

# Using *C. vulgaris* assisted microbial desalination cell as a green technology in landfill leachate pre-treatment: a factor-performance relation study

Wang Jian Hui, Ewusi-Mensah David and Jingyu Huang

## ABSTRACT

Algae biocathodes have become one of the most sustainable replacements for abiotic cathodes which were expensive and had toxic chemical oxidant by-products. In this study, a pure culture of *Chlorella vulgaris* from a photobioreactor was pumped into a photosynthetic microbial desalination cell to treat real landfill leachate (had undergone physical treatment) under varying 'factor-conditions (FC)' to embark on a factor-performance relation (FPR) study. This aimed at determining the relationship between operating factors and to depict the most favourable conditions (and range) in order to boost the overall performance of the reactor/cell. Three groups of FC (A, B and C) were adapted, in that, under FC A external resistance was varied, FC B varied pumping rate and FC C varied temperature, light intensity and dissolved oxygen under conditions flow and recirculation mode. Results showed 95% chemical oxygen demand (COD) removal, a maximum power density of 121.57 mWm<sup>-2</sup> (anodic volume) and an average desalination rate of 3.93 mg/L/h. The varying results at different FC showed the significant impact of operating conditions on performance. Algae biocathodes also proved to be an essential benefit in boosting the sustainable application of microbial desalination cells (MDCs) in wastewater and landfill pre-treatment as well as the generation of bioenergy.

**Key words** | algae biocathode, factor-conditions (FC), landfill leachate, MDC performance, microbial desalination cells

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## ABBREVIATIONS

AEM	Anion exchange membrane
CEM	Cation exchange membrane
HRT	Hydraulic retention time
IEMs	Ionic exchange membranes
MDCs	Microbial desalination cells
METs	Microbial electrochemical technologies
MFCs	Microbial fuel cells
MII	Membrane International Inc.
PMDCs	Photosynthetic microbial desalination cells

FC	Factor-conditions
FPR	Factor-performance relation

## INTRODUCTION

A microbial desalination cell is a bio-based technology that has received attention due to its sustainable potential applications such as bioenergy generation, wastewater treatment, water desalination (Luo *et al.* 2012), remediation of groundwater (Tong & He 2013), water softening (Arugula *et al.* 2012), ammonia recovery and volatile fatty acid recovery (Zhang & Angelidaki 2015). Its mechanism is based on the interactions between living microbial cells and conductive electrodes through the oxidation of organic matter similar

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to microbial electrochemical technologies (METs) from which it evolved (Logan *et al.* 2015; Schröder *et al.* 2015; Hernández 2016; Jingyu *et al.* 2017).

Many studies have been carried out in the past few years to advance this technology and make it more efficient and effective owing to its limitations, similar to other biological and membrane-based technologies (Franks & Nevin 2010; González Del Campo *et al.* 2013). These limitations include the use of expensive catalyst (supply oxygen as an electron acceptor) and toxic chemical oxidants (Logan 2007; González Del Campo *et al.* 2013; Kokabian & Gude 2013, 2015; Luo *et al.* 2017), poor cathode oxygen reduction reaction (ORR) (Franks & Nevin 2010; Zhang *et al.* 2016), difficulty in scale-up (Shehab 2014; Sevda *et al.* 2015), high internal resistance (Zhang 2012), anode material (the need for more conductive material with large surface area) (Gálvez *et al.* 2009; Kalathil *et al.* 2017), cathodic limitation (Rismani-Yazdi *et al.* 2008), the use of membranes (Logan *et al.* 2015), conductivity water loss and anodic pH variation (Zhang & Logan 2009; Chen *et al.* 2012; Ge *et al.* 2014; Ping 2016; Borjas *et al.* 2017) and electron transfer limitations (Watson & Logan 2011). These constraints have been proven experimentally to have a substantial impact on the overall performance of the technology.

Albeit, the impact some operating factors have on the reactor's performance is more significant than others, it is essential to understand that the presence or absence of one factor (or condition) can influence the impact another factor (or condition) has on the reactor's performance and efficiency (Jingyu *et al.* 2017). Fan *et al.* (2008) grouped power output limiting factors including efficiency of electron transfer from microorganisms to electrodes, electrode surface area, the resistance of the electrolyte into the categories of kinetic, and ohmic and transport limitations. They suggested that performance can be improved when these factors are considered in the reactor configuration and operation modification. Other studies have addressed and proven that some factors are responsible for performance decline. They also discovered how these factors and conditions are related to each other. For that reason, numerous techniques, materials and sustainable alternatives including recirculation (Qu *et al.* 2012), biocathodes (Milner *et al.* 2016), algal-biocathode (Kokabian & Gude 2013), stacked structured microbial desalination cell (MDC)

(Kim & Logan 2013), carbon brush (Roy *et al.* 2016; Kalathil *et al.* 2017; Santoro *et al.* 2017a, 2017b), materials with capacitive adsorption properties (Forrestal *et al.* 2012a, 2012b, 2015; Yuan *et al.* 2012), bipolar membranes (Huang & Xu 2006; Mikhaylin 2015; Chen *et al.* 2016; Ghassemi & Slaughter 2017), ion exchange resins (Morel *et al.* 2012; Zuo *et al.* 2016), as well as different reactor configurations (Kim 2009; Logan 2007; Logan *et al.* 2015; Saeed *et al.* 2015; Carmalin Sophia *et al.* 2016; Jingyu *et al.* 2017) have been introduced to address the limitations in MDC.

