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BIOELECTRICITY GENERATION AND TREATMENT OF PETROLEUM REFINERY EFFLUENT BY *BACILLUS CEREUS* AND *CLOSTRIDIUM BUTYRICUM* USING MICROBIAL FUEL CELL TECHNOLOGY

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ABSTRACT
Microbial fuel cells (MFCs) being an emerging technology have been the research focus of increasing interest due to their sustainable capacity for wastewater treatment together with electricity generation. This study investigated the potential use of pure culture Bacillus cereus and Clostridium butyricum as inoculums in MFCs for simultaneous bioelectricity generation and treatment of petroleum refinery effluent. Double-chambered MFCs was used for the study and operated over four-batch cycles for 30 cumulative days but with different external resistances. The influent concentrations of chemical oxygen demand (COD) and total organic compound (TOC) in the petroleum refinery effluent was 970 mg/l (ppm) and 156 g/l, respectively. Experimental results indicated that the MFCs with the use of Bacillus cereus as biocatalyst achieved its maximum COD removal, TOC degradation and coulombic efficiencies of 70%, 88.7% and 19.21%, respectively; while with the use of Clostridium butyricum, it achieved the highest COD removal, TOC degradation and coulombic efficiencies of 54.2%, 68.7% and 17.84%, respectively. A maximum voltage of 450 mV and highest power density of 17066.67 mW/m² with a maximum current density of 1.270 mA/m² was obtained in regard to the external resistor of 1000 Ω using Bacillus cereus as biocatalyst. Similarly, using Clostridium butyricum as biocatalyst the maximum voltage of 370 mV and highest power density of 8816.17mW/m² with a maximum current density of 0.913 mA/m² was achieved. The study demonstrated that both Bacillus cereus and Clostridium butyricum has strong potentials to be used as inoculums for simultaneous bioelectricity generation and treatment of petroleum refinery effluent in MFCs.

Keywords: *Microbial fuel cell; Petroleum refinery effluent; Bacillus cereus; Clostridium butyricum; Bioelectricity; Biodegradation*

1. INTRODUCTION
Combustion of fossil fuels being the major source of global energy requirement has been reported to have serious negative impacts on the environment due to its greenhouse emissions, which has been implicated as one of the main reason for climate change and global warming [1]. Because of the concerns for fossil fuel depletion as its worldwide demand is astronomically increasing and for climate change and global warming, the search for fossil fuels alternatives has intensified in recent times [1] with thenew technologies development of energy production using renewable and carbon-neutral sources [2]. Bioelectrochemical systems (example, microbial fuel cells (MFCs) and microbial electrochemical cells (MECs)) are such systems that exploit the ability of microbial species to respire through the transfer of electrons outside the cell [3]. MFCs are devices that can convert the chemical energy stored in organic or inorganic compounds (matter) present in wastewaters into electricity or electrical current [3–4] while simultaneously treating the wastewater [5].The principle of MFCs is based on the fact that electrical current generation is one of the basic properties of microbes, as they possess the ability to transfer electrons from an oxidized electron donor to an electron acceptor at a higher electrochemical potential [6].

Over the last decade, MFCs have been the research focus of increasing interest due to their sustainable capacity for wastewater treatment together with electricity generation [7]. Many different types of wastewaters such as municipal, brewery, paper recycling and food processing have been examined for treatment as well as for electrical current generation using MFCs[8-11]and their performance has substantially varied depending on the specific wastewater and reactor configuration. Treatability studies are therefore needed to evaluate a

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specific wastewater in an MFC in terms of power generation and the extent of organics removal. The strength and treatability using aerobic processes of wastewaters are often evaluated using chemical oxygen demand (COD), biochemical oxygen demand (BOD), and a BOD/COD ratio [12]. The BOD procedure tests biodegradability under aerobic conditions, and thus a high BOD/COD ratio might not guarantee effective treatment in bioelectrochemical systems where the anode is the electron acceptor instead of oxygen. This suggests that the use of COD and BOD concentrations as indicators of successful treatment of wastewaters in bioelectrochemical systems needs to be evaluated. COD removal in MFCs therefore occurs both through anaerobic processes (by exoelectrogenic microorganisms on the anode) and aerobic degradation sustained by oxygen crossover through the cathode [13].

Refinery operations are quite complex which generates different kinds of waste. These wastes are released into the environment in the form of particulates, gases, and liquid effluent (liquid consisting of surface runoff water, sanitary wastewater, solid waste and sludge) [14]. The effluent released from the refineries are characterized by the presence of large quantity of crude oil products, polycyclic and aromatic hydrocarbon, phenols, metal derivatives, surface active substances, sulfides, naphthalene acids and other chemicals [15]. A typical petroleum refinery effluent treatment process consists of primary method which consists of mechanical and physicochemical methods, such as oil–water separation, followed by secondary method made up of biological treatment [16]. Activated sludge, sequencing batch reactors and membrane bioreactors (MBR) has been some of the employed biological treatment process [17]. All of these current wastewater treatment technologies due to the requirement for aeration of the wastewater as to provide dissolved oxygen for microorganisms are strongly energy intensive. This energy utilization could be avoided by using bioelectrochemical systems such as MFCs which are completely sealed off from air, where the anode is used as the electron acceptor with hydrogen gas produced at the cathode [13].

