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Boosting voltage generation in dual-chambers microbial fuel cells through multi-parametric optimization of effects of selected factors using Box Behnken design

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Abstract

Low electricity output of microbial fuel cells (MFCs) has continued to limit their large-scale applications, as bioenergy sources. Thus effects of surface area of anode (0.005 to 0.015 m^2), surface area of cathode (0.005 to 0.015 m^2) and volume of substrate in anode chamber (750 to 1500 ml), on MFCs voltage output, were optimized. Replicated Box Behnken Design (Minitab) gave 30 runs. After 25 days operation, average voltage generated by MFCs ranged from 7.76±0.28 mV to 34.32±3.2 mV, across 10 kΩ. Response Optimizer (Minitab) indicated 0.011 m^2 as optimal surface area of anode, 0.015 m^2 for cathode and 1500 mL for volume of substrate in anode chamber, with estimated maximum voltage of 41.83 mV, when used. This gives 1:1.3:136,363 ratio for surface area of anode, surface area of cathode and volume of substrate in anode, which could be useful in scaling up the device. On application of these optima, highest and lowest average voltages of 54.5±3.2 mV and 20.1±2.7 mV were generated. This maximum voltage was 30.3% higher than the estimate by Response Optimizer, and 58.8% higher than the highest average voltage recorded without optimization. Again, the lowest average voltage (20.13±2.7 mV) obtained after optimization was 159.4% higher than the lowest voltage (7.76±0.28 mV) recorded without optimization. BOD of piggery wastewater, used as substrate, reduced by 18.9%, while COD declined by 31.4%. Diverse Gram-positive and Gram-negative bacterial isolates were identified in the wastewater. Therefore, Box Behnken design is useful optimization of factors, to boost the output of MFCs.

Keywords: Bioenergy; Optimization; Power density; Wastewater treatment

1. Introduction

Microbial fuel cell (MFC) has been described as a promising renewable energy technology, which can guarantee a sustainable and direct generation of electrical energy from the metabolic activities of microorganisms, coupled to the treatment of wastewater. It was borne out of the need to meet the ever increasing global demand for energy, as well as minimize the present overdependence on fossil fuels as sources of energy [1]. Fossil fuels are nonrenewable, hence are faced with increasing depletion. They are also characterized with release of large amounts of carbon dioxide into the atmosphere, which alters environmental conditions, leading to climate change and its attendant problems [2-5]. Different types of wastewater, including acetate, brewery wastewater, synthetic wastewater, inorganic compounds, and azo-dyes, which may be hazardous to the health of both living and nonliving components of the environment, have served as substrates for generation of electricity using MFCs [6].

Irrespective of its foreseen advantages, poor stability, high costs, and insufficient generation of electricity from MFCs, for practical applications have continued to be major challenges which require further studies in its development, and

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eventual commercial deployment [7-8]. Exploratory efforts to enhance the output of MFCs have largely centered on the electrode materials and reactor designs [9]. For improved efficiency of MFCs, an ideal material for anode must be inexpensive, highly biocompatible, highly conductive, and chemically stable [1].

Anode chamber contains microorganisms, the substrate, mediator, where necessary, and an anode electrode, which accepts electrons [10]. Since anode is the site of bio-electrochemical reactions, the efficiency of MFCs is predominantly dependent on anode performance, including rate of degradation of substrate, and ease of transferring electrons from electrogens to the anode. Thus, all prevalent factors in the anaerobic anode chamber of MFCs must always be compatible and optimal, for effective biomass degradation by microorganisms, and harvest of electrons by anode. Anode materials and designs which determine its surface area, longevity, chemical resistivity, and electrical conductivity, thereby significantly affecting MFC performance require special attention [11]. On the other hand, protons produced in the anode chamber are transferred to the cathode through the proton exchange membrane (PEM). Also, electrons harvested by anode are transferred through external wires to the cathode to complete the circuit. In the cathode chamber, both transferred electrons and protons combine with each other, in the presence of oxygen radical, to form water [10]. This completion of the circuit ensures steady current production [12]. However, amount of electricity generated is affected by the rate of accepting electron species, presence of protons, the performance of catalyst, and structure of electrode. Oxygen is usually the choice last electron acceptor, because it is cheaper, most abundant and ecofriendly, and has high potential of oxidation, which leads to formation of water [13].

Among other researchers, Wang *et al.* [8] have equally identified that the development of electrodes for MFCs still requires further studies to enhance their electrical conductivity, microbial affinity and surface area. However, most studies have always centered on selection of materials and surface modification of electrodes, giving less attention to optimizing their surface areas, which also imparts on overall output and internal resistance of MFCs. Again, some efforts to study the effects of relevant factors have always employed single-factor experiments, which often result in non-reproducible and inconclusive solutions, due to the complexity of MFC systems in which the interaction of multiple parameters determine the overall performance [14]. Consequently, the present study is aimed at undertaking a multi-factors optimization of the effects of surface areas of cathode and anode, together with volume of substrate in anode chamber, on the electrical output of MFCs.

2. Material and methods

2.1. Preparation of component materials for local fabrication of MFCs

The dual chambers type of microbial fuel cells was adopted in this study. The two-chambers of MFCs were fabricated using locally available 2 l capacity plastic containers. Both anode and cathodes were made with aluminum sheets, while Nafion® 117 served as the proton exchange membrane (PEM). One (1) inch PVC adopters were used to join the perforated chambers, and also position the PEM. Then, 1 mm copper wires were used to complete the circuit by connecting the two electrodes to the terminals of a digital multimeters (Alda DT-830D), which was used to record voltage (mV) generated from the MFCs. In order to remove impurities, Nafion® 117, produced by DuPont, USA, was initially pre-treated following the method described by Fan and Zhang [15]. It was first cut into appropriate sizes, which were then immersed in 3% H_2O_2 and boiled at 80 ^oC for an hour, for removal of organic impurities. This was followed by repeated rinsing in deionized water. They were then boiled in 1 M H_2SO_4, at 80 ^oC for an hour, to remove metal impurities on surface of the membrane. Rinsing was done in deionized water by boiling them for an hour, to remove residual H_2SO_4 on the surface of the membrane. Finally, they were preserved in deionized water until used.

