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Research Article

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Posted Date: May 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-421790/v1

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Anaerobic Treatment of Oily Wastewater Using a biofilm-electrode reactor: A Kinetic Study and Energy Consumption

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Abstract

The present study introduced a laboratory-scale, anaerobic treatment system for the removal of oil from synthetic wastewr using a biofilm-electrode reactor (BER). The operating parameters of current intensity, initial concentration, reaction time, and supporting electrolyte were investigated. The results of the present study showed that the optimal conditions were: a current intensity of 15 mA, COD concentration of 1500 mg/L, a reaction time of three days, and a supporting electrolyte (NaCl) of 150 mg/L. The highest efficiency for the removal of COD was 86.7% using the introduced method, while it was 65.9% using biological processes. Increased efficiency was attributed to the employment of the proposed bioelectrochemical system that stimulated bacterial growth. In the present study, the energy consumed by the bioelectrochemical system was 1.914 kWh/m^3. The kinetic study indicated that the removal reaction was more consistent with the modified Stover-Kincannon model. The present study findings indicated that BER is a promising method for the treatment of oil-contaminated wastewaters.

Keywords: Bioelectrochemical, Anaerobic, Oily Wastewater, Kinetic, Stover-Kincannon model

1. Introduction

Recently, oil-contaminated wastewater is considered one of the environmental problems. The large quantity of these wastewaters is generated by various sources due to the rapid growth of industrialization and urbanization over the past decades. Oils can enter the environment at different stages of production, transportation, refining, and use (Zhu et al., 2018). The main sources of oil-contaminated wastewater include food industries, oil refineries, petrochemical companies, and metal, textiles, and leather producing plants, as well as restaurants, kitchens, and vehicles (Mirshafiee et al., 2018). Discharge of oily effluent to the environment may cause irreparable damages. Their very low solubility in water and very poor degradability cause detrimental effects on the environment (Gurd et al., 2020). Layers of oil and grease can threaten aquatic life by reducing oxygen and light penetration (Sharma et al., 2020). Oil, at higher levels, harms aquatic life, creates obnoxious odors and unpleasant sights, reduces tourism activities, and causes economic damage (Brillas et al., 2020). Various physical, chemical, and biological methods are introduced for the removal of oil from wastewater, of which biological systems are significantly used due to their advantages, of which more compatibility with the environment is noteworthy. Moreover, no environmentally harmful chemicals are used in biological systems; therefore, their effluent and sludge are less hazardous to the receiving waters than the chemical systems. These features make bioelectrochemical systems a cost-effective and environmental-friendly approach. Biodegradation of organic matter is triggered by supporting microbial growth and creating optimum environmental conditions to turn pollutants into carbon dioxide and other gases, inorganic matter, water, safe and stable substances, and biomass. Among biological methods, biofilm reactors have advantageous properties. It is well understood that biofilm reactors are suitable for the treatment of effluents containing poorly biodegradable compounds and decomposable organic matter. Biofilm, formed by the fixation on active microorganisms, increases biomass and improves organic and hydraulic loading (Karadag et al.,2015). Fixed biofilm is effective in reducing toxic compounds and the treatment of wastewater containing biodegradable matter (Zhang et al., 2015). Other advantages of biofilm systems are longer shelf life, more diverse microbial species, and increased stability(Tan et al., 2018). Numerous researchers tried to treat oily effluent in anaerobic systems (Chan, et al., 2010; Wang et al., 2016). The anaerobic process, widely used for industrial wastewater treatment, has advantages such as the generation of less sludge, lower costs, less energy consumption, no need for aeration, low nutritional requirements, high organic load tolerance, high shock loading resistance, and production of methane gas. The improvement and repair of this process are faster if it is used in industries closed for a period in the year with no utilization. It also has disadvantages as the quality of wastewater is not high and does not meet the effluent disposal standards (Kong, et al., 2019). Therefore, an anaerobic system needs further measures to be improved and upgraded, for example, increasing the activity of bacteria by electrostimulation (Adibzadeh, et al., 2016). Bioelectrochemical systems (BESs) or biofilm electrode reactors (BERs), as a relatively new technology, are appropriate and promising methods with great potentials for wastewater treatment and are considered clean technology. In this technology, microorganisms are used as an electrode-attached biofilm. In this method, oxidation-reduction (redox) reactions are catalyzed by the interaction between the electrode and the biofilm. In recent years, wastewater treatment by BESs is critically considered by researchers(Cao, et al., 2018). Evidence suggests that induced current can stimulate metabolism and enhance biochemical function in bacteria. The applied current increases the rate of ion migration and reactions on the surface of the electrodes. Lower voltages are used in these systems, which can overcome some problems such as corrosion of the anode and high energy consumption observed in the electrochemical method. The induced current must be adequate, otherwise inverse results are obtained, and the activity of microorganisms is restrained (Liu, et al., 2015). Due to the increasing generation of oil-contaminated wastewater and the benefits of applying the anaerobic method, and that this method needs upgrading and improvement of efficiency, the present study aimed at investigating increased efficiency of anaerobic treatment of oil-contaminated wastewater by electrostimulation and BESs. The main objectives of the present study were: (1) adaptation of bacteria to BES under anaerobic conditions and determination of the effect of changes in the applied electrical current on biomass performance by removal efficiency, (2) investigation of the effect of operational parameters such as initial pollutant concentration, reaction time, and supporting electrolyte, (3) improvement of the anaerobic process by BESs and comparison of the removal efficiency of BES with a conventional method in the absence of applied current, (4) estimation of energy consumed, and (5) kinetic study of the substrate removal process.

2. Materials and methods

2.1. Experimental setup

A cylindrical Plexiglas reactor, with an effective volume of 2.25 L, was used in the present study. It contained stainless steel electrodes fixed by a holder. It was covered to provide anaerobic conditions. The anode electrode was steel mesh, and its cavities were so small to facilitate the loading of biomass. A magnetic stirrer (Alfa, HS-860, Iran) was used throughout the experiments for gently mixing and homogenizing the reactor contents. Direct current was supplied by a power supply (ATTEN APS3005S-3D, China). The electrodes were washed with HCl, rubbed with a sponge, and rinsed with distilled water in order to be prepared.

2.2. Wastewater preparation

Synthetic oil emulsions were prepared by adding edible oil and sodium dodecyl sulfate, as an emulsifier, to distilled water and mixing for one hour at 900 rpm (Mirshafiee et al., 2018).

2.3. Experimental procedure

The current experimental, laboratory-scale study was performed as a batch system in a BER under anaerobic conditions. The seed sludge was taken from a wastewater treatment plant (Tehran, Iran), washed three times with tap water to remove impurities, and used as a microbial inoculant. All experiments were performed at ambient temperature (25±2°C) and neutral pH range. Nutrients, added to enrich the bacteria, were composed of NH_4Cl 0.50 g/L, KH_2PO4 0.25 g/L, K_2HPO_4 0.25 g/L, $MgCl_2$ 0.30 g/L, $CoCl_2$ 25 mg/L, $ZnCl_2$ 11.50 mg/L, $CuCl_2$ 10.50 mg/L, $CaCl_2$ 5 mg/L, $MnCl_2$ 15 mg/L, $NiSO_4$ 16 mg/L, and $FeCl_3$ 25 mg/L.

All chemicals used in the experiments had an analytical degree. After the formation of biofilm and acclimatization of biomass, the effects of different operating parameters, such as changes in the applied current (5-30 mA), initial concentration of COD (1500 - 5000 mg/L), reaction time (1-3 days) and supporting electrolyte (50-200 mg/L), were evaluated. In addition, the bacterial community was investigated under optimal conditions, and the amount of energy consumed was calculated.

