Characteristics and performance of biofilms on the electrode of bioelectrical systems within industrial wastewater in fisheries

Characteristics and performance of biofilms on the electrode of bioelectrical systems within industrial wastewater in fisheries

B Ibrahim[1*], Desniar[1], P Suptijah[1] and C Anwar[1]

[1]Department of Aquatic Product Technology, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia.

*E-mail: bustamibr@yahoo.com

Abstract. Microbial fuel cell (MFC) is a bioreactor which utilizes exoelectrogenic bacteria as electrocatalysis to convert bioenergy from biochemical substances into electrical energy. This research aimed to determine the formation of biofilms attached, the electrical voltage generated from MFC and its effect to reduce biological oxygen demand (BOD), chemical oxygen demand (COD), total ammonia nitrogen (TAN), and to determine the characteristics of the microbes formed. The MFC system has a single chamber using boiled fish processing wastewater as media. Microbial density within the biofilm attached to anode and cathode of the MFC showed different microbial counts. There was quite high density on the cathode but no microbial growth on the anode, either by Nutrient Agar (NA) or deMan-Rogosa Sharpe agar (MRSA) media in plating method. The electricity produced was 0.39 V on average, the highest value was 0.49 V at 42 hours. The MFC system was able to decrease the average value of 83.0% BOD and 83.5% COD. The TAN value increased from 0.063±0.01 to 0.36±0.16 mg/L. There were 12 bacterial isolates, six grown on NA and others on MRSA. Three isolates that grew on NA had different characteristic groups of colony and six on MRSA showed six different characteristics.

Keywords: bacteria characteristics on cathode, biofilm characteristics, microbial fuel cell.

1. Introduction

The production of fish processing industry in Indonesia has increased every year. In 2010 its production amounted to 4,081,668 tons and increased to 5,375,835 tons in 2014 [1]. Water demand for production is $20m^3$ per ton of fishery products produced so that the wastewater produced will also continue to increase. The fishery industry's wastewater is in the form of blood, mucus, and fat and contains a lot of high organic matter which is characterized by high levels of biological oxygen demand (BOD), total suspended solids (TSS), and total Kjeldahl nitrogen [2]. If wastewater with high organic nutrient content is not treated in advance it can lead to a decrease in environmental quality which can reduce water quality, the occurrence of eutrophication, reduced dissolved oxygen, and siltation which ultimately causes the death of aquatic organisms. Treating wastewater so that it can meet quality standard requirements for wastewater requires high costs, and consequently many industries ignore it. Activated sludge treatment requires energy costs of up to 75% of the total energy cost of the processing unit, while the sludge disposal costs can reach 60% of the total cost of its operation [3]. Besides that, the processing of wastewater produces emissions of greenhouse gases such as CO_2, NO_2 and other volatile compounds [4].

The fisheries industry wastewater treatment can reduce organic compounds and can also produce electricity [5-8]. In the process of treating wastewater, microorganisms in activated sludge will break down the complex organic material of wastewater into simple substances as a nutrient for bacteria. Activated sludge is a complex ecosystem consisting of bacteria, protozoa, viruses, and other organisms. The activated sludge usually consists of a combination of decomposing bacteria such as *Aerobacter* sp., *Nitrobacter* sp., *Nitrosomonas* sp., and all will break down the organic material so that it decreases [1], [9]. Organic material that has been broken down will form ions. These ions become a source of electrical energy that is utilized by a bioelectrical system called microbial fuel cell (MFC). The bacteria that have the potential to transfer electrons outside the cell to insoluble electron acceptors is called exoelectrogen. Very few exoelectrogens have been directly isolated from MFCs.

Wastewater treatment combined with the MFC bioelectric system can provide energy recovery from the reaction of organic material contained so that the processing of wastewater can be a sustainable process. The results of this research are also expected to be one step forward to solve two problems at the same time, namely obtaining new and renewable energy sources and an efficient and environmentally friendly water sanitation system. This research aims to determine the characteristics of microbes formed on electrodes in relation to electrical power produced.

