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Performance of Dairy Wastewater Intrinsic Bacteria in Microbial Fuel Cell

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Abstract

The use of wastewater in microbial fuel cell (MFC) simultaneously clean up the waste and generate electricity. While organic matters in wastewater serve well as electron donor, electrode-reducing bacteria are responsible for transferring electron to anode. This study investigated the performance of intrinsic bacteria in dairy wastewater in oxidizing organic content and generating electric current. The experiment was conducted in a dual chamber MFC with graphite electrodes. The variables were electrode surface area and wastewater concentration. An increase in bacterial population, a decrease in organic content (COD) and electric current obtained over the test period confirmed bacterial activity. Electric current generation was found to increase with electrode surface area. In the 1.7 L chamber with initial COD of 2500 mg/L, the maximum current of 307.6 µA and 635.12 µA 307 across a standard 1k ohm were obtained from the 78 and 150 cm^2 electrodes, respectively. Current generation was found to vary with organic concentrations. In the 1 L chamber using 78 cm^2 electrodes with initial COD of 1000 and 400 mg/L, the maximum current were lower, at 42.57 µA and 4.99 µA, respectively. Coulombic efficiency obtained from this study was in the range of 0.13-2.64%. Bacterial identification by PCR-DGGE and DNA sequencing showed that *Acidobacterium sp.* and *Azovibrio restrictus* were the predominant species on the anode with 8 anaerobic species predominated in suspension.

Keywords : microbial fuel cell; dual-chamber; dairy wastewater

Introduction

Microbial Fuel Cell (MFC) is a bio-electrochemical system that generates electric current by electrode-reducing bacteria. The simplest system was dual chamber type, consisting anaerobic anode chamber and aerobic cathode chamber, separated by proton exchange membrane (PEM). Substrates are oxidized in anaerobic anode chamber, electrons are liberated and transferred to anode by electrode-reducing bacteria. Electrons then flow to cathode through external circuit and are transferred to electron acceptor. In the meantime, protons produced at the anode chamber are exchanged to cathode through PEM [1, 2]. Under aerobic condition O_2 accepts electrons from cathode and is reduced to water. Electrode-oxidizing bacteria may accept electrons from cathode to reduce NO_3 or SO_4 to N_2 or sulfur ions or reduce CO_2 to acetate under anaerobic condition [3].

MFC can be classified by methods of electron transfer as mediated or unmediated [4]. Some bacteria poses electrochemically active redox protein (cytochrome) on the outer membrane, electron directly transfer from bacterial cell to anode [5]. Some bacterial cells have nanowire structure (pilli) that electron can pass to anode. Electron can be transferred from bacterial cell to anode through an extracellular mediator [6].

Factors influencing electricity generation in MFC includes substrate, electrode, microorganisms and electron acceptor. Although MFC is still inefficient with low electric current, it is attractive due to different types of substrate can be used. The use of organic matter in wastewater as substrate in MFC benefits in 2 ways, generating electricity and treating wastewater simultaneously. Wastewater from various sources (domestic, refinery, petroleum industry etc.) were used in previous studies [7, 8]. Substrate concentration is also an important factor, too high the concentration may have negative impact to microorganisms.

Microorganisms of exoelectrogenic type plays an important role in electricity generation, acts as biocatalyst in an anode chamber. Diverse groups of exoelectrogenic bacteria have been found in MFC. Different classes of Proteobacteria, Firmicutes and Acidobacteria phyla have shown the ability of generating electric current [9]. Several metal-reducing bacteria such as *Shewanella oneidensis, Shewanella putrefaciens* [10], *Geobactor sulfurreducens* [11], *Geothrix fermentans* [12], *Rhodoferax ferrireducens* [13] *Aeromonas sp.* [14] and *Citrobacter sp.* [15] are able to generate electricity in a mediator-less MFC. Mixed cultures from wastewater treatment sludge constitute a good source of exoelectrogenic bacteria and generate current density higher than those of pure culture [16, 17].

