Carbon Aerogel Coated
Air-Cathode Bioelectrochemical System
for Simultaneous Nitrogen and Phosphorus Removal

Taheri Dezfouli, Tahmineh

Department of Environment, North Tehran Branch, Islamic Azad University, Tehran, I.R. IRAN

Kashefiolasl, Morteza*+; Marandi, Reza

Department of Environmental Engineering, North Tehran Branch, Islamic Azad University, Tehran, I.R. IRAN

Emtyazjoo, Mozhgan

Department of Marine Sciences, North Tehran Branch, Islamic Azad University, Tehran, I.R. IRAN

Javaheri, Maasomeh

Department of Ceramics, Materials and Energy Research Center (MERC), Karaj, I.R. IRAN

ABSTRACT: *Modern bio-electrochemical technologies can convert the energy stored in the chemical bonds of biodegradable organic materials into renewable electrical bioenergy through the catalytic reactions of the microorganisms while treating the wastewaters. The present research has been conducted to study the efficiency of the single-chamber bioelectrochemical system with carbon aerogel catalyst as a new, simple, and inexpensive approach to remove and recover the valuable but polluting nutrients (nitrogen and phosphorus) from municipal wastewaters and also determine the optimal conditions to scale up the system in countries with hot, dry climates. In the present study, the bacterial consortium was isolated from the sediments of local lagoons, and municipal wastewater was used as the substrate. During the six months of cell operation, the effluent of BES showed a 54.9% decrease in nitrate concentration and a 59.8% decrease in total N and 90% of phosphate removed from wastewater, the total nitrogen and total phosphate concentration in effluent were 28.9 ± 24.3 mg/L. and 13 ± 46.8 mg/L, respectively. The maximum removal of COD was 80%, and the maximum power density was $1.82 mW/m^2$. Carbon aerogel, as a novel material with suitable absorbance and resistance to oxidation by urban wastewater pH can be coated on electrodes to facilitate the Oxidation Reduction reactions and electricity transmission.*

KEYWORDS: *Nitrogen removal, Bioelectrochemical system, Bioenergy, Carbon aerogel, Resource recovery.*

INTRODUCTION

With increasing population and demand for resources, the use of sustainable and low consumption technologies in wastewater treatment and the possibility of recovering valuable resources, such as energy, water, and nutrients, is much studied [1]. Current wastewater treatment technologies are based on the activated sludge method, which is quick, but more energy consuming, through a high-cost chemical process [2].

BioElectrochemical Systems (BESs) such as Microbial Fuel Cells(MFCs) and Microbial Electrolysis Cells (MECs), represent novel technologies that can convert the chemical energy in biodegradable organic materials into renewable electrical energy by using anaerobic microorganisms called exoelectrogens as catalysts [3–7]

The basis of these technologies is forcing the bacteria to degrade organic matters on the anode surface, and redox enzymes, such as cytochrome, located on the external membrane of bacteria, which can transfer electrons directly to an electrode [8–11]. As seen in Table 1, the aerobic cathode reduction reaction occurred and oxygen played the role of electron acceptor [11–13] (Table 1, Eqs.1-13). When the redox potential of the cathode side reduction becomes higher than the oxidation half's reaction at the anode ($E_{cell}= E_{cat}-E_{anod}$), electricity will be generated; otherwise, power will be needed to drive redox reactions[14,15].

With BESs, the primary purpose is removing organic carbon, although a substrate of a mixture such as sewage contains other compounds, for example, nitrogen and sulfur [16]. Phosphorus and nitrogen are considered pollutants in urban wastewater, which causes eutrophication in water supplies and reduces water quality [5,17]. Furthermore, a nitrogen-removing process needs more energy than does a carbon-removing process [18].

In the wastewater, nitrogen is most present in the form of ammonium, and the main typical process of its removal is the high energy-consuming nitrification (ammonia oxidation to nitrate) / denitrification (reduction of nitrate to N_2 gas) process because of aeration, but it can remove nitrogen at low concentrations [19,20]. Anaerobic process anammox (the anaerobic process of ammonia oxidation, in which nitrate is the electron acceptor) has been proposed as an energy-efficient alternative. This process relies on the biological conversion of ammonium and nitrate to N_2 gas [21]. Anammox is combined with another biological process such as the SHARON® process (Single reactor system for high activity ammonia removal over nitrite), oxidizing ammonium to nitrite in the presence of nitrite-oxidizing bacteria and ammonium-oxidizing bacteria. The CANON process (Completely Autotrophic Nitrogen removal Over Nitrite) is another approach, through which the aerobic and anaerobic ammonium oxidizers coexist symbiotically [22]. Another process has been reported [23] for wastewater nitrogen removal named CANDO (Coupled Anaerobic-anoxic Nitrous Decomposition Operation), which happens in three steps: partial nitrification of $N-NH_4^+$ to $N-NO_2^-$ (SHARON process), the partially anoxic reduction on $N-NO_2^-$ to N_2O and at last decomposition or combustion of N_2O to nitrogen, oxygen, and energy [23].

P and N can be recovered from wastewater as magnesium ammonium phosphate (struvite, or MAP ($MgNH_4PO_4.6H_2O$), based on the following reaction [5]:

$$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \quad (14)$$
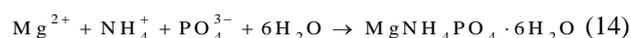
Nitrogen is eliminated by electrochemical denitrification or the transferring of ammonium during electricity production in BES [24]. Removal and recovery of phosphorus in BES depend on high pH conditions due to cathodic reduction reactions [25]. Anammox is the process that theoretically can occur in BESs, even though thermodynamically it is slow and invisible in BESs [26].

The use of a substrate such as wastewater as an electron source is attractive because of the demand for low-carbon water and wastewater treatment technologies [6,27]. Because of their potential for considerable removal of COD and simultaneous production of bioenergy, BESs been used for issues such as water resource recovery [28,29], acid mine drainage treatment [30], and landfill leaches [14]. Several challenges are facing the operational scale-up of BESs, which are required more in situ studies. The significant challenges include low power density, expensive materials (especially the membranes and separators), operating expenses, and the most critical limiting factor, poor kinetic Oxidation-Reduction Reactions (ORR) at the neutral pH required for single-chamber air-cathode cells [31,32]. Enhancing catalyst efficiency can contribute to better system performance. Many studies

Table 1: Oxidation and reduction reaction may occur in bio electrochemical system cathode and anode [15].

Oxidation reactions (anode)	
$C_6H_{12}O_6 + 12H_2O \rightarrow 6HCO_3^- + 30\,H^+ + 24\,e$ ($E° = -.429$ V vs. SHE)	(1)
$C_3H_8O_3 + 6H_2O \rightarrow 3HCO_3^- + 17H^+ + 14\,e$ ($E° = -.289$ V vs. SHE)	(2)
$C_4H_5O_5 + 7H_2O \rightarrow 4H_2CO_3^- + 11H^+ + 12\,e$ ($E° = -.289$ V vs. SHE)	(3)
$HS^- \rightarrow S° + H^+ + 2e$ ($E° = -.230$ V vs. SHE)	(4)
Reduction reactions (cathode)	
$O_2 + 4H^+ + 4e \rightarrow 2H_2O$ ($E° = +1.230$ V vs. SHE)	(5)
$O_2 + 2H^+ + 2e \rightarrow H_2O_2$ ($E° = +0.269$ V vs. SHE)	(6)
$NO_3^- + 2H^+ + 2e \rightarrow NO_2 + H_2O$ ($E° = +0.433$ V vs. SHE)	(7)
$NO_2^- + 2H^+ + e \rightarrow NO + H_2O$ ($E° = +0.350$ V vs. SHE)	(8)
$NO + H^+ + e \rightarrow \frac{1}{2}N_2O + \frac{1}{2}H_2O$ ($E° = +0.175$V vs. SHE)	(9)
$\frac{1}{2}N_2O + 2H^+ + 2e \rightarrow NO_2 + H_2O$ ($E° = +0.355$ V vs. SHE)	(10)
$2NO_3 + 12H^+ + 10e \rightarrow N_2 + 6H_2O$ ($E° = +0.734$ V vs. SHE)	(11)
$Fe_3^+ + H^+ + e \rightarrow Fe_2^{2+} + 1/2\,H_2O$ ($E° = +0.773$V vs. SHE)	(12)
$MnO_2 + 4H^+ + 3e \rightarrow Mn^{2+} + 2\,H_2O$ ($E° = +0.602$ V vs. SHE)	(13)

have been carried out on various metallic and non-metallic catalysts in search of a biocompatible, conductive, porous, and low cost-material [32–34]. Among these, carbon materials are widely considered for their specific surface area and chemical inactivity as a catalyst base [34,35]. Recently, nano and three-dimensional electrodes such as nanographene materials, carbon nanotubes, carbon nanofibers, and nanocomposites are widely used [31,36,37]. The properties of carbon aerogels are high hierarchical porosity (> 80%), high specific surface area (400-1200 m^2/g), and high Total Pore Volume (TPV), demonstrated high electrocatalytic activity and high selectivity, which can facilitate ORR in an air cathode in a neutral pH electrolyte [32]. These structures have low thermal conductivity due to their high porosity. Their nanoparticle structure also controls their behavior as electrodes in electrochemical double-layer capacitors [34,37].