2. Methods

2.1. Materials and tools

The material used in this study is the boiled fish wastewater obtained from CV Cindy Group Bogor, activated sludge obtained from Nizam Zachman Jakarta Ocean Fisheries Port (PPS) wastewater treatment unit, H_2SO_4 (Merck), $K_2Cr_2O_7$ 0.25 N (Merck), Ferrous ammonium sulfate [Fe $(NH_4)_2$ $(SO_4)_2$] 0.2 N (Merck), NaOH (Merck), *phenate* (Merck), *Clorox* (Merck), $MnSO_4$ (Merck), KH_2PO_4 (Merck), *plate count order* (Oxoid). The tools used in this study were container 10 L, 1 L measuring cup, MFC single-chamber cathode-shaped water with dimensions of 7x10x10 cm, cable, aerator (Recent RC-410), hose, pumice, pH meter (TOA HM-30V), DO meter (LT Lutron DO-5510), Winkler bottle 250-300 mL (Durant), Erlenmeyer (Pyrex), UV-VIS spectrophotometer (Optima SP-300), wire scissors, Sudip, volumetric pipettes (Pyrex), multimeter (UK 830LN), Erlenmeyer 125 mL (Pyrex), burette (Pyrex), Kjeldahl tube, heating device, incubator (Memorth Germany), and petri dish (Pyrex).

2.2. Research procedure

The research procedure included four stages, namely the manufacturing of a single vessel microbial fuel cell, the initial characterization of the wastewater of boiled fish processing, testing the performance of MFCs in the form of electrical voltage and reducing pollution by biological oxygen demand (BOD), and total ammonia nitrogen (TAN), and also biofilm characterization on electrodes and waste load of shredded fish stew after MFC testing. Making MFC single chamber without a separator membrane refers to [4] with modifications. A schematic figure of the MFC system used in this experiment is included in figure 1. Rectangular lever made of a 7x10x10 cm acrylic glass. The electrodes used were copper plates and carbon graphites with a size of 7x1x0.1 cm. Electrodes that had been made were then mounted on MFC vessels.

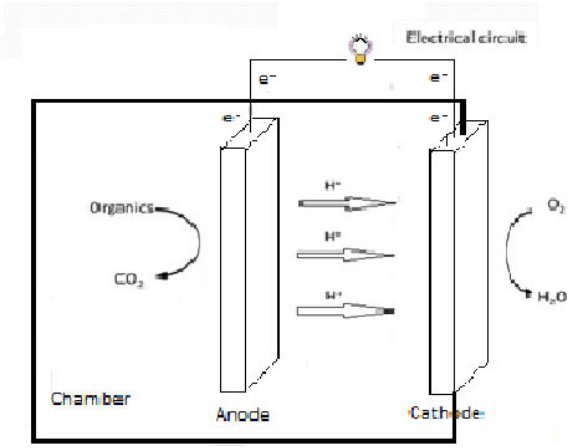

Figure 1. Schematic figure of MFC used in this study.

Activated sludge taken from Nizam Zachman Jakarta Fishing Port's wastewater treatment unit before acclimatization was used. This acclimatization was carried out by adding the activated sludge to the wastewater with a ratio of 1: 3 and giving aeration for 48 hours. Acclimatized activated sludge was put into MFC containing liquid waste with a ratio between activated sludge and liquid waste of 1:10 referring to [10] and its electricity was measured. Electricity generated was measured using a multimeter every hour for 120 hours. Each electrode was connected to a cable and the vessel was closed tightly. Both cables were connected to a multimeter. The multimeter was then set to measure electrical voltage and electric current at the smallest scale first, then the values listed on the multimeter screen were observed at certain intervals. Each analysis was carried out three times [11].

After the aforementioned process was complete, the treated wastewater quality was analyzed in the parameter of pH, BOD [12], COD [12], and TAN [12]. Furthermore, biofilm density formed on the electrodes were tested using plating method [13]. The method used nutrient agar (NA) media and deMan-Rogosa Sharpe agar (MRSA). The method that uses NA was carried out to calculate the density of general bacteria on biofilm formed, while the MRSA was used to calculate lactic acid bacteria. Biofilm formed on the surface of the MFC electrode during the 120 hours was taken using a sterile swab. Swabs and trapped biofilm cells are then transferred into a test tube containing 10 mL of KH_2PO_4 physiological solution and then diluted to obtain a dilution solution of 10^1. The solution was then diluted again with dilutions of 10^2, 10^3, 10^4, and 10^5. Afterwards, 1 mL diluent solution was put into a petri dish then mixed with NA media and incubated at 37◦C for 48 hours, then the number of colonies growing on the cup was calculated.