2.2. Collection and processing of wastewater sample

Piggery wastewater which served as substrate in this study was collected from one of the farms at Piggery Clusters, No 5 Bus Stop, Nekede Old Road, Imo State, Nigeria. The piggery clusters located on the bank of the famous Otamiri River, are notorious for discharging untreated piggery wastewaters into the River. Plastic container for wastewater collection was first surface sterilized by washing with sodium hypochlorite for 30 minutes, followed by rinsing with sterilized deionized water. It was then washed with 95% ethanol for 30 minutes, followed by rinsing thrice with sterilized deionized water. Before collection, freshly discharged wastewater was used to rinse the container thrice, then filled completely, and immediately transported to the laboratory for treatment. Primary treatment of the wastewater was first undertaken by sieving it through a surface sterilized mesh, to removed coarse solid suspensions.

2.3. Design of study and optimization of effects of factors

The study for optimization of effects of surface area of anode (0.005 to 0.015 m^2), surface area of cathode (0.005 to 0.015 m^2) and volume of substrate in anode chamber (750 to 1500 ml), was designed using Box Behnken Design (Minitab® 17), with two replicates. This produced 15 nonrandomized runs, each of which has two replicates, with specific dimensions of the selected factors, as shown in Table 1. Following the specifications of the design (Table 1), a total of 30 units of dual chambers MFCs were coupled. While appropriate volumes of piggery wastewater, as defined in the design, were put into each anode chamber, 1500 ml of distilled water was put into all the cathode chambers, to serve as catholyte. These were followed by insertion of appropriate dimensions of electrodes into their respective chambers. The circuits were completed by means of 1 mm copper wires, which were connected to external digital multimeters, for recording generated voltage. Anode chambers were tightly closed, and all the openings were completely sealed, to promote the development of anaerobic condition. However, the cathode chamber was loosely closed to allow unhindered diffusion of oxygen into it. Open circuit voltage (OCV) and voltage recorded across 10 kΩ resistor, by each MFC, were taken at 6 am and 6 pm daily, for a period of 25 days.

Table 1 Runs and their specific values obtained from design of study using Box Behnken Design

Run Order	Anode Surface Area (m^2)	Cathode Surface Area (m^2)	Anode volume (ml)	Run Order	Anode Surface Area (m^2)	Cathode Surface Area (m^2)	Anode volume (ml)
1	0.005	0.005	1125	16	0.005	0.005	1125
2	0.015	0.005	1125	17	0.015	0.005	1125
3	0.005	0.015	1125	18	0.005	0.015	1125
4	0.015	0.015	1125	19	0.015	0.015	1125
5	0.005	0.01	750	20	0.005	0.01	750
6	0.015	0.01	750	21	0.015	0.01	750
7	0.005	0.01	1500	22	0.005	0.01	1500
8	0.015	0.01	1500	23	0.015	0.01	1500
9	0.01	0.005	750	24	0.01	0.005	750
10	0.01	0.015	750	25	0.01	0.015	750
11	0.01	0.005	1500	26	0.01	0.005	1500
12	0.01	0.015	1500	27	0.01	0.015	1500
13	0.01	0.01	1125	28	0.01	0.01	1125
14	0.01	0.01	1125	29	0.01	0.01	1125
15	0.01	0.01	1125	30	0.01	0.01	1125

At the end, the average voltage recorded across 10 kΩ resistors from each MFC replicates was computed, inputted into Response Optimizer (Minitab® 17) and optimized to derive the optimal values of each factor, for increased electrical output of the MFCs. Subsequently, the optimal values of the factors were used to set up another batch of MFC units, in quadruplets, for confirmation of amount of voltage estimated by Response Optimizer. Average voltage recorded from the MFCs set up with optimal values was compared to those un-optimized MFCs and estimations by the Response Optimizer.

2.4. Physicochemical and bacterial analyses of wastewater samples

Before treatment, samples of original piggery wastewater were analyzed for selected physicochemical and bacterial compositions. Similarly, some samples were put into surface sterilized plastic containers, and left untreated throughout the period of treatment. These served as the control samples for the study. At the end, their physicochemical and bacterial analyses were carried out. Similarly, biofilms on the surfaces of anodes were swabbed at the end of the treatment, and their bacterial diversity, as well as total heterotrophic counts was determined. The analyzed physicochemical factors include pH and total dissolved solid (TDS), determined with Hanna Instrument for pH, TDS, Temperature and EC (Model No.: HI9811-5). Others parameters are NO_3^-, PO_4^{3-} and NH_4^+ contents, measured with Hanna Instrument for Multi-parameter photometer (Model No.: HI83099). BOD_5 and COD were measured by determining and computing the dissolved oxygen content of wastewater (using Dissolved Oxygen meter by LT. Luton, Model No.: DO-5509), before and after 5 days of incubation, or hours digestion respectively.

