JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2024, 25(4), 131–141
https://doi.org/10.12911/22998993/183943
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.01.23
Accepted: 2024.02.17
Published: 2024.03.01

Comparative Biosorption Proficiency in Intact and Autoclaved Biofilm Matrices

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ABSTRACT

The urgent need for technological innovation to combat water pollution underscores the significance of biosorption as a potential solution. The success of biosorption hinges on the careful selection of a suitable biosorbent. Biofilms, composed of microbial communities, emerge as a promising alternative due to their expansive adsorption capacity and ready availability. In practical applications, biosorption is often executed at pollutant concentrations lethal to microbes. Consequently, comprehending the biosorption potential of biofilms with deceased microbes becomes imperative. Notably, biofilms with deceased microbes offer the added advantage of minimizing the risk of pathogenic microbial contamination. Despite this, studies are scarce comparing biosorption between intact biofilms and those with deceased microbes. This comparative analysis could enhance the feasibility of biofilms in biosorption as an eco-aquatic technology for alleviating aquatic pollution. This study aims to scrutinize the biosorption characteristics of intact biofilm (with living microbes) and autoclaved biofilm (with deceased microbes). The methods employed for analyzing biosorption characteristics encompass examining electric charge properties, FTIR spectra analysis, ion adsorption, and ion desorption. The model ions chosen for this study are K^+ (monovalent ion) and Mg^{2+} (divalent ion). Results indicate that the biofilm's electric charge properties and adsorption capacity remain relatively unchanged post-autoclaving. Based on these findings, it can be concluded that biofilms, whether intact or autoclaved, present substantial potential as biosorbents in the advancement of eco-aquatic technology for mitigating water pollution.

Keywords: biofilm, biosorbent, water pollution, aquatic ecosystem, microbial ecology

INTRODUCTION

Water pollution is a global predicament, posing a formidable challenge to attaining Sustainable Development Goals (SDGs) worldwide, including in Indonesia [Yadav et al., 2022; Obaideen et al., 2022]. The primary hurdle in addressing water pollution in developing nations like Indonesia is fostering environmental awareness and devising pragmatic, efficient, and eco-friendly technologies [Garg et al., 2018]. Consequently, the imperative of developing technology for environmental purification emerges to fortify environmental policy and propel technological advancements.

At the crux of steering technological change to combat pollution, particularly within aquatic ecosystems, lies the innovation of eco-friendly technologies [Wang et al., 2022a; Wang et al., 2022b; Ji et al., 2022]. Technologies grounded in ecological principles, leveraging ecosystem components to manage and exploit aquatic resources, are termed eco-aquatic technologies. Biosorption, a technology harnessing various biosorbents from aquatic ecosystems, plays a pivotal role in this paradigm [Jiang et al., 2020; Priya et al., 2022;

Thirunavukkarasu et al., 2021]. Biosorption entails immobilizing pollutants from ecosystems by utilizing organisms or their components as pollutant-binding substances [Kulkarni et al., 2022]. The linchpin to successful biosorption lies in the meticulous selection of a biosorbent [Dey et al., 2022]. An ideal biosorbent should boast a substantial adsorption capacity while readily available in aquatic ecosystems [Pourang and Rezaei, 2021; Michelon et al., 2022; Singh et al., 2020].

Our prior research highlights the microbial community known as biofilm as a promising biosorbent within the aquatic ecosystem [Kurniawan and Yamamoto, 2019; Kurniawan et al., 2022a; Kurniawan et al., 2022b]. Despite studies exploring biofilm applications in biosorption, the focus has predominantly been on intact biofilms [Catania et al., 2020]. In reality, however, biofilm microbes are often rendered lifeless through techniques like autoclaving, primarily to eliminate potentially pathogenic microbes harmful to humans. A pivotal concern arises regarding whether autoclaving alters the biofilm's capacity to adsorb ions. Yet, studies delving into the impact of autoclaving on biosorption by biofilms are conspicuously scarce [Seed et al., 2022].

This study delves into ions' adsorption and desorption characteristics from intact biofilm (biofilm with live microbes) and autoclaved biofilm (biofilm with killed microbes). These characteristics are paramount in shaping biofilm-based eco-aquatic technology for mitigating water pollution. The investigation employs biofilms naturally occurring in the Lahor Reservoir, East Java, Indonesia. The biosorption characteristics are scrutinized by analyzing the biofilm's electric charge properties, ion adsorption, and ion desorption when ions accumulate. The findings affirm that both intact and autoclaved biofilms emerge as potential biosorbents in eco-aquatic technologies, laying the groundwork for a resilient society.

MATERIALS AND METHODS

Sample preparation

Biofilm samples for this study were sourced from stones immersed in the Lahor Reservoir, East Java, Indonesia, situated at a depth of approximately 50 cm. Stones adorned with biofilm were carefully placed in plastic containers filled with reservoir water and transported to the laboratory while maintaining a temperature of 4 °C. To extract the biofilm from the rocks, a soft toothbrush was employed to brush the biofilm into 40 mL of distilled water. The resulting biofilm suspension underwent three rounds of washing with distilled water through centrifugation (8,000 × g at 4 °C for 5 minutes). Subsequently, biofilm pellets were preserved at -40 °C until utilized in experiments. Autoclaved biofilms were prepared by subjecting biofilm pellets to autoclaving at 121 °C and 106 kPa for 30 minutes. Moreover, microscopic examination was conducted using an optical microscope (Olympus BX50 Fluorescence Microscope, Olympus, Japan) to delve into the structural characteristics of both intact and autoclaved biofilms. The biofilm pellet was immersed in distilled water and subsequently spread onto a glass slide for observation.

