Bioelectricity and Biohydrogen Production Using High Solid Content of Oily-Kitchen Wastes in Air Cathode Microbial Fuel Cells

Kafaei, Raheleh
Department of Environmental Health Engineering, School of Public Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, I.R. IRAN

Yazdanbakhsh, Ahmad Reza
Workplace Health Promotion Research Center, Shahid Beheshti University of Medical Sciences, Tehran, I.R. IRAN

Sadani, Mohsen; Alavi Nadali*[+•]
Department of Environmental Health Engineering, School of Public Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, I.R. IRAN

ABSTRACT: *For the first time ability of a microbial fuel cell (MFC) to produce value-added products from the high content of oily kitchen waste was evaluated. A Single-chamber, air-cathode MFCs containing 30% solids was designed for evaluation of the rate of biohydrogen and bioelectricity production. Food wastes were studied in four states: oil-free (0%), containing 3% oil, 6%, and 9% oil during 30 days of operation. Experiments showed with increasing the amount of oil, the amount of biohydrogen produced increased from 0 to 6% of the oil, and with the addition of 9% of oil, no significant change was observed in the biohydrogen production rate. The average daily production of biohydrogen for 0, 3, 6 and 9% of the oil was estimated at 42.5, 58.7, 69.6 and 70.1 mL per day, respectively, which showed that adding oil up to 6% could increase the efficiency of the system for biohydrogen production. On the other hand, with the increase in the amount of oil, the production of bioelectricity decreased, so that the maximum output voltage was recorded for the fourth day of zero state: 472 mV, and the lowest voltage on most of the days recorded for 9% of oil. The results of Chemical Oxygen Demand (COD) removal showed with increase in the amount of oil, although the amount of initial COD increased, the amount of COD removal decreased, which is consistent with the process of electricity production. Volatile fatty acids including acetate, butyrate, and propionate were other valuable products of the system, although the accumulation of VFA was indicated as an inhibitor for biohydrogen production The results showed kitchen waste without oil separation can be used as a useful substrate in MFC systems to produce value-added products, in this way, sewage pollution by oil resulted from food waste could be prevented*

KEYWORDS: *Biohydrogen; Bioelectricity; Microbial fuel cell; Food waste; Volatile fatty acids; Sustainable environment.*

***** *To whom correspondence should be addressed.*
+ E-mail: alavi@sbmu.ac.ir
• Other Address: Environmental and Occupational Hazards Control Research Center, Shahid Beheshti University of Medical Sciences, Tehran, I.R. IRAN
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INTRODUCTION

The increase in demand for energy, along with the decrease in fossil fuel resources, has made the extraction of clean energy from renewable sources a priority. Bioelectrochemical systems are emerging and promising green technologies whose mechanism is based on biomass conversion and the generation of beneficial output from liquid and solid wastes [1-3]. Microbial Fuel Cells (MFCs) are bioelectrochemical systems that oxidize organic substrates through electrochemically active microorganisms and release an electron. Electrons are transferred to an anodic surface to produce bioelectricity, then through an external circuit transferred to a cathode for H_2O_2 or H_2O production by oxygen reduction in a $2e^-$ or $4e^-$ pathway [4, 5]. In addition to the produced electricity, recovery of value-added products such as biohydrogen, bioethanol, and volatile fatty acids, less energy production, recyclability, pollutant degradation with less toxic products, and high efficiency, are other benefits of usage of MFCs [6-9].

Biohydrogen is a useful byproduct from MFCs that has received much attention as an energy source among sustainable biofuels due to its high calorific value, cleanliness, long storage capability, the possibility of use in both fixed and movable forms, and usability as a carrier gas [10, 11]. In MFCs, biohydrogen is produced through electron reduction by oxygen from a water molecule and in the presence of protons in the cathodic chamber. However, most of the biohydrogen production is related to the production of this gas in the acetogenesis of anaerobic fermentation of the substrate in the anode chamber. In the anaerobic conversion of biomass, larger molecules are first hydrolyzed and then converted into molecules accessible to bacteria, later fermented by acidogens and acetogens and converted to Volatile Fatty Acids (VFAs) and hydrogen [12, 13].

The behavior of microorganisms in MFCs includes complex processes. Mixed bacterial cultures often generate much higher power than a single bacterium. Different anodophilic bacteria from the families of Geobacteraceae, Alteromonadaceae Clostridiaceae Desulfuromonaceae, Aeromonadaceae Enterobacteriaceae, Pasteurellaceae, and Comamonadaceae cantransfer electrons to electrodes. Fe(III)-reducing bacteria are important electrogenic microorganisms in MFCs with a high energy conversion efficiency [14].

Among various organic substrates used for biofuel production through MFCs, food waste generated from edible food is a potential substrate due to its high content of energy components including carbohydrates, lipids, amino acids, and so on. On the other hand, converting food waste to energy could be a solution to the treatment and disposal of the huge amount of 1.3 billion tons per year of food waste estimated by the Food and Agriculture Organization (FAO) [15, 16]. Various studies have reported the use of food as a substrate to generate electricity from MFCs [17-19]. Whilst, during biological fermentation, 33% of the available energy is converted to hydrogen and biohydrogen could be considered a byproduct of MFCs [20].

In addition, 4 to 8% of the content of food waste is waste cooking oil, which is not a suitable method for their disposal, and discharging into the sewage collection network causes clogging of the sewage network and the spread of pollution to the environment [21-23]. The acquisition of gas and electricity from an oily substrate is well documented in various studies. *Badia-Fabregat et al.* reported crude oil as a useful substrate for biohydrogen production from microbial electrolysis cells [24]. Biodegradation of oil-contaminated sediments in a sediment microbial fuel cell was reported successfully by Aleman-Gama and the maximum power output measured 178 mW m^{-2} [25]. *Baranitharan et al.* treated palm oil mill effluent in a microbial fuel cell with simultaneous generation of the power density of about 54 mW m^{-2} [26]. However, when using food waste as a substrate, the food waste is pre-refined and the available oil is removed before entering the experiments. Anaerobic digestion [27] and land application [28] are some of the methods are used to treatment of oily food waste.

Although the performance of MFCs has improved in recent years, the low energy efficiency still hinders their widespread application on a real scale. In more recent studies, single-chamber MFCs without proton exchange membranes with air-cathode type cathodes have shown higher efficiency than double-chamber, especially for solid-phase MFCs [29, 30]. In these single-chamber systems, while the anode and cathode are placed close to each other to facilitate electron transfer, the anode is completely immersed in the substrate, but the cathode is selected from cathode-air electrodes, which are It is in contact with the substrate and is directly exposed to air on

the other side, and unlike two-chamber systems where oxygen is supplied through mechanical aeration, the oxygen supply in air-cathode systems is through the passage of atmospheric air through the pores of cathode [31].

