Commagene Journal of Biology

Boran, (2022) *Comm. J. Biol.* 6(1), 1-5.
DOI: 10.31594/commagene.1037538

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

Dye Removing with Dry and Wet Forms of Pure Bacterial Cellulose Produced by *Gluconacetobacter xylinus*

Filiz BORAN

Inonu University, Art and Science Faculty, Department of Biology, 44280, Malatya, TÜRKIYE
ORCID ID: Filiz BORAN: https://orcid.org/0000-0002-8801-7987

Received: 16.12.2021 **Accepted:** 17.02.2022 **Published online:** 30.03.2022 **Issue published:** 30.06.2022

Abstract: Bacterial cellulose is used in different areas. One of these areas is using this material as bioadsorbent for decolorization of different dyes. In the study, *Gluconacetobacter xylinus* was used for obtaining bacterial cellulose (BC). The wet and dry forms of the BC were utilized as bioadsorbent for removal of the Reactive Blue 171 (C.I Chrocion Blue HERD) (200 mg/L), Remazol Brilliant Blue R (150 mg/L) (C. I Reactive Blue 19), and Chrocion Green H-E4BD (100 mg/L) dyes. The highest decolorization rates were obtained with wet BC at the first use as 51%, 52% and 54% for CBHERD, RBBR, and CH GREEN dyes after 24 hours of incubation at 30°C, 150 rpm, respectively. These values were 11%, 21%, and 20% for dry forms of BC at the end of the first use. At the same time, the structure and morphology of bacterial cellulose were determined by SEM, XRD, and FTIR analysis.

Keywords: Bacteria, bioadsorbent, biologic material, bioremediation, textile dye.

Gluconacetobacter xylinus Tarafından Üretilen Saf Bakteriyel Selülozun Kuru ve Yaş Formları ile Boya Uzaklaştırılması

Öz: Bakteriyel selüloz farklı alanlarda kullanılmaktadır. Bu alanlardan biri, bu malzemeyi farklı boyaların renklerinin giderimi için biyoadsorban olarak kullanmaktır. Çalışmada, bakteriyel selüloz (BS) elde etmek için *Gluconacetobacter xylinus* kullanılmıştır. BS'nin yaş ve kuru formları, Reaktif Mavi 171 (CI Chrocion Mavi HERD) (200 mg/L), Remazol Parlak Mavi R'nin (150 mg/L) (C. I Reaktif Mavi 19) ve Chrocion Yeşil H-E4BD (100 mg/L) boyalarının uzaklaştırılması için biyoadsorban olarak kullanıldı. En yüksek renk giderme oranları, ilk kullanımda yaş BS ile 30°C, 150 rpm'de 24 saat inkübasyondan sonra CBHERD, RBBR ve CH Yeşil boyaları için sırasıyla %51, %52 ve %54 olarak elde edilmiştir. Bu değerler ilk kullanım sonunda BS'nin kuru formları için %11, %21 ve %20 idi. Aynı zamanda bakteriyel selülozun yapısı ve morfolojisi SEM, XRD ve FTIR analizleri ile belirlendi.

Anahtar kelimeler: Bakteri, biyoadsorban, biyolojik materyal, biyoremediasyon, tekstil boyası.

1. Introduction

Cellulose is the most abundant macromolecule in the world produced mainly by plants; however, it can also be produced by some bacteria (Kim et al., 2017). Some genera of bacteria such as *Acetobacter, Agrobacterium, Pseudomonas, Rhizobium, Sarcina, Azotobacter, Achromobacter, Aerobacter, Salmonella,* and *Escherichia* can synthesize cellulose but among them a gram negative bacterium *Gluconacetobacter xylinus* (*G. xylinus*) is the most preferred species. This species produces high amount of cellulose with high mechanical resistance (Gromet-Elhanan & Hestrin, 1963; Ross et al., 1991; Shoda & Sugano, 2005; Brown et al., 1996; Keshk et al., 2006; Karahan et al., 2011; Ullah et al., 2016). *G. xylinus* (formerly *Acetobacter xylinum*) is now known as *Komagataeibacter xylinus* after being reclassified depending on the 16s rRNA gene sequence (Yamada et al., 2012).

