Effect of Combining Different Substrates and Inoculum Sources on Bioelectricity Generation and COD Removal in a Two-Chambered Microbial Fuel Cell: A Preliminary Investigation

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Abstract – In recent years, fuel cells have become a renewable source of energy. Among different kinds of fuel cells, microbial fuel cells, which convert organic substrates to electricity by electrogenic bacteria have attracted most attention. In this study, which is preliminary in nature, potential of electricity generation and chemical oxygen demand (COD) removal were studied in a two-chamber microbial fuel cell (MFC) reactor. Effect of type of feedstock and inoculum source on bioelectricity generation and COD removal was studied as well. Brewery wastewater and potato waste were used as substrates while anaerobic sludge and cow dung were used as inoculum sources. The substrate and inoculum sources were in 8.2:1 ratio and a phosphate buffer was added to the anode compartment to regulate the pH. The system was operated at 30 °C and a home-made membrane served as a bridge between the electrodes. A maximum voltage of 3.6 mV was generated from the brewery wastewater sludge and the maximum COD removal after 3 days was 43.7 %. It was further found that the use of animal dung as inoculum source outperformed the use of sludge as regard the bioelectricity generation but not for COD removal. Similarly, the use of the brewery waste as an organic substrate outperformed the use of potato waste as regard the bioelectricity generation but not for COD removal. All experiments yielded a measurable voltage, however, the unsteady behaviour of the voltage output made it difficult to compare substrates in terms of their viability as organic fuel. Therefore, future studies should consider conducting substrate physico-chemical analysis and genomic analysis of the inoculum sources to understand their composition.

Keywords – Anaerobic sludge; animal dung; brewery water; potato waste

Nomenclature

COD	Carbon oxygen demand	g/L
MFC	Microbial fuel cell	–
PEM	Proton exchange membrane	–
<i>R</i>	Resistance	Ω

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I	Current	A
P	Power	W
P_d	Power density	W/m ²
J	Current density	W/m ²
V	Voltage	V
A_s	Surface area of electrodes	m ²

1. INTRODUCTION

Global warming, which causes climate change, a highly discussed topic over the last few decades, has been attributed to CO₂ emissions from the use of fossil fuels [1], [2]. Thus, alternative sources of clean energy technology are becoming ever more vital [3]. A possible solution to the energy debacle and a source for renewable and clean energy is the use of microbial fuel cell (MFC) to generate electricity [4]. It is possible to produce power using this process as certain bacteria have the capacity to transfer electrons outside of the cell itself [5]. MFC is structured like a chemical fuel cell: in the anodic chamber, a microbe culture oxidizes substrate into carbon dioxide, electrons and protons. The electrons are absorbed by the anode while the protons move from the anodic chamber to cathodic chamber through a proton exchange membrane where they react with oxygen to form water [6]. To facilitate the flow of electrons through an external circuit, the anodic chamber must be anaerobic as oxygen is often the final electron acceptor in the microbial respiration process [5]. The electron flow via the external circuit to the cathode creates an electrical circuit and current and the electrical capability of the cell.

Furthermore, the mechanism of power production in MFC depends on the biodegradation of organic molecules and has zero net carbon emissions [6]. Moreover, the ability of the cells to convert chemical energy directly to electrical energy without heat generation makes it unique [1]. There are a large variety of bacteria which act as catalysts and, due to self-replication, are self-sustaining for the electrical production process from renewable energy sources [7]. Lastly, bacterial reactions are highly versatile with regards to substrate utilization [5]. Hence, MFCs use a variety of organic sources such as sewage, cattle, swine dung, and brewery wastewater for bio-electricity generation [8]. However, various challenges such as low power outputs and low power densities are hindrances to generating electricity efficiently from these devices [9]. Thus, maximizing the efficiency and economic potential of MFCs to the point of commercialization is an aspiration of many researchers. Additionally, MFCs could be more economically viable if they combine electricity generation with wastewater treatment. Therefore, in this study, potential application of an MFC as a power generating unit and a wastewater treatment unit was evaluated. In addition, effect of inoculum source and substrate source on bioelectricity generation and chemical oxygen demand (COD) removal, which is an important parameter in wastewater treatment [10] in the MFC was investigated.