So far, very few studies have been performed using oil refinery wastewater in MFC [18-19] and MEC [19]. Majumder et al. [18] used a single pure culture of *Pseudomonas putida* for their study while Zhang et al. [19] and Zhang et al. [20] used the raw oil refinery wastewater with the inherent consortium of microorganisms present in it. In this study, the potential of pure strain of *Bacillus cereus* and *Clostridium butyricum* to simultaneously treat petroleum refinery effluent and generate electricity using microbial fuel cell technology was investigated and evaluated.

2. MATERIALS AND METHODS

2.1 Collection of Petroleum Refinery Effluent

The petroleum refinery effluent was collected from Warri Refining and Petrochemical Company (WRPC), Nigeria. It was stored in a refrigerator at 4 °C. The effluent was diluted with deionised water prior to use.

2.2 Isolation, Characterization and Identification of Microorganisms

Microbial colonies were isolated from freshwater polluted with petroleum refinery effluent using Nutrient Agar medium (Beef Extract-3 g; Peptone - 5g; Sodium Chloride - 5g; Agar – 20 g; Distilled Water -1000 ml, pH 7.0). The bacteria were identified based on colony characteristics, Gram staining and by biochemical tests as given by Bergey's Manual of Determinative Bacteriology and selective media [21].The microbial species isolated from the petroleum refinery effluent were found to be *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Clostridium butyricum*, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* species.

2.3 Microorganisms and Medium

The two microorganisms used as biocatalyst in this study are *Bacillus cereus* and *Clostridium butyricum*. The two microorganisms were separately cultured in a conical flask with 250 ml nutrient broth media and then incubated at temperatures of 35 $\pm$ 2 °C. The nutrient broth media consisted of the following (g/l) peptic digest of animal tissue: 5, sodium chloride: 5, beef extract: 1.5, yeast extract: 1.5. The two bacteria were cultured for 48 - 72 h, and were then inoculated into the reactor.

2.4 Physical and Chemical Characterization of effluent Sample

The physical and chemical characterization of petroleum refinery effluent was carried out according to APHA standard methods [22]. The physical and chemical characterization of the effluent is as follows: pH, 9, Electrical conductivity, 3.7 µS/cm, Total Dissolved Solids (TDS), 3445 mg/l, Turbidity, 1077 NTU, Total Suspended Solids (TSS), 1192 mg/l, Biochemical Oxygen Demand (BOD), 640 mg/l, Chemical Oxygen Demand (COD), 970 mg/l, and Total Organic Compound (TOC), 156 g/l.

2.5 Construction of Microbial Fuel Cell (MFC)

The constructed double-chambered MFC used for this study has been described in one of our previous studies elsewhere [23-24].

2.6 Effluent Treatment and Bioelectricity Experimental Design

The experimental design was carried out according to the method of Aremu and Agarry [23] and Agarry et al. [24]. The cathode chamber which is an aerobic chamber was filled with 100 mM phosphate buffer (catholyte mediator) and pH was adjusted to 7 by the addition of 0.5 M sodium hydroxide. To ensure an effective contact between the proton, electron and catholyte mediator, the solution in the cathode chamber was stirred continuously at 50 rpm agitation speed using magnetic beads. The dissolved oxygen (DO) concentration in the cathode chamber was adjusted to about 4 or 5 mg/l by passing air into it through a 0.45 m pore size filter. For the initial operation of the MFC, an artificial wastewater whose composition has been provided in our previous study [24], having glucose as carbon source was introduced into the anode chamber and the MFC was operated for two cycles.

At the end of two cycles, 50% artificial wastewater and 50% petroleum refinery effluent that makes up the feed solution was inoculated separately into the anode chamber and allowed to operate for another two cycles. At the end of another two cycles, the feed solution was changed to 100% petroleum refinery effluent sample. Throughout the experiment, anode pH was maintained at 6.0 ($\pm$ 0.1). After each feeding cycle, nitrogen gas was sparged into the anode chamber for a period of 4 min to maintain anaerobic microenvironment. MFC performance was evaluated under ambient temperature and ambient pressure. The MFCs were operated for 30 days and readings were taken up to 28 days.

