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INVESTIGATION OF BIOFILM STRUCTURE IN A MEMBRANE BIOREACTOR (MBR)

MEMBRAN BİYOREAKTÖRDE BİYOFİLM YAPISININ İNCELENMESİ

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ABSTRACT

This study investigates in detail the structural and chemical properties of the biofilm formed in a membrane bioreactor (MBR) system used in the treatment of real textile wastewater. The performance of the MBR system was analyzed based on color, chemical oxygen demand (COD), and dissolved organic carbon (DOC) removal. The results showed that COD removal was approximately 50%, while DOC and color removal remained quite limited. The biofilm formed on the membrane surface was characterized by Fourier transform infrared (FT-IR), thermogravimetric analysis-differential thermal analysis (TG/DTA), and differential scanning calorimetry (DSC) analyses. Analysis results revealed that the biofilm structure consists mainly of extracellular polymeric substances (EPS) of protein and polysaccharide origin, along with textile dyes and inorganic components. Heavy metal analyses showed that elements such as Ca, Mg, and Si play a binding role in biofilm formation. These findings indicate that integration of MBR systems with advanced treatment processes is necessary to increase their effectiveness in the treatment of textile wastewater.
Keywords: Textile wastewater, membrane bioreactor, biofilm

ÖZET

Bu çalışma, gerçek tekstil atık suyunun arıtılmasında kullanılan membran biyoreaktör (MBR) sisteminde oluşan biyofilm tabakasının yapısal ve kimyasal özelliklerini ayrıntılı olarak incelemektedir. MBR sisteminin performansı, renk, kimyasal oksijen ihtiyacı (KOİ) ve çözünmüş organik karbon (ÇOK) giderimi açısından analiz edilmiştir. Sonuçlar, KOİ gideriminin yaklaşık %50 olduğunu, ÇOK ve renk gideriminin ise oldukça sınırlı kaldığını göstermiştir. Membran yüzeyinde oluşan biyofilm, Fourier dönüşümlü kızılötesi (FT-IR), termogravimetrik analiz-diferansiyel termal analiz (TG/DTA) ve diferansiyel tarama kalorimetrisi (DSC) analizleriyle karakterize edilmiştir. Analiz sonuçları, biyofilm yapısının esas olarak protein ve polisakkarit kökenli hücre dışı polimerik maddelerden (EPS), tekstil boyalarından ve inorganik bileşenlerden oluştuğunu ortaya koymuştur. Ağır metal analizleri, Ca, Mg ve Si gibi elementlerin biyofilm oluşumunda bağlayıcı bir rol oynadığını göstermiştir. Bu bulgular, tekstil atık suyunun arıtılmasında MBR sistemlerinin etkinliğini artırmak için gelişmiş arıtma süreçleriyle entegre edilmesinin gerekli olduğunu göstermektedir.

Anahtar Kelimeler: Tekstil atıksu, membran biyoreaktör, biyofilm

INTRODUCTION

The textile industry is a significant source of environmental pollution due to its high-water consumption and the generation of wastewater containing complex pollutants during its production processes. Textile industry wastewater is characterized by high organic load, dyes, salinity, and compounds with low biodegradability. Over the years, different treatment methods such as physical, chemical, and biological processes have been effectively used to remove the pollutant from textile wastewaters (Saad et al., 2017). Biological treatment has been reported to be a preferable alternative because it produces less sludge and is of low cost compared to physico-chemical treatments (Kocaer & Alkan, 2002).

Membrane bioreactor (MBR) systems combine the decomposition of organic matter through biological processes with membrane filtration to produce high-quality effluent, offering advantages over conventional activated sludge systems such as high solid-liquid separation, smaller plant footprint, and better discharge water quality (Lin et al., 2023). These features have made MBR technology an attractive option, particularly in the treatment of industrial wastewater and in applications with demanding loads, such as the textile sector (Uddin et al., 2024). However, the high levels of dissolved organic matter, dyes, and complex chemical components in textile wastewater directly affect the performance of MBR systems and may require additional processes, particularly in the removal of dissolved organic carbon and color (Ergül, 2014). Furthermore, membrane fouling and clogging in MBR processes are critical limiting factors determining system efficiency and operating costs, and therefore, understanding biofilm formation and fouling mechanisms is important for improving treatment efficiency (Lin et al., 2023). Recent studies have reported that hybrid systems (e.g., MBBR–MBR, activated carbon-assisted MBR) provide higher COD, color, and dissolved organic matter removal in textile wastewater (Uddin et al., 2024; Kozak et al., 2021). In this context, in addition to the advantages offered by MBR technology, process optimization and advanced treatment integrations should be evaluated to improve its performance.

