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ORIGINAL ARTICLE

Evaluation of dairy industry wastewater treatment and simultaneous bioelectricity generation in a catalyst-less and mediator-less membrane microbial fuel cell

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Abstract Increased human activity and consumption of natural energy resources have led to decline in fossil fuel. These current methods of energy production are not compatible with the environment. In this study catalyst-less and mediator-less membrane microbial fuel cell (CAML-MMFC) represents a new method for simultaneous dairy industry wastewater treatment and bioelectricity generation. The CAML-MMFC used was designed as two chambered that included an anaerobic anode and aerobic cathode compartment and was separated from each other by a proton exchange membrane. The anode and cathode electrodes were made from graphite plate. Current intensity, power density and voltage produced from wastewater as fuel were measured and the effluent from the anode compartment was examined to evaluate pollutant decrease. The maximum current intensity and power density produced were respectively 3.74 mA and 621.13 mW/m^2 on the anode surface, at OLR equal to 53.22 $kgCOD/m^3$ d and at the external resistance of 1 k Ω. The maximum voltage produced was 0.856 V at OLR equal to 53.22 $kgCOD/m^3$ d and at temperature 35°C. The maximum coulombic efficiency of 37.16% was achieved at OLR equal to 17.74 $kgCOD/m^3$ d. The HRT was examined as a factor influencing the power generation and when it was 5 day, maximum voltage and power density were obtained. The maximum removal efficiency of COD,

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BOD_5, NH_3, NH_4^+, dissolved phosphorus, phosphorus in suspended solids, SO_4^{2-}, TSS, and VSS was respectively achieved at 90.46%, 81.72%, 73.22%, 69.43%, 31.18%, 72.45%, 39.43%, 70.17% and 64.6%. The results showed that generating bioelectricity and dairy industry wastewater treatment by CAML-MMFC are a good alternative for producing energy and treating wastewater at the same time.

1. Introduction

Increased human activity and intensive use of natural energy resources, have led to decrease in fossil fuel. The current methods of energy production are not compatible with the environment. Fossil fuels are serious threats to the environment due to the production of greenhouse gases, including carbon dioxide [1,2]. Concerns about climatic change and global warming, increased global demand for oil and the necessity of substituting natural fuel for energy production, require the implementation of new methods for energy production by using natural and renewable carbon resources. In this regard scientists have been through intense efforts in discovering and substituting energy production methods. Many researches have shown that hydrogen and bioelectricity can have an important role as fuel in the future [3]. The technology of Microbial Fuel cells (MFCs) is the latest method for producing electricity from biomaterial by using microorganisms. MFCs are electrochemical converters and convert the chemical energy stored in organic material to current energy by microorganisms which act as biocatalysts in anaerobic conditions [4–6]. Microorganisms in the anode chamber oxidize the substrate added to the system such as wastewater or any other sugary or hydrogenated compound and produce electrons and protons. Free electrons are transferred to the anode electrode and through the external circuit they reach the surface of the electrode cathode. The produced protons pass through the proton exchange membranes or salt bridges and reach the cathode surface and in the presence of oxygen and electrons from water molecules [6,7]. In this process, along with the production of electrical power, the wastewater in the anode chamber is used as a substrate for treatment. Although carbon dioxide is produced during the oxidation process, its dissemination with this technology is low; because the carbon dioxide from renewable substrates originates from photosynthesis in the atmosphere and therefore the amount of carbon dioxide produced is natural [8,9]. The mechanism of electron transfer in MFC is an important topic in their function. Most bacterial species used in MFCs are inactive for electron transfer. Therefore, synthetic and natural compounds such as Thionine, Humic Acid, Neutral Red, Methylene Blue, Methyl viologen, and *Hydroxy naphthoquinone* which are Redox intermediates are used. By adding the mediators the commercial use of MFCs for energy production and wastewater treatment faces trouble, as most of these mediators are expensive and toxic [10,11]. Therefore, nowadays there is a lot of emphasis for improving MFCs without mediators. This method of electricity production leads to improvements in operating costs and increased acceptability of MFCs for wastewater treatment. Recently it has been proved that Iron reducing bacteria such as *Shewanella putrefaciens*, *Shewanella oneidensis*, Geobacteraceae bacteria such as *Geobacter sulfurreducens* and Geobacter metallireducens, Rhodoferax ferrireducens and *Aeromonas hydrophila* which are electrochemically active can transfer electrons to the anode electrode directly, by using Redox enzymes such as cytochrome which are present on the external membrane of bacteria. Soil, marine sediment, fresh water sediment, wastewater and particularly anaerobic and activated sludge are all rich sources for these microorganisms [12,13]. The function of microbial fuel cells is affected by several factors such as the amount of oxidation and electron transfer to the electrodes by microorganisms, loading rate, the nature of the used carbon source, the nature of the proton exchange membrane, proton transfer through the membrane to the cathode chamber, oxygen supply in the cathode, the nature and type of electrodes, circuit resistance, the electrolyte used, operation temperature, pH and sedentary time [14,15]. Among the other uses of this technology is the production of bio- hydrogen and using it as a sensor for pollutant analysis (BOD measurement) [3,10]. MFCs have many advantages, including the cleanliness of the process, efficiency, easy conduct in different circumstances and not producing toxic side by- products; and therefore have shown to be a better option for producing simple and complete renewable energy [13,16]. Industrial dairy wastewater is an important source of organic material for electricity production by using MFCs. In this study for the first time we show electricity production directly from dairy wastewater and its simultaneous treatment by using CAML-MMFC technology.

2. Materials and methods

2.1. Wastewater sample and seeding

Dairy industrial wastewater was collected from Mahtaj Dairy Industry in Zahedan City, Iran and kept in a refrigerator at 4 °C before use. This wastewater is classified as nontoxic due to low hazardous chemicals and high amounts of

Table 1 The characteristic of Mahtaj Dairy factory wastewater in Zahedan.