The use of the passive algae biocathode, as the key supply of oxygen in the process of photosynthesis, is one of the sustainable solutions to the limitation of the use of an expensive catalyst. The chlorophyll-containing algae uptake carbon dioxide from the atmosphere during the process of photosynthesis and release oxygen as a by-product. The oxygen is therefore used as an electron reducing agent at the cathode thereby completing the circuit (Zhao *et al.* 2006; Rismani-Yazdi *et al.* 2008; González Del Campo *et al.* 2013; Kokabian & Gude 2013, 2015; Gajda *et al.* 2015; Jingyu *et al.* 2017; Saba 2017; Zamanpour *et al.* 2017). It has proven to be a direct mediator in electron transfer and reduces ohmic and charge transfer resistance losses which directly boosts general performance in MDC and other related technologies (Demirbas 2011; Kakarla & Min 2014). Algae can be introduced into either the anodic or the cathodic compartments, and in that light, a variety of species have been studied under different electrolytes in various configurations, electrode materials and techniques (Kakarla & Min 2014).

In spite of this, remarkable improvement in finding solutions to some of the limitations in this technology, understanding of the relationship between the operating factors and conditions has been mildly explored. While many studies have discovered the potential of this technology, it is equally important to comprehend the mechanism, operating conditions and performance in detail. In this research, we investigate pre-treatment of real landfill leachate using a *Chlorella vulgaris* aided 'photosynthetic' microbial desalination cell (PMDC) under varying factor-conditions (operations conditions) to determine the influence these conditions have on the general performance (power density, desalination rate and chemical oxygen demand (COD) removal) of the reactor. Our results discuss the influence of inter-membrane distance, membrane surface area and other

prominent factors on performance. To the best of our knowledge, this is the first paper to report the use of *C. vulgaris* biocathode MDC in pre-treatment of real landfill leachate (anolyte) under varying operating conditions as a factor-performance relation (FPR) study.

## MATERIALS AND METHODS

### Microorganisms and electrolyte

Sludge taken from Qijiazhi wastewater treatment plant, Jilin City, was mixed with 2 g/L of glucose and left under standard anaerobic conditions at the Key Laboratory of Songliao Aquatic Environment, Changchun, China. After several days, 200 mL of the liquid culture was separated into a 250 mL baffled conical flask and fed with 0.03 g of glucose daily for a period of time to enhance the growth of microbes under favourable anaerobic conditions. A pure culture of *C. vulgaris* purchased from the Institute of Hydrobiology (Wuhan, Hubei, China), was cultured for the purpose of this study. This species of algae have a good tolerance for high levels of CO<sub>2</sub> and high efficiency in utilizing CO<sub>2</sub> through photosynthesis, and was suitable since we wanted to avoid pumping air continuously into the cathode reservoir (Kokabian & Gude 2013). Blue-green medium (BG11) was chosen as growth media due to its great influence on the growth of *Chlorella* sp. (Chia *et al.* 2013). It contained the following ingredients: NaNO<sub>3</sub> (15 g/100 mL dH<sub>2</sub>O), K<sub>2</sub>HPO<sub>4</sub> (2 g/500 mL dH<sub>2</sub>O), MgSO<sub>4</sub>·7H<sub>2</sub>O (3.75 g/500 mL dH<sub>2</sub>O), CaCl<sub>2</sub>·2H<sub>2</sub>O (1.8 g/500 mL dH<sub>2</sub>O), C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> (0.3 g/500 mL dH<sub>2</sub>O), ferric ammonium citrate (C<sub>6</sub>H<sub>8</sub>FeNO<sub>7</sub>) (0.3 g/500 mL dH<sub>2</sub>O) and EDTA Na<sub>2</sub> (0.05 g/500 mL dH<sub>2</sub>O), Na<sub>2</sub>CO<sub>3</sub> (1.0 g/500 mL dH<sub>2</sub>O). A trace metal solution included H<sub>3</sub>BO<sub>3</sub> (2.86 g/L), MnCl<sub>2</sub>·4H<sub>2</sub>O (1.86 g/L), ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.22 g/L), NaMoO<sub>4</sub>·2H<sub>2</sub>O (0.39 g/L), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.08 g/L) and Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O (0.05 g/L). The liquid pure culture was transferred into the triangular flask, sealed and placed in a light incubator for 2 days, with an incubation time of 12 h day/12 h night. The temperature was kept at an average of 25 °C. The culture was then transferred into a 2 L glass bottle after a period of time as the catholyte (photobioreactor). The

culture medium was prepared at algae to medium ratio of 1:1 mL (both medium and algae were in liquid form).

Real landfill leachate was sampled from Changchun Sandao MSW Landfill in Sandao, Erdao District of Changchun City and pre-treated at Key Laboratory of Songliao Aquatic Environment, Changchun, China. The leachate was poured into a 5 L reservoir where it was stirred continuously for 2 h with an automated stirrer. 3 L of the mixture was sieved twice, to remove the solids and particles, into a clear reservoir and allowed to settle for 48 h. The decantate was skimmed to remove the oil and grease floating on the surface. Finally, 1 L of the physically pre-treated leachate was used as the anolyte.

### PMDC set-up

The reactor was manufactured with cast acrylic cylindrical tube stock with a 4 cm and 5 cm inner and outer diameter, respectively, in our laboratory. The reactor had a working volume of 150 mL (anode chamber), 50 mL (desalination chamber) and 100 mL (cathode chamber), giving the reactor a volumetric ratio of 6:1:4 (V<sub>anode</sub>:V<sub>saline</sub>:V<sub>cathode</sub>). Each chamber had two 8 mm diameter holes connected to a 2.5 cm long cylindrical acrylic tube protruding outside the chambers as inlet and outlets. The reactor was thoroughly washed with hot water (about 60 °C) and allowed to dry in an incubator at 30 °C prior to use. Since the reactor was operated in a continuous mode (with recirculation of electrolytes), there was no need for stoppers at the openings.