Despite the extensive research on MBR systems, most studies in the literature rely on synthetic wastewater or focus primarily on overall treatment efficiency. There is a significant knowledge gap regarding the detailed structural and thermal characterization of biofilms formed under the complex matrix of real industrial textile wastewater, which contains various dyes, surfactants, and inorganic salts. Specifically, the combined use of FT-IR, TG/DTA, and DSC to deconstruct the fouling layer's organic and inorganic synergy remains under-explored in full-scale applications. Understanding these specific fouling mechanisms is essential for transitioning from general maintenance to targeted membrane management. Therefore, this study aims to fill this gap by providing an in-depth analysis of the biofilm/cake layer formed in a real-scale textile MBR, identifying the specific roles of textile dyes and inorganic binders. The findings are expected to contribute to the development of more effective strategies for controlling membrane fouling in textile wastewater treatment.

MATERIALS-METHOD

MBR For Textile Wastewater

The biofilm was collected from the MBR of the wastewater treatment plant of the Textile Industry located in Kahramanmaras, Türkiye. The characteristics of real textile wastewater are presented in Table 1. Since the study was conducted on a full-scale industrial treatment plant, average operating values during the sampling period were used. The HRT and SRT were approximately 18-24 hours and 25-40 days, and the MLSS concentration was maintained around 8000-10000 mg/L, which is typical for industrial textile MBR applications.

Table 1. Characteristics of Real Textile Wastewater

Parameters		Unit	Characteristics of real textile wastewater
pH		-	9-9.5
DOC		mg/L	299
COD		mg/L	1378±100
Color		Pt-Co	2380±100
	λ_{436}	m^{-1}	61.8±10
Color	λ_{525}	m^{-1}	46.5±10
	λ_{620}	m^{-1}	23.6±5

Aeration was provided by a coarse bubble diffuser system located beneath the membrane modules, maintaining a dissolved oxygen (DO) level of 2.0-3.0 mg/L to support biological activity and provide surface scouring to control biofilm growth. The system was monitored over a period of about 3 months to allow for the development of a mature

and stable biofilm layer. The industrial-scale MBR system operated as a submerged configuration where hollow fiber membrane modules were directly immersed in the biological reactor. The membranes are made of polyvinylidene fluoride (PVDF) with a nominal pore size of 0.04 µm. Textile wastewater was continuously fed to the reactor, while the membrane permeate was continuously withdrawn under a flux of 12.40 LMH (L/m^2h). During operation, the transmembrane pressure (TMP), which is an indicator of the extent of membrane fouling, was continuously monitored and reached approximately -348 mbar. The membrane flux was monitored using an online computer connected to an electronic balance.

Analysis

The color measurements were taken using the HACH DR-5000 UV-vis spectrophotometer (Cole Parmer Instrument, Loveland, CO., USA). DOC, TN, TC, and IC were analyzed using the Shimadzu TOC-VCPN/TNM-1 (Kyoto, Japan). COD measurements were conducted using the dichromate-closed reflux Colorimetric Method as defined by Standard Methods (5220 D). pH was measured with a pH meter (Thermo, Orion 4 Star, Indonesia). Heavy metal analysis was performed using Flame Atomic Absorption Spectrometry (FAAS) (AA 680, Shimadzu). Heavy metal levels in the water circulation system were measured monthly. To prevent changes in the parameters, water samples for measurement were collected in PET (polyethylene terephthalate) bottles, and the pH was reduced to 2.5 using 1 N HNO_3. Functional groups on the contaminated membrane surface were identified using FT-IR (Perkin Elmer Spectrum 400, Germany) (Sahinkaya et al., 2018). TG-DTA (SII 6300, Japan) analysis was performed at a heating rate of 10 °C/min and a temperature range of -30 to 600 °C. DSC (Perkin Elmer x DSC-4000) analyses were performed in a nitrogen (N_2) gas environment at a heating rate of 10 °C/min and a temperature range of 30-400 °C.