Number	Parameter	Value
1	COD	3620 mg/l
2	BOD_5	2115 mg/l
3	Total P	187 mg/l
4	NH_3	167 mg/l
5	NH_4^+	174 mg/l
6	TSS	1430 mg/l
7	VSS	647 mg/l
8	SO_4^{2-}	835 mg/l
9	EC	2176 (ms/cm)
10	pH	8.5–10.3

biodegradable organics in comparison to other industrial wastewater. Some of the characteristics of this wastewater are shown in Table 1. The Dairy Industry wastewater was used as fuel and substrate for MFC tests without additions of any other nutrient or trace metals. The inoculated wastewater before injection to the anode MFC chamber was passed through a filter with pore sizes about 2 μm. Samples were deoxygenated with gas of N_2 for 30 min to remain in anaerobic condition before experiments. Also, in order to prevent the activity of methanogenic bacteria (which are electrochemically inactive) the BES solution (2 bromo-ethano-sulfonate) was added to the sample. The container with wastewater was placed on a mixing device with 50 rpm in order to prevent sedimentation. The inoculated sludge used in this study as the initial microbial seed for fortification was activated sludge taken from the Zahedan city treatment facility. These sludge included microorganisms that were electrochemically active and very suitable for MFCs that act without external mediators.

2.2. MFC configuration and operation

The CAML-MMFC used in this study was designed as two chambers. As seen in Fig. 1, this MFC includes two anode and cathode chambers that were separated from each other by a proton exchange membrane make from Nafion 117 (DuPont Co USA). The chambers were made from plexiglass sheets with 2 cm diameter and each was made with a 2 L effective volume that was completely sealed by gaskets. The anode and cathode electrodes were made from a graphite plate with $14 \times 6 \times 0.5$ cm^3 dimensions. The anode electrode was located in 5 cm and the cathode electrode was in 2 cm distance from the membrane. The electrodes were connected with copper wire with 2 mm diameter and 35 cm length through a resistance (resistor substitution box, RS 500, Elenco electronics) of 1–25 kΩ and a digital multimeter (Model 2700, Keithley Instruments Inc., Cleveland, OH, USA). The internal resistance between the electrodes and the copper wire was less than 2 Ω measured by a multitester (Model 2000, Keithley, MA, USA). The electrodes were soaked in deionized water for 24 h. Before starting, the proton exchange membrane was placed in hydrogen peroxide solution 30% in H_2O, deionized water, and then H_2SO_4 0.5 M; and eventually was placed in de-ionized water again before application, to get rid of the extra acid. The whole process was done at 80 °C for one hour. The MFC reactor was operated in batch mode until stable MFC system was established. Afterward, the dairy industrial wastewater as fuel was continuously pumped into the anode chambers using a peristaltic pump (Cornwall, UK 505S, Watson-Marlow, Falmouth). The flow rates for MFC were fixed at 16.7, 11.12, 8.34, 6.67, 5.56, 4.76 and 4.17 ml/min to maintain HRT (hydraulic retention time) of 2, 3, 4, 5, 6, 7 and 8 day, respectively. The cathode chamber was aerobic and was filled with phosphate buffer (di-potassium, hydrogen phosphate 50 mM) as a catalyst and electron receiver. In order to supply oxygen to the cathode chamber at a continuous rate of 2 mg/h an aquarium pump was used and the dissolved oxygen was measured using a DO meter (Model 50B,YSI Incorporated, Yellow Springs, Ohio, USA). The catalytic pH was fixed between 7.0 and 7.5 and the pH was measured using a pH meter (pH/ION Meter DP-880, Dong-Woo Medical System, Seoul, Korea).

2.3. Analyses and calculations

The current was calculated according to Ohm law, $I = V/R$, where I (mA) is the current, V (V) is the voltage, and R (Ω) is the external resistance. Current density was calculated as $i = I/A$, where A (84 cm^2) is the projected surface area of the studied electrode. The power density was calculated according

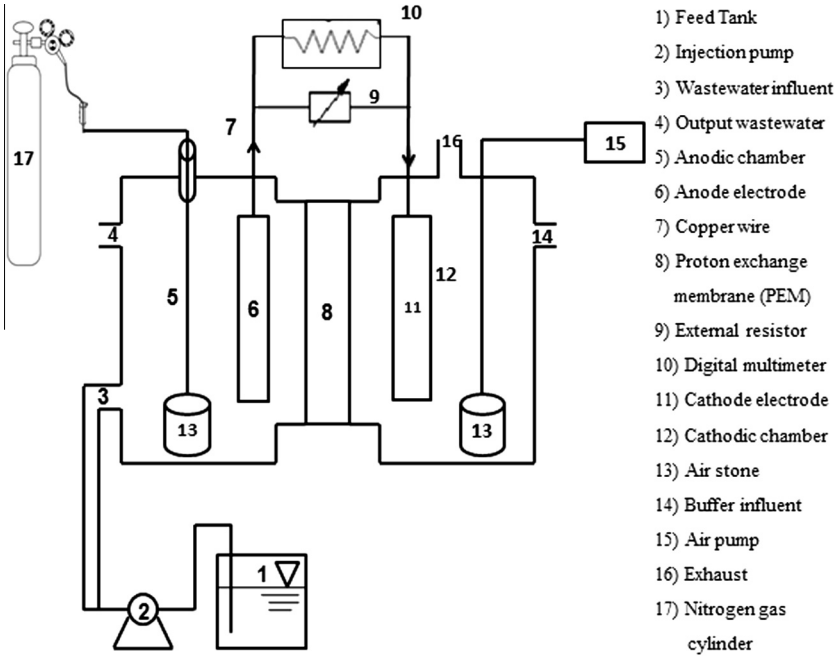

Figure 1 The schematic view of the CAML-MMFC reactor.

to $P = IV/A$, where I, V and A are the same as previously described. The columbic efficiency (CE) is calculated as $CE = (C_P/C_{Ti}) \times 100$ and $C_{Ti} = Fb_iS_iV/M_i$, where CE is the columbic efficiency, C_P is the total Coulombic calculated from the produced current, C_{Ti} is the theoretical amount of colomb that can be produced from wastewater, F is the Faraday constant (98,485 C/mol-e), b_i is the number of mol electrons produced per each mol substrate (4), S_i is the substrate concentration, V is the volume of liquid and M_i is the molecular weight of oxygen (32 g mol^{-1}). In order to evaluate the efficiency of wastewater treatment through the MFC system, the effluent from the anode chamber was examined with regard to COD (Chemical Oxygen Demand), BOD_5 (Biological Oxygen Demand), NH_3 (ammonia), NH_4^+ (Ammonium), dissolved P (Phosphorus), P (Phosphorus) in suspended solids, TSS (Total Suspended Solids), VSS (Volatile Suspended Solids), SO_4^{2-} (sulfate) and pH according to the standard methods in the textbook of standard methods for water and wastewater examination [17]. The MFC function in eliminating substrate during operating time is calculated according to $\xi COD = (CSO - CS)/CSO \times 100$, where ξCOD is the removal efficiency of substrate, CSO is the concentration of initial COD, and CS is the concentration of the remaining COD. The OLR (organic loading rates) in kg COD/m^3 d is also calculated according to $OLR = (CSO \times FreeRate)/(ReactorVolume)$.