The anode and cathode electrodes in the MDC reactor were made from Toray carbon fibre brushes twisted in titanium metalcore (Shenzhen Kang Preston Technology Co. Ltd, China). Anode brushes were 10 cm in length and 4 cm in diameter while the cathode brushes were 8 cm by 4 cm. All electrodes were pretreated as previously described (Feng *et al.* 2010). After pretreatment, the electrodes were inserted into the electrode hole drilled in the reactor and sealed with neutral silicone sealant and allowed to harden. All adhesives were completely dried in 24 h. Between each chamber where two flat gaskets were placed were also membranes: anion exchange membrane (AEM, AMI-7001) (between the anode and the desalination chamber) and cation exchange membrane (CEM, CMI-7000) (between the desalination and cathode chamber). Both were

purchased from Membranes International Inc., USA. Prior to use, both ion exchange membranes were immersed in 5% sodium chloride solution for 24 h and then allowed to dry before installing into the reactor.

### Start-up protocol, inoculation, and operation of MDC

The whole system was fed by three 2 L reservoir tanks containing anolyte, saline and catholyte (simple photobioreactor) solutions. Leachate used as the anolyte had an initial COD of 2,769 mg/L, conductivity of 432  $\mu\text{S}/\text{cm}$ , salinity of 216 mg/L and pH of 4.75, which was adjusted to 8.35 using  $\text{NaHCO}_3$ . The cathode chamber was also fed continuously from a 2 L algal-photobioreactor containing 1 L of concentrated algae water and BG 11 media, with a constant light supply at 5,000 Lm intensity (PL-X300D series xenon lamp, China). 500 mL of 15 g/L NaCl was introduced into the desalination chamber. Saline water was recirculated from a 2 L clear glass bottle as a reservoir.

An efficient start-up protocol, as proposed by Borjas *et al.* (2017), was adopted in that, prior to inoculation  $\text{N}_2$  gas was passed through the anolyte and the anode chamber for 2 h to ensure an anoxic environment. After the reactor was fully assembled, the electrolytes and saline water in the reservoirs were connected to the cell with 8 mm inner diameter tubes. PTFE tape was used at the connecting ends to prevent leakages. Three one-channel peristaltic pumps were used for recirculation. At a flow rate of 70 mL/min, the electrolytes were recirculated to test for leaks and to ensure there were no stagnant zones, and this lasted overnight. Recirculation was stopped as the enriched inoculum of anaerobic microbes was inoculated onto the anode brush and remained overnight without recirculation to enable the microbes to adhere to the anode brush surface. The recirculation of solutions (at an initial flow rate of 50 mL/min) was resumed for a period of time until a more stable current density was attained. Meanwhile, air was constantly supplied to the photobioreactor at 3 L/min. Then the parameter reading devices were connected placed to commence data recording in an open circuit (no external power supply was connected to increase the cell potential since this was to study MDC self-sustenance). After 24 h of operation when a stable voltage was attained, the circuit was closed with an external load of 500  $\Omega$  using a 0.5 mm

**Table 1** | Operating conditions for performance study

Factors	Monitoring action	Factor-condition group (FC)			Duration/ Days
		A	B	C	
Resistance	Resistance box: By varying load value	×			2
Pumping rate	Peristaltic pump: By pumping rate variation		×		7–14
Temperature	Air conditioner control			×	14–21
Light intensity	Xenon light source: By turning source on/off			×	14–21
Dissolved oxygen	Air pump: By turning source on /off			×	14–21

diameter titanium wire connected to the electrodes as electron collectors across a resistance box. PMDC with algae biocathode was operated in a continuous mode with varying operation conditions as summarized in Table 1. Operating conditions influencing performance were varied periodically to enhance the monitoring and interpretation of results, also shown in Table 1. Operating conditions were divided into three (A, B, C) groups according to their relations and altered at: factor study A, day 2; B, day 7; and C, day 14. Figure 1 shows the details of the experimental set-up together with the data collection point and flow directions.

### Monitoring and analysis of cell performance

The voltage ( $V$ ,  $mV$ ) produced across the potential ends of the electrodes was recorded every minute with a VersaSTAT 4 data acquisition system running on a computer software program, Versa studio. The current density ( $I$ ,  $\text{mAcm}^{-2}$ ) and volumetric power density ( $P$ ,  $\text{W}/\text{m}^3$ ) were calculated as previously (Logan 2007). Electrical conductivity, salinity, total dissolved solids (TDS), pH and dissolved oxygen (DO) measurements were carried out using DZS-706 Multi-Parameter (and pH/Oxi 340i) also running on computer software, REX Instrument Analysis software. The desalination rate ( $Q_D$ ,  $\text{mg}/\text{L}/\text{h}$ ) was calculated using Equation (1):