2.4. Analysis

COD concentration was measured by the closed reflux method, described in the standard method. The samples were analyzed using a spectrophotometer (Rayleigh, Vis-7220 / UV-9200). The following equation was used to calculate the removal efficiency (Re%):

$$\text{Removal Efficiency} = \frac{\text{C0}-\text{Ct}}{\text{C0}} \times 100 \qquad (1)$$

where (C0) is the initial concentration (mg.L^{-1}) and (C_t) the concentration at any time (mg.L^{-1}). A portable pH meter (Oaklon, Malaysia) was used to measure the pH. A scanning electron microscopy (SEM, Seron technology, AIS-2100) was used to investigate the biofilm morphology.

2.5. Kinetic modeling

Kinetic models can be useful in the design and modeling of bioreactors, predict their performance, determine the relationship between variables, and optimize the design. In the present study, three common models of first-order, Grau model, and modified Stover–Kincannon were evaluated to investigate the kinetics of the biological reactions.

2.5.1. First-order kinetic model

Assuming that the first-order kinetic reactor is predominant, the changes in substrate concentration in a completely-mixed system were expressed as follows:

$$-\frac{\text{ds}}{\text{dt}} = \frac{\text{Q}\,\text{S}_0}{\text{V}} - \frac{\text{Q Se}}{\text{V}} - \text{K}_1\,\text{Se} \qquad (2)$$

In a biological reactor, under steady-state conditions, changes in the removal of substrate concentrations (-ds/dt) are negligible, so Equation 2 can be modified to Equation 3:

$$\frac{S_0 - Se}{HRT} = K_1\, Se \qquad (3)$$

where S_0 and S_e are the substrate concentrations of influent and effluent (mg/L), Q the flow rate of wastewater (L/d), V effective reactor volume (L), HRT hydraulic retention time (day), and K1 the first-order substrate removal rate constant (1/d).

The K1 can be obtained by plotting [$(S_0$-$S_e)$/HRT] versus S_e. It can also be calculated based on the slope of the line.

2.5.2. Grau model

The Grau model represents the second-order kinetics, which can be expressed by the following equation:

$$-\frac{ds}{dt} = K_2 \cdot X \left(\frac{Se}{S_0}\right)^2 \qquad (4)$$

If Equation 4 is integrated and then linearized, Equation 5 is obtained as follows:

$$\frac{S_0\ HRT}{S_0 - Se} = HRT - \frac{S_0}{K_2\, X} \qquad (5)$$

By holding the second part of the right side of Equation 5 as a constant value of a, Equation 6 is obtained as follows:

$$\frac{S_0\ HRT}{S_0 - Se} = a + bHRT \qquad (6)$$

$(S_0$ - $S_e)/S_0$ expresses the substrate removal efficiency and is symbolized as E. Therefore, the last equation is as follows:

$$\frac{\mathrm{HRT}}{\mathrm{E}} = \mathrm{a} + \mathrm{bHRT} \qquad (7)$$

where a and b are second-order kinetic constants, a S_0/K2X, and b dimensionless.

The kinetic parameters of a and b can be determined by the intercept and slope of the plotline S_0 HRT/(S_0-S_e) versus HRT, respectively(Pahlavanzadeh et al., 2018).

2.5.3. Modified Stover-Kincannon model

Generally, the modified Stover-Kincannon model is widely used to evaluate the kinetic parameters of biofilm reactors. This model is expressed as follows:

$$\frac{\mathrm{dS}}{\mathrm{dt}} = \frac{\mathrm{Q}(\mathrm{S_0} - \mathrm{Se})}{\mathrm{V}} = \frac{\mathrm{U_{max}}\left(\frac{\mathrm{QS_0}}{\mathrm{V}}\right)}{\mathrm{K_B} + \left(\frac{\mathrm{QS_0}}{\mathrm{V}}\right)} \qquad (8)$$

where K_B and U_{max} represent saturation constant (mg/L.day) and maximum substrate removal rate (mg/L.day), respectively. The linear form of this equation is expressed as Equation 9:

$$\left(\frac{\mathrm{dS}}{\mathrm{dt}}\right)^{-1} = \frac{\mathrm{V}}{\mathrm{Q}(\mathrm{S_0} - \mathrm{Se})} = \frac{\mathrm{K_B}}{\mathrm{U_{max}}} \cdot \left(\frac{\mathrm{V}}{\mathrm{QS_0}}\right) + \frac{1}{\mathrm{U_{max}}} \qquad (9)$$

Plotting V/[Q (S_0 - S_e)] versus (V/QS_0) creates a straight line that gives the intercept and slope of the line as 1/U_{max} and K_B/U_{max}, respectively.

3. Results and discussion

3.1. Start-up and biomass adaptation process

Adaptation and acclimation is an important stage in the biological process. It is more sensitive in the BESs since, in addition to pollutants and wastewater, the biomass should also be adapted to electric current. Oil-contaminated compounds usually contain substances that are not easily degraded by bacteria in nature, so the bacteria should be acclimatized to the environment. The gradual increase in pollutant concentration is a method that can be used for better adaptation. This strategy prevents severe shocks, and by gradually adding oil-contaminated wastewater, the bacteria are given the chance to adapt to the environment. Figure 1 shows the biomass adaptation results. COD concentration at this stage was 1500 mg/L, and HRT was considered three constant days. Glucose was first used to acclimatize the bacteria to oil-contaminated wastewater as it is a palatable organic matter for them. During the experiment, glucose levels were reduced and the oil-contaminated wastewater was gradually added. On the other hand, since the electric current causes bacterial lysis, a very low current was used in the adaptation stage in order to acclimatize them over time. The induction current was 1 mA at this stage. The reactor was managed until reaching stable conditions. The removal efficiency decreased to some extent after each increase in the concentration of oil-contaminated wastewater, indicating that the system was shocked. The bacteria then adapted to new conditions, and the system improved over time by continuing the operation. The operation proceeded slowly until the entire influent was just the oil-contaminated wastewater. The adaptation period lasted 105 days, and at the end of the start-up period, the influent substrate was only the oil-contaminated wastewater.

