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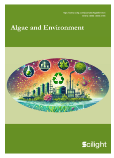

Article

Evaluation and Prevention of Biofilm Formation on Stainless Steel Sensor Mesh for Effective Biofouling Mitigation

Junhui Chen [1], Yuting Li [1], Archer Anderson [1], Shan Liu [1,2], Leilei Dai [1], Lu Wang [1], Mark Gino K. Galang [3], Yuchuan Wang [1], Juer Liu [1], Nan Zhou [1], Yuxi Chen [1], Xiangyang Lin [4], Kirk Cobb [1] and Roger Ruan [1,*]

[1] Center for Biorefining and Department of Bioproducts and Biosystems Engineering, University of Minnesota, 1390 Eckles Avenue, St. Paul, MN 55108, USA
[2] College of Engineering, China Agricultural University, Beijing 100083, China
[3] Sanitary Environmental Engineering Division (SEED), Department of Civil Engineering, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, SA, Italy
[4] College of Biological Science and Engineering, Fuzhou University, Fuzhou University Town, Fuzhou 350108, China
* Correspondence: ruanx001@umn.edu

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Abstract: This study evaluated strategies for preventing biofilm formation on stainless steel sensor mesh screens by integrating anti-fouling approaches and applying mathematical models to stimulate biofilm elimination during anti-fouling processes. Results showed that the proposed sensor mesh housing equipped with an external low-level strength electric field effectively reduced microbial biofilm formation and mitigated sensor mesh biofouling. Additionally, the combined application of the alternating electric field and ultrasonic irradiation significantly enhanced biofilm prevention and control. Anti-fouling tests conducted in natural lake water revealed that microalgae and bacteria can form biofilms on stainless steel screen electrodes in a time-dependent manner, with the 48-h time interval being crucial for cell adhesion and biofilm formation. Moreover, the dynamic process of biofilm elimination during ultrasonic treatment was successfully simulated using different mathematic models, demonstrating their suitability for describing these anti-fouling processes. This work provides valuable insights into the protection of water-monitoring sensors and probes, and to minimize biofouling through the combined use of stainless steel mesh, electric field, and ultrasonic irradiation to ensure long-term performance of sensors in aquatic high-fouling environments.

Keywords: microalgae; biofilm formation; electric field; ultrasonic treatment; biofouling mitigation

1. Introduction

Biofouling is the colonization process by which biological organisms attach to submerged surfaces in an aqueous environment. This poses significant global industrial problems with increased drag and fuel consumption; it may escalate greenhouse gas emissions, transfer, or introduce, invasive aquatic species into an industrial system [1,2]. In generally, biofouling includes macro- and microfouling in which microfouling is mainly due to the attachment of microalgae and other species to form biofilms on submerged surfaces [3]. Under natural conditions, biofilms on submerged surfaces are mainly composed of microalgae, and the microalgal cell undergoes three main fouling stages, i.e., initial adhesion, rapid growth, and stability, to form stable biofouling microenvironment [4,5]. With regard to scientific water monitoring sensors used to advance our understanding of science and natural biogeochemical processes, biofouling, particularly microfouling caused by microbial biofilm, can became a serious concern for the efficient functioning of the sensing equipment [1,6]. These in-situ sensors and probes are essential tools to characterize the physical, chemical, and biological properties of the aquatic environment. They provide valuable scientific data regarding the biogeochemical processes being studied. Thus, it is crucial to mitigate biofouling on these sensors and probes for ensuring accurate measurements, as biofouling adversely affects their performance, maintenance, and data quality [1]. Noticeably, these in-situ devices have been miniaturized, compared with existing lab-scale sensors, since their small size, reduced production cost, and lower energy consumption, make them economical and convenient to use in a natural, or industrial, environment [7]. As a result, traditional biofouling management processes, including antifouling coatings and in-process cleaning, are not suitable to protect these miniaturized sensors from fouling [8].

Among the variety of approaches to avoid fouling from biofilms on the surfaces of sensors immersed in aquatic environments, stainless steel mesh is a viable support material and can be developed into a protective housing to shield sensor devices from fouling, mainly due to its porosity, flexibility, large surface area, and good mechanical strength [6,9]. Among its structural characteristics, aperture size plays a critical role in determining its properties and specific applications [9]. However, different mesh sizes may not provide sufficient antifouling activity, due to a variety of aquatic microalgal and bacterial organisms. This may lead to compromised performance of sensors, and increased maintenance costs [10]. Therefore, if applying stainless steel mesh to minimize biofouling, this technique needs to be integrated with other approaches to remove microbial biofilms which can develop on the metal mesh, to ensure measuring accuracy.

Effective anti-biofouling strategies, such as surface electrical disturbance or ultrasonic irradiation, are potential, viable approaches for preventing microbial biofilm formation on stainless mesh. In combination, these in-process techniques can effectively control bio-fouling, and maintain accurate measurements from sensors and probes. Electric fields have been shown to be a promising anti-biofouling strategy, since they are efficient and environmentally harmless, and do not require the use of additional chemicals to control fouling. Both electric fields and electric currents have been proved effective in preventing and controlling biofouling on different submerged materials and surfaces [8,11,12]. Some conventional electrical techniques may use pulsed or alternating high frequency electric fields. In comparison, a low-level electrical field, with a low energy consumption, has emerged as a practical antifouling approach, since it has sufficient strength to mitigate biofilm fouling on sensor surfaces [13,14]. Similarly, ultrasonic vibration (20~100 kHz) is an alternative physical treatment for biofouling mitigation; it can remove microbial biofilms from adhering to device surfaces [15]. Additional research has shown that low-frequency (~20 kHz) ultrasound can also generate bubble cavitation, and thus prevent and remove microbial biofilms from instrumental surfaces. The antifouling performance of ultrasonic treatment is determined by three key factors: power density, frequency, and extended time of the ultrasonic irradiation [16,17]. This requires further optimization to successfully prevent microbial adhesion at the early stage, and also remove existing biofilms from the probe surfaces. Regarding the process optimization, *Chlorella vulgaris* is a typical model fouling microalgal species and is recognized as one of the primary representative microorganisms contributing to biofouling. It is widely utilized in many previous studies to systematically investigate anti-biofouling performance of different approaches and treatment processes and other anti-fouling coating research [18,19]. Thus, this model microalgal species is suitable to explore efficient technical methods and approaches for the prevention of biofilms and biofouling on device surfaces.

Furthermore, a variety of research studies have employed mathematical models to investigate microbial biofilm detachment and elimination. These studies have addressed biofilm thickness control and removal, to protect the measuring capacities of sensors and probes. These mathematical models include conventional biphasic models, the Weibull model and other models, which all have the capability to evaluate the dynamic processes of microbial biofilm elimination during ultrasonic treatment [20,21]. However, these dynamic models are case-dependent. Their predictive performance can be affected by different factors, including microbial species, operating parameters, and environmental conditions. Therefore, more research efforts are still required to develop suitable models that can effectively describe and address the challenges of efficient mitigation of biofouling, particularly the prevention and removal of microbial biofilms, which adhere to the surfaces of sensors and probes.

The objective of this study is to evaluate the feasibility of using integrated anti-biofouling strategies to prevent biofilm formation on stainless steel mesh sensor screens, and then to compare mathematical models to simulate biofilm elimination during anti-fouling processes. Briefly, a stainless steel sensor mesh housing for anti-biofouling was developed and evaluated to prevent the interference from microalgae and other particulate matter, and minimize biofilm growth on sensor surfaces. The effects of low-level electric field and ultrasonic treatment were investigated to explore their feasibilities for suppressing the adhesion of microalgae and the subsequent formation of biofilms and biofouling on the surfaces of sensor mesh housings. Subsequently, the proposed mesh screen housing device with an alternating electric field was further tested under real environmental conditions to evaluate its effectiveness in mitigating biofouling caused by biofilm formation of microalgae and bacteria in natural lake water. This was followed by model simulations of biofilm elimination using ultrasonic processes to provide theoretical guidance for future applications. This work will provide valuable insights into the protection of water-monitoring sensors and probes, and to minimize biofouling through the combined use of stainless steel mesh and ultrasonic irradiation to assure long-term performance of instruments in aquatic environments.

2. Materials and Methods

2.1. Microalgae and Materials

The green microalga *Chlorella vulgaris* were used in this study to investigate biofilm formation on stainless steel mesh. The microalga *Chlorella vulgaris* was previously purchased from the American Type Culture Collection (ATCC) and maintained on sterile agar slants containing modified Basal medium and stored in the refrigerator (4 °C) for long-term preservation. Microalgal cells were inoculated into 250 mL Erlenmeyer flasks (100 mL sterilized Basal medium with 25 g/L glucose) and cultivated in an orbital shaker at 150 rpm and 26 °C under continuous light irradiation (ca. 10~20 μmol/m^2/s) for subsequent experiments [22]. Stainless steel woven wires with different mesh sizes of 80, 300, and 500 (hole diameters: 177, 55, and 25 μm) were purchased from local companies and used for the fabrication of mesh housing to prevent biofouling on sensor surfaces. Microalgal culture solution and untreated natural water from a local lake were used as sources of microalgae and bacteria for biofilm formation on mesh housings in the subsequent experiments.

