Biotreatment of Slaughterhouse Wastewater Accompanied with Electrcity Generation and Nutrients Recovery in Microbial Fuel Cell

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ABSTRACT

In recent years and decades, there is a great need for developing new alternative energy sources or renewable sustainable energy. On the other hand, new technology approaches are growing towards benefits from the valuable nutrients in wastewater which are unrecoverable by traditional wastewater treatment processes. In the current study, a novel integrated system of microbial fuel cell and anoxic bioreactor (MFC-ANB) was designed and constructed to investigate its potential for slaughterhouses wastewater treatment, nitrogen recovery, and power generation. The system consisted of a double-chamber tubular type MFC with biocathode inoculated with freshly collected activated sludge. The MFC-ANB system was continuously fed with real-field slaughterhouse wastewater, with initial concentrations of COD and ammonium were 990 mg/L and 200 mg-N/L, respectively. The MFC-ANB system was operated for a total period of 43 days. Maximum removal efficiencies of COD, ammonium, nitrate, nitrogen recovery, Columbic efficiency, and power generation were 99%, 99.3%, 100%, 100%, 13.37% and 162.22 mW/m^2, respectively.

Key words: Microbial fuel cell**,** slaughterhouse wastewater, ammonium, and nitrate.

المعالجة البايولوجية للمطروحات السائلة من المجازر مع توليد الطاقة الكهربائية وأسترجاع المغذيات في خلية الوقود الأحيائية

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الخلاصة

خلال السنوات والعقود الماضية ظهرت حاجة ماسة لتطوير مصادر جديدة بديلة للطاقة اواللجوء للطاقة المتجددة او المستدامة. من ناحية اخرى، ان المسلك التكنولوجي الحديث يتجه نحو استغلال القيمة العالية للمغذيات في المياه الملوثة والتي من غيرالممكن استرجاعها بأستخدام الطرق التقليدية في المعالجة. في هذه الدراسة تم تصميم وتنفيذ منظومة معالجة جديدة قائمة على اساس الدمج بين خلية الوقود الاحيائية (MFC) مع المفاعل الاحيائي اللاهوائي (ANB) وهذه المنظومة "MFC-ANB" تقوم بمعالجة مياه المجازر واسترجاع النتروجين بالاضافة الى توليد الطاقة الكهرائية المستدامة في آن واحد. ان شكل وتصميم خلية الوقود الاحيائية (MFC) في هذه الدراسة هو انبوبي ذو حجرتين، حجرة الانود والتي تحتوي على قطب الانود الاحيائي وحجرة الكاثود التي تحتوي على قطب الكاثود الاحيائي ، وقد تم تغذيتها بألحمأة النشطة كمصدر للبكتريا النشيطة. تم تغذية منظومة المعالجة "MFC-ANB" بشكل مستمر بمياه المجازر الحقيقية لمدة 43 يوم. علما ان التركيز الابتدائي لمحتوى الطلب الكيمياوي للاوكسجين (COD) وتركيز الأمونيوم في حدود 990 ملغم/لتر، و 200 ملغم-نيتروجين/

لتر على التوالي. اظهرت النتائج المختبرية ان اقصى ازالة للطلب الكيمياوي للاوكسجين،، الامونيوم، النترات، استرجاع نتروجين، كفاءة كولومبية، وتوليد طاقة كانت 99٪، 99.3٪، 100٪، 100٪ ، 13.37٪ و 162.22 ملي واط/م^2 على التوالي.

الكلمات الدالة: خلية الوقود الاحيائية، مياه المجازر، الامونيوم،النترات، واسترجاع النتروجين.

1. INTRODUCTION

The technology of microbial fuel cells (MFCs) represents the latest approach of generating electricity from biomass using active living bacteria with wastewater treatment. MFC is one of the sustainable alternatives for renewable energy production. This process happens when the biofilm of bacteria on the anode surface oxidizes organic matter in the wastewater and produce electrons and protons, which generates electrical current through an external circuit **Arora, 2012; Janicek, et al., 2014**.

The slaughterhouses are the most important agro-industrial facility that has a direct impact on public health and the environment. Meat processing in slaughterhouses produces large amounts of organic wastewater during the manufacturing process, including, stunning, bleeding, hide or hair removal, carcass washing, cleaning process and trimming **Wang, 2004**; **Yordanov, 2010.** The slaughterhouse wastewater has a high strength, in terms of biochemical oxygen demand (BOD), and chemical oxygen demand (COD), suspended solids (SS), nitrogen and phosphorus, as compared with domestic wastewater **Wang, 2004**.