Electrophoretic mobility

The biofilm fragment suspension was introduced into an electric field to assess electrophoretic mobility, and the fragment velocities were measured across a range of pH conditions. This enabled estimating the functional group(s) responsible for generating electric charges within the biofilm. Following centrifugation of the biofilm suspension at 8,000 × g for 10 minutes at 4°C, the supernatant was discarded, leaving a biofilm pellet. This pellet was reconstituted in 40 mL of a 10 mM NaCl aqueous solution and subjected to centrifugation (8,000 × g at 4°C for 10 minutes), removing the supernatant. The resulting biofilm pellet (approximately 0.03 g) was suspended in 1 mL of a 10 mM NaCl aqueous solution. The suspension underwent vigorous mixing using a Vortex-Genie 2 (M&S Instruments, Inc., Osaka, Japan; 3,000 rpm) for 5 minutes, followed by sonication (2510J-MT, Yamato Scientific, Tokyo, Japan; 42 kHz, 125 W) for 10 minutes, and a brief vortexing for 10 seconds. The biofilm suspension was then blended with 10 mM phosphate-buffered saline (PBS) (NaCl, 0.526 g; $Na_2HPO_4 \cdot 12H_2O$, 0.358 g; distilled water, 1,000 mL) at a ratio of 1:19 for electric charge analysis. The biofilm's electrophoretic mobility (EPM) was measured using a ZETASIZER Nano-Z (Malvern Instruments, Ltd., Worcestershire, England) in PBS, with pH values ranging from 2.0 to 9.0. Buffer pH was adjusted with 20 mM HCl or NaOH aqueous solutions to maintain a consistent ionic strength (10 mM).

FTIR analysis

FTIR spectrum analysis was conducted on both intact and autoclaved biofilms. The biofilm pellets were dehydrated at approximately 60 °C until a constant weight was achieved, and these dried pellets were employed in the subsequent FTIR analysis. For each analysis, 0.01 grams of the dry biofilm pellets were blended with Potassium Bromide (KBr) powder and compressed under high pressure. The application of pressure caused KBr to melt and encapsulate the compound within the matrix. The resulting KBr pellets, each containing a representative portion of the biofilm, were then subjected to analysis using a Shimadzu 84002 FTIR Spectrometer.

Adsorption and desorption of ions

The ions under scrutiny encompassed a monovalent cation (K^+) and a divalent cation (Mg^{2+}). To initiate the adsorption process, 5 mL of 20 mM KCl or $MgCl_2$ aqueous solutions, prepared by diluting reagent-grade chemical compounds in 5 mM phosphate buffer (pH 7), were introduced into 45 mL samples of the biofilm suspension (containing 1 wet-g biofilm). Maintaining the suspension at 25°C through a thermostatic water bath, vigorous mixing was ensured using a magnetic stirrer (approximately 700 rpm). Subsamples of the suspension were extracted at various time intervals (1, 3, 5, 10, 20, 30, 60, 90, 120, and 180 minutes after the addition of the test) and subsequently centrifuged (10,000×g at 4°C for 1 minute) to obtain the supernatant. The ion concentration in the supernatant was determined using capillary electrophoresis (CAPI-3300; Otsuka Electronics, Osaka, Japan).

Control experiments used 50 mL of 5 mM phosphate buffer (pH 7). The quantity of ions adsorbed to the biofilm was computed by discerning the concentration differences in the subsamples and the controls. Additionally, exploration extended to other cations distinct from those initially adsorbed, investigating desorption from the biofilm through ion exchange. The amounts of desorbed ions were calculated by contrasting the measured concentrations of the desorbed ions with the background concentrations following the introduction of the considered ion. Background concentrations were established by resuspending an equivalent amount of biofilm in 50 mL of 5 mM PBS (pH 7), subject to the same procedural steps described above but without ion addition.

RESULTS AND DISCUSSION

The exploration of ion adsorption and desorption within biofilms constituted a thorough examination encompassing electric charge properties, ion adsorption, and ion desorption phenomena. This study focused on modelling the behaviour of monovalent ions (K^+) and divalent ions (Mg^{2+}), utilizing intact and autoclaved biofilm matrices as the subjects of investigation. This comprehensive approach aimed to elucidate the intricate mechanisms underlying ion interaction with biofilm matrices, shedding light on both intact and autoclaved biofilm systems.

Structural characteristics of biofilms

The microscopic observations unveiled a significant alteration in the coloration of the biofilm (Figure 1), transitioning from its original green tint to shades of brown and gray following the autoclaving procedure. This change can be attributed to modifications in cellular pigments and alterations in the biofilm matrix's chemical composition [Han et al., 2018; Dutta and Sit, 2023]. Previous research has highlighted that green

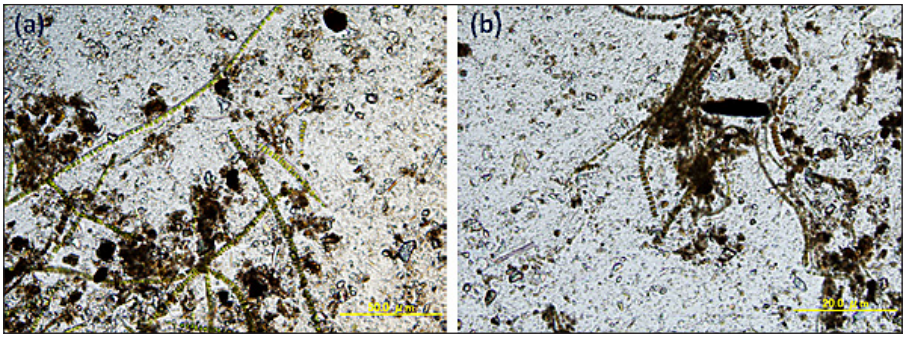

Figure 1. The structural characteristics of intact (a) and autoclaved (b) biofilms were observed using an optical microscope

coloration in biofilms often indicates the presence of photosynthetic pigments, such as chlorophyll, produced by microorganisms like algae or cyanobacteria residing within the biofilm matrix. The shift towards brown and gray hues post-autoclaving suggests a cessation of metabolic activities within the microbial components of the biofilm, leading to alterations in pigment production or degradation.