About 10-40% of the dyes used in the dyeing industries are released into the aquatic environment. This prevents sunlight from entering the water, thus reducing the photosynthetic activity and the amount of dissolved oxygen causing serious environmental problems and affecting the life of living things. Most importantly, some dyes are toxic (Georgouvelas et al., 2021; Rainert et al., 2021). There are several methods for removing of dyes such as adsorption that is economic and effective for treatment of wastewater (Anbia et al., 2010).

Bacterial cellulose (BC) is a biopolymer and used in many applications. One of these applications is using BC as bioadsorbent for decolorization of dyes (Mohite & Patil, 2014). Cellulose-based materials show high affinity for specific contaminants such as dyes (Georgouvelas et al., 2021). Because of the chemical groups on its surface, BC helps the adsorption of the charged pollutants.

The aim of the study was to screen and compare the bioadsorption ability of wet and dry forms of pure BC having different piece sizes during the repeated use.

2. Material and Methods

2.1. Bacterial strain used

Gluconacetobacter xylinus B759 was used as the bacterial strain for BC production. This bacterium was provided by Dr. Aynur Gül Karahan Çakmakçı. It was grown statically on Hestrin-Schramm (HS) agar plates containing (g/L) glucose, 20; peptone, 5; yeast extract, 5; Na_2HPO_4, 2.7; citric acid, 1.15; agar, 15 g at 30°C for 10 days. Then, it was stored at 4°C and subcultivated every 1 month.

*Corresponding author: filiz.kuru@inonu.edu.tr

2.2. Preparing Inoculum Culture Medium and Production of BC

Solid culture of *Gluconacetobacter xylinus* was inoculated into 50 mL HS broth medium composed of (g/L) glucose, 20; peptone, 5; yeast extract, 5; Na_2HPO_4, 2.7; citric acid, 1.15 (Hestrin & Schramm, 1954) and the liquid culture was incubated statically at 30°C for 10 days. After incubation, 1 mL from this culture was inoculated into 50 mL HS medium in 100 mL flask and incubated statically at 30°C for 10 days. This culture was used as stock inoculum.

HS broth was used as the main production medium for BC production. One mL from stock inoculum culture of *Gluconacetobacter xylinus* was transferred into 100 mL flasks with 40 mL HS broth. These cultures were incubated statically at 30°C for 10 days. Then the BC pellicles formed were filtered and washed three times using distilled water. NaOH at the concentration of 0.1 N was added onto BC pellicles that were kept in the water bath at 75°C for 2 hours. After this process, the pure BC pellicles were washed for a few times in distilled water.

2.3. Obtaining Wet and Dry Pure BC Samples

Some of the pure BC pellicles were kept in distilled water in the refrigerator at 4°C and used as wet BC. The others were lyophilized for 16 hours after freezing at -20°C for obtaining dry BC pellicles.

2.4. Dyes

In the study, Reactive Blue 171 (C.I Chrocion Blue HERD), Remazol Brilliant Blue R (C. I Reactive Blue 19), and Chrocion Green H-E4BD dyes were used at 200, 150, and 100 mg/L concentrations, respectively.

2.5. Dye Decolorization by Wet and Dry Pure BC Samples

The CB HERD, Remazol Brilliant Blue R, and Chrocion Green H-E4BD (the dyes mentioned above) decolorization ability of BC pellicles was tested during repeated-batch studies. The dry and wet BC samples were cut in 0.5 and 1.0 cm sizes. Then, 10 mL from each dye solution was transferred onto 10 or 20 BC pieces. The BC pieces were used for 3 times with the residence time of 24 hours. In each cycle, the medium was replaced with 10 mL fresh dye solution and incubated with the same BC pieces. The incubation condition was 30°C and 150 rpm. Dye decolorization was detected spectrophotometrically (Shimadzu-UV-1601, UV/Visible) for each dye at their maximum wavelengths. All experiments were performed in three replicates and dye removal percentages were calculated with SPSS 15.0 package program.