2.2. Effects of Different Mesh Sizes and External Electric Field on Biofilm Formation on Sensor Mesh Housing

An innovative mesh housing for anti-biofouling was developed and evaluated to prevent the interference from microalgae and other particulate matter, and minimize biofilm growth on sensor surfaces. In this section, considering that microalgae is the main species in biofilms and for the simplification of experiments, *Chlorella vulgaris* culture solution was used in the study for investigating the effects of different treatments on biofilm formation on sensor mesh housings. This device was based on electrochemical techniques, and developed as an effective antifouling method, based on electrochemical characteristics of biofilm components and their interaction. The housing consisted of a set of mesh screen electrodes with low DC voltage (1.5 V) to create a low electric field, to attract the algae and biofilms onto the outside screen electrode. This design was used to prevent the biofilm from getting onto the inside screen electrode for 24 h, to protect the sensor inside the inner screen electrode from any interference from these microorganisms or other particulate matters. These screen electrodes were made of stainless steel woven wires with different mesh sizes (80, 300, and 500 mesh). The purpose of mesh screens with electric field was to minimize biofilm formation, and compare their performance, in order to select the optimal screen mesh size, and best protect the sensor surface.

2.3. Effect of Alternating Electric Field on Biofilm Prevention on Mesh Screen Electrodes for Biofouling Mitigation

To explore the feasibility of surface electrical disturbance for suppressing the initial adhesion of microbes and the subsequent formation of biofilms and biofouling, a low-intensity alternating electric field (3.6 V/cm) with a frequency of 60 Hz was evaluated for 12 h, to investigate their effects of biofilm prevention on mesh screen electrodes made of stainless steel woven wires with different mesh sizes (80, 300, and 500 mesh). The low-level electric field applied here offers several advantages over conventional electric anti-biofouling techniques which typically use strong electric fields (1–100 kV/cm) at high frequencies (~1000 Hz) [13]. The effects were investigated and compared through evaluating changes of microalgal cell density in the solution throughout the treatment processes.

2.4. Effects of Different Time Intervals of Ultrasonic Treatment on Biofilm Elimination from Mesh Screen Housing for Antifouling

Ultrasonic irradiation is an effective method to reduce and control biofouling on sensors immersed in aquatic environmental conditions. 300-mesh stainless steel woven wires was selected to fabricate mesh screen housing for the following ultrasonic irradiation experiments. To facilitate biofilm formation on these mesh screen electrodes, an external DC electric field at 1.5 V was applied to the screen electrodes for more than 12 h. Subsequently, an ultrasonic sonicator operating at a frequency of 30 kHz and a working voltage of 12 V, was employed to treat the screen electrodes for 0, 30, 60, 120, 180, and 240 s to evaluate the effects of high-frequency ultrasound on biofilm removal and biofouling prevention. After ultrasound treatment at different time intervals, changes in OD_{680} of the microalgal solution within inner screen electrodes, with and without the electric field, as well as the external microalgal solution, were compared to assess the feasibility of low-frequency ultrasound for biofilm removal.

2.5. Investigation and Prediction of Biofilm Formation by Microalgae and Microbes from Local Lake Water on Stainless Steel Sensor Mesh

In order to realize the effective protection of the sensor from biofouling, the proposed mesh screen housing device with the external electric field, was further tested in real environmental conditions, for actual field testing of the device. Given the detrimental effects of biofilms on the accuracy and reliability of sensor measurements, early-stage detection and mitigation are essential for effective biofilm suppression and sensor anti-biofouling. Thus, an early-stage biofilm formation on stainless steel screen electrodes was simulated and conducted in this section over a period of five days. This study aimed to assess the viability and technical feasibility of using different anti-biofouling approaches for biofilm mitigation in real environments. Briefly, stainless steel screen housings made of 500-mesh stainless steel woven wires submerged for 5 days, in bottles containing the natural lake water, to allow the initial biofilm formation. Meanwhile, a low-intensity alternating electric field (3.6 V/cm) was applied to evaluate its effects on biofilm prevention on the mesh screen housings. Changes of temperature, pH, water conductivity, and cell densities of microalgae and bacteria, during the treatment processes were measured and compared, to evaluate the effects of the low-frequency electric field for biofilm prevention. Additionally, the correlation analysis was conducted to evaluate cell densities of total cells and microalgae that adhered to the surfaces of stainless steel electrodes for biofilm formation.

2.6. Low-Frequency Ultrasonic Treatment for the Elimination of Formed Biofilm on Stainless Steel Sensor Mesh

Ultrasonic process is an effective method for biofilm elimination from electrode surfaces. In this section, an ultrasonic sonicator operating at a frequency of 30 kHz, was tested to evaluate the effects of high-frequency ultrasound on the removal of biofilms generated in the above experiments. After ultrasound treatment, variations of optical densities of the solution at a wavelength of 680 nm for microalgae and 580 nm for bacteria and/or cyanobacteria [19,23], as well as cell number concentration changes of microalgal cells and total cells, were compared to assess the feasibility of low-frequency ultrasound for biofilm elimination [24]. Subsequently, in order to evaluate the dynamic processes of biofilm elimination, and changes to the biofilm cells, mathematical models, including Polynomia, Weibull, and Biphasic models [21,25], were compared to describe biofilm elimination processes. The adjusted determination coefficient (adj-R^2) and the root mean square error (RMSE) were used to assess and determine which mathematical model best predicted the changes to the biofilm cells during the ultrasonic treatment.

2.7. Analytical Methods

To determine the cell densities of microalgae and bacteria, the optical densities at 680 nm and 580 nm were measured using the Hach DR 5000 spectrophotometer (Hach Company, Loverland, CO, USA). The cell number concentrations of total cells were measured using an automated Nexcelom Cellometer Auto X4 cell counter (Nexcelom Bioscience, Lawrence, MA, USA). Microalgal cell concentrations were determined using the same counter equipped with a fluorescence optics module XB-535-401 to measure the counts of fluorescent cells.

2.8. Data Analysis

Experimental data shown in the figures are presented as the mean value ± standard deviation (SD). All experiments were performed in triplicate. One-way analysis of variance (ANOVA) and the Student's *t*-test were used to analyze the statistical significance of the results with the significant levels of $p < 0.05$ or $p < 0.01$. Values marked with asterisks indicate statistically significant differences compared to the control group (* $p < 0.05$; ** $p < 0.01$). Data analysis was performed using the Origin software (OriginLab Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. Effects of Different Mesh Sizes and External Electric Field on Biofilm Formation on Stainless Steel Screen Mesh Housings

Microfouling primarily the attachment of microalgal biomass to solid surfaces is a widespread phenomenon and remains one of the key challenges to the long-term deployment of submerged water sensors and autonomous environmental monitoring in aquatic environments [26]. To mitigate and control biofouling on the surfaces of sensors immersing in water, the application of an electric field is also considered a promising anti-biofouling strategy for preventing biofilm formation on the surfaces of these sensors [8,27]. Thus, this study developed and evaluated an innovative housing design (Figure 1) consisted of a set of mesh screen electrodes with different mesh sizes of 80, 300, and 500 for such submersed sensors, to prevent the interference of microalgae and eliminate the microalgal biofilms onto sensor surfaces. It is based on electrochemical techniques and developed as an effective antifouling method based on electrochemical characteristics of biofilm components and their interaction. Besides, the biofouling of sensor mesh housings made of stainless steel woven wires by the microalga *Chlorella vulgaris* was investigated, mainly due to that microalgae are generally the first colonizers of submerged materials. They can sense and respond to different surfaces by secreting polysaccharides, which help regulate biofilm dynamics and promote initial cell attachment [28].

