removal capacities (Q_{eq}) of free-bacteria cells and bacteria immobilized webs were also calculated and are presented in Table 1. Both webs have shown efficient removal yields at each concentration, while there were slight differences between them (Fig. 3, Table 1). Statistical analysis of this experiment by one-way ANOVA test for free-bacteria, bacteria/PCL and bacteria/PLA samples has shown that the variations among them are not significant ($p = 0.914$). For 50 mg/L of initial dye concentration, free-bacteria sample has shown the highest removal yield with 95.56%, while bacteria/PLA and bacteria/PCL webs followed this with the removal yields of 89.57% and 87.88%, respectively. The Q_{eq} values of the samples were in the same sequence, 119.56 mg/g for free-bacteria, 112.15 mg/g for bacteria/PLA web and 109.75 mg/g for bacteria/PCL web samples. For 100 mg/L of initial dye concentration, bacteria immobilized webs have shown higher removal yields than free-bacteria sample, where bacteria/PCL web has shown 92.52% removal with a Q_{eq} value of 307.89 mg/g, bacteria/PLA web has shown 91% removal with a Q_{eq} value of 301.65 mg/g, and free-bacteria sample has shown 89.04% removal with a Q_{eq} value of 296.56 mg/g. For 200 mg/L of initial dye concentration, bacteria immobilized webs have shown once more higher removal yields than free-bacteria sample, where bacteria/PCL web has shown 93.18% removal with a Q_{eq} value of 621.52 mg/g, bacteria/PLA web has shown 93.60% removal with a Q_{eq} value of 623.28 mg/g, and free-bacteria sample has shown 90.49% removal with a Q_{eq} value of 603.23 mg/g. Pristine PCL and PLA webs were also tested as negative controls for dye removal, and the results revealed that PCL and PLA webs had negligible effects on dye removal, which suggests that the dye removal capabilities of bacteria immobilized webs were primarily based on the bacterial cells. The results of dye

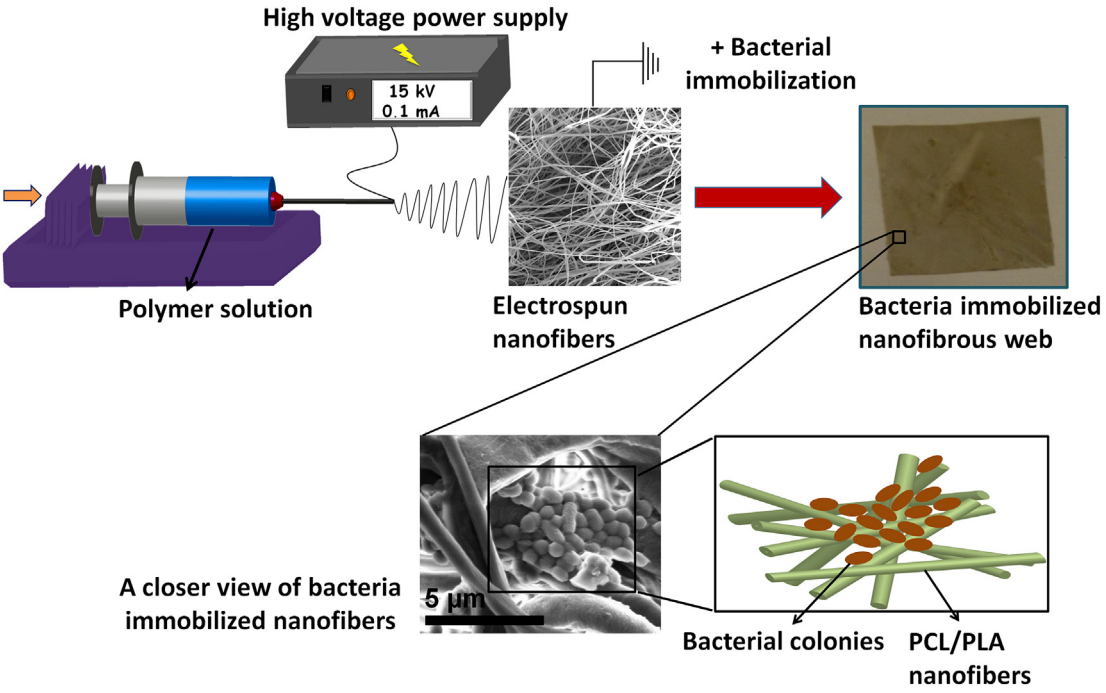

Fig. 1. Schematic representation of the electrospinning process and representative images for bacterial immobilization including a photograph of bacteria immobilized (*Clavibacter michiganensis* cells) electrospun nanofibrous web, a SEM micrograph of bacteria immobilized nanofibers and a schematic representation of the immobilized cells on electrospun nanofibers.