The reduction of nitrate in the wastewater treatment process reduces the aeration time and energy consumption increases the efficiency of the treatment facility and decreases BOD and COD. The goal of the present study is to examine a new, simple and inexpensive approach to remove and recover the valuable but polluting nutrients (N and P) from municipal wastewater in countries with a hot, dry climate, by using the native bacterial consortium in the bio-electrochemical system with carbon aerogel coated cathode.

EXPERIMENTAL SECTION
Designing and starting up the microbial fuel cell

The designed BES consisted of two parts, an anaerobic single-chamber MFC and an aeration chamber. Two cylindrical glass chambers with a capacity of 1500 mL, each one contained a cylindrical anode with 6 cm distance around the tubular air cathode, and an independent aeration bottle was used for each system. Since the anaerobic bacteria grow very slowly, the pilot was designed as a sequencing batch reactor to prevent the biofilm from being rinsed, with an external resistance of 100 Ω. The structure of single-chamber cell electrodes of the air-cathode was made based on the method developed previously (*Cheng et al.,* 2006)

The 200 g/m^2 (ISO3374) double-sided carbon cloth (Iran Composites Institute, Tehran, Iran) with a width of 0.2 mm was used as the anode electrode with 432 cm^2

projected area, and T-304 stainless steel mesh was used as the electricity collector. The materials used as the cathode in microbial fuel cells are similar to the materials used in the anode, these materials differ only in that when they are used in a cathode, usually, there is also a need for a catalyst, to increase the cathode surface area and facilitate the ORR [27,32]. Since the microorganisms can act as the catalyst in the cathode and speed up the transfer and consumption of electrons, the present study has been conducted without a catalyst [39,40].

To increase the cathode surface area and facilitate the ORR, a layer of carbon aerogel from a previous study coated on the cathode as a catalyst base [34]. The catalyst ink was prepared by adding Perfluorosulfonic acid (Nafion) 5% suspension to carbon aerogel (with a loading of 1 mg /cm^2 of cathode surface) and brushed onto the carbon cloth [38,41]. Polytetrafluoroethylene (PTFE) was used as the binder in the fuel cell [42,43]. The municipal wastewater from Ekbatan wastewater treatment plant (Tehran, Iran) with the 7.81 average influent pH,1120 µs/cm conductivity, and 677.23 mg/L COD used as the substrate.

All cells were inoculated with the electrogenic bacterial consortium that isolated from the anaerobic sediments of the Anzali Lagoon in Gilan Province and Eshgh Abad Lagoon in Varamin County, Tehran Province. The bacterial suspension to inoculate the cell was cultured both aerobically and anaerobically in Luria-Bertani (LB) and BHl nutrient medium in an incubator at a temperature of 35.5 ℃, after 48 hours for aerobic types and after 96 hours for anaerobic microorganisms [44]. To ensure the entire biofilms formation, the two cells operated for one month.

Analytical methods

The design of the experiment and statistical analysis of data was carried out by the Response of Surface Method (RSM) with Design-Expert 11 (Stat-Ease, Inc., Minneapolis, MN, USA). So air temperature at three levels (15, 25, and 35 ° C), pH at three levels (6, 7.5 and 9), and hydraulic retention time (0 to 120 hours) were selected as three independent effective variables; and responses including concentrations of ammonium, nitrate, and nitrite, and COD were analyzed by Box-Bunken's method. P-value with a 95 % confidence level was considered for the evaluation of the effectiveness of

the model terms. It should be noted HRT was calculated based on 1.5 L feed volume (the volume which was filled and discharged).

According to the number of factors (3 independent variables including temperature, hydraulic retention time, and pH), the number of experiments was 20 runs. The COD, cations, and anions concentrations were analyzed using the standard method (APHA 2011) [45]. Each test was repeated twice. The cell voltage data were recorded every 10 min by a multimeter (MAS830L Digital Multimeter, MASTECH, Shenzhen, China). New feed (wastewater) was added to the cell whenever the voltage was below 50 mV [5]. The current (I=V/R) and power (P=IV) were according to Ohm´s law, power density (Pd=IV/A), where A is the area of the operational surface, and Coulombic Efficiency (CE) drawn based on the method proposed by *Chen et al.* [46], and the polarization curve added by exerting external resistance from 2000 Ω to 10 Ω in 10-minute intervals till reaching a constant voltage. The internal resistance and the maximum power density were obtained by analyzing the polarization curve [5]. The electrochemical tests were conducted by a potentiostat (ILIUM Technology, Inc., Medford, MA, USA), with the cathode in the middle of the single-chamber BES facing the air from the inside as working electrode, and the reference electrode Ag/AgCl (3m KCl, + CO.21 V versus a standard hydrogen electrode), in the potential range 4 to -4 mV with a scan rate of 10 mV/s. Chronoamperometry tests were performed by setting a potential in a stepwise manner after the reactor operated in an open circuit condition for three hours. The morphology and structure of the electrodes before and after biofilm formation were observed by a Scanning Electron Microscope (SEM) (Stereoscan S-360 SEM, CAMBRIDGE, Leica Group, München, Germany).

RESULTS AND DISCUSSION

The effects of the critical parameters and optimum conditions for N and P removal by BES were examined using the RSM method after the constant voltage was observed. With multiple responses, it is possible to locate the pre-decided criteria for different responses. The desirability contour of pH, temperature, and HRT shows the optimal region obtained for each response in the specified intervals for factors and responses (Fig. 1).
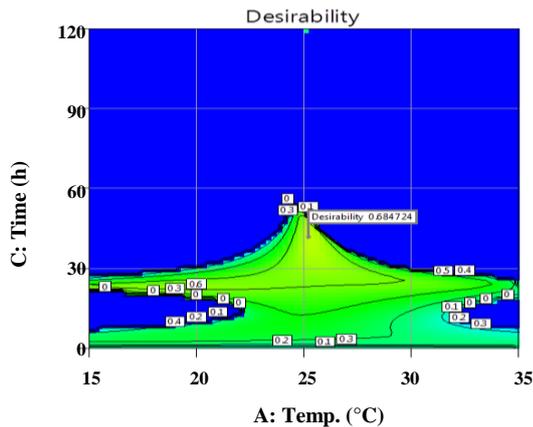
Fig. 1: Determination of optimal region for single chamber BES with RSM method.

The suggested HRT was 40 hours at neutral pH, and the optimal ambient temperature (25 ºC) was determined, with 0.68 desirabilities in specified factor ranges (Table 2).

DO´s concentration, pH, carbon/nitrogen ratio, and electricity generation in anode are the main factors that can affect nitrogen removal in BESs [18]. Neutral pH is suitable for nitrification processes, while higher DO and pH conditions can be inhibited for the denitrification process [6].