3. Results and Discussion

3.1. Characteristics of wastewater

The condition of organic pollution load on fishery wastewater used in MFC reactors was tested before and after 5 days of processing. This is to indicate whether or not the wastewater treatment process was going well. Characteristics of the wastewater can be seen in table 1. The results of the characteristics of the wastewater of boiled fish decoction in Table 1 shows the pH value before the process was 5.76±0.09. After a 5-day process in the MFC reactor, the pH of the wastewater increased to 7.88±0.07. An increase in pH value may be caused by the occurrence of alkaline compounds such as ammonia, trimethylamine and other volatile compounds due to protein restraint by microorganisms [14]. Based on the Ministry of Environment Regulation [15] regarding Wastewater Quality Standards, an increase in pH value is still within the threshold of fisheries wastewater quality standards namely 6-9.

Table 1. Characteristics of wastewater.

Parameter	Unit	Boiled fish wastewater before treatment	Wastewater after treatment	Standard quality of fisheries wastewater [15]
pH	-	5.76±0.09	7.88±0.07	6-9
COD	mg/L	4,256±150.26	703.54±360.38	Max 150
BOD	mg/L	468.4±1.8	79.96±4.09	Max 75
TAN	mg/L	0.063±0.01	0.36±0.16	Max 5

From testing the COD and BOD parameters, the wastewater of boiled fish decoction shows that the decrease in the parameter value was still good. COD value decreased by 83.5% from 4256±150.26 mg/L to 703.54±360.38 mg/L and BOD value decreased by 83% from 468.4±1.8 mg/L to 79.96±4.09 mg/L. Although the decline was quite high, the COD and BOD values still did not meet the quality standards. Single Chamber MFC (SCMFC) removed COD of up to 80% while generating electrical power until the maximum of 26 mW/m^2 [16]. But low coulombic efficiency in the system has indicated that a substantial fraction of the organic matter was lost without current generation.

TAN concentration is influenced by the N-ammonia oxidation process and hydrolysis of nitrogen-containing organic compounds by the microorganism [17]. The increase in TAN value is caused by the hydrolysis process of organic compounds greater than the N-ammonia oxidation process found in wastewater. Although the TAN concentration increased during the transformation process, it was still in accordance with the quality standard for fisheries wastewater which is a maximum of 5 mg/L based on the Ministry of Environment Regulation [15].

3.2. Electricity from MFC of boiled fish processing wastewater

The electrical value of the microbial fuel cell (MFC) reactor in the treatment of boiled fish processing wastewater with added activated sludge can be seen in figure 2.

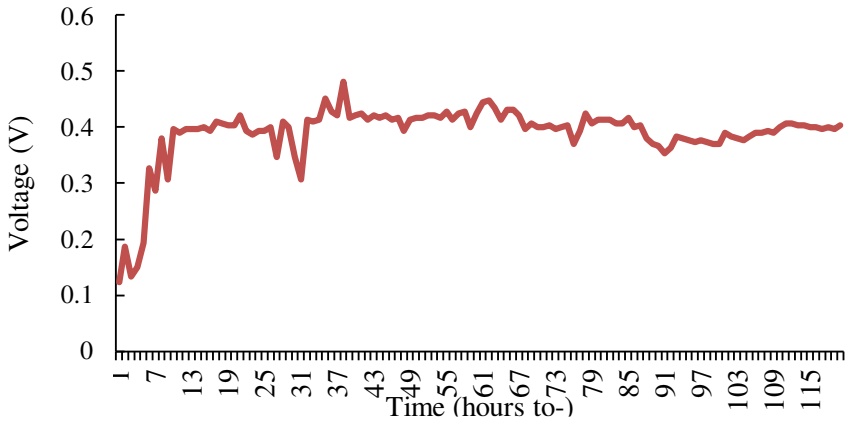

Figure 2. The electricity value of the microbial fuel cell reactor.

The results of the electrical microbial fuel cell system with the substrate wastewater of boiled fish decoction and the addition of activated sludge fluctuate are shown in figure 2. The generated electricity tends to increase in the first 10 hours. Electrical fluctuations tend to be stable in the 10th to 120th hours. The highest electricity obtained is found in the 42nd hour with a value of 0.48V and the lowest is in the 1st hour with a value of 0.12V. The average electricity produced was 0.39V.

