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Growth Energy of Bacteria and the Associated Electricity Generation in Fuel Cells

Emad Y. Moawad

Dept. of Engineering, Ain Shams University, Cairo, Egypt
*Corresponding Author: emadmoawad@hotmail.com

Abstract This paper aims to determine the mechanism by which microbial fuel cells (MFCs) convert the chemical energy into electrical energy by the catalytic reaction of microorganisms in waste water/sewage treatment plants, to be predictable and controllable so as to economize the project. The wide range of soluble activated sludge in waste streams as a source of bacteria - from low to very high-estimated statistically is responsible for risks of electric current inhibition. For optimizing electricity generation in a continuous process and a large-scale production; a mathematical model is presented to describe the bacteria growth energy (BGE) consumed from the amounts of the biochemical oxygen demand (BOD) contained in fed wastewater along with predicting the amounts of electricity generated by the MFCs. Simulations of the presented model showed that the Input Energy (IE) of the BOD is always balanced with its subsequent from the BGE and the concomitant generated electrical energy according to law of conservation of energy.

Keywords Sewage Sludge, Wastewater Treatment, Oxidation, Energy Balance, Renewable Energies, Electricity

1. Introduction

The use of microorganisms in fuel cell as a catalyst for electricity generation was known 40 years ago [1, 2]. When micro-organisms consume a substrate such as sugar in aerobic conditions they produce carbon dioxide and water, while when oxygen is not present they produce carbon dioxide, protons and electrons: $C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^-$ (Eqt. 1) [3]. As organic material is used to 'feed' the Microbial Fuel Cell (MFC), MFCs are suggested to be installed to wastewater treatment plants [4]. The bacteria would consume waste material from the water and produce supplementary power for the plant, and use inorganic mediators to tap into the electron transport chain of cells and obtain the produced electrons [5]. This process has to be accommodated in fuel cells in which the organisms capable of producing an electric current are termed Exoelectrogens. The use of MFCs in diverse research projects is helping explain the mechanism by which the bacteria shuttle electrons externally. New forms of interactions between bacteria have been discovered demonstrating how multiple populations within microbial communities can co-operate to achieve energy generation [6]. Since the anode is the terminal electron acceptor recognized by bacteria in the anodic chamber, therefore the microbial activity is strongly dependent on the redox potential of the anode [7]. The principle behind generating a flow of electrons from most micro-organisms is to turn this into a usable supply of electricity. Generating a useful current requires creating a complete circuit, but not just shuttle electrons to a single point [8]. In fact, it was recently published that a Michaelis-Menten curve was obtained between the anodic potential and the power output of acetate driven microbial fuel cell. Cheng et al., 2008 has showed that the critical anodic potential seemed to exist at which a maximum power output of a microbial fuel cell is achieved [9]. Allen and Bennetto have showed that mediators can be used to facilitate the transfer of electrons between microbial cells and an electrode, as the mediator crosses the outer cell lipid membranes and plasma wall and then begins to liberate electrons from the electron transport chain that would normally be taken up by oxygen or other intermediates [10, 11]. Alternatively, Park et al. has showed that bacterial cells can be modified with hydrophobic conducting compounds to increase electrochemical activity [12, 13]. The now-reduced mediator exits the cell laden with electrons that it shuttles to an electrode where it deposits them. This electrode becomes the electro-generic anode (negatively charged electrode). The release of the electrons means that the mediator returns to its original oxidized state ready to repeat the process. It is important to note that this process can only happen under anaerobic conditions. If oxygen is present then it will collect all the electrons as it has a greater electronegativity than the mediator [14]. Connecting the two electrodes allows the protons produced as described in Eqt1 to pass from the anode chamber to the cathode chamber. The reduced mediator carries electrons from the cell to the electrode. Here the mediator is oxidized as it deposits the electrons. These electrons then flow across the wire to the second electrode, which acts as an electron sink. From here they pass to an oxidizing material [15]. Several researches investigated how to improve the performance of the MFC using the biochemical oxygen demand (BOD) sensor to measure the real time BOD values [16, 17]. BOD value is determined usually by incubating samples of activate sludge collected from sewage works for 5 days with proper source of microbes. When the BOD value is used as a real time control parameter, the 5 days incubation is considered too long [18]. Oxygen and nitrate are preferred electron acceptors over the electrode reducing current generation from the MFC. But MFC-type BOD sensors which is commercially available, underestimate BOD values in the presence of these electron acceptors. This can be avoided by inhibiting aerobic and nitrate respirations in the MFC using terminal oxydase inhibitors such as cyanide and azide [18]. Many studies dealing specifically with some of related problems like optimizing generating electricity have conducted such topic. Most of them have not introduced a conceptual reasoning to this issue for its statistical analysis nature. Latter could not show how to predict the electricity yield for large-scale production, and pointed out that despite rapid progress, many questions remain unanswered [19]. Yet, none of these latter-day scientists could propose a theory or a concept for the mechanism of the current kinematics of such an unlikely appearing event; the amount needed of input energy (IE) of the organic matter versus that resulted in as an electrical or output energy (OE) in the form of almost steady electric current due to organism metabolic process. An understanding of the physiological characteristics of microorganisms, including factors that regulate carbon and energy metabolism during growth, can furnish useful information when engineering a bioconversion process involving different substrates [20, 21]. Hence, growth yield measurements can provide useful information concerning the relationship among substrate utilization, consumed energy and energy production. Kim et al. go on to argue that the residual of contained organic materials could be attributed to the occurrence of cell death or inactive conditions including nutritional limitations or high toxic metabolite accumulates without presenting the constraints of such factors [22]. In an effort to assist in the understanding of electricity generating and the energy balance that mediate this process, current approach provides a framework for using mathematical techniques to study novel industrial strategies aimed at controlling generating electricity, and tries to relate the IE course of the treatment to bacteria response during it, and presents the first experimentally driven mathematical model designed to investigate interplay between the possible mechanisms of generating electricity.