Typical concentration parameters of slaughterhouse wastewater such as BOD, COD, and nitrogen are 1900, 2579-6650, and 80 mg/L, respectively **Wiesmann, et al., 2007**.

However, primary and/or chemical treatments are not appropriate to reduce the pollutants in slaughterhouse wastewater to meet the discharge standards. Therefore, biological treatment is widely used for partial or complete stabilization of biologically degradable substances **Cheremisinoff, 1996**. On the other hand, anaerobic-aerobic treatment is an efficient way to treat highly COD loaded industrial and municipal wastewater **Chan, et al., 2009.**

Nitrification and denitrification are common processes to remove ammonia from slaughterhouse wastewater **Mousavi et al., 2012**. MFCs can be applied as a novel technique for bioelectrochemical nitrogen recovery, which can be accomplished by the nitrification process in an aerated biocathode **Yan, et al., 2012.**

Malaeb et al., 2013, developed a system consisting of a microbial fuel cell combined with membrane bioreactor (MFC-MBR) for simultaneous wastewater treatment and power generation. The achieved COD and NH_3−N removals were 97% and 91%, respectively.

Lin, et al., 2015 studied high-strength animal manure wastewater treatment and electrical energy recovery by air-cathode microbial fuel cells (MFCs). The MFC system was fed with swine wastewater and under external resistor of 2.2 kΩ, the observed energy production efficiency, columbic efficiency, and generated power were 0.37%, 1.5% 28.2 μW, respectively. **Zhu et al., 2016,** investigated the simultaneous nitrification and denitrification of cyanobacteria solution accompanied with power generation using a dual-chamber MFC. Removal of 0.064 ± 0.005 kg TN/m^3.day and 0.063 ± 0.005 kg NH_4^+/m^3.day were achieved. **Hiegemann et al., 2016,** evaluated the performance of pilot scale MFC system of 45 L volume consisting of 4 single-chamber membrane-less MFCs integrated into a full-scale wastewater treatment plant. Results revealed that COD, TSS, nitrogen removal, and columbic efficiency of 24.8% of 24%, 40% and 28%, respectively were achieved.

The present study looks forward to experimentally and theoretically investigate the performance of a sequential-flow tubular microbial fuel cell integrated with external anoxic bioreactor (MFC-ANB) for simultaneous treatment of real-field slaughterhouse wastewater, sustainable power generation, and nitrogenous-nutrient recovery. The MFC-ANB system was fed with real-field slaughterhouse wastewater and inoculated with activated sludge.

2. MATERIALS AND METHODS

2.1. MFC-ANB integrated system

MFC

An integrated complete system mainly consisted of tubular type microbial fuel cell associated with internal aerobic bioreactor and external anaerobic bioreactor was set up as given in Fig. 1. The tubular dual-chamber MFC consisted of three concentric Plexiglas cylinders. The internal, mid, and external cylinders represent the anodic section, cathodic compartment, and the extended aerobic bioreactor, respectively. The diameter of anode, cathode and bioreactor chambers were 60 mm, 160 mm, and 200 mm, respectively with height of 420 mm. The anode compartment was occupied with graphite rod. The graphite rod diameter and the effective length were 20 and 290 mm, respectively, resulted in a total effective surface area of 185.35 cm^2. The cylindrical perforated anode chamber had 169 holes, each hole of 11 mm diameter. The effective volume of anode was 680 mL. The anodic chamber was wrapped with a rectangular (410 mm x 200 mm) sheet of cationic membrane (CMI7000s). Graphite granules were used as contact material in the cathode compartment. This material had a bulk density of 1660 kg/m^3, surface area of 0.0832 m^2/g, and granular size diameter range of 2-4 mm. The total mass of the granular graphite electrode in the cathode compartment was 2200 g. It was placed in a perforated basket made of stainless steel (304) mesh 14.

Denitrification bioreactor

Continuous up flow mixed bed bioreactor was designed and installed to carry out the denitrification process, the bioreactor was made of Plexiglas cylinder (outside diameter 60 mm, height 420 mm).The active volume of the reactor was 680 mL.

