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Determination of dye biosorption capacity of lichens and reusability of wastes as antimicrobial agents

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ABSTRACT

Determination of dye biosorption capacity by lichen biosorbent and reusability of the waste lichen biosorbent as an antimicrobial agent are the aims of this study. The study has three stages: 1. to carry out dye biosorption assays, 2. to scan antimicrobial activity of waste lichen biosorbent after dye loaded, 3. to examine the antimicrobial function of a fabric which absorbed waste lichen extract. The optimal conditions for the best dye biosorption were determined. After the biosorption experiments, the waste lichen biomass was harvested from the working solution and the extraction of dye-loaded waste biomass was done using methanol and ethanol. The extracts obtained from both dye-loaded and un-loaded biosorbents were scanned for antimicrobial activity potential by the disk diffusion method. The results of this study showed that the waste lichen biosorbent of biosorption processes can be reused as antimicrobial agents.

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Introduction

The lichens are represented by about 20,000 species all over the world. Although an estimated 2–3 thousand species are grown in Turkey, only more than 1000 species were recorded. In recent years, the studies focused on lichen flora of Turkey and also aimed to highlight the ecological roles of lichens in medical and economic applications (Güvenç et al., 2018; Sezer, 2016).

Lichens are the mutualistic organisms composed of a mycobiont (fungus) and a photobiont (cyanobacteria or algae). There is a close physiological relationship between symbiotic partners in lichens. The more dominant heterotroph fungus meets carbonic nutrient requirements with photosynthesis products of photobiont (Nash, 1996). Besides, in the case of mycobacterial nitrogen-fixing cyanobacteria, it obtains a nitrogen source from the other biont. However, it is not known whether there is a food transfer from fungus to photobiont cells, but it is assumed that fungus can function as an inorganic food reservoir for photobiont as in other fungal symbioses (Brown, 1985; Rai, 1988; Rai et al., 1980). Another consequence of the close physiological relationship is the secondary metabolites found in lichens as crystals outside the cell, most of which are not found in free-living fungi (Culberson & Elix, 1989). Different types of lichen metabolites, antagonist effects on yeast and algae (antibiotic), have been known for many years. More than 50% of the lichen samples tested antibiotic effect, the most effective substances such as usnic acids,

pulvunic acid derivatives (e.g. vulpinic acid), and aliphatic acids. Most of the recent studies focus on the development of new antimicrobial agents due to the antibiotic resistance of pathogens (Duygu et al., 2019). Lichens are favorable organisms to use as an antimicrobial agent because they are not pathogenic or toxic to living organisms (Shahi & Patra, 2003). Also, it is reported that some of the lichen species had also antitumor activity (Fernández-Moriano et al., 2016). Lichens are used as important sources to produce cosmetic and medical treatment products from the past to the present (Amberg & Fogarassy, 2019). Recent researches focused on the utilization of lichens in biological wastewater treatment technologies (Gül et al., 2019; Şenol et al., 2019). The use of biological materials to remove dyes from wastewater is both inexpensive and effective (Liu et al., 2018). It is reported that lichens were very effective and inexpensive biosorbents to treat uranyl or lead-contaminated water (Gül et al., 2019; Şenol et al., 2019). However, there is not any study that reuses the waste of lichen biomass after the biosorption process in the literature.

This study aims to determine the potential use of the lichen biosorbent for removal of commonly used textile dye and to test the re-use of the extraction of waste biomass as an antimicrobial agent after the biosorption process. This antimicrobial agent was scanned to determine the antimicrobial effect on *Escherichia coli* and *Staphylococcus aureus*, which are the most common pathogens used in hospitals (Jones, 2010). Some studies reported that antimicrobial resistance of *E. coli* became a significant problem

to treat infections originated from the hospital (Norgaard & Roslev, 2016). *S. aureus* caused several skin diseases such as atopic dermatitis, and ongoing researches are done to find some agents against this bacterium (Amagai et al., 2018). Also, the antibiotic resistance problem of *C. albicans* was reported previously and it was difficult to struggle with infections caused by this yeast due to its eucaryotic cell structure (Vannini et al., 2018).