2. MATERIALS AND METHODS

2.1. Inoculum and Substrate Source

Mixed culture sludge was used as the first inoculum source of bacteria. The anaerobic sludge was taken from Bushkoppies wastewater treatment plant, South Africa. The sludge in the form of raw sewage was always shaken before use to ensure a consistent mix and to avoid a separation

between the solid and liquid particles. Mixed culture of bacteria found in animal dung was used as the second inoculum source. 100 g of the animal dung was weighed out, mixed with 200 ml of ultra-pure water and stirred. This mixture was further diluted with 200 ml in order to free the particles inside and make for better mass transfer and surface area of the bacteria and substrate contact. The inoculum sources were kept in a refrigerator at 4 °C to keep the bacteria deactivated. No adjustments were made to the inoculum samples with regards to pH, nutrients, presence of mediators, or trace metals [11].

Brewery wastewater was acquired from the University of the Witwatersrand Microbrewery located in the School of Chemical and Metallurgical Engineering. This waste was specifically extracted from the mash tailing. The waste was treated by leaving it for 24 h then straining it. Next the brewery wastewater was put to boil to 120 °C at 0.1 MPa in an autoclave to get rid of any yeast or bacteria still active. Potato waste was the second substrate. Most waste from potato production are from the peels and damaged potatoes [12]. In this study, damaged potatoes were mostly used. Both substrates were kept in a refrigerator at 4 °C.

2.2. MFC Design and Construction

The MFC used in this study was constructed in-house. The microbial fuel cell (MFC) was constructed as a two-chamber cell in an H shape. Two Erlenmeyer flasks (250 ml) were modified by a glass blower to attach a bridge between them. The working volume of the anode and cathode were 250 ml and 240 ml, respectively. The bridge was constructed out of glass (length = 90 mm, 45 mm each side, diameter = 22 mm). A homemade, nanocomposite proton exchange membrane (PEM) made from polystyrene rubber and carbon nanospheres and silica was used in the MFC. The membrane was placed in the middle of the bridge, 45 mm, this was secured by a clamp and plastic paraffin (Parafilm 'M'). Circular (rod-shaped) electrodes were made of graphite (length = 40 mm, diameter = 5 mm) and were submerged in the center of each compartment. Their surface area was 0.001257 m².

Fig. 1. Fuel cell experimental set up.

The electrodes were pretreated as described in Sekoai and Gueguim-Kana [13]. Copper wires and crocodile claws were connected to the electrodes and then to a variable resistor (96 kΩ) to make the external circuit. An air-tight seal was made with a rubber stopper and Parafilm 'M' with a sample point to have anaerobic conditions in the anode. The cathode was left open as it operates under aerobic conditions. The physical model is presented in Fig. 1.

2.3. Operation of the MFC

The anodic chamber was operated under anaerobic conditions with working volume of 240 ml for Combination 1 (i.e. experimental run 1.1) and 250 ml for Combination 2 (i.e. experimental runs 2.1 and 2.2.) and Combination 3 (i.e. experimental runs 3.1 and 3.2), respectively. 205 ml of substrate was added as the carbon source followed by 25 ml of the inoculum source for the bacteria. An overall ratio of 8.2:1 of substrate to inoculums was used. Three separate runs were conducted using a different pairing of substrate and inoculums. Combination 1 used brewery wastewater along with sludge. Combination 2 used potato waste and sludge. Combination 3 used potato waste and animal dung mixture. No mediator was used for these runs. A buffer was used to adjust the pH of the mixture, phosphate buffer solution purchased from Merck of 1.0M pH 7.4 at 25 °C. 10 ml of the buffer was added to combination 1 and 20 ml to combination 2 and combination 3. This was done with the aim of getting the pH close to 7. Anaerobic conditions were initiated by pumping nitrogen gas into the anode chamber for 4 minutes to flush the oxygen and then sealed with a rubber stopper. The sample port was connected to a syringe and clamped. The cathode chamber was filled with 240 ml of distilled water. Air was pumped into the cathode via a fish pump for aerobic conditions. The entire rig was placed inside a water bath of constant temperature of 30 °C and monitored for 72 h. Each combination was run in duplicate except combination 1 which was run only once due to time constraint and technical issues during the experimentation.