3. Results

The two chamber catalyst and mediator less membrane microbial fuel cell used in this study, operated continuously for 30 days by dairy industrial wastewater. The results of this research are shown in Figs. 2–12. Fig. 2 shows the polarization curves, which show how the power density and the voltage changed when the current intensity changed. When the current intensity increased from 0.1 to 2.9 mA, the voltage decreased from 0.856 to 0.178 V. The power density increased with current intensity to a maximum power point (621.13 mW/m^2). Fig. 3 shows power density and columbic efficiency in pH 7 and different OLRs. As it can be seen in the Fig. 3, in the OLR of 53.22 kgCOD/m^3 d, the maximum power density of 621.13 mW/m^2 was achieved. In OLR of 17.74 kgCOD/m^3 d, the maximum columbic efficiency (37.16%) was achieved. Figs. 4 and 5, show the effect of temperature on the voltage and current intensity of the microbial fuel cell. The maximum voltage (0.856 V) and current intensity (3.74 mA) were achieved at 35 °C. The effect of OLR on the current density produced by dairy industrial wastewater is shown in Fig. 6. The maximum current density (795.74 mA/m^2) was achieved at OLR of 53.22 kgCOD/m^3 d. Fig. 7, shows the effect of microbial fuel cell external resistance on the power density and voltage produced as it can be seen that the maximum power density and voltage were achieved at 1 kΩ external resistance. Fig. 8, shows the changes in outlet wastewater (anolyte) pH from the anode chamber and the catholyte in the cathode chamber. The maximum pH changes of the anolyte from the anode chamber of the microbial fuel cell were 1.05 and for the catholyte was 0.39. The other important parameter in the operating process of the microbial fuel cell was the hydraulic retention time. The maximum voltage and power density were achieved at the HRT of 5 days (Fig. 9). Figs. 10–12, show the treatment rate of wastewater at a fixed pH = 7.0 and in completely anaerobic conditions. The maximum removal efficiency of COD, BOD_5, NH_3, NH_4^+, dissolved phosphorus, phosphorus in suspended solids, SO_4^{2-}, TSS, and VSS was respectively 90.46%, 81.72%, 73.22%, 69.43%, 31.18%, 72.45%, 39.43%, 70.17% and 64.6%. In this study the effect of inlet wastewater concentration as fuel was compared with other studies done in relation to this technology and is shown in Table 2.

4. Discussion

4.1. The concentration of fuel, and the current intensity, voltage and power density

Wastewater concentration is one of the most important factors that affect MFC function. Many systems showed that electricity production in both MFCs with closed systems and MFCs with continuous systems depends on the concentration of the fuel and usually fuels with higher concentrations have a higher voltage. Also in low concentrations of substrate, more time is needed for reaching the maximum current intensity and voltage, however in higher concentrations of substrate the maximum current intensity and voltage are achieved in a shorter time [25,26]. Fig. 2, shows the polarization curves. The polarization curves are used for determining the dependency of

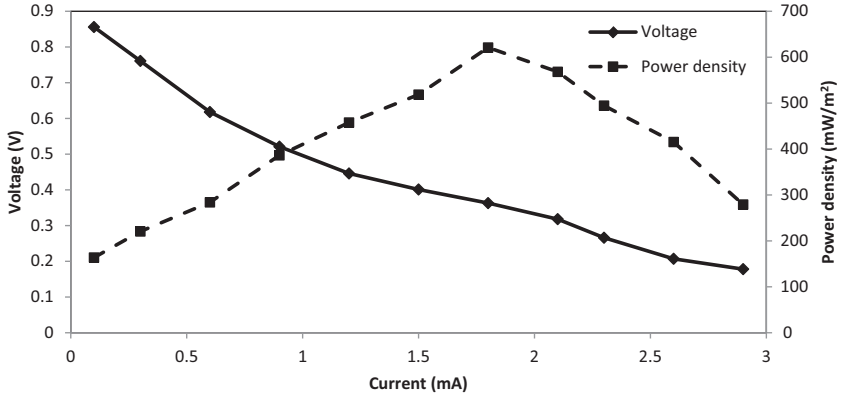

Figure 2 The voltage and power density produced as a function of current intensity (polarization curves) from dairy industry wastewater used as fuel by CAML-MMFC.

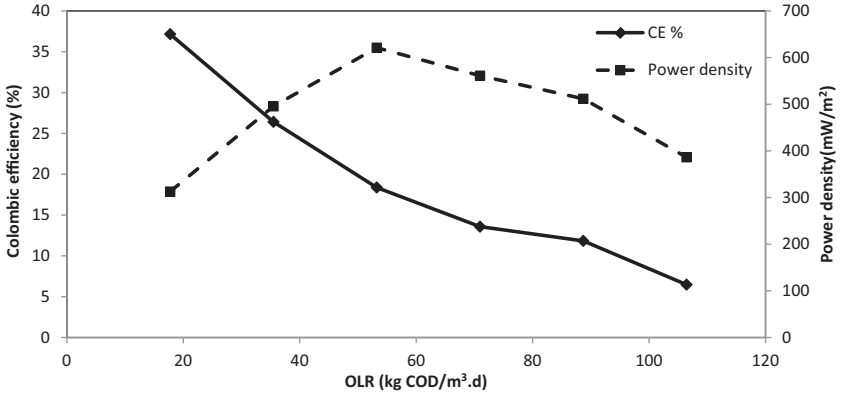

Figure 3 The columbic efficiency and power density in different OLR of CAML-MMFC by using dairy industry wastewater as fuel.

