Nanoremediation of Toxic Dyes Using a Bacterial Consortium Immobilized on Cellulose Acetate Nanofiber Mats

Esra ERKOÇ[1], İmre TÜZÜN[2]; Filiz KORKMAZ[3], Nalan Oya SAN KESKİN[2], Nur Koçberber KILIÇ[1ID]

[1]Department of Biology, Faculty of Science, Ankara University, Ankara, Turkey

[2]Polatlı Science and Literature Faculty, Biology Department, Nanosan Laboratory, Ankara Hacı Bayram Veli University, Ankara 06900, Türkiye

[3]Biophysics Laboratory, Faculty of Engineering, Atilim University, Ankara, Turkey

Correspondence

Department of Biology, Faculty of Science, Ankara University, Ankara, Turkey.

Email: nrkilic@ankara.edu.tr

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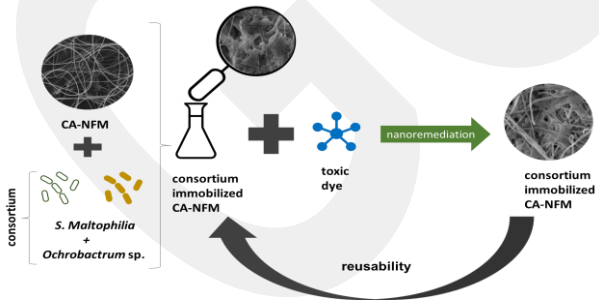
Graphical Abstract



Abstract

The bacterial consortium consisting of *S. maltophilia* and *Ochrobactrum* sp. demonstrated the highest rates of dye bioremediation. The trials were performed at pH 8, which resulted in the highest bioremediation rate of 64.6% in media containing 21.2 mg/L Setazol Black B. As the dye concentration increased, the pollutant removal rate decreased, with the maximum bioremoval rate of 70.3% observed in media containing 8.8 mg/L dye. The removal capacity was increased with an increase in biomass concentration; the highest yield of 91.3% was obtained in media containing 14.2 mg/L dye and 12% (v/v) biomass. In nanoremediation studies, the bacterial consortium was immobilized on cellulose acetate nanofiber mats (CA-NFM). SEM micrographs showed that bead-free nanofiber mats were effective in immobilizing bacterial cells. Moreover, nanofiber structures were capable of supporting EPS formation, as confirmed by FTIR analysis. The bacterial consortium immobilized on CA-NFM showed a maximum bioremoval rate of 56.5%. Reusability tests demonstrated that the consortium immobilized CA-NFM could be used at least five times. Furthermore, after leaving the mat for one month at 4°C, it was still usable, and the removal efficiency was found to be 45.4%. Based on our findings, bacteria immobilized on CA-NFM have the potential to be used as highly effective and versatile nanobiotechnological biological sorbents in the treatment of wastewater containing dyes.

Highlights

- *S. maltophilia and Ochrobactrum sp*. were used as a bacterial consortium.
- Bacterial consortium immobilized on CA-NFM was tested.
- EPS production by the consortium was confirmed by FTIR analysis.

- CA-NFM could be used at least five times.

KEYWORDS

bacteria, cellulose acetate nanofiber, dye, wastewater, nanoremediation

1 INTRODUCTION

As industrialization continues to grow, wastewater pollutants have become a concerning issue. Industrial wastewater contains a vast array of organic and inorganic compounds, including synthetic dyestuffs used in the textile industry. Unfortunately, these dyestuffs pose a risk to numerous living organisms due to their toxic, mutagenic, and carcinogenic properties. The colored nature of this wastewater also impacts the aquatic environment by reducing light transmittance and ultimately affecting the vitality of the ecosystem.

Untreated wastewater of this kind can result in numerous environmental issues. Fortunately, textile wastewater can undergo treatment through both chemical and biological methods. However, chemical treatment techniques have their drawbacks, including the production of unwanted byproducts, high costs, and risk of secondary pollution. Consequently, biological treatment has become the preferred option. By utilizing microorganisms with bioremediation abilities, biological methods offer a simpler, safer, cost-effective, and more practical alternative to chemical treatment.[1]

Microorganisms, including bacteria, fungi, microalgae, and cyanobacteria, have proven valuable in treating wastewater containing dyes.[2] Researchers have found that these microorganisms can be used in two ways: as pure cultures or as a consortium.[3] When used in a mixed population, they can accomplish tasks that

might be otherwise difficult or impossible for a single culture, often requiring multiple steps. This cohabitation approach can provide stability in the face of environmental changes, the ability to share metabolites, and resistance to nutrient restrictions and invasion by other species.[4]

Chaieb et al. conducted a study on using halotolerant bacteria for the removal of Congo red and malachite green dyes through bioremediation.[5] Three bacteria strains were tested: *Klebsiella pneumoniae* K2, *Enterobacter* sp. K16b, and *Vibrio tritonius* K20. The highest efficiency for Congo red removal was achieved by *Enterobacter* sp. K16b with 85.71% and for malachite green removal, by *K. pneumoniae* K2 with 82.73%. In another study, *Enterococcus faecium* and *Pantoea* spp. were examined, and *E. faecium* was found to remove 19.79% of 100 mg/L Sudan Black dye at pH 8 and temperature of 50 °C.[6] *Bacillus cohnni* (RKS9) isolated from textile wastewater was tested by Kishor et al. for the bioremoval of textile wastewater and different dyes.[7] It was found that the bacterium removed the color in real wastewater with 93.87% efficiency and Congo red dye with 99% efficiency.