Five-fold serial dilution of wastewater samples was first aseptically carried out. Bacterial analysis of the samples was then undertaken using Eosin Methylene Blue Agar (EMBA), Nutrient agar (NA), Mannitol salt agar (MSA) and Salmonella-Shigella agar (SSA). The total heterotrophic count on each media was determined. Following the observation of distinct colonies, pure colonies were prepared by sub-culturing them on freshly prepared media. Then, basic biochemical tests were carried out and together with cultural characteristics of colonies, identities of the isolates were determined, as described by Cheesbrough [16].

2.5. Data analysis

The design and optimization of the study were done with Minitab® 17. All the data generated from the study were statistically analyzed with Minitab® 17 and Microsoft Excel 2010 to obtain the mean, standard deviation etc. Statistical charts were prepared using Microsoft Excel 2007.

3. Results and discussion

3.1. Optimization of effects of selected factors on voltage generation

Over a period of 25 days, the average open circuit voltage (OCV) recorded from the 15 MFCs used in optimization study, ranged from 220.62±109.76 mV to 502.96±37.62 mV (Fig. 1). Average voltage (mV) across 10 kΩ, recorded from individual 30 units of MFCs, set up according to the specifications of the experimental design in Table 1, was computed after 25 days of record-taking, and shown in Table 2. Taken the average of the two replicates, voltage ranged from 7.76±0.28 mV to 34.32±3.2 mV. The wide variation of voltage recorded among these MFC units implies that different combinations of these selected factors produced appreciable effects on the voltage output of the devices. This supports the need for optimization of the factors, to derive their optimums maximum voltage generation. In comparison to the voltage recorded in the present study, Fan and Zhang [15] had revealed that steady state voltage generated by MFCs using Nafion membrane and membrane made with a composite of ZrP nanoTiO_2/SiO_2 were 0.0072 V and 0.0082 V respectively. These voltages are lower than the average voltages recorded in the present study. On the other hand, the average voltages in the present study are lower than 969.6 mV, 1228.5 mV and 1338.5 mV reported by Anuforo *et al.* [17], in their study, which was undertaken with copper electrodes. This higher voltage is attributable to their use of potassium permanganate ($KMnO_3$), instead of water used as the catholyte in the present study. Permanganate is known to better recover electrons than potassium ferricyanide and water [5]. However, it has the problem of creating environmental problems with its disposal, unlike water [17].

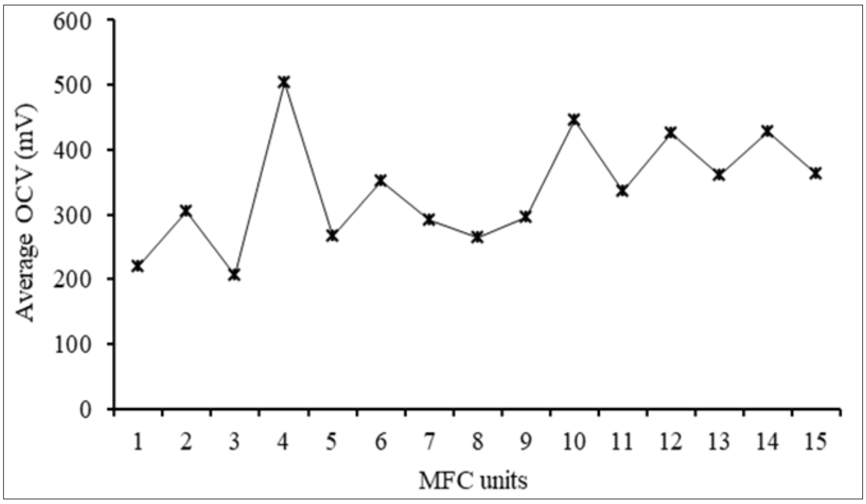

Figure 1 Average OCV from MFC units set up with un-optimized parameters

Table 2 Average voltage (across 10kΩ) produced by MFCs set up according to experimental design and operated for 25 days

Run Order	Anode SA (m^2)	Cathode SA (m^2)	Anode Vol. (ml)	Average voltage (mV)	Run Order	Anode SA (m^2)	Cathode SA (m^2)	Anode Vol. (ml)	Average voltage (mV)
1	0.005	0.005	1125	7.56	16	0.005	0.005	1125	7.96
2	0.015	0.005	1125	10.98	17	0.015	0.005	1125	12.82
3	0.005	0.015	1125	17.52	18	0.005	0.015	1125	11.88
4	0.015	0.015	1125	26.64	19	0.015	0.015	1125	41.16
5	0.005	0.01	750	20.06	20	0.005	0.01	750	8.44
6	0.015	0.01	750	16.02	21	0.015	0.01	750	20.22
7	0.005	0.01	1500	17.12	22	0.005	0.01	1500	16.10
8	0.015	0.01	1500	13.64	23	0.015	0.01	1500	10.88
9	0.01	0.005	750	31.94	24	0.01	0.005	750	28.22
10	0.01	0.015	750	27.76	25	0.01	0.015	750	7.10
11	0.01	0.005	1500	20.42	26	0.01	0.005	1500	24.10
12	0.01	0.015	1500	41.00	27	0.01	0.015	1500	20.80
13	0.01	0.01	1125	32.06	28	0.01	0.01	1125	36.58
14	0.01	0.01	1125	43.80	29	0.01	0.01	1125	9.68
15	0.01	0.01	1125	19.98	30	0.01	0.01	1125	23.98

Fig. 2 shows the main effects plot of interactions among the selected factors on voltage generated by the MFCs. Here, it is evident that increase in the surface area of anode, from 0.005 m^2, resulted in drastic increase in average voltage generated. This scenario continued until the surface area of anode approached 0.01 m^2 when voltage generated gradually plateaued. The plot indicated that further increase in surface area, with other factors remaining constant, resulted in sweeping decline in the voltage generated. On the other hand, increasing the surface area of cathode from 0.005 m^2 caused only a gradual, but steady increase in average voltage generated. This outcome was sustained up to when the cathode surface area of 0.015 m^2 was attained. In the case of volume of anode, initial volume of 800 ml produced an average voltage of about 25 mV. As it increased to about 1300 ml, the average voltage gradually decelerated and eventually leveled off on attainment of 1500 ml mark.