2. Method and Materials

Some parameters are associated with generating electricity in MFCs, such as number of existed bacteria, consumed energy, metabolism inhibitors and electron acceptors. Recent studies have studied the interaction effects of these parameters on the generated electricity by MFCs [23]. One of the main constraints in obtaining higher rates of electric current is the inhibition of bacteria metabolism by both high concentration of organic substrate as well as the electrons acceptors [24]. Generally in industrial bioelectricity production, an initial of 0.16-0.18% BOD is used and when substrate concentration increases, osmotic pressure becomes pronounced which seriously effects MFC efficiency [25]. Temperature exerts a profound effect on all aspects of growth, metabolism, and survival of organism [24]. Metabolism in industry is usually carried out at ambient temperature (25 – 35$_o$C). A high temperature led to a decrease in the current produced and bacteria death. The inhibition effect of the initial BOD concentration on cell growth was clearly observed. The adopted mathematical model could describe very well the dynamics of generating electricity from the beginning up to the stationary phase [6, 24]. Also, pH of 5.0, 6.0, 7.0 and 8.0 had been investigated for MFCs and it was concluded that low pH inhibits the microorganism multiplication [26], while the inhibitory effect of pH (at the high level) on the electrical current produced could be due to the lower ATP production during the metabolic changes in microorganism [24]. The controversial objectives of the current approach are to (1) estimate the maximum potential of inoculated wastewater with activated sludge, (2) minimizing Loss energy (LE) and (3) understand the main and interactive effects of Bacteria growth energy (BGE) on generating electricity and its role in the administered sludge content that could lead to minimize its residual wastes and to optimize the produced electrical power as well. It is evident that increasing organic materials (IE) leads to the increase of BGE and consequently the produced current until metabolism process affected due to the osmotic stress of the higher fed rates of EI. The metabolism process is affected also for low fed rates of IE where it is stopped as soon as IE becomes smaller than that of BGE which cause substrate inhibition due to bacteria death [6, 24]. The relation between Cell Growth Energy (CGE) and cell Doubling Time (t_D) which is known by Emad formula has been derived and presented by Moawad as follows:

$$\text{CGE} = \ln\left[\ln\frac{\ln 2}{t_D}\right]^2 \text{ Emad (1), where Emad} = 23234.59$$

MeV (2) in which CGE increases by the increase of cell t_D [27-32].