2.7 Measurements and Calculations

To obtain the polarization curves, polarization tests were performed using the multi-cycle method in which different external loads (resistance) were applied for a complete batch cycle, in order to obtain the treatment efficiencies at different current conditions, and to minimize the possibility of power overshoot [18]. The external resistances (load) were varied from 20000 to 20 Ω in a decreasing order over successive fed-batch cycles. All the reactors were left open circuit for a cycle. The MFC was then operated under a single constant external resistance ($R = 1000$ Ω), and the current was measured with respect to time. Voltage potential across the external resistance in the MFC circuit was measured at 30 min intervals using an auto-range digital Multimeter (made by Kusam, model DT-830D) connected to the external resistance. The current was calculated according to Ohm law, $I = V/R$, where I (mA) is the current, V (V) is the voltage, and R (Ω) is the external resistance. Current density was calculated as $i = I/A$, where A (84 cm^2) is the projected surface area of the studied electrode. The power density was calculated according to $P = IV/A$, where I, V and A are the same as previously described. The open circuit voltage (OCV) was the voltage obtained at zero current. The Coulombic efficiency was calculated as [18]:

$$CE = \frac{\int Idt}{\frac{\Delta COD}{32 \times 1000} \times 4 \times V \times 96480} \times 100 \quad (1)$$

In Eq. (1), ΔCOD is the difference in the COD value between the initial and final of the anode chamber, V is the volume of liquid waste treated, 4 is the number of mole of 1 mole O_2-related electrons, 32 is the molecular weight of oxygen (O_2) and I is the current.

2.8 Data Analyses

All experimental runs were conducted using four separate microbial fuel cells. When a single MFC was used, the experiments were carried out in triplicates and results are presented as average values. We found that the all data presented were statistically significant.

3. RESULTS AND DISCUSSION

3.1 Current Generation

Electricity was generated over a period of four batch cycles with a fixed external resistance (1000 Ω)as shown in Figure 1.

During the start-up stage, a lag period of two days was observed and this was followed by inoculation and the course lasted for a total of 28 days from the beginning of the first cycle. An initial low peak current of 0.1 mA and 0.077 mA was obtained in the first cycle with the use of *Bacillus cereus* and *Clostridium butyricum*, respectively. The bacterial population was restored by the addition of fresh substrate at the beginning of the second cycle and this resulted in an immediate electricity generation with a current of 0.085 mA and 0.11 mA using *Bacillus cereus* and *Clostridium butyricum*, respectively. Thereafter, a sharp increase in current was observed to reach 0.22 mA and 0.31 mA with the use of *Bacillus cereus* and *Clostridium butyricum*, respectively, which might indicate the bio-electrochemical activity of the two bacterial species; it then gradually began to decrease after 11 days. The third and fourth cycles were performed as per the second cycle. Higher current output was observed in the third and fourth cycles (feed batches) as compared with the first cycle. The initial lag of current generation as observed for *Bacillus cereus* and *Clostridium butyricum*, respectively may be due to the adjustment and adaptation period of the two different bacteria to the new environment in which it has not easily been able to utilize the total organic compounds present in the liquid waste. The mechanism of anodic bacterial electron transfer is governed by three different mechanisms (6, 18). One is the direct electron transfer between the electrode surface and bacterial membrane. Second is the mediated electron transfer that uses a redox active compound for the electron shuttle between the electrode and bacteria. The third one is wire electron transfer, which uses facilitated nanowire by bacteria for the electron transfer to electrode. In the case of the low current in first cycle, the biofilm has not properly developed and still immature and was thus considered not to contribute much to the electron transfer. The higher current output, especially in the third and fourth cycle may be as a result of the development and maturity of the biofilm after a long incubation time.

Each of the four cycles observed for *Bacillus cereus* and *Clostridium butyricum*, respectively can be divided into two phases-ascending and descending. The ascending phase indicates that the *Bacillus cereus* and *Clostridium butyricum* was respectively able to utilize (biodegrade) the organic compounds (substrates) existing in the liquid waste for generation of bioelectricity as long as the bacteria was still viable while the descending phase (i.e. drop or decrease in voltage, current and power density) might be due to the fact that the easily degradable organic substrates and nutrients were getting exhausted. Also, it could probably be due to the inhibition of electron transfer from the bacteria to the anode surface as a result of biofilm formation in the reactor by the electrochemically-active bacteria.

3.2 Polarization Curve and Power Density

One of the most important parameters for the characterization of the MFC is the polarization curve. This is used to evaluate performance on the basis of current generation. A polarization curve represents voltage as a function of current. In a single batch cycle, polarization data were obtained by varying the circuit external resistance, and the power densities curve was calculated from the polarization data.