Afrin et al. conducted a study on textile wastewater and isolated four different bacteria, including *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Enterococcus faecium*, and *B. thuringiensis* to test their effectiveness in removing various types of dyes.[3] The study found that when these bacteria were used in a consortium, they were able to perform bioremediation in a significantly shorter time and with higher efficiency compared to when used in pure cultures. Similarly, Ayed et al. discovered that a consortium of *Pseudomonas putida*, *Chlorella,* and *Lactobacillus plantarum* was successful in removing COD and reactive blue 40 dye.[8] In a separate study on the removal of a dye mixture of Reactive Blue (RB 221), Reactive Yellow (RY145),

and Reactive Red (RR195) by three different consortia (alkalophilic, thermophilic, and sludge), the highest dye removal rate was achieved by the sludge consortium (91.9%), with the alkalophilic consortia it was 85.3% and thermophilic consortia achieving 93.5% removal.[9]

Achieving efficient and cost-effective wastewater treatment is crucial, and advanced technologies are necessary to meet this objective. Recent advancements in nanomaterials have piqued the interest of scientists due to their potential benefits. Nanoremediation is a fast and effective technology that leverages engineered or biogenic-product nanomaterials in treatment processes.[10] Numerous studies have been conducted on nanoremediation using different types of nanomaterials, including carbon, silica, metal, and polymers.[11,12] Additionally, studies involving nanomaterials have produced modified structures like microorganism-integrated materials.

In some recent studies, researchers integrated nanomaterials produced using various polymers through electrospinning with microorganisms to remove different pollutants.[13,14] The produced nanomaterials are effective in bioremediation as they form a sturdy surface where microorganisms can effectively adhere, form a biofilm, and can be reused.

Our study aimed to demonstrate the effectiveness of bacterial bioremediation in removing different dyestuffs from industrial wastewater. We identified the most efficient bacteria and formed a consortium to improve dye bioremediation. Our objectives were to determine the best conditions for the consortium to achieve maximum dye bioremediation and to test its effectiveness in nanoremediation using cellulose acetate nanofiber mats (CA-NFM). We also conducted reusability tests for

the CA-NFM with the immobilized consortium. This report represents the first investigation of bacterial consortium immobilized CA-NFM in nanoremediation of textile dye-contaminated wastewaters.

2 MATERIALS AND METHODS

2.1 Dye solutions, microorganisms, and culture condition

Pure forms of pollutants Setazol Black B, Setazol Blue BRF-X, Setazol Turquoise Blue G, Setazol Navy Blue SBG were purchased from SETAS, Chemistry Factory (Tekirdag, Turkey), whereas Acid Red1 was purchased from Sigma.

Pseudomonas aeruginosa, *Stenotrophomonas maltophilia*, *Ochrobactrum* sp., and Isolate 1 were provided by Bioremediation Research Laboratory (Ankara University, Faculty of Science, Department of Biology).[15,16]

Bacteria were grown at pH 7 in a mineral salt medium (MSM) containing 5 g/L glucose and 25 mg/L dyestuff.[17] The content of the minimal medium was KH_2PO_4, 1.7 g/L; $(NH_4)_2SO_4$, 2.69 g/L; $MgSO_4$, 0.2 g/L; and $CaCl_2$, 0.03 g/L. One mL of each sample was inoculated into 50 mL flasks containing 20 mL of medium and were grown at 30 °C (NB-205V, Korea).

2.2 Selection of bacteria that bioremediate dyes with the highest capacity

Bacteria performing the maximum removal were selected in MSM media (pH 7) containing different dyestuffs such as Acid Red1, Setazol Black B, Setazol Blue BRF-X, Setazol Turquoise Blue G, and Setazol Navy Blue SBG.

For this purpose, 25 mg/L contaminant was added to MSM media as 100 mL in 250 mL flasks. Bacteria [2% (v/v)] were grown in prepared media in a shaking

incubator (NB-205V) with a rotation speed of 100 rpm at 30 °C for seven days. The remaining dye concentration was measured spectrophotometrically (BioDrop, UK).

At the end of this series of experiments, bacteria with the highest capacity to biodegrade pollutants and the highest tolerance to dyestuff were selected.

2.3 Effect of different environmental conditions on dye bioremediation

In these trials, the bacteria with the highest dye bioremediation performance were mixed and used as a consortium. Media containing the selected dyestuff were used to determine the conditions under which the consortium achieves the maximum dye bioremoval. The effect of different pH, initial dye, and biomass concentrations on the dye removal efficiency was tested.

2.4 Effect of pH on dye bioremediation

MSM media were prepared at pH 6 (23.5 mg/L dye), pH 7 (20.1 mg/L dye), pH 8 (21.2 mg/L dye), and pH 9 (25.2 mg/L dye) as 100 mL in 250 mL flasks to test the effect of pH on the dye bioremoval efficiency. 10 g/L glucose was added into the media to support the bacterial growth. These media were inoculated with 2% (v/v) for each microorganism. The pH of the media where the consortium bioremediated with the highest capacity was determined with these trials.

2.5 Effect of increasing dye concentration on dye bioremediation

To investigate the effect of increasing concentrations of pollutants on bioremoval efficiency, the media was prepared in 250 mL flasks containing 100 mL of the optimum pH solution and different concentrations of dye as 8.8 mg/L, 18.4 mg/L, 41.0 mg/L, 57.2 mg/L, and 76.9 mg/L. The activated bacterial consortium was inoculated into the prepared media at a concentration of 2% (v/v) for each

microorganism. These experiments aimed to determine the amount of pollutant that the bacterial consortium could remove from the environment.

2.6 Effect of increasing biomass concentration on dye bioremediation

To investigate the effect of different biomass concentrations on pollutant removal, the dye concentration resulting in the best removal rate was added to the media at optimum pH in 250 mL flasks with a total volume of 100 mL. Biomass concentrations of 4%, 8%, and 12% (v/v) were tested for dye removal efficiency. The concentration showing the highest efficiency was determined.