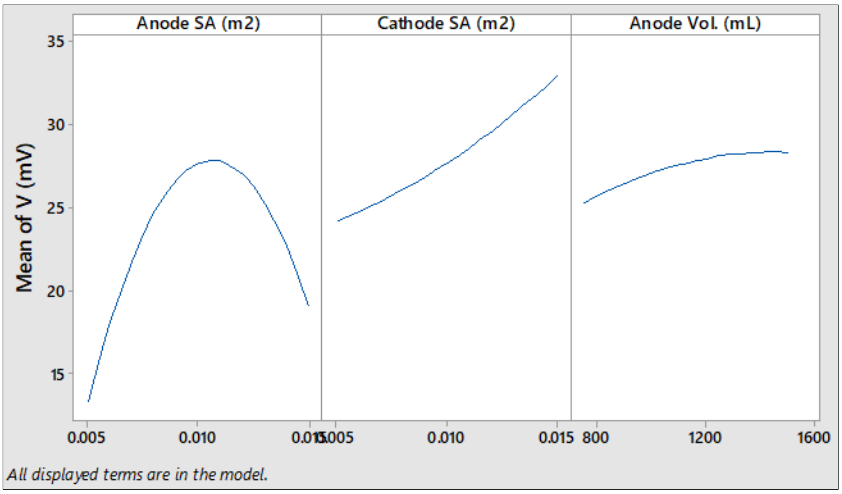

Figure 2 Main effects plot for voltage (mV)

The results of this study have shown how these factors interact with each other to affect the performance of microbial fuel cell. In their reports, Mardanpour *et al.* [18] have implicated substrate injection, electrode surface area, and substrate concentration as some of the factors which affect the power generation and internal resistance of MFCs. Therefore, there is need determine their optimum levels, to maximize the potentials of MFCs. Beside the nature of electrode material, higher surface area is required at both anode and cathode of MFCs. At the anode, increased surface area will allow more bacterial biofilms to form, thereby increasing the metabolic degradation of wastes and release of electrons. However, the increase is not infinite, as it also imparts on internal resistance of MFCs. This is in line with the findings of Wei *et al.* [19], which revealed that high surface area of electrodes with relatively rough surface is an essential surface property in MFC, because it helps to retain bacteria to the surface. Furthermore, the surface plots of these interactions are depicted in Fig. 3.

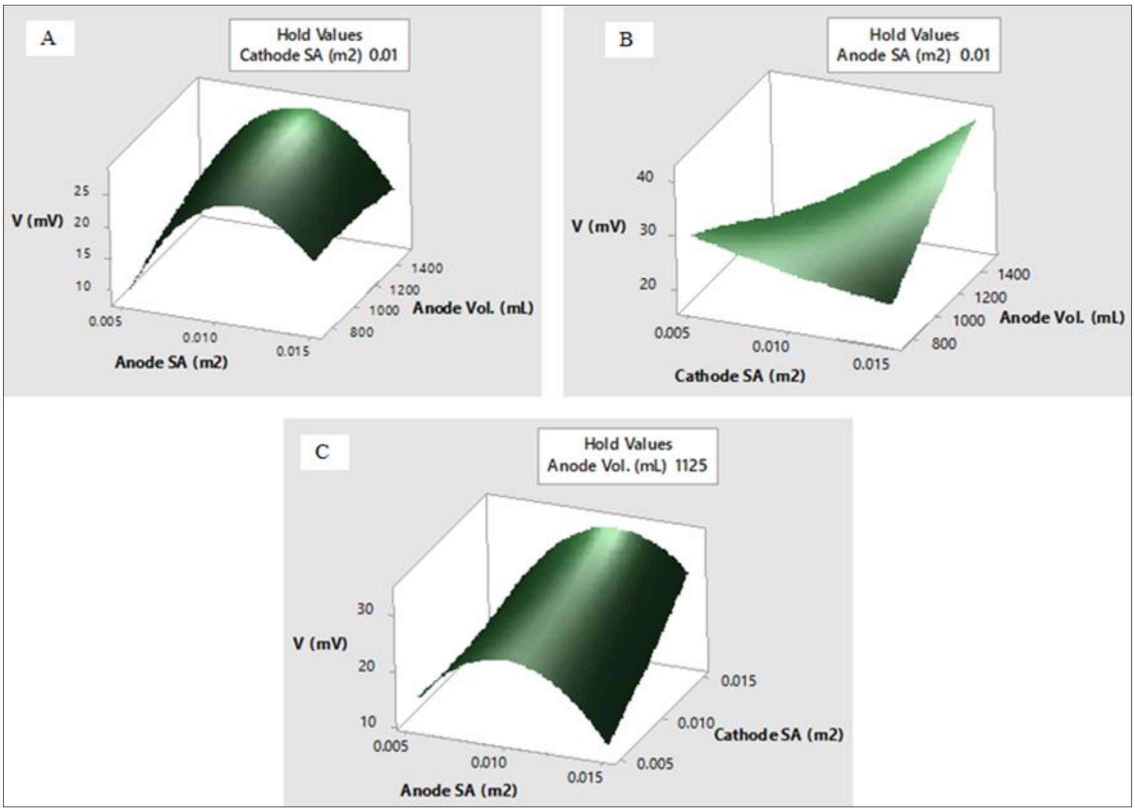

Figure 3 Surface plots of voltage (mV) against; (a) Anode volume and Anode surface area (SA) (b) Anode volume and cathode surface area (SA) (c) Cathode surface area and Anode surface area (SA)

Then, the resulting average voltages were then fed into Response Optimizer (Minitab® 17), and optimized. Results obtained (Fig. 4) indicated that the optimal surface area of anode, optimal surface area of cathode and optimal volume of substrate in anode chamber were 0.011 m^2, 0.015 m^2 and 1500 ml respectively, with an estimated maximum voltage of 41.83 mV, when used.