2.2. Preparation of the MFC integrated system

Prior to construction and set up of the MFC integrated system, all its components were cleaned well with an appropriate detergent, then repeatedly rinsed with tap water and deionized water. Cation exchange membrane (CEM) was subjected to a course of pre-conditioning by immersion in a 5% sodium chloride solution for 24 h to allow for membrane hydration and expansion, and then washed with deionized water.

2.3. Acclimation and enrichment of biomass

To start up and operate the MFC, 680 mL of the activated sludge was placed in the anode compartment, and was sparged with nitrogen gas for a period of 10 min to maintain anaerobic environment. During the enrichment period, the anode was periodically fed by nutrient salt solution. After 45 days, the MFC was fed with a real field slaughterhouse wastewater at a rate of 0.38 mL/min.

2.4. Substrate, Inoculum and Chemicals

The real slaughterhouse wastewater was freshly collected from Al-Shoula slaughterhouse in Baghdad. The quality of wastewater is given in Table 1. The activated sludge which was obtained from the aeration tanks of Al-Rustamia third extension municipal treatment plant, Baghdad was used to inoculate the anode, cathode, and the denitrification bioreactor.
In order to develop and enrich the growth of microorganisms in the MFC, a mineral salt media was used for this purpose. This MSM solution was prepared according to the procedure outlined in **Ghangrekar, et al., 2005**. The media solution was prepared by dissolving; 0.56 g $(NH_4)2SO_4$, 0.20 g MgSO4•7H_2O, 15 mg $CaCl_2$, 1 mg $FeCl_3$•6H_2O, 20 mg $MnSO_4$•H_2O, 0.42g $NaHCO_3$ in liter distilled water, and then the solution was autoclaved at 121°C for 20 minutes and cooled under oxygen-free nitrogen gas before use.

2.5. Start up and operation of MFC integrated system

After 45 days of inoculating the MFC, wastewater was continuously fed to the anodic chamber of the MFC at a flow rate of 0.38 mL/min to achieve hydraulic retention time (HTR) of 30 h. Nitrogen gas was purged into the wastewater feed tank to eliminate oxygen content and provide anaerobic environment. At the same time, an air compressor with a maximum flow rate of 10 mL/min was connected to the cathode compartment to supply oxygen in a continuous manner. Also, the pH of the solution in the MFCs was monitored continuously and adjusted to 7-7.2 using 1M HCl or 1M NaOH solution. The MFC integrated system was operated at ambient temperature.

3. Data acquisition system and analysis

3.1. Wastewater and treated effluent analysis

Chemical oxygen demand (COD), biological oxygen demand (BOD), dissolved oxygen (DO), pH, NO_3^-, NH_4^+, and TDS tests were carried out by the researcher on a daily basis. The tests were conducted every day in accordance to the procedures outlined in the *Standard Methods* **APHA, 1998,** and the **ASTM Manual of Water and Environmental Technology, D1426-92.** Instruments and measuring devices used in this study were; COD reactor (model RD 125, Lovibond, Germany, COD test (model MD 200 COD vario, Lovibond, Germany), Multidirect (Lovibond,Germany), pH meter (model portable HI-83141, Hanna Instruments, Romania), Voltage data logger (model: Lascar EL-USB-3, USA), Multimeter (model MT1233C, pro'skit, Taiwan), Peristaltic pump (Model Mini-Pump Variable Flow Control, USA), Air compressor (model ACO-318, HAILEA, China), Resistance box (0-100,000 Ohm), Multiparameter Photometer bench type (Model C200, Hanna Instruments, Romania), BOD Measurement System (Model OxiDirect, Lovibond, Germany), Dissolve Oxygen meter (model HI9147-04, Hanna Instruments, Romania),Gas Chromatographs (Packard Models 438A, Packard instrument company, USA), Micromeritics analyzer (model: Micromeritics ASAP 2020, Micromeritics Instrument Corporation, USA).

3.2. Power density

The MFC voltage was continuously monitored via USB data logger. This data was converted to current by using Ohm's law Eq. (1):

$I = E_{cell}/R_{ext}$ (1)

The power density was calculated according to Eq. (2):

$P = (E_{cell})^2/R_{ext}*A_{an}$ (2)

where: P is the power (W/m^2), I is the current (Amp.), R_{ext} is the external resistance (Ω), and E_{cell} is the cell voltage (V), A_{an} is the projected surface area of anode (m^2).

