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Potentiality of petrochemical wastewater as substrate in microbial fuel cell

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Abstract. The petrochemical wastewater (PCW) from acrylic acid plant possesses very high chemical oxygen demand (COD) due to presence of acrylic acid along with other organic acids. The treatment of PCW by conventional methods is energy intensive. The treatment of PCW with concurrent power generation by employing microbial fuel cell (MFC) could be a potential alternative solving the problem of energy and environment. The goal of the present paper is to evaluate the viability of treating the wastewater using anaerobic sludge as biocatalyst in a dual-chamber MFC for simultaneous power generation and wastewater treatment. This study demonstrates that anaerobic sludge (AS) could work as a biocatalyst producing maximum power density of 0.75 W/m³ at current density and open circuit voltage (OCV) of 412 mA/m² and 0.45 V respectively using PCW with an initial COD of 45,000 mg/L. The COD removal efficiency and the coulombic efficiency (CE) were found 40% and 13.11%, respectively. The mechanism of electron transfer in the anode was analyzed by cyclic voltammetry (CV) and the resistances across the electrode/biofilm/solution interface were investigated by employing impedance spectroscopy (EIS). The current work proves the capability of the MFC for the treatment of acrylic acid plant PCW using anaerobic sludge (AS) as biocatalyst.

1. Introduction

The global demand for fossil fuel continues rising due to the ever-increasing population as well as industrialization leading to the generation of huge amount of wastewater. In an effort to support energy demand, research initiatives are focused on alternate and renewable energy sources[1]. Microbial fuel cell (MFC) is a bio-electrochemical system (BES) that has the potentiality to solve the problem of



present wastewater treatment and energy crisis using microorganisms as biocatalyst. Dream of massive power generation by MFC definitely has faded away but reality of a self-sustained wastewater treatment plant is still achievable[2]. Through metabolic activity, microbes can breakdown the organic compounds present in the substrate and directly convert chemical energy into electricity[3]. Different types of complex wastewaters, such as food wastewater[4], domestic wastewater[5], starch wastewater[6], POME[7] etc. have already been investigated in MFCs and have demonstrated their potentialities to be used in MFCs achieving efficient wastewater treatment with concurrent power generation. However, petrochemical wastewater (PCW) from petroleum industries has rarely been used in MFC due to their complexity in biodegradation. So far, only few literatures have shown to use PCW containing phenolic compounds along with other aromatic and aliphatic hydrocarbons[8, 9] but the performance was not satisfactory due to the complex interaction of microbes and substrates [10]. Besides, different conventional methods like adsorption[11], coagulation[12], biofiltration[13] and flocculation[14] have already been used to remove chemicals from PCW. However, those are highly energy intensive and not effective in meeting the stringent environmental standards. As far as the authors are concerned, none of them have used the PCW from acrylic acid plant in double chamber MFC. Therefore, the goal of the present study is to examine the possibilities of MFC technology to treat the PCW from acrylic acid plant by using anaerobic microorganisms. The reason for choosing the anaerobic sludge (AS) is due to its availability and its capability to host wide range of microorganisms. The overall performance of AS driven MFC fed with PCW was correlated with power generation, coulombic efficiency and COD removal efficiency. Additionally, the electron transfer mechanism was elucidated by using cyclic voltammetry (CV) and the resistances across the electrode/biofilm/electrolyte interface were determined by employing electrochemical impedance spectroscopy (EIS).

2. Materials and methods

2.1. Sample collection and inoculum preparation

Petrochemical wastewater (PCW) was obtained from local acrylic acid plant. The sample was filtered by using Whatman no.1 filter paper to remove the suspended particles and stored at 4 °C. Anaerobic sludge (AS) was collected from sampling port at the bottom of an operational anaerobic digester of a palm oil mill industry named LKPP Corporation Sdn. Bhd. (Latitude: 3.709472, Longitude: 103.102686), Gambang, Kuantan, Pahang. The AS was stored at 4 °C and used as biocatalyst in the anode of MFC.