Despite the pronounced color change, the fundamental structural integrity of the biofilm remained unaltered. This observation implies that the autoclaving process transforms the biofilm matrix, potentially associated with the inactivation or death of living organisms residing within the biofilm while preserving the underlying structural framework [Han et al., 2018]. Additionally, autoclaving may lead to denaturation of proteins and alterations in cell membrane integrity, contributing to the observed changes in coloration [Dutta and Sit, 2023].

The persistence of the biofilm's structural morphology post-autoclaving underscores its resilience to withstand thermal treatments while retaining its basic architectural framework. This resilience is crucial for potential applications where the biofilm's structural integrity is paramount, such as in biofilm-based bioremediation or biosorption processes. Moreover, the observed color changes following autoclaving provide valuable insights into the physiological state of the biofilm, reflecting alterations in metabolic activities and pigment production in response to thermal stress.

The shift in coloration may be attributed to various factors, such as alterations in cellular pigments or chemical composition within the biofilm matrix. The observed change from green to brown and gray hues indicates a shift in the biochemical composition, possibly linked to the cessation of metabolic processes within the microbial components of the biofilm. While the color transformation signifies a significant impact on the biofilm's biological constituents, preserving its essential structural integrity underscores the resilience of the biofilm matrix to withstand the autoclaving process without substantial alterations in its physical form.

These findings align with the expected outcome of autoclaving, known primarily for its sterilization effect by eradicating living microorganisms. The observed color changes reflect the cessation of metabolic activities, suggesting a shift in the physiological state of the biofilm. Notably, the maintained structural integrity of the biofilm post-autoclaving signifies its potential suitability for further applications, emphasizing the enduring stability of its foundational architecture despite the induced changes in color.

Electric charge properties of biofilm

The analysis of intact and autoclaved biofilms' electric charge properties involved the measurement of electrophoretic mobility (EPM) across various pH levels 2–9 (Figure 2). EPM values demonstrated a polarity spectrum, showcasing negative and positive electric charges. Negative EPM values diminished with decreasing pH, culminating in a positive value at pH 2. The negative EPM indicated the presence of negatively charged sites within the biofilm polymer, likely stemming from functional groups

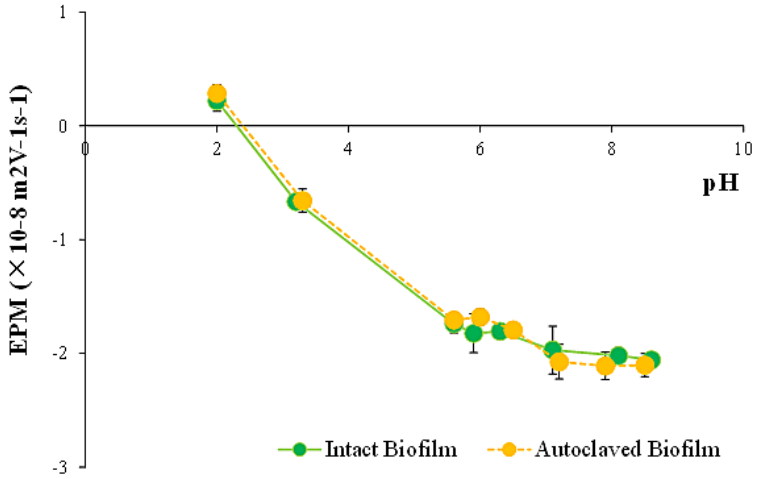

Figure 2. Electrophoretic mobility of intact biofilm (green circle) and autoclaved biofilm (orange circle) in various pHs. Bars represent standard errors

like carboxyl groups [Anggayasti et al., 2023]. As H^+ concentration increased, a deionization process unfolded, facilitating the binding of H^+ to these negatively charged sites. A pivotal EPM shift around pH 4 suggested the existence of highly protonated functional groups, notably those with pKa 4 values, such as carboxyl groups [Kurniawan and Fukuda, 2023].

The transition to positive EPM at pH 2 signified the emergence of positively charged sites, potentially associated with amino groups. Biofilms, therefore, exhibited both positive and negative charges. At pH levels above 2, the abundance of negatively charged sites surpassed that of positively charged sites, resulting in an overall net negative EPM [Herzberg et al., 2020]. The intricate balance between positive and negative charges influenced the biofilm's behavior in EPM measurements. Notably, when negative and positive charges equaled, the biofilm exhibited a neutral charge, while an excess of negatively charged sites, as observed at pH > 2, led to negative EPM values. Furthermore, the capture of H^+ by negatively charged functional groups neutralized electrical charges, causing the biofilm to manifest positive EPM values at pH 2.

The dynamic interplay of electric charges within biofilms, as revealed through EPM measurements, is integral to understanding their electrochemical behavior. The pH-dependent shifts in charge properties, notably the transition from negative to positive EPM at pH 2, point to distinct functional groups, such as carboxyl and amino groups. These findings resonate with existing literature highlighting the importance of pH in influencing biofilm charge characteristics [Xu et al., 2020]. The discussion underscores the complex nature of biofilm electric charge dynamics, laying the foundation for the subsequent exploration of adsorption and desorption processes.

The efficacy of adsorption and desorption processes in biofilms is intricately linked to electrostatic interactions between charged sites in the biofilm and oppositely charged substances in the surrounding water. This electrically charged property is a crucial determinant enabling biofilms to capture or release ions. Notably, even after autoclaving, where the microbial component of the biofilm is eradicated, the electrical charge property of the biofilm remains unaltered. This implies that autoclaved biofilms retained adsorption sites, showcasing their potential as biosorbents despite the absence of live microbes [Wang et al., 2021]. The steadfastness of biofilm electrical charge properties post-autoclaving emphasizes their resilience and utility in ion adsorption applications.