2.7 Bacterial consortium and nanofiber use in dye bioremediation: Nanoremediation

After determining the optimum conditions for the consortium in dye bioremediation, bacteria were immobilized on nanofiber mats. This step of the study was carried out under the optimized dye bioremoval conditions determined in the previous steps. Bacteria-free nanofiber mats were also tested for their possible function in dye removal (control group).

2.8 Electrospinning of cellulose acetate nanofibrous mat (CA-NFM)

The electrospinning of porous CA-NFM was carried out as described in our previous study.[14] The chemicals were purchased from Sigma-Aldrich (Germany) and used without further purification (dichloromethane, DCM, 99% (GC); acetone, 99% (GC); cellulose acetate, (CA, Mw: 30.000 g/mol, 39.8 wt% acetyl)). A clear electrospinning solution was prepared by dissolving CA in a DCM/acetone (2/1 (v/v)) binary solvent mixture at 7.5% (w/v) polymer concentration. Next, this solution was taken in a 3 mL syringe fitted with a 0.6 mm inner diameter metallic needle. Electrospinning

parameters were arranged as follows: feed rate of solutions 1 mL/h, applied voltage 15 kV, tip-to-collector distance 10 cm. The electrospinning apparatus was enclosed in a Plexiglas box, and the electrospinning was carried out at 2˚C at 20% relative humidity. At least 100 points were measured from the nanofibers and analyzed with the ImageJ (ver. 1.53n) program for the diameter measurements of the produced nanofibers.

2.9 Immobilization of bacterial consortium on CA-NFM, nanoremediation, and reusability of the consortium immobilized on CA-NFM

Bacteria grown and reached the exponential growth phase in the MSM were incubated for two days by inoculating with cut-sterilized nanofibers (20 mg/100 mL) into the medium at 1%. In this process, bacteria were immobilized onto the nanofiber surface and inside the fibers.

To assess the reusability of bacteria immobilized on CA-NFM for dye removal, nanofibers were first added to the optimized medium (pH 8; C_o: 10 mg/L; incubation period: seven days). The reusability was tested for five cycles. These trials were also performed after storing the mats at 4˚C for one month. During each cycle, the flasks were incubated in a shaking incubator for seven days. After incubation, samples were collected and centrifuged, and the dyestuff in the supernatant was analyzed.

2.10 Scanning Electron Microscopy

The elemental analysis of materials, morphology of nanofibers, and corresponding diameters were investigated using a scanning electron microscope (SEM). For sample fixation, bacterial consortium immobilized on mats were washed with PBS buffer and placed overnight in 2.5% glutaraldehyde solution prepared in PBS buffer.

The mats were then rewashed with PBS buffer and dehydrated using a series of EtOH solutions (ranging from 30% to 96%). Finally, the samples were coated with 5 nm Au for SEM imaging (ZEISS EVO 40, SEM).

2.11 FTIR analysis

Cellulose Acetate Nanofibers were of dry form and measured directly by placing onto the ATR diamond. Nanofibers with bacteria had to be dried with a gentle stream of N_2 gas for 45 min to remove excess water. Infrared spectra were obtained using a Thermo-Scientific Nicolet 6700 FTIR spectrometer, equipped with a diamond attenuated total reflection (ATR, ConcentratIR2, Harrick) accessory. A clean diamond surface was used as the background interferogram. Absorbance spectra were recorded in the 800-4000 cm^{-1} range by collecting and averaging 64 scans at 4 cm^{-1} resolution using the instrument software OMNIC (v. 8.2.388). Spectra are ATR corrected for the visual presented in the manuscript.

2.12 Statistical analysis

The results were analyzed for remarkable differences using a variance method (ANOVA) and compared by standard deviations (±S.E.) The trials were done with two repetitions**.**

2.13 Analytical methods

Samples of 3 mL were taken daily from each Erlenmeyer flask. These samples were centrifuged to harvest the biomass at 6000 rpm for 10 minutes (Hermle Z207A, Germany). The growth of bacteria was measured by the dry weight of washed biomass after the incubation period. The amount of pollutant concentration remaining in the medium was determined spectrophotometrically (BioDrop, UK).

Wavelengths were 600 nm for Acid Red1, 595 nm for Setazol Black B, 609 nm for Setazol Blue BRF-X, 669 nm for Setazol Turquoise Blue G, and 602 nm for Setazol Navy Blue SBG. Medium without inoculation was used as the blank.

Dye bioremoval yield (*Y%*) was studied as a function of the pH, dye concentration, and biomass concentration. Co is the initial, and Cf is the dye's final concentration (mg/L).

The percentage of dye removal yield was found with Equation (1):

$$Y\% = \left(\frac{Co - \mathrm{Cf}}{Co}\right) x100$$

3 RESULTS AND DISCUSSION

3.1 Selection of Bacteria with the Highest Bioremediation Capacity

P. aeruginosa, *S. maltophilia*, *Ochrobactrum* sp., and Isolate 1 were tested to find the bacterium that bioremoved the applied pollutant (Setazol Black B, Setazol Blue BRF-X, Setazol Turquoise Blue G, Setazol Navy Blue SBG, and Acid Red1) with the highest efficiency. The results obtained from this series of experiments are given in Table 1. The tested bacteria had no removal capacity for Acid Red1 dye. On the other hand, *P. aeruginosa* removed the applied 24.7 mg/L Navy Blue SBG with a yield of 10.5%. *S. maltophilia* removed Setazol Black B and Setazol Blue BRF-X with the highest removal efficiency of 18% and 19.4%, respectively. *Ochrobactrum* sp. had the maximum bioremediation capacity for the Setazol Black B (27%) and Setazol Navy Blue SBG (13%). Isolate 1 removed the applied Setazol Turquoise Blue G with a removal efficiency of 9.9%.