As illustrated in the perturbation diagram, total nitrogen concentration had a significant inverse relationship with time, and the total nitrogen concentration decreased with time in the effluent. The slope of the temperature distribution diagram is shown a little significant direct correlation, with a decrease in nitrogen removal as the temperature increases. The pH factor had no significant impact on nitrogen removal within the specified range (Fig. 2-A). For TPO$_4$ the significant effect of time on phosphate concentration was observed. There was a positive effect of temperature on phosphorous removal (Fig. 2-B)

It is clear that the pH of the electrolyte in BES is a crucial parameter because it affects the metabolism of the microorganisms, and excess nitrogen compounds can affect the electrolyte´s pH.

Nitrogen and phosphorus removal

In this study, nitrogen concentration was measured in forms of N-NH$_4^+$, N-NO$_3^-$ and N-NO$_2^-$. Total N was calculated as the sum of the above quantities. The three-

dimensional diagram shows the interaction of temperature and HRT on the total nitrogen concentration in the effluent of the MFC part. According to the figure, the highest amount of total nitrogen (ie, the lowest amount of nitrogen removal) was in the temperature range of 30 to 35 °C, and the least in the temperature range of 16 to 25 °C. The optimal performances for nitrogen removal were performed at 25 °C and 40 hour residence time (Fig. 3).

In the beginning, the concentrations of N-NH$_4^+$, N-NO$_3^-$ and N-NO$_2^-$ were 63.8± 27.2, 6.66±4.6, and 1.4±2.46 mg/L, respectively. The measured concentrations in the MFC chambers anolyte and the aeration chamber are plotted in Fig. 4 as a function of time.

N-NH$_4^+$ dropped to 11.5 ± 27.2 mg/L before aeration, while the N-NO$_2^-$ concentration in anolyte increased. Nitrates decreased after 24 h in the single-chamber MFC. The lowest nitrate concentration was 2.76±4.6 mg/L, although nitrate increased significantly after aeration, probably because of N-NH$_4^+$ nitrification in the presence of oxygen. A downward trend was observed in ammonium concentration during the entire process (from 63.8± 27.2 mg/L to 7.9 ± 27.2 mg/L); however, there was a sharp decrease after 3 hours in anaerobic condition. The effluent of the anaerobic single-chamber MFC showed a 54.9% decrease in nitrate concentration, with a 59.8% decrease in total N concentration after anaerobic treatment in the MFC and then the aeration process.

In evaluating the performance of the BES in nitrogen removal, it was observed that, to an acceptable extent, BES was capable of nitrogen removal from raw wastewater. N-NO$_3^-$ and N-NO$_2^-$ concentrations were very insignificant in the MFC chamber´s anolyte throughout the process, indicating there was almost no activity of nitrifying bacteria or anaerobic ammonia oxidation bacteria in the anaerobic chamber. This conclusion is consistent with the findings of *Zhang et al.* [32] that the microbial community of nitrifying bacteria or anaerobic ammonia oxidation bacteria could not be detected in the anode chamber. As illustrated in the time-based changes diagram (Fig. 4), as ammonium decreased, there was an increase in nitrate concentration; meanwhile, the nitrate concentration was reduced by nearly 50%, and after aeration nitrification has occurred, so there was a sharp increase in nitrate concentration.

Table 2: The selected optimal factors for nutrient removal in BES (The Design Expert output).

temp	pH	time	N-NH4*	N-NO3*	N-NO2*	TN	tpo4*	COD *	Desirability	Desirability (w/o Intervals)	
25.213	7.489	41.654	4.136	4.219	0.157	55.625	11.148	30.776	0.685	0.833	Selected
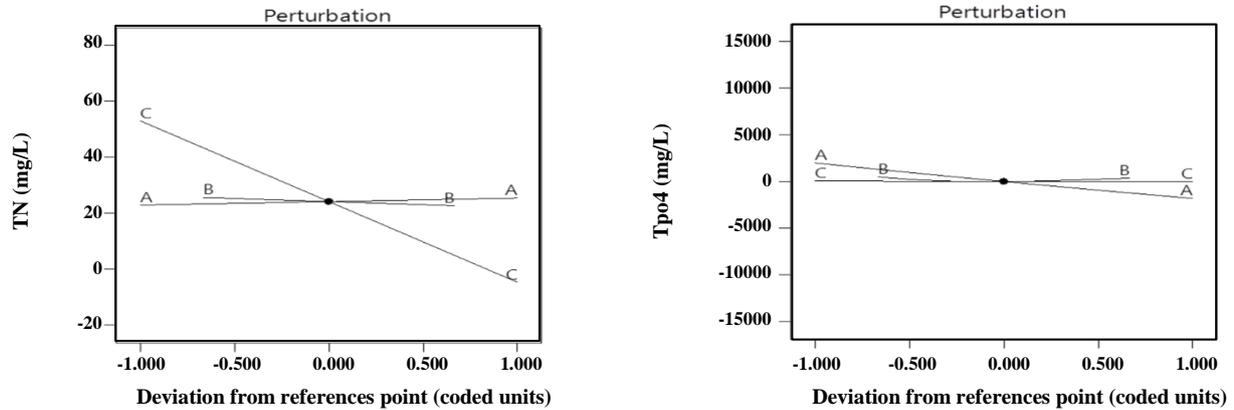
Fig. 2: A) The perturbation diagram of temperature, pH, and HRT effect on TN. B) The perturbation diagram of key parameters effect on TPO₄.
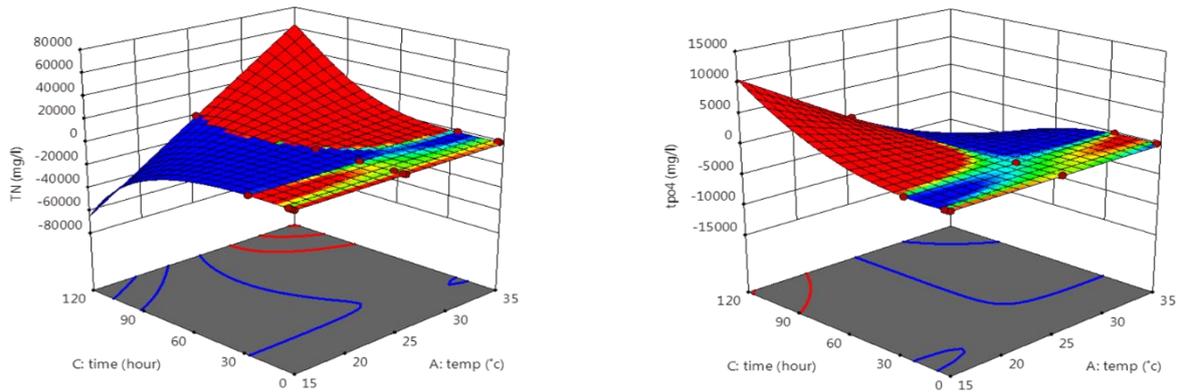
Fig. 3: A) The three-dimensional diagram of time and temperature with TN´s concentration .B) The three-dimensional graph of time and temperature with TPO₄ concentration.

In fact, most of the $N-NH_4^+$ was converted to $N-NO_2^-$ and the $N-NO_3^-$ concentration was deficient. The following points may explain the above results: some of $N-NH_4^+$ was first oxidized to $N-NO_2^-$ by oxygen from the cathode side, then to $N-NO_3^-$. Nitrite and nitrate can serve as the energy source of chemotrophic bacteria that have started to grow under the presence of hydrogen carbonate in the fuel cell [47]. $N-NO_3^-$ was used as the electron acceptor in biocathode and acetate as the electron donor in bio anode for electricity generation, and nitrate was reduced to $N-NO_2^-$ afterward (Eq. (7)).

Denitrification was less likely to occur because the aeration chamber´s DO was at a high level (12 mg/L).