2.2. MFC operation

The MFC setup was assembled as reported in our previous study. Briefly, cubic chambers made of plexi glass (Sunny Scientific, Shanghai, China) with a dimension of 8.3 cm × 8.3 cm × 8.3 cm were used as anode and cathode. The porous polyacrylonitrile coated carbon felt (2 cm×2 cm) was used as electrode material in all the experiments. The electrodes were pre-treated with HCl solution (1 M) and successively washed with DI water. The anode and cathode chambers were separated by Nafion117 membrane (Dupont Co., Wilmington, DE) which was pre-treated with diluted H₂SO₄ at 80 °C for 2 hr followed by washing with deionized water at the same temperature for 1.5 hrs and stored in normal deionized water before using. The electrodes were connected with titanium wire and both chambers were tightened together with screws to prevent the leakage. The anode chamber was filled with 35mL substrate (50% PCW) in accordance to the following compositions: NaHCO₃ (8mg), NH₄Cl (4mg), CaCl₂ (0.52 mg), MnCl₂·4H₂O (0.044 mg), CoCl₂·2H₂O (0.02 mg), CH₄N₂O (20 mg) and Na₃PO₄ (20 mg). The fixed amount of inoculum (5 mL) was introduced to the anode under purging with N₂ for 15 min and thereafter sealed properly to maintain the anaerobic condition. The cathode chamber was filled with 40mL KMnO₄ (0.1M) as an electron acceptor. The MFC was operated for 11days at 1 kΩ of external resistance.

2.3. Characterization of biofilm

The anode biofilm formation was analyzed by employing FESEM (JEOL JSM7800F microscope) at 5kV. 1cm² of carbon felt was cut off from the anode chamber at different days of operation and gently washed with distilled water for 10min. Then, it was soaked in 3% glutaraldehyde solution and maintained anaerobic condition for 2 hr. Thereafter, the sample was dehydrated with 10%, 20%, 40%, 60%, 70%, 80% and 100% ethanol. Finally, the sample was dried at room temperature for 2 hrs and prior imaging, it was sputtered with Pt using ion-sputtering system (10nm).

2.4. Data acquisition and calculations

The current generation data was acquired using digital multimeter with continuous data acquisition (True RMS multimeter, Fluke 289) over an external resistance of 1kΩ at regular time interval. The polarization curve was obtained using resistance from 50-20000Ω at different days of operation. The power density was calculated by Eq. 1:

$$P = \frac{VI}{v} \quad (1)$$

where, P is the power density (W/m³), V is the potential (V), I is the current (A) and *v* is the volume of anode chamber (m³).

COD of the anolyte was measured at different days of operation using was determined using a COD reactor (HACH DRB 200, Loveland, CO).The COD removal efficiency (η) was calculated using Eq. 2[15]:

$$\eta = \frac{COD_i - COD_t}{COD_i} \times 100\% \quad (2)$$

where, COD_i = initial COD (mg/L) and COD_t= COD at any time (mg/L).

The columbic efficiency (CE) was calculated by Eq.3 [15]:

$$CE = \frac{8 \int_0^t Idt}{FV_{AN}\Delta COD} \quad (3)$$

where, I= current (A), t = change in time (s), F = Faraday's constant (96485 C/mol), (A), V_{An} = Volume of substrate and inoculum in anode compartment (g/L), and ΔCOD = Change in COD concentration (mg/L) Here,8 is a constant.

2.5. Cyclic voltammetry (CV) and Electrochemical Impedance spectroscopy (EIS)

The electron transfer mechanism in anode was investigated by CV. The data was acquired by connecting the MFC with potentiostat (Autolab compact PGSTAT 204, Netherland) where the anode, cathode and Ag/AgCl (1.0 M KCl) electrode were used as working, counter and reference electrode respectively. The reference electrode was placed in the anode chamber through a luggin capillary. The potential window for CV was maintained in the range of -1.0 to +1.0 V and it was scanned at a scan rate of 1 to 20 mV/s.EIS was done to observe interfacial charge transfer resistances of the MFC anode. Similar configuration was used to record the EIS data. The EIS was conducted at OCV in the frequency range of 100 kHz–5 mHz with an applied ac signal of an amplitude of 10 mV. Results and discussion

2.6. Current vs. Time curve and polarization and power density curve

The MFC was operated with PCW as anolyte with different initial COD (100000 - 26000 mg/L) for 11 days at an external resistance of 1 kΩ. The current generation vs. time curve is presented in Figure 1(a). For all CODs the trend of current generation with time is similar which an initial increase followed by stable and decay phases. However, the overall current generation is significantly lower while using 100000 and 75000 mg/L COD. With further dilution of the anolyte to 45000 and 26000 mg/L, the current generation is significantly higher. For MFC operating with an initial COD of 45000 mg/L, there is a

steady increase in current generation until 7 days of operation where an apparent stable phase producing around $150 \pm 10 \mu\text{A}$ current is observed, however it starts declining from 9th day. The trend continues till the end of operation.