2.6. Characterization of BC Samples

X-ray diffraction (XRD), Scanning electron microscope (SEM), and Fourier transform infrared spectrometer (FTIR) analysis of dry BC samples were performed at Inonu University Scientific and Technological Research Center Laboratories.

3. Results and Discussion

3.1. BC Production and Dye Removal by Wet and Dry Bacterial Cellulose

One of the bacterial cellulose applications is the use of cellulose as a bioadsorbent. For this aim, in the study, wet and dry forms of pure BC pellicles obtained from *Gluconacetobacter xylinus*, an efficient BC producer, were used as bioadsorbents for testing their dye decolorization activity. Firstly, BC pellicles were obtained after incubation of *G. xylinus* in HS medium for 10 days (Fig. 1a), secondly the BC samples were filtered (Fig. 1b) and; then, they were washed for 2 times (Figs. 1c, d).

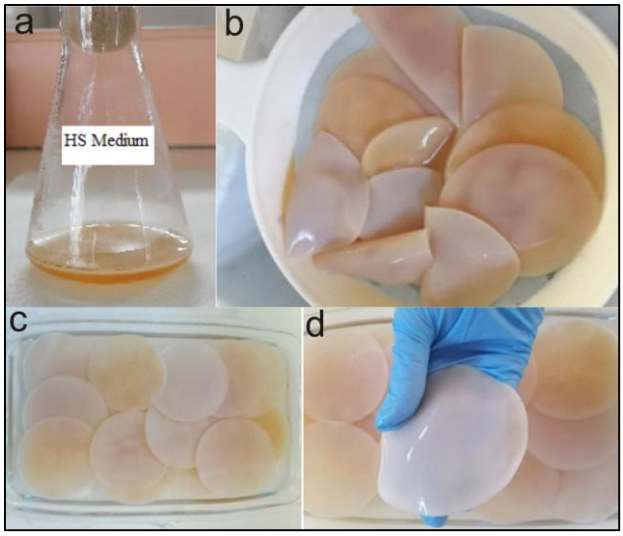

Figure 1. Production and obtaining of BC: a- Production in HS medium for 10 days, b- Filtering of the BC, c- BC after first wash with distilled water d- BC after second wash with distilled water.

For obtaining pure BC pellicles, they were placed in the containers containing 0.1 N NaOH and the samples were kept in a water bath at 75°C for 2 hours to remove bacteria (Fig. 2a). Then, they were filtered and washed. Figures 2b-e show the pure BC pellicles after filtration and washing for a few times for neutralization.

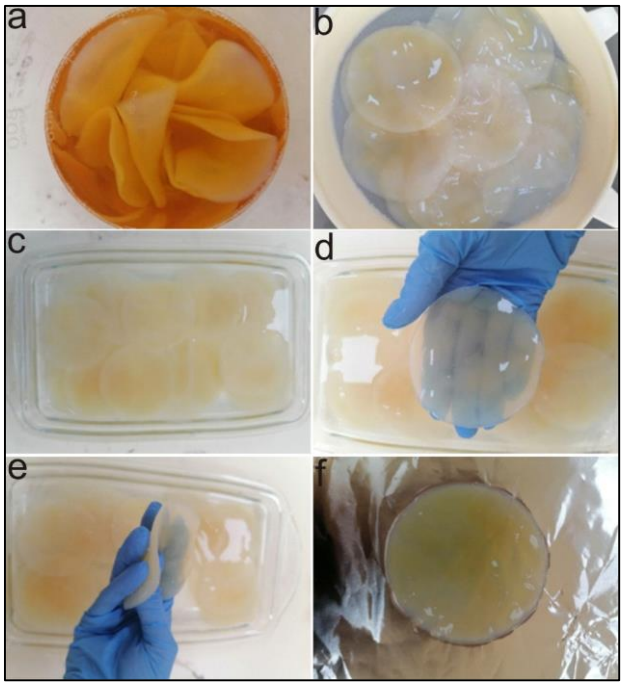

Figure 2. Obtaining pure bacterial cellulose **a-** Pure cellulose pellicles in 0.1 N NaOH, **b-** Straining of the pure cellulose pellicles, **c-** First wash with distilled water **d-** Second wash with distilled water **e-** Third wash with distilled water **f-** Pure and wet cellulose pellicles.