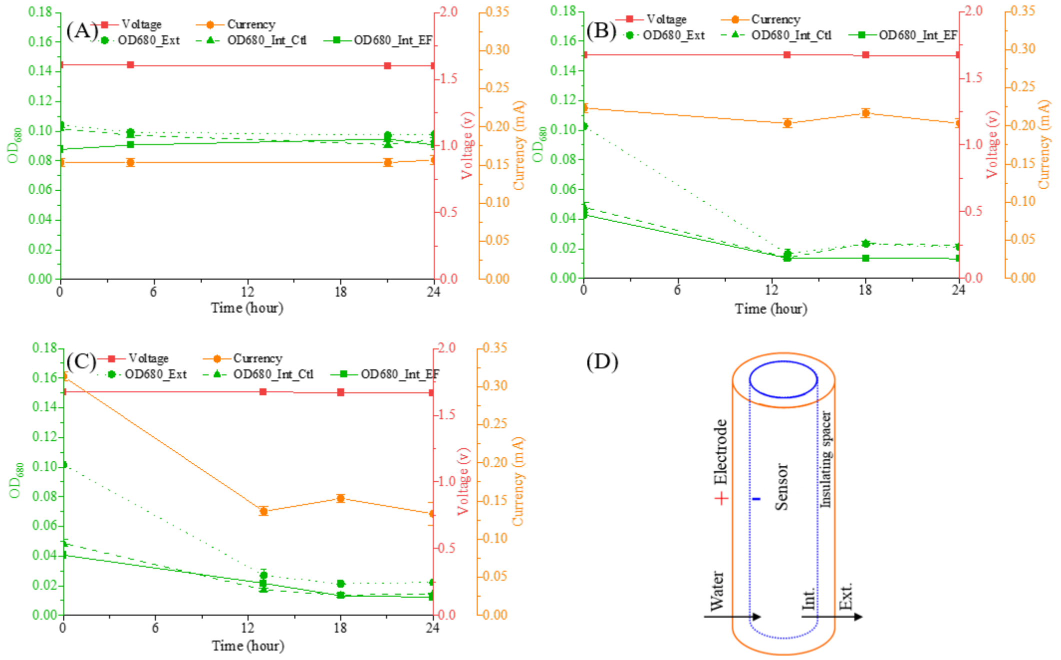


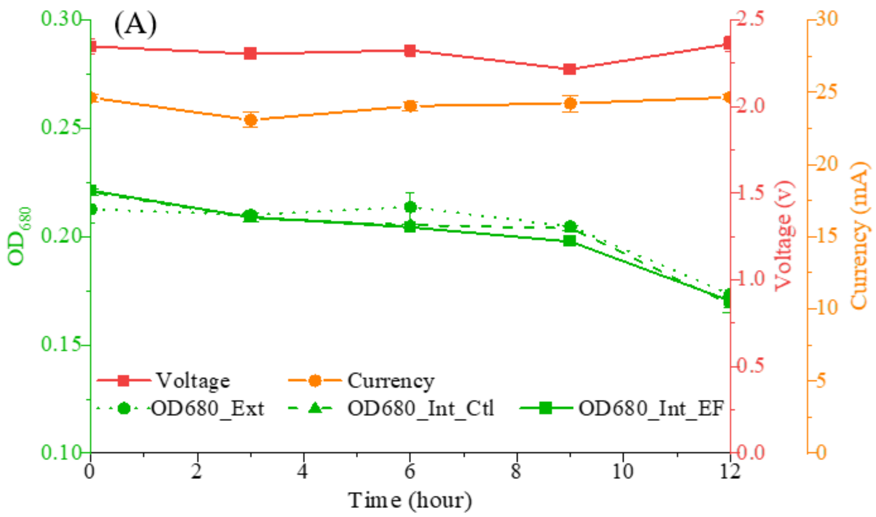
Figure 1. Voltage and current changes of the electric field and variations in OD_{680} of microalgal solutions within inner screen electrodes with electric field (EF), inner screen electrodes without electric field (Control), and the external microalgal solution throughout the treatment processes. Schematic diagram (**D**) of the mesh housing composed of two layers of stainless steel woven wires used for preventing biofouling on sensor surfaces. Three different stainless steel woven wires with mesh size of 80 (**A**), 300 (**B**), and 500 (**C**) were used as screen electrodes and connected to a DC voltage to create an electric field (2.6 V/cm) for the protection treatment.

As shown in Figure 1A–C, during the electric field treatment, the voltages were slowly decreased. The current was very small, in the range of several microamperes, and basically remained unchanged during the process. After 24-h treatment of external electric field, biofilms particularly microalgae can accumulate on, or be attracted to, the surfaces of outside screen positive electrodes with 300 and 500 mesh sizes, thus effectively protecting the inside screen electrode and also sensors inside from biofouling. Similarly, a previous study reported that 316 stainless steel mesh with the smallest pore mesh with 800# demonstrated the most effective antifouling performance [10], which suggested that the pore/mesh size of stainless steel mesh may significantly influence its antifouling capability when used in sensor housings. The variations of OD_{680} within different parts of this device, clearly demonstrated that both the mesh size of the screen housing and the electric field treatment played important roles in protecting the internal sensor through absorbing microalgal cells or other biofilm components, onto the surfaces of the outside screen electrode. The results was partly consistent with a previous study, which reported that the type of sensor housing can affect sensor measurements. However, that study recommended using protective housing with large openings or holes to ensure accurate sensor readings [29]. In contrast, our findings suggest that, in terms of biofouling prevention, mesh housings with smaller pores provided more effective antifouling protection for the sensor. Thus, to effectively prevent biofilm formation, the stainless steel mesh screen housing should be made of stainless steel woven wires with 300 or more meshes. This type of mesh allowed only specific particles smaller than 55 μm to pass through its openings, effectively minimizing the entry and attachment of potentially biofouling microorganisms into the sensor area. In addition, the application of electric field treatment successfully inhibited biofilm formation, thereby preventing biofouling on sensor surfaces. It was reported that applying an electric field at extreme potentials of ±0.9 V could alter the redox potential across bacterial cell membranes, disrupt redox homeostasis, and reduce exopolysaccharides (EPS) production, thereby ultimately inhibiting bacterial growth and biofilm formation for anti-biofouling [30]. Unlike conventional electrical techniques that rely on high-frequency, high-strength electric fields [13,14], the results demonstrated that the proposed sensor housing with an external electric field can prevent biofouling of sensors since a low-level strength of electric field is sufficient to mitigate biofilm fouling on sensor surfaces.

3.2. Effect of the Alternating Electric Field on Biofilm Formation on Sensor Mesh Screen Housings

Electric field with a low-level strength and energy consumption has emerged as a practical antifouling approach [31,32]. However, considering that the continuous persistence of the electric field would lead to the accumulation of biofilms on the surface of outside screen electrode, it is better to use an alternating electric field and then evaluate its electric field intensity for the best treatment performance of antifouling. In this section, surface electrical disturbance was evaluated and tested to prevent microbial adhesion at the initial stage and disturb existing biofilms already on the electrode housing for antifouling of sensors and probes immersed in aquatic environments.

As shown in Figure 2, due to discrepancies in the mesh size of the stainless steel woven wires used as screen electrodes, the initial OD_{680} of the microalgal solution may vary. The 500 mesh size was more favorable for the prevention of microalgal adhesion and biofilm formation. The variations of OD_{680} within different parts of this device further clearly demonstrated that the alternating electric field treatment effectively prevents biofilm growth and adhesion. Unlike the external DC electric field preventing biofilms on the inside of the screen electrode, the application of an alternating electric field, after 12-h treatment, can destroy microalgal cells and thus decrease cell densities both within and outside the screen electrodes. Previous studies have reported that surface electrical disturbance can effectively suppress the initial adhesion of microbes and eliminate those in the proximity of sensor mesh surfaces [8,33]. This is achieved through direct electrification, which transfers electrons between the electrodes and microbial films, leading to cell inactivation and thereby preventing the subsequent formation of biofilms and biofouling. Additionally, an alternating electrical field at low voltage can repel microbes through the dielectrophoresis effect, and can even kill microbial cells via membrane electroporation [13,14]. It was consistent with a previous study reported that increasing the intensity of a low voltage electric field (0.6~1.5 V) gradually reduced bacterial cell attachment to carbon nanotubes membranes with a concomitant high inactivation (95.3%) of attached bacteria achieving at 1.5 V [32]. Additionally, it has been reported that electric fields ranging from 2 to 6 V/cm with corresponding electric potentials between 1 and 3 V, can effectively protect the surfaces of two epoxy-encapsulated aluminum wires from biofouling [13]. Overall, the results proved that the alternating electric field with the mesh screen housing can effectively prevent microbes from attaching to the surfaces of the screen electrodes and the sensor inside the screen electrodes.



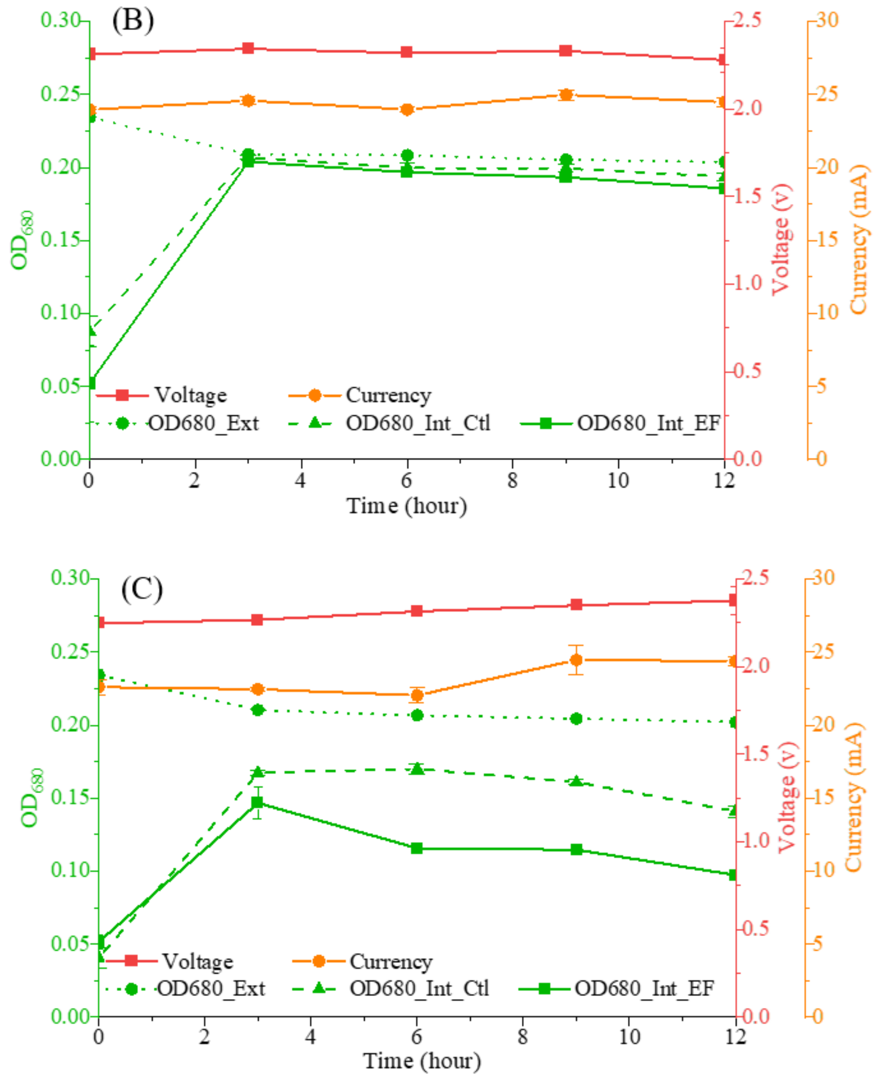


Figure 2. Voltage and current changes of the alternating electric field between screen electrodes composed of different stainless steel woven wires with mesh size of 80 (**A**), 300 (**B**), and 500 (**C**) for biofilm prevention, and variations in OD_{680} of microalgal solution within inner screen electrodes with alternating electric field (EF), inner screen electrodes without alternating electric field (Control), and the external microalgal solution.