The constancy of biofilm electrical charge properties post-autoclaving holds significant implications for environmental applications, particularly in water purification technology. Preserving adsorption sites in autoclaved biofilms indicates their potential as effective biosorbents, even in scenarios where the microbial component is inactive. This resilience expands the scope of biofilm utilization in eco-aquatic technologies for water pollution mitigation, offering a sustainable solution for wastewater treatment and environmental remediation [Jasu and Ray, 2021]. The constancy of biofilm charge properties highlights the importance of understanding the underlying mechanisms of ion adsorption and desorption in biofilms for optimizing their performance in water treatment processes [Bhagwat et al., 2021; Qiongjie et al., 2022]. The discussion prompts further exploration into the specific mechanisms behind ion adsorption and desorption by biofilms, shedding light on their potential role in sustainable water purification strategies.

Functional groups at biofilm matrices

A critical aspect of the biofilm matrix, facilitating the accumulation of diverse ions, is the presence of charged sites within biofilm polymers. These electrically charged sites stem from the ionization of various functional groups in biofilm polymers [Kurniawan and Fukuda, 2023]. To assess the composition of functional groups in biofilm matrices, both intact and autoclaved biofilms were subjected to FTIR spectral analysis in this study (Figure 3).

The FTIR spectral analysis revealed that intact and autoclaved biofilms exhibited nearly identical FTIR profiles. Peaks around 1650 cm^{-1} in the spectra indicated the presence of carboxylic groups (C=O) within the biofilm matrix. These carboxylic groups are known to ionize, contributing to the negatively charged electrical sites in the biofilm matrix [Kurniawan and Fukuda, 2023]. The spectrum's region at 3600–3100 cm^{-1} suggested the existence of hydroxyl groups (-OH), a finding supported by the stretching of C=O due to the presence of a carboxylic skeleton. A sharp peak at 2926 cm^{-1} indicated the characteristic C-H stretching from the methyl (-CH3) or methylene ($-CH_2-$) group. Peaks around 1700 cm^{-1} hinted at

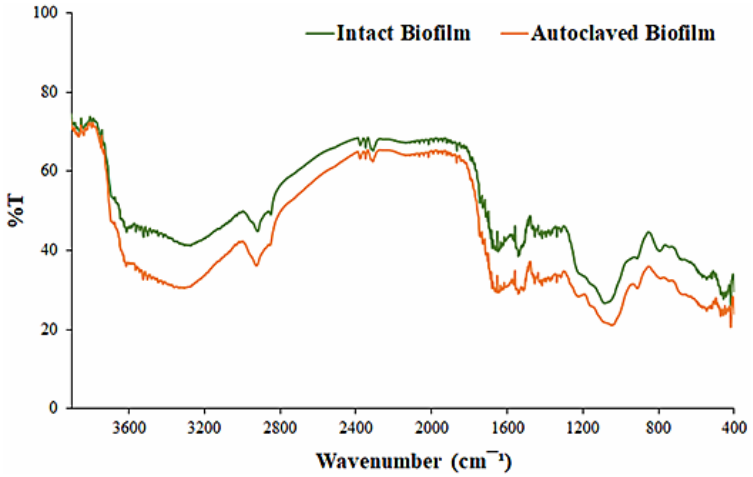

Figure 3. FTIR spectra analysis of intact biofilm (green) and autoclaved biofilm (orange)

an aliphatic carboxyl group (-COO-), while those around 1400 cm^{-1} indicated the possibility of an amino or amide group. The 1300–1000 cm^{-1} broadband was likely attributed to C-O vibrations from the ester group.

The FTIR spectra analysis provided valuable insights into the impact of the autoclaving process on the functional groups within the biofilm matrix. Remarkably, the results revealed that the autoclave treatment did not induce significant changes in functional groups' properties in the biofilm matrix. This observation suggests that the autoclaving process, while effective in sterilizing the biofilm by eliminating microbial components, did not alter the chemical composition or integrity of the biofilm matrix itself [Dutta and Sit, 2023].

The persistence of functional groups, particularly those responsible for providing electrically charged sites, post-autoclaving underscores the biofilm's robustness and resilience. These charged sites are crucial in facilitating ion adsorption, enabling biofilms to effectively capture and retain ions from surrounding aqueous environments [Qiongjie et al., 2022]. The consistent presence of charged sites, as evidenced by FTIR analysis, aligns with the biofilm's ability to maintain its ion adsorption properties even after autoclaving.

The observed resilience of functional groups in the biofilm matrix post-autoclaving not only highlights the structural stability of the biofilm but also reinforces its potential as an effective biosorbent. This resilience suggests that autoclaved biofilms retain their capacity to adsorb ions, thereby offering a viable solution for water purification and environmental remediation applications [Catania et al., 2020]. By maintaining their ion adsorption properties, autoclaved biofilms can contribute to sustainable water treatment strategies, mitigating pollution and promoting environmental sustainability.

The discussion further explores the interplay between biofilm composition, functional groups, and ion adsorption mechanisms. Understanding biofilms' underlying mechanisms governing ion adsorption is essential for optimizing their performance in water purification processes [Jasu and Ray, 2021]. By elucidating these mechanisms, researchers can develop tailored strategies to enhance biofilm-based biosorption technologies, ultimately contributing to more efficient and cost-effective water treatment solutions.

Adsorption ability

This study delved into the adsorption ability of intact biofilms (Figure 4) and autoclaved biofilms (Figure 5), scrutinizing their capacity to adsorb ions. Adsorbates, or the adsorbed ions, exhibited stability in their binding to the biofilm over time, with the number of ions adsorbed in 1 minute remaining constant throughout the experiment. Intact biofilm could adsorb Mg^{2+} and K^{+} at approximately 0.24 mmol/dry-g and 0.13 mmol/dry-g, respectively. The negatively charged sites in intact and autoclaved biofilms, as confirmed by EPM measurements at pH 7, seem to facilitate the attraction and storage of positively charged ions such as Mg^{2+} and K^{+}.