$$Q_D = \frac{C_0 - C_t}{t} \quad (1)$$

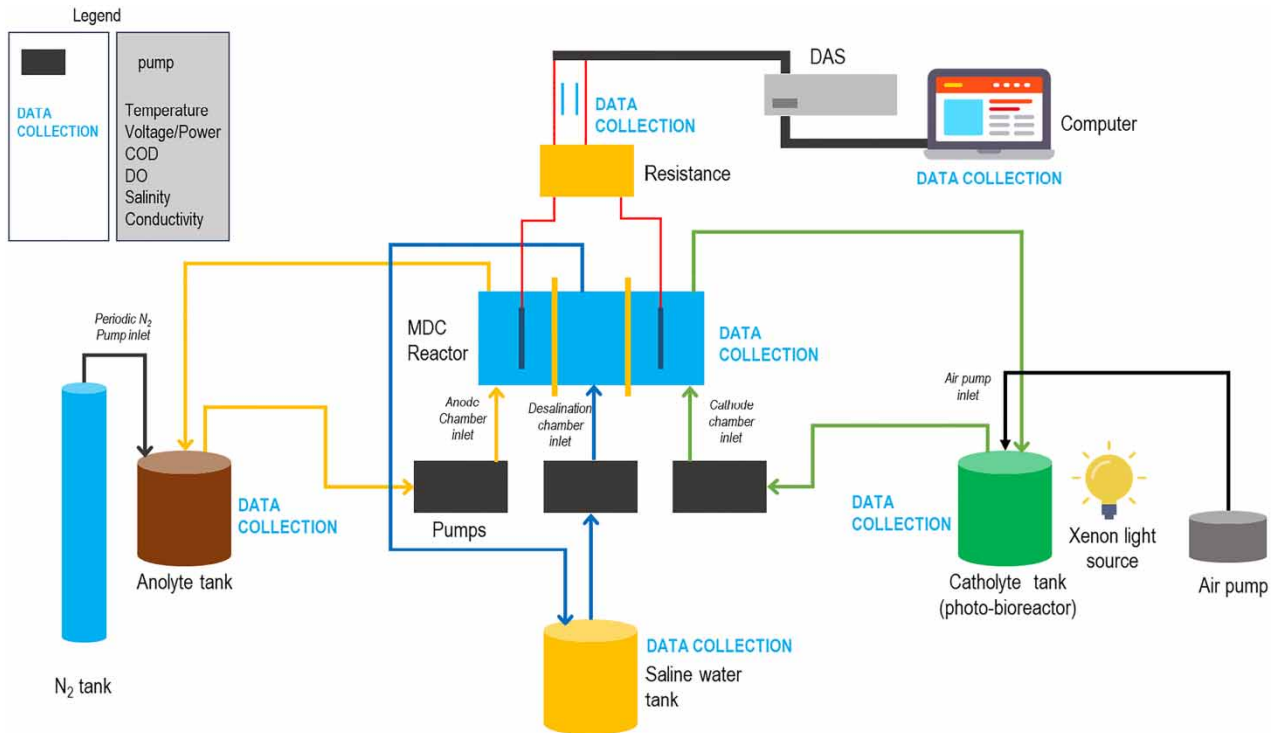


Figure 1 | Details of the experimental set-up.

where  $C_0$  and  $C_t$  are the initial and the final TDS of saltwater in the desalination chamber and  $t$  is the time period (h).

A step test was conducted to determine the performance of the cell by analysing a polarization curve from the data obtained. Using the resistance box, voltage rate of change was recorded over 20 min of each step from 9,000  $\Omega$  reducing to 10  $\Omega$ , repeatedly. These data helped to determine where voltage or power losses may have occurred and also served as a reference for how much current the cell can generate at a given voltage. By analysing these data, the maximum power possibly generated by the cell was determined as well as the corresponding current at which it was attained. We could also approximately determine the internal resistance of the MDC cell (since maximum power occurs when the external resistance is approximately equal to the internal resistance in a simple resistive circuit). Power density and current density were plotted against current to enhance the above-mentioned analysis. The COD removal efficiency was calculated by the COD difference between the influent and effluent divided by hydraulic retention time (HRT); where HRT is

the volume of the reactor (anode chamber) divided by feed rate in a continuous operation.

## RESULTS AND DISCUSSION

Performance of the PMDC was analysed in terms of electricity generation (current density,  $\text{mAcm}^{-2}$ ; power density,  $\text{mWm}^{-2}$ ), desalination or salt removal (desalination rate,  $\text{mg/h}$ ) and COD removal (COD removal and rate). Operating conditions were varied, as shown in Table 1, to enhance the analysis of the relations between factors affecting the performance of the cell in a continuous mode of operation.

### FCs influence and changes in electricity generation (voltage)

The first couple of data produced by the cell prove the efficiency of the start-up protocol adapted. After the microbes inoculated on the anode were allowed to adhere overnight, the peristaltic pumps were activated allowing monitoring

of performance parameters in an open circuit. Exoelectrogenic microbes in the anode need good contact with the anode in order to boost the electron transfer (Das 2018). It should be noted that to access the 'self-sustenance' ability of the cell, the external power source was not applied to maintain cell potential.

With an initial voltage of 388.596 mV, open circuit voltage (OCV) readings steadily increased until they reached a stable range over 24 h (1 day) of operation. Basically, at the point of OCV, no external electric current flows through the terminals (Girme et al. 2014). The highest OCV was recorded at 528 mV (Figure 2). This can be attributed to the high initial COD of the leachate available for microbial metabolism, which was reduced by 22.32% within 48 h of operation (Figure 3). The anaerobic microbes in the anode practically take some time to grow and adapt to environmental conditions and this process ideally influences the performance of the cell. Similarly, in other studies, the lag phase influenced the *in situ* oxygen generation by algae and the electron generation by anode biofilm (Kokabian & Gude 2013; Zamanpour et al. 2017). The gradual increase in voltage over time (during 48 h of operation) indicated the acclimatization of the microbial consortia forming the biofilm (Christgen 2010). FC A condition therefore proved to be very conducive as it provided a suitable temperature (25 °C), continuous air supply in the photobioreactor, light and a steady continuous flow rate (50 mL/min).