3.3. Effects of Different Time Intervals of Ultrasonic Treatment on Biofilm Elimination from Protective Sensor Mesh

Fouling from microorganisms and particulate matters can clog the mesh screen in practical applications, which necessitated effective maintenance practices [9]. Ultrasonic irradiation is an effective method to reduce and control biofouling on sensors under environmental conditions. It is well known that high-intensity ultrasound induces cavitation, which generates vapor bubbles capable of disrupting microbial cells and biofilm structures before they form [1,31]. In this study, an ultrasonic sonicator operating at a frequency of 30 kHz and a working voltage of 12 V, was employed and tested to evaluate the effects of high-frequency ultrasound on biofouling control. As shown in Figure 3, the ultrasonic sonicator was used to treat the screen electrodes for 0, 30, 60,120, 180, and 240 s to investigate the effects of low-frequency ultrasound on biofilm removal and biofouling prevention. The variations of OD_{680} of microalgal solution within inner screen electrodes with/without electric field, as well as the external microalgal solution, clearly demonstrated the feasibility of low-frequency ultrasound for biofilm removal. It was illustrated that, after 240 s of ultrasonic treatment, the OD_{680} values of both the external microalgal solution and the solution within the control inner screen electrode increased significantly ($p < 0.05$). It was indicated that microalgal cells had detached from screen electrodes. However, the OD_{680} within the inner screen electrode, with the applied DC electric field, remained stable, showing no significant changes. The above results demonstrated that surface electrical disturbance and ultrasonic irradiation are two effective anti-biofouling strategies for preventing biofilms and biofouling on both the protective housing and the sensor. These strategies can be combined with our proposed housing, which utilizes screen electrodes and an external electric field, to enhance the prevention and control of biofilms and biofouling.

However, further research is still needed to optimize the conditions of the alternating electric field and low-frequency ultrasound, for improved anti-biofouling performance.

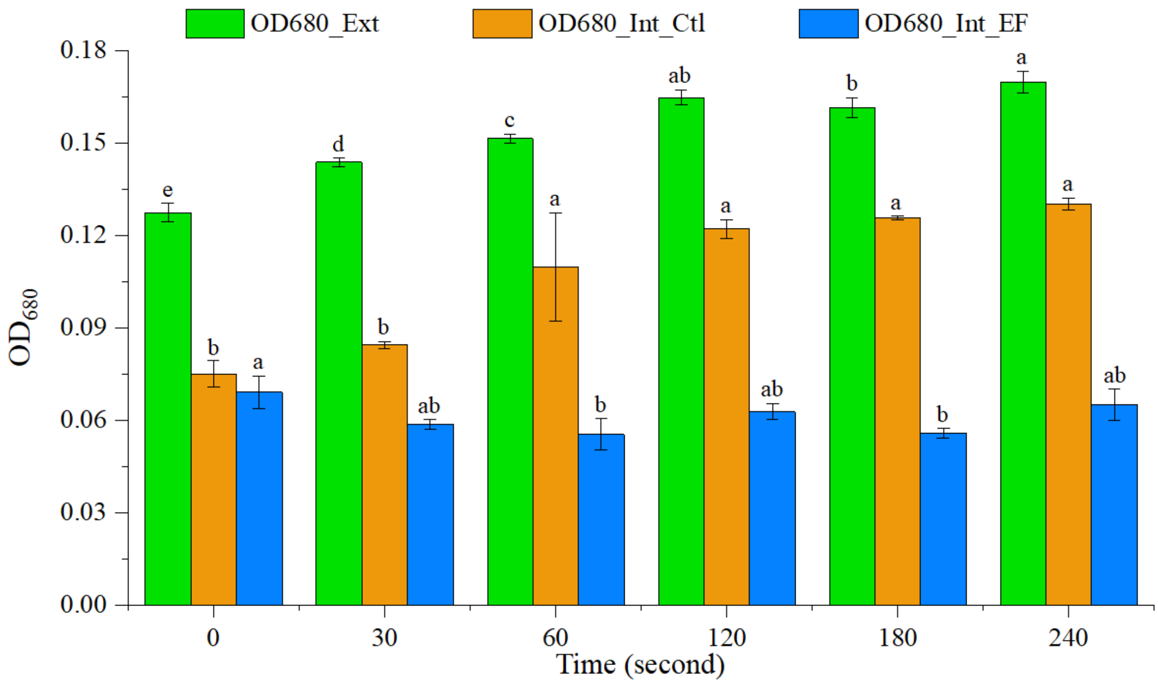


Figure 3. Changes in OD_{680} of the microalgal solution within inner screen electrodes, with and without the electric field, as well as the external microalgal solution during the low-frequency ultrasonic treatment for biofilm removal from the housing device to assess the feasibility of low-frequency ultrasound for biofilm removal. Bars with different letters are significantly different from each other ($p < 0.05$).

3.4. Investigation and Prediction of Biofilm Formation by Microalgae and Microorganisms from Local Lake Water on Stainless Steel Sensor Mesh Housings

Biofilm formation and development are dynamic, multi-step processes involving five main stages: reversible attachment, irreversible attachment, microbial cells synthesize and secrete EPS, maturation, and dispersal [34,35]. Of these, the initial three stages are particularly critical for biofilm control. They are influenced not only by intrinsic microbial properties but also various environmental factors. These changes in environmental conditions can considerably affect both microbial behavior and surface properties [34]. Therefore, in order to realize the effective protection of the sensors from biofouling, the proposed housing device with the external electric field was further tested in real environmental conditions for actual field testing of the device. Briefly, biofilm formation on stainless steel screen electrodes in local natural lake water was simulated and investigated for performance evaluation. Based on our previous experiments in microalgal culture solution, microalgae can form biofilm on stainless steel screen housings in a time-dependent manner, with cell adhesion and early-stage biofilm development occurring predominantly within the first one or two days. Therefore, screen electrodes made of 500-mesh stainless steel woven wires were submerged in the nature lake water for 5 days to allow biofilm formation. Moreover, our previous studies have already demonstrated that the alternating electrical field at low frequency can repel microbes through the dielectrophoresis effect and even kill microbial cells via membrane electroporation. Thus, in this study, a low-intensity alternating electric field (3.6 V/cm) was applied to evaluate its effects on biofilm prevention on the sensor mesh housings.

As shown in Figure 4, crucial environmental factors including temperature and pH remained relatively stable throughout the period, while only water conductivity exhibited significant changes compared to the control group ($p < 0.05$). Prior research has reported that, during the early stage of biofilm formation, the absorption of organic compounds onto the surfaces reduces hydrophobicity and leads to partial pore wetting. This, in turn, results in a sharp increase of permeate conductivity [36]. Such a mechanism could also clearly explain the observed sudden rise in water conductivity observed at the 12 h in the present study. Overall, these environmental conditions remained relatively stable throughout the process and appeared to exert limited influence on microbial activity and biofilm development. With regard to other factors not investigated in the study, dissolved oxygen levels likely remained stable due to continuous mixing during the early stages of biofilm formation. A previous study reported that dissolved oxygen had a lesser effect on biofilm formation compared to more viable factors such as temperature and nutrient availability [37]. Nutrient concentrations, particularly phosphorus, are considered important for microbial adhesion and biofilm development; however, their levels in the natural, healthy shallow lake water used in our study were low (e.g., ~60 μg/L of phosphorus). During the initial stages of biofilm development, particularly the reversible and irreversible attachment phases, nutrient availability may have a limited effect. It was reported that, at this early stage, microorganisms can produce extracellular polymeric substances (EPS) that mediate strong attachment to surfaces, and microalgal cells can aggregate and adhere irreversibly, independent of external environmental conditions [34,35]. Therefore, it was inferred that the changes of nutrient concentration during the treatment process likely had limited impact on biofilm formation particularly in the early attachment stages. Altogether, these environmental factors appeared to exert limited influence on microbial activity and biofilm development throughout the treatment process.