CANDO is the most probable process which occurred in this BES system. About 59.8% of TN was removed in an anaerobic chamber, which may be due to consumption of microorganisms' growth and reproduction, ammonium ion diffusion, or volatilization. In the microbial fuel cell with a diverse microbial consortium, we realize the consumption of wastewater organic matters and electricity generation in the early hours of operation. These reactions provide
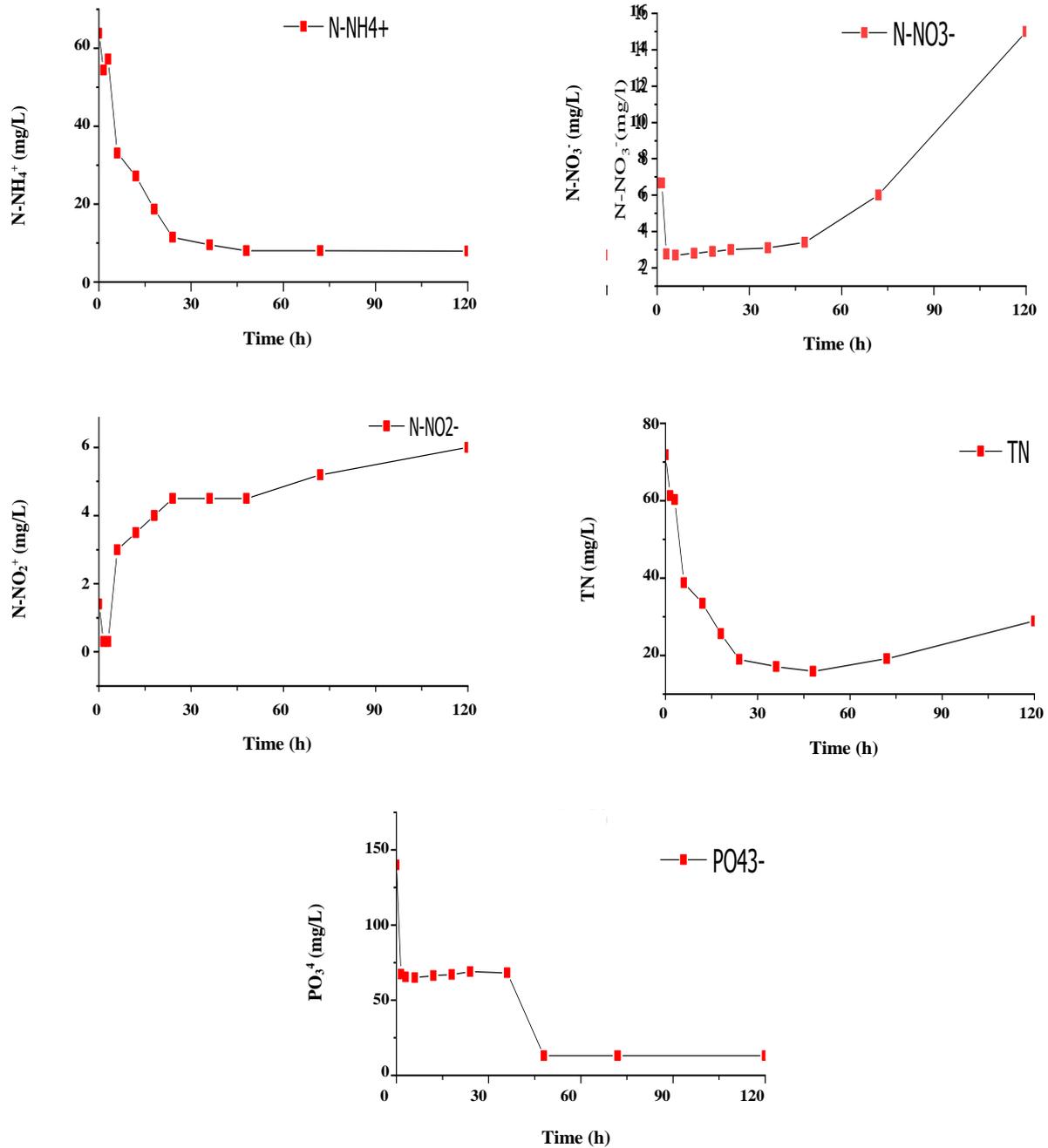
Fig. 4: Nitrogen and Phosphorus removal in BES anolyte and aeration chamber as a function of time (p< .05)

the nitrogen needed for the organic carbon and provide the conditions for the conversion of nitrate to N_2 gas (Eq. (11)). Electricity production was coupled to nitrate reduction, which is consistent with Clauwaerts study on MFC [48].

Finally, more ammonia removal was achieved in the aeration chamber, and 87.6% and 77.8% of ammonia and TN removal, respectively, were obtained after 72 hours,

compared to previous researches with enriched pure nitrifying bacteria and a specially prepared situation only for nitrifying bacteria [49].

As illustrated in Fig. 4, an immediate decrease in TP concentration was observed in the first two hours after wastewater fed into the system (from 140 ± 46.8 mg/L to 65.5 ± 46.8 mg/L), which can occur through struvite crystallization being near the aerobic cathode (Eq. (14)).

Then, a steady-state and another decrease after aeration occurred, presumably because of phosphorus-accumulating bacteria activity. Phosphorus-accumulating bacteria can release phosphorus under anaerobic conditions and uptake phosphorus under aerobic conditions [5]. More than 50% of total phosphate was removed in the anaerobic single-chamber MFC. Then there was another sharp drop observed after aeration to the lowest concentration (13±46.8 mg/L) of phosphate in the aeration chamber effluent. Finally, 90% of the phosphate was removed from the wastewater after 48 hours' treatment, and it stayed constant through the next 24 hours of aeration (Fig. 4).

Struvite crystallization occurs when Mg^{2+}, NH_4^+ and PO_4^{3-} concentrations exceed their solubility limits, which are highly pH-dependent (minimum solubility at pH>8 or near 10) [25,50]. The effluent's average pH was near 7.5. No needle-shaped prismatic morphology typical of struvite described in the literature [51] was found in cathode SEM (Fig. 7). Two possible reasons are as follows. First, by-products of struvite crystallization such as $Mg(H_2PO_4)_2$, $Mg_3(PO_4)_2$, and $Mg(OH)_2$ may be obtained at an importer pH. Second, aeration in the aeration chamber may have a negative effect on struvite crystallization.

Electricity production

Fig. 5 shows the electricity generated from the time the cell started to operate over 15 operating cycles.

The current generation was deficient during the first three days; however, it was observed from the beginning moments, and a constant voltage was observed for the five times the system was rebuilt after five to seven days. The maximum generated voltage was 304 mV, comparable to that of previous findings [52]. The results show that electricity was generated by the organic compounds of the municipal wastewater as the substrate, without using the initial starting current; therefore, BES can enter the municipal wastewater system without auxiliary devices.

Each time the cell was fed, there was a sudden drop in voltage (on average from 53 mV to 11 mV), which peaked afterward. Low pH in the anode chamber had an inhibitory effect on exoelectrogenic bacteria activities and resulted in decreasing the electricity production during the last days of each batch cycle [53].
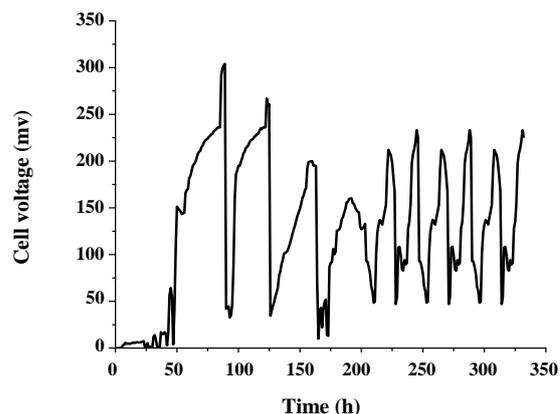

Fig. 5: Electricity generation of the single chamber air cathode BES, recorded for 15 days of operation

The polarization curve has been illustrated in Fig. 6A. The maximum obtained power density, and current density were 346.7 mW/m^2 and 2.73 mA/m^2, respectively (Fig. 6B).

The relationship between pH and cell performance has been illustrated in Figure 7. A decrease in pH at each run was observed along with an increase in the voltage (from 7.6 to 6.8 on average). The high voltage was observed at lower pH (Fig. 7).

The voltage rose for 72 hours, and then a sudden increase in voltage (probably because of consuming acetate for generating electricity) was observed, followed by the reduction in voltage resulting from the organic substrate in the cell. The sudden drop in voltage curve and then increases after each feed indicate that electricity generation is affected by the chemical reactions. The initial voltage drop (activation overpotential) indicates energy consumption to activate the oxidation and reduction reactions in cathode and anode, respectively. The less the pH in air-cathode reactors, the more H^+ is provided for the cathodic reduction reaction. A decrease in pH at each run.