TABLE 1 Selection of bacteria that bioremediate dyes with the highest capacity (pH 7; incubation period of seven days at 30 ˚C)

Dye		Bacterium			
		P.aureginosa	***S.maltophilia***	***Ochrobactrum sp.***	**Isolate 1**
	C_o (mg/L)	**Y%**	**Y%**	**Y%**	**Y%**
Setazol Black B	22.2	8.6±1.0	18.0±1.5	27.0±1.2	9.0±1.0
Setazol Blue BRF-X	24.7	8.1±1.5	19.4±1.0	7.7±1.0	7.7±0.8
Setazol Turquoise Blue G	21.3	8.3±0.5	2.3±0.7	4.1±1.1	9.9±1.1
Navy Blue SBG	24.7	10.5±1.1	9.7±0.9	13.0±1.6	4.9±1.2
Acid Red1	23.1	-	-	-	-

Based on the findings, *S. maltophilia* and *Ochrobactrum* sp. were identified as the bacteria with the highest pollutant removal rates when grown in media containing Setazol Black B. These two bacteria were combined to form a consortium and used for further experiments. Previous studies have used either *S. maltophilia* cells with laccase activity or *Ochrobactrum* sp. cells to treat RB5.[18,19] To the best of our knowledge, this study is the first to investigate the use of the selected bacteria as a consortium.

3.2 Effect of pH on bioremediation by bacterial consortium

The effect of pH on dye bioremoval by the consortium is summarized in Table 2. The microorganisms were found to remove the dye with the highest yield of 64.6% at pH 8. However, at pH 6, the yield was only 33.6%, and at pH 7 and 9, the highest

pollutant removals were 30.9% and 30%, respectively. Therefore, based on this data, further investigations were conducted with the media at pH 8.

TABLE 2 Effect of pH on Setazol Black B bioremoval by the bacterial consortium of *S. maltophilia* and *Ochrobactrum* sp. (incubation period of three days at 30 ˚C)

pH	**C_o (mg/L)**	**Y%**
6	23.5	33.6±1.3
7	20.1	30.9±4.0
8	21.2	64.6±1.4
9	25.2	30.0±1.0

Among the tested bacteria in the current study, *Ochrobactrum* sp. was previously reported as the best at pollutant bioremoval at pH 8, while *S. maltophilia* was best for bioremediating heavy metals at pH 7.[15,16] These results agree well with previous studies investigating the role of pH on dye-bioremoval efficiency. For example, a report by Khan et al. showed that living *O. intermedium* BS39 cells effectively removed RB5 in the pH range of 5-9.[20] Cheng et al. also reported that bioremoval of RB5 was performed by *O. anthropi* with the highest efficiency at pH 7 and 9.[19] Alaya et al. reported that *S. maltophilia* had an efficient malachite green removal yield at pH 8.[21] Johansson et al. concluded that pollutant reduction efficiency was dye-specific and pH-dependent due to dye-enzyme interactions.[21,22] In this study, the highest Setazol Black B removal rate was achieved at pH 8, likely due to the pH-dependent nature of dye-enzyme interactions

3.3 Effect of initial dye concentration on bioremediation by bacterial consortium

The experiments were carried out in media containing approximately 10–80 mg/L dyes to investigate the Setazol Black B bioremediation by *S. maltophilia* and *Ochrobactrum* sp. consortium (Figure 1).

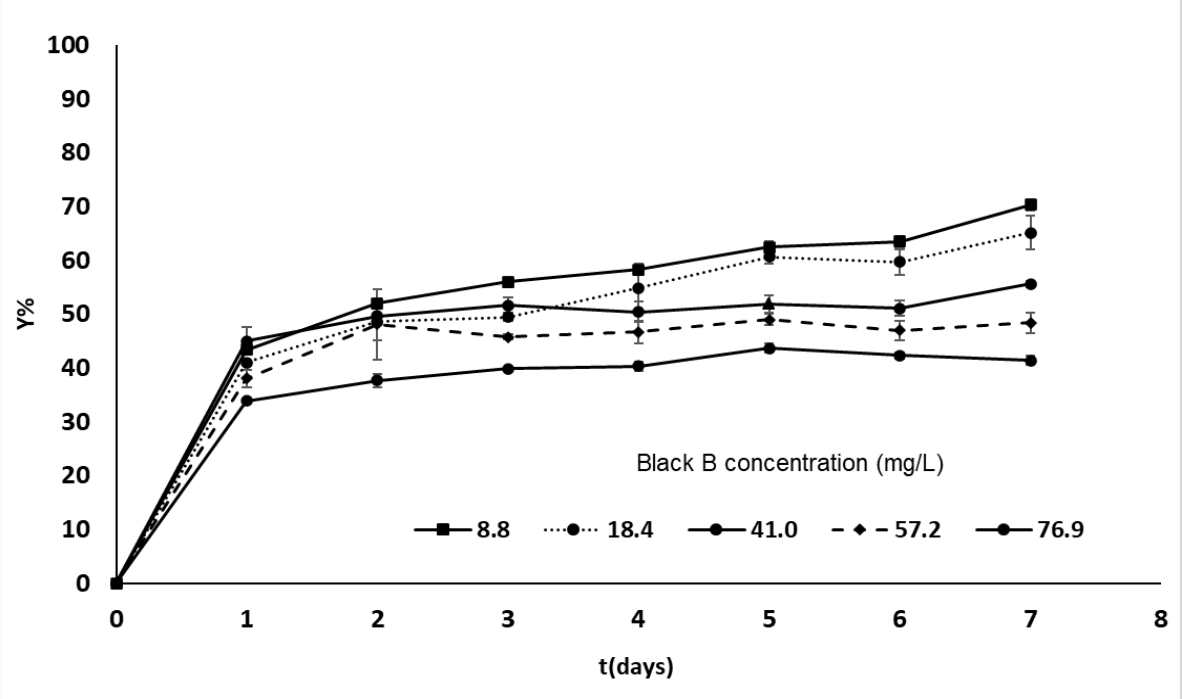


FIGURE 1 Effect of increasing dye concentration on dye bioremediation efficiency by the consortium of *S. maltophilia* and *Ochrobactrum* sp. (pH 8, incubation period of seven days at 30°C).