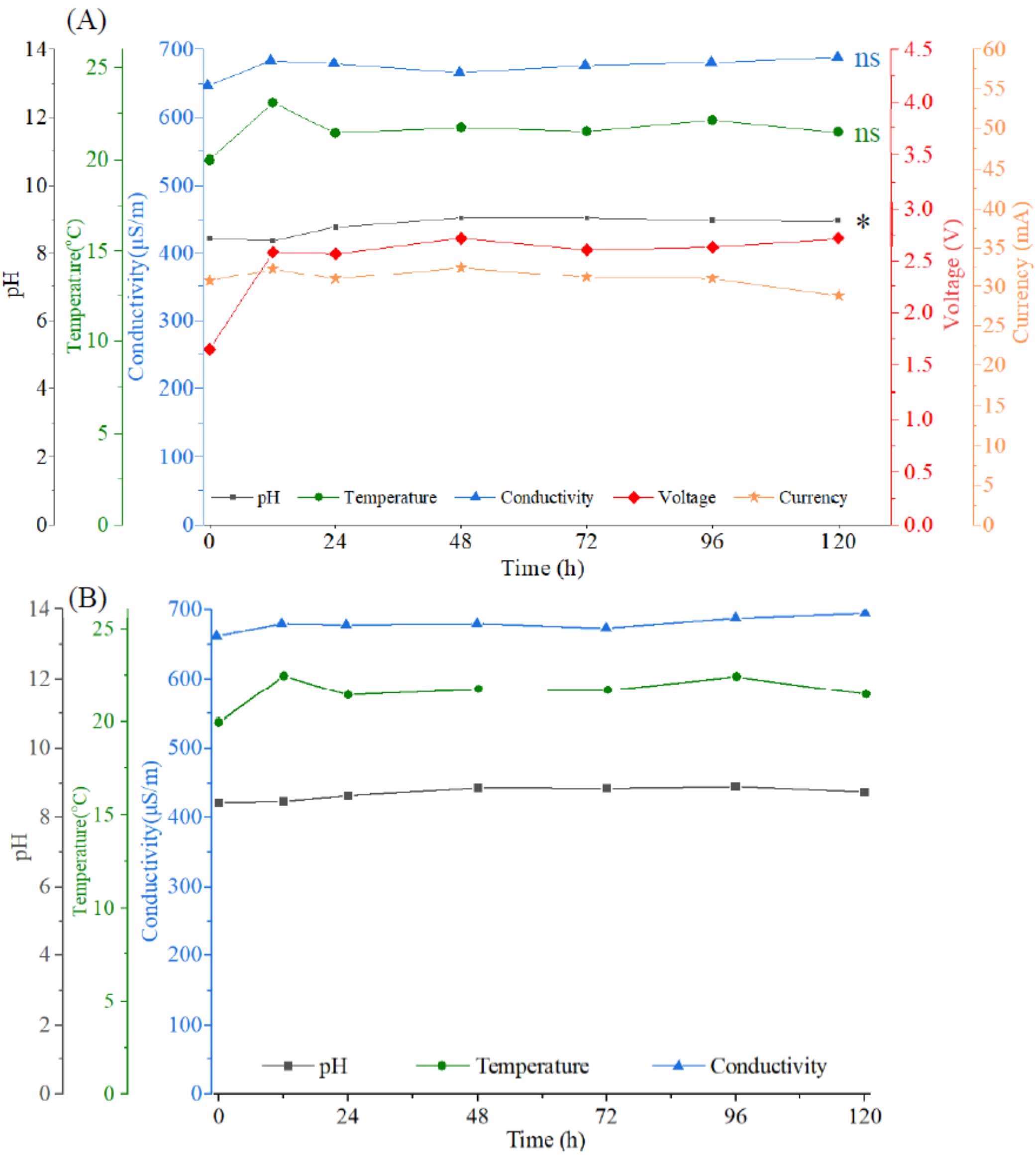


Figure 4. Changes in cultural parameters during biofilm formation by microalgae and microorganisms from local lake water on stainless steel screen mesh with (**A**) and without (**B**) a low-frequency electrical field for biofilm prevention. Values marked with an asterisk indicate statistically significant differences compared to the control group (* $p < 0.05$). ns: no significance.

Figure 5 demonstrates the variations of OD_{680} and OD_{580} of the solution within inner screen electrodes with alternating electric field (EF) and the external microalgal solution throughout the treatment processes. Optical density at a certain wavelength is generally a widely used method to measure microbial population density. In this study, OD_{680} was used for microalgal cell density determination for investigating electric field effects. OD measurements in the range of 580 ~620 nm are theoretically valid for bacterial density determination. Considering that certain materials such as exopolysaccharide may affect the absorbance values of microbial cells and cyanobacteria usually measured at the wavelength of 580 nm [19,23,35], OD_{580} measurement was used for population density estimation of bacteria including cyanobacteria. The results indicated that the alternating electric field can destroy microbial cells and thus decreased cell densities both within the screen electrodes. Although OD_{680} and OD_{580} of the solution may vary, it was shown that the alternating electric field effectively prevented microbes from attaching to the surfaces of the screen electrodes and also the sensor inside the screen electrodes particularly during early stages of treatment.

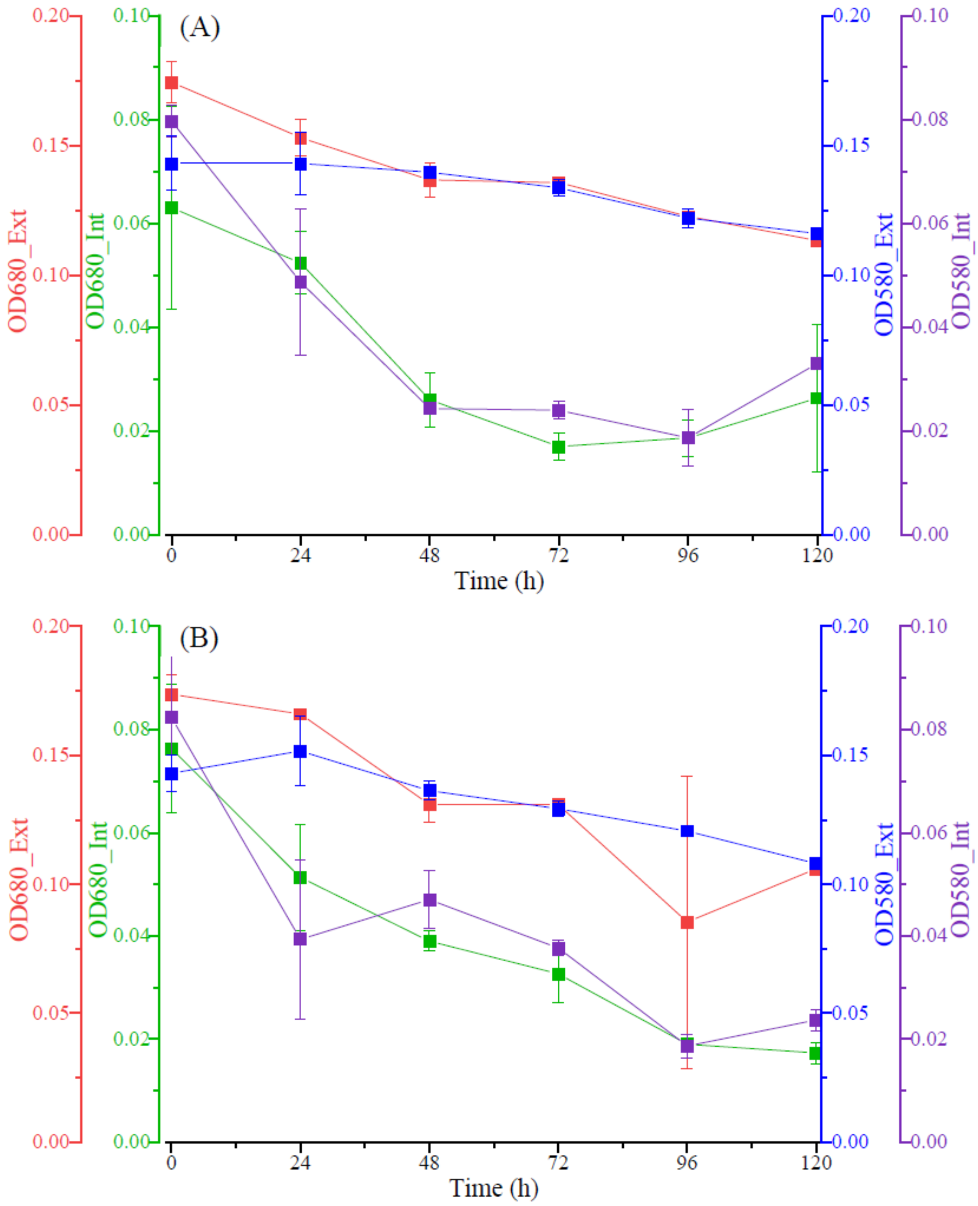


Figure 5. Variations in OD_{680} (microalgal density) and OD_{580} (bacterial density) within inner screen electrodes with alternating electric field (EF) and the external lake water were investigated and compared throughout the treatment processes. (**A**): screen electrodes with alternating electric field; (**B**): screen electrodes without alternating electric field as the control.