3.3. Coulombic efficiency

The columbic efficiency was carried out according to Eq. (3) for continuous flow:

$$\epsilon_{cb} = \frac{MI}{Fbq\Delta COD} \quad (3)$$

Where: M is the molecular weight of oxygen (32 g/ mol), F is the Faraday constant (96485 C/mol of e^-), I is the current (Amp.), b is the number of electrons exchanged per mole of oxygen (4 mol/mol), q is the volumetric influent flow rate (m^3/sec), ΔCOD is the difference COD between the influent and effluent.

4. RESULTS AND DISCUSSION

4.1. Substrate (as COD) removal

The MFC-ANB system was continuously fed for 43 days with real-field slaughterhouse wastewater having average COD concentration of 1000 mg/L at organic loading rate of 0.8 kg COD /m^3.d. As shown in Fig. 2. , steady state condition was directly achieved in MFC-ANB fed with real-field slaughterhouse wastewater. This behavior could be due to the favorable and significant growth of previous acclimated microorganisms in the anodic and cathodic biofilm.

The slight fluctuation in COD removal was observed and was well expected when using real-field wastewater due to the variation in COD concentration, as well as the existence of different species which may affect the substrate biodegradation. Inspite of this fluctuation, maximum and average COD removal efficiencies were 98% and 94%, respectively. These results were comparable, or in fact relatively higher than 92% reported by **Min et al., 2005,** for the treatment of swine wastewater by MFC.

4.2. Ammonium ions removal

The ammonium concentrations in each part of the MFC–ANB system were examined during the entire period of the system operation. As given in Fig. 3, the influent average ammonium concentration was 200 mg-N/L. The removal efficiency of ammonium ions achieved 97.9% after 14 days of continuous operation, and then a steady state condition was observed with a maximum ammonium removal of 99.3%. However, a relatively moderate fluctuation in the ammonium removal ranged from 88.5% to 99.3% was observed. The removal of NH_4^+ occurred in the biocathodic section by the activity of nitrifying bacteria growing within the cathodic biofilm. The transfer of ammonium ions from the anodic section to the biocathodic section occurred by diffusion through the cation permeable membrane (CMI7000s). On the other hand, the small remaining concentrations of NH_4^+ ions will transfer from the anode via the sequential flow of the anodic effluent to the cathode.

4.3. Nitrate removal

The ammonium ions were converted to nitrate ions by the nitrifying bacteria in the aerobic biocathodic section and the subsequent extended aerobic bioreactor. Then after, the resulted nitrate ions were converted to nitrogen in the anoxic bioreactor (ANB) by the activity of denitrifying bacteria at steady state of continuous operation. Nitrate removal efficiency increased achieving 92.69% after19 days as shown in Fig.4. After 21 days, maximum removal efficiency of nitrate achieved 100%. The slight fluctuation in nitrate removal could be attributed to the limited supply of COD to the denitrifying bacteria to reduce nitrate to nitrogen in the anoxic bioreactor, **Schipper, et al., 2010**. The nitrate removal efficiency remained within a range from 90.3% to 95.5% after 35days.

4.4. MFC Voltage

The MFC voltage was regularly monitored and directly measured using the data logger during the entire period of continuous operation. As shown in Fig.5, the voltage sharply increased to achieve a maximum value of 350 mV after the first day of operation. Then, a decline of voltage was observed to a value of 300mV which lasted for about 8 days, and then the voltage was re-stabilized at 350 mV until the end of the operation period at the day 43. This short period-voltage drop could be related to the sudden existence of complex organic matter accompanied with other forging species and high ammonium concentration that may inhibit and adversely affect the growth and reproduction of bacteria and a subsequently lower electrons generation. The re-stabilization of voltage to its original maximum value was most likely due to the re-acclimation of active microorganisms to this type of substrate.

4.5. Polarization curve

In order to investigate the performance of MFC during a stable phase of operation, the output voltage was recorded under various external loads from 10 to 1000 Ω. In order, to get the polarization curve, the current density was plotted against voltage and power density. As given in Fig.6, maximum power and current densities were 165.22 mW/m² and 472 mA/m^2, respectively obtained at 40 Ω external resistances.