The main purpose of this study was to utilize wastewater from dairy industry as substrate or electron donor for generating electric current in MFC and investigate performance of intrinsic bacteria. The batch experiment was conducted in a dual chamber MFC with graphite electrode. Electric current was measured, organic content and bacterial population determined at varying operation conditions of electrode surface area and wastewater concentrations. Identification of bacterial species was carried out by molecular technic (Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) and DNA sequencing). Results from the study would reveal the potential of intrinsic bacteria in dairy wastewater treatment and utilization.

Material and Methods

Wastewater

Wastewater was collected from Kasetsart University Dairy Product Center. The organic content in the form of COD was in the range of 990-3300 mg/L, BOD 880-2570 mg/L with BOD:COD ratio of 0.7-0.8.

Dual Chamber MFC reactor

The experiments were conducted in MFC reactors of dual chamber type using graphite as electrodes. Anode chamber and cathode chamber are separated by proton exchange membrane (PEM – Nafion 117, 3cm. diameter). Two reactors of different sizes, namely MFC1 and MFC2, were employed (**Figure 1**). MFC1 consisted of 1.0 L anode and cathode chambers (9.5 cm. diameter x 18.5 cm. high) with graphite electrodes of 78 cm^2 surface area (9 cm. x 3cm. x 1 cm.). MFC2 was double in chamber volume of 1.7 L and electrode surface area of 150 cm^2. Anode chamber was in anaerobic condition while cathode chamber was aerated. Anode and cathode connected to form an external circuit with external resister (R_{ext}, 1 kΩ). Multimeter (UNI-T UT136D) was connected across the resister for voltage monitoring.

(a) (b) (c)

Figure 1 Dual chamber MFC reactor (a) schematic diagram of MFC1 (b) MFC1 (c) MFC2

Prior to starting of the experiment, the chambers were sterilized with 95% ethyl alcohol and sterile distilled water [18]. The electrodes were washed and stored in sterile deionized water. PEM was immersed in 30% hydrogen peroxide at 80°C for 1 hour, washed with deionized water, heated in sulfuric acid at 80°C for 1 hour then final washed with deionized water [19]. Wastewater in the anode chamber was purged with nitrogen gas for 30 min. to provide anaerobic condition, while water in the cathode chamber was aerated to provide O_2.

Experimental procedure on MFC

The effect of substrate concentration

To investigate the effect of substrate concentration, the experiment was conducted in MFC1 reactor. Raw wastewater (973 mgCOD/L – C2) and diluted wastewater (50% raw wastewater, 406 mgCOD/L – C1) were compared. After filling in the wastewater, voltage (E, mV) was monitored, current (I, mA) ($I = E/R_{ext}$) and power density (mA/m^2) calculated. Organic content (COD and BOD) and biomass (VSS) were determined [20] every 4 days.

The effect of electrode surface area

To investigate the effect of electrode surface, the experiment was conducted in MFC2 reactor. Electrodes of 78 (G1) and 150 cm^2 (G2) surface area were compared at 2400 mgCOD/L. In addition, the experiment with wastewater of 970 mgCOD/L was also run in MFC2 reactor to compare with experiment C2 (run in MFC1 reactor).

Performance evaluation and calculations

MFC performance in generating electric current was evaluated by Coulombic Efficiency (CE) and Power curve.

CE is defined as the ratio of total Coulombs transferred from substrate (electron donor) to the anode to maximum possible Coulombs if all substrate produced current. The total Coulombs obtained is determined by integrating the current over time [2]. The value can be calculated by the following equation.

$$CE = \frac{\int Idt}{\frac{\Delta COD}{32 \times 1000} \times 4 \times V \times 96480} \times 100\%$$

where I = current (Amp), 32 is molecular weight of oxygen, 4 is the number of electrons exchanged per mole of oxygen, 96480 is Faraday’s constant and V is the volume of liquid in anode chamber.