2.4. Process Monitoring and Calculations

Voltage (V) in the MFC was monitored using a digital multimeter with a USB interface (*Mustool MT826*). This was connected to a personal computer which recorded the voltage every hour. The voltage was recorded in hourly increments. The voltage and variable resistor's resistance (R) were used to determine current (I), power (P), power density (P_d) and current density (J). All density and area values were calculated and normalized as described in [1], [14] according to the surface area of the electrodes (A_s) which was 0.001257 m². The calculations were performed using Eq. (1) to Eq. (4).

$$V = I \cdot R \quad (1)$$

$$P = V \cdot I = \frac{V^2}{R} \quad (2)$$

$$P_d = \frac{P}{A_s} \quad (3)$$

$$J = \frac{I}{A_s} \quad (4)$$

Samples were taken at the start of each run and then once daily from the sample point (5 ml each). For each sample, the pH and the COD were measured. The pH was measured using a bench

top pH meter (OHAUS, Starter 3100C). The COD was measured using standard methods with a kit (Merk). The overall removal was calculated using Eq. (5) [14]:

$$\text{COD}_{\text{removal}} = \frac{\text{COD}_i - \text{COD}_f}{\text{COD}_i} \cdot 100\% \quad (5)$$

where

COD_i is initial concentration of the sample, mg/l;

COD_f is final (observed) concentration of the sample (mg/l) compared from day to day.

The system was monitored for over 72 h where the results for these various parameters were recorded.

3. RESULTS AND DISCUSSION

3.1. Analysis of Bioelectricity Generation

Electricity generation is a key part of what makes microbial fuel cells so useful. Electricity is generated from the digestion reaction where the bacteria and microbes break down the carbon source producing electrons which travel via the external circuit. Therefore, chemical energy is transformed into electrical energy. This is very efficient as no heat is generated from the reaction along with the fact that this can illuminate the Carnot cycle which hinders efficiency. Efficiency of over 70 % is thus achievable [15]. The real-time voltage generation for the combinations of inoculum and substrates studied is presented in Fig. 2 and voltage generation is shown in Table 1.

Very different behaviour and no commonality can be seen from the three combinations (see Fig. 2). This can be attributed to the behaviour of the bacteria in the mixed culture and the sludge used in the study. In combination 1 (i.e. experimental run 1.1.), there is a large initial spike and fluctuation to the point where the maximum voltage was achieved of 3.6 mV (see Fig. 2(a)).

a)

b)

c)

Fig. 2. Real-time voltage generation as a function of time. (a) brewery wastewater and sludge mixture (combination 1); (b) potato wastewater and sludge mixture (combination 2); and (c) potato waste and animal dung mixture (combination 3).

For combination 2, potato waste was used as the substrate which had a lower COD. This is reflected in a lower overall voltage among the combinations. Steady-like behaviour can be seen for both runs 2.1 and 2.2 of this combination, straight lines and little fluctuation are visible until the later stages where the voltage decreases and reaches almost zero (see Fig. 2(b)). The trend downwards indicates an exhaustion of nutrients [13]. In Run 2.1, from the 58th hour there were large ripples, and this can be attributed to the nature of bacteria (see Fig. 2(b)). Bacteria undergo a boom-bust relationship where they start up with exponential growth, reach a static phase and then die out when there are not enough nutrients [16]. The peaks can be seen to depict new bacteria life cycles and different bacteria reacting with different organic nutrients.