In this study, we intend to design a series of experiments that use oil-containing food waste as a substrate to produce biohydrogen and bioelectricity through MFCs. Most MFC studies have been performed on liquid substrates [32-34] the solid phase has been less studied, and food substrates have been used after extensive dilution. Due to the difficulty of electron transfer in the solid phase substrate, the cell faces problems like high internal resistance, long operational time, and low energy efficiency. However, because solid wastes are rich in organic matter, a study on microbial cells operated in the solid phase seems valuable [35]. Therefore, the substrate used in this study was selected from oily food waste with a high content of solids. Accordingly, this study aimed to evaluate the potential of biohydrogen and bioelectricity production from oily food waste with a high content of solids through an air cathode of a single-chamber microbial fuel cell for the first time. In addition, the rate of COD removal and the production of volatile fatty acids was also examined.

The increased amount of oil showed a higher ability to the generation of biohydrogen in single-chamber air-cathode MFC. However increasing levels of oil reduced generated bioelectricity, and oily kitchen waste was able to produce bioelectricity in different oil concentrations.

EXPERIMENTAL SECTION

MFC construction

Four of the same single chamber cubic-shaped reactors air cathode MFCs, a net volume of 630 mL, were constructed and operated simultaneously. The anodes were made of carbon cloth and the cathode electrodes were made of carbon cloth containing platinum catalyst (0.5 mg/cm^2). The projected surface area of each anode and cathode was 25 cm^2 with a 3 cm spacing between the electrodes used in Plexiglas reactors. The external circuit between electrodes was connected through copper wire.

Matters and reagents

Food groups from four categories of bread and cereals, grains and meat, vegetables, and fruits were selected as substrates. Anaerobic sludge had been collected from the sludge thickening tank in the South wastewater treatment plant. Analytical grades of potassium dichromate, mercuric acid, sulfuric acid, ferroin, ferrous ammonium sulfate, sodium hydroxide-sodium thiosulfate, borate buffer, sodium carbonate, sulfuric acid, sodium bicarbonate were used to analysis of COD, nitrogen content and alkalinity from Merck Co.

Substrate preparation

After drying at 105°C for 24 h, the raw materials were powdered and then mixed in equal amounts. Waste of sunflower edible oil was prepared from a restaurant Oil was added to the food in proportions of 0, 3, 6, and 9% of the total weight of solids (W/W).In 162 g of the prepared composition, the amount of carbohydrate, protein, and fat of the composition according to the FAO [36] was estimated to be 42, 14.7, and 2.09 g, respectively. The amount of carbon, based on burning at 550°C for 20 min was measured as 50-55% of total solids. The nitrogen content was measured by equal to 1-2% of total solids. The C/N ratio of 1/35 was estimated to be the optimal condition for anaerobic bacteria to produce gas. Finally, 30% food containing 162 g solids with oil in proportions of 0, 3, 6, and 9% of oil, and 70% of 0.01 M phosphate buffer solution (PBS) (pH=7.4) containing 1.4g Na_2HPO_4, 0.2g NaH_2PO_4, and 8.5g NaCl were selected as input feedstock.

The alkalinity of the prepared matrix (input feedstock) was measured as 1100 mg/L, and to create suitable conditions for gas production in anaerobic conditions, alkalinity reached 3000 mg/L using 1 g of sodium bicarbonate and pH was adjusted to 7.2.

To break the compounds with low degradability, the matrix was incubated at 15°C for 7 days and then transferred to the reactor.

MFC start-up and operation

All bioreactors were inoculated with anaerobic sludge. Before inoculation sludge was filtered to acquire a homogenous solution and was heated for 15 min at 100 °C to enrich H_2-producing bacteria. 12.5 mL/L trace minerals and 10 mL/L vitamins (Wolfe's vitamin solution and mineral solution) were added to 100 mL sludge. The exact value of 2 g sodium acetate (which generated the COD value of 1560 mg/L) was used as a source of the substrate. The matrix was sparged with nitrogen gas for 10 min to remove oxygen and exposed to the anode. After starting

the work, with the current dropping below 80 mV, the anode was re-fed until the maximum voltage obtained was repeated in at least 3 consecutive cycles. After this step, the anode was ready for operation. The reactor was operated at laboratory temperature (25 °C). The duration of operation of each run was equal to 1 month. The experiments were repeated 3 times.

Analysis

The output voltage was measured in millivolts using a multimeter. The amount of gas produced was measured daily using the water displacement method. The volume of water displaced in the column after connecting the gas bag reflected the volume of biogas produced. The composition of the produced gas, including biohydrogen, was determined using a Gas Chromatography (GC) device equipped with a thermal conductivity detector (Extended Agilent Fast Refinery Gas Analyzer, Agilent 7890A). Samples were brought to atmospheric pressure before injection. Sample collection container connected to inlet tube. Gas flowed from the collection tube through the valve to purge dead air space and fill the sample tube. About 15 mL normally is sufficient to clear the lines and to provide a sample of 1 to 2 mL. The sample transferred from the loop into the carrier gas stream (#2720) [37].

Volatile fatty acids in the initial feed as well as at the end of each run were measured using a GC device equipped with a flame ionization detector (Varian 3800). Effluent samples were kept at 4°C and 30 mL of sample was transferred to a glass vial and acidified to pH 2 with phosphoric acid. The acidified sample was centrifuged into a polycarbonate tube until the center was separated from the supernatant. The supernatant was placed in a 3.5 mL glass vial. And 1 µL of the sample was injected for analysis. For each set of 10 samples, at least one blank sample was analyzed (#5560) [37].

COD was measured using the 5220-Standard Method with the Open Reflux Method at the beginning and end of each run. A portion of the sample was diluted to 50 mL. 1 g $HgSO_4$ and 5 mL sulfuric acid were added. 25 mL potassium dichromate was added and the mixture was refluxed for 2 hours [37]. The percentage of COD removal was calculated as follows:

$$COD\ Removal\ \% = \frac{COD_0 - COD_e}{COD_0} \times 100$$

COD_0 = influent COD (mg/l)

COD_e = effluent COD (mg/l)

Nitrogen content was determined according to the standard method (Norg-4500). 10 mg of sample homogenized and after digestion through sulfuric acid measured by titration [37]. Alkalinity was measured using Standard Method (#2320) by titration method [37].

RESULTS AND DISCUSSION

Biohydrogen production

Figure 1 shows the amount of hydrogen produced during the operation period daily. The reason for choosing these 4 weeks for operation is related to the high concentration of the substrate that makes the reaction time longer, although it does not affect the efficiency [38].

As shown in Fig. 1, for all matrices, the process of hydrogen production starts on the second day and increases rapidly. In the first week, the process reached the maximum amount of biohydrogen production, and then until the end of the period, the cycle of reduction-increase was encountered frequently. This indicates gas production in the first days of fermentation and the passage of time and excessive consumption of food (substrate) have reduced the efficiency of gas production. The very high start of gas production in the microbial fuel cell also indicates the high performance of gas production by the biomass inside the cell [39]. In *Chookaew et al.* study also maximum hydrogen production during dark fermentation occurred at the first hours of operation due to the abundance of nutrients at first and the consumption of hydrogen by methanogens at the final [40].