There are 2 potential losses, namely: (1) Loss of bacterial metabolism, and (2) loss of activation [18]. Loss of bacterial metabolism is caused by differences in pH and redox ratio between products and reactants, so this is difficult to know. Fluctuations in pH and redox ratio associated with biodegradation of wastewater have the potential to cause voltage loss. Activation loss can be reduced by lowering the activation energy which then continues to increase the surface area of the anode and cathode, increase the number of catalyst electrodes (especially platinum), increase the temperature, and stabilize the amount of biofilm at each electrode.

Electricity fluctuations can be caused by interactions and competition between bacteria in utilizing organic material substrates as growth. Bioelectric energy is highly dependent on the availability of substrate [19]. Electrical energy increases with the oxidation process of the substrate until the availability of substrate is reduced. Bacteria break down organic compounds contained in waste as a source of nutrients to produce energy. Bacterial metabolic activity converts biomass in waste into electricity through a microbial fuel cell system that acts as a bioelectrochemical system. An increase or decrease in electricity is related to the number of free electrons produced by bacteria. The free electrons (apart from the valence band) that move are caused by the bonding of vibrating atoms. If inside the material is given an electric field,that is by giving a potential difference, the free electrons will move into an electrical current.

3.3. Biofilm density on MFC electrodes

Microorganisms play an important role in the microbial fuel cell system that forms the biofilm on the surface of the electrode. Biofilm is a community of microorganisms that can transfer electrons to other materials that function as recipients of electrons. To see the role of bacteria in the formed biofilm, bacterial density is calculated on the surface of the electrode. The results of the calculation of bacterial density at the electrode can be seen in table 2.

Table 2. Bacterial density in anode and cathode.

Sample	Unit	TPC	LAB Total
Activated sludge	CFU/mL	$58.5x10^7$	-
Anode	CFU/cm^2	-	-
Cathode	CFU/cm^2	$1.5x10^7$	$1.2x10^7$

Table 2 shows that there was a density of bacteria on the cathode. Whereas at the anode there was no microbial density. Meanwhile, from observations, it appears that there was a biofilm on the surface of the anode, which means there was a high probability of microbial density. The activity of bacteria in the anode takes place anaerobically. While the method of incubation of biofilm bacteria on the anode in this study was carried out under aerobic conditions, namely the total plate count method and for facultative anaerobic bacteria with a total method of lactic acid bacteria, the availability of oxygen affected the growth of aerobic and anaerobic bacteria. Bacteria that live around the anode are active bacteria in environments with low oxygen or no oxygen.

The density of bacteria on the cathode biofilm shown in table 2 based on the total plate count testing was $58.5x10^7$ CFU/cm^2 and the total lactic acid bacteria was 1.2×10^7 CFU/cm^2. This indicated that there were microbial activity and growth in the biofilm formed on the cathode.

Microorganisms that play a role in MFC are aerobic, facultative anaerobes or obligate anaerobes [20]. The density of bacteria on the cathode in total plate count testing and the total acid lactic bacteria were different. Bacteria that grow on NA media are not necessarily able to grow in total lactic acid bacteria with MRSA media and vice versa. The growth of microorganisms was influenced by the degree of acidity (pH) and organic substrates [21]. Biofilm growth on the cathode was formed homogeneously in a single

chamber microbial fuel cell system [22]. Biofilm formation on the cathode can reduce power because it inhibits proton transfer, but increases coulomb efficiency because it prevents oxygen from crossing to the anode.

3.4. Isolates and characteristics of bacteria at cathodes

Microorganisms accumulated as an anode biofilm oxidize the available substrate such as boiled fish processing wastewater (i.e., the electron donor) at the anode chamber anaerobically and release electrons from the cellular respiratory chain ultimately to the anode. The anode acts as an artificial external electron acceptor. The electrons pass through an external circuit across a resistor and reach the cathode where they combine with protons and an electron acceptor like oxygen molecule in the presence of catalyst such as platinum. This flow of electrons generates electricity [23].

The bacterial isolates found in cathode biofilms were obtained from *total plate count* testing (PCA) and the total lactic acid bacteria (MRSA) were isolated in the media. Isolates were purified and characterized in the form of gram stain, motility test, protease test, and catalase test. Results of isolates and characteristics of bacteria on the cathode can be seen in table 3.

Table 3. Isolates and characteristics of bacteria on the cathode.