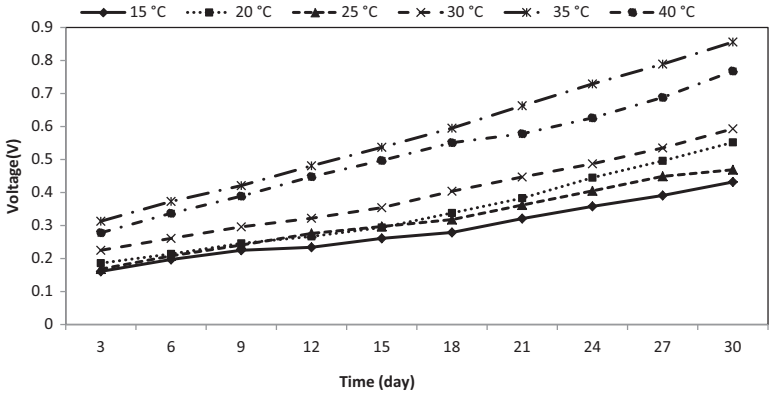

Figure 4 The effect of different temperatures on the amount of voltage produced from dairy industry wastewater used as fuel by CAML-MMFC.

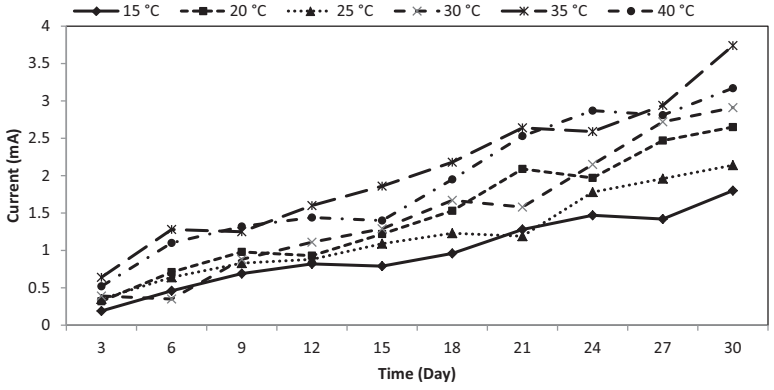

Figure 5 The effect of different temperatures on the current intensity produced by dairy industry wastewater used as fuel by CAML-MMFC.

microbial fuel cell function of external resistance and changes in power density and voltage with changes in current intensity [27]. According to these curves when the current intensity increased (from 0.1 to 2.9 mA), the voltage decreased (from 0.856 to 0.178 V) and the power density increased to a maximum power point (621.13 mW/m^2). Beyond this point, the power density dropped due to the increasing ohmic losses and high electrode potentials. The experimental results with dairy industry wastewater used as fuel in Fig. 2, showed that when the MFC system was launched with this wastewater, maximum voltage of 0.856 V was produced. During the operating time of the CAML-MMFC, fluctuations were seen in voltage production. These sudden fluctuations in voltage may be due to potential differences between electrodes based on chemical and biological factors [28]. Therefore, due to biological activity, voltage increases and after 30 days it stands at 0.856 V. The voltage was recorded after reaching a steady state. Electricity production in each step includes three phases:

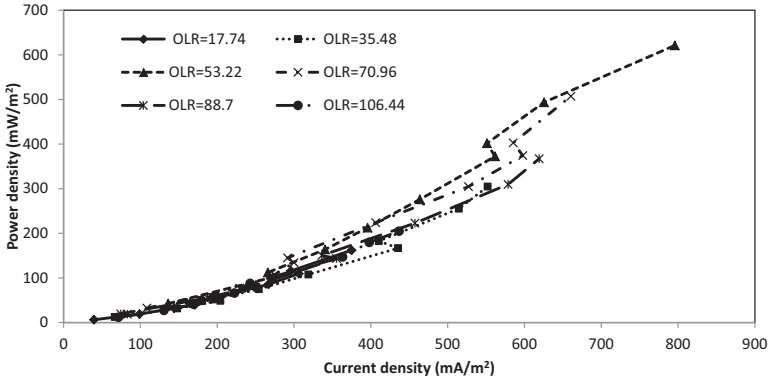

Figure 6 The current density and power density produced from CAML-MMFC in different OLRs from dairy industry wastewater used as fuel.

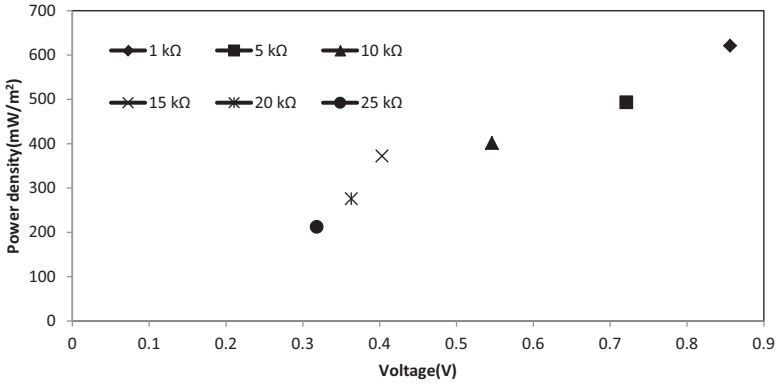

Figure 7 The voltage and density of electricity produced at different external resistances of CAML-MMFC from dairy industry wastewater used as fuel.