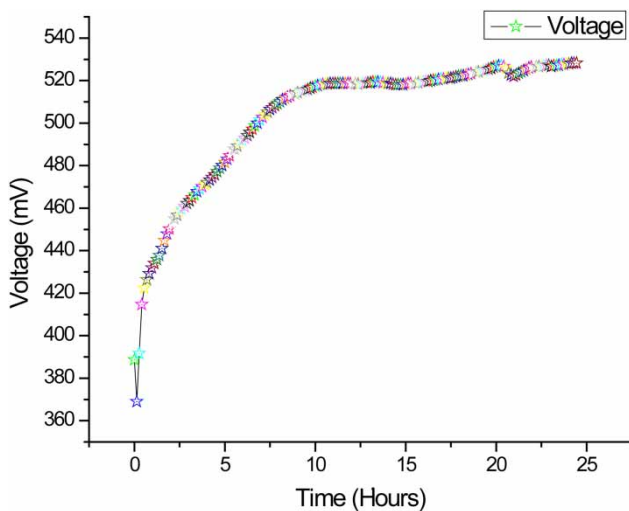


Figure 2 | OCV readings under FC A.

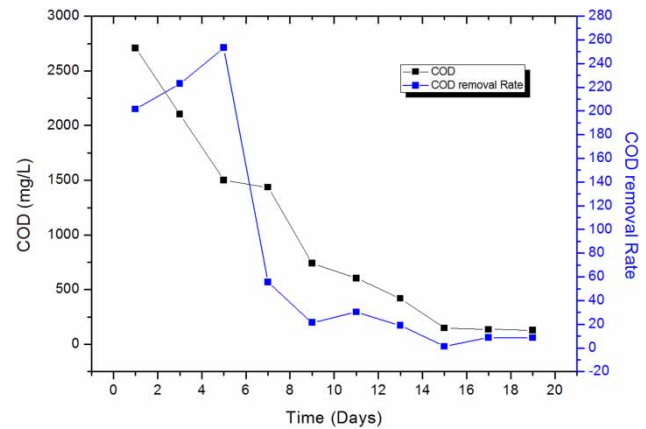
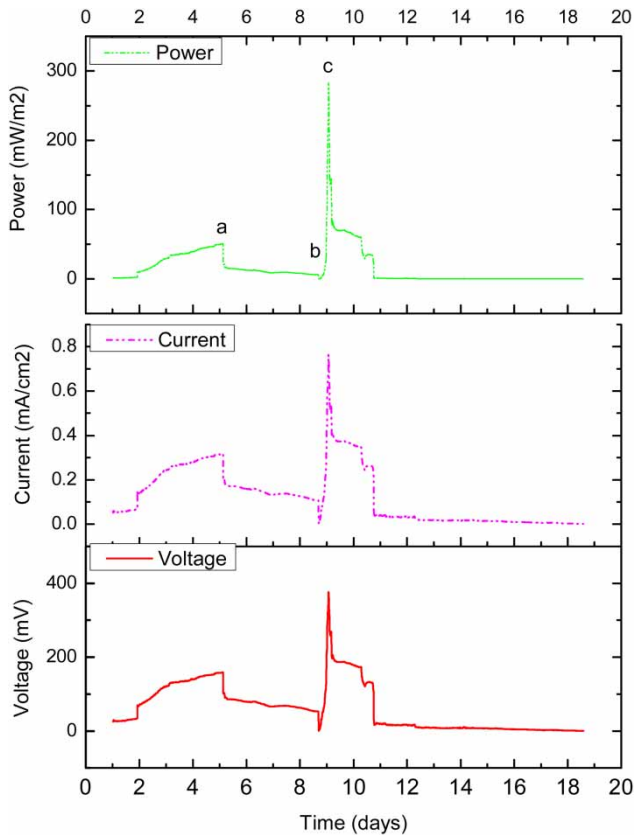


Figure 3 | The COD removal reading under FC A, B and C.

Contrarily, when the external resistance was loaded, the potential declined even further as a function of the generated current. Voltage dropped to a new starting point of 27.91 mV when the cell's circuit was closed with an external resistance of 500  $\Omega$  (under FC A conditions) after voltage readings attained stability. As seen in other studies, it might be due to (i) activation, (ii) ohmic and (iii) mass transport losses (Kumar et al. 2013; Kim et al. 2016). The current generated from the closed circuit steadily increased until it hit its two peaks at 'b' and 'c' (between days 4 and 10) of operation recording a maximum current density and volumetric power density of 0.806  $\text{mWm}^{-2}$ , 50.68  $\text{mWm}^{-2}$  and 1.924  $\text{mWm}^{-2}$ , 121.57  $\text{mWm}^{-2}$ , respectively, as shown in Figure 4. After reaching these high readings under FC A and B, voltage readings gradually declined continuously under FC C after 14 days of processing. Compared to other studies with leachate anolyte, these results are lower, probably due to the high value of the external resistance (Jambeck 2015; Sonawane et al. 2017). Higher external resistance value demands smaller current on the anode leading to rapid culture growth and acclimatization (Christgen 2010).