Polarization curve is a plot of electrode voltage against expended current density. Power curve is a plot of power density and current density. To construct the curves, external load (external resistance, R_{ext}) was varied from 62K to 0.3K ohms, the voltage across the resistance was measured for each value of R.

Molecular identification of bacteria

In this study, intrinsic bacteria was responsible for organic removal and electricity generation. In addition to determining biomass (VSS), molecular identification of the population in the reactor, both suspended and attached on anode, was also carried out. Bacterial DNA was extracted using FavorPrep™ Soil DNA Isolation Mini Kit according to the manufacturer's instruction. The 16srRNA was amplified by polymerase chain reaction (PCR) using primers 968F-GC clamp (5' CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC 3') and 1401R (5' CGG TGT GTA CAA GGC CC 3') [21]. The condition of PCR in thermal cycle consisted initial denaturation - 94°C 5 min and 30 cycles of denaturation - 94°C 1 min, annealing - 53°C 1 min, elongation - 72°C 2 min, then final extension 72°C 10 min. PCR products were separated by agarose gel electrophoresis and DNA size (base pairs) checked with DNA marker. The selected DNA bands was then run in Denaturing Gradient Gel Electrophoresis (DGGE) for separation of different DNA sequences, the individual bands were sequenced at Macrogen Inc. Co. Ltd. (Seoul, Korea) and analyzed using NCBI Blast.

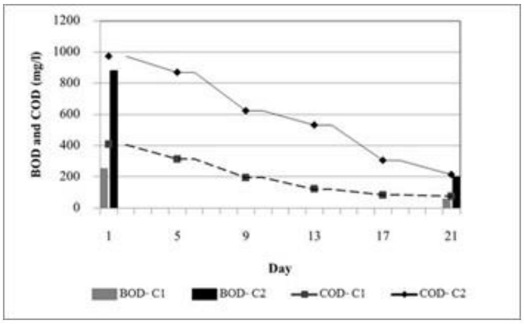
Results

1. The effect of substrate concentration

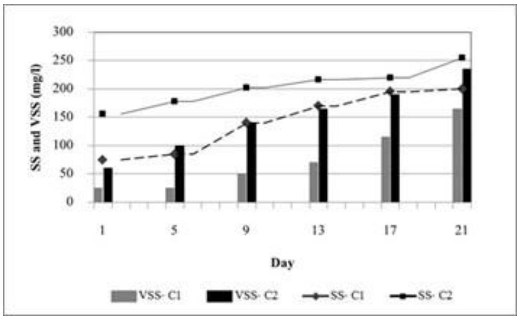
The experiment was conducted in MFC1 reactor to compare 50% diluted wastewater (C1) with 100% raw wastewater (C2), the results are presented in **Figure 2**.

In both concentrations of wastewater, organic degradation and bacterial growth proceeded in the same trend but at different rates. As shown in **Figure 2a**, in C1 initial organic content of 406 mgCOD/L decreased to 75 mgCOD/L (81.53% removal) while in C2 starting with 973.3 mgCOD/L decreased to 213.3 mgCOD/L (78.08% removal). BOD result was in agreement with COD, 76.47% and 77.30% removal achieved.

The increase in biomass (dry weight – VSS) over the test period suggested the organic removal was by bacterial activity. **Figure 2b** shows the increase in bacterial population in suspension. At the end of test period, suspended population were 165 and 235 mgVSS/L. The initial increase in population was due to the abundance of organic



(a)



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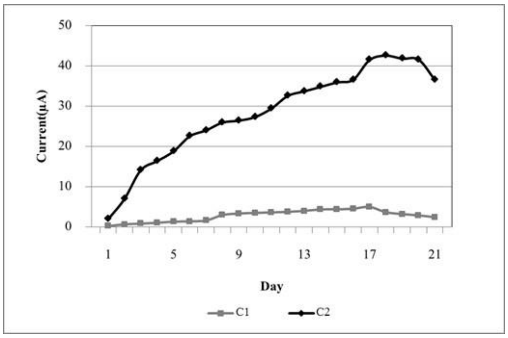


(c)

Figure 2 The performance of MFC with 78 cm^2 surface area, 1 kΩ external resistance, comparison of C1 (406 mgCOD/L) and C2 (973 mgCOD/L). (a) COD (b) dry weight of bacteria in suspension (c) electric current matter (high COD). The leveling off of the population in later stage was due to the significant lowering of COD. The difference in bacterial population in C1 and C2 showed the effect of substrate concentration on bacterial growth.