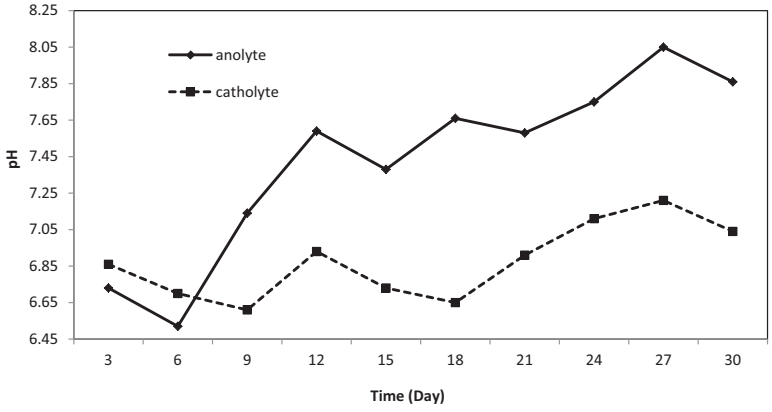

Figure 8 The change in the pH catholyte and anolyte during the operation of CAML-MMFC with dairy industry wastewater used as fuel.

increasing, constant and decreasing phase. MFCs that are operating with wastewater including complex chemicals as substrate have a lengthier constant phase in comparison to mono carbon sources such as glucose and acetate. Results showed that using the wastewater of this industry for producing an acceptable voltage is practical [29]. Electricity production is based on the transfer of microbial electrons in graphite electrodes of MFC. In some studies it has been reported that the production of biofilm happens between 6 and 15 days until a stable amount of electricity is produced. This can be the reason for a considerable voltage production after 5–6 days [4,30]. However we think that due to the observed higher voltage on day 5, the production of biofilm happens in a shorter time. This is probably due to the presence of the required bacteria as a biocatalyst in this wastewater in addition to inoculated sludge. The sudden increase in voltage

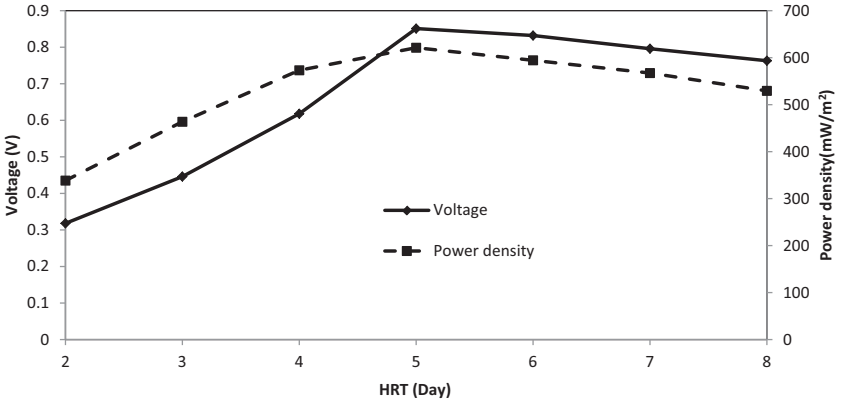

Figure 9 The effect of hydraulic retention time on the voltage and density of electricity produced from dairy industry wastewater as fuel by CAML-MMFC.

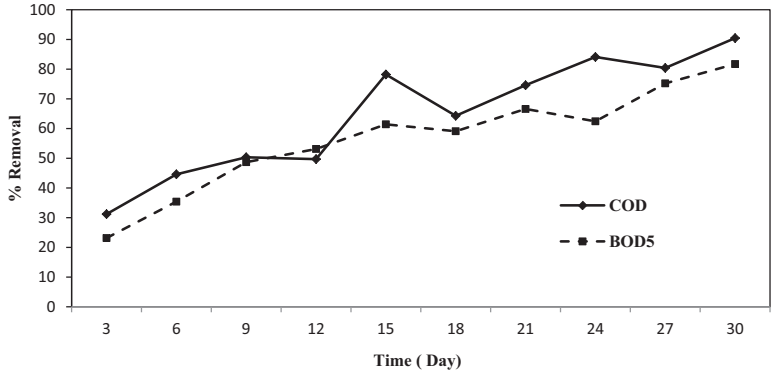

Figure 10 The removal efficiency of COD and BOD_5 of dairy industry wastewater used as fuel by CAML-MMFC at pH = 7.0.

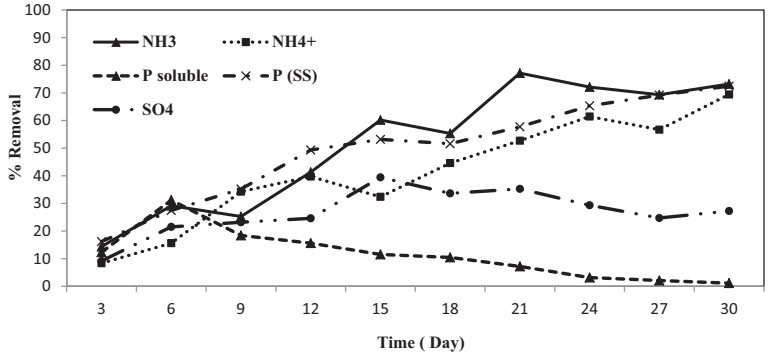

Figure 11 The removal efficiency of NH_3, NH_4^+, dissolved phosphorus, phosphorus in suspended solids and SO_4^{2-} from dairy industry wastewater used as fuel in CAML-MMFC at pH = 7.0.

may be due to the presence of chemicals in dairy industrial wastewater which is easily used by anode bacteria. After using substrate the voltage gradually decreases. The maximum current intensity that was 3.74 mA and power density that was 621.13 mW/m^2 was achieved on the surface of the anode. These results are in line with the results from Lu et al. in 2009 about electricity generation from starch processing wastewater, Lefebvre et al. in 2013 about optimization of a microbial fuel cell for wastewater treatment and Cha et al. in 2010 about applicable microbial fuel cells in aeration tank for wastewater treatment [31–33].