The experiments conducted showed that a removal efficiency of more than 30% was achieved for all concentrations of Setazol Black B dye tested after being incubated for one day. In media that contained 8.8 mg/L dye, the bioremoval yield was 58.3% at the end of the four-day incubation period. For the same incubation period, the yield was 54.9%, 50.4%, 46.7%, and 40.3%, respectively, for dye concentrations of 18.4 mg/L, 41.0 mg/L, 57.2 mg/L, and 76.9 mg/L. After seven days of incubation, the highest bioremediation of dye was 70.3% in media containing 8.8

mg/L dye. As the concentration of Setazol Black B dye increased, the removal percentages decreased due to the toxicity of the dye to the consortium. At the end of the incubation period, the highest concentration of dye (76.9 mg/L) had a removal yield of only 41.4%. As the amount of pollutant increased, the toxic effect of the dye on the tested bacteria became more apparent. Our findings are consistent with those of Afrin et al., who also found that bioremediation yield decreased as the pollutant concentration increased and that the highest yield was obtained at the lowest dye concentration.[3]

3.4 Effect of initial biomass concentration on bioremediation by the bacterial consortium

To investigate the effect of biomass concentration on dye bioremediation by the consortium, two dye concentrations (15 mg/L and 40 mg/L) and three biomass concentrations [4-12% (v/v)] were used.

The experiments shown in Figure 2 compared the removal yield of different biomass concentrations (4%, 8%, and 12% v/v). The results showed that the higher the concentration of biomass, the better the removal yield. For example, in media containing 14.2 mg/L of dye, increasing the biomass concentration from 4% to 8% or 12% (v/v) resulted in a higher bioremediation yield of 60.7%, 89.1%, and 91.3%, respectively. In addition, the study found that when the pollutant concentration was 41.3 mg/L, the bioremoval efficiency increased to 85% at a 12% (v/v) biomass concentration.

Based on these findings, the 12% (v/v) biomass concentration was selected for further experiments. Similar results were obtained in Gomaa's study, where the bioremoval efficiency of dye increased in proportion to the inoculum size, and the

maximum dye removal was achieved with a 20% (v/v) inoculum size.[23] However, no significant difference in dye removal efficiency was observed with a further increase in the inoculum size. Another study showed that increasing the biomass concentration from 2% to 5% and then to 10% resulted in a clear improvement in dye removal efficiency. However, beyond 10% biomass concentration, the efficiency decreased.[3]

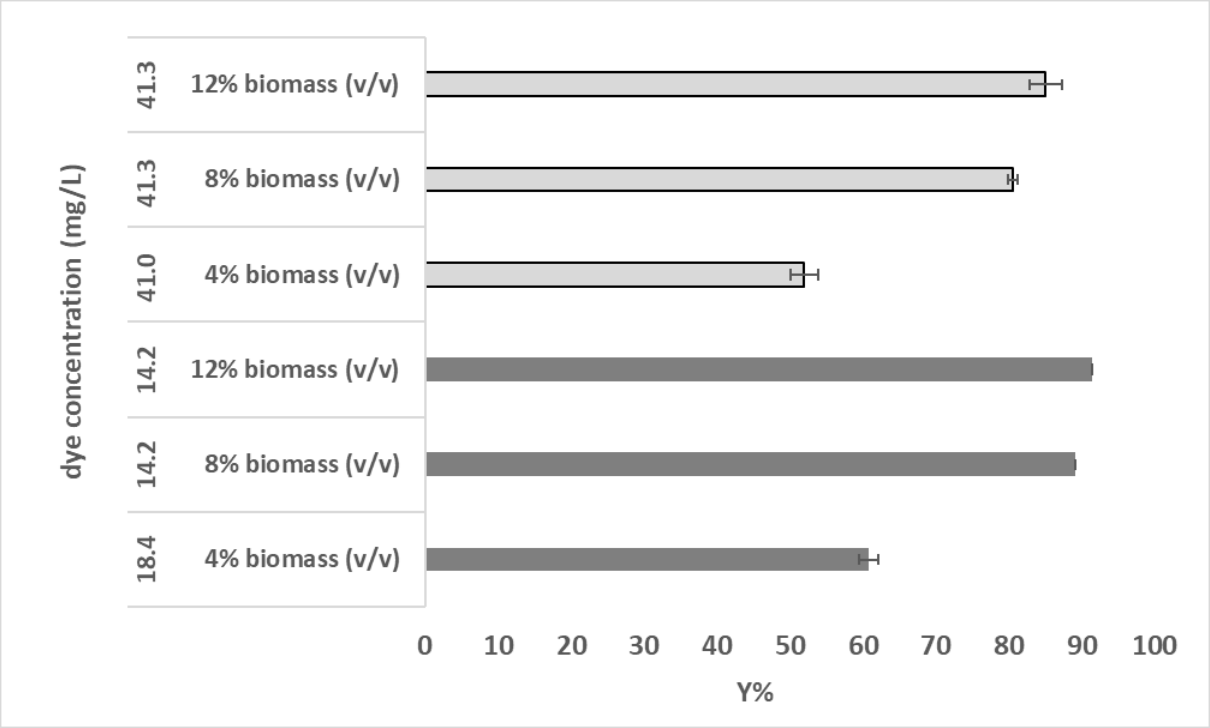


FIGURE 2 Effect of increasing biomass concentration on dye bioremediation by *S. maltophilia* and *Ochrobactrum* sp. consortium (pH 8; incubation period: 5 days, T: 30 ˚C) C_o:18.4 mg/L (4% biomass v/v); C_o: 14.2 mg/L (8% and 12% biomass v/v); C_o:41.0 mg/L (4% biomass v/v); C_o: 41.3 mg/L (8% and 12% biomass v/v).