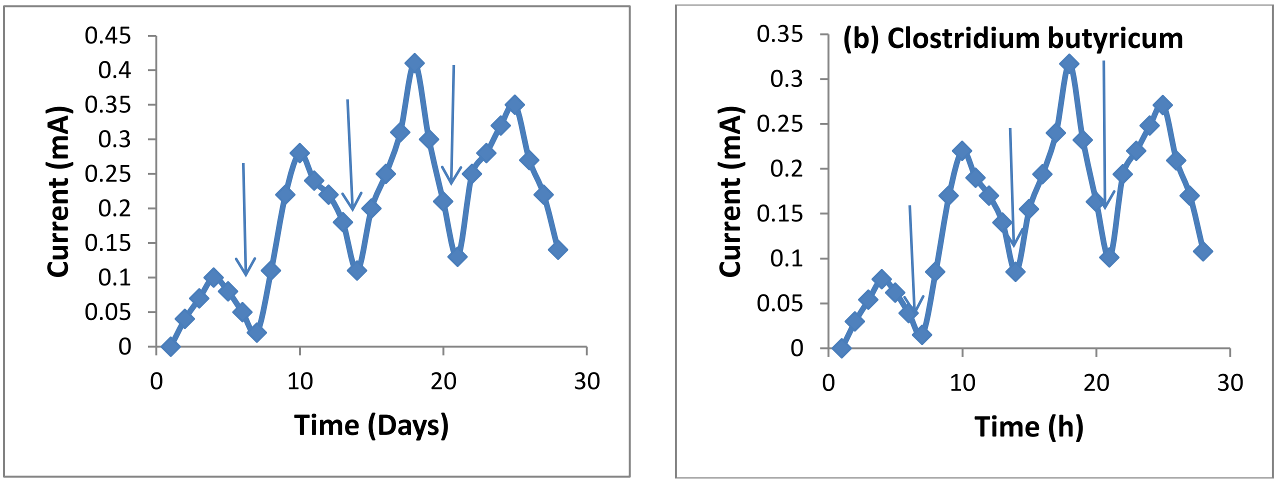

Figure 1: Current generation in MFC for four fed-batch cycles. The arrows indicate the substrate inoculum addition as an end of each cycle.

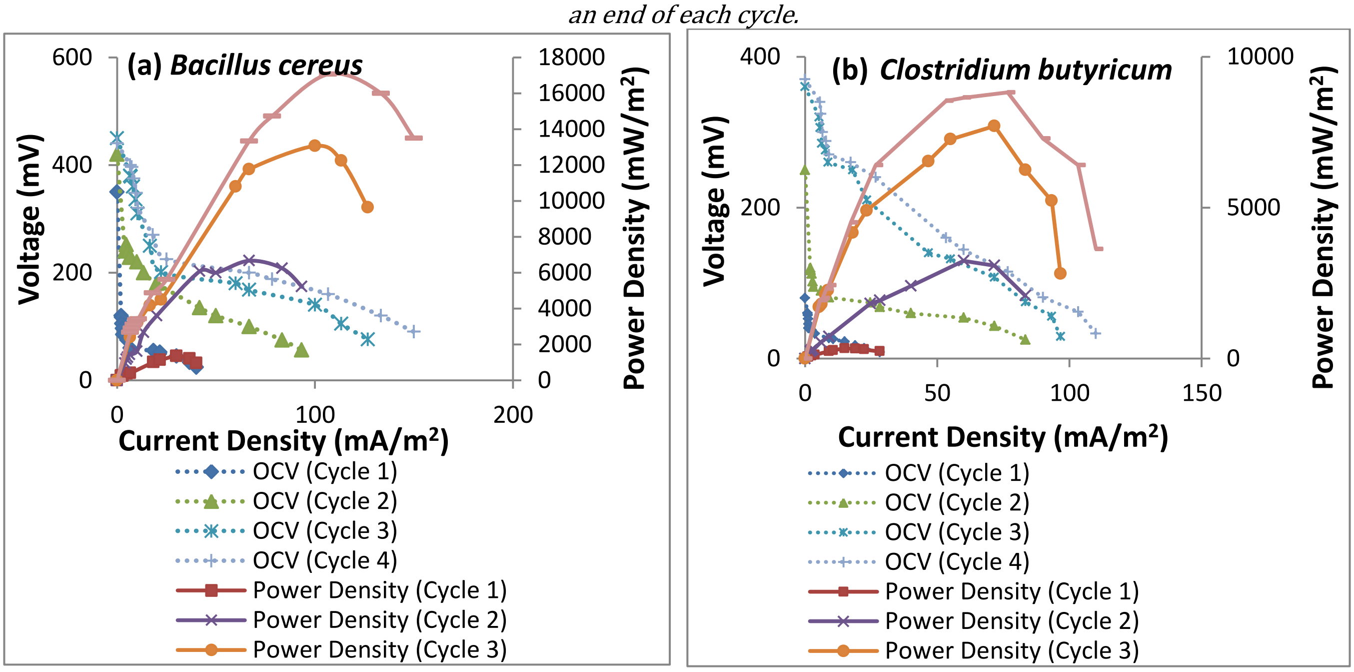

Figure 2: Power density and polarization curves of the MFC

Figure 2 shows the polarization curves of voltage, current and power density obtained for using *Bacillus cereus* and *Clostridium butyricum*. The curves followed a similar pattern for all four cycles. According to the polarization curve, the optimum resistance for our study was 1000 Ω, which applies to all cycles. The polarization curves for *Bacillus cereus* and *Clostridium butyricum* shows three phases, respectively (Figure 2(a) and 2(b)): Activation losses, ohmic losses and mass transport limitation [1, 3, 18]. For both cases, during the phase 1; there is a voltage drop at the beginning of power generation, which may be attributed to activation loss of substrate diffusion. This observation is in agreement with the observations of Logan et al. [3], Venkata Mohan et al. [25], Wen et al. [1], and Majumder et al. [18]. Similarly, as the resistance increased there was a decrease in the current. A similar observation has been reported [1, 3, 18, 26]. In the second phase of ohmic losses, there was an increase in current and thus a linear relationship of voltage and current was exhibited. This observation may be due to the resistance of electron and ion movement. This phase formed an overshoot and hence reached their maximum power densities. During the third phase, there is a steep drop of power density near to the maximum cell voltage, which may be attributed to mass transport losses respective of all cycles. The third cycle and fourth cycle produced better power curves than the two initial ones (Figure 2).