4.2. Fuel concentration, organic loading and Coulombic efficiency

The ratio of the number of electrons transferred to the external circuit to the number of electrons from the oxidation of the substrate is called the Coulombic efficiency [34]. Obviously

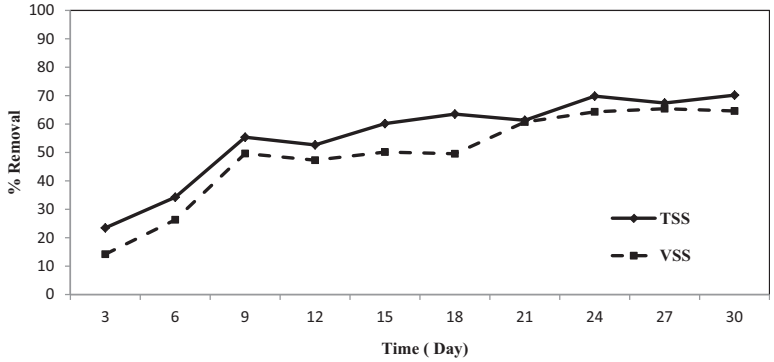

Figure 12 The removal efficiency of TSS and VSS from dairy industry wastewater used as fuel by CAML-MMFC at pH = 7.0.

with an increase in the Columbic efficiency in a battery, electricity production increases. In this study as it can be seen in Fig. 3, the columbic efficiency achieved has a range between 6% and 37% and the highest amount that is 37.16% was achieved at OLR equal to 17.74 kgCOD/m^3 d and decreased with an increase in the OLR. The main reason for this inverse association between the coulombic efficiency and fuel concentration and OLR was that high fuel concentration and organic loading factors decrease bacterial activity and therefore CE decreases. There may be two factors related to small amounts of CE from organic substrates in MFCs. First instead of producing electricity from the substrate, only bacterial growth happens. Second, organic substrates and electrons are used by the other electron receptors available in the solution such as oxygen, nitrate and sulfate, and decreased CE [35,36]. Also the columbic efficiency has an inverse relation with the removal efficiency of COD. The low amount of columbic efficiency shows the inability of bacteria in converting all organic chemicals to electricity. Among the other reasons is the inappropriateness of a big part of products from food degradation for producing electricity in the MFC systems. Therefore the remaining substrate in the system prepares a suitable condition for the growth of methanogens in favorable environmental conditions. Therefore, the columbic efficiency has an inverse relation with COD removal efficiency and with an increase in the percent of COD removal, a lower percentage of columbic efficiency is expected. These results are compatible with the results of the studies done by Kim et al. in 2005, Aelterman et al. in 2008 and Liu et al. in 2007 [2,37,38].

4.3. The effect of temperature

The two chamber MFC used in this study operated continuously in 6 temperatures (15, 20, 25, 30, 35 and 40 °C) and its voltage and current intensity efficiency were read in a 30 day period. The operating time in high temperatures was longer than low temperatures, but the voltage produced at high temperatures (30 and 35 °C) was more. This result was similar to the results from Jadhav et al. about the performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration and Wei et al. about effects of temperature and ferrous sulfate concentrations on the performance of microbial fuel cell [13,39]. The voltage and current intensity produced in different temperatures are shown in Figs. 4 and 5. The maximum voltage and current intensity were achieved at 35 °C. Decrease in the voltage efficiency and current intensity may happen due to several reasons. As temperature increases, the rate of biochemical reactions in cells and the rate of bacterial growth intensify and therefore the metabolism rate of bacteria increases that leads to the quick growth of bacteria and higher voltage efficiency. However, during long operating times when bacteria are in high temperatures, important compounds in cells including nucleic acid and other material susceptible to temperature may get damaged irreversibly and this will lead to an intense deterioration of cell function or cell death. In this situation the voltage and current intensity decrease dramatically. Also the slow growth rate of bacteria in low temperatures leads to decrease in the population of bacteria in the anode chamber and operation in these low temperatures is not successful and the voltage efficiency decreases [40–42]. This point can be concluded from this study and other studies that each type of microorganism can only live in a specific temperature. The growth and reproduction rates of microorganisms will be the fastest and the cell function is not affected only if they live in the optimum temperature [32,38].

4.4. The effect of organic loading rate (OLR)

Many studies about electricity production by MFCs have shown that the amount of current produced both in closed MFCs and those with continuous current depend on the organic loading [4,43]. In this study CAML-MMFC was examined in different organic loadings and their effects on current density and power density as parameters in evaluating the function of MFC during operation are shown in Fig. 6. As it can be seen the highest current density and power density during the 30 days of operation were achieved in OLR equal to 53.22 kgCOD/m^3 d. The reason for this is the fact that in low OLR the fuel cells need more time to reach the maximum current density and power density. But in higher OLR the system reaches the maximum current density and power density in a shorter time. The other reason is the compatibility of microorganism present in wastewater and inoculated sludge [44]. These results are also in line with the results of Mohan et al. in the years 2007 and 2008 and Huang et al. in 2008 about MFCs which reported that the anodic reactions depend on the ease of accessibility to the carbon present in the fuel [12,20,45].

Table 2 Comparison of the produced electricity and the function of microbial fuel cells in different concentrations of fuel.

Membrane use	Catalyst used	Cathodic system	Reactor structure	Substrate	OLR (kg COD/m^3 d)	Removal Efficiency of COD	Maximum current intensity (mA)	Maximum voltage produced (V)	Source
Yes	No	Airing	Two chambers	Industrial dairy wastewater	17.74–106.44	89%	3.74	0.856	This Study
Yes	No	Airing	Two chambers	Glucose	1.404–1.165	39–55.4	2.97	0.7	[18]
Yes	No	Aerial Cathode	Single chambers	Glucose	2.64–3.54	43.78–43.06	0.11–0.98	0.24	[19]
Yes	No	Airing	Two chambers	Glucose	0.517–1.033	60–62%	1.66	0.423	[20]
Yes	No	Airing	Two chambers	Synthetic waste	0.753	74.20	2.37	0.586	[21]
No	No	Aerial Cathode	Single chambers	Sludge	4.3	58%	3.5	0.49	[22]
Yes	No	Airing	Two chambers	Chocolate Industry wastewater	–	75%	3.02	0.398	[23]
Yes	No	Aerial cathode	Single chambers	Urban wastewater	0.13–0.7	40–80%	3	0.4	[24]