Recent studies aimed to produce fabrics with antimicrobial properties (Tan et al., 2019). It is desirable to have antimicrobial functions, especially in textiles used in medical and hygienic areas, since microorganisms attach to the surface, transport, and transmit the diseases caused by them. In the studies on bacterial growth in textiles, it was observed that commonly used textile materials host a high amount of pathogens (Morais et al., 2016). Also, it has been determined that infections of hospitals are spreading in hospitals due to the hands or clothes of the hospital personnel (Chastre & Luyt, 2010). Also, it is intended to examine the antimicrobial property of a medical fabric after treated with the reused lichen extract in this study. The objectives of the current work were listed as determining the biosorption properties of lichen *Pseudovernia furfurace*, examining the antimicrobial activities of the waste lichen biomass formed after the biosorption process and testing the antimicrobial effects of the medial fabric pieces treated with waste lichen extracts. The lichen species called *P. furfurace* was obtained from the local environment of Bilecik, Turkey, and recent studies focused on the dye removal properties of lichen species (Bayazit et al., 2019; Koyuncu & Kul, 2020) but there is not any published work about textile dye biosorption properties and antimicrobial activities of lichen species called *P. furfurace* obtained from Bilecik, Turkey. To our present knowledge, this is the first paper showing the usage of waste lichen biomass obtained from dye biosorption in order to produce a medical fabric having an antimicrobial function. Also, this is the first preliminary study tested the antimicrobial function of medical fabric pieces treated with the waste lichen biomass revealed after the biosorption process

Materials and methods

Medium for microbial incubation in antimicrobial assays

Bacteria and yeast were incubated on nutrient broth (NB) and Sabouraud's dextrose broth (SDB) at 37 °C for 24 h, respectively. The used NB (pH 6.9 without NaCl) and SDB (pH 5.6) were purchased from Merck (Germany). The antibiotics used for control in antimicrobial assays were obtained from Bioanalyse (Turkey).

Biosorbent preparation for biosorption assays

The samples of lichen called *Pseudovernia furfurace* were collected from Bilecik province (N 400 11.5262', E 0290 57.962') in September 2019 and used in biosorption experiments. The lichen samples cleaned using double distilled

water. The lichen samples were dried at room temperature for 2 days and used as biosorbent in the experiments.

Dye solution

Acid Red P-2BX (AR) dye was obtained from the textile factory in Turkey. The stock AR solution was prepared as 1000 mg l⁻¹ in distilled water. The desired concentrations of diluted stock solutions were used in the biosorption experiments.

Biosorption assays and analysis

Experiments were carried out at the batch scale level to determine the influence of different parameters such as pH, dye concentration, and biosorbent dosage. Erlenmeyer flasks (100 mL) were used in all series of the biosorption experiments. The conditions of pH influence experiments were listed as pH (2.0–10.0), 1 g l⁻¹ biosorbent dosage, 100 mg l⁻¹ initial dye concentration. Initial dye concentration influence was tested at pH 2 and 25–200 mg l⁻¹ dye concentrations with 1 g l⁻¹ biosorbent dosage. The influence of contact time was investigated at 0–2880 min.

In the experiments, 2 mL of samples was taken from flasks and centrifuged at 6700 g for 15 min. The supernatants were analyzed at a 535-nm wavelength spectrophotometer. During the analysis, the flasks contained distilled water used as controls. An Agile spectrum uv/vis spectrophotometer (Delta Electronics, China) and Beckman Coulter (Beckman Coulter, Germany) model centrifuge were used for absorbance measurements and centrifugation, respectively. The dye biosorption percentage was calculated from Equation (1).

$$\text{Biosorption (\%)} = (C_o - C_f) / C_o \times 100 \quad (1)$$

The definitions for Equation (1) are as follows: q_m is the maximum specific dye uptake (mg g⁻¹), X_m is the maximum dried cell mass (g l⁻¹), C_o is the initial dye concentration (mg l⁻¹), and C_f is the final dye concentration (mg l⁻¹).

Biosorption isotherms and kinetics

The most common isotherm and kinetic models were used in this study. The equations used for calculations were given in Equations (2)–(8):

$$\begin{aligned} \text{Langmuir Isotherm Model : } C_e / q_e \\ = (1/q_m) C_e + 1/K_L q_m \quad (2) \end{aligned}$$

$$\begin{aligned} \text{Freundlich Isotherm Model : } n(q_e) \\ = \ln(K_F) + 1/n \ln(C_e) \quad (3) \end{aligned}$$

The definitions for Equations (2) and (3) are as follows: q_e is the amount of dye adsorbed per unit weight of adsorbent at equilibrium (mg g⁻¹), q_m is the maximum dye uptake per unit mass of adsorbent (mg g⁻¹), K_L is the Langmuir isotherm constant (L mg⁻¹), and K_F is the Freundlich isotherm constant (L mg⁻¹).