4.6. Power and current density

Profiles of the generated current and power are presented in Fig. 7. A sharp increase in the current and power were observed after the first day of operating the MFC-ANB system. After 10 days, the current and power densities were stabilized at 472 mA/m^2 and 165.22mW/m^2, respectively and steady state conditions were observed. Then the current and the corresponding power exhibited unsteady state condition for a period of 40 days.

5. CONCLUSIONS

This study demonstrated and evaluated the performance of an integrated system of dual chamber tubular microbial fuel cell (MFC) with extended aerobic bioreactor and anoxic bioreactor for real field slaughterhouse wastewater treatment, ammonium removal, and power generation. Results revealed that maximum efficiencies of COD removal, ammonium oxidation, nitrate reduction, Columbic efficiency, and power generation were 99%, 99.3%, 100%, 13.37% and 162.22 mW/m^2, respectively.

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7. NOMENCLATURE

BOD =biochemical oxygen demand, mg/l
COD= chemical oxygen demand, mg/l
SS =suspended solids, mg/l
MFC= microbial fuel cell.
MFC-ANB= microbial fuel cell integrated with external anoxic bioreactor
MCL=maximum contamination level, mg/l
TDS= total dissolved solid, mg/l

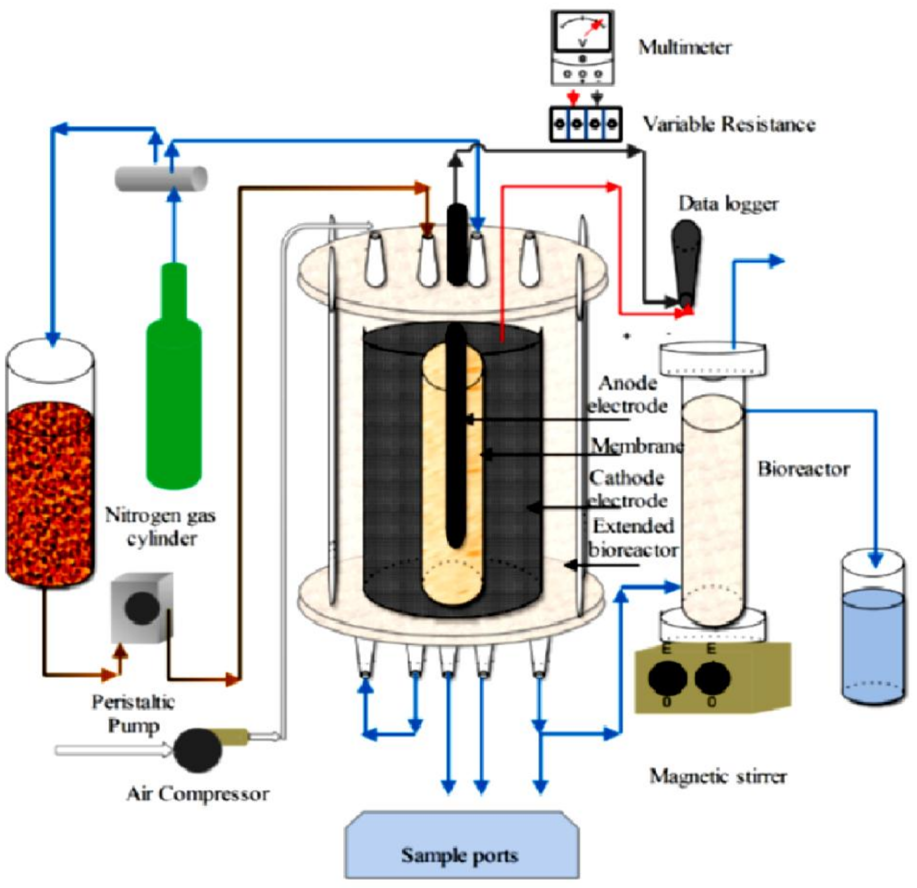

Figure 1. Schematic diagram of microbial fuel cell integrated with anoxic bioreactor .