Combination 3 produced contrasting trends with run 3.1 having a large initial spike while for run 3.2 the voltage increased gradually. This combination produced higher voltage than

combination 2. This is significant as a different source of inoculum was used. This shows that a change in inoculum brings new bacteria which are more effective in electricity generation than those in the anaerobic sludge. A higher voltage of 2.79 mV was achieved run 3.1. and 2.48 mV in run 3.2. for combination 3 (see Fig. 2(c) and Table 1). Combination 2 and combination 3 only achieved a maximum of 1.16 mV and 2.79 mV, respectively (see Table 1). This difference can be accredited to the nature of the substrate used in combination 1, brewery wastewater. The brewery wastewater was found to have higher carbon content as can be seen from its COD. This means there is more food for the bacteria to feed on and subsequently a higher voltage. The initial spike is also related to the more volatile and easier to metabolize carbon materials are broken down first while some take longer. This goes hand in hand with the mixed culture of bacteria. It has been found that mixed cultures are better for more complex substrates such as those from industry waste such as wastewater. This is because the mixed range has a larger range of bacteria which can break down more organic compounds and at different rates [9]. It was found in a short preliminary study before Run 1 that a voltage of 7 mv was achieved where sucrose was used. This backs the higher carbon understanding. Subsequently, as the substrate gets depleted so does the voltage as it decreases steadily from 12 h. At hour 50 and onwards the graph reaches a steady state like behaviour.

TABLE 1. SUMMARY OF MAXIMUM VOLTAGES AND POWER DENSITY ACHIEVED FOR THE VARIOUS PAIRINGS OF THE THREE COMBINATIONS

Combination	Run	Substrate	Inoculum	Max Voltage, mV	Power density mW/m ²
1	1.1	Brewery wastewater	Anaerobic sludge	3.6 ± 0.00	0.001 ± 0.00
	2.1	Potato		1.16 ± 0.00	0.0001 ± 0.00
2	2.2	waste	Anaerobic sludge	2.48 ± 0.00	0.0003 ± 0.00
	3.1	Potato		2.79 ± 0.15	0.0006 ± 0.00
3	3.2	waste	Animal Dung	2.64 ± 0.15	0.0005 ± 0.00

The inconsistent pattern in the two graphs recorded in the combination 3 (i.e. for run 3.1. and run 3.2.) can also be attributed to the nature of the bacteria in the combination. The bacterium is clumped up inside the animal dung, and despite being diluted it is hard to break down and release. This creates an issue with mass transfer and contact time between the bacteria and substrate. Therefore, the bacteria can be released at different times leading to different peaks and trends observed in run 3.1. and run 3.2. for combination 3. This also correlates to the COD for these experimental runs. Run 3.1 displayed similar steep-increase behaviour as run 1.1. in combination 1. The reason can be similar with substrate usage at the beginning of the experiment and then moves towards stabilization; and then cell death before picking up again. Run 3.2, however, shows the typical behaviour of bacteria life cycle with the lag phase and gradual growth phase indicated by a gradual increase between 30 h to 60 h with some form of steady-state taking place before the hump peaking at hour 68 h; and then dropping to the steady state level (see Fig. 2(c)).

Overall there are considerations to be taken for all the graphs. When pH was taken on the daily mark this unsettled the reactor and created peaks like that in Runs 2.1 and 3.1 at 24 hours and 1.2 and 2.2 at 48 hours. Moreover, the nature of the bacteria and substrate need to be considered with both been of mixed nature with unknown compositions. This is both a positive and negative as on the one hand, one does not know what the bacteria are consuming and what organic source is. But on the other hand, there is a better chance of one of the bacteria to digest the substrate. This is

because the mixed range has a larger range of bacteria which can break down more organic compounds [9].