3.5 Immobilization of *S. maltophilia* and *Ochrobactrum* sp. consortium on CA-NFM

SEM micrographs of CA-NFM and its average fiber diameter are shown in Figure 3 (a, b, c). *S. maltophilia* and *Ochrobactrum* sp. consortium immobilized on CA-NFM is shown in Figure 3 (d, e).

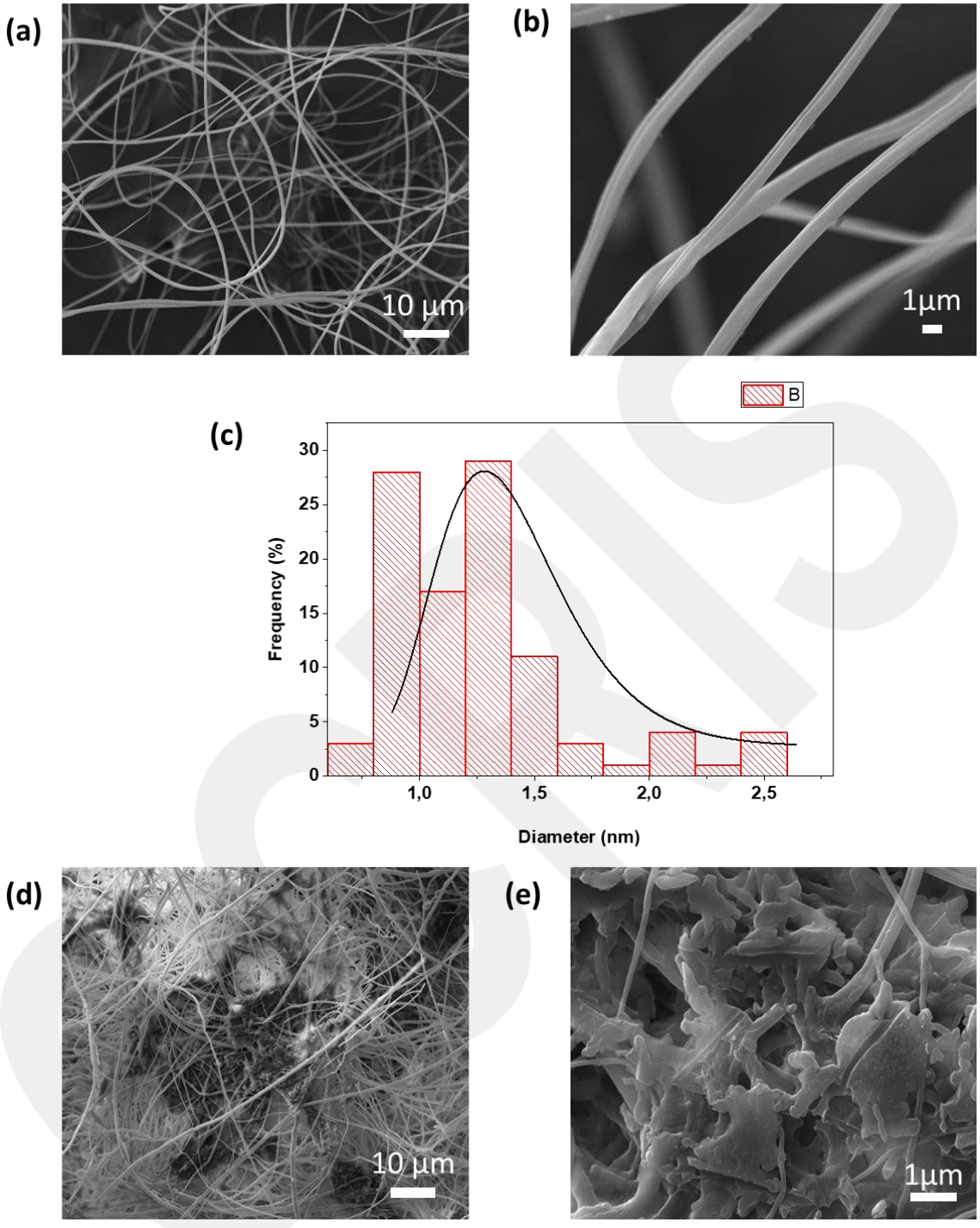


FIGURE 3 SEM micrographs of CA-NFM (a, b), average diameter of CA-NFM (c), SEM micrographs of *S. maltophilia* and *Ochrobactrum* sp. consortium immobilized CA-NFM (d, e).

Diameter measurements were taken from 100 different points, revealing that the diameter dimensions of CA-NF were 1.25 ± 0.4 µm (Figure 3(c)). SEM analysis was conducted to inspect bacterial attachment onto the nanofiber mats after seven days of incubation. Micrographs showed that the consortium produced a biofilm layer to attach and form a matrix required for bioremediation (Figure 3(d, e)). Previous studies have reported that *Ochrobactrum* sp. and *S. maltophilia* produced exopolysaccharides (EPS) to defend themselves in stress conditions and bioremediate different pollutants.[15,16] EPS can play a crucial role in reducing the surface and interfacial tension of bacteria and enhancing the dispersion, emulsification, and degradation of hydrocarbon pollutants by increasing the cell surface hydrophobicity of bacteria.[24] The consortium in the current study also displayed such key structural properties as shown by the SEM micrographs. It appears that EPS produced by immobilized bacteria on mats may have contributed to the removal of the applied dye through these mechanisms.