For obtaining the dry and pure BC pellicles, firstly they were frozen at -20°C and then these frozen pellicles were lyophilized. In addition, pure wet BC samples were kept in the refrigerator until used. The obtained dry and wet cellulose pellicles were cut in 0.5 and 1.0 cm sizes as shown in Figure 3a-d.

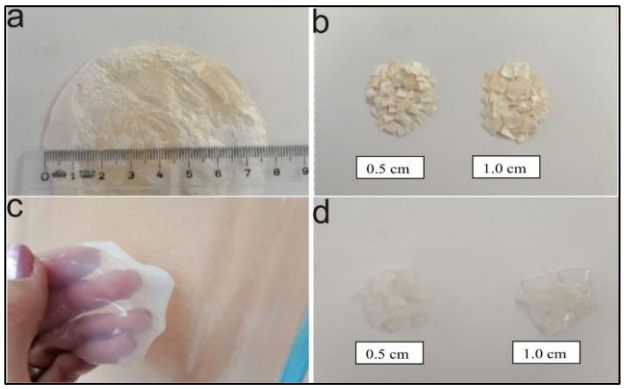

Figure 3. a- Dry and pure cellulose pellicle b- Dry and pure cellulose pellicles in 0.5 and 1.0 cm sizes c- Wet and pure cellulose pellicle d- Wet and pure cellulose pellicles in 0.5 and 1.0 cm sizes.

After obtaining the pure wet and dry BCs, they were used for biosorption of the textile dyes. Firstly, the Reactive Blue 171 dye biosorption ability of the two BC forms was tested. When 10 and 20 pieces of the 0.5 cm size wet and dry BCs were used, no color removal was observed during the first use. Similarly, 10 pieces of wet and dry BCs in 1.0 cm size showed no color removal. However, when 20 pieces of the 1.0 cm size wet or dry BCs used, dye removal was achieved during the first use and 10% and 51% dye decolorization rates were obtained with dry and wet BCs, respectively. While no color removal was observed with the dry BC during the second use, with wet BC samples 22% and 16% dye removals were determined, respectively (Fig. 4). The photographs and spectrophotometric scans of the Reactive Blue 171 dye solutions incubated with wet and dry BCs were given in in Figure 5.

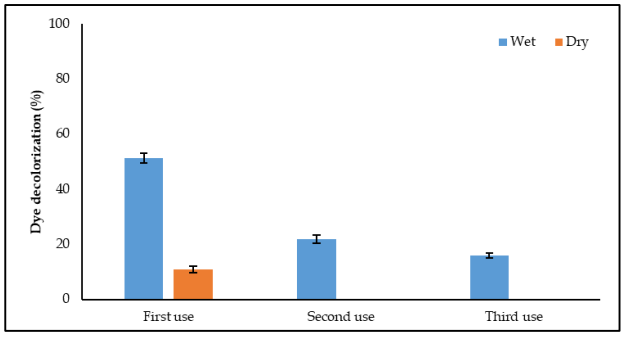

Figure 4. Reactive Blue 171 dye removal activity of the wet and dry BCs during the repeated use (Black line- control, Red line- dry BS, Blue line- wet BS).