3.2. pH Analysis

When dealing with an MFC, pH is a key factor. This affects the way the reactants behave as well as how the bacteria operate because this is pH dependent like temperature. If the system is left untreated the two chambers should have the same pH since production rates will be the same [6]. However, since there is a PEM or some form of a membrane there will be a transfer of protons to the cathode side where the limiting reaction takes place resulting in a pH difference since both sides are not the same [17]. This unbalance of reaction rates does have a positive effect, as it creates a driving force for the protons to diffuse from the anode to the cathode through the membrane.

Combination 2 and combination 3 displayed similar behaviour regarding increase in pH. Both started at 7.5 with combination 2 and combination 3 terminating at 7.403 and 7.565, respectively (see Fig. 3).

Fig. 3. pH vs time. (a) brewery wastewater and sludge mixture (combination 1); (b) potato wastewater and sludge mixture (combination 2); and (c) potato waste and animal dung mixture (combination 3).

However, for combination 1 (i.e. run 1.1) there was a large decrease in pH from 6.9 to 4.34. The largest decrease can be seen over the first 24 hours (see Fig. 3(a)). This can be attributed to the type of substrates used. In both cases, the system tends to adapt to the pH of the substrate at the end of the runs, where the brewery wastewater had a pH of 5 and the potato waste 8.08. Therefore, there is a clear shift towards the original state with some changes as a result of the electricity generation. The phosphate buffer did act as a regulator initially in both experiments bringing the starting conditions as close to pH 7. This case of pH change also highlights the results seen in the voltage and COD. It has been shown that a more neutral pH between the two chambers gives higher COD and power generation.

This implies that a pH of 7 is best when distilled water is used in the anode as in the case of this study. In a study where pH was varied between 6–8 for varied concentrations of brewery wastewater, a pH of 7 yielded the best results overall. Current generation of 10.89 mA and 93.8 % COD removal was achieved [18]. In another study with a phosphate buffer, pH 7 was used, and the current increased two-fold compared to when there was no addition of buffer [19]. This shows the need for the buffer and the role pH plays in all aspects.

3.3. COD Analysis

COD is an important parameter as there is regulation for discharge limits in terms of COD. There are also increased tariffs depending on the effluents containing high level of COD. What makes the MFC a desirable technology is its ability to decrease COD. Combination 1 displayed the smallest decrease in COD despite the substrate having the largest original COD of 2426 g/L. The percentage removal was found to be 8.62 % from the initial mixture. Previous studies using sludge and solid municipal waste reported COD removal of 50.1 % after 5 days [13]. In the same vein, Mathuriya and Sharma [18] reported that duration of operation enhances efficiency of COD removal in MFC. In their studies, COD removal of 71.52 % was achieved after 15 days. However, another study using brewery waste achieved similar result after three days where their COD removal was 14.85 %, but when the system was left for 15 days and the authors achieved 71.52 % [18]. Results obtained in this study, especially for COD removal of 43.71 % for Combination 2 (i.e. experimental run 2.1), although the MFC was operated for 3 days, is consistent with literature. It is expected that higher COD removal could be obtained if the MFC is operated for more than 3 days. Combination 3 achieved a COD removal of 6.62 % and 18.61 %, respectively.

respectively, for experimental runs 3.1 and 3.2 (see Table 2). The difference can relate to the higher COD at $t = 0$ for run 3.2. This may be because of the varying nature of the substrate used and inoculums as they are mixed. It implies that there was not a set uniformity between them, and like the voltage generation for Combination 3 the results can vary due to numerous factors. Some explanations are extra solids, entrapment of bacteria and more bacteria in one sample. Overall, Combination 2 displayed the best COD removal, despite running it for 3 days, indicating the promising potential of this technology to treat wastewater (see Table 2).