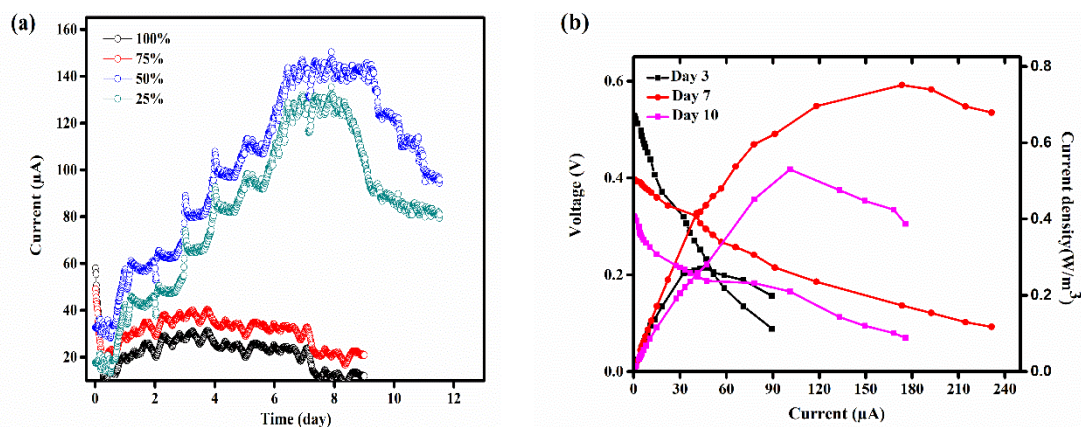


Figure 1. (a) The current vs. time curve and (b) The polarization and power curve in respect of current [PCW (PH= 7, initial COD= 45,000 mg/L)]

At high initial COD, microbial cell deactivation could occur leading to inefficient consumption of organic compounds in PCW [16]. On the other hand, at lower concentration, all the organic matters available in the substrates were consumed resulted the cell continued its stability for longer periods [16]. The MFC with 45000 mg/L COD containing PCW showed a better performance than the others and was used as substrate in the further study. The polarization curves of the MFC are presented in Figure 1(b). From Figure 1(b), it can be seen that after the 3rd day of operation, maximum power density was found to be low which might be due to the lower abundance of microbial biofilm on the anode surface [17]. After 7 days of operation, highest power was obtained. However, on day 10, power generation was dropped due to the inefficient biofilm formation comprising with large number of dead cells [18].

2.7. The COD removal efficiency and the columbic efficiency

The COD removal efficiency and the columbic efficiency as a function of time are presented in Figure 2. From Figure 2, the COD removal efficiency (η) increased steadily with time. However, the CE increased until 7 days of operation and thereafter slightly decreased. The maximum CE after 7 days of operation indicated the efficient utilization of the substrates by the microbes leading to high current generation. The drop of CE after 10 days of operation revealed the formation of inefficient biofilm, where the electrons generated by the metabolic pathway of the microbes were not reached the anode surface and the higher COD removal was due the microbial activities in the bulk. The concurrent increase of COD removal efficiency and CE with respect to time [19] suggesting that the microbial cell growth in the bulk analyte as well as on the electrode surface occurred at similar rate. However, from 7th to 10th day the increase in COD removal efficiency was not in accordance with the CE suggesting the less efficiency of the charge transfer through biofilm. The biofilm formation in the anode of the MFC was evaluated by FESEM, and presented in Fig 3(a-d).