RESULTS AND DISCUSSION

Performance Of The MBR

The color removal performance in the MBR is shown in Table 2. The color removal efficiency was 14.7%, 6.9 %, 5.1%, and 12.6% for Res 436, 525, 620, and Pt-Co, respectively. These results are consistent with prior studies, which reported that MBR systems alone demonstrate limited efficacy against dissolved and, in particular, refractory azo and anthraquinone-based dyes (Hai et al., 2007; Holkar et al., 2016). While membrane processing is effective in removing particulate and colloidal color-forming substances, it may be insufficient for retaining low molecular weight and water-soluble dye fractions. Forgacs et al. (2004) reported that the presence of aromatic structures and chromophore groups resistant to biological degradation is associated with low color removal efficiencies. The results indicate that integrating MBR systems with complementary processes such as advanced oxidation, adsorption, or coagulation may be necessary to enhance color removal in textile wastewater.

Table 2. The Color Removal Performance of MBR

Color	Influent	Effluent	Removal efficiency (%)
λ_{436} (m^{-1})	61.8±10	52.7±10	14.7±1
λ_{525} (m^{-1})	46.5±10	43.3±10	6.9±1
λ_{620} (m^{-1})	23.6±5	22.4±5	5.1±1
Pt-Co	2380±100	2080±100	12.6±5

The COD and DOC removal efficiency of the system was about 50% and 3.5%, respectively (Table 3). The limited COD removal in the present system likely reflects the high proportion of non-biodegradable, recalcitrant organics typical of textile wastewater, which are known to challenge biological treatment processes unless supplemented by advanced or combined treatment stages (Uddin et al., 2024). In line with COD results, DOC showed minimal reduction, indicating that the MBR predominantly removed particulate and readily biodegradable organic fractions while solution-phase refractory organics persisted in the effluent (Yang et al., 2020).

Table 3. The COD and DOC Removal Performance of MBR

Parameters	Influent	Effluent	Removal efficiency (%)
COD (mg/L)	1378±100	692.5±10	50±5
DOC (mg/L)	299±10	288.4±10	3.5±1

Heavy Metal Analysis

Table 4 shows the heavy metal concentrations in textile wastewater. The wastewater contained 4.4% Ca, 0.07% Al, 0.38% Mg, 0.24% Si, and 0.008% Cr. Inorganic elements with positive divalent properties, such as Ca, Si, and Mg, are known to act as binding elements in biofilm formation. In addition, these elements are effective in biofilm

formation by creating bridges between cells and biopolymers (Çiftçioğlu, 2021). Mg and Ca indicate that alkaline earth metal ions, originating from softening, fixing, and auxiliary chemicals commonly used in textile wastewater, accumulate in the biofilm. Calcium and magnesium are known to increase inorganic fouling by contributing to precipitation and scaling processes on the membrane surface (Le-Clech et al., 2006). Al is commonly found in textile dyeing stabilizers and flocculant additives. Silica-containing fillers, surfactants, and silicates from textile fibers condense on the membrane. Silicate species are an important indicator of fouling, particularly because they crystallize at high temperatures, contributing to inorganic fouling (Guo et al., 2012).

Table 4. Heavy Metal Concentration in Textile Wastewater

Heavy metal	Unit	Concentration
Cr_3/Cr_4 (Chromium-III/ Chromium-VI)	mg/kg	0.88
Mg (Magnesium)	mg/kg	38.87
Ca (Calcium)	mg/kg	446.1
Al (Aluminium)	mg/kg	7.139
Si (Silisium)	mg/kg	2476

FT-IR Analysis

The biofilm FT-IR analyses are shown in Figure 1. The broad, distinct band observed in the FT-IR spectrum, near 3300-3400 cm^{-1}, is attributed to hydroxyl groups (-OH) in the biofilm and to bound water adsorbed onto the structure. Peaks around 2920-2850 cm^{-1} are associated with aliphatic C-H stretching vibrations, indicating the presence of aliphatic chains from textile dyes, along with lipids and cellulosic structures in the biofilm matrix. The peak at 1711 cm^{-1} is attributable to amine groups on the surface of the contaminated membrane. The peaks at 1407 cm^{-1} and 1711 cm^{-1} are indicative of amide I and a carboxylic acid group (Liu et al., 2015). The peaks at 1407 cm^{-1}, 1336 cm^{-1}, and 1258 cm^{-1} originate from Amide I (CO stretching), Amide II (D-H in the plane), and Amide III (C-N stretching) in proteins. The peak at 1336 cm^{-1} arises from the C=O bond, indicating the presence of a carboxylate group. The two characteristic bands at 1407 cm^{-1} and 1336 cm^{-1} originate from C-H out-of-plane deformation vibrations. The band at 1083 cm^{-1} is related to C-F stretching, consistent with the PVDF membrane structure. The strong peak detected at 1414 cm^{-1} in the FT-IR analysis signifies the presence of proteins within the biofilm. The 800 cm^{-1} and 464 cm^{-1} regions are referred to as fingerprint zones. The peak observed at approximately 900 cm^{-1} (870 cm^{-1}) has been shown to characterize phosphate stresses (P-OH) (Duyar et al., 2021).