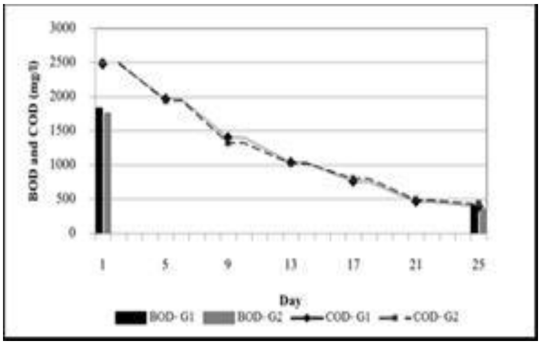
A thin layer of biofilm was found attached to the anode surface. Bacterial population on the anode was also influenced by substrate concentration. The population density of C1 anode was 1.92 mgVSS/cm^2, lower than that of C2 anode, at 3.08 mgVSS/cm^2.

Current generation in **Figure 2c** showed that electric generation began early in the experiment, requiring no noticeable acclimatization period. The current increased with time, at different rates, reaching the maximum values Since electricity generation was by bacterial activity, so it was influenced by substrate concentration, the maximum current of 4.99 µA at day 10 in C1 was much lower than 42.57 µA at day 17 in C2.

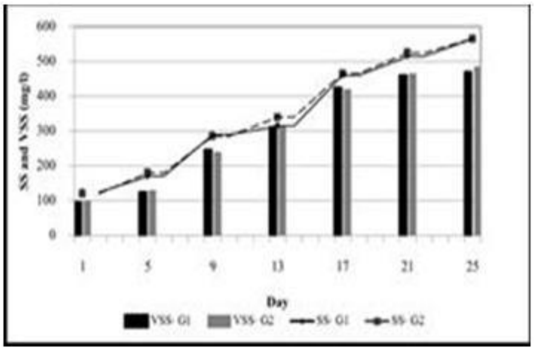
2. The effect of electrode surface area

To investigate the effect of electrode surface area, the experiment was conducted in MFC2 reactors with G1 (78 cm^2 electrode) and G2 (150 cm^2) using wastewater of the same COD range. The changes in COD, as shown in **Figure 3a**, was found to decrease continuously from the initial values of 2473 mg/L and 2499 mg/L to the final of 391 mg/L and 428 mg/L or 84.19% and 82.86% removal in G1 and G2, respectively. BOD results were in agreement with those of COD, 77.47% and 79.62% removal achieved at the end of the experiment.

Increase in bacterial population in suspension was substantial at early period, due to the abundance of organic matter (high COD). After 17 days, the population leveled off at 470 and 485 mgVSS/L (**Figure 3b**), due to the significant lowering of COD. The degrees of changes in COD and bacterial population were very much the same for G1 and G2 both of which were identical in volume. This was due to the fact that bacteria in suspension played a major role in organic (COD) removal. Despite the difference in surface area, population density of biofilm on the G1 and G2 anode were about the same, with the value of 5.1 and 5.4 mgVSS/cm^2, respectively.