The results highlighted a noteworthy distinction in the adsorption capacities, with intact biofilm adsorbing nearly twice the amount of Mg^{2+} compared to K^{+}. Divalent cations, such as Mg^{2+},

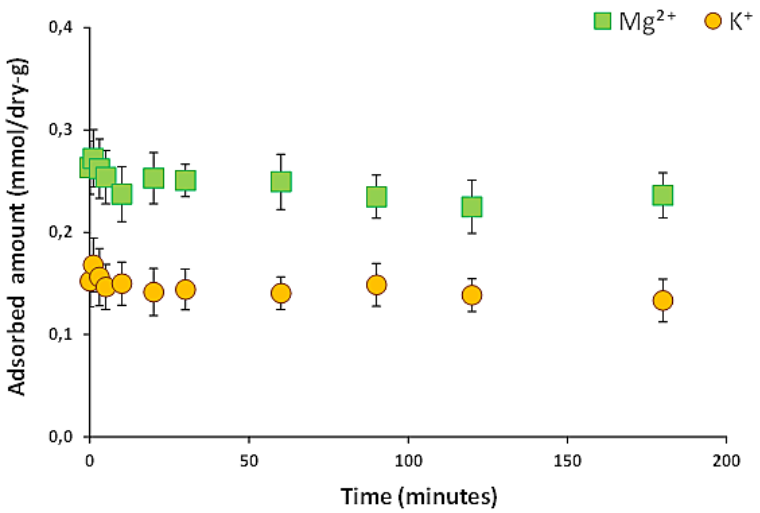

Figure 4. Adsorption of Mg^{2+} (green square) and K^{+} (orange circle) to the intact biofilms. Bars represent standard error

exhibited a stronger binding affinity than monovalent cations, a phenomenon attributed to the higher electric charge on divalent cations, promoting more robust electrostatic interactions with the negatively charged sites in intact biofilm. The adsorption process for divalent and monovalent cations appeared through physicochemical mechanisms, particularly electrostatic interactions between charged sites in the biofilm and oppositely charged ions in the surrounding water.

Despite undergoing a microbial eradication process, Autoclaved biofilms demonstrated similar adsorption capabilities for Mg^{2+} and K^{+} (Figure 5). The autoclaved biofilm adsorbed Mg^{2+} and K^{+} at approximately 0.22 mmol/dry-g and 0.12 mmol/dry-g, respectively, with stable adsorption observed throughout the experiment (1–180 minutes). This resilience suggested that the autoclaving process did not significantly impact the biofilm's ability to adsorb ions. Even after autoclaving, the functional groups in the biofilm polymer remained intact, serving as binding sites for ions through a passive uptake process.

The results from the FTIR analysis provide valuable insights into the effects of autoclaving on the biosorption capabilities of biofilms. The comparable amounts of Mg^{2+} and K^{+} adsorbed by both intact and autoclaved biofilms underscore the resilience of the biofilm structure and its capacity to function as a biosorbent even after undergoing thermal treatment. This finding suggests that while autoclaving effectively

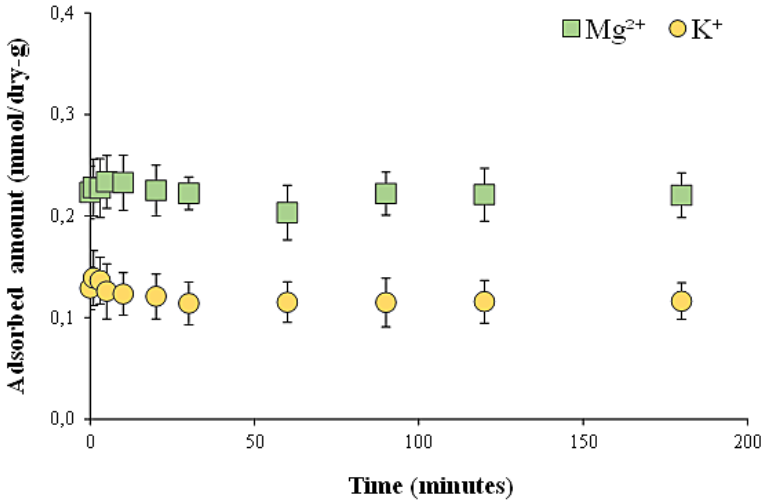

Figure 5. Adsorption of Mg^{2+} (light green square) and K^{+} (light orange circle) to autoclaved biofilms. Bars represent standard errors.

eliminates live microbial components, it does not significantly alter the biofilm's ability to bind ions [Manobala et al., 2021].

The preservation of ion-binding active sites within the biofilm matrix and microbial cell walls post-autoclaving contributes to the sustained biosorption capabilities observed in autoclaved biofilms. This preservation highlights the robustness of the biofilm's chemical composition and structural integrity, further supporting its potential as a reliable biosorbent for both monovalent and divalent cations [Tores, 2020]. The study's findings endorse the autoclave process as a practical and cost-effective method for biofilm preparation in eco-aquatic technologies aimed at environmental pollution mitigation.

The consistent biosorption performance observed in intact and autoclaved biofilms underscores the versatility of biofilms in environmental purification applications. These results emphasize the feasibility of utilizing biofilms as effective biosorbents even in scenarios where microbial activity is limited or inactive. The study contributes to the growing body of evidence advocating for integrating biofilms into eco-friendly approaches for addressing environmental pollution challenges [Sharma et al., 2020]. Further research efforts are warranted to elucidate the underlying mechanisms governing ion adsorption by biofilms and assess their long-term stability in real-world environments. Such investigations can enhance our understanding of biofilm-mediated processes and optimize their utilization in sustainable environmental remediation strategies.