The electrochemical tests were conducted to examine the kinetics of cathodic ORR, two peaks observed on the LSV curve in 17 and -13 mV vs. Ag/AgCl (vs. SHE), which presents the reduction of two elements, namely oxygen and sulfur in the aerobic cathode as the final acceptor, compared to the redox table [54]. The reduction peaks were also observed on the CV curve, and the reactions in this potential range were considered to be completely forward and backward reactions. The chronopotentiometry curve in the first 20 seconds
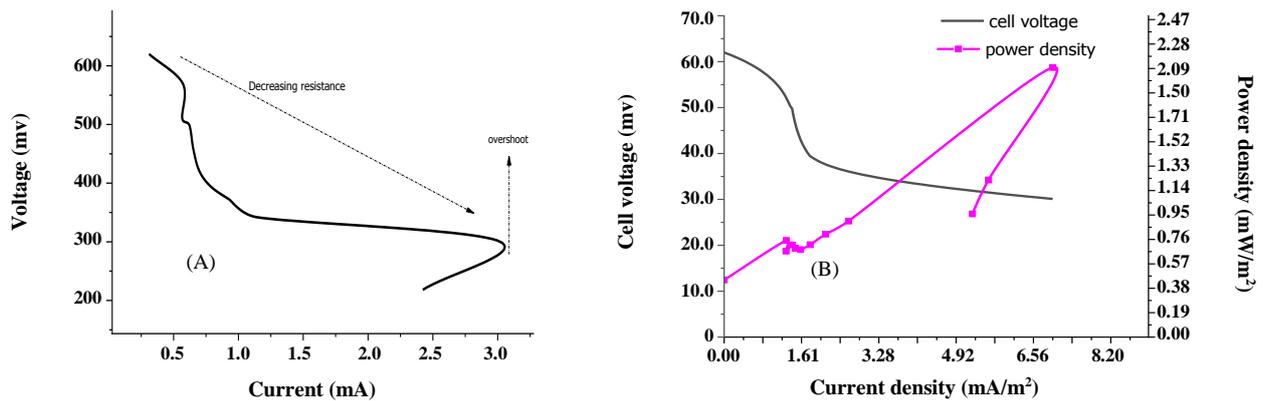
Fig. 6: (A) Polarization curve of the single chamber air cathode BES, (B) Comparison curve of the cell voltage, obtained power density and current density of the single chamber air cathode BES.
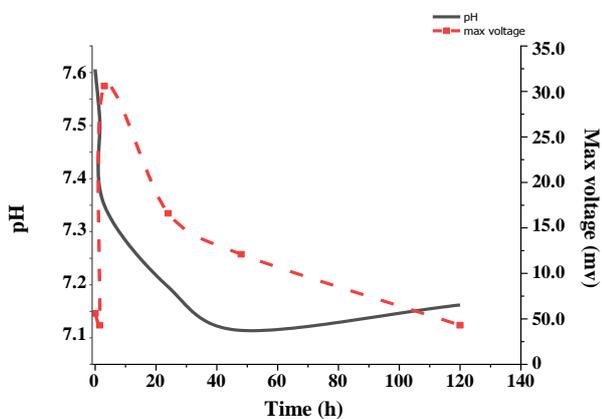

Fig. 7: The relationship between pH and cell performance of the single chamber air cathode BES

of these two reactions reveals the adjacent reduction cathode (Fig. 8).

was observed along with the increase in the voltage. According to *Zhang, et al.*[32], the decrease in the maximum current after some system operation can be caused by the development of biofilm on the cathode and its effect on the catalytic activity of carbon. Bionitrification can buffer the pH increase of catholyte by reducing oxygen[5], as seen in this study.

The maximum generated voltage was 304 mV. The low operating voltage (Vop) concerning the predicted thermodynamic potential (E_{thermo}) may originate from different factors such as reactions to activation-related losses in electrodes and transfer of electrons to the anode, metabolism of bacteria, losses of mass transfer (due to finite flux from the reactors to electrodes), and the ohmic losses [55].

The square shape of the CV curve indicates the excellent catalytic activity of carbon aerogel in the aerobic cathode, which will continue the reduction reactions [32] and the sufficient existence of oxygen as the final receptor of protons. Although the good selectivity of carbon aerogel and reducing oxygen to water through a four-electron process ($O_2 + 4H^+ + 4e^-$ $2H_2O$) was concluded through cronoamprometric tests. The catalyst activity of carbon aerogel nanocatalyst (N-CNT-TiO$_2$) has been studied in the oxidation of H$_2$S to sulfur, which shows the highest catalyst activity due to its higher surface area and pore volume [56].

Electrodes were analyzed by SEM before biofilm formation and after the operation for 60 days. The SEM images of the cathode electrode showed biotic biofilm formation and its proper microbial attachment to the air cathode (Figure 9). Long rod-shaped bacteria (about 5.00 μm in length) and other coccial bacteria could be observed upon the close examination of the cathode.

COD removal and coulombic efficiency

The operational efficiency of the bioelectrochemical system, which includes the potential efficiency, coulombic efficiency, and energy conservation, and efficiency, were calculated for the wastewater effluent with an average 580 mg/L COD and 100 Ω resistances over 120 hours. As shown in Figure 10, a relationship has been observed between the current generation and organic matter removal.

By numerical integration, with the surface area under the current curve through time, the coulombic efficiency can be calculated. Power densities are inversely proportional
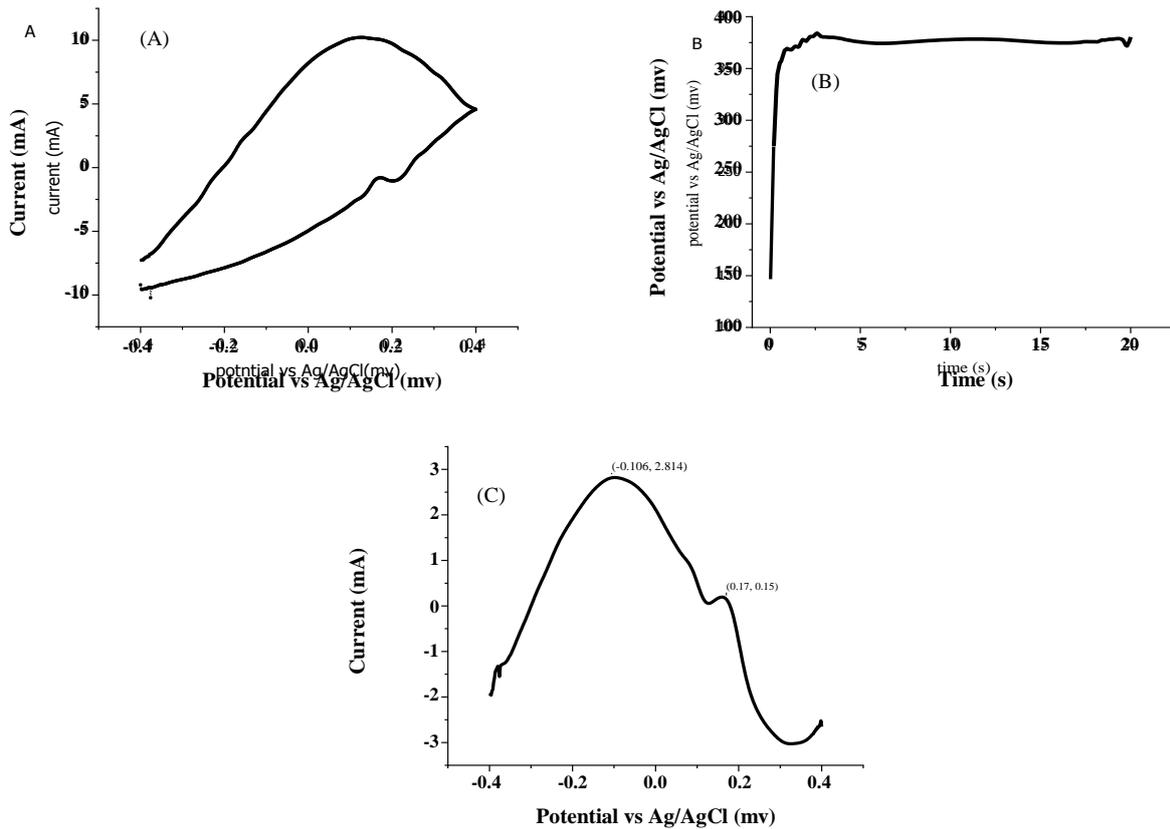
***Fig. 8: Potentiometric curves of the aerobic bio-cathode with carbon aerogel at -0.4 - 0.4 mV (vs. Ag/AgCl) potential
A) CV curve B) The chronopotentiometry curve of the single chamber air cathode BES .c)LSV curve vs. Ag/Agcl (vs. SHE)
to examine the kinetics of cathodic oxygen reduction reaction (ORR).***

to the logarithm of the surface area of the anode. The average COD removal efficiency was about 85.92% in single-chamber BES. The coulombic efficiency of the the bioelectrochemical system was 28.5%. The minimum effluent total COD in this study was 94 ± 53.7 mg/L.