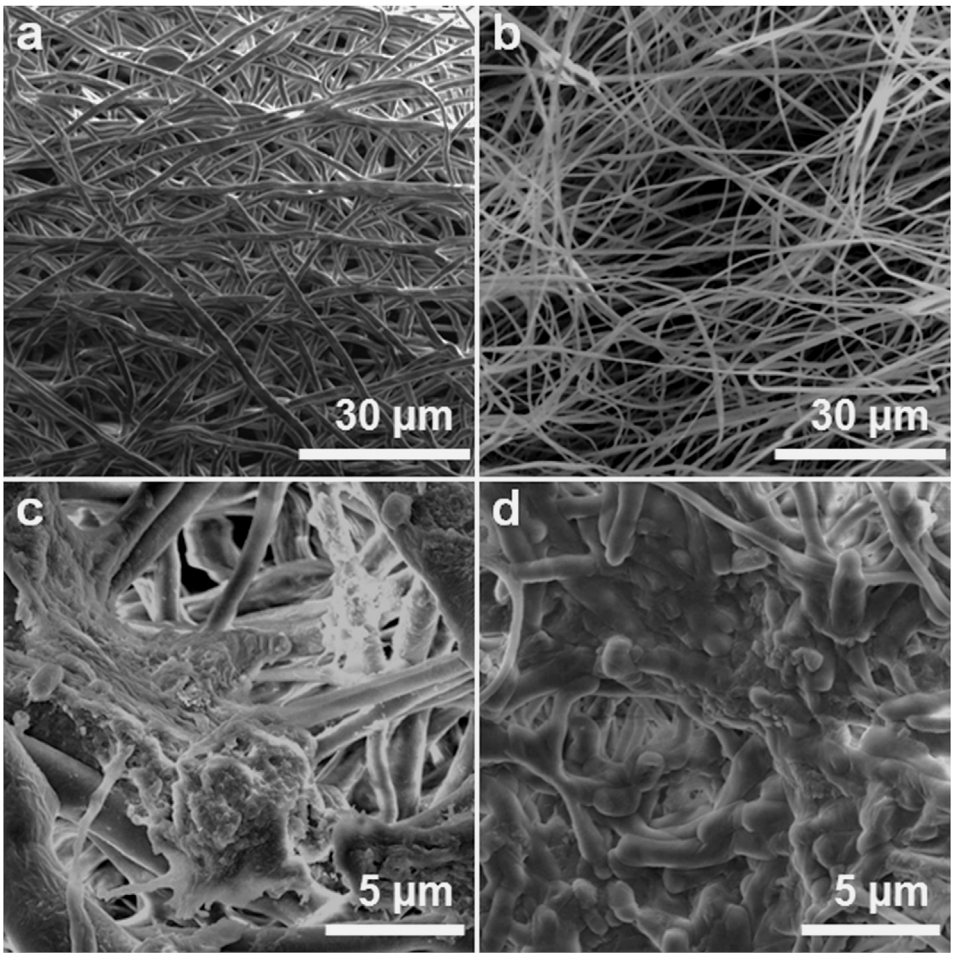

Fig. 2. SEM micrographs of (a) pristine PCL (b) pristine PLA (c) bacteria/PCL and (d) bacteria/PLA webs.

removal experiments were found as promising and henceforth bacteria immobilized web samples were further tested for their potential reusability.

3.4. Evaluation of adsorption isotherm coefficients and reaction kinetics

Adsorption isotherm coefficients and their estimated values are given in Table S1. All of the samples have shown good fits for each isotherm model. No distinctive fitting was observed for the Langmuir model in each sample, suggesting the dye removal process might be heterogeneous and multilayeric through bacteria (Ergul-Ulger et al., 2014). The maximum removal capacities (Q_{max}) were estimated to be 1.41×10^4 mg/g for free-bacteria, 1.77×10^3 mg/g for bacteria/PCL web and 3.24×10^3 mg/g for bacteria/PLA web samples under the Toth model.