3.2. Effect of applied current

Figure 2 shows the effect of the intensity of different applied currents on the bioelectrochemical removal rate. In the present study, the current intensities of 5, 10, 15, 20, 25, and 30 mA were investigated. Hydrolic retention time and initial COD concentration were considered constant. Evaluation of the effect of changes in the applied current on the removal efficiency using BES indicated that by applying a current intensity of 5 mA, the removal efficiency was 72.3%, and the applied current intensity increased after reaching stable conditions. With increasing current intensity above 15 mA, the removal efficiency decreased, and the decreasing trend even continued by increasing the current intensity. In other words, a very high increase in current intensity reduced efficiency. As shown in Figure 2b, maximum efficiency was obtained at 15 mA that was 83.4%. Therefore, it was selected as the optimum current and used in the experiment. The obtined results show that the optimum current has a beneficial effect on bacteria and their enzymatic activity. When an electric field is applied to the microbial system, the permeability of the cytoplasmic membrane increases, and as a result, nutrients better pass through the cell membrane. Increasing the electrical field can also affect the activation of species carrying electrons. These species directly affect enzymes and promote bacterial metabolism. The activity and metabolism of most microorganisms increase by electrostimulation or biostimulation (Zhang et al., 2014). Electrostimulation of cells improves DNA and protein synthesis, cell membrane permeability, and growth and can promote the biological removal of COD (Wei et al., 2011). Likewise, the direct oxidation can affect the removal of contaminants, in which the organic matter is adsorbed on the surface of the anode and is removed (García-García et al., 2015). Over-optimum current intensities have adverse effects on bacteria. High current intensity can cause direct oxidation in cell structure and death. In addition, it makes alterations in the permeability of the cell wall so that the molecules diffuse to the outside (Adibzadeh, et al., 2016). High electric current can cause water electrolysis and the generation of H_2O_2 and radicals, such as $\bullet OH$ and $\bullet O_2$, which are harmful and have a detrimental effect on microbial metabolism, and ultimately inhibit microbial growth. Over-optimum currents can cause irreversible permeability of cell membranes and subsequently lead to leakage of essential cytoplasmic contents and lower cell respiration (Wei, et al. 2011). In the indirect oxidation process, strong oxidants, such as hypochlorite/ chlorine, ozone, and hydrogen peroxide, are produced based on electrochemical reactions. All these oxidants are produced in situ and used immediately; they can also be harmful to the biofilm in higher concentrations (García-García et al., 2015). Zhang et al., (2011) studied BER, in which microorganisms specifically attach to the electrode as a biofilm and play a pivotal role in biodegradation. They reported that applying over-optimum currents causes separation and fall-off microorganisms from the electrode (Zhang et al., 2011).

3.3. Effect of the initial concentration of COD

Change in influent concentration is one of the factors affecting biological treatment processes. After determining the optimum current intensity, the efficacy of the bioreactor in the removal of influent was evaluated at different concentrations. Figure 3 shows the removal efficacy of BER at different COD concentrations. According to Figure 3, after increasing the COD concentration, the system efficacy decreased due to the shock caused by the applied concentration, and a few days later, the efficiency gradually increased and approached the previous state. When the system stabilized, the influent concentration was suddenly increased. When the reactor faced an organic shock loading, the biomass tried gradually be adapted to it to regenerate the system; however, at higher concentrations, the improvement remained incomplete as, under stable conditions and high concentrations, the removal efficiency decreased. The average removal efficiency of oil-contaminated wastewater by bioelectrochemical method at different initial concentrations is shown in Figure 3b. The maximum COD removal efficiency was obtained at 1500 mg/L. With further increase in the influent concentration, shock loadings imposed on the bioreactor increased so that the average removal efficiency decreased with concentration increase. The results showed that increased concentrations exceeded the maximum biodegradation capacity of microorganisms and played a limiting role in biomass. Therefore, to prevent biomass loss, continue the experiment, and investigate the effect of retention time on process efficiency, the influent concentration returned to the baseline state (1500 mg/L). Different concentrations can directly affect the biomass activity and ohmic resistance of BES. The reason for a decrease in removal efficiency with an increase in influent concentration may be due to the point that higher oil-contaminated wastewater as substrate concentrations can act as a limiting factor on the biofilm (Mudliar et al., 2008). High concentrations create a critical state in microorganisms. With an increase in initial concentration, the time required to achieve the latest removal efficiency also increases. Therefore, with an increase in concentration, bacteria need more time for degradation. Wen et al. (2013), in a study on BES, reported that at the reaction time of 24 hours, under the same conditions, the removal efficiency of the system decreased from 82.3% to 50.3% with increasing the initial concentration of pollutants from 0.19 to 0.78 mM (Wena et al. 2013). Xuena et al., (2009) concluded that in BESs, increasing the concentration of influent can inhibit the growth of bacteria on biofilm (Xuena et al., 2009).

3.4. Effect of hydrolic retention time

The reaction time is one of the major parameters in BESs. After examining different concentrations and applying organic shock loading, the COD concentration was set again at 1500 mg/L, and when the system stabilized, the effect of hydrolic retention time (HRT) changes on the optimal current intensity was investigated. At this stage, HRT was reduced step by step while the concentration was constant. As shown in Figure 4, HRT was studied at different reaction times of 1, 1.5, 2, 2.5, and 3 days. Changes in reaction time increased organic loads. As shown in Figure 4a, the efficiency decreased with decreasing reaction time from 3 to 2.5 days. However, the bioreactor efficiency improved somewhat over time, although it did not reach the initial state. As soon as the system stabilized, the retention time was reduced again from 2.5 to 2 days, in which an immediate decrease was observed in efficiency, but improved over time, although it did not reach the initial state. The greater reduction in retention time, the greater reduction in efficiency. Figure 4b shows the average removal efficiency of COD by BES after reaching stable conditions at different time points. It can be concluded that time reduction adversely affects COD removal efficiency. The maximum efficiency was 84.2% in the best conditions in terms of reaction time. Organic loading increases with a reduction in HRT. Therefore, it can be concluded that the removal efficiency increases at higher HRTs, which is proportional to the lower load on the system. Investigation of residuals in the system showed that the residual COD increased with decreasing reaction time in BES. The reason for decreased efficiency versus reduced time is mainly related to reduced contact time between the substrate and biomass, which does not provide sufficient time for conveying the materials of liquid mass to biomass. As a result, the COD concentration increases in the effluent, and the removal efficiency decreases. In other words, a shorter contact time between biomass and oil-contaminated wastewater reduces biodegradation. In addition, a decrease in efficiency due to a reduction in retention time results from the fact that, despite a constant concentration of the substrate, microorganisms encounter an increase in organic load (Dareioti et al., 2014). Mohanakrishna et al., (2018) conducted a study on the treatment of oil-contaminated wastewater in an oil refinery by BES. They utilized a reactor with an effective volume of 1.13 L and two 5×5×1-cm electrodes. Four different HRTs were considered in the study, and they concluded that the conditions for electrostimulation are more suitable at higher HRTs than shorter times in BESs. That is, the removal efficiency is higher at higher HRTs. They also found that the oil and grease removal efficiencies were significantly higher than that of COD, which could be due to the degradation of oil complex molecules into simpler ones still occurring as total COD (Mohanakrishna et al., 2018). Zhuang et al. (2014), in a study on biological treatment of wastewater, concluded that as the retention time decreases, the efficiency of denitrification decreases, and the reason can be attributed to insufficient contact time between biomass and substrate, as well as the separation of biofilm from media and its wash-out (Zhuang et al., 2014). Guo et al. (2017), in a study on the effect of HRT on denitrification performance, indicated that the critical point of HRT effect on microbial process performance is the provision of contact time between biomass and substrate for microbial reaction. With an increase in retention time (within a particular range), the removal efficiency also increases. This increase in time leads to sufficient contact time between bacteria and wastewater. Sufficient contact time between the microbial population and substrate is not provided at shorter retention times for complete degradation, which leads to a reduction in removal efficiency. In addition, HRT can affect the secretion of extracellular polymeric substances (EPS) and the activity and accumulation of microorganisms. These materials also have a protective effect; they hold the biofilm-forming bacteria together and protect them against toxins and sudden increase in concentration to prevent them from wash-out (Guo, et al., 2017). HRT is a key parameter in BERs. Increased retention time persuades bacteria to excessively consume and degrade organic matter. It can also affect the type of microbial population. The longer retention time helps bacteria to more acclimatize to biodegradation-resistant and toxic substances. They can repair their enzyme system to acclimatize to these compounds or biodegrade them. Large bacterial populations may also develop enzyme systems suitable for degrading such organic matters. Therefore, researchers believe that higher HRTs are beneficial for the removal of poorly degradable or biodegradation-resistant compounds. In a bioreactor that refines poorly-degradable materials, a longer HRT can help to achieve high biodegradation efficiencies. Increased HRT gives more chance to substances to better contact microorganisms that increases the biodegradation rate. In lower HRTs, organic loading increases, leading to incomplete biodegradation so that the degradation process remains incomplete. In addition to influencing the efficiency of the process, HRT affects reactor volume and manufacturing costs. Therefore, determining the optimum time for an acceptable and satisfactory efficiency is one of the main stages in the bioreactor design, considering the minimum bioreactor volume required (Shi et al., 2017). To prevent the loss of system biomass, resulting from shock loading, and improve the reactor performance, HRT increased at the initial stage (three days).