Furthermore, in order to assess the differences in microalgae and bacteria adhering to the surface of electrodes during biofilm formation, the changes in cell densities of microalgal and bacterial cells in the solutions were monitored throughout the treatment. As shown in Figure 6, the changes in the cell densities of total microbial cells, microalgae, and bacteria in the solution were investigated and compared throughout the treatment processes. The results indicated that the cell densities of total cells and microalgae in the solution declined a little more rapidly under the treatment with an external alternating electric field, exhibiting a significant difference compared to the control ($p < 0.05$). This may be attributed to the alkalinity of natural lake water and the disruption of microbial cells caused by the alternating electric field. The results were in accordance with previous studies reported that the application of the alternating electric field can generate disinfecting effects through electrochemical reactions and effectively kill or suppress biofilm formation [38]. Considering that biofilm formation as well as the adhesion of microbial cells to the surface are always time dependent, the correlation analysis was conducted to evaluate cell densities of total cells and microalgae adhered to electrode surface during the period. Figure 7 obviously demonstrated that there was a cell-adhesion pattern between electrode surfaces. The counts of total cells and microalgal cells that are adherent to electrode surface increased along with the time. It was consistent with that the adhesion and formation of biofilms are time dependent. Generally, stainless steel is a durable and antifouling material with a surface roughness in the range of 0.5~3 μm, which is not particularly prone to biofouling. However, it is still possible for microalgae and bacteria to attach to the relatively smooth surface of stainless steel and establish a foothold for growth [6]. Additionally, the persistence of external electric field kindly accelerated cell adherence and biofilm formation particularly at the first 48 h. Altogether, the results demonstrated that microalgae and bacteria can from biofilm on stainless steel screen electrodes in a time-dependent manner, with the 48-h time interval being crucial for cell adhesion and biofilm formation.

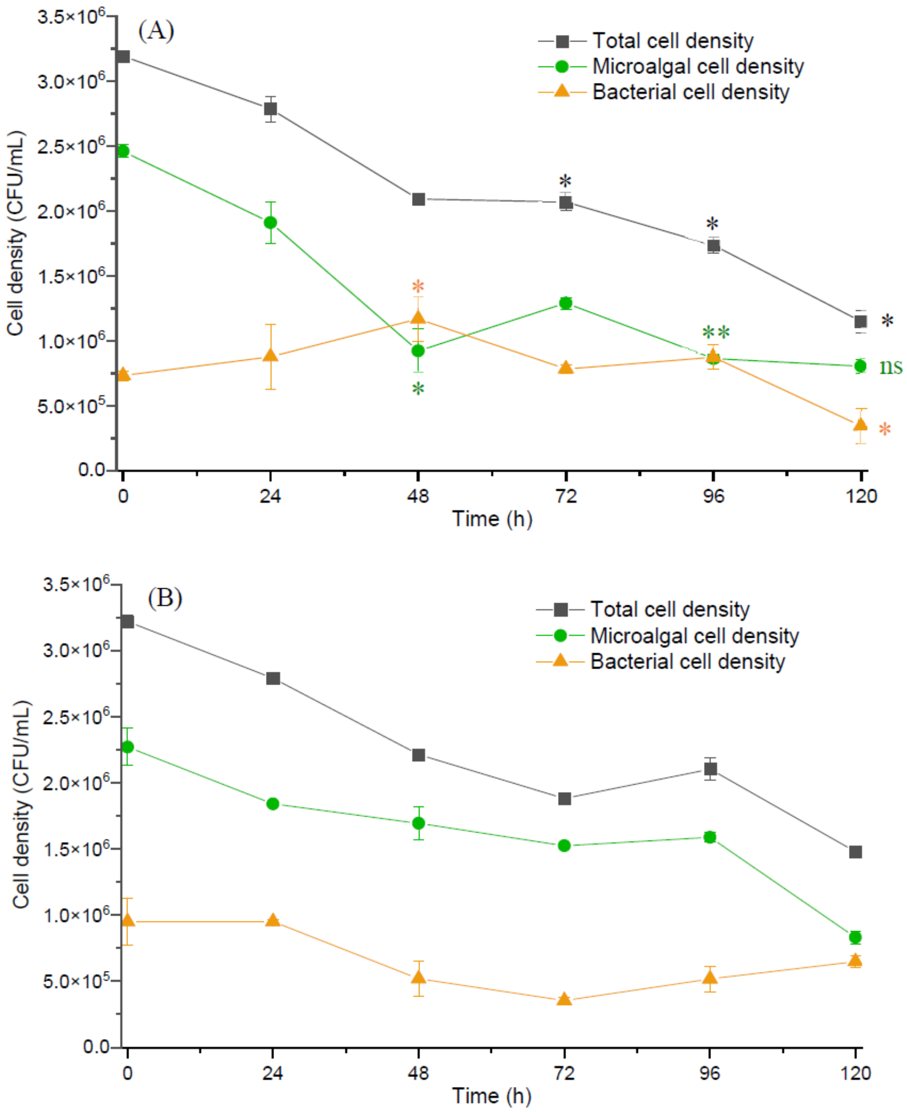


Figure 6. Time changes in cell densities of total cells, microalgae, and bacteria in lake water during the treatment processes with (**A**) and without (**B**) alternating electric field (EF). (**A**): screen electrodes with alternating electric field; (**B**): screen electrodes without alternating electric field as the control. Values marked with asterisks indicate statistically significant differences compared to the control group (* $p < 0.05$; ** $p < 0.01$). ns: no significance.

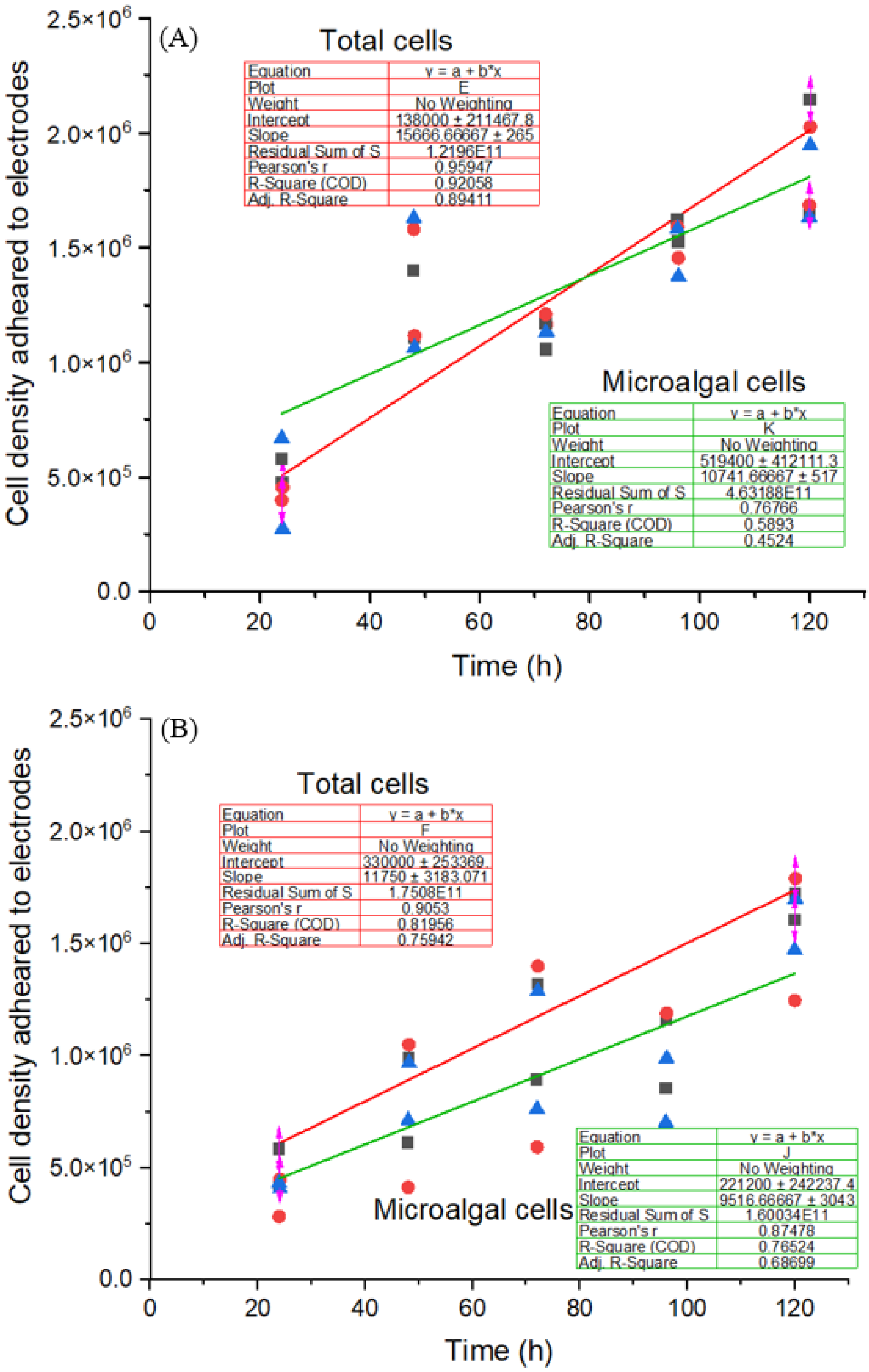


Figure 7. Time-dependent linear relationships of total cells and microalgal cells adhered to the surfaces of stainless steel screen electrodes in lake water. (**A**): screen electrodes with alternating electric field; (**B**): screen electrodes without alternating electric field as the control.