(a)



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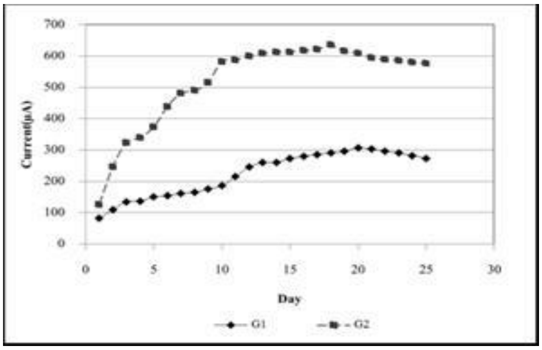


(c)

Figure 3 The performance of MFC with 1 kΩ external resistance, comparison of G1 (78 cm^2 surface area) and G2 (150 cm^2). (a) COD (b) dry weight of bacteria in suspension (c) electric current

Current generation in **Figure 3c** exhibited the same pattern as in **Figure 2c** with the current being generated from the very beginning. It gradually increased and reached maximum at 307.6 μA at day 12 in G1. The rate was significantly higher in G2 and the current reached 600 μA at day 10.

3. Comparison of MFC performance under different test conditions

Experimental data obtained from all test conditions were compared in **Table 1**. It should be noted that, under all conditions in the study, overall removal of organic matter brought about by population both in suspension and attached on cathode, were within the range of 78-84%. Capodaglio et al [22] conducted a study on single cell MFC using urban wastewater inoculated with mixed sludge. They found the average COD removal efficiency was 86%, about the same value obtained in this study. Although all the organic removal results in this study were about the same, results of electricity generation were substantially different.

Increase in population density of bacteria, both suspended and attached, correlated well with that of wastewater concentration, while the variation in electrode surface did not. G1 and G2 with CODi in the range of 2400 mg/L but different electrode surface (78 and 150 cm^2) had the same population density. The population density in C2 (CODi 973 mg/L, 78 cm^2) was also close to that of MFC2 (CODi 970 mg/L, 150 cm^2). Meanwhile those in C1 (50% wastewater) was lower than in C2 (100% wastewater) with the same electrode size were much different.

For electricity generation, the effect of electrode surface area was investigated by comparing the runs of different electrode sizes but the same range of initial COD, G1 with G2 (2400 mgCOD/L) and MFC2 with C2 (970 mgCOD/L). It can be seen that doubling of the electrode area resulted in doubling in maximum current generation and Coulombic Efficiency (CE). Maximum current and CE of 307.6 μA and 1.14 obtained in G1 (78 cm^2) was lower than 635.12 μA and 2.64, in G2 (150 cm^2). Meanwhile maximum current and CE of 42.57 μA and 0.56 obtained in C2 (78 cm^2) was lower than 118 μA and 1.03 in MFC2 (150 cm^2).

The effect of wastewater concentration on current generation seemed to be stronger than that of electrode size. Comparing the runs of different concentrations but the same electrode size, C1 with C2 (78 cm^2 electrode area) and G2 with MFC2 (150 cm^2), it can be seen that doubling in initial COD resulted in more than 5 times increase in maximum current and CE.

Table 1 Summary of MFC performance under different conditions

	G1	G2	MFC2	C1	C2
Volume (L)	1.7	1.7	1.7	1.0	1.0
Electrode size (cm^2)	78	156	156	78	78
CODi (mg/L)	2473.5	2499	970	406	973.3
CODf (mg/L)	391	428	213	75	213
%RemovalCOD	84.19	82.86	78.08	81.53	78.08
%RemovalBOD	77.47	79.62	79.38	76.47	77.30
Suspended biomass (VSS-mg/L)	470	485	282	165	235
Biofilm biomass (VSS-mg/L)	5.1	5.4	4.27	1.92	3.08
maxCurrent (μA)	307.6	635.12	118	4.99	42.57
Coulombic Efficiency	1.14	2.64	1.03	0.13	0.56

Maximum current and CE of 4.99 μA and 0.13 obtained in C1 (406 mgCOD/L) was much lower than 42.57 μA and 0.56 in C2 (973.3 mgCOD/L), while 118 μA and 1.03 of MFC2 (973.3 mgCOD/L) was much lower than 635.12 μA and 2.64 of G2 (2499 mgCOD/L).