Allocations of open circuit voltage (OCV), maximum power density, and maximum current output are presented in Table 1.

The maximum power density achieved was 13066.67 and 17066.67 mW/m^2 respectively for the third and fourth cycle, and the associated currents were 100 and 106.67 mA for *Bacillus cereus* (Table 1) while for *Clostridium butyricum*, the maximum power density attained was 7704.17 and 8816.17 mW/m^2 respectively for the third and fourth cycle, and the associated currents were 71.67 and 76.67 mA (Table 1). According to Table 1, the OCVs of the four cycles for *Bacillus cereus* were consecutively at 350 mV for the first cycle, 420 mV for the second cycle, 450 mV for third cycle and 440 mV for fourth cycle while for *Clostridium butyricum* they were at 80 mV for the first cycle, 250 mV for the second cycle, 360 mV for third cycle and 370 mV for fourth cycle. In the status of open circuit, no current is circuited through the circuit and hence power production is null. Mujumder et al. [18] reported that a maximum voltage of 355 mV was produced from MFC operation of oil refinery wastewater using a pure culture of *Pseudomonas putida* BCRC 1059. Dalvi et al. [27] and Fatemi et al. [28] have correspondingly reported that MFCs using single or pure culture of *Klebsiella pneumonia* and *Saccharomyces cerevisiae* generated 453 mV and 290 mV from autoclaved dairy waste water, respectively. While Fatemi et al. [28] and Siva et al. [29] have correspondingly reported that mixed culture in dairy effluent generated 500 mV and 450 mV without mediators, respectively.

Furthermore, Siva et al. [29] have reported that mixed culture in sugar waste water produced 400 mV in MFC without mediators. Mathuriya and Sharma [2] used Sugar Industry waste water and Dairy waste water in their MFC without mediators and reported that a maximum current output of 11.39 mA and 8.39 mA was generated on the 5th day from sugar waste water and dairy waste water, respectively. While Siva et al. [29] obtained a maximum current output of 0.957 mA and 0.851 mA respectively for Dairy and Sugar Industry waste water.The above reports suggest that it is the capacity of the individual organism to effectively transfer electrons to anode to generate more voltage irrespective of the presence/absence of other factors. Mujumder et al. [18] observed that *Pseudomonas putida* BCRC 1059 generated a maximum power density of 0.005 mW/cm^2 from oil refinery wastewater using an air-cathode chamber as MFC while Zhang et al. [19] reported that MFCs with the SEA configuration produced a maximum power density of 280 $\pm$ 6 mW/m^2 with current density of 16.3 $\pm$ 0.4 W/m^3 and the MFCs with SPA arrangement produced a maximum power density of 255 $\pm$ 2 mW/m^2 from oil refinery wastewater.

Table 1.Polarization and current output values for four fed-batch cycles during operation with different external resistance (20000 – 20 Ω).

Microorganism	Cycle number	Open circuit potential (mV)	Maximum current (mA)	Maximum power density (mW/m^2)
Bacillus cereus	Cycle 1	350	30	1350
	Cycle 2	420	66.67	6667
	Cycle 3	450	100	13066.67
	Cycle 4	440	106.67	17066.67
Clostridium butyricum	Cycle 1	80	15	337
	Cycle 2	250	40	2400
	Cycle 3	360	71.67	7704.17
	Cycle 4	370	76.67	8816.67

In addition, Wen et al. [30] obtained a maximum power density of 669 mW/m² from the MFC treatment of continuous brewery wastewater while Agarry et al. [24] obtained a maximum power density of 189 mW/m² using a salt bridge for cassava mill effluents. Siva et al. [29]also obtained a maximum power density of 143 mW/m² and 113 mW/m² using a salt bridge for dairy and sugar industry waste waters while Nor et al. [21] obtained a maximum power density of 85.11 mW/m² from MFC operation of palm oil mill effluent using its natural microflora and 451.26 mW/m² using pure culture of *Pseudomonas aeruginosa* ZH1. Thus, these reported power density values are lower than the power density value of 17066.67 mW/m² and 8816.17 mW/m² obtained in this study suggesting that a single pure culture of *Bacillus cereus* and *Clostridium butyricum* are able to produce more power than some single and/or microbial consortium (mixed culture). This also proves the fact that performance of MFCs with respect to bioelectricity generation is dependent on availability of various types of microbes found in biological wastes or effluents. Electrode fouling was not observed and the electrodes could be used in further experiments without remarkable activity loss.