RBBR and Chrocion Green dye removal activities of the 20 pieces of the 1.0 cm size wet and dry BCs were also tested. The wet BCs showed 52%, 35%, and 23% RBBR dye decolorization activity during the first, second, and third uses, respectively. On the other hand, these values were 21%, 18%, and 11% for dry BCs (Fig. 6). Vyjayanthi and Suresh (2010) used palladized bacterial cellulose for decolorizing of Drimarene Red at 100 mg/L dye concentration in a rotating catalyst contact reactor (RCCR) and it was stated that approximately 90% of the dye was discolored in 25 minutes at pH 2 in RCCR.

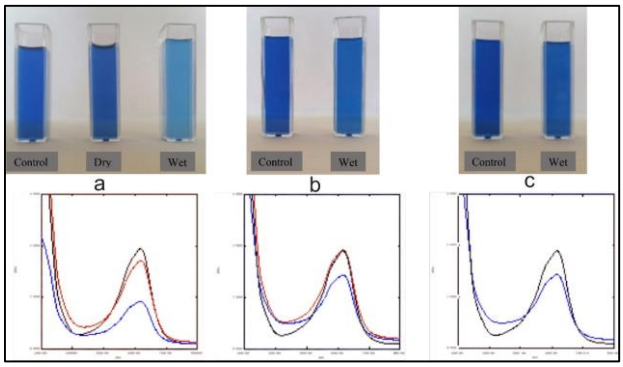

Figure 5. The photographs and spectrophotometric scans of the Reactive Blue 171 dye solutions incubated with wet and dry BCs: a- First use, b- Second use, c- Third use (Black line- control; Red line- dry BS; Blue line- wet BS).

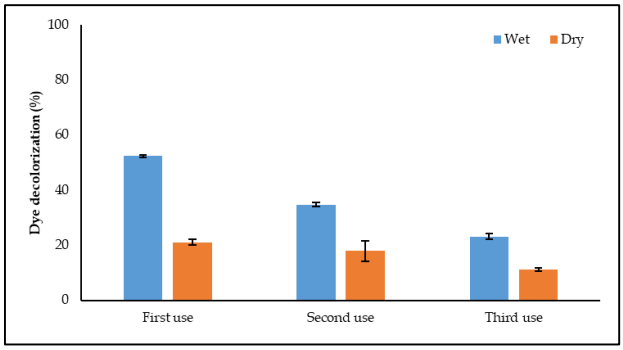

Figure 6. RBBR dye removal activity of the wet and dry BCs during the repeated use

The photographs and spectrophotometric scans of the RBBR dye solutions incubated with wet and dry BCs were given in in Figure 7.

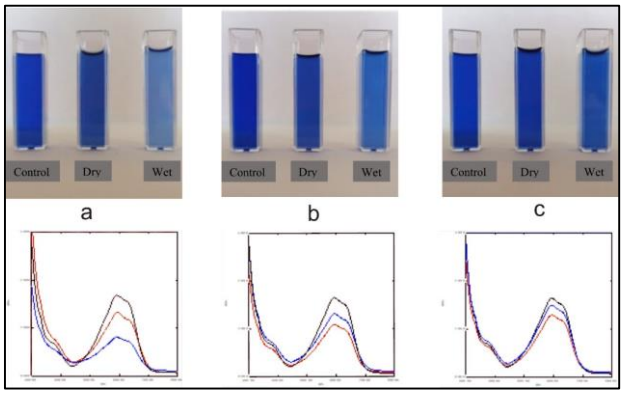

Figure 7. The photographs and spectrophotometric scans of the RBBR dye solutions incubated with wet and dry BCs a- First use, b- Second use, c- Third use (Black line- control; Red line- dry BS; Blue line- wet BS).