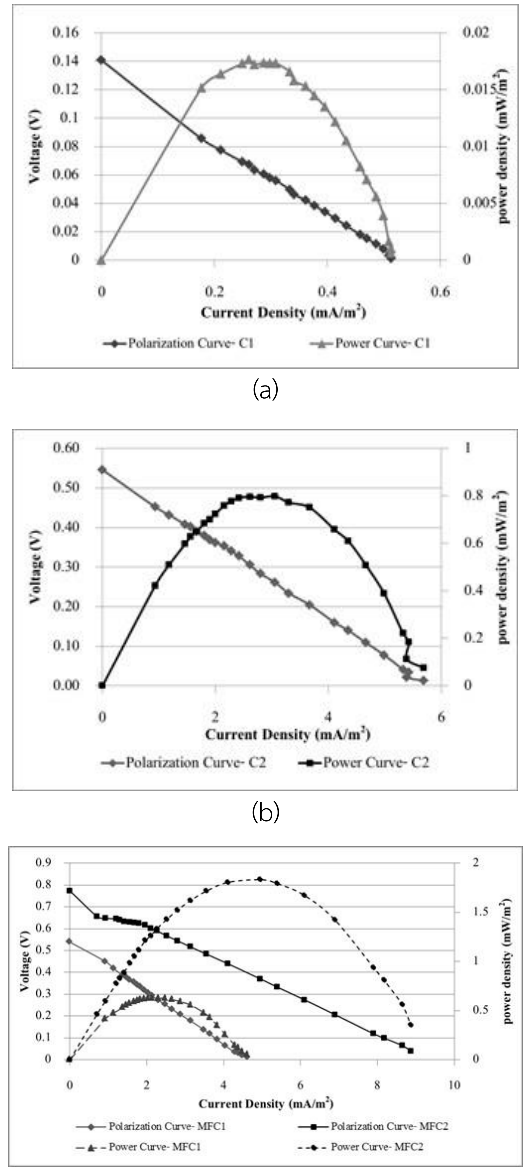
Polarization curve presents voltage as a function of current density. Current drawn from the cell to the external load (R_{ext}) entails a drop in the electrode voltage. The slope of curve indicates how well the cell can supply current (and energy) to the external load or how much the cell electrical generation capacity is affected by the external load. The steeper the curve, the more affected it is. Another indicator on the performance of MFC is the plot of power against current density. The plot shows how much power can be drawn from the cell, and at what current level.

Comparing the curves of C1 and C2 in **Figures 4a** and **4b**, large difference in the magnitude of voltage and power can be observed. The maximum power of 0.799 mW/m^2 was reached when the current density was 3.05 mA/m^2 and the cell voltage was 0.26 V. These were several times higher than the 0.018 mW/m^2, 0.33 mA/m^2 and 0.05 V obtained from C1. Electrode size also enhanced MFC performance. MFC2 (**Figure 4c**), with the same COD as C2 but larger electrode size, yielded the highest power of 1.833 mW/m^2 at 4.94 mA/m^2 and 0.37 V.

4. Identification of bacteria in biofilm

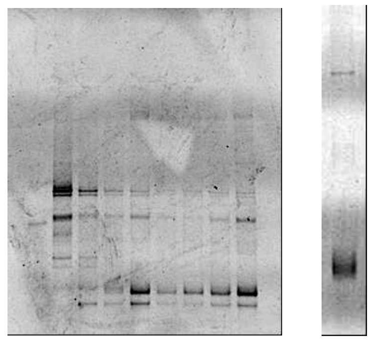
Using DGGE techniques to separate bacterial consortium in MFC (samples were taken from suspension every 4 days over the test period and biofilm at the end of the test period), different DNA bands were observed. DNA bands obtained from the samples in suspension (**Figure 5a**) shows that some bands existed throughout the test period while some diminished before the test ended. Less bands were obtained from biofilm (**Figure 5b**).