3.3 Removal of COD and Effluent Treatment Efficiency

One of the main purposes of the MFC system is the treatment of wastewater by removing the COD load. The effluent COD concentrations and its removal efficiencies for all of the four cycles are shown in Figure 3.

The COD removal efficiency increased as a function of time, ranging from 23.3% to 70% for *Bacillus cereus* (Figure 3a), and from 18% to 54.2% for *Clostridium butyricum* (Figure 3b). A similar observation has been reported for the use of *Pseudomonas putida* in the simultaneous generation of electricity and treatment of oil refinery wastewater by Majumder et al. [18]. The workers reported a low COD removal efficiency ranging from 10 to 30%. While Zhang et al. [32] reported COD removal efficiencies of 86% and 63% using MFCs configured with separator electrode assembly (SEA) or spaced electrode (SPA) configurations, respectively in the oil refinery wastewater treatment that contained mixed culture of microorganisms. The columbic efficiencies were calculated for each cycle as shown in Figure 4.

According to Figure4 (a), the coulombic efficiencies of the four cycles for *Bacillus cereus* were consecutively at 4.07% for the first cycle, 12.19% for the second cycle, 15.64% for third cycle and 19.21% for fourth cycle while for *Clostridium butyricum* they were at 2.65% for the first cycle, 9.45% for the second cycle, 14.48% for third cycle and 17.84% for fourth cycle (Figure4 (b)). The computed coulombic efficiency data for *Bacillus cereus* and *Clostridium butyricum* showed that coulombic efficiency increased with the number of cycles. Similar observations have been reported [31]. Majumder et al. [18] reported very low CE for the use of *Pseudomonas putida* in electricity generation from oil refinery wastewater. In addition, the coulombic efficiency increased with increase in maximum power densities as shown in Figure 4 (a) for *Bacillus cereus* and Figure 4 (b) for *Clostridium butyricum*. Zhang et al. [19] have reported an increase in coulombic efficiency with increase in current density.

Furthermore, the coulombic efficiency increased with increased COD removal by *Bacillus cereus* (Figure 4 (c)) as well as by *Clostridium butyricum* (Figure 4 (d)). That is, at higher COD removal there was a higher coulombic efficiency. A COD removal efficiency of 70.0% and 54.2% in MFC by *Bacillus cereus* and *Clostridium butyricum* respectively and the relatively high corresponding coulombic efficiencies of 19.21% and 17.84% indicated that the major consumption of soluble/insoluble organic chemicals (substrates) in refinery liquid waste was associated with electricity generation..

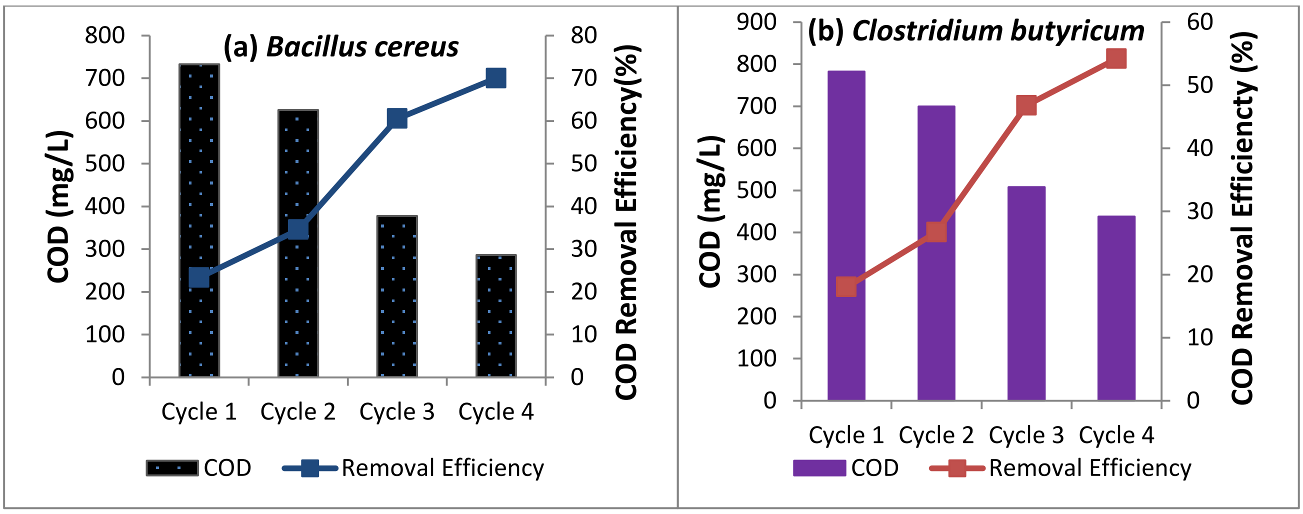

Figure 3: COD removal and its removal efficiency for (a) Bacillus cereus (b) Clostridiumbutyricum