The large surface area of the anode brush (125.71  $\text{cm}^2$ ) provided a more thriving space for more microorganisms to adhere and minimized internal resistance of the cell. Kumar et al. (2013) observed similar results and concluded that microbes are effectively immobilized over the extended cavities of electrodes and ensure the effectual and direct electron transfer from the biological catalysts. Ultimately, it is responsible for the gradual increase in current and



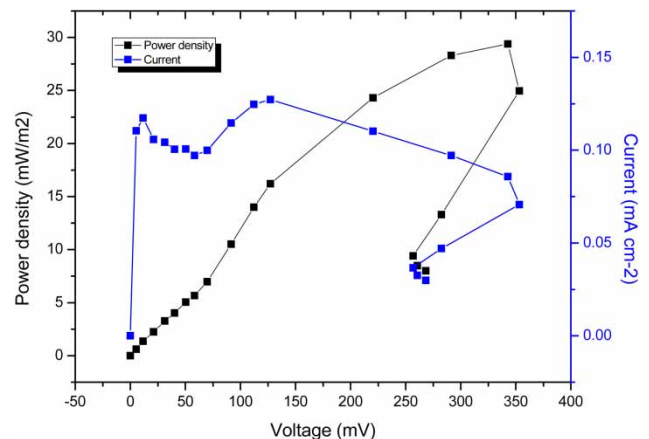
**Figure 4** | Variation of generated current, power and voltage by time in a closed circuit under FC A, B and C.

power density reading (Gálvez *et al.* 2009; Kumar *et al.* 2013; Logan *et al.* 2015) (Figure 4, label 'a' shows a break in the gradual increase). Microbial population and its metabolic activities at the anode after 5 days of continuous operation, although under other favourable conditions of FC A and B, was very low due to the high salinity of the leachate, obviously proving microbes' low tolerance to the high saline environment and complex organic matter profile in feed (Hashad *et al.* 2006; Mirbolooki *et al.* 2017). At this period of the continuous operation of the cell, about 50% of the COD concentration had been removed and this could also contribute to the low performance since microbial activities were inhibited (Dong *et al.* 2016). Therefore, general performance including current density and desalination was affected significantly, after attaining a peak at 'b' (Figure 4). Another important contributing factor to the low performance of the cell was higher internal resistance, which was approximated to be about 4,000  $\Omega$ , as seen in other studies (Rabaey *et al.* 2004; Logan *et al.* 2006;

Ye *et al.* 2013; Abourached *et al.* 2014). A number of factors contributed to the high internal resistance of the reactor. Studies have proved that the small surface area of the membrane exposed for desalination, horizontally wide reactors, and the large inter-membrane distance are contributors to the high internal resistance of the cell (Kim & Logan 2011; Taylor *et al.* 2014; Chen *et al.* 2016; Jingyu *et al.* 2017).

### Polarization characteristics

Polarization curve was measured on the 13th day of continuous operation. Figure 5 shows the electrochemical parameters obtained from the polarization curves, such as the maximum power density ( $\text{mWm}^{-2}$ ), the current density obtained at maximum power density ( $\text{mAcm}^{-2}$ ) and the internal resistance ( $\text{k}\Omega$ ). Current density increased sharply from 10 to 100  $\Omega$  and then steadily decreased until 700  $\Omega$ . It increased again reaching its maximum value and steadily decreased between 1 k $\Omega$  and 9 k $\Omega$ . The maximum power density and current density observed were 29.4  $\text{mWm}^{-2}$  and 0.127  $\text{mAcm}^{-2}$ , respectively. The maximum power density was attained at 4,000  $\Omega$  which is equivalent to an estimate of the internal resistance of the reactor. Numerous factors, already discussed in previous sections, might have contributed to the high internal resistance of the cell. Mass transfer limitation within the anode and cathode might have also contributed to this effect (concentration polarization). Concentration losses might have caused a sudden drop in the polarization curve at high current density



**Figure 5** | Polarisation curve: voltage, current density and power density.

when chemical species diffusion to the electrode surface is limited (Logan *et al.* 2006). Results were similar to studies where high internal resistance resulting in low performance was attributed to inter-membrane distance, membrane surface area, changes in medium conductivity, continuous operation, membrane, and electrolyte resistance (Huang *et al.* 2012; Ge *et al.* 2014; Taylor *et al.* 2014; Ebrahimi *et al.* 2017). This is, therefore, a major contributor to the generally low performance of the reactor in this study.

### FCs influence on microalgae performance

Algae autotrophic microorganisms that do not need to consume other microorganisms for food make them a sustainable and economic alternative biocathode (González Del Campo *et al.* 2013). The photobioreactor was a simple reservoir with algae culture with a constant air supply at 3 L/min, steady room temperature and direct light supply (5,000 Lm) under FC A and B, which supplied the cathode chamber continuously. Power density steadily increased under conditions FC A and B, until conditions under FC C which altered temperature, light source/intensity and DO/air pump. The results showed the influence of such factors in the metabolism of algae and general output, similar to the results of Commault *et al.* (2017) where they concluded that the presence of algae boosts power generation. Temperature, air (CO<sub>2</sub>) and length intensity interdependently affect the rate of photosynthesis which is the baseline process for the production of oxygen in the cathode chamber. The algae-assisted cathode is efficient *in situ* oxygenators and a facilitator of the cathodic reaction/oxygen reduction reaction (Bin Wang *et al.* 2014). In the absence of such conditions, as in FC C, the results showed a decline in DO, which was reflected in the steady decline in voltage and power reading after 14 days of operation (as shown in Figure 4). González del Campo *et al.* (2013) observed a close relationship between dissolved oxygen in the cathode and cell voltage. Therefore, the absence of favourable conditions for algae growth and metabolism, as in FC C, significantly affect the performance of algae assisted biocathode MDC. It has to be noted that during the study, the medium for algae was not replenished through the continuous process in order to observe its self-sustenance and adaptation to environmental variation. Luo *et al.* (2017)

reports the variation in performance with different species of algae, and *C. vulgaris* has performed well among other species with its strong adaptation features.