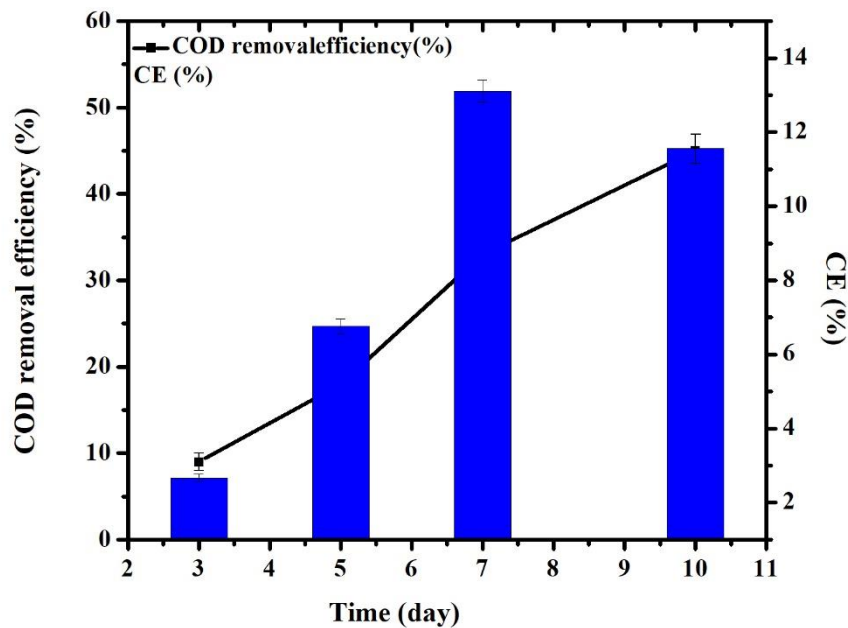


Figure 2. COD removal efficiency (%) and CE (%) with respect to time

2.8. Field Emission Scan Electron Microscopy (FESEM)

The biofilm formation in the anode of the MFC was evaluated by FESEM, and presented in Fig 3(a-d). From Figure 3 (b), it can be seen that, microbes started to form the colony on the anode surface after 3 days of operation. Due to the steady growth of the microbes in the bulk with time, after 7 days of operation, a good population of electroactive microbes were successfully attached themselves on the anode surface forming an effective electroactive biofilm leading to the drastic increase in current generation as depicted from Figure 3 (c) and Figure 1 (b). After 10 days of operation, the microbial population on the electrode surface was further increased leading to the formation of multilayer biofilm (Figure (d)). However, the power generation at this stage was reduced which might be due to the presence of outnumbered dead cells caused by the accumulation of the toxicants in the biofilm due to the diffusional resistances[20].

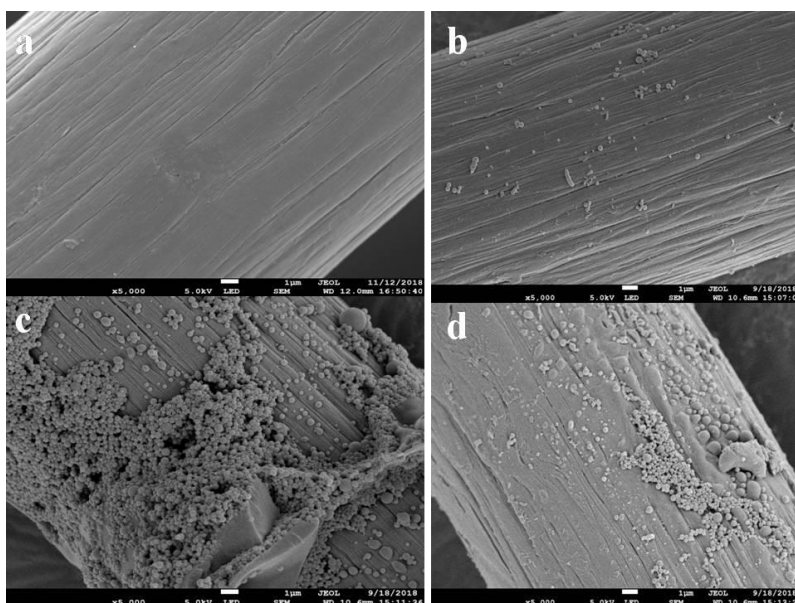


Figure 3. (a) FESEM analysis on control, (b) day 3, (c) day 7 and (d) day 10

2.9. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS)

The mechanism of electron transfer in the anode was evaluated by cyclic voltammetry (CV) and presented in Figure 4(a-c). CV provides a clear explanation of the presence of redox mediators responsible for charge transfer in anode [21]. Previous literatures reported that, microbes are capable of producing the metabolites that can transfer the electrons to the anode surface [22, 23]. Figure 4(a), represents the CV after 3, 7 and 10 days of operation along with the control (before adding the inoculum). After operation for 3 days, a redox peak (0.721 V and 0.21 V) was observed. The redox peak intensity was increased after 7 days of operation (0.76V and 0.2V) and thereafter dropped. These results suggest the production of mediators by the microbes and the very high intensity of the peaks indicates the presence of high concentration of the mediators. The presence of the redox peak suggests the indirect electron transfer via metabolites produced by the biocatalysts [24]. CV after 7 days of operation was done at different scan rates and presents in Figure 4b. The peak current is plotted vs. the square root of the scan rate (Figure 4c) where a linear dependence for both oxidation and reduction current is observed indicating the occurrence of the surface confined redox process of the metabolite which is limited by diffusion [25]. Nyquist plot (Figure 4d) represents a complex plane plot for the anode of the MFC expressing the real (X-axis) and imaginary part impedance (Y-axis) [26]. The Nyquist plot formed a semicircle at higher frequency region followed by a tail at medium to lower frequency region. The anode impedance data was fitted with an equivalent circuit $[R_s(C[R_{ct1}([R_{ct2}T]Q)))]$ shown in the inset of Figure 4(a) which present the complex behaviour of the bio-electrochemical reactions. The components of the equivalent circuit, R_{ct1} , R_{ct2} , T, C and Q describes the ohmic resistance (R_s), charge transfer resistances (R_{ct1} and R_{ct2}), tangent-hyperbolic element (T), Capacitance (C) and constant phase elements (Q). The charge transfer resistance at the electrode/biofilm interface is represented by R_{ct1} , whereas the charge transfer resistance across the biofilm/solution interface is represented by R_{ct2} [27]. The diffusion process can be characterized by diffusion resistance (R_d) which is the portion of the real axis between the vertical capacitance line and the high-frequency tail of the semicircle surface [27]. R_d is calculated by the following equation [28].