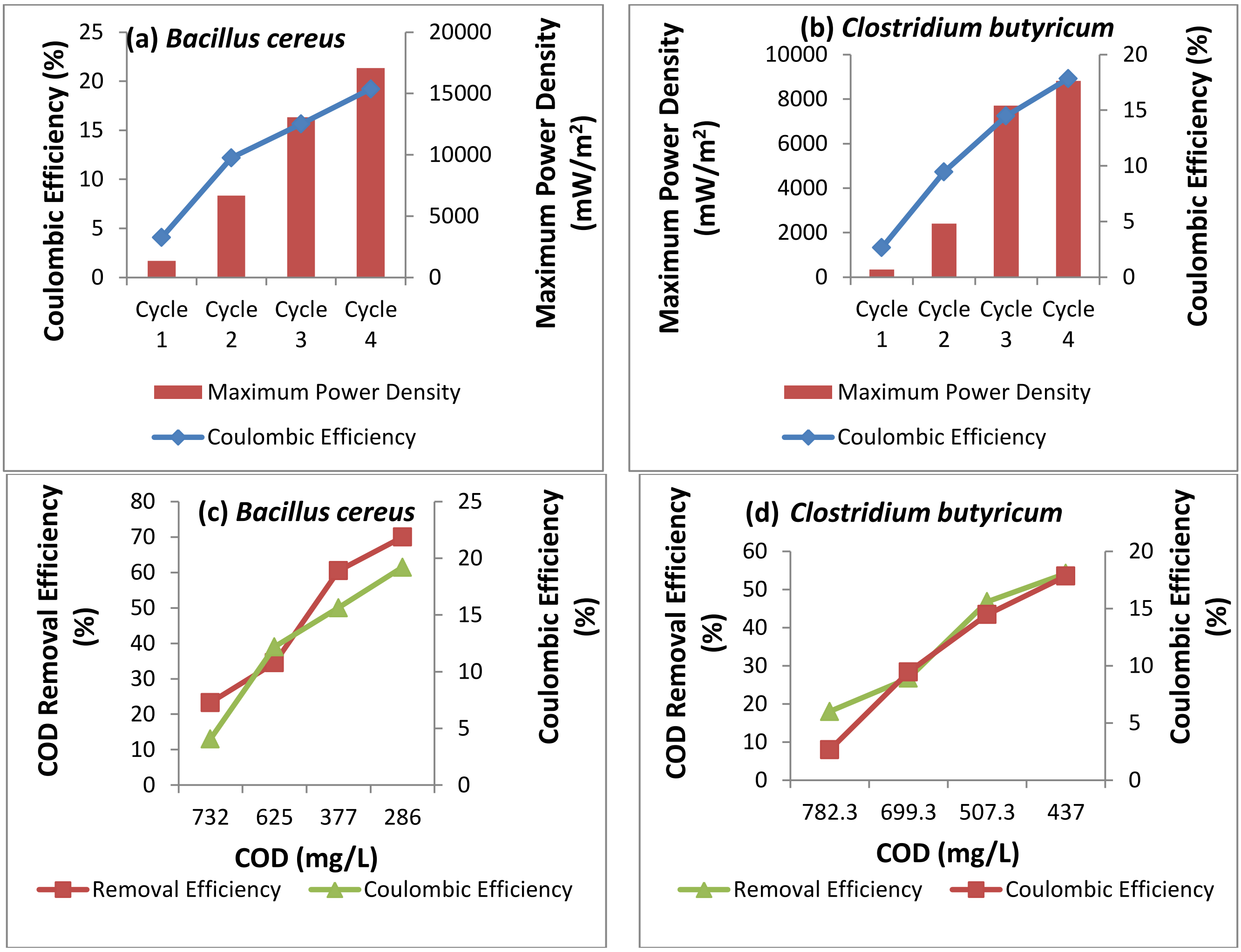

Figure 4: (a) Coulombic efficiency and maximum power density obtained in four fed-batch cycles for Bacillus cereus (b) Coulombic efficiency and maximum power density obtained in four fed-batch cycles for Clostridium butyricum (c) COD removal and coulombic efficiencies as function of COD for Bacillus cereus (d) COD removal and coulombic efficiencies as function of COD for Clostridium butyricum

The liquid waste treatment efficiency achieved is sufficient as compared with other studies. Nevertheless, the results or data revealed that there is a linear or proportional relationship between the COD removal efficiencies and the coulombic efficiencies (plot not shown)

3.4 TOC Biodegradation

During operation, all MFCs were continuously monitored for total organic compound (TOC) removal or degradation to evaluate the potential of MFC to act as liquid waste treatment unit. Continuous TOC removal or biodegradation was observed in all MFCs. Figure5 shows the biodegradation profile of TOC with time.