Consequently, increasing CGE can be achieved through agents that lead to the increase of the cell t_D or in other words cause the cell to divide more slowly that if severe or prolonged increases CGE. Current approach aims to investigate the interaction of BGE in generating electricity process where its increase is favorable and vice versa to avoid osmotic stresses taking into consideration the correlation between growth and aging in the micro-organism;

whenever bacteria grow larger they grow older, typically divides asymmetrically to give a large mother cell and a smaller daughter cell [33]. As mother cells become old, they enlarge and produce daughter cells that are larger than daughters derived from young mother cells. Like large mothers, large daughter cells have shorter replicative life span [34]. And which confirm that, IE calorie restriction in which nutrient intake is restricted to 60-70% that of voluntary levels increases life span in most species including mammals [35-37]. Then, targeting the increase in CGE by increasing the cell tD should not lead to aging of cells through EI calorie restriction. Consequently, controlling the increase of BGE to avoid the osmotic stress should be through number of bacteria (n) but not the CGE as:

$$BGE = CGE \times n \qquad (3).$$

2.1. MFC Mathematical Model

Evidence is beginning to show that excess of IE may increases generating electricity. The main idea of such hypothesis was concluded from consistent observations have confirmed that energy restriction reduces BGE by inhibiting substrates and vice versa [26]. But this interaction between IE and BGE would not last in either direction due to cell death for shorter replicative life span or/and osmotic stress as previously reported [31]. Thus, administering an appropriate IE for MFC that minimizes Residual Energy (RE) and maximizes produced electrical energy (OE) can be performed by considering the interaction between the consumed amount of organic material and the bacteria growth in addition to their subsequent of the generated electricity as an isolated system. Current approach presents a mathematical model of MFC describes the BGE during generating electricity process, for which efficiency is measured by comparing results of OE to amounts of IE but not for consumed energy (CE). Since, maintenance of high cell viability is a major characteristic of MFC to get high current yield, an important aspect of the model is that BGE should be less RE to avoid risks of the osmotic stresses. The hypothesis of our model in all cases is that electric current generation by MFC is an energy balance process in which summation of BGE, OE are always balance with the consumed energy (CE). In same time summation of CE and RE are balance with IE. Accordingly the above mentioned conditions and constraints can be expressed mathematically as follows:

Due to balance of energy:

$$IE = CE + RE = [BGE + OE] + RE \qquad (4),$$

To avoid the osmotic stress:

$$BGE > RE \qquad (5)$$ [31]

While the OE (Joules) gained of the electrochemical process can be calculated based on the output power (Watt) and time of the process duration (Second) as follows:

$$OE = Power \times Time \qquad (6)$$ [39],

The power can be determined by knowing the current intensity (I) in Ampere, MFC resistance (R) in Ohm and the potential difference generated (V) in Volts according to Ohm's law

$$V = I \times R \ (7),\quad P = I \times V \qquad (8)$$ [39].

2.2. Checking the MFC Model Efficacy

Kim et al. showed [38] that a fuel cell was used to enrich a microbial consortium generating electricity, using organic wastewater containing 5-20 ml sludge before the potential was recorded as the fuel and wastewater fuel was fed continuously at the rate of 0.3 mL / min for continuous enrichment culture. The biochemical oxygen demand (BOD) value of the wastewater was around 1700 mg/L. The wastewater contained 25 ± 7.7 mg / L total nitrogen and 10.7 ± 1.7 mg/L total phosphorus, respectively. Within 30 days of enrichment the steady current of background level of 0.02 mA was generated with a resistance of 1 k Ω. Current generation was associated to a fall in the BOD over 30 d from 1,700 mg/L down to 50 mg /L [28].

3. Results and Physical Analysis

BOD decreased exponentially within 30 d from 1700mg/L to 50mg/L by half-life time of $\left(t_{1/2}\right) = \frac{30}{\log_2\left[1700/50\right]}$ =5.897d . Accordingly, growth of bacteria would follow a growth constant equivalent to BOD decay constant ($\ln 2/5.897d$).