Dubinin–Radushkevich (D–R) Isotherm Model : q

$$= X_{DR} e^{-K_{DR} \varepsilon^2} \quad (4)$$

The definitions for Equation (4) are as follows: q is the amount of adsorbed dye (mol kg^{-1}), X_{DR} is a measure of adsorption capacity, K_{DR} is the activity coefficient ($\text{mol}^2 \text{KJ}^2$), and ε is Polanyi potential.

$$\text{Polanyi potential} : \varepsilon = RT \ln (1 + 1/C_e) \quad (5)$$

The definitions for Equation (5) are as follows: R is the ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$) and T is the absolute temperature (K).

$$\text{Adsorption energy (E)} : E = (2K_{DR}) - 0.5 \quad (6)$$

The value of E (kJ mol^{-1}) indicates the adsorption mechanism physically or chemically. If the adsorption energy is $8 < E < 16 \text{ kJ mol}^{-1}$, the adsorption is physically controlled and $E < 8 \text{ kJ mol}^{-1}$ indicates that the adsorption proceeds physically (Dubinin et al., 1947).

$$\text{Pseudo–First–Order Kinetic Model} : \log (q_e - q_t)$$

$$= -k_1/2.303t + \log q_e \quad (7)$$

$$\text{Pseudo–Second–Order Kinetic Model} :$$

$$t/q_t = 1/k_2 q_e^2 + 1/q_e \cdot t \quad (8)$$

The definitions for Equations (7) and (8) are as follows: q_e is the adsorption capacity, k_1 is the rate constant of the pseudo-first-order kinetic model, and k_2 is the rate constant of the pseudo-second-order kinetic model

Extraction from lichen waste for antimicrobial activity

The dried and ground lichen (*Pseudoevernia furfuracea*) samples (8 g) were extracted with two different organic solvents (ethanol and methanol) using a Soxhlet apparatus. Subsequently, the organic solvents were removed using a rotary evaporator. The extracts were weighed and stored in dark glass vials in the refrigerator for use in subsequent studies.

Scanning of antimicrobial activity

In order to determine the antimicrobial activities of ethanol and methanol extracts of lichen samples, the test microorganisms called *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *Bacillus cereus* ATCC 10876, and *Candida albicans* ATCC 90028, and the agar diffusion method according to the criteria of the Clinical Laboratory Standards Institute (CLSI) were used (CLSI M02-A12, 2015; CLSI M44-A, 2004). For the scanning antimicrobial activity of extracts obtained from dye-loaded and un-loaded biomass, four different groups of pathogenic microorganisms were tested. *E. coli* represents the gram-negative bacterium, *S. aureus* is a gram-positive bacterium, *B. cereus* represents gram-positive forming endospore bacterium, and *C. albicans* is a yeast. The purpose of the antimicrobial activity tests in this study is to perform preliminary tests showing which microorganism is prevented more effectively by lichen extracts. It is suggested

that subsequent studies should be performed with microorganisms affected negatively by the extracts to develop treatment agents against these microorganisms.

The modified agar diffusion method was used to determine the antimicrobial activities of ethanol and methanol extracts of lichen samples (CLSI M44-A, 2004; Dubinin et al., 1947). The test organisms used for this purpose were *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *B. cereus* ATCC 10876, and *C. albicans* ATCC 90028.

Bacteria and the yeast from the test microorganisms were respectively incubated in NB and SDB at 37°C for 24 h. The densities of these cultures were set to the same turbidity of the 0.5 McFarland standard (at 625 nm, 0.08 to 0.1 absorbance) with sterile saline water.

The adapted cell suspensions were spread on the whole surface of Mueller-Hinton agar (MHA) plates for bacteria and potato dextrose agar (PDA) plates for yeast prepared in 90-mm Petri dishes using sterile swabs. MHA was used for bacteria, and PDA was used for yeast. Wells were made on the agar plates with sterile 6-mm cork borer. The $50 \mu\text{L}$ of extract solutions prepared with DMSO (0.2% w/v) was poured to the wells. The plates were kept in the refrigerator for 1 h to diffuse and then incubated at 37°C for 24 h. Cefotaxime (Bioanalyse, 30 mcg), Gentamicin (Bioanalyse, 10 mcg), and Fluconazole (MP Biomedicals, 1.25 mg ml^{-1}) were used as positive control and DMSO as the negative control. The plates were observed for the zone clearance around the wells. All assays were done in duplicate.

Testing the antimicrobial property of medical fabrics treated with lichen extracts

The non-woven fabric for medical usage was purchased from the medical market in Turkey. To provide antimicrobial property, the sterilized 1 cm^2 of fabric pieces was prepared. The extracts that were diluted with their organic solvents (ethanol and methanol) were absorbed into 1 cm^2 sterile pieces of fabric and then allowed to remove organic solvents. The prepared fabric pieces were placed in the inoculated Petri dishes. The negative control was used as a blank fabric, ethanol-impregnated fabric, and methanol-impregnated fabric. After 1 h in the refrigerator to diffuse, they were allowed to incubate at 37°C for 24 h. Clear zones formed after incubation were evaluated. All assays were done in duplicate.