The wet BCs showed 54%, 34%, and 23% Chrocion Green dye decolorization during the first, second, and third uses. On the other hand, while 20% color removal was detected with dry BC during the first use, a little color removal was obtained during the second and third uses (Fig. 8). Figure 9 shows the photographs and spectrophotometric scans of the Chrocion Green dye solutions incubated with wet and dry BCs. Dye removal activity of microbial cellulose from *Komagataeibacter saccharivorans* LN886705, a cellulose producing bacterium,

was tested against various dyes (Malachite Green, Bromophenol Blue, Bismark Brown Y, Orange G, Reactive Blue 221, Acridine Orange, Tryphan Blue, Reactive Green 19, and Indigo Carmine). Obtained cellulose was added into the dye solution media at a ratio of 2% and incubated at 30°C and 150 rpm. The dye removal percentages were determined by taking samples from the dye solutions at the 4th, 24th, and 72nd hours. For indigo carmine dye (50 mg/L), only about 5% color removal was observed at the 24th hour and only about 13% at the 72nd hour. On the contrary, the best dye removal rates were seen in Tryphan Blue and Acridine Orange dyes. (Birben, 2019).

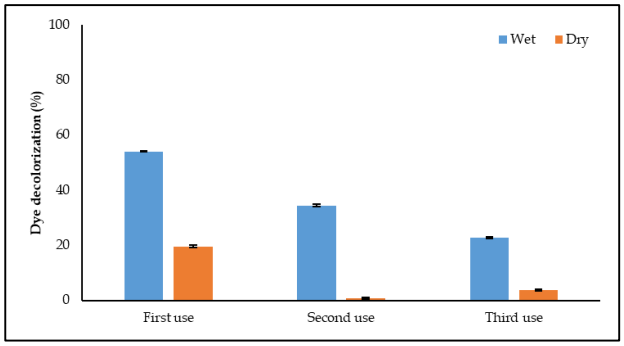

Figure 8. Chrocion Green dye removal activity of the wet and dry BCs during the repeated use

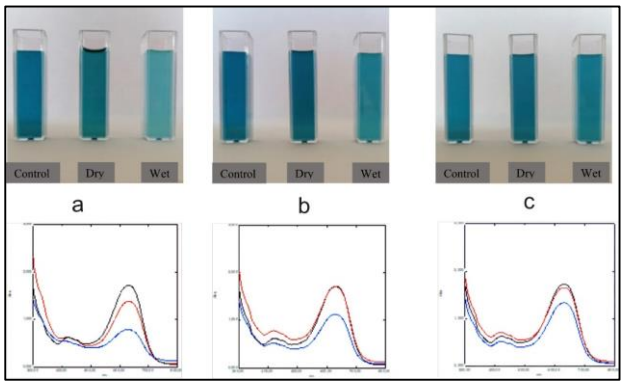

Figure 9. The photographs and spectrophotometric scans of the Chrocion Green dye solutions incubated with wet and dry BCs a- First use, b- Second use, c- Third use (Black line- control; Red line- dry BS; Blue line- wet BS).

3.2. Characterization of Bacterial Cellulose

The characteristics of BC were determined by SEM, FTIR, and XRD. Figure 10 shows the SEM image of BC. SEM image shows the fibrilar structure of bacterial cellulose. According to this image, the fibril widths of the sample was determined as 37.72 nm. Ruka et al. (2012) reported the fibril width as 40 nm for BC. XRD analysis (Fig. 11a) showed two peaks that refer to the presence of cellulose type 1 and 2 for BC as 22°≤ 2θ ≤23°and 13°≤2θ ≤15° (Leal et al., 2021). Figure 11b shows the FTIR spectrum of bacterial cellulose with characteristic peaks at around 3300, 2880, 1640, and 1057 cm^{-1} (Lin et al., 2013; Yim et al., 2017).

4. Conclusions

Dyes are used by many industrial fields such as the textile industry and approximately 10-20% of these dyes are discharged into wastewater. Textile wastewaters, which are released into the environment without being sufficiently treated, contain dyes with a high pollution load. The release of wastewater containing dyes into aquatic environments is aesthetically undesirable. However, it is also very harmful for the aquatic ecosystem and human health. Degradation of dyes is very difficult with traditional methods. Therefore, it is very important to find effective and new methods for removing dyes. The results of this study show that bioadsorption of dyes by bacterial cellulose may be an effective and promising solution to solve this environmental pollution problem with such a cheap, easy, and environmental friendly application.