4.5. The effect of external resistance

High external resistance leads to decrease in the produced power density. Therefore, in order to have a high output, MFC has to be designed with low external resistance [13]. In order to find out in what external resistance the highest power density happens, the circuit external resistances were changed (Fig. 7). According to Ohm's law, voltage and resistance have a direct relationship. In other words, as resistance increases, voltage increases and current decreases. As external resistance increases from 1 to 25 kΩ the generated voltage decreases from 0.856 to 0.318 V. This decrease in voltage means that some electrons have been used by mechanisms other than cathodic reactions [46,47]. In high external resistance, low voltage may be due to the lower speed of using electrons in the cathode in comparison to its transfer rate from the external circuit. Also due to the less conductive external path at higher external resistance, the demand for electrons is lower than what microbes can produce. It is acceptable that when the resistance in the circuit increases, the ejection of electrons through the circuit decreases. The electrons in the cathode are used to decrease other electron acceptors such as sulfate, nitrate or permeable oxygen from the cathode [47,48]. At low external resistance, electrons easily pass through the external circuit and oxidize the electron carriers on the external membrane of the microorganisms on the anodic chamber. Therefore more fuel oxidation for removing organic material by microorganisms and with high speed and in low external resistance happens. Also in the MFC systems, the maximum power density is achieved when the internal and external resistances are equal [48,49]. The differences in the MFC performance with different external resistances may be attributed to the variations in the activation losses at the anode, which is a function of electrochemical activity of anode reducing microorganisms [31].

4.6. The effect of pH

The other important parameter in MFC systems that affects both the current intensity and the treatment rate of wastewater entering the anode chamber as fuel, is the pH of the wastewater entering the anode chamber and the phosphate buffer entering the cathode chamber [4]. The anode chamber is anaerobic and microorganisms transfer electrons from the degradation of organic material to the anode electrode. Despite the presence of oxygen due to the high electronegative power, all of the produced electrons are caught and reduced [23]. The best pH for microorganism activity in these anaerobic conditions is pH 6.5–7.0 [50]. The pH of dairy wastewater used in this study was 8.5–10.3 and its value was adjusted with NaOH or H_2SO_4. The electrolyte used in this study was phosphate buffer whose pH was set at 7.0 and the function of MFC as a new technology in producing electricity and wastewater treatment in this pH was evaluated. In a study done by Li et al. in 2008 and Jadhav et al. in 2009, the maximum efficiency of MFC was seen in pH 7.0 [13,51]. The result of this research and other studies done by other researchers showed that microbial activity is slower in pH less than the optimum pH in comparison to optimum pH. Low pH in the anode chamber deactivates microorganisms and decreases the MFC efficiency. The low current intensity around the optimum pH of the anode chamber may be due to weak transfer of protons to the other side of the membrane. Changes in pH were seen more in the initial steps of MFC operation, and this is due to the slower transfer of protons through the proton exchange membrane in comparison to its production rate in the anode chamber and its consumption rate in the cathode chamber [13,52]. The maximum pH changes in the anolyte from the anode chamber of the MFC was 1.05 and in the catholyte from the cathode chamber was 0.39 (Fig. 8).

4.7. The effect of hydraulic retention time (HRT)

Hydraulic retention time (HRT) is an important parameter in wastewater treatment and determines the concentration of the remaining substrate and the amount of dissolved oxygen in the reactor. In the MFC system the substrate concentration and the amount of dissolved oxygen for electricity production is controversial [53]. When HRT decreases, the substrate concentration increases and leads to consumption of the whole substrate and increased voltage and power density. On the other side, high concentrations of dissolved oxygen in the influent wastewater lead to increase in the oxidation reduction potential (ORP) and this leads to decrease in the voltage and power density produced in the MFC [10,41]. In order to understand the influence of HRT on bioelectricity production, the CAML-MMFC was operated continuously with dairy

industry wastewater under seven different HRTs (2, 3, 4, 5, 6, 7 and 8 day). As it can be seen in Fig. 9, the highest voltage and power density in HRT were achieved on 5 day and were respectively 0.851 V and 621.17 mW/m^2. As it can be seen, when HRT changed from 3 to 5 day, the efficiency voltage increased and reached 0.851 V. The reason for voltage increase as the HRT increases may be due to the longer contact time between biofilm and organic material that can show the advantage of biofilm, substrate degradation, electron production and transfer to the anode surface. Despite this, when HRT increased (8 days), the voltage slightly decreases. These results are in line with the results of electricity production by single chamber MFC with the aerial cathode in the presence and absence of proton exchange membrane and also the results of electricity production and wastewater treatment by using the single chamber MFC by Liu et al. in 2004 [54].

4.8. The efficiency of wastewater treatment

The MFC system used in this study was able to produce electricity continuously and was able to simultaneously treat the wastewater used as fuel. The different parameters of dairy wastewater that have been used in this study as a fuel are mentioned in Table 1. The concentration of the effluent pollutants was measured after each cycle and dramatic changes were seen in the amount of different parameters in wastewater. The concentration of COD and BOD_5 in wastewater and the removal efficiency is shown in Fig. 10. The results show that removal efficiency, increases simultaneously with the operation time from 78.21% to 90.46% for COD and from 61.43% to 81.72% for BOD_5. This amount of removal happened in OLR equal to 53.22 kg COD/m^3 d and one reason for this may be the high compatibility of the microorganisms present in the anode chamber in this OLR. This shows the high potential and function of the MFC system as a substitute for the usual biological treatment processes of wastewater [10,55]. The high removal rate of COD and BOD_5 in this study is probably related to the long operation time of this method, although the Coulombic efficiency decreases continuously. This shows that the consumption of organic material in dairy wastewater is not entirely related to electricity production and there may be other reasons such as biomass and fermentation. Although the efficiency of converting organic material into electricity is low, the organic material in dairy wastewater is abundant and free and the aim of treating wastewater can be achieved along with electricity production or other processes [16,56]. These cells can be used as a new technology for biological treatment and electricity production simultaneously. Among the benefits of using these cells instead of the routine systems for wastewater treatment are electricity production, in the necessity of aeration of the air–cathode system, decrease in sludge production in comparison to aerobic systems and the possibility of stench control [31,32].