Results and discussion

This study composed of three stages of experiments. In the first stage, dye biosorption experiments were carried out and optimal dye biosorption conditions were determined. Also, the calculations of the biosorption isotherm and kinetic model were done in the first step. In the second stage, the waste lichen biomass (dye loading) after biosorption was scanned in order to examine the changes in antimicrobial properties. The un-loading lichen biomass was used as control. The extraction of both dye-loading and un-loading lichen biomasses was done with ethanol and methanol solutions. The antimicrobial activities of these extracts were tested. In the last stage, the medical fabrics were treated

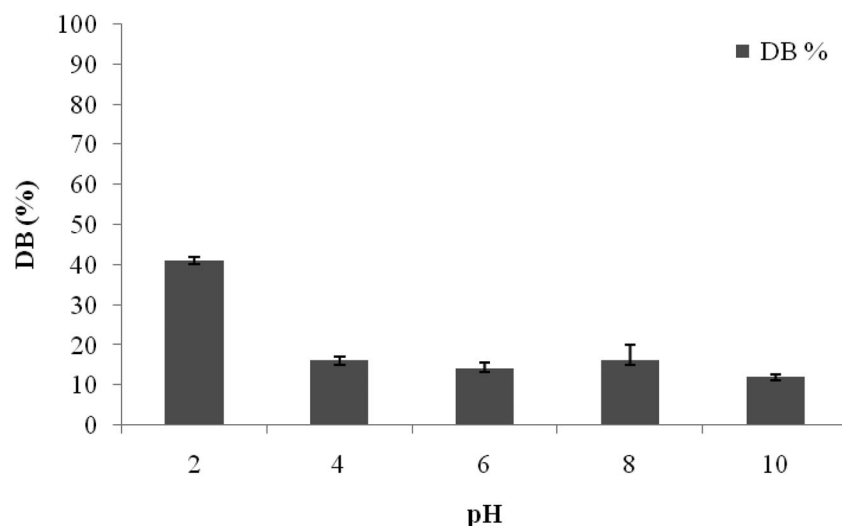


Figure 1. The influence of pH on the percentage of dye biosorption (DB%).

with the extracts of dye-loading and un-loading lichen and tested the antimicrobial function of these fabric pieces.

The influence of pH on dye biosorption

The pH of the solution is an important parameter for the biosorption process. The dye removal percentage was decreased by the increasing of the pH value. The optimal pH for dye biosorption by lichen biosorbent was found as 2 (Figure 1). Similarly, previous studies showed that other lichen biosorbents performed the highest anionic dye removal at pH 2 due to the positively charged lichen surface (Bayazit et al., 2019).

The influence of initial dye concentration on dye biosorption

The influence of different dye concentrations was examined, and it was observed that the increment of dye concentration caused a decrease in the dye biosorption process (Figure 2). Recently, biosorption studies reported that the pollutant biosorption percentage of lichen samples was decreased by increasing pollutant concentration (Dubinin et al., 1947; Gül et al., 2019; Şenol et al., 2019). It was also reported that the adsorption process was affected by the concentration of adsorbant negatively due to the saturation of the adsorbent surface (Crini et al., 2018).

The influence of contact time

To determine the influence of contact time, the dye concentrations in the solution were measured from 30 to 2880 min. Figure 3 shows that the biosorption percentage was reached a maximum value of 1440 min and the percentage did not increase after 1440 min. In Figure 3, there was an increase in the percentage of biosorption up to 1440 min and reached the highest value in 1440 min. After 1440 min, since the active groups on the biosorbent surface reached saturation, there was no increase or significant change in the percentage of biosorption (CLSI M02-A12, 2015; Gül et al., 2019;

Şenol et al., 2019). Therefore, the optimal contact time was determined as 1440 min in this study. Similarly, most of the recent studies about biosorption by lichens showed that the optimal contact time was 24 h in the literature (Dubinin et al., 1947; Gül et al., 2019; Şenol et al., 2019).

The influence of biosorbent dosage on dye biosorption

The influence of biosorbent dosage on dye biosorption was also examined. The results are given in Figure 4. An optimal biosorbent dosage was found as 2 g l^{-1} . Similarly, Kulkarni et al. (2014) reported that the dye removal rate was increased by the improvement of biomass dosage (Kulkarni et al., 2014). The results of this study were suitable with the results published in the literature previously (Sharma et al., 2019).