Desorption of ions

In the process of ion adsorption into the biofilm matrix, both intact and autoclaved biofilms exhibited subsequent desorption of ions. The capacity of the biofilm to bind ions is contingent upon the quantity of electrically charged sites, and the measured ions, expressed in terms of electric charges (meq/dry-g), are detailed in Table 1. Notably, Mg^{2+}, possessing twice the electric charge of K^+ for an equivalent number of molecules, revealed a pronounced role in this dynamic. Desorbed ions during Mg^{2+} adsorption included K^+, Na^+, NH_4^+, and Ca^{2+}. Conversely, K^+ adsorption resulted in the desorption of Na^+, NH_4^+, Ca^{2+}, and Mg^{2+}. The consistency in the type of ions desorbed from both intact and autoclaved biofilms underscored that the autoclaving process did not induce significant alterations in the biofilms' characteristics and ion adsorption capacities.

The detection of desorbed ions pointed towards ion exchange as a primary mechanism in the adsorption process of ions by biofilms. In this mechanism, ions previously bound in the biofilms are replaced by ions adsorbed by the biofilm. This process aligns with increasing ion concentrations in the water surrounding the biofilm. Common ions such as K^+, Na^+, NH_4^+, Ca^{2+}, and Mg^{2+}, prevalent in aquatic ecosystems like the Lahor Reservoir, were identified both in the biofilm and its surroundings. The close connection between ions in the biofilm and those in the environment indicated that the biofilm acts as a nutrient-rich microhabitat from its early stages of formation.

In terms of electric charge, the amount of Mg^{2+} adsorbed by the biofilm (0.48 meq/dry-g and 0.44 meq/dry-y for intact and autoclaved biofilms, respectively) surpassed the amount of K^+ adsorbed (0.13 meq/dry-g and 0.12 meq/dry-g for intact and autoclaved biofilms, respectively). Divalent cations, with their doubled electrical charge compared to monovalent cations, exhibited stronger binding in the biofilm matrix. This finding reinforced the role of electrostatic bonds in the adsorption process of ions into biofilms.

Despite the clear presence of ion exchange mechanisms, the total number of ions desorbed from intact biofilm (0.25 meq/dry-g and 0.101 meq/dry-g for Mg^{2+} and K^+ adsorption, respectively) was less than the number of ions adsorbed (0.48 meq/dry-g and 0.13 meq/dry-g for Mg^{2+} and K^+, respectively). This departure from the characteristic of ion exchange resins, where the number

Table 1. Comparison of ion adsorption and desorption by biofilms

Biofilm	Adsorbed (meq/dry-g)		Desorbed (meq/dry-g)					
			K^+	Na^+	NH_4^+	Ca^{2+}	Mg^{2+}	Total
Intact	Mg^{2+}	0.48	0.01 ± 0.001	0.13 ± 0.2	0.01 ± 0.003	0.1 ± 0.02	-	0.25
	K^+	0.13	-	0.002 ± 0.001	0.003 ± 0.0004	0.08 ± 0.003	0.016 ± 0.002	0.101
Autoclaved	Mg^{2+}	0.44	0.01 ± 0.002	0.11 ± 0.2	0.01 ± 0.004	0.1 ± 0.01	-	0.23
	K^+	0.12	-	0.004 ± 0.003	0.002 ± 0.001	0.08 ± 0.001	0.016 ± 0.003	0.102

of desorbed and adsorbed ions is equal, suggested that the adsorption of ions into the biofilm occurs through a combination of ion exchange and electrostatic bonding mechanisms. Consequently, the sites of ion deposition within the biofilm matrix encompass both electrically charged sites and the interstitial spaces between biofilm polymers.

The intricate balance between ion exchange and electrostatic bonding mechanisms uncovered in this study sheds light on ion interactions within biofilms [Hansima et al., 2021]. These nuanced findings offer valuable insights into the dynamic processes that govern ion dynamics within biofilms, highlighting the multifaceted nature of these interactions [Li et al., 2023].

Further investigation into the specific characteristics of ion-binding sites, including their spatial distribution and chemical composition, can provide a deeper understanding of the mechanisms underlying ion adsorption and desorption in biofilms [Luo et al., 2022]. Additionally, exploring the temporal dynamics of ion exchange processes and their implications for biofilm-mediated environmental remediation strategies can enhance our ability to harness biofilms for water purification applications [Syed et al., 2023].

This study underscores biofilms' adaptive and versatile nature as effective biosorbents capable of dynamically responding to changes in environmental conditions [Mishra et al., 2022]. By elucidating the fundamental processes driving ion adsorption and desorption in biofilms, this research contributes to developing innovative eco-aquatic technologies for sustainable water purification [Flemming et al., 2023].

Moving forward, further research endeavors should focus on elucidating the intricate mechanisms governing ion dynamics within biofilms and exploring novel approaches for enhancing their efficacy in environmental remediation [Leininger et al., 2021]. By leveraging the insights gained from this study, future investigations can optimize the design and implementation of biofilm-based systems for water purification, ultimately contributing to developing more efficient and sustainable solutions for addressing water pollution challenges [Philipp et al., 2023].

This study underscores the critical role of biofilms in eco-aquatic technologies and emphasizes the importance of ongoing research efforts to unlock their full potential for environmental remediation [Dar et al., 2020]. By advancing our understanding of biofilm-mediated processes, this research paves the way for developing innovative strategies for sustainable water purification and environmental protection.