$$R_d = \frac{B}{3Y_0} \quad (4)$$

where, B= Admittance parameter and Y_0 = Time constant are obtained from EIS fitting.

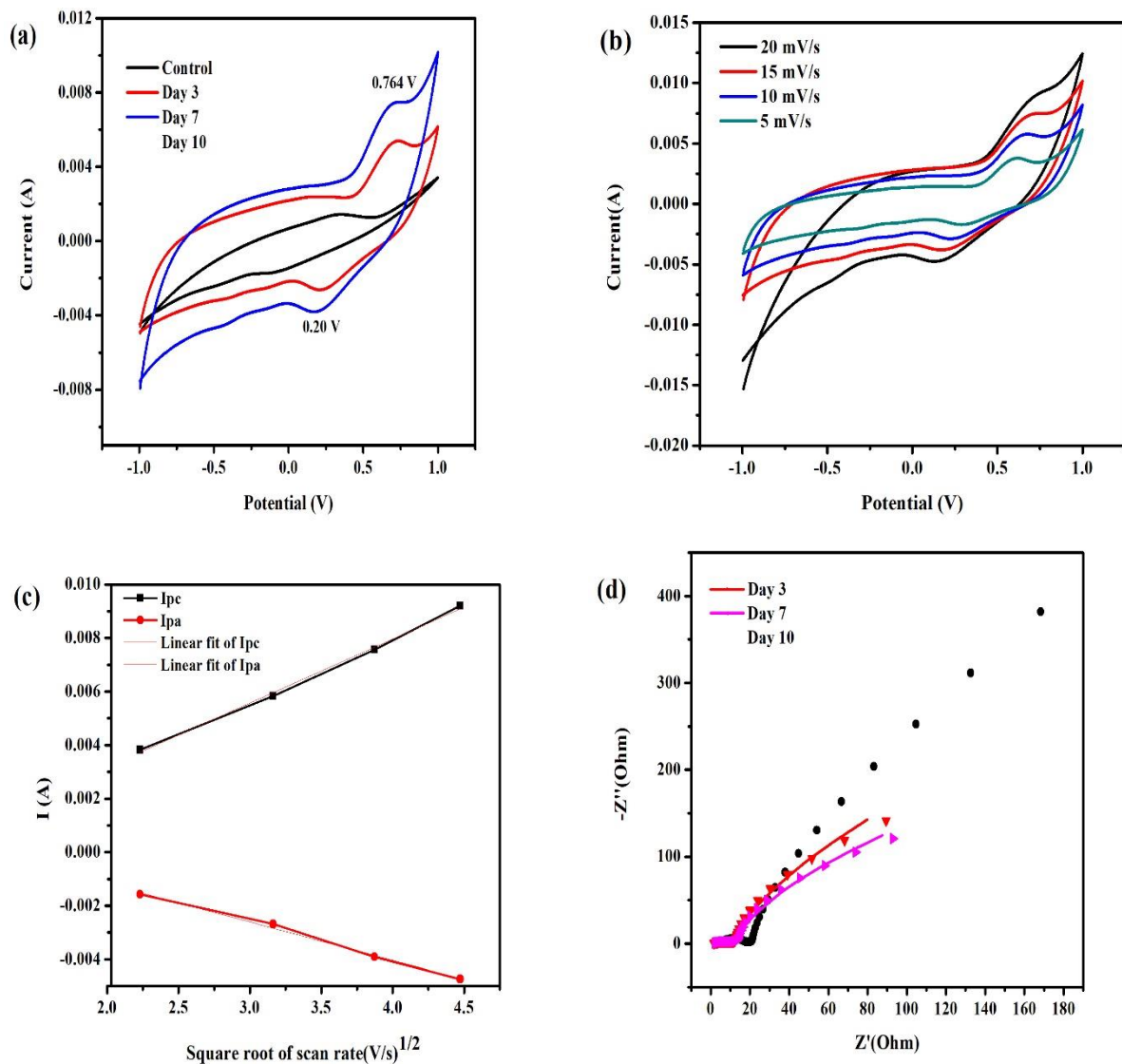


Figure 4. (a) Cyclic voltammetry on day 3, day 7 and day 10, (b) CV at different scan rates (on day 7), (c) Peak current vs. square root of the scan rate on day 7 and 10 and (d) Nyquist plot for anode 0n day 3, day 7 and day 10 respectively of the MFC

The resistances determined by the EIS data fitting with the equivalent circuit are presented in Table 1.

Table 1. EIS data fitting parameters for DC-MFC

Time	R_s (Ω)	R_{ct1} (Ω)	R_{ct1} (Ω)	R_{ct2} (Ω)	C (μF)	Q (μF)	R_d (Ω)	Total resistance (Ω)
3 d	5.62	10.7	10.7	3.16	3.64	4.2	2.88	22.36
7 d	1.50	1.77	1.77	15.7	9.08	8.81	0.08369	15.51
10 d	2.20	2.48	2.48	18.8	7.06	5.87	0.1255	18.64

The charge transfer resistances R_{ct1} for day 3 was found slightly higher than the R_{ct2} indicating a slow charge transfer between the microbes cells at biofilm as the electrode [27]. Besides, on day 7 achieved a maximum capacitance and constant phase element (which are inversely proportional to charge transfer resistance) proved that enhanced charge holding capacity on day 7 [29]. The mass transfer resistance was found to be significantly lower for day 7 compared to the other days indicating good diffusivity might be due to the lower diffusion path [30]. The total resistance on day 7 decreased than other days and this result is consistent with polarization curve and CV data. The maximum power density (W/m^3) in the present study was compared with other literature and is shown in Table 2

Table 2. Performance comparison of treatment of wastewater in MFC with the literature

Type of substrate	Chamber	Initial COD conc. (mg/L)	Electrode	Microbes	Maximum power density (W/m ³)	(COD removal efficiency) (%)	CE (%)	Ref.
PCW	Double	45,000	Carbon felt	AS	0.75 W/m ³	33	13.11	This study
PRW	Single	2,213	Carbon cloth	<i>Pseudomonas putida</i>	0.005 mW/cm ²	30	6×10 ⁻³	[8]