TABLE 2. COD REMOVAL, FINAL AND INITIAL VALUES FOR THE THREE COMBINATIONS

Combination	Run	Substrate	Inoculum	COD at $t = 0$, g/L	COD at $t = 72$ h, g/L	COD Removal, %
1	1.1	Brewery wastewater	Anaerobic sludge	1931.47 ± 0.00	1764.93 ± 0.00	8.62 ± 0.00
2	2.1	Potato	Anaerobic sludge	27.65 ± 0.00	15.56 ± 0.00	43.71 ± 0.00
	2.2	waste		23.65 ± 0.00	18.65 ± 0.00	21.14 ± 0.00
3	3.1	Potato	Animal Dung	26.92 ± 0.00	25.13 ± 0.00	6.62 ± 6.00
	3.2	waste		33.95 ± 0.00	27.63 ± 0.00	18.61 ± 0.00

3.4. Substrate and Inoculum Comparisons

For the influence of the substrate, it could be speculated that a comparison of Combination 1 and Combination 2 gives a clear picture of the better substrate, although effect of physico-chemical properties of these substrates on the observation cannot be ruled out. However, in terms of voltage generation brewery wastewater displayed a higher value with 3.6 mV compared to the lowest of 1.16 mV for potato waste. The maximum current density of 0.3 mA/m² and power density of 0.001 mW/m² were recorded in Combination 1 from all three combinations (see Table 1).

Though the initial values of COD for these combinations are different, but when Eq. (5) is considered in the calculation of the COD removal comparison of COD removal is possible. It is noteworthy to mention that influence of initial COD concentration cannot be overlooked. Thus, it can be speculated that Combination 2 was better with a maximum of >40 % COD removal compared to that of 8.6 % COD removal in combination 1 (i.e. run 1.1).

For the influence of the inoculum, it could be speculated that a comparison of Combination 2 and Combination 3 gives a clear picture of the influence of inoculum. Though the influence of bacteria population on this observation cannot be overlooked. However, in terms of voltage generation animal dung provides a higher value with 2.79 mV compared to the 1.16 mV of sludge. The higher current density and power density is recorded in Combination 3 as 0.23 mA/m² (for run 3.1) and 0.0006 mW/m² (for run 3.2) (see Fig. 2(c)). In terms of COD, Combination 2 is better with a maximum of > 40 % COD removal when compared to that of 12 % COD removal in Combination 3. These observed differences could be attributed to behaviour of the bacteria whereby one substrate is favoured over the other.

4. CONCLUSION

Overall a preliminary study focusing on different combinations of substrate and inoculum in a two-chamber microbial fuel cell was carried out. This was investigated to evaluate the potential of the MFC to operate simultaneously as a power generator and as a wastewater

treatment unit. Operation of the unit for these purposes seems promising. However, types of inoculum source and substrate source influence the performance of the MFC. Furthermore, the study reveals that the system could be improved on and optimized for enhanced performance. For instance, the use of substances like mediators in the MFC and a better understanding of bio-profile of the mixed culture and physico-chemical property of the substrate could be instrumental to enhancing the performance. Moreover, the use of an agitator could enhance mass transfer in the MFC, thereby enhancing COD removal efficiency. Despite the challenges faced during the study, this preliminary study has highlighted technical issues confronting the use of microbial fuel cell architecture simultaneously as a power generator and as a wastewater treatment unit. In batch operation, mixing is very important to enhance mass transfer. Furthermore, the use of an efficient proton exchange membrane is very important for efficient electron transfer. Also, shifting mode of operation from batch to continuous/semi-continuous such as a fed-batch mode could increase the throughput. At the same time, conduct of substrate physico-chemical analysis, and genomic analysis of the inoculum sources are suggested as well to understand their composition. Study reveals that composition of substrate has a multifaceted influence on power density and efficient electron removal of an MFC; so is the composition of the microbial community of the inoculum [20]. For instance, electrochemically active bacteria exist in large quantities in inoculum source such as wastewaters and activated sludge [21].

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