The result of DNA sequencing was summarized in **Table 2**. The dairy wastewater intrinsic species predominating in the suspension throughout the test period were identified



(a) (b) (c)

Figure 4 Polarization and power curves at varying external resistance of 62-0.3 kΩ.
(a) C1- 406mgCOD/L, 78 cm^2 electrode
(b) C2- 973.3mgCOD/L, 78 cm^2
(c) MFC2 - 970mgCOD/L, 156 cm^2 compared to C1



D1 ---------------------- D33 D33

(a) (b)

Figure 5 DNA bands separated by DGGE (a) in suspension at different times from initial to the end of test period (b) on biofilm at the end of the test period

Table 2 Summary of MFC performance under different conditions

	% Similarity	D_1	D_5	D_9	D_{13}	D_{17}	D_{21}	D_{25}	D_{29}	D_{33}
In suspension										
1. *Chryseobacterium sp.*	98%	✓	-	-	-	-	-	-	-	-
2. *Propionispira sp.*	98%	-	✓	✓	✓	✓	✓	✓	✓	✓
3. *Zymophilus sp.*	93%	-	✓	✓	✓	✓	✓	✓	✓	✓
4. *Azospira sp.*	92%	-	✓	✓	✓	✓	✓	✓	✓	✓
5. *Paenibacillus lactis*	84%	-	✓	✓	-	-	-	-	-	-
6. *Streptococcus sp.*	93%	-	✓	✓	-	-	-	-	-	-
7. *Azovibrio sp.*	98%	-	✓	✓	✓	✓	✓	✓	✓	✓
8. *Bacteroides sp.*	95%	-	-	✓	✓	✓	✓	✓	✓	✓
On biofilm										
1. Acidobacterium sp.	83%									
2. *Azovibrio restrictus*	83%									

as *Propionispira sp., Zymophilus sp., Azospira sp., Azovibrio sp.* and *Bacteroides sp.* On biofilm, the predominating species were *Acidobacterium sp. and Azovibrio restrictus str.* Both species have flagella or nanowire facilitating electron transfer from cytochrome to anode. The study, by Zhang et al. (2011), suggested that the type of substrate fed to MFC was a very important parameter for reactor performance and microbial community, and significantly affects power generation in MFCs [23]. However, *Azovibrio sp.* and *Bacteroides sp.* were the species in common with those found in MFC using UASB sludge as starter culture by Zhang et al. (2013) [24].

Conclusion

The use of dairy wastewater intrinsic bacteria in microbial fuel cell (MFC) to simultaneously treat wastewater and generate electricity were investigated in dual chamber reactor with graphite electrodes. The variables were electrode size and wastewater concentration.

1. Electric generation increased with the initial wastewater concentration. With initial COD of 2473, 973 and 406 mg/L (using 78 cm^2 electrode surface), the maximum current of 307, 42.57 and 4.99 µA were obtained, respectively.

2. Electric generation increased with the electrode surface area. The maximum current of 307.6 µA and 635.12 µA across a standard 1k ohm were obtained from the 78 and 150 cm^2 electrodes, respectively.

3. Bacterial identification showed that *Acidobacterium sp.* and *Azovibrio restrictus* were the predominant species on the anode with 8 anaerobic species predominating in suspension.

Acknowledgement

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References

[1] Du, Z., Li, H., Gu, T.A. 2007. A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. Biotechnology Advances. 25(5): 464-482.

[2] Logan, B.E., Hamelers, B., Rozendal, S., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K. 2006. Microbial fuel cells: methodology and technology. Environmental Science & Technology. 40: 5181-5192.

[3] Berchmans, S. Microbial Fuel Cell as alternate power tool : potential and challenges. In: Das, D. Microbial Fuel Cell, Springer Cham, 2018: 403-419.

[4] Pandi, S., Kuppam, C., Kakarla, R., Kadier, A., Jeevitha, V. Basic Principles of Microbial Fuel Cell: Technical Challenges and Economic Feasibility. In: Kalia, V.C., Kumar, P. Microbial Application, Springer, 2017: 165-188.

[5] Bretschger, O., Gorby, Y.A., Nealson, K.H. A survey of direct electron transfer from microbes to electronically active surfaces. In: Rabaey K. Bioelectrochemical Systems: from extracellular electron transfer to biotechnological application, IWA publishing; 2010.