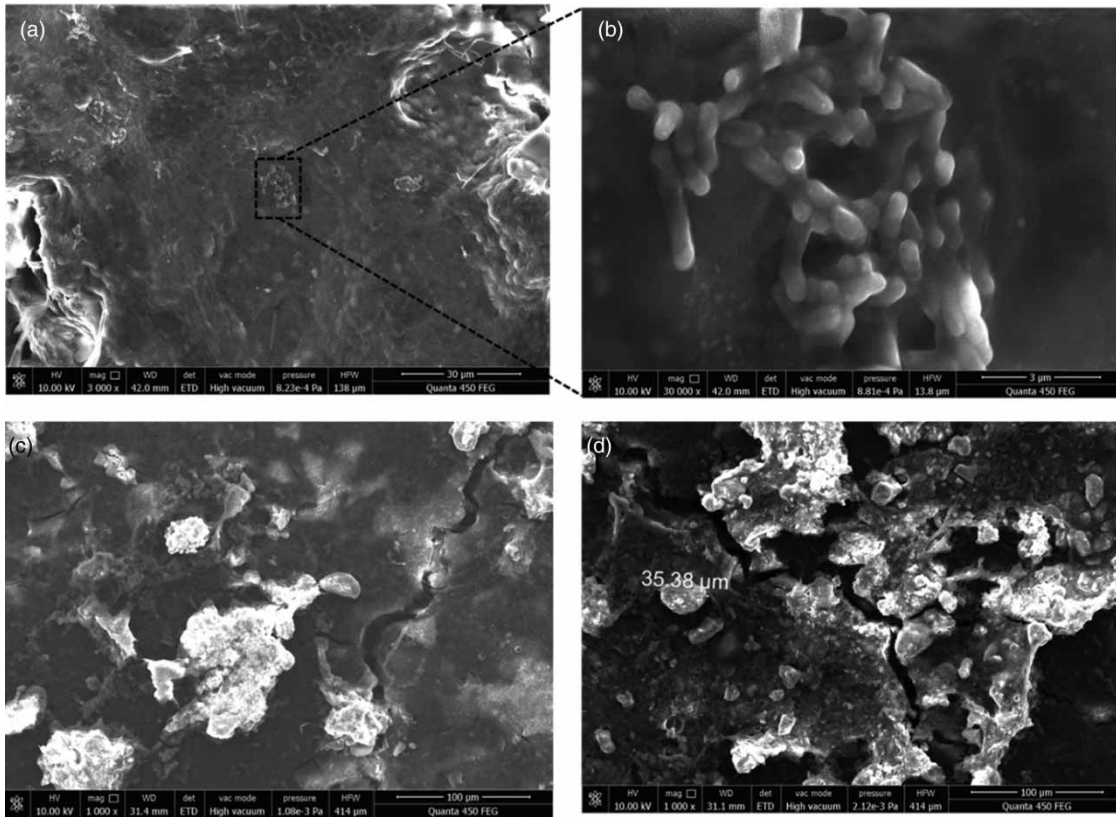
### FCs influence on COD removal/organic matter removal

The continuous operation under FC A, B and C achieved 95% COD removal (see Figure 3). The COD removal rate was high under FC A, probably due to the HRT (Ebrahimi *et al.* 2017). The COD removal rate was very high with FC A, where the pumping rate was 50 mL/min until it was changed in FC B at 10 mL/min as retention time was increased in the chamber. COD removal rates generally increase with shorter HRTs, although longer HRT is needed for efficient COD removal. Results showed (as mentioned in an earlier section) 22.32% COD removal within 2 days of continuous operation proving the influence of microbial influence and the significance of the efficient start-up protocol adapted. This start-up protocol, according to Borjas *et al.* (2017), is not only optimized for a time but also simplifies operational procedures, making it a more feasible boost to MDC potential applications and performance, especially at initial stages of performance (Mirbolooki *et al.* 2017).

### FCs influence on desalination or salt removal

The results showed a low desalination rate and efficiency as well as conductivity readings, especially at the initial stage of the experiment (data not shown). This low performance was mainly attributed to back diffusion from the anolyte chamber into the desalination stream. Apparently, the migration of ion through the AEM is mainly driven by concentration gradient which is caused by water transport, molecule transport and Donnan effect (Milner *et al.* 2016; Santoro *et al.* 2017a, 2017b). Figure 6(c) shows fouled ions on the AEM side facing the middle chamber, similar to the results of Luo *et al.* (2017), which was attributed to back diffusion, also proving the migration of ions to the middle chamber. Layers of biofouling on the AEM side facing the anode chamber are observed in Figure 6(d), which might be as a result of the organic matter profile (thus volatile fatty acid (VFA), complex organic matter, ammonia, etc.) in the landfill leachate in the anode chamber. This is a contributor to the relatively high internal resistance of the