Code Isolate	Shape	Gram staining	Katalase analyse	Protease analyse	Motilitase analyse
IM K1 10^2 1B	Coccus	Gram Postitive	-	+	Non-motile
IM K1 10^2 2B	Coccus	Gram Postitive	-	-	Non-motile
IM K2 10^2 3B	Bacilli	Gram Postitive	+	+	Non-motile
IM K2 10^3 4B	Coccus	Gram Postitive	-	-	Non-motile
IM K3 10^1 5B	Coccus	Gram Postitive	-	-	Non-motile
IM K3 10^1 6B	Coccus	Gram Postitive	-	-	Non-motile
IM K1 10^1 7T	Coccus	Gram Postitive	+	+	Non-motile
IM K1 10^1 8T	Coccus	Gram Negative	+	-	Non-motile
IM K1 10^2 9T	Bacilli	Gram Negative	+	-	Non-motile
IM K3 10^2 10T	Bacilli	Gram Postitive	-	-	Motile
IM K2 10^1 11T	Coccus	Gram Postitive	+	-	Motile
IM K2 10^1 12T	Bacilli	Gram Postitive	+	-	Motile

There were 12 isolates produced in the study. The isolation was carried out on the plate count (PCA) to produce 6 isolates and MRSA as many as six isolates. Different isolate characteristics show different types of microorganisms. The type of microorganisms isolated on MRSA based on table 3 shows three different types of microorganisms, namely:

1) Characteristics of 1B isolate namely gram-positive with coccus shape, non-motile, protease activity and no catalase activity.
2) Characteristics of 3B isolates namely gram-positive with bacilli form, non-motile, have catalase and protease activity.
3) Isolates 2B, 4B, 5B, and 6B have the same characteristics, namely gram-positive with coccus, non-motile, no catalase and protease activity.

The type of microorganisms isolated from PCA based on Table 3 shows six different types of microorganisms, namely:

1) 7T isolates have gram-positive characteristics with coccus, non-motile forms, protease, and catalase activity.
2) Isolate 8T has gram negative characteristics with coccus, non-motile, catalase activity and no protease activity.

3) Isolate 9T has gram negative characteristics with bacilli, non-motile, catalase activity and no protease activity.
4) Isolate 10 T has gram-positive characteristics with bacilli, motile, no catalase and protease activity.
5) Isolate 11 T which is gram-positive with coccus, motile, catalase activity and no protease activity.
6) Isolate 12 T which is gram negative with bacilli form, motile contained catalase activity, and no protease activity.

Most isolated bacteria have gram-positive characters. The dominant genus found in cathodic biofilms was Nitincola due to alkaline conditions near the cathode [24]. The community of microorganisms at the cathode in the form of aerobic and facultative anaerobic bacteria which show oxygen concentration affects the composition of bacteria in biofilms. Bacteria that grow in the electrodes will be further screened in order to determine those with an electrogenic character.

4. Conclusion

The MFC reactor which was affixed to boiled fish processing wastewater treatment with activated sludge still ran well as seen from the decrease in COD by 83.5% and BOD by 83%. The electricity power produced fluctuated with the highest electrical value of 0.48V occurring in the 42nd hour. The average electricity power produced was 0.39V. Bacterial growth in PCA and MRSA media from the anode was not found. Twelve isolates from the cathode were found, six isolates from PCA and six isolates from MRSA. The six isolates from PCA formed three groups of bacteria with different characteristics, while the other six isolates from MRSA also formed six groups of bacteria with different characteristics. The bacterias' names were not known. Within ongoing research the species of found bacteria have to be identified.