Among the other parameters measured in assessing the function of MFC as a new technology in wastewater treatment was ammonia. As it can be seen in Fig. 11, ammonia decreased by 76.12% and this high removal rate of ammonia was astonishing. This ammonia removal efficiency was achieved at OLR equal to 35.48 kgCOD/m^3 d and is attributed to biofilm activity, biological processes such as denitrification, anaerobic oxygenation, physicochemical processes and some other methods that previously have not been seen in the oxidation of ammonia with electricity production [26,57,58]. Ammonium is a common problem in many industrial wastewaters especially for those that contain high concentrations of organic nitrogen. As it can be seen during the anaerobic degradation process, a considerable part of organic nitrogen is converted to ammonium [59]. In this study the concentrations of ammonium in wastewater in comparison to the inlet wastewater decreased to 69.43% (Fig. 11). These amounts of ammonium decrease are attributed to the transfer of ammonium through the membrane to the cathode and its evaporation. Ammonium distribution through a membrane to the cathode chamber is mainly responsible for the decrease of ammonium in the anode because evaporation of high amounts of ammonium due to the relatively low pH of wastewater is difficult and blocks the anode chamber [60]. Min et al. in 2005 managed to remove high amounts of ammonium (83%) from animal wastewater by using single chamber MFCs with aerial cathodes [61]. In this study it was proven than decrease in ammonia in the single chamber MFC with aerial cathode and without membrane is mainly due to the evaporation of ammonium. The concentration of nitrate during these experiments increased from 4 to 7.8 mg/l. This increase in oxidized nitrogen may be due to nitrification, which is probably due to the penetration of oxygen through the cathode [62]. Seems like the increase in nitrate cannot improve the removal of ammonium nitrogen, however, other processes such as denitrification, which has an important role in degrading nitrogen in anaerobic conditions and anaerobic oxidation of ammonia similar to anammox that converts ammonium directly to nitrogen gas happens. In addition to confirming these results, the removal of ammonia nitrogen similar to the ammonia in MFC is the result of complicated biologic and physicochemical processes and there is a need for further studies in order to better understand the mechanisms of nitrogen removal and its conversion in MFC [59,63,64].

Phosphorus is another important parameter in treating wastewater. The total concentration of phosphorus in dairy wastewater is 187 mg/l and from this amount 21 mg/l is dissolved phosphorus and 166 mg/l of it is suspended solids. The average concentration of phosphorus in wastewater was 46 mg/l and its removal rate was 72.45% (Fig. 11). This removal rate for phosphorus was along with suspended solids and the concentration of dissolved phosphorus in wastewater on the initial days of MFC operation decreased to 31.18% and as time passed its ratio to influent wastewater increased. These amounts were achieved at OLR equal to 35.48 kgCOD/m^3 d. These results show that during the operation time phosphorus dissolved in suspended solids in the liquid phase and the amount of dissolved phosphorus increased. Also the other reason of this phenomenon is probably due to the low Redox potential that leads to stimulation of microorganisms for releasing phosphorus or converting the organic phosphorus in the wastewater to orthophosphate [65,66]. The concentration of sulfate in the effluent from CAML-MMFC was evaluated and the results are shown in Fig. 11. After 30 days operation, the highest rate of sulfate removal was 39.43% and was achieved at OLR equal to 53.22 kgCOD/m^3 d. Some of the microorganisms in sludge and wastewater have the capability to use sulfate in wastewater as the eventual receiver of electrons and after that sulfate is reduced to sulfide [23,39]. Sulfide is electrochemically active

at the anode and is oxidized on the electrode surface and loses its electrons and is reduced again to sulfate and this causes the low removal of the sulfate ion. This type of fuel cell has a higher current density in comparison to other types and does not need other heterogeneous mediators, because Redox sulfate or sulfides act as a mediator [32,33].

Decrease in the TSS (70.17%) and VSS was 64.4% and was achieved at OLR equal to 88.7 kg COD/m^3 d and shows that microorganisms use it and the wastewater is being treated (Fig. 12). One probable reason is the degradation of colloidal and complex organic material due to the biological catalysis process. The results of these studies are in accordance with findings of Mansoorian et al. in 2013, about bioelectricity generation using two chamber microbial fuel cell treating wastewater from food processing [67]. However, several studies have to be conducted in order to understand the mechanism of removal of these parameters through this technology. The increase in current conductivity of the anode samples after performing the experiments was 11%, which may be due to the presence of electrochemically active microorganisms. By continuous operation of MFC there is a possibility to increase the efficiency of wastewater treatment along with an increase in electricity production. In MFC technology the current efficiency is still relatively low. There are also limitations in its application for treating wastewater and this technology is still not ready for marketing [59,68]. According to the voltage difference between the electron provider and receiver, the maximum expected voltage in MFC was 1.13 V, which was higher than the 0.856 V produced in this study. The current intensity efficiency and power density are less than what is theoretically expected. In order to make MFCs capable of treating wastewater the production and operation costs must decrease. Also the electron transfer rate should be improved, that this may be possible by choosing an appropriate compatible endophilic microbial population and optimizing the operation conditions. In this system higher voltages may require stronger wastewater [8,63]. Using wastewater as an electrolyte in MFC due to its lower cost in comparison to ferricyanide may be effective as an electrolyte. Chemical or microbial analysis of wastewater can help us in understanding the microbial reactions that happen in the cathode and their role in increasing current efficiency [33,66].

5. Conclusions

In this study for the first time electricity production directly from the dairy industry waste water and simultaneously treating it with CAML-MMFC technology has been shown. The maximum current intensity and power density production were achieved respectively at 3.74 mA and 621.13 mW/m^2 on the anode surface. The maximum voltage produced was 0.856 V and the maximum coulombic efficiency was 37.16%. The maximum removal efficiency for COD, BOD_5, NH_3, NH_4^+, dissolved phosphorus, phosphorus in suspended solids, SO_4^{2-}, TSS and VSS was achieved at 90.46%, 81.72%, 73.22%, 69.43%, 31.18%, 72.45%, 39.43%, 70.17% and 64.6% respectively. Also, this study proved that MFC function is affected by many factors including oxidation rate, transfer of electrons to the electrode by microorganisms, the OLR, the nature of the carbon source under use, good biofilm formation, the nature of the proton exchange membrane, proton transfer through the membrane to the cathode chamber, oxygen supply on the cathode, the nature and kind of electrodes, the operating temperature, pH and retention time.

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