According to Fig. 1, the lowest amount of biohydrogen produced belongs to the oil-free matrix (figure 1a), and by increasing the amount of oil to 6% (figure 1c), the amount of biohydrogen produced also increases, and then by adding the amount of oil up to 9% (figure 1d), there was no difference in the amount of biohydrogen produced.

As can be seen, with increasing the amount of oil, the time to reach the process of reducing the production of biohydrogen is also delayed, so that for the matrix without oil after the third day the reduction process begins, and for matrices containing 3 and 6% oil was observed after the sixth day.

For the oil-free matrix (figure 1a), an average of 11 mL of biohydrogen was observed on the second day, and on the third day, it reached its maximum production level of 58 mL and continued to decrease. However, compared

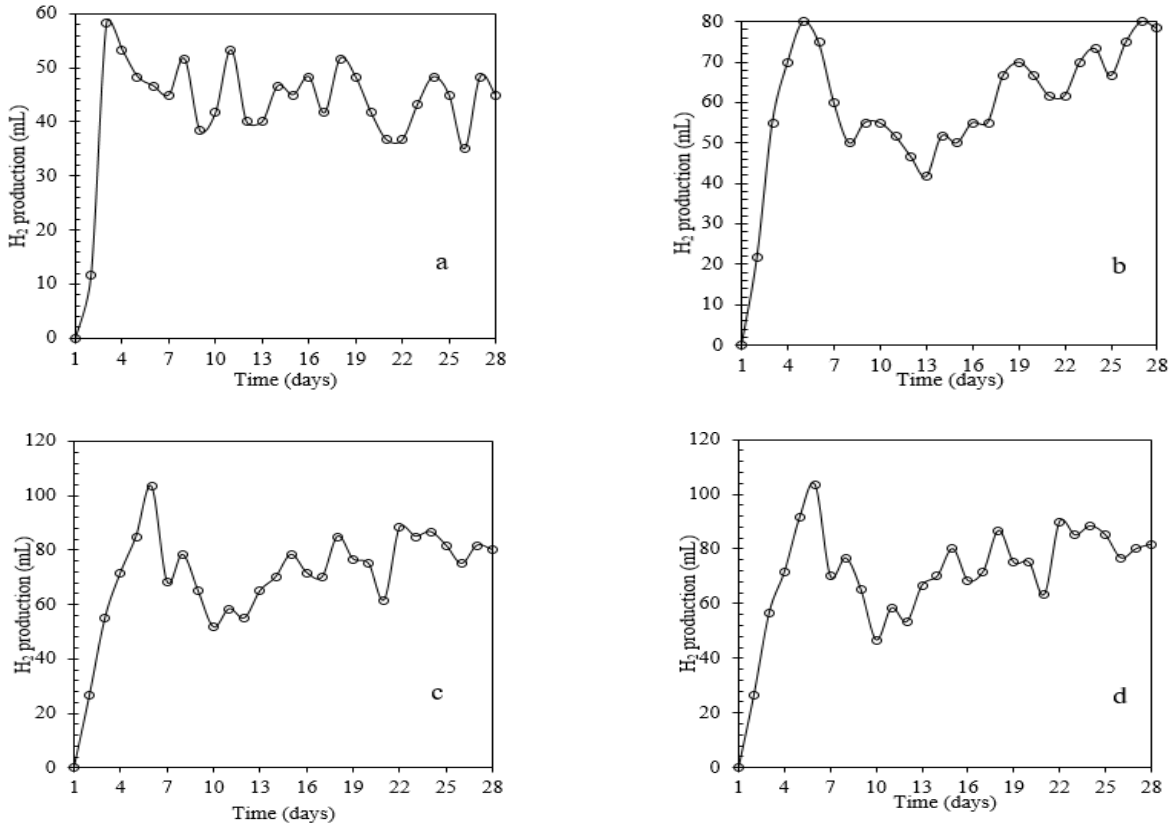

Fig. 1: Daily H_2 production rate (a) without oil, (b) 3% oil, (c) 6% oil, and (d) 9% oil.

to the others, it showed a more uniform trend, and the amount of biohydrogen produced varied in the range of 34-53 mL per day.

For the matrix containing 3% oil (Fig. 1b), the maximum biohydrogen production was obtained on the fifth day with an average of 80 mL, and then faced a decreasing trend from the eleventh day, and reached its minimum level of 41 mL on the thirteenth day, which compared to the amount the maximum was reduced by half. After that, the production process increased and reached its maximum in the last days of operation.

As can be seen in the figure (figure 1c,d), the graphs containing 6% and 9% are tangential to each other at most points, and the amount of produced biohydrogen on most days was measured close to each other and more than the other two matrices. This means that adding oil up to 6% increases the production of biohydrogen, but then adding oil has no effect on the amount of biohydrogen produced. The maximum amount of biohydrogen produced for both matrices was observed on the sixth day and was equal to 103 mL, which was the highest amount of biohydrogen produced among the studied matrices. After reaching the maximum level, these two matrices also experienced a decreasing trend and reached their minimum level and less than half of the maximum level on the tenth day. After that, the process of increasing biohydrogen production was observed and from the 22nd day onwards, it reached close to the maximum production of the first days.

According to our observations, the process of hydrogen production is a function of oil hydrolysis. In this way, the matrix without oil reached its maximum hydrogen production faster than other matrices and continued to show a more uniform trend than other matrices. The delay of other matrices to reach the maximum voltage indicates that longer oil hydrolysis time affects hydrogen production and in the whole process showed more fluctuations than the oil-free matrix. Also, matrices containing 6 and 9% of oil showed more hydrogen production than other matrices, which indicates an increase in hydrogen production due to the increase in oil content.

In the study of *Meng et al.*, in the process of anaerobic digestion, gas production from oil wastes was reported to be more than from food wastes, although the digestion time was longer [41]. An oil or fat subjected to anaerobic digestion is first hydrolyzed to glycerol and long-chain free fatty acids (LCFAs). LCFAs have been reported to be the main mediators of the oil breakdown process and are subsequently converted to acetate and hydrogen by acetogenic bacteria. Theoretically, oil produces more gas than other waste products such as carbohydrates and proteins, and is sometimes added to anaerobic digesters to produce more biomethane. Oil hydrolysis is a limiting step in anaerobic digestion [41].

It should be noted that the main product of MFCs is bioelectricity and biohydrogen is considered a byproduct of the process, so the reporting of biohydrogen extraction results from MFCs is very limited. One of the few studies in this field is the study of *Chandrasekhar et al.*, who reported the cumulative amount of biohydrogen produced from an oil-free food substrate in a single-chamber MFC at the end of 30 days of 5400 mL [42].

Besides hydrogen, carbon dioxide was other main gas that was produced during the operation of the system. Generally, it was observed that oily kitchen waste can serve as a substrate of MFC for biohydrogen production and generation of clean gas.