Subsequently, these optimal values were used to set up MFCs, in quadruplicates, and average open circuit voltage (OCV), together with average voltage recorded across 10 kΩ resistors was computed. From the results obtained (Fig. 5), average OCV obtained was higher than average voltage recorded across 10 kΩ resistor. Generally, average OCV was 232.9±9.7 mV on day one, from which it maintained gradual increment until it peaked at 364.1±5.5 mV on day 11. This was followed by continuous decrease from day 12 to 25, when it recorded an average of 269.6±3.01 mV. On the other hand, voltage recorded across 10 kΩ gradually increased from an average of 20.13±2.65 mV on day 1, until it peaked at 54.5±3.2 mV on day 19. Then, it consistently began to decline from day 20, until day 25, when it recorded 32.5±0.71 mV. Fig. 5a shows that the highest OCV of 368±23 mV was recorded in the morning, on day 11 of operation of MFCs, while the lowest OCV of 226±20.6 mV was recorded in the evening of day 1 of operation. From day 2 to 9 of operation, the average voltage recorded in the evening was higher than that of morning. From day 10 to 16, voltage generated in the morning was higher than what was generated in the evening. However, the remaining days of the duration recorded mixed episodes between the two sessions. In Fig. 5b, the highest average voltage across 10 kΩ was 56.75±8.5 mV recorded in the evening of day 19 of operation of MFCs, while the lowest was 18.25±2.6 mV recorded in the morning of day 1. Similar to the observation made in OCV. Average voltage recorded in the evening took the lead over that recorded in the morning, from day 2 to 9 of operation. The observation reversed from day 10 to 14. Beyond day 14 there was mixed swap in highest voltage recorded between morning and evening.

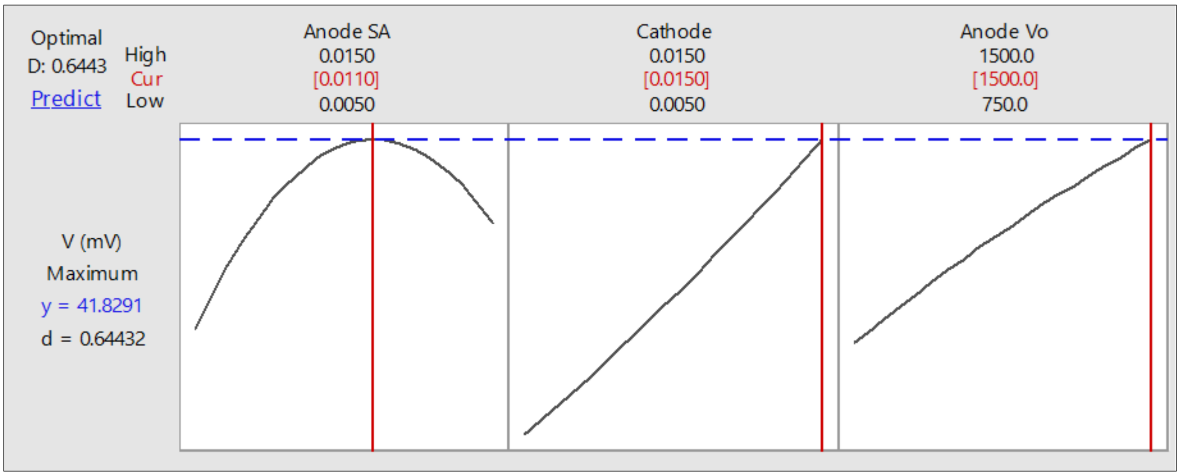

Figure 4 Optimal values of selected factors and the estimated maximum voltage obtained using Response Optimizer

In line with observation in the present study, Griškonis *et al.* [1] have reported that there was a sudden rise in voltage generated from MFCs, with maximum stable average of about 177 mV over 24–72 h after setting it up. This was attributed to complete inoculation of bacteria and formation of active biofilms on the surface of anodes. As shown in the present study, voltage generated by MFCs is not constant, but varies with time and other factors. This was corroborated by the report which indicated that after 1 h of setting up MFCs, the OCP generated by bare GF electrode in the aerated PBS, was about 20 mV which is higher than the one generated in de-aerated PBS. This voltage increased to 76.8 mV in the MFC set up with ethylenediamine modified-graphite felt (GF) anode. The control MFC with bare GF as the anode generated only 10.7 mV [10]. A related report also showed that all MFCs set up generated an open-circuit voltage in the range of 782±12.2 mV, after 30 days of operation, irrespective of the treatment [20]. Average OCV generated by using optimal values of selected factors, in this study, is comparable to the highest OCVs of 969.6 mV, 1228.5 mV and 1338.5 mV recorded after 25 days of operation of MFCs [17]. In consonance with the observation of the present study, the voltage recorded across MFCs decrease with addition of, and decreasing external resistance [17].