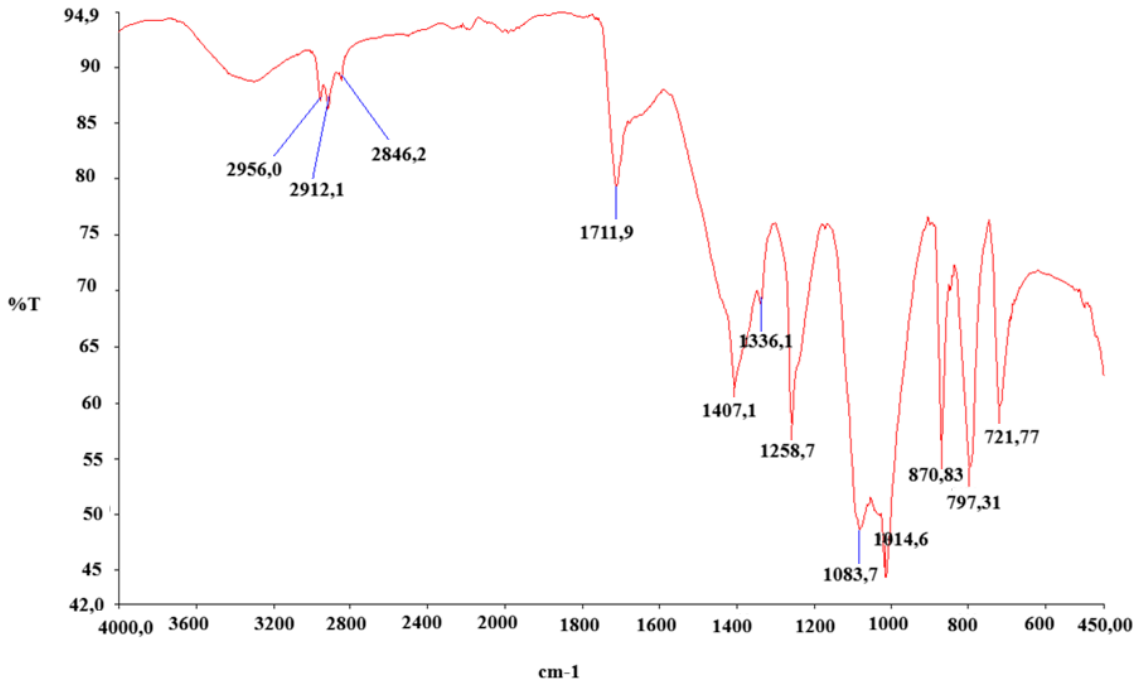

Figure 1. FT-IR Spectra Analysis on The Biofilm

TG/DTA Analysis

The biofilm TG/DTA analyses are shown in Figure 2. To better evaluate the thermal stability of the biofilm, Derivative Thermogravimetry (DTG) was employed. While the TG curve indicates the cumulative weight loss, the DTG curve (the first derivative of the TG weight loss) provides the rate of mass loss, allowing for a more precise identification of the temperatures at which individual decomposition steps occur. As seen in the results, the DTG peaks clearly distinguish between the evaporation of bound water, the degradation of organic EPS (proteins and polysaccharides), and the subsequent decomposition of more stable aromatic textile dyes. The initial mass loss (~10-15%) observed in the 50-150 °C range corresponds to the removal of free and weakly bound moisture present in the biofilm, and the weak endothermic DTA peak in this region is consistent with water evaporation. Similarly, it has been previously reported that the initial mass loss at low temperatures in biofilm is due to moisture and volatile components (Meng et al., 2009; Drews, 2010). The second significant mass loss occurred in the range of approximately 200–350 °C (~25–30%), supported by a prominent peak around 260–290 °C in the Derivative Thermogravimetry DTG curve. This region is attributed to the thermal decomposition of polysaccharides and proteins, the main components of extracellular polymeric materials (EPS) (Huang et al., 2022). The decomposition of EPS content in this temperature range indicates that the biofilm structure observed in MBR systems is biopolymer-dominant.