3.5. Low-Frequency Ultrasonic Treatment for the Elimination of Formed Biofilm on Stainless Steel Sensor Mesh Housings

Ultrasonic process is an effective method for biofilm elimination from sensor surfaces because ultrasonic waves with frequencies ≥ 18 kHz can create oscillating regions with the support of cavitation and generate vapor bubbles capable of disrupting biofilm structures for biofouling mitigation [8]. Based on the aforementioned experiments, the biofilm generated on sensor meshes can be further treated by the low-frequency ultrasonic exposure to eliminate the formed biofilms on the stainless steel screen electrodes. In this study, an ultrasonic sonicator operating at a frequency of 30 kHz, was employed and tested to evaluate the effects of low-frequency ultrasound on the removal of biofilms generated in above experiments. After ultrasound treatment, the variations of OD_{680} and OD_{580} of the solution were investigated and compared to assess the feasibility of low-frequency ultrasound for biofilm elimination. These results will be compared to the mathematical model simulations for biofilm elimination using ultrasonic process, thereby providing theoretical guidance for future applications.

As shown in Figure 8, after 180 s of ultrasonic treatment, the OD_{680} and OD_{580} values of the external solution increased significantly ($p < 0.05$) at 120 h, which indicated that microalgal and bacterial cells had detached from the mesh housings. These results were consistent with the data presented in Figure 9. The cell densities of total cells increased significantly at the first 120 h ($p < 0.05$). The results demonstrated that microbial cells were much easier to be detached from the biofilms which were adhered to the screen electrodes with alternating electric fields. Additionally, the results indicated that, compared to high-frequency ultrasound (>200 kHz), low-frequency ultrasound (20~100 kHz) has a low energy consumption but still has sufficient strength to generate bubble cavitation and induce a range of mechanical stresses and effects for mitigating biofilm fouling. These characteristics make it suitable for antibacterial applications and biofouling prevention [17,39]. These findings provide valuable guidance for future applications of the proposed housing device for biosensor protection and biofouling prevention under real environmental conditions.

Furthermore, ultrasonic treatment is a widely-used approach for the inactivation of microorganisms over a variety of conditions. Various mathematical models can be used to analyze the inactivation kinetics and to optimize the best treatment condition for microorganism inactivation [25,40]. In this study, in order to evaluate the dynamic processes of biofilm elimination and number changes of biofilm cells, the advanced Polynomia, Weibull, and Biphasic models were used to compare the biofilm elimination processes data, and better predict the number changes to the biofilm cells throughout the ultrasonic treatment.

As shown in Figure 10, all these models can fit the observed experimental data of total cells detaching from the biofilms. The fitted parameters of the Polynomia, Weibull, and Biphasic models were calculated and presented in Table 1. These models can be used to evaluate the dynamic processes of biofilm elimination, optimize the treatment conditions, and further reduce the biofilms formed on sensor mesh housings for long-term anti-biofouling. According to the fitted lines of both models, no significant decrease was observed in the first 60 s of the process, and most of the removal of biofilm occurred during the 60 to 120 s. These results indicate that the resistance of biofilms to intervention, occurred during the initial period. These findings further underscored the importance and value of predictive modeling of biofilm elimination kinetics, which can be used to compare and optimize ultrasonic treatment parameters.

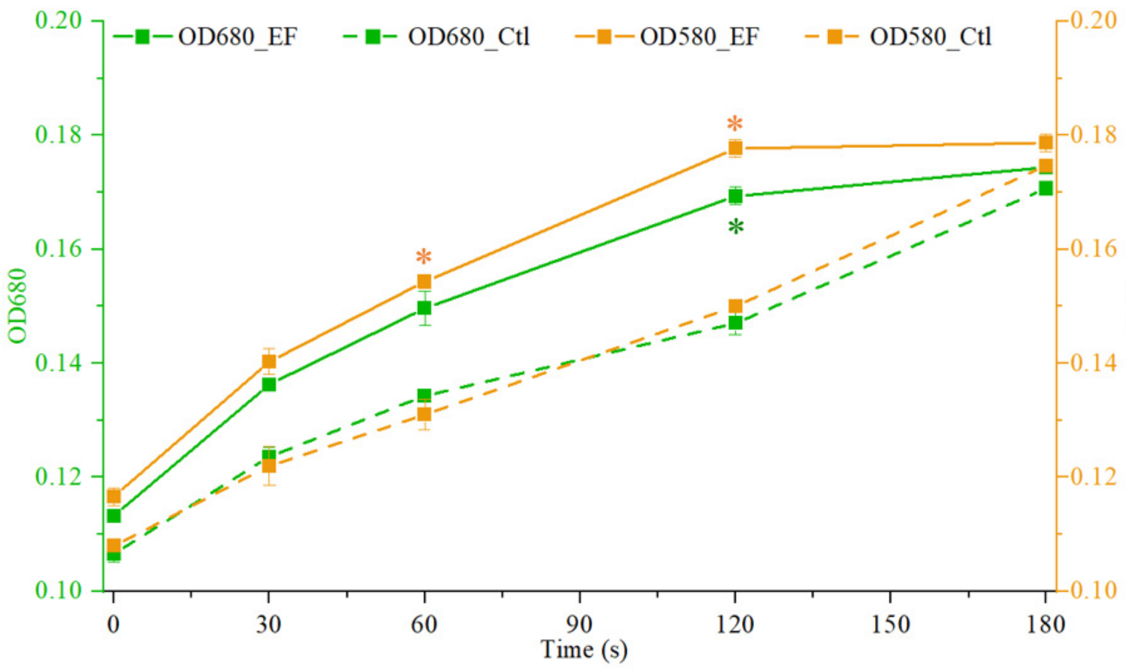


Figure 8. Changes in OD_{680} and OD_{580} in lake water throughout the ultrasound treatment for comparisons of the feasibility of low-frequency ultrasound for biofilm removal. Solid lines: screen electrodes with alternating electric field; dash lines: screen electrodes without alternating electric field as the control. Values marked with an asterisk indicate statistically significant differences compared to the control group (* $p < 0.05$).

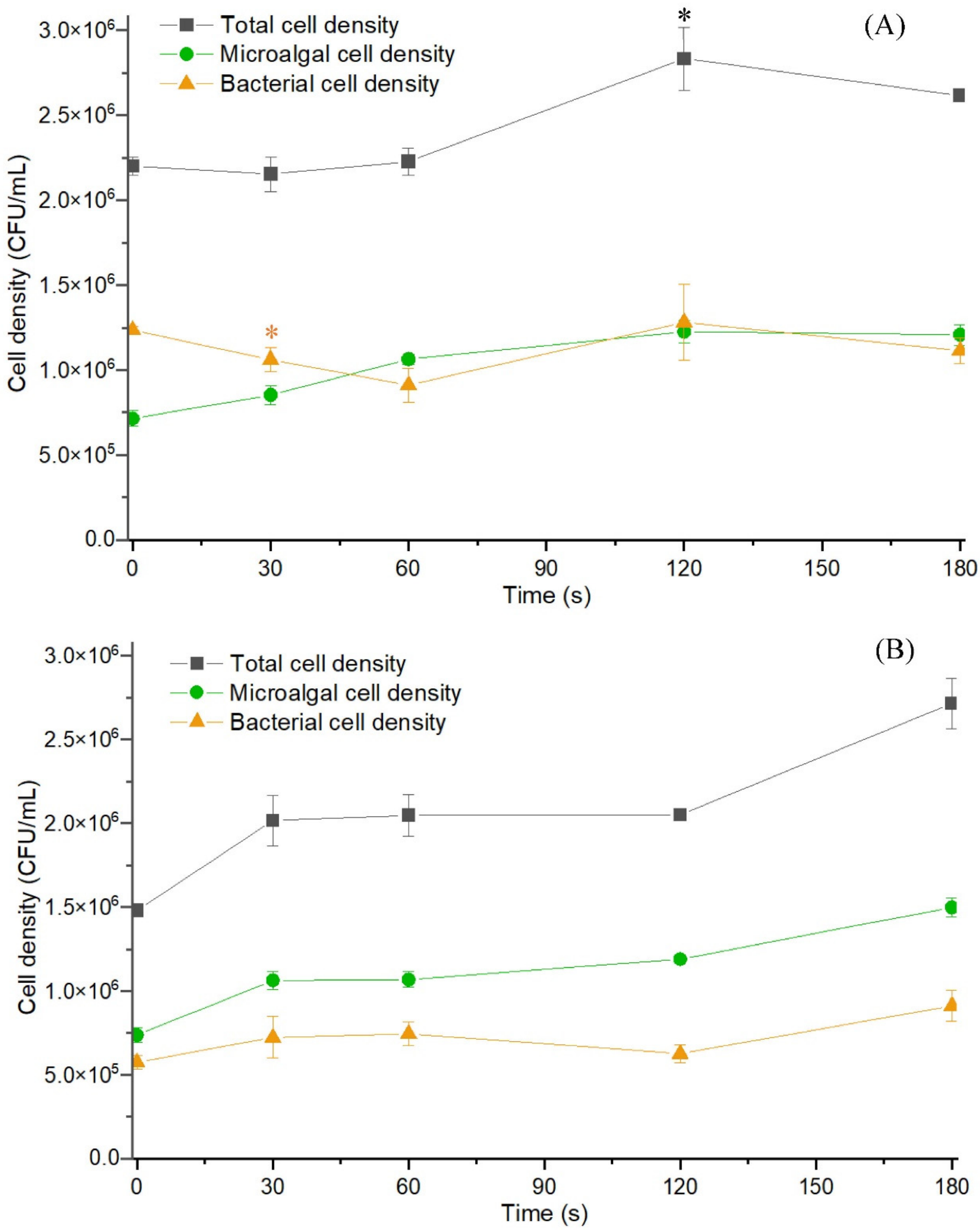


Figure 9. Time changes in cell densities of total cells, microalgae, and bacteria in lake water during the ultrasonic treatment. (**A**): screen electrodes with alternating electric field; (**B**): screen electrodes without alternating electric field as the control. Values marked with an asterisk indicate statistically significant differences compared to the control group (* $p < 0.05$).