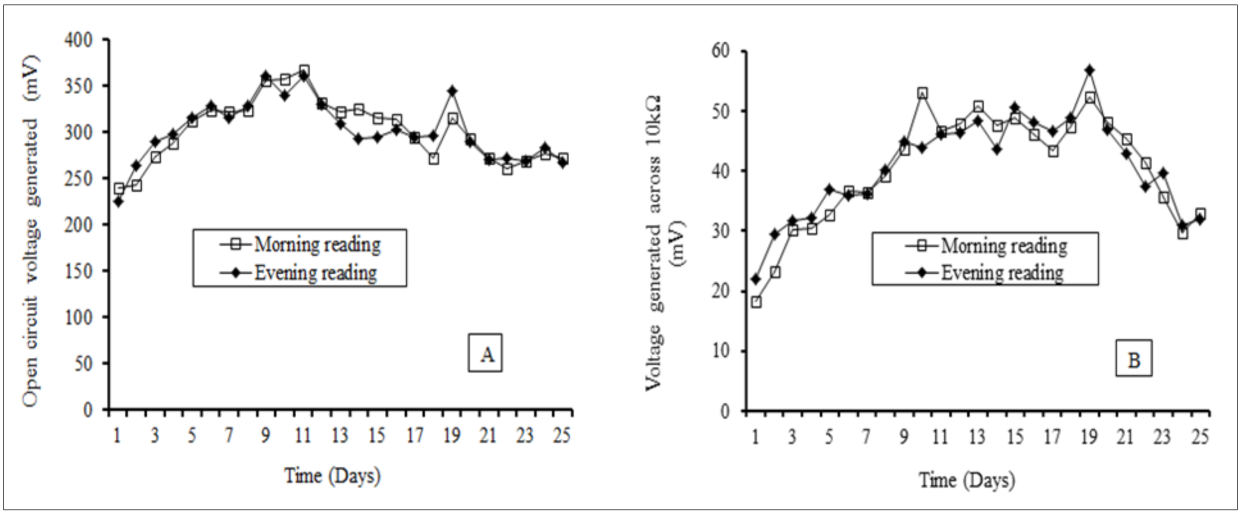

Figure 5 Average voltages recorded from MFCs on daily basis (a) open circuit voltage (b) voltage across 10kΩ resistance.

Results obtained (Figure 6) indicated that when the optimal values of selected factors obtained from optimization in this study, were used to set up MFCs, the highest average voltage (across 10 kΩ) was 54.5±3.2 mV, while the least was 20.1±2.7 mV. This generated highest average voltage is 30.3% higher than 41.83 mV which was estimated by the

Response Optimizer used in this study. Also, this 54.5±3.2 mV highest average voltage, generated with optimal factors obtained in this study, was 58.8% higher than the highest average voltage (34.3±3.2 mV) recorded without optimization of effects of the selected factors. Similarly, the lowest voltage of 20.13±2.7 mV, obtained following optimization was 159.4% higher than the 7.76±0.28 mV recorded without optimization. The improvement in electricity output of MFCs recorded in this study lends credence to the earlier report that incorporation of multiple input parameters into optimization of MFC systems is essential for enhanced performance [21]. Also, Bataillou *et al.* [22] have also reported that the surface area of electrodes, coupled to other surface properties affect the attachment of bacteria to the electrode surface, as well as the electron transfer kinetic between microbes and electrodes. Following the modification of graphite felt (GF) anode with p-phenylenediamine, approx. 32% higher voltage was generated compared to the control MFC [1]. This further emphasizes the importance of surface area, in addition to the conductivity and biocompatibility of electrode materials in biofilm attachment and MFC performance [23]. Simeon *et al.* [20] suggested that the better performance recorded in their SEC–MFCs is due to their lower internal resistance. From the reported results, it was shown that a decrease in the surface area of anode from 2×63 to 2×38 cm, caused a reduction in current density produced by about 34% [18].

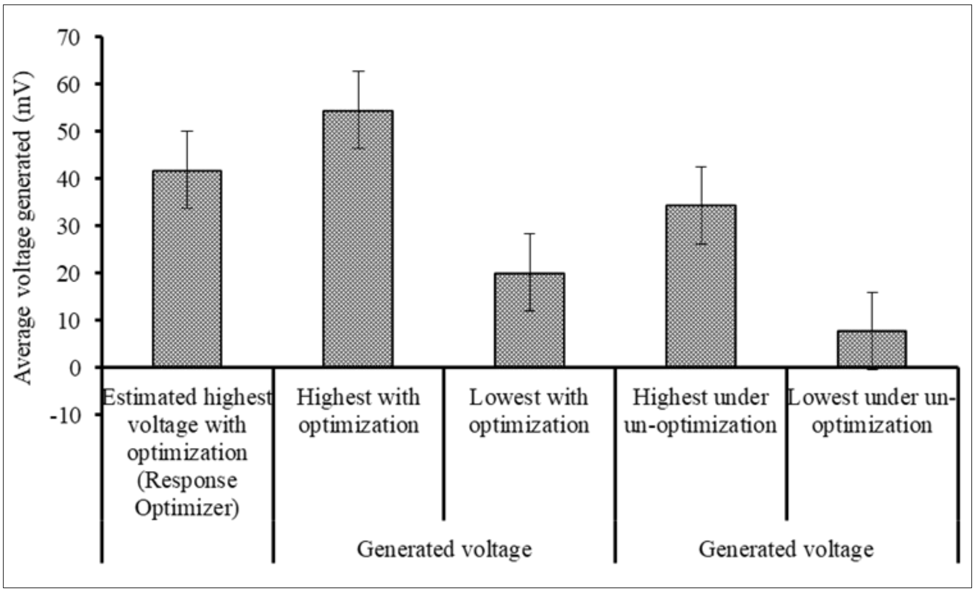

Figure 6 Comparison of estimated voltage, and average voltages recorded before and after optimization of effects of selected factors