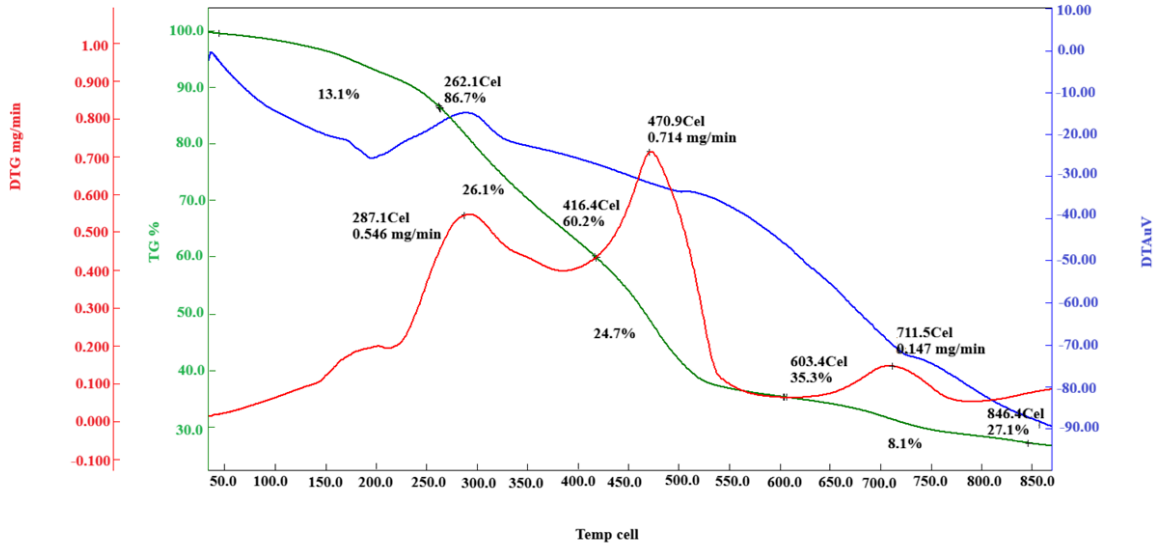

Figure 2. TG/DTA Analysis on The Biofilm

The dominant DTG peak, located in the range of approximately 400–500 °C and reaching a maximum around 470 °C, when considered together with the distinct exothermic peaks in the DTA curve, corresponds to the oxidative degradation of complex organic compounds with aromatic rings and cross-links (Holkar et al., 2016; Hai et al., 2007). The lower rate of mass loss above 600 °C and the plateauing of the curve indicate that the organic components have largely decomposed, and the remaining fraction consists mainly of inorganic ash and metal oxides. These results reveal that a hybrid fouling structure consisting of organic EPS + inorganic accumulation is dominant in the system. TG/DTA results generally indicate that the biofilm formed in the MBR contains a significant amount of organic matter of biological origin, along with significant amounts of aromatic dyes and inorganic salts specific to the textile process. These findings are consistent with FT-IR analyses (Figure 1) and parallel to the typical MBR fouling characterization reported in the literature (Meng et al., 2009; Drews 2010; Li et al., 2006).

DSC Analysis

The biofilm DSC analyses are shown in Figure 3. The first distinct endothermic transition observed in the curve between 228–260 °C ($\Delta H \approx 8.6$ J/g) is associated with the thermal denaturation and degradation of protein and polysaccharide-based extracellular polymeric substances (EPS) found in the biofilm structure. Breaking of hydrogen bonds and disruption of macromolecular structural order within EPS have been commonly reported in this temperature range (Sheng et al., 2010; Aquino et al., 2004). Consistency with TG/DTA results in the same temperature range indicates that the biofilm contains a significant amount of biopolymer.