The higher the coulombic efficiency, the more microorganisms were found in ideal conditions, and optimal conditions and nutrition have been provided for them. The results showed system efficiency to COD degradation; but most of the removal was before aeration, probably because of phosphorus-accumulating bacteria in the anaerobic chamber, which assimilated organic matter in their cells in polyhydroxybutyrate form and used it as a carbon source in aeration conditions [5].

Total sediment volume after 30 min was 50 mL of cell effluent. Compared to other biological wastewater treatment processes, sludge production was less clear [57] because a significant amount of organic matter was converted into electricity and microbial population growth. Besides, as explained in the previous section, the process of nitrification and conversion of ammonium to nitrate was observed in the early hours after the new feeding. Nitrification bacteria, unlike common heterotrophs, use carbon dioxide and carbonate hydrogen as carbon sources [5]. This factor, in addition to the activity of electrogenic bacteria on the anode, can cause a severe reduction in COD in the first 3 hours.

The maximum removal of COD was 80%. The maximum power density was 54 mW/cm^3 and the minimum 1.82 mW/cm^2, which was similar to the results obtained by the *Niyom* study on MFC [58].

These results are considered acceptable considering the use of municipal wastewater instead of the synthetic wastewater employed in most of the previous researches and wide-ranging microbial consortiums.

The wastewater treatment efficiency of the BES system was presented in table3. According to the results, the final effluent concentration of COD could meet Iran´s Environmental
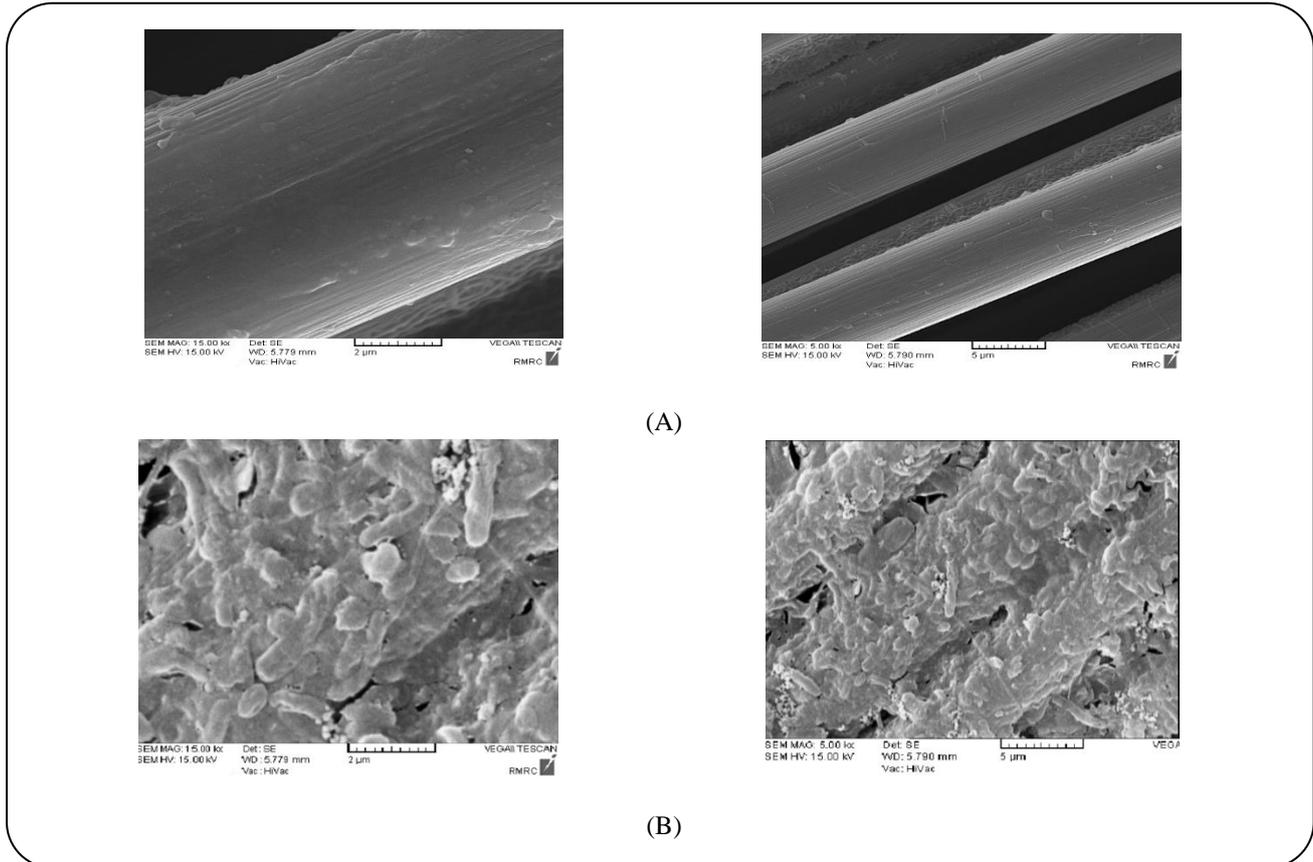
Fig. 9: SEM images of air cathode. (A) The carbon cloth cathode before biofilm formation (B) Biofilm formation, and microbe attachment to the carbon aerogel air cathode, the cathode compartment showing the long rod-shaped bacteria
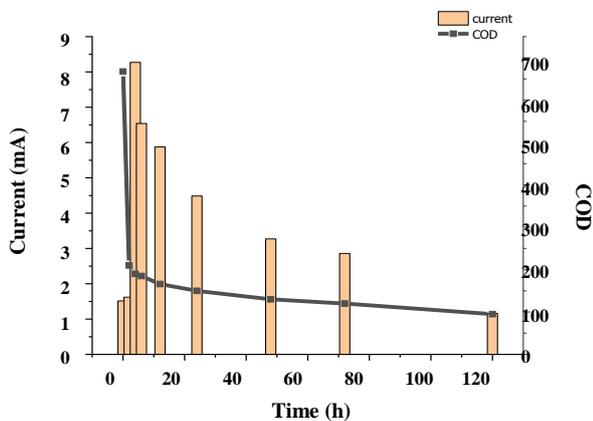

Fig. 10: The relationship has been observed between the current generation and organic matter removal in 120 h of operation of the single-chamber air cathode BES.

Protection Organization Standard for agricultural reuse; still, the phosphate concentration is not (COD and phosphate lower than 200 mg/L and 6 mg/L respectively).

This correlation proves that electricity generation in the microbial fuel cell can remove the nitrogen and phosphorus compounds or control them in wastewater treatment.

CONCLUSIONS

The present study has been conducted to examine the efficiency of electricity generation along with the removal of nitrogen and phosphorus compounds or control them by the bioelectrochemical system, as an energy-efficient technology from municipal wastewater, while all current treatment technologies require energy for nutrient removal mainly through aeration. No active oxidation is required for ammonium oxidation; instead, the movement of electrons from the anode to the cathode drives ammonium recovery at the cathode.