3.2. Treatment of piggery wastewater

Selected physicochemical parameters of piggery wastewater samples were studied before and after treatment using MFCs. Results obtained showed that the pH, NO_3^-, PO_4^{3-}, and NH_4^+ contents of the original wastewater sample were 7.1±0.78, 28±3.5 mg/l, 2.34±0.2 mg/l and 2.77±0.4 mg/l respectively. After treatment, only pH of the wastewater increased to 8.33±1.4, while the concentrations of NO_3^-, PO_4^{3-}, and NH_4^+ decreased to 19.33±0.8 mg/l, 1.83±0.1 mg/l and 1.52±0.1 mg/l respectively. In control samples, only slight reduction in concentrations of these parameters was observed, except for pH. The NO_3^- content reduced to 23.33±1.5 mg/l, PO_4^{3-} content reduced to 2.77±0.4 mg/l and NH_4^+ content reduced to 2.23±0.15 [Figure 7]. These were generally lower than the level of reduction recorded in the treated samples. Similar to the observation in this study, earlier reports have shown that the decomposition of organic substrates in the anode chamber of MFCs leads to changes in pH values of anode and cathode chambers. In another study, it was reported that anode pH increased as the reaction progressed, from 6.32 to 6.65 and 6.42 to 6.78 for α-ZrP nano membranes and ZrP MFCs respectively [8]. In contrast to the observation in this study, another study reported that the concentration of NH_4^+ remained almost the same, because it was slightly, or not reduced in the MFC substrates studied. On the other hand, the report showed there was about 90% reduction in the concentration of NO_3^- after treatment of substrate with MFC [24].

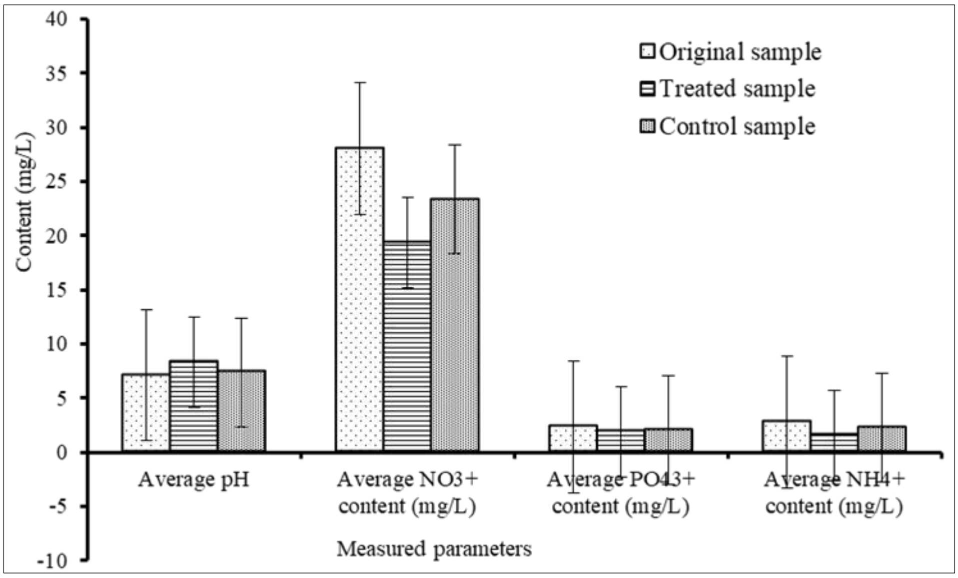

Figure 7 pH, nitrate, phosphate and ammonium contents of original, treated and control wastewater samples

Furthermore, the total dissolved solid (TDS) content of the original wastewater was 1010±45.1 mg/l, the biochemical oxygen demand (BOD) was 1705.33±255.8 mg/l, while the chemical oxygen content (COD) content was 5311.67±313.2 mg/l. After treatment, TDS increased by 25.2% to 1350±17.3 mg/l. However, BOD declined by 18.9% to 1383.3±76.4 mg/l, while COD reduced by 31.4% to 3643.3±160.1 mg/l [Figure 8]. In the control samples, TDS increased by 15.6% to 1196.7±41.63 mg/l. BOD content in control samples reduced by 7.15% to 1583.3±78.4 mg/l, while COD content declined by 11.52% to 4699.7±57.5 mg/l. These percentage reductions were lower than those recorded in treated samples. Earlier report has shown that MFCs set up with three different proton exchange membranes significantly reduced the COD content of wastewaters compared with the influent wastewater, such that 38.1%, 70.98%, 79.25% were achieved in MFCs made with Nafion membrane, ZrP nanoTiO$_2$ and SiO$_2$ membrane, and α-ZrP nanoTiO$_2$ and SiO$_2$ membrane respectively [15]. The result that is more comparable to the present study showed that COD removal rates of 24.15%, 24.91%, and 35.53% were achieved in MFCs used to treat molasses wastewater [25]. On the other hand, other reports have indicated that dual-chamber MFCs achieved 79.8% [26], 83% [27], and 94.6% [28] COD removal efficiency from sugar wastewater, seafood processing wastewater, and brewery wastewater, respectively. Furthermore, COD removal efficiencies of 65%, 51% and 47% were recorded in MFCs after 25 days of operation [17].