COD removal

Since the organic and carbon parts of the feed act as an electron-producing factor in the anode chamber and in addition to the decomposition of the substrate, causes the production of bioelectricity, the COD changes of the cell are measured (Fig. 2). In our study, the rate of COD removal at the end of the process was measured relative to the inlet COD concentration, because in fed-batch reactors, sampling during the period and returning the solution to the chamber, due to disturbing operating conditions such as air entering the chamber, will affect the performance of the reactor [43].

The initial COD of our study in oil-free foods was 55.700 mg/L, which increased to 66900, 82533.33, and 107466.66 mg/L, respectively, with an increase in oil values of 3, 6, and 9%. Our data were consistent with the values reported in the literature. *Liu et al.* reported the COD of food containing oil, 70000 mg/L [44], and *Wu et al.* measured the amount of COD of food waste containing

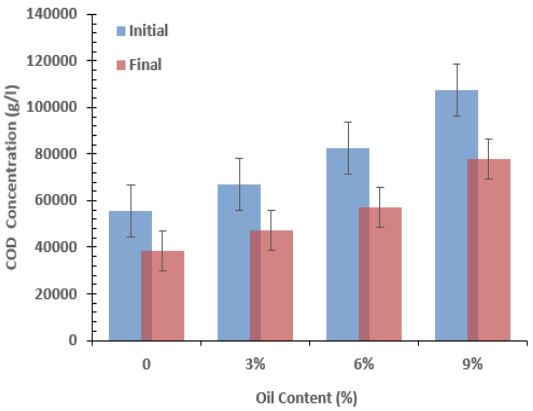

Fig. 2. The COD value at the start and end of the process for different studied conditions (without oil, 3% oil, 6% oil, 9% oil).

14 g/L of fat, about 101 g/L [27]. However, the rate of COD removal for different matrices showed little difference, and for oil-free matrices, 3, 6, and 9% oil, 31, 29, 31, and 27% were read, respectively. both biohydrogen and bioelectricity production are affected by microbial metabolism that is paused by a high concentration of substrate, although COD increase due to an increase of oil content could be effective on the amount of biohydrogen production [45].

It should also be noted that the initial concentration of the substrate of more than 20 g COD /L may reduce hydrogen production, so the low amount of biohydrogen obtained can correspond to the high load of COD [46]. On the other hand, if the bioelectricity decreases with increasing COD removal (increasing the COD removal in the matrix of 6% oil compared to 3% versus decreasing the generated electricity), this could be related to biohydrogen production, which is consistent with our study [26].

Compared to studies in MFCs with high solids content, our results were almost close to the study of *Lee et al.* who reported a COD reduction of about 40% during 40 days in an MFC containing 24.5 g/L solids and COD of livestock waste from 7000 mg/L reached 4000 mg/L. According to these researches, hydrolysis of cattle manure particles leads to an initial increase in COD, which eventually turns into VFA [29].

However, compared to other similar studies, a lower rate of COD removal was obtained in our study. In the study on MFC with a ratio of 270 mL solids to 30 mL water, the initial COD of the inlet food before oil removal (oil content 38 g/L) was measured at 380 g/L, which on 30^{th} day reached

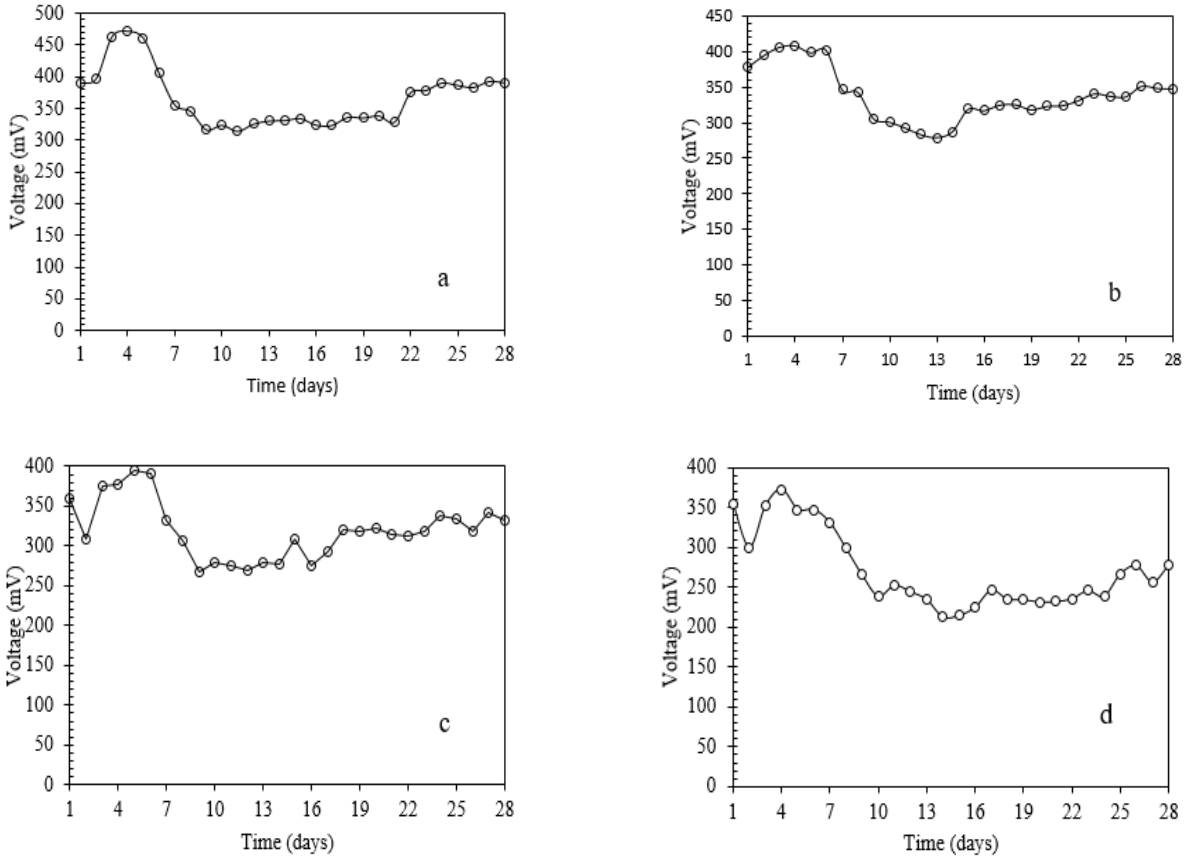

Fig. 3: Daily produced voltage (mV) (a) without oil, (b) 3% oil, (c) 6% oil, (d) 9% oil..

30th day reached 106 g/L and showed a 72% removal [42]. In another investigation, in an MFC containing 450 mL of oil-free food and 50 mL of water, at the end of 29 days of operation, 73% removal of COD was reported [47].

The most important point in COD removal efficiency is that the rate of COD removal in MFC is affected by the microbial growth rate and the surface of the electrodes, especially the anode, and the removal rate increases with increasing electrode surface [43].