Thus from Eqt 1, CGE = $\ln\left[\ln\left(\frac{\ln 2}{5.897?\ \ 4\times 60?\ \ 0}\right)\right]^2$ =5.2 Emad.

The average capacity along the 30 d period of the used MFC after the first fed of wastewater on first minute was $\frac{1}{2}\times 20$ ml sludge before the potential +0.3ml = 10.3 m L. Such volume would contain a final number of bacteria after 30 days growth equivalent to: n= $10.3\times 10^{-3}\times e^{\frac{\ln 2}{5.897}\times 30}\times 10^{9}$ cells, taking that number of cells are 10^9 per liter.

Consequently from Eqts 2 and 3:

$$BGE = .10.3\times 10^{-3}\times e^{\frac{\ln 2}{5.897}\times 30}\times 10^{9}\times 5.2\times\frac{23234.59}{6.242\times 10^{12}} = 6.8J$$

As organic materials that fed MFC had been nearly consumed completely then the CE along the 30 days was the total organic material contained in the wastewater which was of volume equivalent to 10.3 mL /1000+ 0.3 mL× 60× 24× 30/1000/ 1000=12.9703 L. The wastewater was containing 25 ± 7.7 mg / L total nitrogen and 10.7 ± 1.7 mg/L total phosphorus. Thus, the total organic material per liter of the used waste water was (25mg +10.7mg)/L=35.7mg/L.

Taking into account that energy per one gram of organic material is 17J/gm [31] , then the total

IE along the 30 days was:

$$IE = 35.7/1000\times 12.9703\times 17 = 7.87 \text{ J}.$$

Thus, BGE was less than of the IE to comply with law of energy conservation. According to the hypothesis of the introduced mathematical model in Eqt.4, the Generated Electrical Energy (OE) during the same period of metabolism suppose to be less than IE – BGE

$$\text{I.e. } OE< (7.87 – 6.8)\ 1.07 \text{ J}$$

While from the recorded observations; the generated electric current intensity (I) was 0.02mA with 1k Ω resistance, then applying ohm's law in Eqt 7; the generated electric current potential difference was equivalent to:

$$V= IR= 0.02\times 0.001 \times 1000= 0.02 \text{ Volt}.$$

From Eqt 8, this potential difference of the generated electricity had a power equivalent to:

$$P=IV= 0.02\times 10^{-3} \times 0.02= 4.0 \times 10^{-7} \text{ Watt}.$$

From Eqt 6, the amount of OE corresponds to this amount of the generated power was equivalent to:

$$OE = 4.0 \times 10^{-7} \times 30\times 24\times 60\times 60 =1.04 \text{ J}$$

This is 97.2% of the available part of the IE (1.07 J) to be converted into electric energy as the major part of the IE (6.8 J) was consumed in increasing the BGE.

Accordingly from Eqt 4, CE = BGE + OE = 6.8 +1.04 =7.84 J <IE to comply with law of energy conservation. Also with respect to the residual energy, from Eqt 4:

$$RE = IE – CE = 7.87 \text{ J} – 7.84 \text{ J} = 0.03 \text{ J}<BGE$$

as postulated by our model in Eqt 5 to negate the exposure of the bacteria inside the MFC to the osmotic stress for the ideal enrichment of MFCs [31].