Figure 5 (a) and Figure 5 (b) showed that there was a lag phase for about 24 h during which there was no TOC biodegradation. After 24 h of lag phase TOC biodegradation by *Bacillus cereus* and *Clostridium butyricum*started and decreased with time from 156 g/l to 17.6 g/l and 48.83 g/l at 144 h with a corresponding biodegradation efficiency of 88.7% and 68.7%, respectively.

The initial lag of TOC biodegradation may be due to the adjustment and adaptation period of the bacteria (*Bacillus cereus* and *Clostridium butyricum*) to the new environment in which it has not readily been able to utilize the TOC present in the liquid waste. High degradation rates and removal efficiency observed in this study for the use of *Bacillus cereus* and *Clostridium butyricum* could be attributed to the presence of petroleum hydrocarbon degrading enzymes present in both microbial species and the presence and availability of an insoluble electron acceptor in the anodic chamber. A similar observation has been reported for naphthalene using anaerobic sludge inoculums [32]. The presence of these enzymes may have facilitated increased cell metabolic rate that could have resulted into higher substrate utilization.

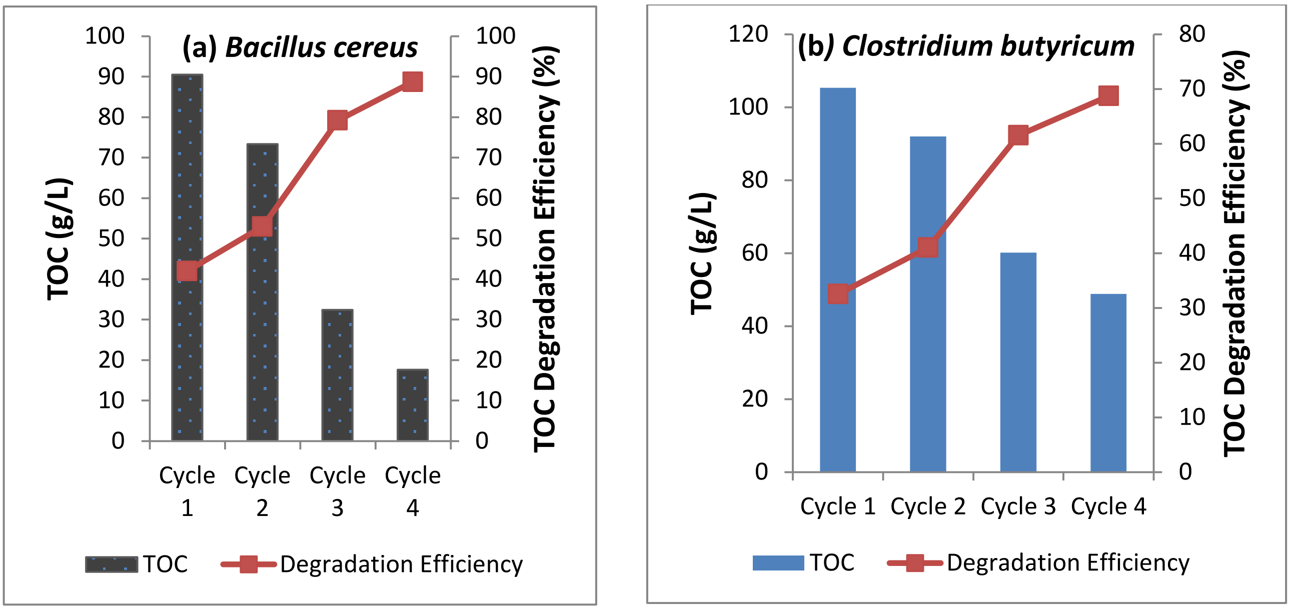

Figure 5: (a) Variation of TOC biodegradation by Bacillus cereus with time (b) Variation of TOC biodegradation by Clostridium butyricum with time

4. CONCLUSION

In this study, the MFC with carbon cathode has shown its great promise as an emerging biotechnological process for treating petroleum refinery effluent and producing electricity. The fuel cell achieved its maximum COD removal, TOC degradation and coulombic efficiencies of 70%, 88.7% and 19.21%, respectively using *Bacillus cereus* while it achieved the highest COD removal, TOC degradation and coulombic efficiencies of 54.2%, 68.7% and 17.84%, respectively using *Clostridium butyricum* as biocatalyst. A maximum voltage of 450 mV and highest power density of 17066.67 mW/m^2 with a maximum current density of 1.270 mA/m^2 was obtained in regard to the external resistor of 1000 Ω using *Bacillus cereus* as biocatalyst. Similarly, using *Clostridium butyricum* as biocatalyst the maximum voltage of 370 mV and highest power density of 8816.17mW/m^2 with a maximum current density of 0.913 mA/m^2 was achieved. However, the power generation was still in a low range; this is a common weakness exhibited by most MFCs that still remains to be solved. If power generation in these systems can be increased, M FC technology may provide a new technology to offset the operating cost of liquid waste or wastewater treatment plant, making liquid waste (wastewater) treatment more affordable for developing and developed nations. Thus, the combination of liquid waste treatment along with electricity generation may help in reducing the cost of liquid waste treatment at present.

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