Bioelectricity production

According to Fig. 3, the process of bioelectricity production was observed in contrast to the process of biohydrogen production. In other words, with increasing the amount of oil, the amount of produced bioelectricity decreased. On most days, the maximum voltage read belonged to the oil-free matrix (Fig. 3 a) and the minimum to the matrix containing 9% oil (Fig. 3 d). From the second day onwards, the bioelectricity production process increased rapidly and the maximum output voltage was measured in the first week of the reaction. The plausible reason for this high power in the first days is due to the availability of sugars and feed in a suitable amount around the anode surface space [48, 49]. After that, until the last week of the reaction, a steady trend was recorded in almost all cases. This constant trend in voltage for the middle days can be due to the less availability of simple monomeric sugars and the poor performance of the electrochemically active biocatalyst [50].

In general, the relationship between voltage generation and the electrical power of a microbial fuel cell can be related to the amount of carbon source (sugar) consumed within the cell. Whenever the carbon source is consumed and a small amount of it remains, the amount of output voltage of the cell will decrease and eventually remain constant, this has been proven for all cases studied in this study [51]. Also, based on the results, it can be seen that the amount of increase in oil content inside the MFC has the opposite effect on producing the output voltage of the cell. As the oil content increased, the output voltage of the cell

decreased, and at 9% content, the lowest output voltage of the cell was recorded during the 28 days of the process. This may be because the oil content prevents the carbon source (sugar) content from reaching the anode surface, which reduces the consumption of sugar and, as a result, reduces the voltage produced by the MFC [51-53]

The concentration of COD and the amount of bioelectricity produced showed an opposite trend and with increasing the concentration of COD, a decrease in output voltage was observed. The reason for this has been attributed to the fact that higher concentrations of the substrate may inhibit microbial growth and distort the metabolism of microorganisms [45].

Various studies have investigated the generation of electricity from the oil substrate in MFCs, which have reported different efficiencies depending on the oil concentration and operating conditions. In a study by *Majumder et al*. on a single-chamber MFC using a substrate taken from the wastewater of an oil refinery containing 2,213 mg/L COD, a maximum voltage of 355 mV was reported [54]. *Srikanth et al*. studied the generation of electricity from petrochemical wastewater containing 720 mg/L oil and fat and 1040 mg/L COD in a single-chamber MFC and reported a maximum output voltage of 318 mV in the batch system [55]. The results of a study by *Guo et al.* on a sample of petroleum sludge containing 40% oil showed that this sludge in a single-chamber MFC is capable of producing 299 mV [56]. Therefore, it can be concluded that by optimizing the test conditions, oily substrates can be considered a source of electricity generation. In studies conducted on food waste, *Chandrasekhar et al*. in a single-chamber MFC reported maximum voltage on the 9th day of about 443 mV, produced from food waste with oil separation that indicated the inhibitory effect of oil on bioelectricity production in our study [42]. In the MFC fed with food waste studied by *Asefi et al*. immediate generation of bioelectricity was observed in the early days and stable voltage production was obtained in the third month. It was close to our results however maximum voltage reported by their study was 600 mV which was higher than our data. Similar to our study they reported a gradual decrease in voltage due to the depletion of nutrients in the anodic chamber [18].

Volatile fatty acids

VFAs are important intermediates with 6 carbon atoms or less, and the major end products of the solution include acetate, butyrate, and propionate. Biological wastes, such as food, are converted to VFAs through acid fermentation processes. VFAs are then oxidized in the MFC by biofilm grown on the anode to generate electricity [57]. VFAs are also commonly used as indicators for monitoring the dark fermentation process of H_2 production [42]. The composition of VFAs during the hydrogen fermentation process depends on operating conditions such as pH, hydraulic retention time, type, and concentration of substrate and microorganisms [58].

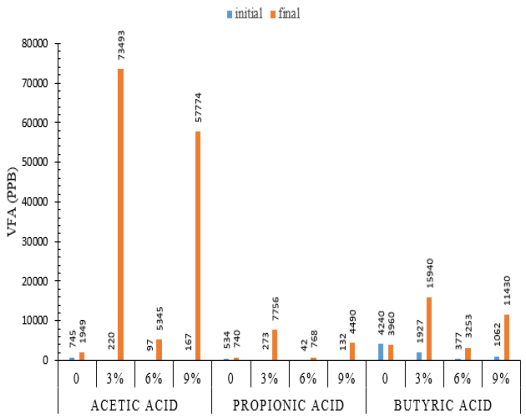

Fig. 4: VFA values (ppb) at the start and end of the operation.

As shown in Fig. 4, the concentration of input VFA compounds in the oil-free matrix was measured higher than in the oil-containing samples, but at the end of the process, the concentration of these compounds in the oil-free matrix was read less than in the other samples and reflected lower VFA production. In the end, except for the amount of acetate, which had almost doubled growth, the concentration of butyrate and propionate was observed close to the initial concentration.

With the addition of oil, the initial concentration of VFA compounds decreased to a minimum of 6% but slightly increased at 9%. Therefore, the decreasing trend of initial concentrations of acetic acid, propionic acid, and butyric acid was ranked as follows: without oil> 3% oil> 9% oil> 6% oil. This trend was also observed for the final concentration of compounds in the samples containing oil and the concentrations of acetate, butyrate, and propionate in the sample containing 3% oil had the highest, and the samples containing 6% oil had the lowest values, and therefore with the values initial showed compliance (except free oil case).

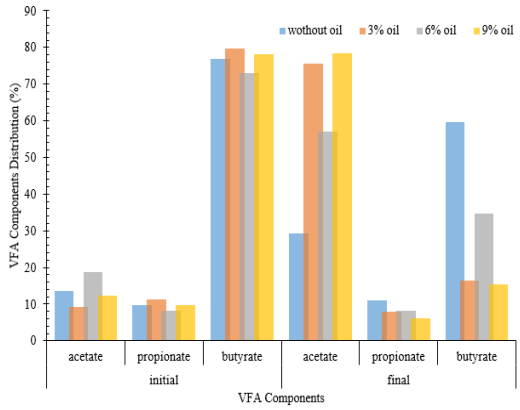

Fig. 5: VFAcompounds distribution (%) in input and output samples.

Fig. 5 shows the distribution of acetate, butyrate, and propionate compounds in the studied samples. As we can see, the percentage of constituents, especially in samples containing oil, shows a uniform distribution. In all input samples, acetate accounted for 9-19%, propionate for 8-11%, and butyrate for 73-80% of the total. In the output samples (containing oil) the distribution of compounds was similar and the acetate value was 57-78%, propionate 6-8% and butyrate accounted for 15-36% of the total composition. Therefore, a decreasing trend of concentration was observed in input samples as butyrate> acetate> propionate, in output samples containing oil as acetate> butyrate> propionate, and in output samples without oil as butyrate> acetate> propionate, respectively.