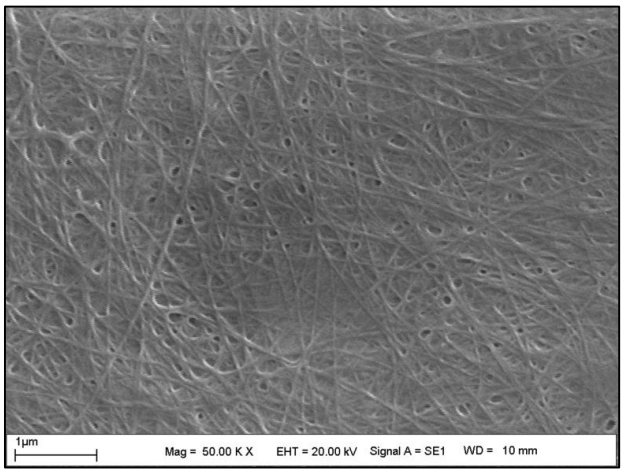

Figure 10. The SEM image of bacterial cellulose

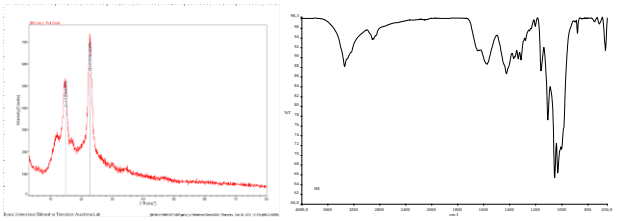

Figure 11. The physio-chemical characterization of bacterial cellulose by XRD (a) and FTIR (b) analyzes

Acknowledgements: This study was supported by Inonu University Scientific Research Projects Coordination Unit (Grant No: FBA-2019-1797).

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The author declares that there is no conflict of interest.

References

Anbia, M., Hariri, S.A., & Ashrafizadeh, S.N. (2010). Adsorptive removal of anionic dyes by modified nanoporous silica SBA-3. *Applied Surface Science*, 256(10), 3228–3233. https://doi.org/10.1016/j.apsusc.2009.12.010

Birben, M. (2019). Investigation on the use of microbial cellulose for dye decolorization. Master of Science, Department of Biology, Hacettepe University. Retrieved from: http://www.openaccess.hacettepe.edu.tr:8080/xmlui/bitstream/handle/11655/9389/10268015.pdf?sequence=1&isAllowed=y

Brown, R.M., Saxena, I.M., & Kudlicka, K. (1996). Cellulose biosynthesis in higher plants. *Trends in Plants Science*, 5(1), 149-156. http://doi.org/10.1016/S1360-1385(96)80050-1

Georgouvelas, D., Abdelhamid, H.N., Li, J., Edlund, U., & Mathew, A.P. (2021). All-cellulose functional membranes for water treatment: Adsorption of metal ions and catalytic decolorization of dyes. *Carbohydrate Polymers*, 264, 1-10. http://doi.org/10.1016/j.carbpol.2021.118044

Gromet-Elhanan, Z., & Hestrin, S. (1963). Synthesis of cellulose by *Acetobacter xylinum* VI. Growth on citric acid-cycle intermediates. *Journal of Bacteriology*, 85(2), 284-292. http://doi.org/10.1128/jb.85.2.284-292.1963

Hestrin, S., & Schramm, M. (1954). Synthesis of cellulose by *Acetobacter xylinum* 2. preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *Biochemical Journal,* 58(2), 345-352. http://doi.org/10.1042/bj0580345

Karahan, A.G., Akoğlu, A., Çakır, İ., Kart, A., Çakmakçı, M.L., Uygun, A., & Göktepe, F. (2011). Some properties of bacterial cellulose produced by new native strain *Gluconacetobacter* sp. A06O2 obtained from turkish vinegar. *Journal of Applied Polymer Science,* 121(3), 1823-1831. http://doi.org/10.1002/app.33818

Keshk, S.M.A.S., Razek, T.M.A., & Sameshima, K. (2006). Bacterial cellulose production from beet molasses. *African Journal of Biotechnology*, 5(17), 1519-1523.