CONCLUSIONS

This study illuminates biofilms' robust ion adsorption capabilities, showcasing their possession of ionizable functional groups that confer electric charges. The biofilm matrix exhibits a nuanced interplay of negatively and positively charged sites, transforming into dynamic binding sites when ion concentrations rise in the adjacent water. Biofilms adeptly employ electrostatic bonding and ion exchange mechanisms to facilitate ion adsorption. Notably, the biofilm's proficiency in adsorbing ions endures through a physicochemical process, remaining resilient even in the absence of live microbes within the biofilm. Crucially, the commonly employed autoclave process, intended for microbial sterilization, emerges as a non-detrimental treatment for biofilms. This study unequivocally demonstrates that the autoclave process does not compromise the biofilm's ion adsorption capacity. The ion adsorption and desorption characteristics observed in both intact and autoclaved biofilms showcase remarkable similarity, underscoring the viability of autoclaves as a preparatory step for biofilm utilization in biosorption applications. This finding is particularly pertinent for scenarios where concerns about pathogenic microbes within biofilms necessitate mitigation without compromising ion adsorption capabilities. The implications of this study extend beyond laboratory contexts, suggesting that biofilms hold immense potential as biosorbents in sustainable eco-aquatic technologies. The consistently preserved ion adsorption ability of biofilms, whether intact or autoclaved, positions them as promising assets in the endeavor to mitigate pollution in aquatic environments. This study contributes valuable insights into the fundamental mechanisms governing biofilm-ion interactions and paves the way for practical applications in eco-friendly water purification technologies.

Acknowledgments

The authors would like to thank Professor Hisao Morisaki from Ritsumeikan University, Dr. Yuki Tsuchiya from Nihon University,

Luthfia Ayu Dhea, Rachmad Dian Kuncoro and this work was supported by the Hibah Kelompok Kajian, Graduate School of the University of Brawijaya, with contract number: 2995/UN10. F40/PT.01/2022 for their support and assistance in carrying out this research

REFERENCES

1. Anggayasti W.L., Salamah L.N.M., Rizkymaris A., Yamamoto T., Kurniawan A. 2023. The role of ion charge density and solubility in the biosorption of heavy metals by natural biofilm matrix of polluted freshwater: the cases of Mg(II), Cr(VI), and Cu(II). Environmental Pollutants and Bioavailability, 35(1), 2220571.
2. Bhagwat G., Tran T.K.A., Lamb D., Senathirajah K., Grainge I., O'Connor W., Palanisami T. 2021. Biofilms enhance the adsorption of toxic contaminants on plastic microfibers under environmentally relevant conditions. Environmental Science & Technology, 55(13), 8877-8887.
3. Catania V., Lopresti F., Cappello S., Scaffaro R., Quatrini P. 2020. Innovative, ecofriendly biosorbent-biodegrading biofilms for bioremediation of oil-contaminated water. New Biotechnology, 58, 25-31.
4. Dar S.A., Bhat R.A. 2020. Aquatic pollution stress and role of biofilms as environment cleanup technology. Fresh Water Pollution Dynamics and Remediation, 293-318.
5. Dey S., Charan S.S., Pallavi U., Sreenivasulu A., Haripavan N. 2022. The removal of ammonia from contaminated water by using various solid waste biosorbents. Energy Nexus, 7, 100119.
6. Dutta D., Sit N. 2023. Comparison of properties of films prepared from casein modified by ultrasound and autoclave treatment. Journal of Food Measurement and Characterization, 17(5), 5426-5439.
7. Flemming H.C., van Hullebusch E.D., Neu T.R., Nielsen P.H., Seviour T., Stoodley P., Wuertz S. 2023. The biofilm matrix: Multitasking in a shared space. Nature Reviews Microbiology, 21(2), 70-86.
8. Garg T., Hamilton S.E., Hochard J.P., Kresch E.P., Talbot J. 2018. (Not so) gently down the stream: River pollution and health in Indonesia. Journal of Environmental Economics and Management, 92, 35-53.
9. Han A., Tsoi J.K., Matinlinna J.P., Zhang Y., Chen Z. 2018. Effects of different sterilization methods on surface characteristics and biofilm formation on zirconia in vitro. Dental Materials, 34(2), 272-281.
10. Hansima M.A.C.K., Makehelwala M., Jinadasa K.B.S.N., Wei Y., Nanayakkara K.G.N., HLerath A.C., Weerasooriya R. 2021. Fouling of ion exchange membranes used in the electrodialysis reversal advanced water treatment: A review. Chemosphere, 263, 127951.
11. Herzberg M., Pandit S., Mauter M.S., Oren Y. 2020. Bacterial biofilm formation on ion exchange membranes. Journal of Membrane Science, 596, 117564.
12. Jasu A., Ray R.R. 2021. Biofilm mediated strategies to mitigate heavy metal pollution: A critical review in metal bioremediation. Biocatalysis and Agricultural Biotechnology, 37, 102183.
13. Ji J., Zhao T., Li F. 2022. Remediation technology towards zero plastic pollution: Recent advance and perspectives. Environmental Pollution, 313, 120166.
14. Jiang R., Huang S., Wang W., Liu Y., Pan Z., Sun X., Lin C. 2020. Heavy metal pollution and ecological risk assessment in the Maowei sea mangrove, China. Marine Pollution Bulletin, 161, 111816.
15. Kulkarni R.M., Dhanyashree J.K., Varma E., Sirivibha S.P. 2022. Batch and continuous packed bed column studies on biosorption of nickel (II) by sugarcane bagasse. Results in Chemistry, 4, 100328.
16. Kurniawan A., Fukuda Y. 2023. Analysis of the electric charge properties of biofilm for the development of biofilm matrices as biosorbents for water pollutant. Energy, Ecology and Environment, 8(1), 62-68.
17. Kurniawan A., Ulfa S.M., Chamidah C. 2022. The biosorption of copper (II) using a natural biofilm formed on the stones from the Metro River, Malang City, Indonesia. International Journal of Microbiology, 2022.
18. Kurniawan A., Yamamoto T. 2019. Accumulation of and inside biofilms of natural microbial consortia: implication on nutrients seasonal dynamic in aquatic ecosystems. International Journal of Microbiology, 2019.
19. Kurniawan A., Yamamoto, T., Ekawati A.W., Salamah L.N.M., Amin A.A., Yanuar A.T. 2022. Characteristics of Cd(II) biosorption into streamer biofilm matrices. International Journal of Technology, 13(2), 367-377.
20. Leininger A., Yates M.D., Ramirez M., Kjellerup B. 2021. Biofilm structure, dynamics, and ecology of an upscaled biocathode wastewater microbial fuel cell. Biotechnology and Bioengineering, 118(3), 1305-1316.
21. Li Y., Qu Y., Yang H., Zhou X., Xiao P., Shao T. 2023. Combatting biofilms in potable water systems: A comprehensive overview to ensuring industrial water safety. Environmental Microbiology Reports, 15(6), 445-454.
22. Luo X., Yang Y., Xie S., Wang W., Li N., Wen C., Chen L. 2022. Drying and rewetting induce changes in biofilm characteristics and the subsequent release of metal ions. Journal of Hazardous Materials, 433, 128832.
23. Manobala T., Shukla S.K., Rao T.S., Kumar M.D. 2021. Kinetic modelling of the uranium biosorption