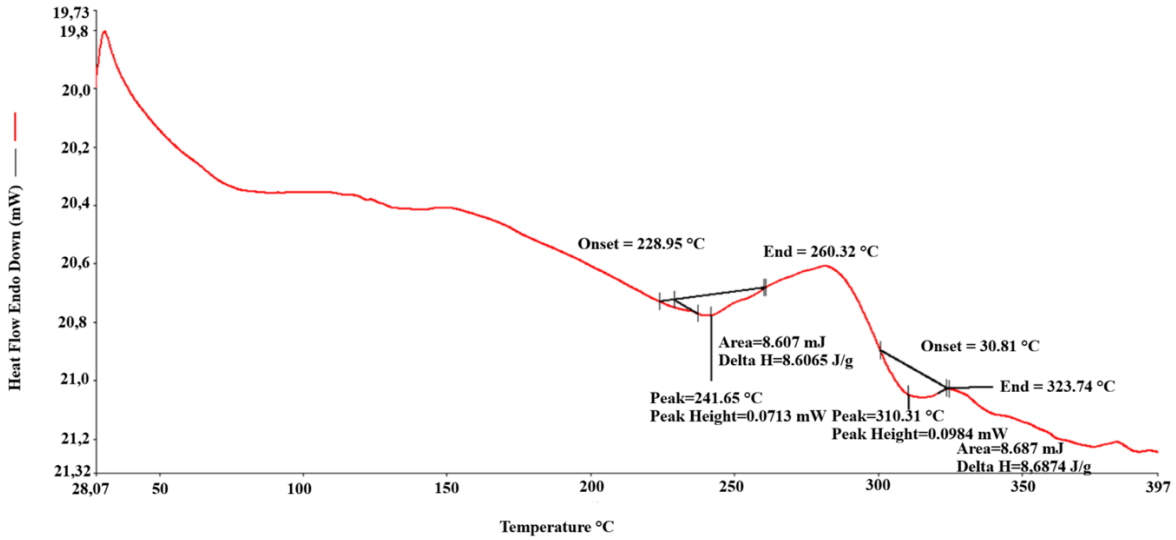

Figure 3. DSC Analysis on The Biofilm

The second endothermic transition, observed at approximately 300-325 °C (ΔH ≈ 8.7 J/g), corresponds to the decomposition of more densely structured organic compounds - particularly aromatic dyes, humic/fulvic substances, and cross-linked EPS fractions. It has been previously reported that azo and reactive dyes in textile wastewater undergo endothermic degradation at high temperatures (Hai et al., 2007; Holkar et al., 2016). The presence of this transition supports the accumulation of dye-induced resistance in organic compounds during biofilm formation. The gradual decrease in heat flux observed along the DSC curve indicates the presence of both weakly bound water and organic fractions with varying stabilities in the biofilm matrix. The occurrence of multiple thermal transitions due to the multi-component structure of EPS (proteins, polysaccharides, lipids, and nucleic acids) has been widely reported in the literature (Sheng et al., 2010; Li et al., 2006). These findings demonstrate that membrane fouling observed in MBR systems is associated not only with physical clogging but also with the development of a biopolymer-rich condensed biofilm structure. When the obtained DSC results are evaluated together with FT-IR and TG/DTA analyses, it is revealed that the resulting fouling layer exhibits a complex structure consisting of a combination of organic (EPS + dyes) and inorganic components. This is consistent with typical MBR membrane fouling mechanisms reported in the literature (Meng et al., 2009; Drews, 2010).

CONCLUSION

This research is rooted in the detailed structural and thermal deconstruction of a biofilm developed in a real-scale industrial MBR. While general fouling mechanisms are known, this study provides a specific 'thermal fingerprint' of fouling caused by real textile wastewater. The findings show that system performance is strongly dependent on the characteristics of the wastewater, and that dissolved organic fractions, particularly those containing dyes, limit the treatment efficiency. It was found that the biofilm structure developing on the membrane has a multi-component fouling mechanism supported not only by biological but also by inorganic components. This reveals that membrane fouling is an inevitable process regardless of operating conditions and cannot be limited solely by hydraulic or biological control strategies. Also, the treated textile wastewater COD and color are not within the discharge limits. The results indicate that integrating MBR systems with complementary processes such as advanced oxidation, adsorption, or coagulation may be necessary to enhance color removal in textile wastewater.

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Artificial Intelligence Contribution Statement

This manuscript was entirely written, edited, analyzed, and prepared without the assistance of any artificial intelligence tools. All content, including text, data analysis, and figures, was solely generated by the authors. (If used must be stated in details)

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