3.5. Effect of supporting electrolyte

Considering a relatively high solubility, availability, low cost, and less toxicity, NaCl was used in the present study as a supporting electrolyte. The effect of NaCl at different concentrations of 50, 100, 150, and 200 mg/L was investigated, and the results are shown in Figure 5. According to Figure 5, by increasing NaCl concentration from 50 to 100 mg/L, the efficiency slightly increased and the trend continued by increasing to 150 mg/L. When the system stabilized, the NaCl concentration was increased to 200 mg/L, along which the efficiency decreased as the bioreactor was shocked. The removal efficiency somewhat improved by continuing the system exploitation in the next days but did not return to the initial state even after the system stabilization. According to the results, a threshold could be considered for the system biomass. As shown in Figure 5b, the highest removal efficiency was 86.7% at 150 mg/L. When concentration increases, the system bacteria are shocked, and the efficiency decreases.

Solution resistance (R) can be calculated using Ohm's law as Equation 10:

$$\eta_{ohm} = IR \tag{10}$$

where η_{ohm} is ohmic overpotential (V), I current intensity (A), and R the local resistance of the electrochemical cell (ohm).

Also, the resistance of the solution can be calculated using Equation 11:

$$R = \rho \frac{d}{A} \tag{11}$$

Accordingly, Equation 10 would be:

$$\eta_{ohm} = \rho \frac{d}{A} I \tag{12}$$

where ρ is the electrical resistivity (Ω m) and the electrical conductivity (σ) is its inverse, d the distance between the anode and the cathode (m), and A the surface area between the anode and the cathode (m^2). One of the features of electrochemical and bioelectrochemical systems is that with an increase in the NaCl concentration and increase in the ionic conductivity of the solution, the ohmic resistance of the electrochemical cell reduces significantly, leading to improved efficiency. Considering equations 10 and 12, an increase in supporting electrolyte reduces energy consumption. Therefore, the presence of NaCl in the solution is economically advantageous over the electrochemical process and reduces costs. Increased NaCl concentration in the solutions of electrochemical and bioelectrochemical systems facilitates the electric current and reduces energy consumption. Likewise, many researchers suggested the addition of NaCl to increase electrical conductivity in such systems (Alam et al., 2016). Kokabian et al., (2013) stated that the effect of NaCl on the performance of anaerobic treatment systems depends on the nature of the microbial population. The study results showed that with increasing salinity, the removal efficiency of anaerobic bacteria increased initially but reduced with a further increase (exceeding the recommended threshold) due to its adverse effects on the system (Kokabian et al., 2013). Aslan et al. (2012), in a study entitled “The Influence of Salinity on Partial Nitrification in a Submerged Biofilter", concluded that with adding NaCl, the removal efficacy increased initially but reduced with further increase. Different studies reported the stimulation of various bacterial species in low salinity. Although the low salinity of influent stimulates bacteria and increases their activity, higher concentrations act inversely. The effect of shock loading is evident at higher salt concentrations. High salinity causes plasmolysis and decreased bacterial activity. The susceptibility of microorganisms to salinity- e g, bacterial tolerance to NaCl- is not the same, and some bacteria are less sensitive than others. Even laboratory conditions, such as pH, temperature, solid retention time (SRT), HRT, and suspended or attached growth system, can affect bacterial strains' susceptibility to salinity (Aslan et al., 2012). Lefebvre et al., (2012) concluded that salinity is generally useful for power production in the microbial fuel cell (MFC) process because an increase in ionic conductivity improves proton transfer and reduces the internal resistance of the system. However, excess salinity has inverse consequences on the physiology of the anaerobic microbial consortia (Lefebvre et al., 2012). Electrolyte and electrode ohmic losses are the two major types of ohmic resistance in BESs. The first one represents the voltage drop due to the movement of ions through weak electrolytes (usually low-conductivity wastewater), and the second refers to the movement of electrons through electrodes and wires connected to them. Many real wastewaters encounter significant ohmic losses since their conductivity is low. Therefore, in the treatment of wastewaters with ohmic resistance, approaches to overcome this dilemma should be considered.To overcome the ohmic resistance, first, it is recommended to reduce the distance between the electrodes as much as possible; however, it is impossible in most cases. Second, increase the conductivity or ionic strength by adding the required amounts of NaCl to the solution (Rozendal et al., 2008). Gui et al. (2017), in a study on the effect of NaCl on the denitrification process, indicated different effects of salinity across various concentrations and its inhibitory and toxic role in concentrations exceeding threshold limits. The toxicity of NaCl in low salinity is related to the hindrance of the enzymatic activity of denitrifiers; in high salinity, however, in addition to the mentioned reason, it is also related to bacterial death (Gui et al., 2017). Ahmadi et al. (2017), in a study on the treatment of petrochemical wastewater by a salt-tolerant bacterial consortium, investigated the effect of salinity and determined the threshold under an HRT of three days and the initial COD concentration of 1240 mg/L. The results showed that salinity above the threshold caused a sharp and sudden decrease in COD removal efficiency, which is related to the loss and death of biomass due to the harmful effects of high salinity on the enzymatic activity of bacteria and their plasmolysis. At this time, the turbidity and TSS of the effluent increase significantly due to bacterial death and minimal biomass deposition (Ahmadi et al., 2017). The survival of the microbial population usually depends on adequate osmotic pressure in the environment. The concentration of the solution is correlated with the osmotic pressure so that if the mineral salt concentration is high in a solution, the osmotic pressure increases. Under isotonic conditions (e g, 0.85 wt.% NaCl), microbial metabolism and growth are optimum. Since water molecules penetrate microorganisms, they may swell and even burst in pure or low salinity waters (e g, 0.01 wt.% NaCl). But bacterial plasmolysis occurs in environments with very high salinity (e g, 2 wt.% of NaCl) because water molecules diffuse to the outside, hindering microbial growth and even causing death. At this time, the concentration of suspended solids increases in the effluent due to microbial death. If salinity increases slowly in the system, the bacteria can reduce its adverse effects and acclimatize to the environment as much as possible. The reason may be that bacteria can regulate osmotic pressure by the efflux pump mechanism (e g, contractile vacuole) or synthesis of compatible salts when salinity increases. The higher salinity leads to more energy consumption by bacteria to maintain osmotic pressure. As a result, the energy accessible to microbial synthesis and function reduces. Microorganisms need organic matter for growth, the process performed with the contribution of enzymes. Microbial enzymes are sensitive to toxins as they are typically proteins. If wastewaters contain some toxic substances, the enzymes are inactivated, and as a result, the removal efficiency of wastewater treatment reduces. The right amount of mineral salts improves microbial metabolism, but exerts toxic effects in excessive amounts, reduces the activity of enzymes, and destructs them. However, the bacteria probably can trigger a new enzyme system in the saline environment that helps to acclimatize to the new environment (He et al.,2017). Indirect oxidation at the anode is also one of the reactions happening during electrochemical and bioelectrochemical processes in the presence of chlorine ions. In indirect oxidation, strong oxidants, such as hypochlorite/chlorine, hydrogen peroxide, and ozone are generated by electrochemical reactions. All these oxidants in high concentrators are harmful to the biofilm. Indirect reactions of chlorine can be triggered by NaCl decomposition, based on the following equations, and its overproduction can cause microbial death.