Biosorption isotherms and kinetics

Biosorption isotherm and kinetic models are given detailed information about the biosorption mechanism. The calculation of the most common models called Freundlich and Langmuir isotherm models was done in this study. The dye biosorption on lichen biosorbent was suited with the Langmuir model due to the highest R^2 value as 0.999 (Table 1). In order to get more detailed information about the dye biosorption mechanism, Dubinin-Radushkevich (D-R) isotherm model was studied. According to the D-R, there is a relationship between adsorption and surface porosity and pore volume. This model tested the adsorption from the energetic point of view. D-R model calculations showed that the biosorption energy was 5.30 kJ mol^{-1} , which revealed that the biosorption process was physical.

The pseudo-first-order and second-order kinetic models were used to find a suitable model for dye biosorption on lichen. As seen in Table 1 the biosorption of dye by lichen biosorbent was fitted with a pseudo-second-order kinetic model via the highest R^2 value of 0.998 and the closest values of $q_{e \text{ cal}}$ and $q_{e \text{ exp}}$.

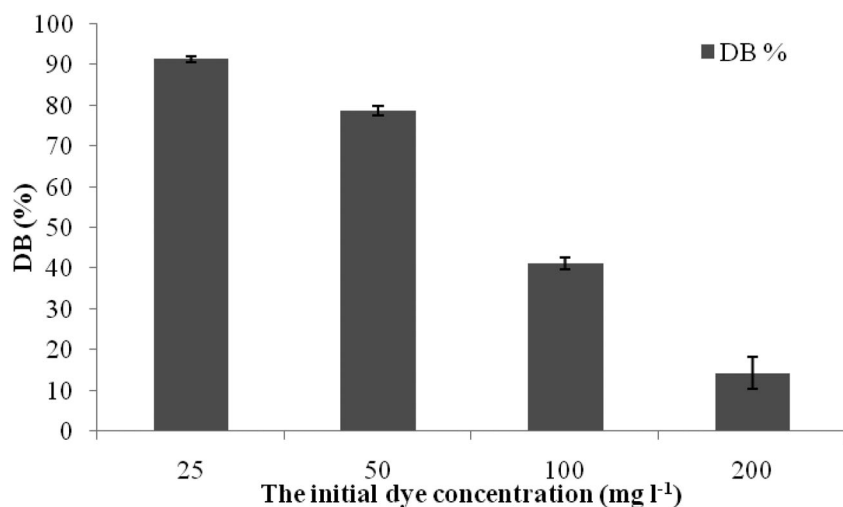


Figure 2. The influence of dye concentration on the percentage of dye biosorption (DB%).

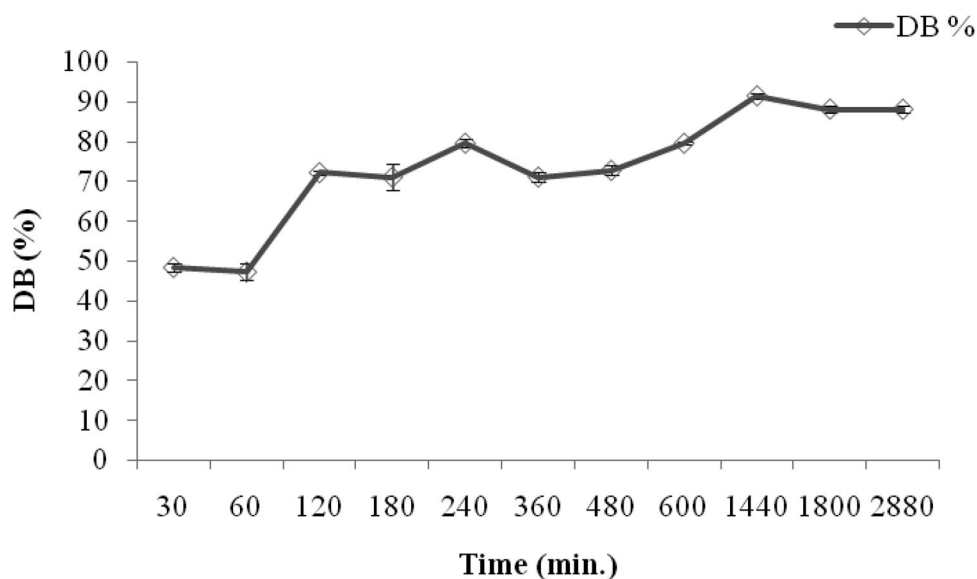


Figure 3. The influence of contact time on the percentage of dye biosorption (DB%).