The results prove that electricity generation in a microbial fuel cell can remove the nitrogen, and phosphorus compounds from wastewater, the TN, and P concentrations in effluent were 28.9 ± 24.3 mg/L, and 13 ± 46.8 mg/L, respectively. Electricity was generated

Table 3: The average values of COD, BOD, TN, and phosphate removal efficiency of BES from wastewater.

	COD mg/L	BOD mg/L	TN mg/L	PO_4^{3-} mg/L	Turbidity NTU
Influent	677.23±157.998	353.12±117.734	71.86± 24.3	140 ±46.8	160 ±31.415
Anaerobic cell effluent	150±157.998	97.89 ±117.734	19± 24.3	65.5±46.8	60±31.415
Aeration chamber efflent	95 ±157.998	67.1±117.734	28.9 ± 24.3	13±46.8	14.2±31.415

by microorganisms as biocatalysts from the organic compounds of the municipal wastewater without using activation current. With a 304 mV Open Circulate voltage, the maximum removal of Chemical Oxygen Demand was 80%, and the maximum power density was 1.82 mW/m^2. Such instruments can be used in municipal wastewater systems, such as manholes, rainwater-harvesting canals, and sewers. The next step is to scale up BESs and bring them into a general application, the results of which will be present in another report. The significant observations in a tubular up-flow BES were high internal resistance, limited conductivity of electrodes, and contact between the electrodes; the current collector was often suboptimal. To reduce operational expenses, we limited the separators, which decreased the treatment and electricity production efficiencies. More pilot studies are essential to demonstrate the practical feasibility of BESs.

Nomenclature

AC	Activated Carbon
BHl	Brain Heart Infusion
BES	Bio Electrochemical System
CB	Carbon Black
CE	Coloumbic Efficiency
COD	Chemical Oxygen Demand
I	Current
LB	Luria-Bertani
MFC	Microbial Fuel Cells
ORR	Oxidation-Reduction Reactions
OCP	Open Circulate Voltage
Pd	Power Density
P	Power
R	Resistance
SHE	Standard Hydrogen Electrode
SOB	Sulfur Oxidizing Bacteria
SBR	Sequencing Batch Reactor
SEM	Scanning Electron Microscope
TPV	Total Pore Volume

REFERENCES

[1] Zhang X., Xia X., Ivanov I., Huang X., Logan B.E., Enhanced Activated Carbon Cathode Performance for Microbial Fuel Cell by Blending Carbon Black, *Environ. Sci. Technol.*, **48**: 2075–2081 (2014).

[2] Sustarsic M., Wastewater Treatment Understanding the Activated Sludge. *CEP, AIChE Publ.:* 26–29 (2009).

[3] Li X., Zheng Y., Nie P., Ren Y., Wang X., Liu Y. Synchronous Recovery of Iron and Electricity Using a Single Chamber Air-Cathode Microbial Fuel Cell, *RSC Adv.,* **7**:12503–12510 (2017).

[4] Logan BE., Wallack MJ., Kim KY., He W., Feng Y., Saikaly PE., Assessment of Microbial Fuel Cell Configurations and Power Densities, *Environ. Sci. Technol. Lett.*, **2**:206–214 (2015).

[5] Tao Q., Zhou S., Luo J., Yuan J., Nutrient Removal and Electricity Production from Wastewater Using Microbial Fuel Cell Technique, *Desalination,* **365**: 92–98 (2015).

[6] Kelly PT., He Z. Nutrients Removal and Recovery in Bioelectrochemical Systems: A Review, *Bioresour. Technol.* **153**: 351–360 (2014).

[7] Logan B.E., Exoelectrogenic Bacteria that Power Microbial Fuel Cells, *Nat. Rev. Microbiol.,* **7**: 375–381 (2009).

[8] Logan B.E. Simultaneous Wastewater Treatment and Biological Electricity Generation, *Water Sci. Technol.,* **52**: 31–7 (2005).

[9] Rabaey K., Boon N., Siciliano SD., Verhaege M, Verstraete W. Biofuel Cells Select for Microbial Consortia That Self-Mediate Electron Transfer, *Appl. Environ. Microbiol.,* **70**: 5373–5382 (2004).

[10] Verstraete W., Rabaey K. Critical Review Microbial Fuel Cells : Methodology and Technology, *Environ. Sci. Technol.,* **40**:5181–5192 (2006).

[11] Angelov A., Bratkova S., Loukanov A. Microbial Fuel Cell Based on Electroactive Sulfate-Reducing Biofilm, *Energy Convers. Manag.,* **67**:283–286 (2013).

[12] Seo Y., Kang H., Chang S., Lee YY., Cho KS. Effects of Nitrate and Sulfate on the Performance and Bacterial Community Structure of Membraneless Single-Chamber Air-Cathode Microbial Fuel Cell, *J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng.*, **53**: 13-24 (2018).

[13] Barua P.K., Deka D. Electricity Generation from Biowaste Based Microbial Fuel Cells, *Int. J. Energy Inf. Commun.*, **1**: 77–92 (2010).

[14] Li S., Chen G., Factors Affecting the Effectiveness of Bioelectrochemical System Applications: Data Synthesis and Meta-Analysis, *Batteries,* **4**: 34 (2018).

[15] Gude VG., Wastewater Treatment In Microbial Fuel Cells - An Overview, *J. Clean. Prod.,* **122**: 287–307 (2016a).

[16] Rabaey K., Van de Sompel K., Maignien L., Boon N., Aelterman P., Clauwaert P., De Schamphelaire L., Pham H.T., Vermeulen J., Verhaege M., Lens P., Verstraete W., Microbial Fuel Cells for Sulfide Removal, *Environ. Sci. Technol.* **40**:5218–5224 (2006).

[17] Domagalski J., Lin C., Luo Y., Kang J., Wang S., Brown L.R., Munn M.D., Eutrophication Study at the Panjiakou-Daheiting Reservoir System, Northern Hebei Province, People's Republic of China: Chlorophyll-a Model and Sources of Phosphorus and Nitrogen." *Agricultural Water Management.,* **94(1–3)**: 43–53 (2007).

[18] Gude, V.G. Wastewater Treatment in Microbial Fuel Cells – an Overview, *Journal of Cleaner Production* **122**: 287–307 (2016b).

[19] Rodríguez Arredondo M., Kuntke P., Jeremiasse A.W., Sleutels T.H.J.A., Buisman C.J.N., ter Heijne A., Bioelectrochemical Systems for Nitrogen Removal and Recovery from Wastewater, *Environmental Science: Water Research & Technology,* **1(1)**: 22–33 (2015).

[20] Knowles R., Denitrification. *Microbiol. Rev.,* **46**:43–70 (1982).

[21] Jetten M.S.M., Cirpus I., Kartal B., van Niftrik L., van de Pas-Schoonen K.T., Sliekers O., Haaijer S., van der Star W., Schmid M., van de Vossenberg J., Schmidt I., Harhangi H., van Loosdrecht M., Gijs Kuenen J., Op den Camp H., Strous M., 1994–2004: 10 years of Research on the Anaerobic Oxidation of Ammonium, *Biochem. Soc. Trans.* **33**: 119–123 (2005).

[22] Jetten MS., Wagner M., Fuerst J., van Loosdrecht M., Kuenen G., Strous M., Microbiology and Application of the Anaerobic Ammonium Oxidation ('Anammox') Process, *Curr. Opin. Biotechnol.,* **12**: 283–8 (2001).

[23] Scherson Y.D., Wells G.F., Woo S.-G., Lee J., Park J., Cantwell B.J., Criddle C.S., Nitrogen Removal with Energy Recovery Through N_2O Decomposition, *Energy Environ. Sci.,* **6**: 241–248 (2013).

[24] Kuntke P., Śmiech K.M., Bruning H., Zeeman G., Saakes M., Sleutels T.H.J.A., Hamelers H.V.M., Buisman C.J.N., Ammonium Recovery and Energy Production From Urine by a Microbial Fuel Cell, *Water Res.* **46**:2627–2636 (2012).