[6] Shi, L., Dong, H., Reguera, G. 2016. Extracellular electron transfer mechanisms between microorganisms and minerals. Nature. Review Microbiology. 14: 651-662.

[7] Logan, B.E., Rabaey, K. 2012. Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies. Science. 337: 686-690.

[8] Lovley, D. R. 2008. The microbe electric: conversion of organic matter to electricity. Current Opinion in Biotechnology. 19: 564-571.

[9] Kim, B.H., Park, H.S., Kim, H.J. 2004. Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. Applied Microbiology Biotechnology. 63: 672-81.

[10] Kim, H.J., Park, H.S., Hyun, M.S., Chang, I.S., Kim, M., Kim, B.H. 2002. A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. Enzyme Microbial Technology. 30: 145-152.

[11] Bond, D.R., Lovley, D.R. 2003. Electricity production by *Geobacter sulfurreducens* attached to electrodes. Applied and Environmental Microbiology. 69(3): 1548-1555.

[12] Bond, D.R., Lovley, D.R. 2005. Evidence for involvement of an electron shuttle in electricity generation by *Geothrix fermentans.* Applied Environmental Microbiology. 71: 2186-2189.

[13] Chaudhuri, S.K., Lovley, D.R. 2003. Electricity generation by direct oxidation of glucose in

mediatorless microbial fuel cells. Nature Biotechnology. 21: 1229-1232.

[14] Chung, K., Okabe, S. 2009. Characterization of electrochemical activity of a strain ISO2-3 phylogenetically related to *Aeromonas sp*. isolated from a glucose-fed microbial fuel cell. Biotechnology & Bioengineering. 104: 901-910.

[15] Xu, S., Liu, H. 2011. New exoelectrogen *Citrobacter sp. SX-1* isolated from a microbial fuel cell. Applied Microbiology. 111(5): 1108-1115.

[16] Watanabe, K. 2008. Recent developments in microbial fuel cell technologies for sustainable bioenergy. Bioscience and Bioengineering. 106(6): 528-536.

[17] Kan, J., Hsu, L., Cheung, A., Pirbazari, M., Nealsin, K. 2011. Current production by bacterial communities in Microbial Fuel Cells enriched from wastewater sludge with different electron donors. Environmental Sciences and Technology. 45(3): 1139-1146.

[18] Juang, D. 2012. Organic removal efficiencies and power production capabilities of Microbial Fuel Cell. Process Biochemistry. 39: 1007-1012.

[19] Norgaard, C.E., Nielsen, U.G., Skou, E.M. 2012. Preparation of Nafion 117™-SnO_2 composite membranes using an ion-exchange method. Solid State Ionics. 213: 76-82.

[20] Baird, R.B., Eaton, A.D., Rice, E.W. 2017. Standard Methods for the examination of water and wastewater 23rd ed. American Water Works Association.

[21] Felske, A., Engelen, B., Nubel, U., Backhaus, H. 1996. Direct ribosome isolation from soil to extract bacterial rRNA for community analysis. Applied and Environmental Microbiology. 62(11): 4162-4167.

[22] Capodaglio, A.G., Molognoni, D., Dallago, E., Liberale, A., Cella, R., Longoni, P., Pantaleoni, L. 2013. Microbial Fuel Cells for Direct Electrical Energy Recovery from Urban Wastewaters. The Scientific World Journal [Internet] [cited 2013 Oct 5]. Available from: http://dx.doi.org/10.1155/2013/634738

[23] Zhang, Y., Min, B., Huang, L., Angelidaki, I. 2011. Electricity generation and microbial community response to substrate changes in microbial fuel cell. Bioresource Technology. 102(2): 1166-1173.

[24] Zhang, B., Zhang, J., Liu, Y., et al. 2013. Identification of removal principles and involved bacteria in Microbial Fuel Cells for sulfide removal and electricity generation. International Journal of Hydrogen Energy. 38: 14348-14355.