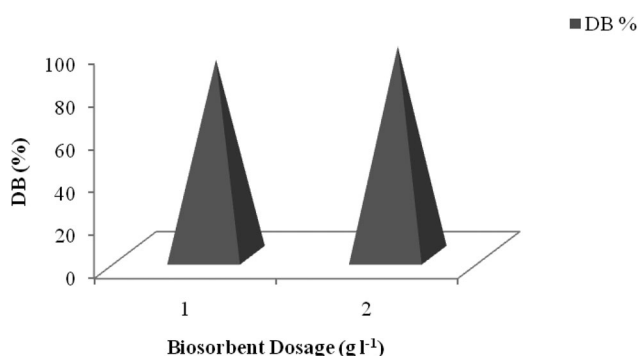


Figure 4. The influence of biosorbent dosage on the percentage of dye biosorption (DB%).

Scanning of antimicrobial activity

The results of *P. furfurace* extracts obtained by the agar well diffusion method are given in Table 2 and Figure 5. Several studies have been conducted on the antimicrobial activity of

Table 1. The isotherm and kinetic model calculations of dye biosorption on lichen biosorbent.

Model	Parameter	Value	
Isotherm	Langmuir	q_{max} (mg g ⁻¹)	
		K_L	
		R^2	
	Freundlich	K_F	
		n^{-1}	
Dubinin-Radushkevich		R^2	
		X_{DR}	
		$-K_{DR} \times 10^9$	
		E_{DR}	
		R^2	
Kinetic	Pseudo First-Order	$q_{e_{cal}}$	
		$q_{e_{exp}}$	
		k_1	
		R^2	
	Pseudo Second-Order	$q_{e_{cal}}$	
		$q_{e_{exp}}$	
			k_2
			R^2

various lichen species (Jha et al., 2017). It is understood that dyed lichen biomass released after dye biosorption does not lose its antimicrobial properties. In this study, *E. coli*

Table 2. The antimicrobial activity of *P. furfurace* extracts obtained from agar well diffusion method.

Well numbers Test organisms	Diameter of zone (mm)					
	1	2	3	4	5	6
<i>Escherichia coli</i> ATCC 25922	18	16	17	16	31	0
<i>Bacillus cereus</i> ATCC 10876	17	16	16	17	0	0
<i>Staphylococcus aureus</i> ATCC 29213	18	18	19	18	0	0
<i>Candida albicans</i> ATCC 7644	18	15	23	25	0	0