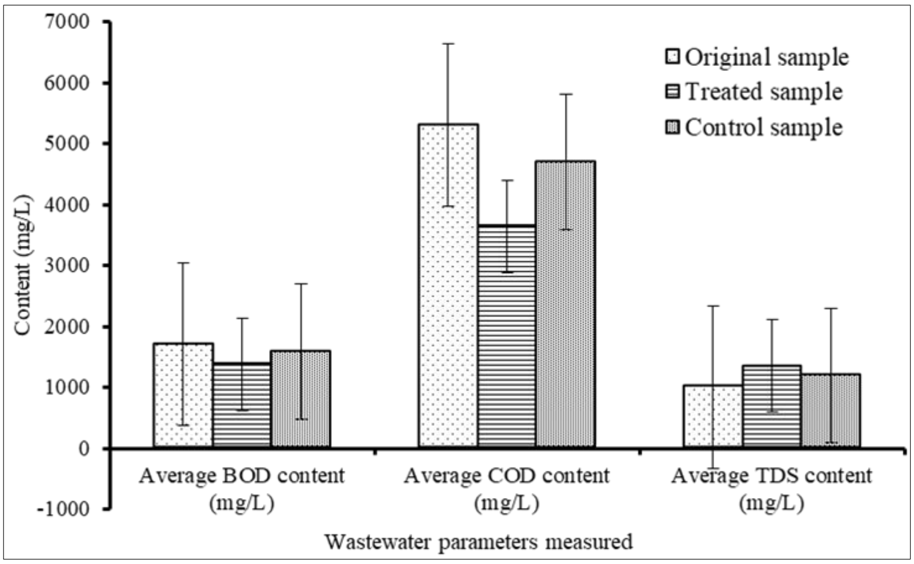

Figure 8 TDS, BOD and COD contents of original, treated and control wastewater samples

3.3. Bacterial analysis of wastewater samples

Results obtained from bacterial analysis of original piggery wastewater samples, using different culture media, revealed the presence of *Escherichia coli, Enterobacter* sp, *Klebsiella* sp, *Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp, *Serratia* sp, *Staphylococcus* sp, *Pseudomonas* sp, *Corynebacterium* sp, *Salmonella* sp and *Shigella* sp. The average total bacterial count recorded were 1.27x10^8 cfu/ml, 9.75x10^7 cfu/ml, 1.75x10^{11} cfu/ml, and 3.0x10^5 cfu/ml, on EMBA, MSA, NA, and SSA respectively. After treatment, the isolates found on the swabbed biofilm on the surface of anode included *Escherichia coli, Enterobacter* sp, *Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp, *Micrococcus* sp, and *Salmonella* sp. The total bacterial counts recorded were 3.67x10^6 cfu/ml, 2.33x10^4 cfu/ml, 3.9x10^9 cfu/ml and 1.0x10^5 cfu/ml on EMBA, MSA, NA and SSA respectively. This showed the absence of *Shigella* sp, *Corynebacterium* sp, *Serratia* sp, *Pseudomonas* sp, and *Klebsiella* sp in the biofilm on the surface of anodes, hence they could not have participated as electrogens.

A related report had earlier revealed the presence of *Lactobacillus* sp*., Escherichia coli, Bacillus* sp., *Streptococcus* sp., *Proteus mirabilis, Corynebacterium* sp., *Pseudomonas* sp., *Enterobacter* sp., *Micrococcus luteus*, *Aeromonas* sp., *Corynebacterium* sp., *and Salmonella* sp. in the original piggery wastewater [17]. Furthermore, Sedky *et al.* [29] have identified the predominance of *Enterobacter cloacae* strain FR in enriched culture, which degraded cellulose as substrate of MFC to produce maximum power density of 4.9 mW/m^2. Also, Bahaa *et al.* [30] have reported that the ten predominant genera of bacteria isolated from wastewater which served as substrate in MFC were *Enterobacter, Bacillus, Pseudomonas, Clostridium, Trabulsiella, Ochrobactrum, Achromobacter*, *Paenibacillus* and *Stenotrophomonas*. Most of these bacterial isolates are known electrogens. Electrogens are electrochemically active microbes, usually bacteria, which are capable of producing electrical energy in an MFC by breaking down organic compounds in the substrate, and transferring the resulting electrons to the anode [31]. Most electrogens form biofilm on the surface of anode, which enhances maximal harvest of electrons arising from metabolic oxidation of organic compounds in the wastewater. These electrons are used to generate power in MFCs [32]. This study has proven that the performance of MFCs can be improved by optimization of effects of factors affecting it.

4. Conclusion

The study was aimed at simultaneously optimizing the effects of surface area of anode, surface area of cathode and volume of substrate in anode chamber, on voltage output produced by MFCs. Results obtained indicated that the optima were 0.011 m^2, 0.015 m^2 and 1500 ml, for surface area of anode, surface area of cathode and volume of substrate in anode chamber, respectively, with estimated maximum voltage of 41.83 mV, when applied. This gives a ratio of 1:1.3:136,363 for surface area of anode, surface area of cathode and volume of substrate in anode. Maximum voltage obtained on application of these optima was 30.3% higher than the estimate by Response Optimizer, and 58.8% higher than the maximum average voltage recorded without optimization. The lowest average voltage (20.13±2.7 mV) generated after optimization was 159.4% higher than the lowest voltage (7.76±0.28 mV) recorded without optimization. BOD content of piggery wastewater, which served as substrate in anode chamber, decreased by 18.9%, while COD content reduced by 31.4%. Among the bacterial isolates identified in the wastewater are *Escherichia coli, Enterococcus* sp, *Klebsiella* sp, *Staphylococcus* sp, *Enterobacter* sp, *Bacillus* sp, *Serratia* sp, *Staphylococcus* sp, *Corynebacterium* sp, *Pseudomonas* sp, *Salmonella* sp and *Shigella* sp.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships regarding the publication of this manuscript.

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