According to the literature, the compounds produced in the fermentation process at pH 4 are mainly hydrogen, acetate, and butyrate [59]. In the *Chandrasekhar et al*. study, the intrinsic VFA of the tested food containing oil was measured at 8.4 g/L. In this study, the initial and final concentrations of acetic acid and butyric acid were measured at 2,000 and 8,500 mg/L and 2,500 and 14,500 mg/L, respectively, which was much higher than the values measured in our study. According to their study, the accumulation of volatile fatty acids reduced the pH and thus reduced the efficiency of hydrogen production in the last few days [42].

As we see in our study, for matrixes containing oil, with increasing of oil from 3 to 6%, VFA content (acetate, propionate, and butyrate) was decreased, then increased in 9% oil was exactly versus the trend of biohydrogen production. It indicated that the accumulation of VFA prevented the production of biohydrogen.

pH variations

According to the diagram drawn in Figure 6, although the pH was set at 7.2 at the beginning of the process, with the start of the process, a decrease in the pH was observed and after the first week to its minimum level in the range of 4 to 4.5 for different matrices were read. At these points of maximum pH drop, minimum voltage and biohydrogen production were measured. Changes in pH during the process of microbial fuel cell function indicate the metabolic activity of biocatalysts within the cell [42]. Decreasing the pH value at any point indicates a decrease in the gas and voltage produced during the process [60]. It in was accordance with our observations on the middle daysof the operation. During the process, in the first week, a decrease in pH is observed, which indicates a rapid metabolic function in the microbial fuel cell. Stable pH in the middle weeks indicates feed inactivity and low gas production during this period [61].

In this study we evaluated biohydrogen and bioelectricity production ate from oily kitchen waste in a single-chamber MFC. Beside of oil content, the effect of pH variation and VFA generation was studied on the efficiency of MFC. It was indicated that various factors can affect on biohydrogen and bioelectricity generation and if they control can make oily waste as a potential substrate for MFCs.

The strength of this work was the simultaneous production of biohydrogen and bioelectricity in a single-chamber air-cathode MFC from a high content of solid substrate that could be a solution to the treatment of solid waste with the acquisition of clean energy. But the high cost of construction and low efficiency of the reactor has hindered commercialization and use of MFCs on a real scale. It seems that by simplifying the structure of the reactor using cheap materials in the construction, and optimizing the operating conditions to be more productive at a lower cost, MFCs are the main tools to achieve clean energy and waste treatment in the future world.

CONCLUSIONS

The ability of biohydrogen and bioelectricity from food waste substrate containing oil was examined in a single-chamber air-cathode MFC. Biohydrogen production increased with an increase in oil content but the trend of bioelectricity generation was the opposite and decreased with the increase in oil content. An increasing amount

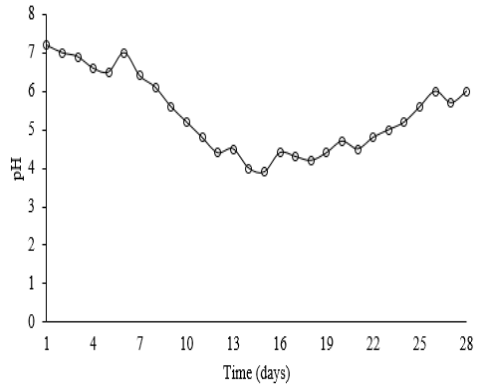

Fig. 6: pH variation during operation time.

of oil until 6% increased the rate of biohydrogen production and at 9% we didn't see an obvious change in the generation of biohydrogen. Bioelectricity production was affected by the content of input COD and the higher content of oil prevented anodic the activity of microorganisms that were responsible for bioelectricity production. Also, pH variation was an important factor in determining of MFC efficiency. In optimized conditions, biohydrogen and bioelectricity production rates were higher than 100 mL and 400 mV, respectively. Volatile fatty acids including acetic acid, propionic acid, and butyric acid were valuable by-products that were generated during the operation of the reactor. We showed in this study that kitchen waste without oil separation could be a proper substrate for biohydrogen and bioelectricity production in MFCs, also using food waste in this way could save sewage from oil pollution distributed by kitchens.

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REFERENCES

[1] Pasupuleti S.B., Srikanth S., Venkata Mohan S., Pant D., Development of Exoelectrogenic Bioanode and Study on Feasibility of Hydrogen Production Using Abiotic VITO-CoRE™ and VITO-CASE™ Electrodes in A Single Chamber Microbial Electrolysis Cell (MEC) at Low Current Densities, *Bioresour. Technol.*, **195**: 131-138 (2015).

[2] Sekar N., Ramasamy R.P., Electrochemical İmpedance Spectroscopy for Microbial Fuel Cell Characterization, *J Microb Biochem Technol S*, **6**: 1-14 (2013).

[3] Palanisamy G., Jung H.-Y., Sadhasivam T., Kurkuri M.D., Kim S.C., Roh S.-H., A comprehensive Review on Microbial Fuel Cell Technologies: Processes, Utilization, and Advanced Developments in Electrodes and Membranes, *J. Cleaner Prod.*, **221**: 598-621 (2019).

[4] Zhang Y., Liu M., Zhou M., Yang H., Liang L., Gu T., Microbial fuel Cell Hybrid Systems For Wastewater Treatment and Bioenergy Production: Synergistic Effects, Mechanisms and Challenges, *Renewable Sustainable Energy Rev.*, **103**: 13-29 (2019).

[5] Li M., Zhou M., Tian X., Tan C., McDaniel C.T., Hassett D.J., Gu T., Microbial Fuel Cell (MFC) Power Performance İmprovement Through Enhanced Microbial Electrogenicity, *Biotechnol. Adv.*, **36**: 1316-1327 (2018).

[6] Wu Q., Jiao S., Ma M., Peng S., Microbial Fuel Cell System: A Promising Technology for Pollutant Removal And Environmental Remediation, *Environ. Sci. Pollut. Res.*, **27**: 6749-6764 (2020).

[7] Munoz-Cupa C., Hu Y., Xu C., Bassi A., An Overview of Microbial Fuel Cell Usage in Wastewater Treatment, Resource Recovery and Energy Production, *ScTEn*, **754**: 142429 (2021).

[8] Lakshmidevi R., Nagendra Gandhi N., Muthukumar K., Bioelectricity and Bioactive Compound Productionin an Algal-Assisted Microbial Fuel Cell with Immobilized Bioanode, *Biomass Convers. Biorefin.*, (2020).

[9] Asunis F., De Gioannis G., Dessì P., Isipato M., Lens P.N.L., Muntoni A., Polettini A., Pomi R., Rossi A., Spiga D., The Dairy Biorefinery: Integrating Treatment Processes for Cheese Whey Valorisation, *J. Environ. Manage.*, **276**:111240 (2020).

[10] Lu C., Li W., Zhang Q., Liu L., Zhang N., Qu B., Yang X., Xu R., Chen J., Sun Y., Enhancing Photo-Fermentation Biohydrogen Production by Strengthening the Beneficial Metabolic Products with Catalysts, *J. Cleaner Prod.*, **317**:128437 (2021).