by Deinococcus radiodurans biofilm. Chemosphere, 269, 128722.

24. Michelon A., Bortoluz J., Raota C.S., Giovanela M. 2022. Agro-industrial residues as biosorbents for the removal of anti-inflammatories from aqueous matrices: An overview. Environmental Advances, 9, 100261.
25. Mishra S., Huang Y., Li J., Wu X., Zhou Z., Lei Q., Chen S. 2022. Biofilm-mediated bioremediation is a powerful tool for the removal of environmental pollutants. Chemosphere, 294, 133609.
26. Obaideen K., Shehata N., Sayed E.T., Abdelkareem M.A., Mahmoud M.S., Olabi A.G. 2022. The role of wastewater treatment in achieving sustainable development goals (SDGs) and sustainability guideline. Energy Nexus, 7, 100112.
27. Philipp L. A., Bühler K., Ulber R., Gescher J. 2023. Beneficial applications of biofilms. Nature Reviews Microbiology, 1-15.
28. Pourang N., Rezaei M. 2021. Biosorption of copper from aqueous environment by three aquatics-based sorbents: A comparison of the relative effect of seven important parameters. Bioresource Technology Reports, 15, 100718.
29. Priya A.K., Gnanasekaran L., Dutta K., Rajendran S., Balakrishnan D., Soto-Moscoso M. 2022. Biosorption of heavy metals by microorganisms: Evaluation of different underlying mechanisms. Chemosphere, 307, 135957.
30. Qais F.A., Khan M.S., Ahmad I., Husain F.M., Al-Kheraif A.A., Arshad M., Alam P. 2021. Plumbagin inhibits quorum sensing-regulated virulence and biofilms of Gram-negative bacteria: in vitro and in silico investigations. Biofouling, 37(7), 724-739.
31. Qiongjie W., Yong Z., Yangyang Z., Zhouqi L., Jinxiaoxue W., Huijuan, C. 2022. Effects of biofilm on metal adsorption behavior and microbial community of microplastics. Journal of Hazardous Materials, 424, 127340.
32. Saeed M.U., Hussain N., Sumrin A., Shahbaz A., Noor S., Bilal M., Iqbal H. M. 2022. Microbial bioremediation strategies with wastewater treatment potentialities – A review. Science of the total environment, 818, 151754.
33. Sharma A., Jamali H., Vaishnav A., Giri B.S., Srivastava A.K. 2020. Microbial biofilm: An advanced ecofriendly approach for bioremediation. In: New and Future Developments in Microbial Biotechnology and Bioengineering: Microbial Biofilms. 205-219.
34. Singh S., Kumar V., Datta S., Dhanjal D.S., Sharma K., Samuel J., Singh J. 2020. Current advancement and future prospect of biosorbents for bioremediation. Science of the Total Environment, 709, 135895.
35. Syed Z., Sogani M., Rajvanshi J., Sonu K. 2023. Microbial biofilms for environmental bioremediation of heavy metals: a review. Applied Biochemistry and Biotechnology, 195(9), 5693-5711.
36. Thirunavukkarasu A., Nithya R., Sivashankar R. 2021. Continuous fixed-bed biosorption process: A review. Chemical Engineering Journal Advances, 8, 100188.
37. Torres E. 2020. Biosorption: A review of the latest advances. Processes, 8(12), 1584.
38. Wang R., Zhang Q., Zhan L., Xu Z. 2022. Urgency of technology and equipment upgrades in e-waste dismantling base: Pollution identification and emission reduction. Environmental Pollution, 308, 119704.
39. Wang Z., Luo P., Zha X., Xu C., Kang S., Zhou M., Wang Y. 2022. Overview assessment of risk evaluation and treatment technologies for heavy metal pollution of water and soil. Journal of Cleaner Production, 308, 134043.
40. Wang Y., Samaranayake L.P., Dykes G.A. 2021. Plant components affect bacterial biofilms development by altering their cell surface physicochemical properties: a predictability study using Actinomyces naeslundii. FEMS Microbiology Ecology, 97(1).
41. Xu Y., Ou Q., Zhou X., He Q., Wu Z., Huang R., Huangfu X. 2020. Impacts of carrier properties, environmental conditions and extracellular polymeric substances on biofilm formation of sieved fine particles from activated sludge. Science of the Total Environment, 731, 139196.
42. Yadav A.K., Yadav H.K., Naz A., Koul M., Chowdhury A., Shekhar, S. 2022. Arsenic removal technologies for middle-and low-income countries to achieve the SDG-3 and SDG-6 targets: A review. Environmental Advances, 9, 100262.