4. Discussion

The aim of current approach is to introduce an overview of MFC technology that may help to get more understanding in current biotechnology and environmental technology. Pham et al. showed that the sustained and reproducible exploitation of this energy will require better understanding and careful management of the microbial communities in the MFS in terms of biomass density per unit volume and specific activity of the biomass [40]. MFCs have mainly been applied to soluble waste streams with low to medium loading, targeting recoveries of at least 1 kWh of useable electricity per kilogram of organic matter removed, and generation of up to 1 kW/ m^3 reactor volume. [23]. In the present study, bacteria were the electricity – producing microbial strain, its growth in gradually increasing concentrations of organic materials showed an increase in generating electricity up till 35.7 mg/L. Microbes enriched for 30 days in the fuel cell almost completely consumed the organic contaminants in wastewater with the concomitant generation of electricity. The BGE was 86.4% of the IE, whereas the OE was 13.2% only of the IE, leaving a small value of RE which was 0.4% of the IE. These results confirmed that the major amount of the organic materials contained in the fed wastewater was consumed by microorganism in increasing the BGE during metabolism, whereas the minor amount of the IE only was converted into electricity. Thereby, it should be understood that the organic materials in the added wastewater continuously is essentially to produce a steady electric current from MFCs and the yield highly depends on the metabolism conditions [35]. Based on the law of conservation of energy the established model described the current yield by MFC efficiently. The high similarity between the experimental value and the predicted ones (R^2 =0.98) suggested that the model was a good fit; the conducted experiment at 35.7 mg/L of the organic materials demonstrated that the electric power from the recorded observations was 4×10^{-7} Watt which was 98% identical to the predicted value by the model [Power = 1.07 J (Predicted OE) /(30× 24× 60 ×60) $=4.1\times10^{-7}$ Watt]. Such accuracy showed that the model was useful to predict the electricity yield so that it could be easily applied for scale - up to produce value - added electricity. Furthermore, it provides a clear cut criterion to accept the hypotheses of the current thesis about the energy conversions take place inside the MFCs, and strengthens the confidence in identifying the BGE mathematically by Emad formula using the rate of the BOD consumption to determine t_D of the bacteria growth as described in an earlier study [31]. There are several advantages of MFCs which make such technique attractive. The ability to reach usable electricity targets from wastewater will make MFC technology comparable to the standard procedure of anaerobic digestion to methane in terms of cost and environmental soundness. Corresponding to the MFC efficiency compared to the other applications of converting energies to electrical energy is considered promising as the fixed current produced was of 0.02mA; the electricity yield was calculated as 1.12×10^{-5} Watt/g BOD. Since, knowledge of how bacteria interact with insoluble electron donors and acceptors is rapidly increasing, thus it can be concluded that parameters which interact the performance of MFCs are (1) OE that can be generated in a microbial fuel cell which is dependent on both biological and electrochemical processes, (2) BGE which interpreted in terms of substrate conversion rate which depends on the amount of bacterial cells, the mixing and mass transfer phenomena in the reactor, the bacterial kinetics such as the maximum specific growth rate of the bacteria, and the bacterial affinity constant for the substrate [23],(3) IE which is the biomass organic loading rate (g substrate per g biomass present per day) as the current generated from the fuel cell is directly proportional to the amount of electron donor utilized, the MFC can be used as a sensor to determine BOD which is considered another area of application of this microbial device[41], (4) the efficiency of the proton exchange membrane for transporting protons [42] and the potential over the MFC.(5) Over potentials at the anode, measured when circuit is open which is affected by the electrode surface, the electrochemical characteristics of the electrode, the electrode potential, and the kinetics together with the mechanism of the electron transfer and the current of the MFC. (6) Over potentials at the cathode similar to the losses observed at the anode, the cathode exhibits significant potential losses can be minimized by reoxidizing by oxygen in air where MFC cathodes preferably should be open-air cathodes [43-45]. (7) The proton exchange membrane performance which has a great deal in decreasing MFC internal resistance as it inhibits bacteria growth at the cathode, where proton migration significantly influences resistance-related losses [46]; adequate mixing could minimize these losses. In addition, internal resistance of MFCs is dependent on both the resistance of the electrolyte between the electrodes and by the membrane resistance (Nafione has the lowest resistance). Thus, for optimal operation, previous parameters should be considered besides taking into account that anode and cathode need to be as close together as possible [40], which would contribute to convert the major part of the energy of the organic contaminants into electricity, leading to a significant reduction in sludge production [38]. Developing the presented mathematical model has showed the importance of estimating the growth energy of such life organisms, where understanding how the microbial community develops and changes over time with organic material substrate will assist in the optimization of MFC technology. This revealed the importance of Emad formula, which calculates the growth energy of the growing systems that follows the exponential growth by knowing its growth constant [27-32]. Emad formula allows measuring the BGE in MeV or Joules, to asses the limits of energy that suitable for generating electricity using MFCs.

Conflict of Interest

The author declares that there is no conflict of interest concerning this paper.

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