[11] Mona S., Kumar S.S., Kumar V., Parveen K., Saini N., Deepak B., Pugazhendhi A., Green Technology for Sustainable Biohydrogen Production (Waste to Energy): A Review, *ScTEn*, **728**: 138481 (2020).

[12] Ferraren-De Cagalitan D.D.T., Abundo M.L.S., A Review of Biohydrogen Production Technology for Application Towards Hydrogen Fuel Cells, *Renewable Sustainable Energy Rev.*, **151**: 111413 (2021).

[13] Baeyens J., Zhang H., Nie J., Appels L., Dewil R., Ansart R., Deng Y., Reviewing the Potential of Bio-Hydrogen Production by Fermentation, *Renewable Sustainable Energy Rev.*, **131**: 110023 (2020).

[14] Scott K., Yu E.H., Microbial Electrochemical and Fuel Cells: Fundamentals and Applications, Woodhead Publishing, (2015).

[15] Hou Q., Yang Z., Chen S., Pei H., Using an Anaerobic Digestion Tank as the Anodic Chamber of an Algae-Assisted Microbial Fuel Cell to İmprove Energy Production from Food Waste, *Water Res.*, **170**: 115305 (2020).

[16] Srivastava N., Srivastava M., Abd_Allah E.F., Singh R., Hashem A., Gupta V.K., Biohydrogen Production Using Kitchen Waste as the Potential Substrate: A Sustainable Approach, *Chemosphere*, **271**: 129537 (2021).

[17] Yaqoob A.A., Bin Abu Bakar M.A., Kim H.-C., Ahmad A., Alshammari M.B., Yaakop A.S., Oxidation of food Waste as an Organic Substrate in a Single Chamber Microbial Fuel Cell to Remove the Pollutant with Energy Generation, *Sustain. Energy Technol. Assess.*, **52**: 102282 (2022).

[18] Asefi B., Li S.-L., Moreno H.A., Sanchez-Torres V., Hu A., Li J., Yu C.-P., Characterization of Electricity Production and Microbial Community of Food Waste-Fed Microbial Fuel Cells, *Process Saf. Environ. Prot.*, **125**: 83-91 (2019).

[19] Tremouli A., Karydogiannis I., Pandis P.K., Papadopoulou K., Argirusis C., Stathopoulos V.N., Lyberatos G., Bioelectricity Production from Fermentable Household Waste Extract Using a Single Chamber Microbial Fuel Cell, *Energy Procedia*, **161**: 2-9 (2019).

[20] Venkata Mohan S., Lenin Babu M., Dehydrogenase Activity in Association with Poised Potential During Biohydrogen Production in Single Chamber Microbial Electrolysis Cell, *Bioresour. Technol.*, **102**: 8457-8465 (2011).

[21] Huang H., Qureshi N., Chen M.-H., Liu W., Singh V., Ethanol Production from Food Waste at High Solids Content with Vacuum Recovery Technology, *J. Agric. Food. Chem.*, **63**: 2760-2766 (2015).

[22] Chen Y.-C., Hsu Y.-C., Wang C.-T., Effects of Storage Environment on the Moisture Content and Microbial Growth of Food Waste, *J. Environ. Manage.*, **214**: 192-196 (2018).

[23] Reddy M.V., Chandrasekhar K., Mohan S.V., Influence of Carbohydrates and Proteins Concentration on Fermentative Hydrogen Production Using Canteen Based Waste under Acidophilic Microenvironment, *J. Biotechnol.*, **155**: 387-395 (2011).

[24] Badia-Fabregat M., Rago L., Baeza J.A., Guisasola A., Hydrogen Production From Crude Glycerol in an Alkaline Microbial Electrolysis Cell, *IJHE*, **44**: 17204-17213 (2019).

[25] Aleman-Gama E., Cornejo-Martell A.J., Ortega-Martínez A., Kamaraj S.K., Juárez K., Silva-Martínez S., Alvarez-Gallegos A., Oil-Contaminated Sediment Amended with Chitin Enhances Power Production by Minimizing the Sediment Microbial Fuel Cell Internal Resistance, *J. Electroanal. Chem.*, **894**: 115365 (2021).

[26] Baranitharan E., Khan M.R., Prasad D.M.R., Salihon J.B., Bioelectricity Generation from Palm Oil Mill Effluent in Microbial Fuel Cell Using Polacrylonitrile Carbon Felt as Electrode, *Water Air Soil Pollut.*, **224**:1533 (2013).

[27] Wu L.-J., Kobayashi T., Li Y.-Y., Xu K.-Q., Comparison of Single-Stage and Temperature-Phased Two-Stage Anaerobic Digestion of Oily Food Waste, *Energy Convers. Manage.*, **106**: 1174-1182 (2015).

[28] Rashid M.T., Voroney R.P., Land Application of Oily Food Waste and Corn Production on Amended Soils, *Agron. J.*, **96**: 997-1004 (2004).

[29] Lee Y., Nirmalakhandan N., Electricity Production in Membrane-Less Microbial Fuel Cell Fed with Livestock Organic Solid Waste, *Bioresour. Technol.*, **102**:5 831-5835 (2011).

[30] Florio C., Nastro R.A., Flagiello F., Minutillo M., Pirozzi D., Pasquale V., Ausiello A., Toscano G., Jannelli E., Dumontet S., Biohydrogen Production from Solid Phase-Microbial Fuel Cell Spent Substrate: A Preliminary Study, *J. Cleaner Prod.*, **227**: 506-511 (2019).

[31] Rathinavel L., Jothinathan D., Sivasankar V., Agastian P., Mylsamy P., Algal Microbial Fuel Cells—Nature's Perpetual Energy Resource, in: Sivasankar V., Mylsamy P., Omine K. (Eds.) Microbial Fuel Cell Technology for Bioelectricity, Springer International Publishing, *Cham*, 81-116 (2018).

[32] Choudhury P., Ray R.N., Bandyopadhyay T.K., Basak B., Muthuraj M., Bhunia B., Process Engineering for Stable Power Recovery from Dairy Wastewater Using Microbial Fuel Cell, *IJHE*, **46**: 3171-3182 (2021).

[33] Walter X.A., Madrid E., Gajda I., Greenman J., Ieropoulos I., Microbial Fuel Cell Scale-Up Options: Performance Evaluation of Membrane (c-MFC) and Membrane-less (s-MFC) Systems under Different Feeding Regimes, *JPS*, **520**: 230875 (2022).

[34] Jiang J., Wang H., Zhang S., Li S., Zeng W., Li F., The Influence of External Resistance on the Performance of Microbial Fuel Cell and the Removal of Sulfamethoxazole Wastewater, *Bioresour. Technol.*, **336**: 125308 (2021).

[35] Wang C.-T., Liao F.-Y., Liu K.-S., Electrical Analysis of Compost Solid Phase Microbial Fuel Cell, *IJHE*, **38**: 11124-11130 (2013).

[36] Chatfield C., Food Composition Tables for İnternational Use, Food and Agrıculture Organızatıon of the Unıted Natıons, Italy, (1953).