Table 1. Polynomia, Weibull, and Biphasic models for fitting experimental data of biofilm elimination using low-frequency ultrasonic treatment.

Models	Equations	k	α	β	SSE	RMSE	Adj. R-Square
Polynomia	$N(t) = k + \alpha * t^1 + \beta * t^2$	2.04×10^6	4.27×10^3	−79.30	9.30×10^{11}	4.65×10^{11}	0.8184
Weibull	$N(t) = N_0 - k * t^\alpha$	8.16×10^{-4}	4.28	/	4.38×10^{13}	2.19×10^{13}	0.8296
Biphasic	$N(t) = N_0 * (k * \exp(-\alpha t) + (1 - k) * \exp(-\beta t))$	−1.97	-8.35×10^{-3}	-5.94×10^{-3}	4.38×10^{13}	1.46×10^{13}	0.8183

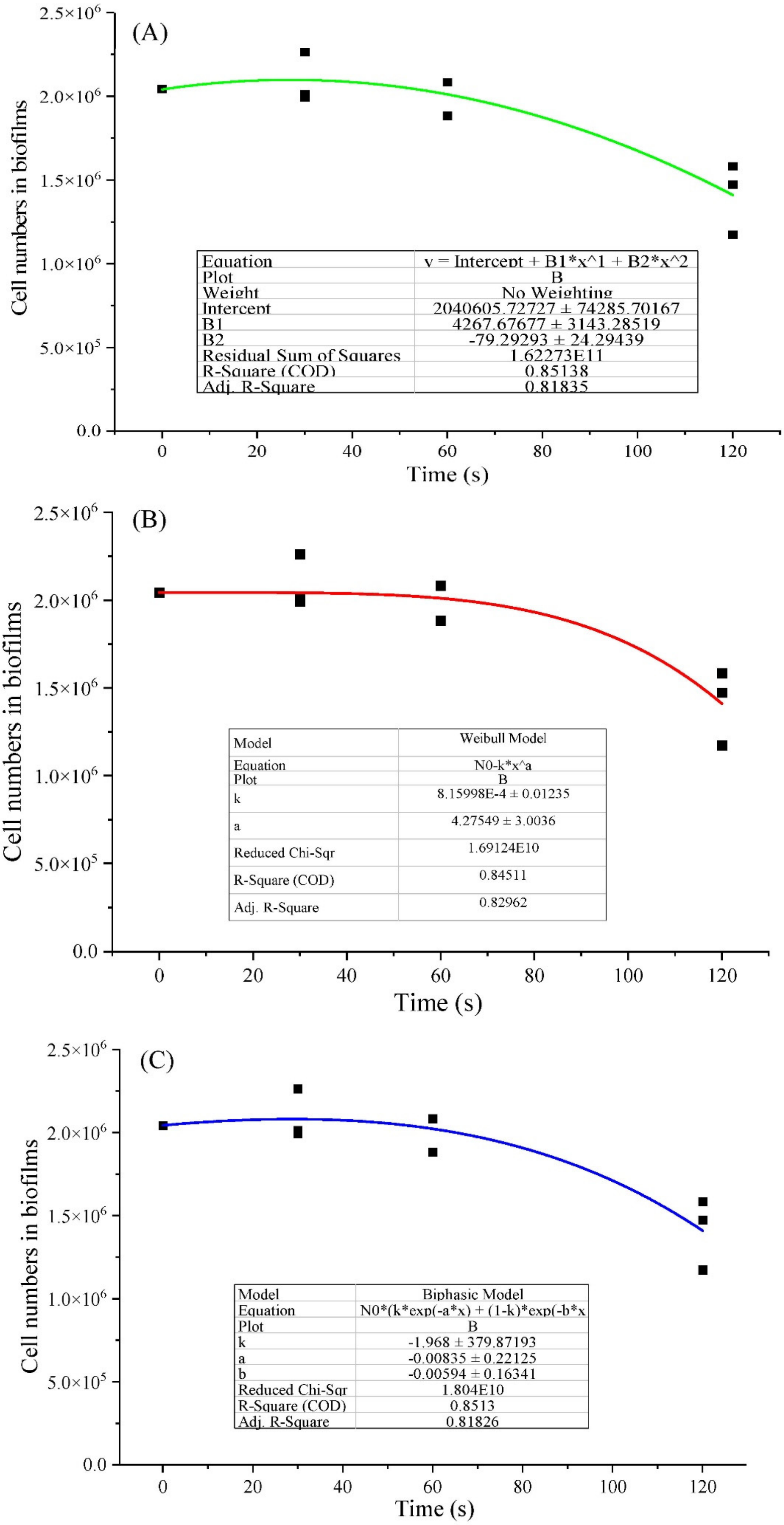


Figure 10. Polynomia (**A**), Weibull (**B**) and Biphasic (**C**) models fitting the experimental data of cell numbers in biofilms on screen electrodes in lake water under ultrasonic treatment.

Furthermore, the values of RMSE and adj. R-square of these kinetic models were calculated to examine the accuracy of the fitting procedure. The adjusted R^2 values for all kinetic models were above 0.81, which indicated that most of the variation in the outcome variable can be accurately predicted by these models with high overall accuracy. Additionally, based on the RMSE parameters, it was concluded that these three models were all suitable for fitting the biofilm elimination processes. Consistent with previous studies that used different kinetic models to simulate the inactivation processes of different microorganisms, this study demonstrated that such models can also be effectively applied to estimate the performance of biofilm elimination processes using ultrasonic treatment. The suitability of mathematical models for predicting the biofilm elimination kinetics of microorganisms during ultrasonic treatments remains to be fully explored. As well known, the second order Polynomia equation describes a quadratic dependence between time and changes in the observed parameter, while the Weibull model is characterized by its ability to capture both upward and downward concavity, reflecting variations in the resistance of surviving microorganisms to ultrasonic treatment [20,41]. In contrast, the Biphasic model, also known as the two-population model, is based on the assumption of the coexistence of two population groups with different resistance levels and/or elimination behaviors under treatments [20]. Therefore, considering current knowledge of microbial fouling, biofilm elimination kinetics, and principles of mathematical models [20,42,43], the Biphasic model may be the most suitable model for reliably illustrating and predicting the dynamic processes of biofilm elimination during ultrasonic treatments [41]. This model can be further fitted with process conditions for evaluating the process parameters involved in biofilm elimination. Overall, the findings of this study provides valuable fundamental insights and theoretic support for the development of integrated anti-biofouling strategies aimed at ensuring long-term environmental monitoring in aquatic environments.

4. Conclusions

This study evaluated the feasibility of using integrated anti-biofouling strategies to prevent biofilm formation on the proposed stainless steel sensor mesh housings and compared mathematical models to describe biofilm elimination during the anti-fouling processes. Applying an external electric field to the mesh housing was proved to effectively prevent biofilm formation and biofouling on mesh surfaces. Furthermore, the application of alternating electric field and ultrasonic irradiation significantly enhanced biofilm prevention and control. Anti-fouling tests conducted in natural lake water revealed that microalgae and bacteria can form biofilms on stainless steel screen electrodes in a time-dependent manner, with the 48-h time interval being crucial for cell adhesion and biofilm formation. The dynamic process of biofilm elimination during ultrasonic treatment was successfully simulated using different mathematic models, demonstrating their suitability for describing these anti-fouling processes. This study offers valuable insights into protecting water-monitoring sensors and minimizing biofouling by integrating stainless steel mesh with electric field and ultrasonic irradiation, thereby supporting long-term sensor performance in aquatic environment.

Author Contributions

J.C.: Conceptualization, Methodology, Writing—Original Draft, Visualization. Y.L.: Conceptualization, Methodology, Writing—Review & Editing. A.A.: Conceptualization, Methodology, Writing—Review & Editing. S.L.: Writing—Review & Editing. L.D.: Writing—Review & Editing. L.W.: Writing—Review & Editing. M.G.K.G.: Writing—Review & Editing. Y.W.: Writing—Review & Editing. J.L.: Writing—Review & Editing. N.Z.: Writing—Review & Editing. Y.C.: Writing—Review & Editing. X.L.: Writing—Review & Editing. K.C.: Writing—Review & Editing. R.R.: Conceptualization, Resources, Writing—Review & Editing, Supervision, Project administration, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The datasets generated during and/or analyzed during the current study are present in the paper. Additional data are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no conflict of interest. Given the role as Editor-in-Chief and Editorial Board Member, Roger Ruan and Shan Liu had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

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