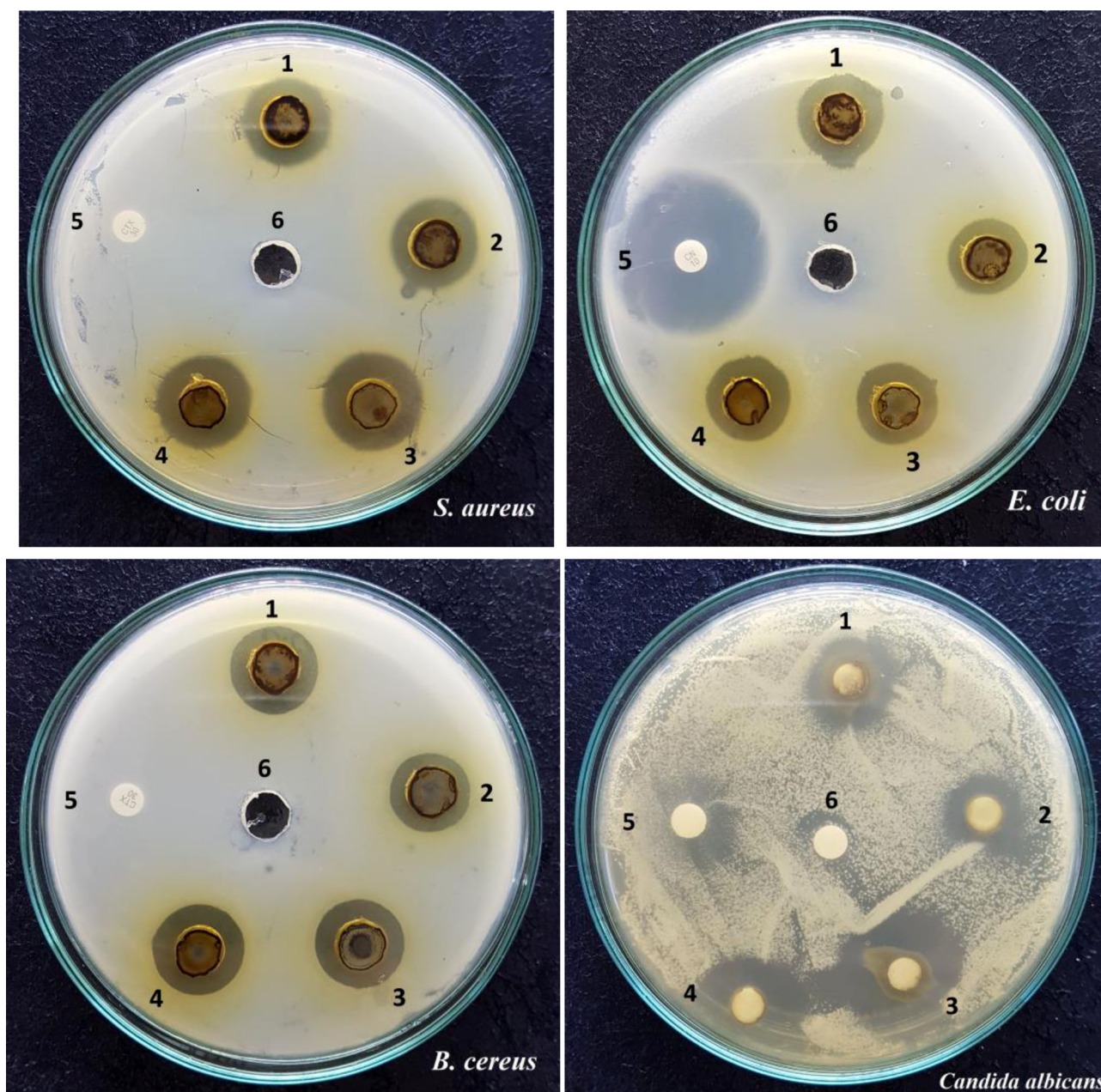


Figure 5. 1) Dyed sample, ethanol extract 2) Undyed sample, ethanol extract 3) Dyed sample, methanol extract 4) Undyed sample, methanol extract 5) Antibiotic 6) DMSO, * Antibiotic used as a positive control for *S. aureus* and *B. cereus*; Cefotaxime (Bioanalyse, 30 mcg), *The antibiotic used as a positive control for *E. coli*; Gentamicin (Bioanalyse, 10 mcg), *The antibiotic used as a positive control for *C. albicans*; Fluconazole (MP Biomedicals, 1,25mg ml⁻¹).

represented gram-negative, and *B. cereus* and *S. aureus* were gram-positive prokaryotic bacteria, while *C. albicans* was a eukaryotic yeast. The lichen extract components were found to be effective against prokaryotic and eukaryotic pathogenic microorganisms. The fact that lichen extract, which has such a broad spectrum effect, continues its effect after dye biosorption renders lichen biomass in waste condition

valuable. With the idea that this biomass can be reused, it has been designed to color the medical fabrics and at the same time to provide antimicrobial activity. However, further studies are needed on this subject.

After biosorption, the waste lichen extract-adsorbed fabrics were tested for their antimicrobial function. Also, the only lichen (not loaded with dye) extracts were used to