The R^2 values of different order plots for dye removal are listed in Table S2. All of the samples have shown the highest correlation with the zero order model with the R^2 values of 0.9909 for free-bacteria, 0.9877 for bacteria/PCL web and 0.9944 for bacteria/PLA web samples. It has been reported that enzyme-catalyzed reactions often fall under the zero order model (Tinoco et al., 1995), hence the dye removal process by bacterial cells was supposed to be enzymatic.

Table 1
Removal capacities of free-bacteria, bacteria/PCL web and bacteria/PLA web samples at equilibrium at the end of the removal process. T = 30 °C, agitation rate: 150 rpm, incubation time: 48 h.

Sample name	Initial concentration (C_0)	Removal (%)	Q_{eq} (mg/g)
Free-bacteria	50 mg/L	95.56 ± 3.16	119.56 ± 5.6
	100 mg/L	89.04 ± 3	296.56 ± 8
	200 mg/L	90.49 ± 0.31	603.23 ± 11.64
Bacteria/PCL web	50 mg/L	87.88 ± 2.79	109.75 ± 3.92
	100 mg/L	92.52 ± 1.98 307	89 ± 6.05
	200 mg/L	93.18 ± 1.29	621.52 ± 20.86
Bacteria/PLA web	50 mg/L	89.57 ± 4.08	112.15 ± 8.6
	100 mg/L	91 ± 3.6301	65 ± 9.67
	200 mg/L	93.60 ± 3.13	623.28 ± 20.25

3.5. Reusability and applicability of bacteria/PCL and bacteria/PLA webs

Bacteria/PCL and bacteria/PLA web samples were tested for reusability in five consecutive cycles (Fig. 4). Statistical analysis of this experiment by Student's t-test for bacteria/PCL and bacteria/PLA samples has shown that the variations between them are not significant ($p = 0.973$). At the end of the reusability test, the average removal capacities of these two webs were found as very similar, 95.36% for bacteria/PCL and 95.41% for bacteria/PLA web samples, showing that both webs retained their dye removal capacities during the test period. In addition, it was found that, while bioremediation needed more time in initial cycles, it decreased after the second run from 48 h to 24 h, implying that the number of

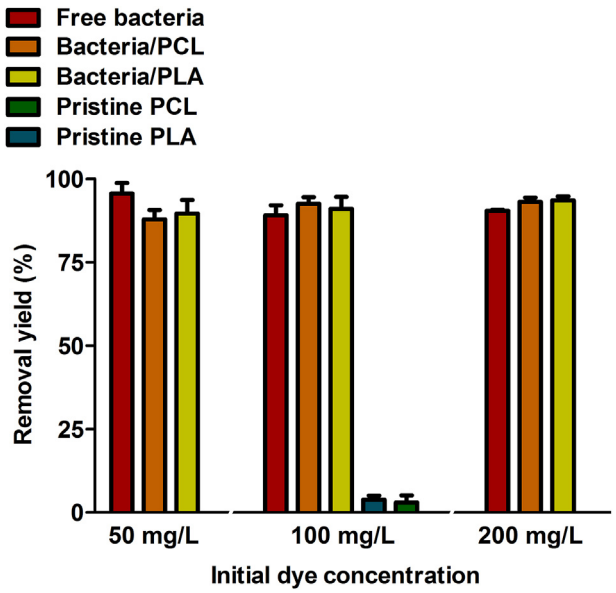

Fig. 3. (a) Setazol Blue BRF-X dye removal profiles of free-bacteria, pristine PCL web, pristine PLA web, bacteria/PCL web and bacteria/PLA web samples at initial concentrations of 50, 100 and 200 mg/L. Error bars represent standard error of mean (SEM) of three independent replicates.

immobilized bacteria increased during the test. This deduction was also supported by OD_{600} measurements, in which OD_{600} values of detached bacteria for equivalent web samples were measured before and after the reusability test. As shown in Fig. 5, the bacterial numbers highly increased during the test for both web samples, which might be the primary reason for faster bioremediation by bacteria immobilized webs after the second run. Furthermore, since the immobilized bacteria grow in dye containing media during the reusability test, they might be better adapted to the dye contaminated environment by changing the expression of genes for dye–metabolizing enzymes. Statistical analysis of this experiment by Student's t-test has shown that the variations in bacteria/PCL