3.6 Nanoremediation by bacterial consortium immobilized on CA-NFM and reusability of this mat

The ability of mats to be reused in studies involving bacteria immobilized on nanofiber mats is crucial. To assess this, the consortium immobilized on CA-NFM was exposed to Setazol Black B for five cycles, as shown in Figure 4. The results indicate that after each cycle, at least 25.7% of the dye was removed after incubation for one day. The maximum dye removal efficiency was observed to be 50.1% in the third cycle after incubation for three days. As the incubation period increased, the dye removal yield increased in each cycle, with the maximum yield of 56.5%

observed in the fifth reuse cycle. Overall, the consortium immobilized on CA-NFM effectively treated the applied pollutant with yields ranging from 50.1% to 55.1%.

To determine the efficiency of dye removal, control group experiments were conducted using bacteria-free nanofibers. The dye removal values obtained from the bacteria-free nanofibers were subtracted from those obtained from bacteria-immobilized nanofibers. The difference between the two values revealed that the consortium immobilized on CA-NFM was responsible for the removal of Setazol Black B. The data obtained from bacteria-free nanofibers was not shown.

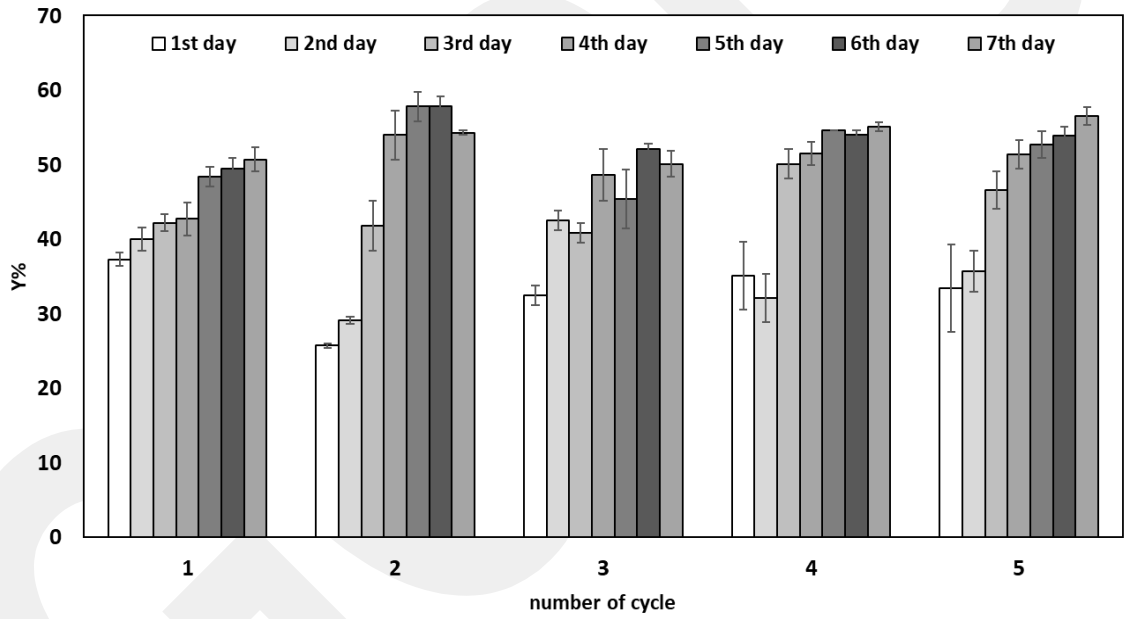


FIGURE 4 Reusability test data of the consortium immobilized CA-NFM for Black B nanoremediation in MSM with 10 mg/L pollutant (number of cycles: 5; pH 8; incubation period: 7 days, T: 30°C).

Figure 5 (a, b) shows SEM micrographs of the consortium immobilized on CA-NFM after the fifth reuse cycle. SEM analysis confirmed that CA-NFM was still usable, and the immobilized bacteria were active for bioremediation. It was clearly seen from SEM micrographs that the consortium produced EPS.

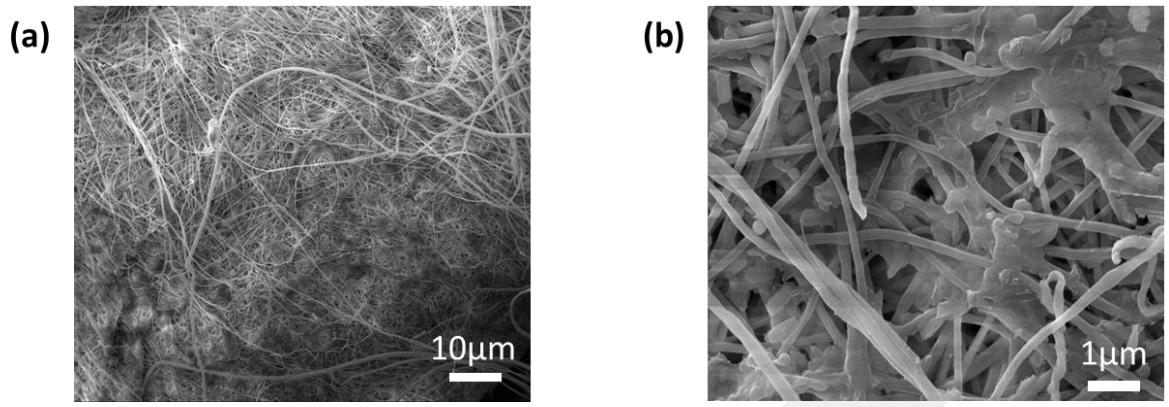


FIGURE 5 *S. maltophilia* and *Ochrobactrum* sp. consortium immobilized on CA-NFM after the fifth reuse cycle.