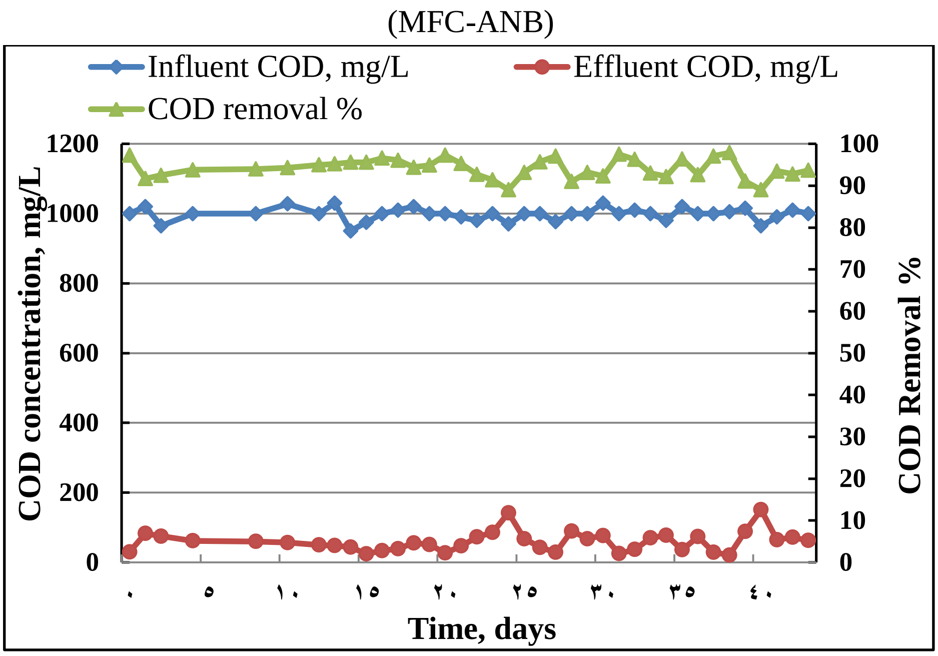

Figure 2. Profile of COD removal in the MFC-ANB system.

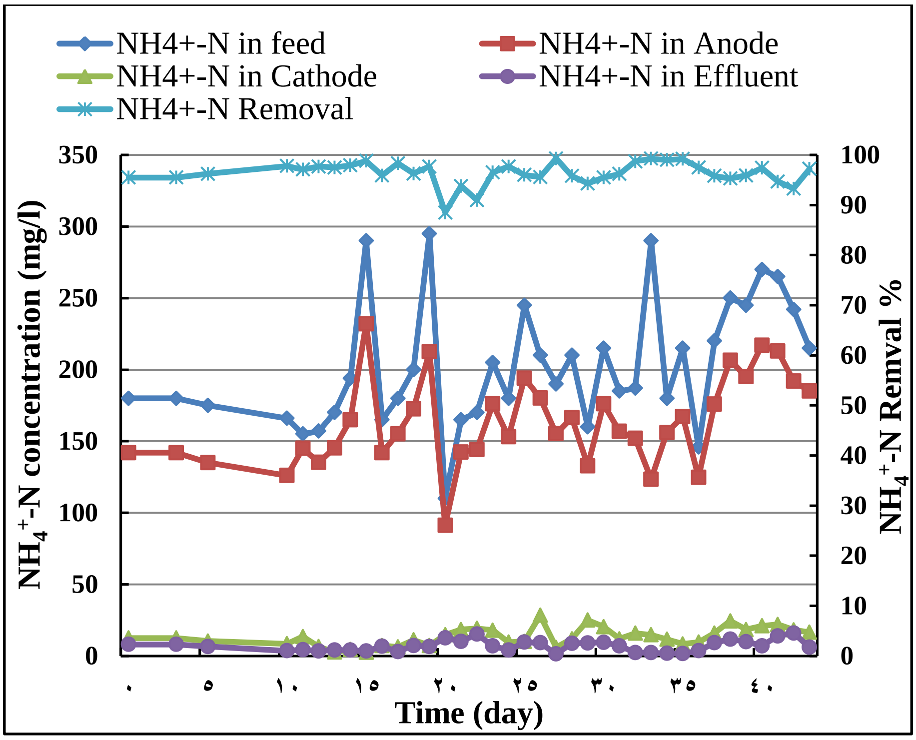

Figure 3. Profile of ammonium ions removal in MFC-ANB.