These reactions occur when chlorine ions are the structural component of the salts used.

$$2Cl^{-} \rightarrow Cl_2 + 2e^{-} \tag{13}$$

$$Cl_2 + H_2O \rightarrow HOCl + H^{+} + Cl^{-} \tag{14}$$

$$HOCl \leftrightarrow ClO^{-} + H^{+} \tag{15}$$

In addition to ClO^-, side reactions generate ClO_2^-, ClO_3^-, and ClO_4^- through anodic oxidation, which can be hazardous to bacteria in high concentrations (Bassyouni et al., 2017).

3-6- Evaluation of bacterial community

Today, various methods are employed to identify biofilms, one of which is microscopic examination. In this method, magnified images are taken from the specimen. The SEM was utilized in the present study to investigate the bacterial community of the biofilm. According to Figure 6, the predominant bacteria in the bioreactor were rod-shaped (bacilli) and cocci. The biofilm includes a bacterial consortium and is composed of a dense bacterial population. In terms of structure, the biofilm has channels allowing the transfer of substrates to the inner part for bacterial accessibility.The results of the present study were consistent with those of Luo et al. (2005). It was reported that at the optimum current intensity, the biofilm-holding bacteria were rod-shaped and cocci, which their natural shape might be changed at different current intensities (Luo et al.,, 2005). Figure 6 illustrates the presence of EPS secretions between bacteria. A biofilm is a community of bacteria attached to a surface and covered by EPSs. Huang et al., (2013) concluded in a study that an increase in electric current can trigger EPS efflux, which ultimately leads to the better formation of biofilm in BES. However, the microbial activity varies across electric currents. The biofilm formation in a BES is of great importance for the removal of pollutants or the production of electric current. They found that at optimum current intensities, the biofilm formation is promoted and improves. However, when the current intensity exceeds the optimum level affects the biofilm adversely and diminishes its formation. They also concluded that the biofilm in BESs nurtured with organic matter is better formed near the anode than the cathode. They reported that EPSs are essential for biofilm formation. Their structural composition depends on the growing conditions of the biofilm. EPSs are composed of polysaccharides, proteins, nucleic acids, and DNAs; however, they are mostly constructed from proteins and carbohydrates. Studies on the composition of EPSs led to a variety of results. Some studies concluded that polysaccharides predominate in the EPS layer of biofilms, while others reported proteins as the main constituent. These contradictory findings indicate that the composition of biofilms depends on growth conditions. In the study by Huang, with excessing the optimum electric current, the EPS also increased and decreased with a further increase, which disrupted the formation of biofilms and inhibited microbial growth (Huang et al.,, 2013). Some cavities and channels seen in Figure 6 are related to the inlet of nutrients and outlet of substances generated by bacteria; they allow substrates to transfer through the inner part of the biofilm (Muda et al., 2010). The results of Gram staining are shown in Figure 6b. According to Figure 6b, the effluent was treated with a consortium, including Gram-positive and -negative bacteria. Simmilarly, Poh et al. (2010), in a study on the treatment of palm oil mill effluent by an anaerobic method, reported that most bacteria detected in the consortium were rod-shaped and cocci, and Gram staining proved that both Gram-positive and -negative species were involved in the process (Poh et al.,, 2010).

3-7- The effect of electric current

An experiment was performed to evaluate the effect of induction and electrostimulation on the removal efficiency of COD. It was performed under the same conditions. Accordingly, BES was compared with a similar bioreactor in the absence of DC (Figure 7). The removal efficiency at the biological state without DC was 65.9%, but it was 86.7% at the BES state. Based on the obtained results, the electric current could positively affect BES, leading to higher removal efficiency than other reactors since it stimulates bacterial growth. In the electrostimulation or biostimulation process, enzymatic activity, cellular biopolymers synthesis, membrane transfers, and reproduction are influenced by the process and can improve the removal efficiency. Cardenas-Robles et al. (2013), in a study on azo dye degradation by BES, concluded that the application of electrostimulation increases the dye removal efficiency. The results also showed that electrostimulation, in addition to an increase in efficiency, reduces the time required for the complete removal of pollutants. This reduction in time also decreases the reactor volume, which leads to the lower design and implementation costs (Cardenas-Robles et al., 2013).

Zhang et al. (2011), in a study on the degradation of 2,4-dichlorophenol by BES in an anaerobic process, reported good results and high efficiency. They compared the removal efficiency of BES and biological (in the absence of electric current) and electrochemistry methods and showed that the bioelectrode process had higher efficiency than the other two methods, and the removal efficiency of pollutants was lower in pure biological and pure electrochemical processes. The removal efficiency of the investigated processes was 100%, 42%, and 61%, respectively. This improvement and increase in removal efficiency were due to the electrostimulation of bacteria (Zhang et al., 2011).

3.8. Kinetic study

3.8.1. First-order kinetic model

The steady-state data of each stage were used to determine the kinetic coefficients. As shown in Figure 10a and Table 1, the first-order kinetic constant (K1) was 0.485 (1/d). Also, the correlation coefficient (R^2) was 0.85.

3.8.2. Second-order kinetic model (Grau model)

The R^2 for the second-order kinetic model was 0.961, indicating that the COD removal process could also follow the second-order model (Figure 8).The kinetic constants of a and b were 1.338 and 0.694 day^{-1}, respectively. Besides, increasing a or b parameters had a direct and adverse effect on efficiency, the removal efficiency increased in the system.

3.8.3. Modified Stover-Kincannon model

In the present study, U_{max} was 1.106 and K_B 0.767 g/ L.d. Both KB and U_{max} play a pivotal role in determining the volume of a bioreactor. The results showed that among the three proposed models, the R^2 of the modified Stover-Kincannon model was higher (0.975), indicating that the model was more fitted with bioreactor performance and the COD removal reaction was more consistent with it. Therefore, the modified Stover-Kincannon model can be used for the accurate prediction of the removal of biodegradable organic matter.

3-9- Energy consumption

Cost is one of the most important factors in choosing water and wastewater treatment methods. The consumed energy plays a particular role in the costs of electrochemical processes. Therefore, energy consumption was calculated using the following equation:

$$\mathrm{E}\left(\frac{\mathrm{Kwh}}{\mathrm{m3}}\right)=\frac{\mathrm{U}\times\mathrm{I}\times\mathrm{t}}{\mathrm{V}} \qquad (16)$$

where U is the applied voltage (V), I the current intensity (A), t the reaction time (h), and V the volume of the effluent (L). According to the applied optimum current intensity (15 mA) and reaction time (three days), the amount of energy consumed by BES was 1.9145 kWh/m^3. The electrochemical process was used to obtain the same removal efficiency, which according to the applied current intensity (1 A) and reaction time (2 hours), the energy consumed by the electrochemical process was 45.33 kWh/m^3. The comparison of the above results indicated that the cost of energy consumed by BES was much lower than that of the electrochemical system. In addition, the cost of electrodes was another factor affecting the cost, which considering higher current intensity and voltage in the electrochemical method, corrosion and consumption rate of electrodes was higher in this method than BES. Jinyou Shen et al., (2012) reported similar results in a study on BES. They concluded that the cost of energy consumed by the BES was much lower than that of the electrochemical one. Excessive energy consumption, as an important drawback, limits the use of the electrochemical system on a large scale. BESs can be used to reduce energy consumption in the electrochemical method, which significantly reduces energy consumption in the system compared to other ones (Shen et al., 2012). Wen et al., (2013) investigated the degradation of 4-chlorophenol by BES in an anaerobic process. They estimated that the energy consumed by BES was 5 to 30 times lower than that of the electrochemical one. They concluded that this technology would be a method of choice for the removal of many pollutants due to its higher efficiency and lower energy consumption (Wena et al., 2013).