After conducting five cycles of reusability tests, the CA-NFM hosting the consortium was kept at 4 ˚C for a month, following which it was tested again for the consortium's ability to remove the dye. The results showed that the dye bioremoval yield was 45.4% after seven days of incubation, indicating that the fiber was still active even after being stored at 4˚C for a month. These experiments demonstrated that the consortium immobilized on CA-NFM has great potential for reusability in nanoremediation of the dye-contaminated wastewater.

In certain studies, it has been reported that copolymers and nanofibrous mats can effectively remove pollutants.[25,26,27] The studies have demonstrated that using bacteria-free nanofibers can remediate pollutants effectively. The use of a consortium immobilized on CA-NFM offers several advantages for bioremediation processes, including occupying less space, being portable, and reusable multiple times. It has also been shown that these mats can be stored at 4°C conditions until the next use. Compared to using free bacteria for the bioremediation of dyes, a consortium immobilized on CA-NFM is more practical and cost-effective. Therefore, these mats are a significant contribution to biological treatment systems.

3.7 FTIR analysis

The infrared spectrum of cellulose acetate nanofibers shows characteristic peaks at 1739 cm^{-1} assigned to C=O stretching mode, 1377 cm^{-1} assigned to C-CH_3 stretching mode, 1229 cm^{-1} assigned to C-O-C stretching mode originating from the acetate group, and at 1039 cm^{-1} assigned to C-O stretching.[28,29] The spectrum of bacterial consortium hosted by the nanofibers shows the same characteristic peaks in addition to the peaks of bacteria (Figure 6). CH_2 and CH_3 stretching modes of membrane lipids are observed in the range 3000-2800 cm^{-1}. Amide I and II bands due to proteins are observed at 1646 and 1547 cm^{-1}, respectively. The peaks at 1377, 1229, and 1039 cm^{-1} that are common between the two spectra are more pronounced with the bacterial consortium. Elevated absorbance at 1039 cm^{-1} argues for an increased number of polysaccharides, showing EPS formation.[30]

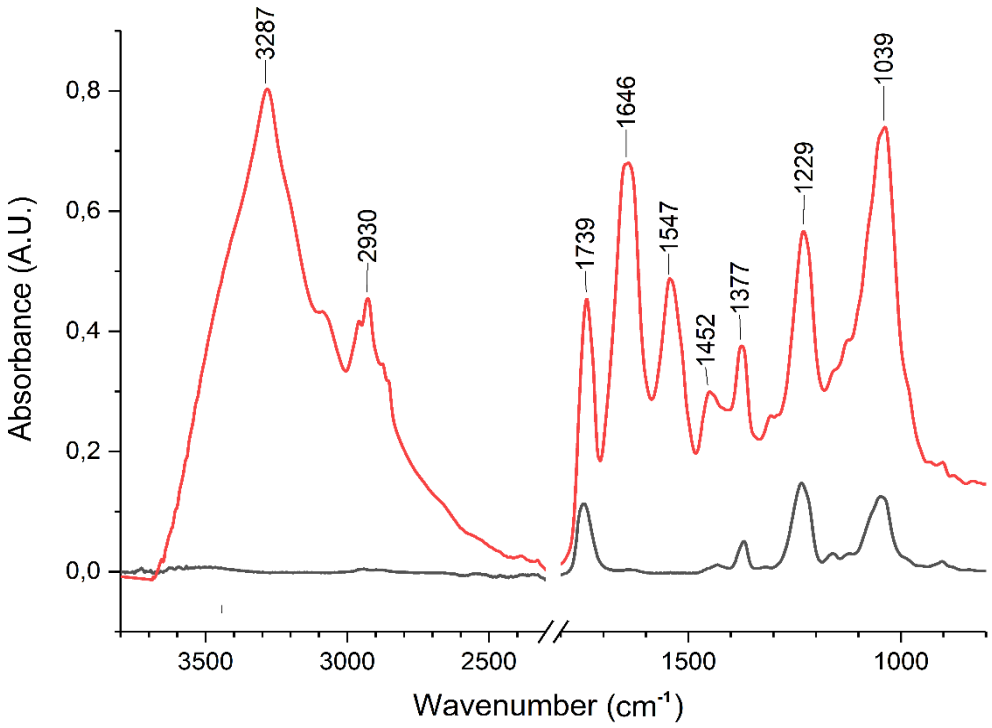


FIGURE 6 Infrared spectra of cellulose acetate nanofibers (grey) and bacteria grown in the nanofibers in the presence of dye (red).

4 CONCLUSIONS

The study aimed to determine the dye bioremoval capacities of four bacterial strains. The results showed that *Ochrobactrum* sp. and *S. maltophilia* had the highest pollutant removal capacity when used in MSM with Setazol Black B and were thus used for nanoremediation studies. Optimization studies were also conducted to determine the best removal efficiency under specific conditions. While there are existing reports on pollutant removal by microorganisms immobilized on nanofiber mats, there is no study on Setazol Black B removal through a bacterial consortium immobilized on CA-NFM. SEM images revealed an average fiber diameter of 1.25 µm for CA-NF, while FTIR analysis confirmed the production of EPS by the bacterial consortium. The current study found that the bacterial consortium (*Ochrobactrum* sp. + *S. maltophilia*) immobilized on CA-NFM exhibited high reusability for at least five cycles while maintaining its bioremediation efficacy even after being kept at 4°C for 1 month. These results suggest that a bacterial consortium immobilized on CA-NFM is an effective biosorbent candidate with strong nanoremediation capability and can be used in various biological treatment systems.

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DATA AVAILABILITY

The data supporting this study's findings are available from the corresponding author upon reasonable request**.**

ORCID

Filiz KORKMAZ https://orcid.org/0000-0003-3512-3521

Nur Koçberber KILIÇ https://orcid.org/0000-0003-2668-3789

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