[25] Ichihashi O., Hirooka K. Removal and Recovery of Phosphorus as Struvite from Swine Wastewater Using Microbial Fuel Cell, *Bioresour. Technol.,* **114**: 303–307 (2012).

[26] He Z., Kan J., Wang Y., Huang Y., Mansfeld F., Nealson KH., Electricity Production Coupled to Ammonium in a Microbial Fuel Cell, *Environ. Sci. Technol.,* **43**: 3391–3397 (2009).

[27] Pant D., Van Bogaert G., Diels L., Vanbroekhoven K. A Review of the Substrates Used In Microbial Fuel Cells (MFCs) for Sustainable Energy Production, *Bioresour. Technol.,* **101**:1533–1543 (2010).

[28] Min H., Kim H. Application of Microbial Fuel Cell for the Monitoring and Control of Sulfur-Related Odor Generation, *Proc. Int. Conf. Biol.* **1**: 102–104 (2010).

[29] Wu S., He W., Yang W., Ye Y., Huang X., Logan BE. Combined Carbon Mesh and Small Graphite Fiber Brush Anodes to Enhance and Stabilize Power Generation in Microbial Fuel Cells Treating Domestic Wastewater. *J. Power Sources* **356**:348–355 (2017).

[30] Pozo Zamora G. Bio-Electrochemical Process for Metal and Sulfur Recovery from Acid Mine Drainage. (The University of Queensland, (2017).

[31] Perazzoli S., De Santana Neto JP., Soares H.M. Prospects in Bioelectrochemical Technologies for Wastewater Treatment, *Water Sci. Technol.* **78**: 1237–1248 (2018).

[32] Zhang X., He W., Zhang R., Wang Q., Liang P., Huang X., Logan B.E., Fellinger T.P., High-Performance Carbon Aerogel Air Cathodes for Microbial Fuel Cells. *ChemSusChem* **9**: 2788–2795 (2016).

[33] Lee D-J., Liu X., Weng H-L., Sulfate and Organic Carbon Removal by Microbial Fuel Cell With Sulfate-Reducing Bacteria and Sulfide-Oxidising Bacteria Anodic Biofilm, *Bioresour. Technol.* **156**: 14–19 (2014).

[34] Tabrizi NS., Yavari M. Methylene Blue Removal By Carbon Nanotube-Based Aerogels, *Chem. Eng. Res. Des.,* **94**:516–523 (2015).

[35] Kalathil S., Patil SA., Pant D., Microbial Fuel Cells: Electrode Materials , "Encyclopedia of Interfacial Chemistry"*, Elsevier.* 309–318 (2018).

[36] You S., Song Y.S., Bai S.J., Characterization of a Photosynthesis-based Bioelectrochemical Film Fabricated with a Carbon Nanotube Hydrogel, *Biotechnol. Bioprocess Eng.,* **24**: 337–342 (2019).

[37] Arenillas A., Menéndez J.A., Reichenauer G., Celzard A., Fierro V., Maldonado Hodar F.J., Bailón-Garcia E., Job N., "Organic and Carbon Gels", Springer International Publishing (2019).

[38] Cheng S., Liu H., Logan BE., Increased Performance of Single-Chamber Microbial Fuel Cells Using an Improved Cathode Structure, *Electrochem. Commun.* **8**: 489–494 (2006).

[39] Song H.L., Zhu Y., Li J., Electron Transfer Mechanisms, Characteristics and Applications of Biological Cathode Microbial Fuel Cells - A Mini Review, *Arab. J. Chem.* (2015).

[40] Gude V.G., Kokabian B., Beneficial Bioelectrochemical Systems for Energy, Water, and Biomass Production. *J. Microb. Biochem. Technol.* (2013).

[41] Wang Z., Mahadevan GD., Wu Y, Zhao F. Progress of Air-Breathing Cathode in Microbial Fuel Cells. *J. Power Sources* **356**:245–255 (2017).

[42] Zhang T., Bain T.S., Barlett M.A., Dar S.A., Snoeyenbos-West O.L., Nevin K.P., Lovley D.R., Sulfur Oxidation to Sulfate Coupled with Electron Transfer to Electrodes by Desulfuromonas Strain TZ1, *Microbiol. (United Kingdom)* **160**: 123–129 (2014).

[43] Zhao F., Rahunen N., Varcoe J.R., Chandra A., Avignone-Rossa C., Thumser A.E., Slade R.C.T., Activated Carbon Cloth as Anode for Sulfate Removal in a Microbial Fuel Cell. *Environ. Sci. Technol.* **42**:4971–4976 (2008).

[44] Koleva R., Yemendzhiev H., Nenov V. Microbial Fuel Cell as a Free-Radical Scavenging Tool. *Biotechnol. Biotechnol. Equip.* **31**: 511–515 (2017).

[45] Editors Rice E.W., Baird R.B., Eaton A.D., Clesceri L.S., "Standard Methods for the Examination of Water and Wastewater" 22nd Edition. American Public Health Association, American Water Works Association, Water Environment Federation (2012).

[46] Chen S., Liu G, Zhang R, Qin B., Luo Y., Hou Y. Improved Performance of the Microbial Electrolysis Desalination and Chemical-Production Cell Using the Stack Structure, *Bioresour. Technol.*, **116**: 507–511 (2012).

[47] Zhu T., Zhang Y., Bu G., Quan X., Liu Y., Producing Nitrite from Anodic Ammonia Oxidation to Accelerate Anammox in a Bioelectrochemical System with a Given Anode Potential, *Chem. Eng. J.* **291**:184–191 (2016).

[48] Clauwaert P., Rabaey K., Aelterman P., De Schamphelaire L., Pham T.H., Boeckx P., Boon N., Verstraete W., Biological Denitrification in Microbial Fuel Cells. *Environ. Sci. Technol.* **41**: 3354–3360 (2007).

[49] Yang Y., Li X., Yang X., He Z. Enhanced Nitrogen Removal by Membrane-Aerated Nitritation-Anammox in a Bioelectrochemical System, *Bioresour. Technol.* **238**: 22–29 (2017).

[50] Ohlinger K.N., Young T.M., Schroeder E.D. Predicting Struvite Formation in Digestion, *Water Res.* **32**:3607–3614 (1998).

[51] Cusick R.D., Bruce E.L., Phosphate Recovery as Struvite within a Single Chamber Microbial Electrolysis Cell, *Bioresource Technology,* **107**: 110–15 (2012).

[52] Jiang D., Curtis M., Troop E., Scheible K., McGrath J., Hu B., Suib S., Raymond D., Li B., A Pilot-Scale Study on Utilizing Multi-Anode/Cathode Microbial Fuel Cells (MAC MFCs) to Enhance the Power Production in Wastewater Treatment, *Int. J. Hydrogen Energy* **36**: 876–884 (2011).

[53] Razavi M., Yousefi Kebria D., Electrokinetic and Sediment Remediation in Microbial Fuel Cell. *Int. J. Eng.* **32**, (2019).

[54] David R. Lide, "CRC Handbook of Chemistry and Physics", Taylor & Francis Inc. 88th ed. Bosa Roca, United States, (2007).

[55] Du Z., Li H., Gu T., A State of the Art Review on Microbial Fuel Cells: A Promising Technology for Wastewater Treatment and Bioenergy, *Biotechnol. Adv.*, **25**: 464–482 (2007).

[56] Daraee M., Baniadam M., Rashidi A., Maghrebi M. Synthesis of N-CNT-TiO$_2$ Nanocatalyst: Application in Direct Oxidation of H$_2$S to Sulfur, *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, **39(5)**;133-144 (2020).

[57] Zang G.-L., Sheng G.-P., Li W.-W., Tong Z.-H., Zeng R.J., Shi C., Yu H.-Q., Nutrient Removal and Energy Production in a Urine Treatment Process Using Magnesium Ammonium Phosphate Precipitation and a Microbial Fuel Cell Technique, *Phys. Chem. Chem. Phys.* **14**, 1978 (2012).

[58] Niyom W., Komolyothin D., Suwannasilp B.B. Important Role of Abiotic Sulfide Oxidation in Microbial Fuel Cells Treating High-Sulfate Wastewater. *Eng. J.* **22**, 23–37 (2018).