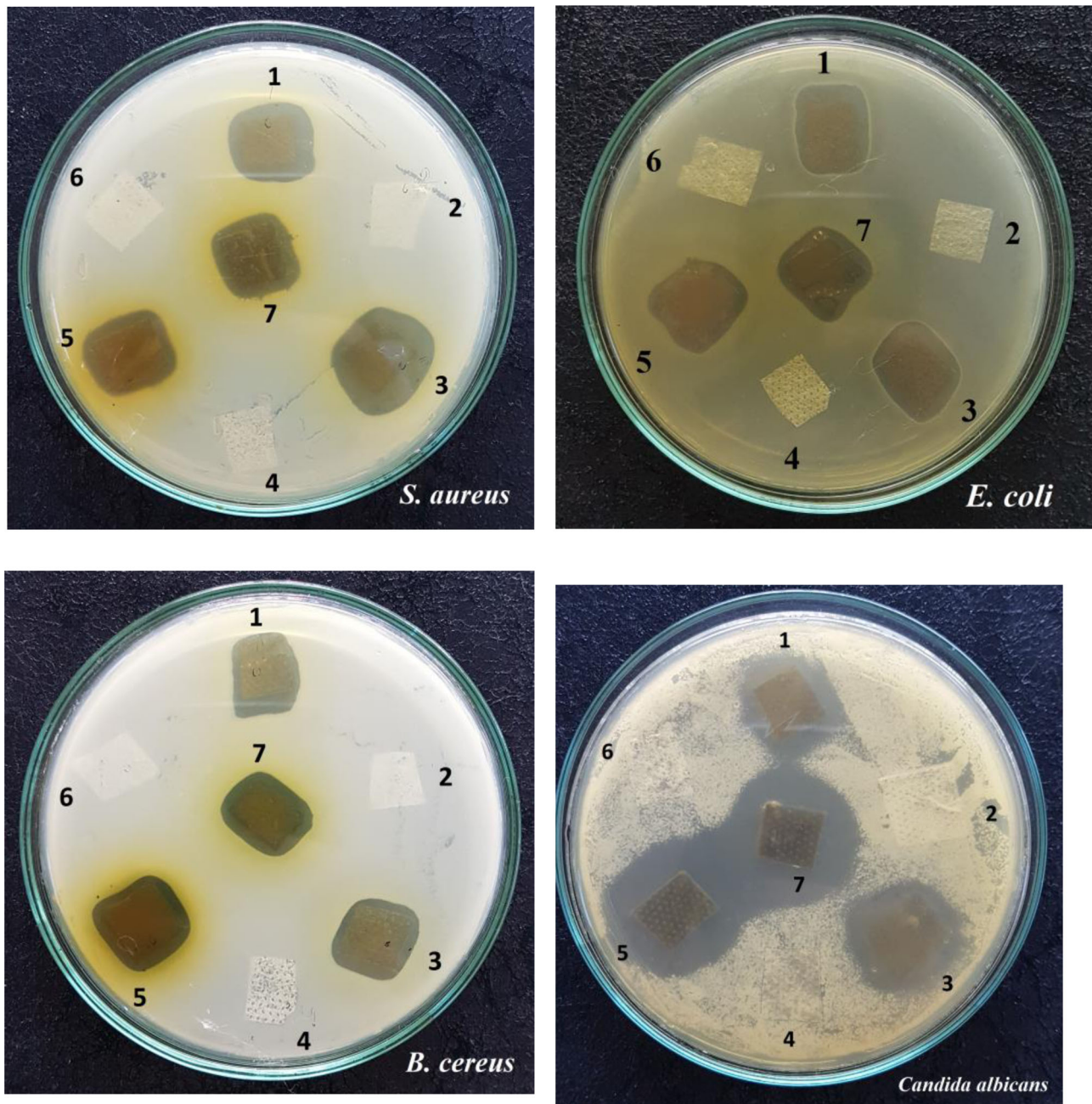


Figure 6. 1) Dyed sample, ethanol-impregnated fabric 2) Blank piece of fabric 3) Undyed sample, ethanol-impregnated fabric 4) Ethanol-impregnated fabric 5) Undyed sample, methanol-impregnated fabric 6) Methanol-impregnated fabric 7) Undyed sample, impregnated methanol extract fabric (* Fabric pieces are cut to 1 cm² size and used sterile).

observe any changes in the antimicrobial activity. As seen in Figure 6, the similar results occurred on the antimicrobial functions of fabrics that were dye-loaded (waste) and unloaded.

Similar results were showed in a previous study. The antimicrobial effectiveness of fabrics functionalized with the absorption of red pepper seed oil was determined, and this nonwoven fabric showed successful antimicrobial activity against *E. coli*, *S. aureus*, and *C. albicans* (Özyildiz et al., 2013). It is also reported that the growth of pathogenic microorganisms during the use and storage of textile products is an important problem. So the consumers especially working in hospitals tend to wear textile products functioning antimicrobial property (Gao & Cranston, 2008). To improve the

production of the textile industry, interest in the production of antimicrobial fabric is expected to increase.

Conclusion

This study composed of three experimental sections such as determination of textile dye removal potential of lichen biomass, examination of antimicrobial activity of waste lichen biomass formed after dye biosorption assays, and investigation of the antimicrobial properties of lichen extract-treated non-woven medical fabric. The results of commonly used textile dye biosorption experiments show that the lichen biomass was a very effective biosorbent removing 97.7% of dye from aqueous solution. Also, the calculations of

biosorption isotherms and kinetics were done. The adsorption of dye on lichen biosorbent was suitable with Langmuir isotherm and pseudo-second-order kinetic models. After biosorption, the waste lichen biomass loaded with dye was used to obtain lichen extract and this extract was scanned its antimicrobial property. At last, this extract was adsorbed by a medical cloth piece in order to examine antimicrobial functions. According to the results of this study, it is considered that after biosorption the antimicrobial effect of waste lichen biomass can be evaluated in different applications such as the production of fabrics having an antimicrobial function. Besides, this study is a preliminary study to show the use of the extracts from waste lichen biosorbents for dyeing and antimicrobial properties of medical fabrics. Also, the results open the door to the reusability of waste biosorbents. The results obtained from this preliminary study showed that waste lichen extracts can be used to produce medical fabrics having antimicrobial properties. For further studies, it is recommended to focus on the more detailed examinations and analyze the procurement of the medical garments and to develop more quality medical fabrics.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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