Dairy wastewater	Double	175.8	Carbon cloth	AS	0.038 W/m ³	-	0.6	[31]
Synthetic substrate	Double	59,500	Carbon fiber paper	AS	0.08 W/m ³	100	1.9	[32]
POME	Double	34,180	Carbon brush	AS	1.82 W/m ³	35	12	[7]

From the Table 2, it can be seen that the PCW driven MFC with anaerobic sludge as biocatalyst produced significantly higher power compared to PRW using *P. putida*, Dairy wastewater using AS and dairy wastewater using AS. PRW contains mainly phenolic compounds, whereas the PCW mainly contains the organic acids. In other kinds of wastewater such as POME the AS produced more power compared to the present study may be due to higher biodegradability of the POME substrates compared to the PCW. The CE efficiency in the present study is found higher compared to the other studies, demonstrated the capability of the AS microbes to form effective and electroactive biofilm in the PCW environment [33].

3. Conclusion

The potentiality of the treatment of PCW in MFC using mixed culture inoculum as biocatalyst was evaluated in the present study. Different concentrations of initial COD were investigated where it was found that the power generation was reduced with the increase of initial COD. Hence MFC could not be sustained while operating with raw PCW. However, the present study reveals the potentiality of lower concentration (45000 mg/L) of PCW to be treated in MFC with concurrent production of electricity (0.75 W/m³). After 10 days of operation the COD removal efficiency and CE were 45 % and 13.12% respectively. However, the maximum current generation was achieved after 7 days of operation due to the formation of an efficient biofilm which was evidenced by FESEM, CV and EIS data analysis. The presence of the redox peak in CV analysis further revealed the indirect electron transfer mechanism which could be accomplished by the metabolites produced by the microbes.

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