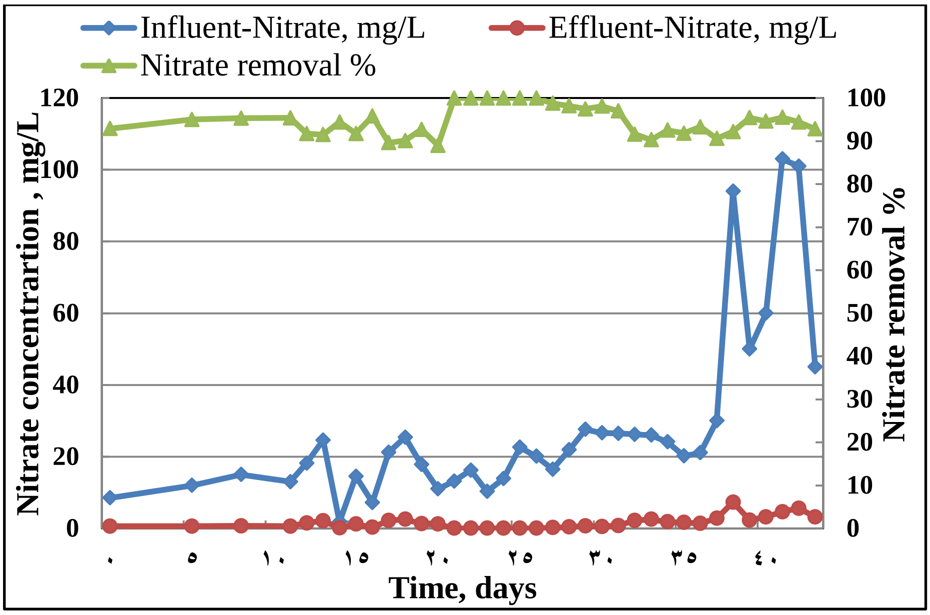

Figure 4. Profile of nitrate ions removal in the MFC-ANB.

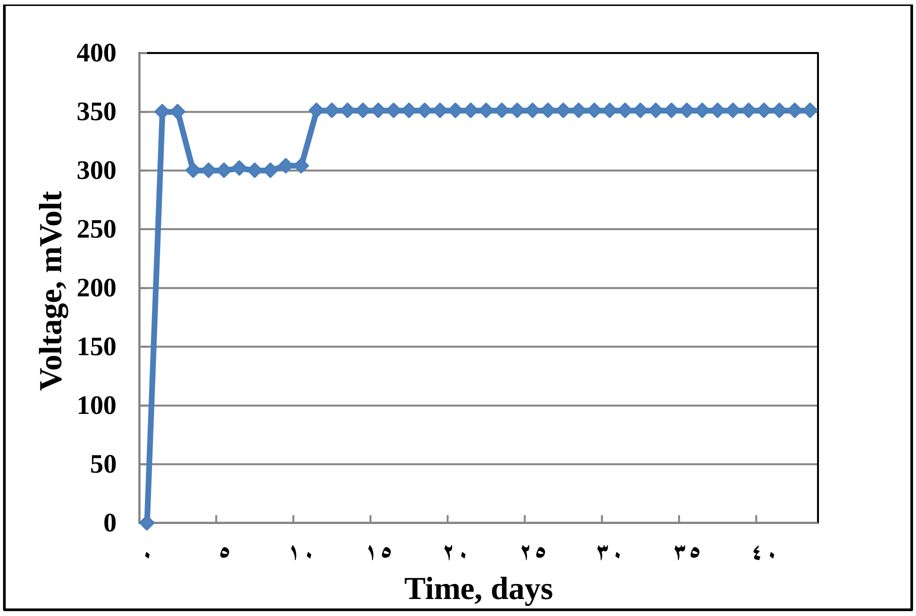

Figure 5. Voltage profile in the MFC at external resistance of 40 Ω.