References

[1] Pusat Data Statistik 2015 Kelautan dan Perikanan dalam Angka 2015 (Jakarta: Kementerian Kelautan dan Perikanan)
[2] McCarty P L, Bae J and Kim J 2011 Domestic wastewater treatment as a net energy producer–can this be achieved *Environ. Sci. Technol.* **45(17)** 7100-7106
[3] Ibrahim B, Suptijah P and Prantommy 2009 Pemanfaatan kitosan pada pengolahan limbah cair industri perikanan *JPHPI.* **12(2)** 154-166
[4] Ibrahim B, Suptijah P and Agung BS 2017 Jarak elektroda *microbial fuel cell* pada limbah cair pemindangan ikan terhadap elektrisitas dan beban pencemaran *JPHPI.* **20(3)** 561-569
[5] Ibrahim B, Trilaksani W and Apriyani D 2013 Potensi biolistrik dari limbah cair industri perikanan dengan *microbial fuel cell* satu bejana *J.Dinamika Maritim.* **3(2)** 45-55
[6] Ibrahim B, Salamah E and Alwinsyah R 2014 Pembangkit biolistrik dari limbah cair industri perikanan menggunakan *microbial fuel cell* dengan jumlah elektroda yang berbeda *J. Dinamika Maritim.* **4(1)** 1-9
[7] Ibrahim B, Suptijah and Rosmalawati S 2014 Kinerja rangkaian seri sistem microbial fuel cell sebagai penghasil biolistrik dari limbah cair perikanan *JPHPI.* **17(1)**71-79.
[8] Du Z, Li H and Gu T 2007 A state art review on microbial fuel cells: a promosing technology for wastewater treatment and bioenergy *Biotechnol. Adv.* **25** 464-482
[9] Ibrahim B 2007 *Studi penyisihan nitrogen air limbah agroindustry hasil perikanan secara biologis dengan model dinamik Activated Sludge Model (ASM)* 1. [Disertasi] (Bogor: Bogor Agricultural University)
[10] Patil S A, Surakasi V P, Koul S, Iljuwar S, Vivek A, Shouche YS and Kapadnia B P 2009 Electricity generation using chocolate industry wastewater and its treatment in activated sludge

based Microbial Fuel Cell and analysis of developed microbial community in the anode chamber *Bioresour. Technol.* **100** 5132-5139
[11] Suyanto E, Mayangsari A, Wahyuni A, Zuhro F, Isa S, Sutariningsih SE and Retnaningrum E 2010 Pemanfaatan limbah cair domestik ipal kricak sebagai substrat generator elektrisitas melalui teknologi *microbial fuel cell* ramah lingkungan. *Prosiding Seminar Nasional Biologi* (Yogyakarta: UGM)
[12] American Public Health Association 1999. *Standard Methods for the Examination of Water and Wastewater 20th Edition* (Washington: American Public Health Association)
[13] Food and Drug Administration *Bacteriological Analytical Manual Online* (Florida Food and Drug Administration)
[15] Kementrian Lingkungan Hidup 2014 *Peraturan Menteri Lingkungan Hidup Republik Indonesia Nomor 5 Tahun 2014 tentang Baku Mutu Air Limbah Cair Industri Pengolahan Perikanan* (Jakarta: KLH)
[16] Liu H, Ramnarayanan R and Logan B E 2004 Production of Electricity during Wastewater Treatment Using a Single Chamber Microbial Fuel Cell *Environ. Sci. Technol* **38** 2281-2285
[17] Dwijani W S, Budiarsa I W S and Indra N M W 2010 Efektivitas sistem pengolahan air limbah instalasi pengolahan air limbah Suwung Denpasar terhadap kadar BOD, COD, dan ammonia *J. Kimia.* **4(2)** 141-148
[18] Logan BE. 2008. *Microbial fuel cells*. Wiley (USA): Hoboken
[19] Sharma Y and Li B 2010 The variation of power generation with organic substrates in single-chamber microbial fuel cells (SCMFCs) *Bioresour. Technol.* **101** 1844–1850.
[20] Kim G T, Webster G, Wimpenny J W T, Kim B H, Kim H J and Weightman A J 2006 Bacterial community structure, compartmentalization and activity in a microbial fuel cell. *J. App. Microbiol.* **101(3)** 698-710
[21] Agustiyani D, Imamuddin H, Faridah E N and Oedjijono 2004 Pengaruh pH dan subtrat organik terhadap pertumbuhan aktivitas bakteri pengoksida ammonia *Biodiversitas* **5(2)** 43-47
[22] Ahmed J and Kim S 2011 Effect of cathodic biofilm on the performance of air cathode single chamber microbial fuel cells *Bull. Kor. Chem. Soc.* **32(10)** 3726-3729
[23] Scott K and Yu E H 2016 *Microbial Electrochemical and Fuel Cells. Fundamental and Application* (Cambridge: Elsevier) p 411
[24] Daghio M, Gandolfi I, Bestetti G, Franzetti A, Guerrini E and Cristiani P 2015 Anodic and cathodic microbial communities in single chamber microbial fuel cells *J. New Biotechnol.* **21(1)** 79-84