4. Conclusions

In the present study, BERs were used to treat oil-contaminated wastewater. The results showed that this process could be very advantageous for the treatment of such wastewaters. The study also confirmed that BERs have higher efficiencies than pure electrochemical ones under the same conditions, indicating that electrostimulation under optimal conditions can increase removal efficiency. However, if the current intensity exceeds optimal levels, the biofilm biomass is lost, and thus the removal efficiency decreases. The results showed that the removal of biodegradable organic matter could accurately be predicted by the modified Stover–Kincannon model. Based on the findings, the cost of energy consumed by BERs was much lower than that of the electrochemical one. The study also indicated that BERs are a promising method with good prospects for the treatment of oil-contaminated wastewaters.

Authors' contributions: A. Mirshafiee performed experiments, analyzed data and co-wrote the paper; A. Rezaee designed experiments and supervised the research.

Funding: This study was supported by TarbiatModares University, Ph.D. thesis support.

Data availability: All data generated or analyzed during this study are included in this published article.

Compliance with ethical standards: Competing interests The authors declare that they have no conflict of interest.

Ethics approval and consent to participate: This research did not involve any human participants and/or animals.

Consent for publication: The authors accepted the publication.

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Figure and Table captions

Fig. 1. Adaptation and acclimation period of biomass (pH 7.0 and induced current = 1 mA).

Fig. 2. (a) Effect applied current on removal efficiency and (b) COD removal efficiencies under different applied currents (COD = 1500 mg/L; pH 7.0; reaction time = 3 day).

Fig. 3. (a) The effect of changes in the initial concentration of COD on the removal efficiency by the bioelectrochemical process; (b) the removal efficiency of COD at different initial concentrations (applied current intensity of 15 mA at ambient temperature).

Fig. 4. (a) The effect of HRT on removal efficiency of the bioelectrochemical process; (b) COD removal efficiency at different reaction times (COD = 1500 mg/L and applied current intensity = 15 mA).

Fig. 5. (a) Effect of different concentrations of NaCl on removal efficiency; (b) COD removal efficiency at different NaCl concentrations (COD = 1500 mg / L and applied current intensity = 15 mA).

Fig. 6. a) Scanning electron microscopy photographes of bacteria in the biofilm, b) image of Gram-stained bacteria.

Fig. 7. Comparison of COD removal efficiency with and without electric field.

Fig. 8. Kinetic plots of the COD removal through the BER process: (a) the first-order model; (b) the second-order (Grau) model; (c) the modified Stover-Kincannon model.

Table 1. Summary of Kinetic Parameters Based on the First-order, Second-order, and Stover-Kincannon Models.

Figures

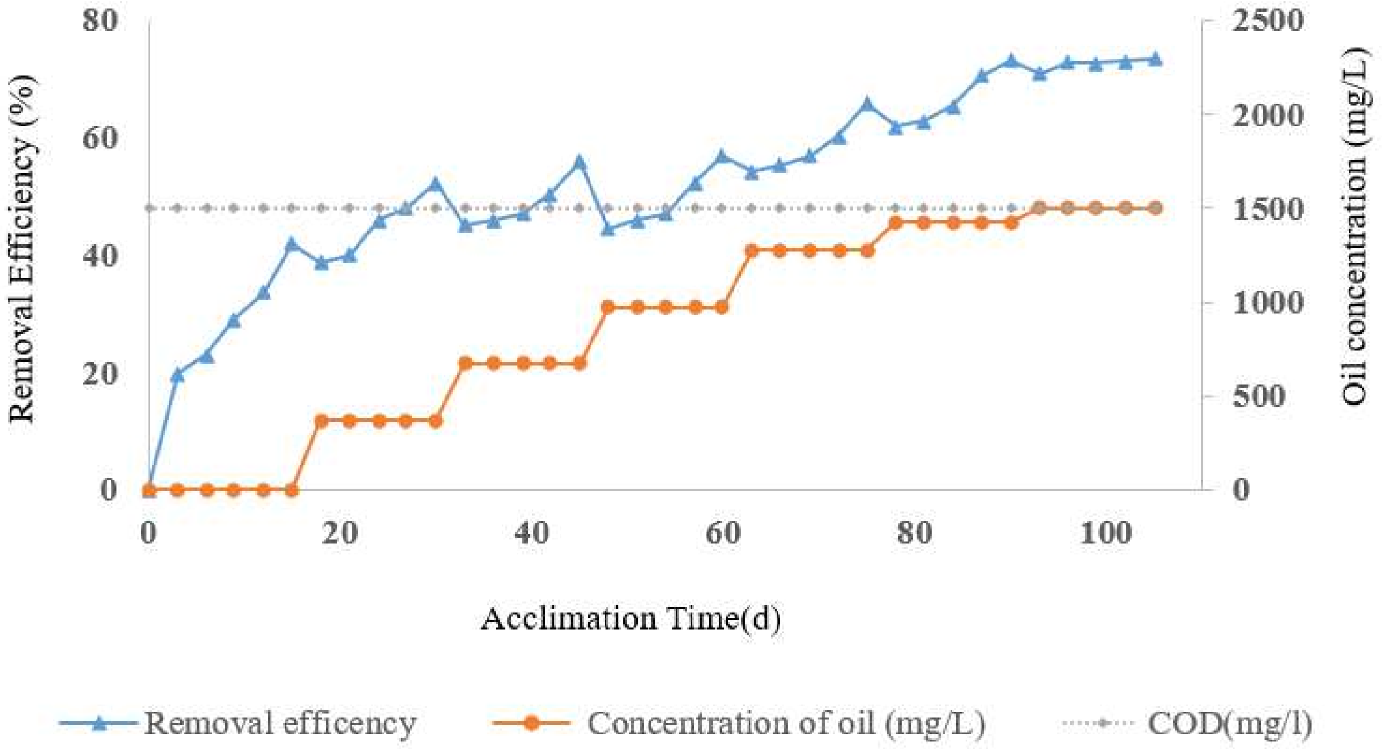

Figure 1

Adaptation and acclimation period of biomass (pH 7.0 and induced current = 1 mA).