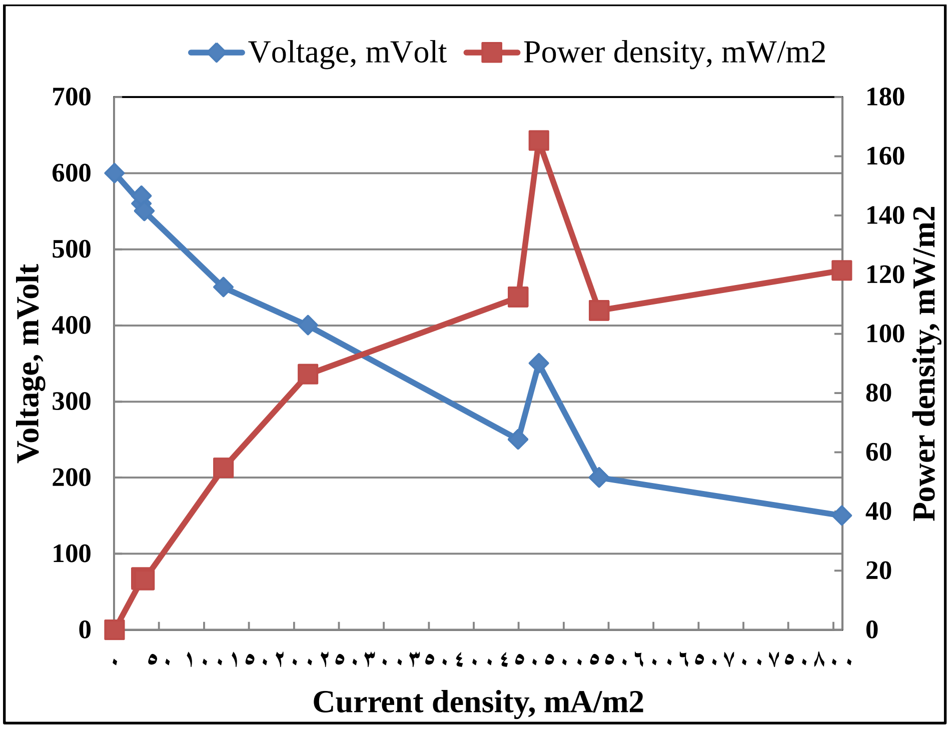

Figure 6. Polarization curve in MFC.

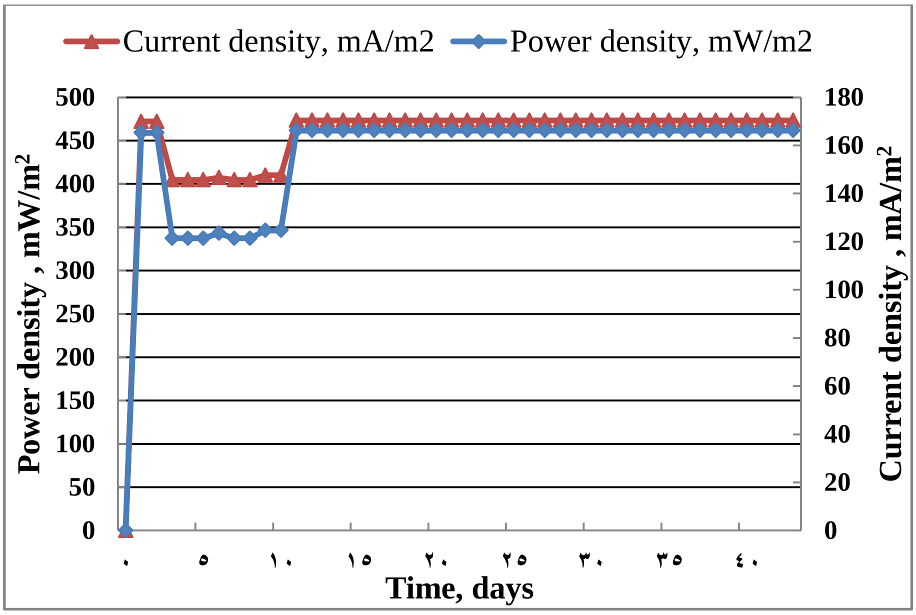

Figure 7. Power density and current density profiles in MFC.

Table 1. Quality of real-field slaughterhouse wastewater.

Constituents	Units	Average concentration
COD	mg/L	1000
BOD	mg/L	550
Ammonium (NH_4^+)	mg/L	75.05
Sulfate(SO_4^{-2})	mg/L	69.5
Nitrate (NO_3^-)	mg/L	13.5
Nitrite (NO_2^-)	mg/L	9.0
Chloride (Cl^-)	mg/L	49.0
Total dissolved solids (TDS)	mg/L	501
Phosphate (PO_4^{-3})	mg/L	3.07
pH	-	6.7