Kim, J.H., Park, S., Kim, H., Kim, H.J., Yang, Y.H., Kim, Y.H., …. & Lee, S.H. (2017). Alginate/bacterial cellulose nanocomposite beads prepared using *Gluconacetobacter xylinus* and their application in lipase immobilization. *Carbohydrate Polymers*, 157, 137–145. https://doi.org/10.1016/j.carbpol.2016.09.074

Leal, A.N.R., Lima, A.C.A., Azevedo, M.G.F.A., Santos, D.K.D.N., Zaidan, L.E.M.C., Lima, V.F., & Filho, I.J.C. (2021). Removal of Remazol Black B dye using bacterial cellulose as an adsorbent. *Scientia Plena*, 17, 3, 034201. http://doi.org/10.14808/sci.plena.2021.034201

Lin, W.C., Lien, C.C., Yeh, H.J., Yu, C.M., & Hsu, S.H. (2013). Bacterial cellulose and bacterial cellulose–chitosan membranes for wound dressing applications. *Carbohydrate Polymers*, 94, 603– 611. https://doi.org/10.1016/j.carbpol.2013.01.076

Mohite, B.V., & Patil, S.V. (2014). A novel biomaterial: bacterial cellulose and its new era applications. *Biotechnology and Applied Biochemistry,* 61(2), 101-110. http://doi.org/10.1002/bab.1148

Rainert, K.T., Nunes, H.C.A., Gonçalves, M.J., Helm, C.V., & Tavares, L.B.B. (2021). Decolorization of the synthetic dye Remazol Brilliant Blue Reactive (RBBR) by *Ganoderma lucidum* on bio-adsorbent of the solid bleached sulfate paperboard coated with polyethylene terephthalate. *Journal of Environmental Chemical Engineering*, 9(2), 1-9. http://doi.org/10.1016/j.jece.2020.104990

Ross, P., Mayer, R., & Benziman, M. (1991). Cellulose biosynthesis and function in bacteria. *Microbiological Reviews*, 55(1), 35-58. http://doi.org/10.1128/mr.55.1.35-58.1991

Ruka, D.R., Simon, G.P., & Dean, K.M. (2012). Altering the growth conditions of *Gluconacetobacter xylinus* to maximize the yield of bacterial cellulose. *Carbohydrate Polymers*, 89, 613–622. https://doi.org/10.1016/j.carbpol.2012.03.059

Shoda, M., & Sugano, Y. (2005). Recent advances in bacterial cellulose production. *Biotechnology and Bioprocess Engineering*, 10(1), 1-8. http://doi.org/10.1007/BF02931175

Ullah, H., Santos, H.A., & Khan, T. (2016). Applications of bacterial cellulose in food, cosmetics and drug delivery. *Cellulose*, 23, 2291–2314. http://doi.org/10.1007/s10570-016-0986-y

Vyjayanthi, J.P., & Suresh, S. (2010). Decolorization of drimarene red dye using palladized bacterial cellulose in a reactor. *Water Environment Research,* 82(7), 601-609. http://doi.org/10.2175/106143009x12529484816114

Yamada, Y., Yukphan, P., Vu, H.T.L., Muramatsu, Y., Ochaikul, D., Tanasupawat, S., & Nakagawa, Y. (2012). Description of *Komagataeibacter* gen. nov., with proposals of new combinations (*Acetobacteraceae*). *The Journal of General and Applied Microbiology,* 58, 397-404. http://doi.org/10.2323/jgam.58.397

Yim, S.M., Song, J.E., & Kim, H.R. (2017). Production and characterization of bacterial cellulose fabrics by nitrogen sources of tea and carbon sources of sugar. *Process Biochemistry*, 59, 26–36. https://doi.org/10.1016/j.procbio.2016.07.001