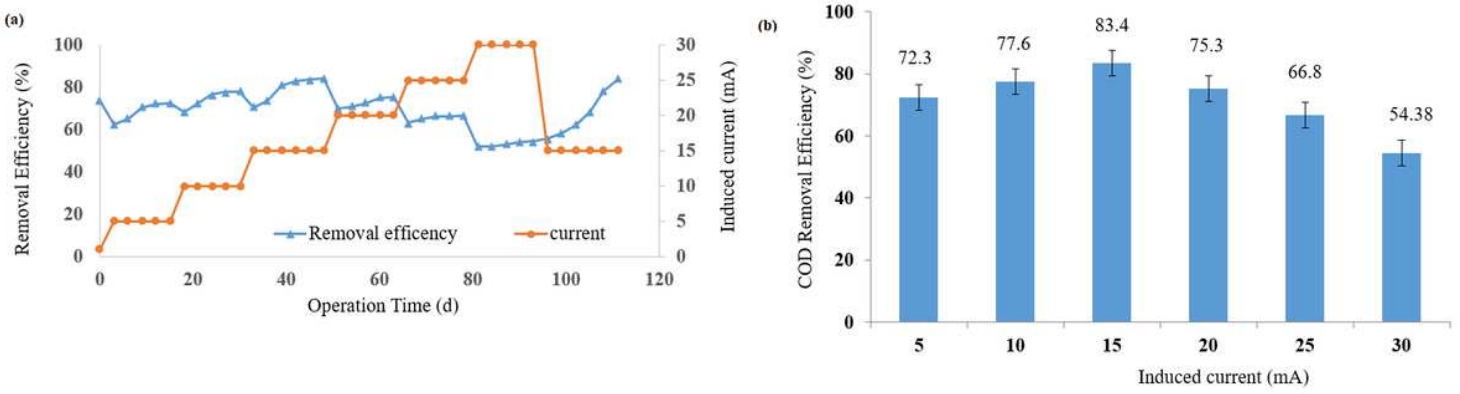

Figure 2

(a) Effect applied current on removal efficiency and (b) COD removal efficiencies under different applied currents (COD = 1500 mg/L; pH 7.0; reaction time = 3 day).

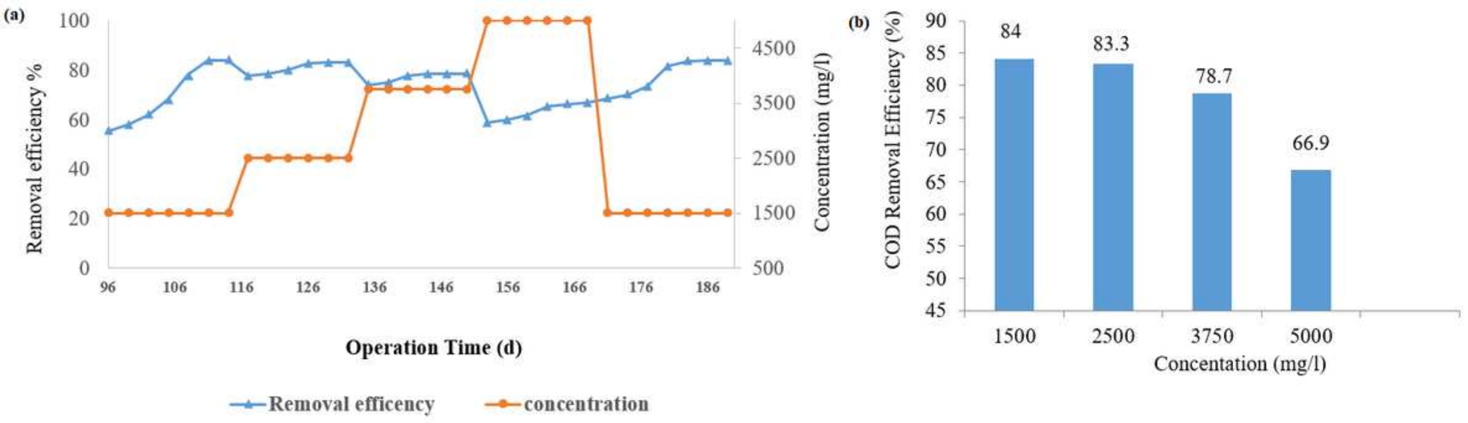

Figure 3

(a) The effect of changes in the initial concentration of COD on the removal efficiency by the bioelectrochemical process; (b) the removal efficiency of COD at different initial concentrations (applied current intensity of 15 mA at ambient temperature).

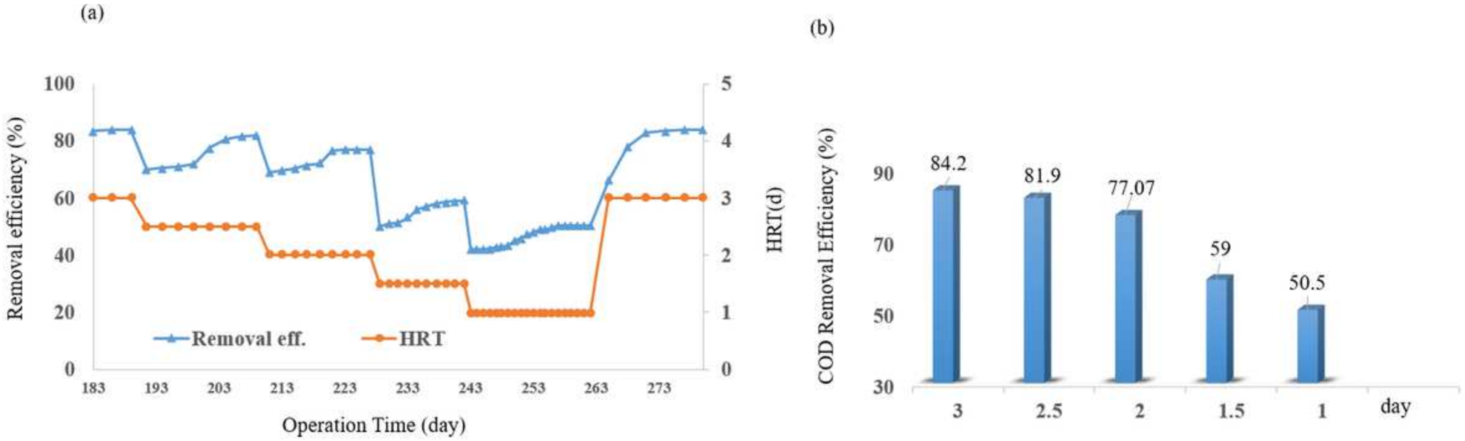

Figure 4

(a) The effect of HRT on removal efficiency of the bioelectrochemical process; (b) COD removal efficiency at different reaction times (COD = 1500 mg/L and applied current intensity = 15 mA).

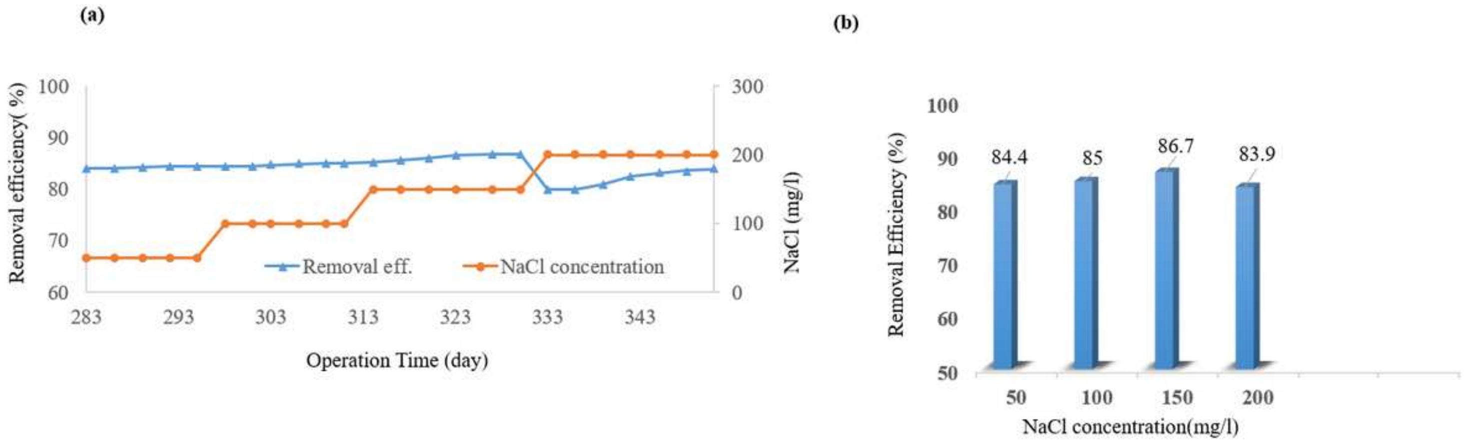

Figure 5

(a) Effect of different concentrations of NaCl on removal efficiency; (b) COD removal efficiency at different NaCl concentrations (COD = 1500 mg / L and applied current intensity = 15 mA).