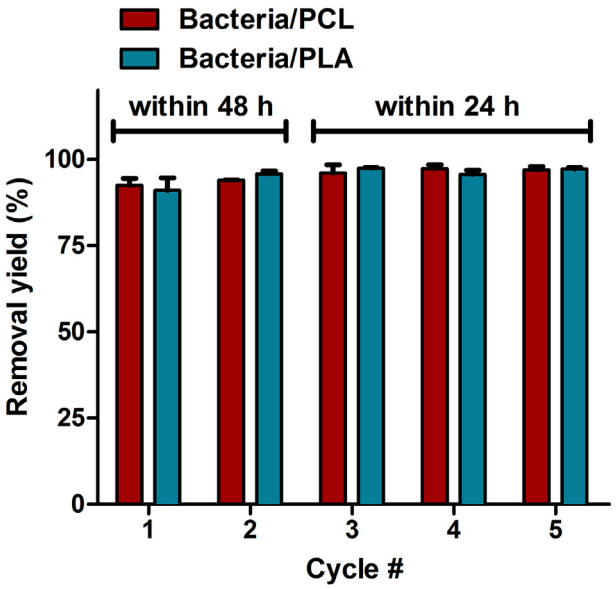

Fig. 4. Reusability test results of bacteria/PCL and bacteria/PLA webs at an initial dye concentration of 100 mg/L. Error bars represent standard error of mean (SEM) of three independent replicates.

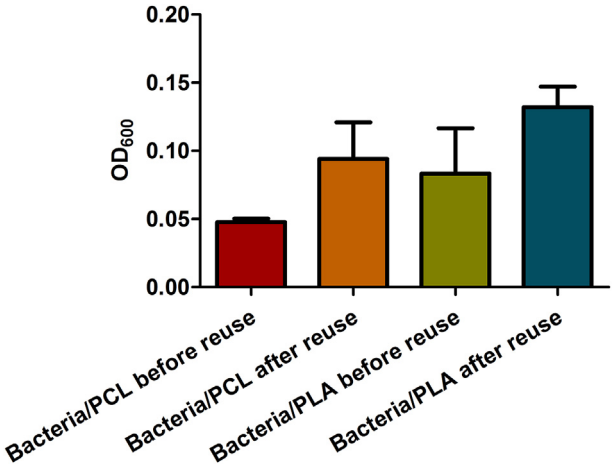

Fig. 5. Comparison for OD_{600} values of bacteria/PCL and bacteria/PLA webs before and after the reusability test.

and bacteria/PLA samples for before and after reuse are not significant ($p = 0.334$ and $p = 0.282$, respectively). Following the reusability test, bacteria/PCL and bacteria/PLA webs were evaluated for bacterial adhesion by SEM imaging. Fig. S2 shows immobilized bacteria retained on both PCL and PLA webs, indicating that bacterial cells have survived and grown throughout the reusability test. These results were found as promising for continuous remediation of textile dyes from aqueous environments by use of bacteria immobilized electrospun fibrous webs.

Dye bioremoval in aqueous solutions by use of different organisms have been extensively studied in the literature. (Asad et al., 2007; Deepa et al., 2013; Deniz and Tezel-Ersanli, 2016; El-Sersy, 2007; Ertugrul et al., 2009; San-Keskin et al., 2015a; Tastan et al., 2010). On the other hand, alternative approaches as in this study are relatively few, hence the objective of this study is the development of novel bio-hybrid materials with distinct features for dye bioremoval. The bacteria immobilized webs have lower space and weight requirements compare to free-bacteria in liquid media, thereby providing lower transportation costs and easier handling as in lyophilized bacteria. In addition, as demonstrated, bacteria immobilized webs are potentially reusable for continuous remediation of contaminated areas. By optimization of environmental parameters and increasing the number of immobilized bacteria, more efficient biocomposites can be produced for continuous, environmentally friendly and cost-effective dye bioremoval in wastewater.

4. Conclusions

In the present study, bacteria immobilized electrospun fibrous materials were developed for textile dye bioremoval in wastewater. Immobilization of bacterial cells was confirmed by SEM imaging and OD_{600} measurements of the pre-immobilized bacteria. The results of dye removal experiments demonstrated that dye removal capabilities of bacteria immobilized webs were primarily based on the bacterial cells. Furthermore, very similar and efficient removal performances were observed between bacteria immobilized webs and free-bacteria. The results of reaction kinetics studies suggest that, dye removal by bacteria immobilized webs is based on biological removal rather than adsorption. The reusability test results revealed that bacteria immobilized webs are potentially reusable for continuous remediation and their removal performances can be improved by increasing the number of immobilized bacteria. In brief, bacteria immobilized electrospun nanofibrous webs are

promising for remediation of textile dyes in wastewater with reusable and improvable properties.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.06.020.

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