[37] Rice E.W., Baird R.B., Eaton A.D., Clesceri L.S., Standard Methods for the Examination of Water and Wastewater, American Public Health Association Washington, DC (2012).

[38] Ye Y., Wang L., Chen Y., Zhu S., Shen S., High yield Hydrogen Production in a Single-Chamber Membrane-Less Microbial Electrolysis Cell, *Water Sci. Technol.*, **61**: 721-727 (2010).

[39] Kim I.-S., Chae K.-J., Choi M.-J., Verstraete W., Microbial Fuel Cells: Recent Advances, Bacterial Communities and Application Beyond Electricity Generation, *Environ. Eng. Res.*, **13**: 51-65 (2008).

[40] Chookaew T., Prasertsan P., Ren Z.J., Two-Stage Conversion of Crude Glycerol to Energy Using Dark Fermentation Linked with Microbial Fuel Cell or Microbial Electrolysis Cell, *N. Biotechnol.*, **31**: 179-184 (2014).

[41] Meng Y., Li S., Yuan H., Zou D., Liu Y., Zhu B., Chufo A., Jaffar M., Li X., Evaluating Biomethane Production from Anaerobic Mono- and Co-Digestion of Food Waste and Floatable Oil (FO) Skimmed from Food Waste, *Bioresour. Technol.*, **185**: 7-13 (2015).

[42] Chandrasekhar K., Amulya K., Venkata Mohan S., Solid Phase Bio-Electrofermentation of Food Waste to Harvest Value-Added Products Associated with Waste Remediation, *Waste Manage.*, **45**: 57-65 (2015).

[43] Zhang X., He W., Ren L., Stager J., Evans P.J., Logan B.E., COD Removal Characteristics in Air-Cathode Microbial Fuel Cells, *Bioresour. Technol.*, **176**: 23-31 (2015).

[44] Liu N., Wang Q., Jiang J., Zhang H., Effects Of Salt and Oil Concentrations on Volatile Fatty Acid Generation in Food Waste Fermentation, *J. Renewable Energy*, **113**: 1523-1528 (2017).

[45] Islam M.A., Karim A., Woon C.W., Ethiraj B., Cheng C.K., Yousuf A., Rahman Khan M.M., Augmentation of Air Cathode Microbial Fuel Cell Performance Using Wild Type Klebsiella Variicola, *RSC Advances*, **7**: 4798-4805 (2017).

[46] Elbeshbishy E., Dhar B.R., Nakhla G., Lee H.-S., A Critical Review on İnhibition of Dark Biohydrogen Fermentation, *Renewable Sustainable Energy Rev.*, **79**: 656-668 (2017).

[47] Mohan S.V., Chandrasekhar K., Solid Phase Microbial Fuel Cell (SMFC) for Harnessing Bioelectricity from Composite Food Waste Fermentation: Influence of Electrode Assembly and Buffering Capacity, *Bioresour. Technol.*, **102**: 7077-7085 (2011).

[48] Shah S., Venkatramanan V., Prasad R., Microbial Fuel Cell: Sustainable Green Technology for Bioelectricity Generation and Wastewater Treatment, in: Shah S., Venkatramanan V., Prasad R. (Eds.) Sustainable Green Technologies for Environmental Management, Springer Singapore, Singapore, pp. 199-218 (2019).

[49] Kumar P., Chandrasekhar K., Kumari A., Sathiyamoorthi E., Kim B.S., Electro-Fermentation in Aid of Bioenergy and Biopolymers, *Energies*, **11**: 343 (2018).

[50] Fischer F., Photoelectrode, Photovoltaic and Photosynthetic Microbial Fuel Cells, *Renewable Sustainable Energy Rev.*, **90**: 16-27 (2018).

[51] Kumar S.S., Basu S., Gupta S., Sharma J., Bishnoi N.R., Bioelectricity Generation Using Sulphate-Reducing Bacteria as Anodic and Microalgae as Cathodic Biocatalysts, *Biofuels*, **10**: 81-86 (2019).

[52] Baranitharan E., Khan M.R., Prasad D.M.R., Teo W.F.A., Tan G.Y.A., Jose R., Effect of Biofilm Formation on the Performance of Microbial Fuel Cell for the Treatment of Palm Oil Mill Effluent, *Bioprocess Biosyst. Eng.*, **38**: 15-24 (2015).

[53] Zhang G., Jiao Y., Lee D.-J., Transformation of Dissolved Organic Matters in Landfill Leachate–Bioelectrochemical System, *Bioresour. Technol.*, **191**: 350-354 (2015).

[54] Majumder D., Maity J.P., Tseng M.-J., Nimje V.R., Chen H.-R., Chen C.-C., Chang Y.-F., Yang T.-C., Chen C.-Y.J.I.j.o.m.s., Electricity Generation and Wastewater Treatment of Oil Refinery in Microbial Fuel Cells Using Pseudomonas Putida, *Int. J. Mol. Sci.*, **15**: 16772-16786 (2014).

[55] Srikanth S., Kumar M., Singh D., Singh M.P., Das B.P., Electro-Biocatalytic Treatment of Petroleum Refinery Wastewater Using Microbial Fuel Cell (MFC) in Continuous Mode Operation, *Bioresour. Technol.*, **221**:70-77 (2016).

[56] Guo H., Xie S., Deng H., Geng X., Wang P., Huang C., Tang S., Electricity Production and Bacterial Communities of Microbial Fuel Cell Supplied with Oily Sludge, *Environ. Prog. Sustain. Energy*, **39**: e13409 (2020).

[57] Choi J.-d.-r., Chang H.N., Han J.-I., Performance of Microbial Fuel Cell with Volatile Fatty Acids from Food Wastes, *BiotL*, **33**:705-714 (2011).

[58] Teng S.-X., Tong Z.-H., Li W.-W., Wang S.-G., Sheng G.-P., Shi X.-Y., Liu X.-W., Yu H.-Q., Electricity generation from Mixed Volatile Fatty Acids Using Microbial Fuel Cells, *Appl. Microbiol. Biotechnol.*, **87**: 2365-2372 (2010).

[59] Atasoy M., Owusu-Agyeman I., Plaza E., Cetecioglu Z., Bio-Based Volatile Fatty Acid Production and Recovery from Waste Streams: Current Status and Future Challenges, *Bioresour. Technol.*, **268**: 773-786 (2018).

[60] Cappai G., De Gioannis G., Friargiu M., Massi E., Muntoni A., Polettini A., Pomi R., Spiga D., An Experimental Study on Fermentative H2 Production from Food Waste as affected by pH, *Waste Manage. (Oxford)*, **34**:1510-1519 (2014).

[61] Chandrasekhar K., Venkata Mohan S., Bio-Electrohydrolysis as a Pretreatment Strategy to Catabolize Complex Food Waste in Closed Circuitry: Function of Electron Flux to Enhance Acidogenic Biohydrogen Production, *IJHE*, **39**: 11411-11422 (2014).