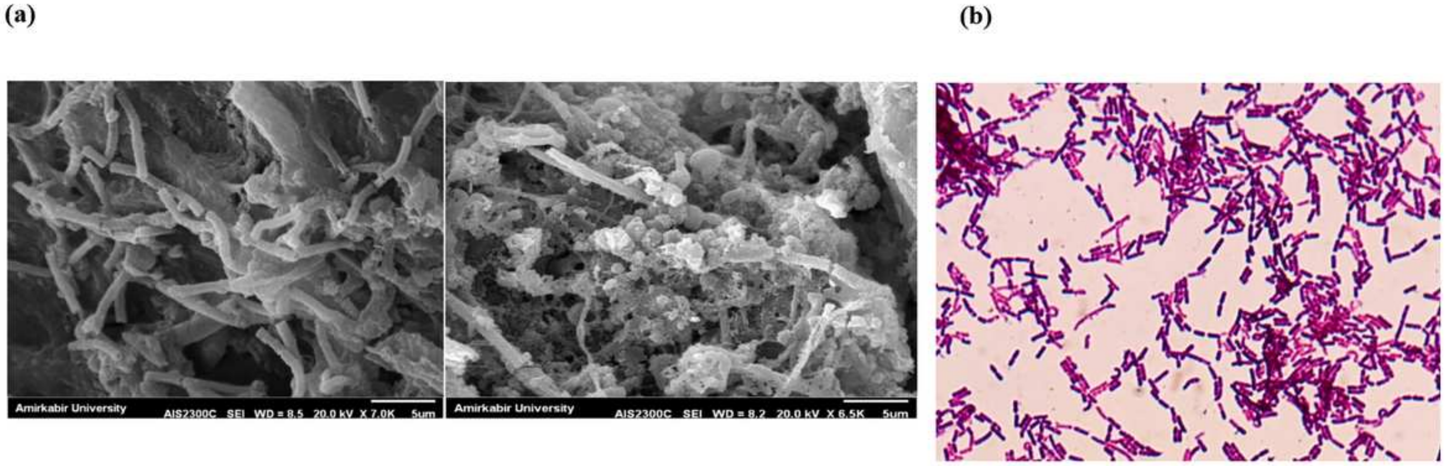

Figure 6

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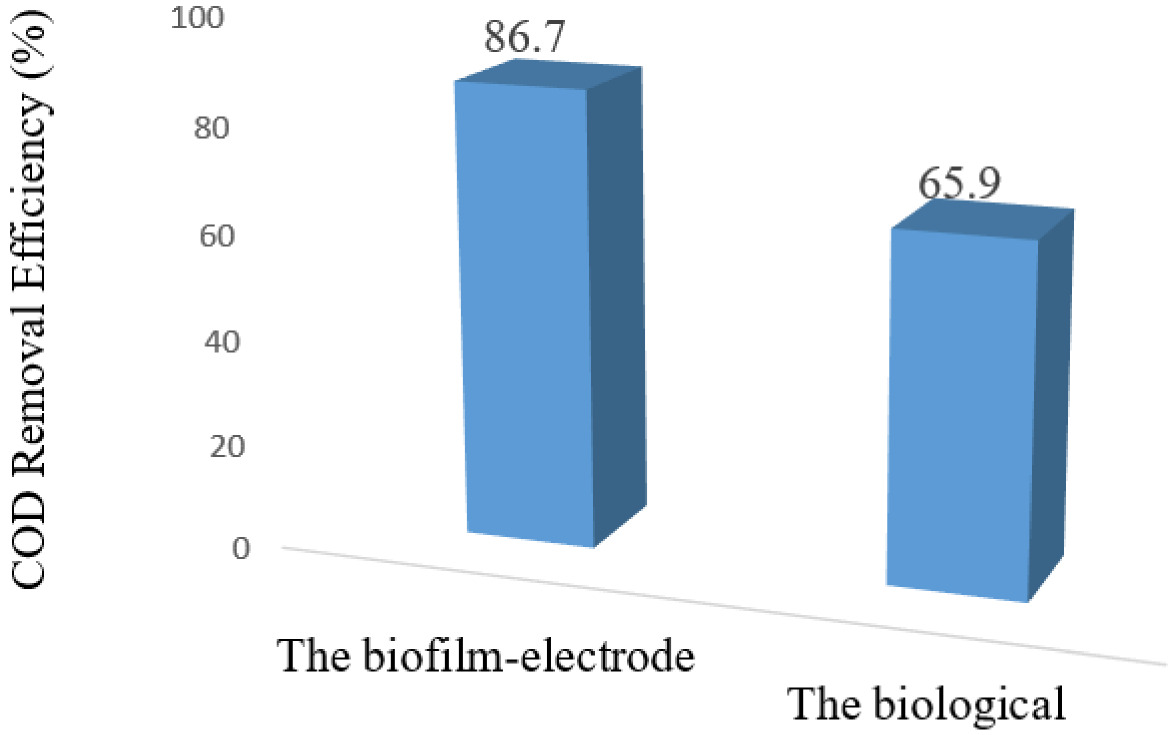

Figure 7

Comparison of COD removal efficiency with and without electric field.

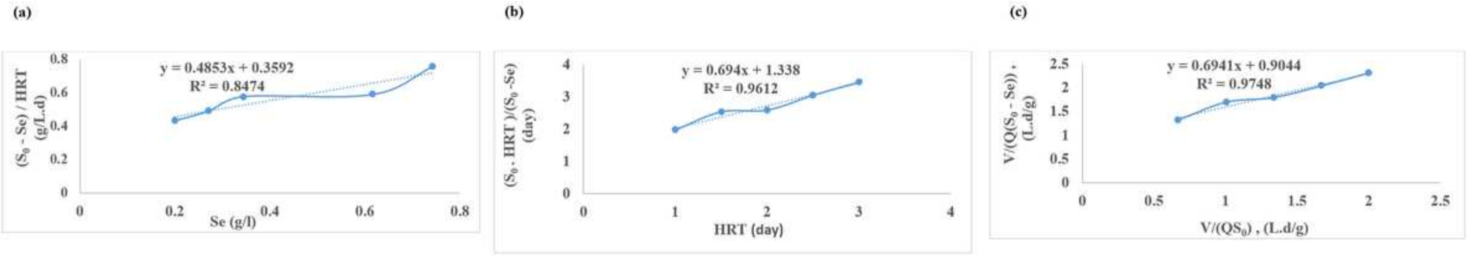

Figure 8

Kinetic plots of the COD removal through the BER process: (a) the first-order model; (b) the second-order (Grau) model; (c) the modified Stover-Kincannon model.