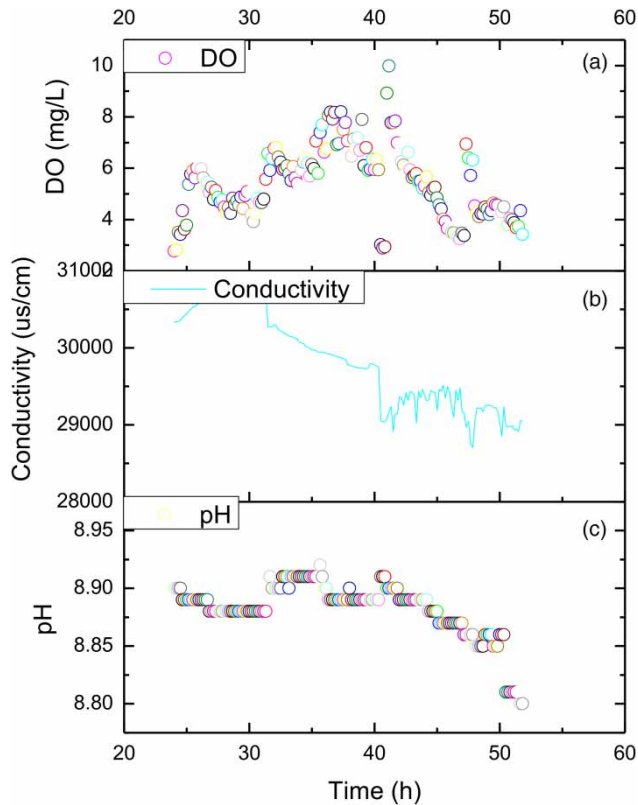
**Figure 6** | Scanning electron microscope (SEM) image: (a) CEM side facing the cathode chamber; (b) insert from (a); (c) AEM side facing desalination chamber; (d) AEM side facing chamber after 21 days of continuous study.

reactor as observed by Luo *et al.* (2012) as well as Werner *et al.* (2013), which in turn, negatively affects performance. Future studies should consider the concentration gradient comparing the anolyte and the saline water to enhance the desalination process.

Again, the surface area of membrane available and exposed to the saline water and the inter-membrane distance plays an essential role in desalination. The reactor used in this study had a membrane surface area of 5.5 cm<sup>2</sup> and an inter-membrane distance of 23 mm. Santoro *et al.* (2017a, 2017b) deduced from their study that inter-membrane was important and consequently high ohmic resistances occurred and negatively affected the overall system electrochemical performances. Ultimately, these are major challenges faced in long-term continuous operation as confirmed by Ping *et al.* (2016). Parallel studies adopting the continuous operation mode of MDC attest to the fact that it comes with various limitations which need more studies. Zhang & Angelidaki (2015) observed consistent conductivity

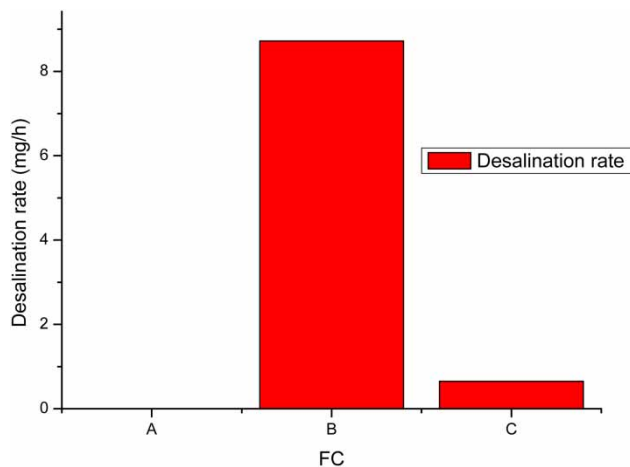
reduction with time in batch operation rather than back diffusion, proving the influence of continuous long-term operation on performance.

Nevertheless, in spite the increase in conductivity at the initial days of operation, the results showed a drastic reduction in conductivity under FC B (see Table 1 and Figure 7). HRT was increased by decreasing the pumping rate of all the solution from 50 mL/min to 10 mL/min. This is evident in the increase in salinity and conductivity of the leachate from the initial 216 to 226 mg/L and 432 to 448  $\mu\text{S}/\text{cm}$ , respectively, over 20 days of continuous operation. Similar results were seen by Taylor *et al.* (2014) as they compared the relation between inter-membrane distance and HRT, in that increasing HRT can improve desalination efficiency. Ebrahimi *et al.* (2017) indicated in their results that the type of hydraulic flow can affect the performance of an MDC. Desalination rates, as shown in Figure 8, differ at each FC. It was as high as 8.73 mg/L/h at FC B, proving the importance of such operating conditions



**Figure 7** | (a) DO, (b) conductivity and (c) pH readings.

for desalination. FC B kept an average temperature of  $27 \pm 2$  °C, 24/12 h day and night together with an external air pump. Obviously, these conditions are suitable for algae growth and metabolism, thereby proving their influence on



**Figure 8** | Desalination rate under FC A, B and C.

performance. Also, HRT was increased, which significantly boosted the desalination process.

The steady increase in the current generation a few days after the circuit was closed with an external resistance could be a contributing factor to the steady decrease in the conductivity of the saline water. Ping *et al.* (2016) observed that the current generation would inhibit the Donnan effect. pH change was not significant due to the effectiveness of the recirculation process and also less migration of ions from the desalination chamber into the anode and cathode chambers. As shown in Figure 7, their pH changes within the anolyte and catholyte (data not shown) were between 7.14 to 8.96 and 8.34 to 9.22, respectively. Similar results were observed in other studies eliminating pH imbalances using recirculation (Qu *et al.* 2012; Zhang & He 2015).

### Relationship between operating factors

From the study, a significant relationship can be deduced between the operating factors (and conditions) that were varied during the studies, some of which have been confirmed by other studies. Understanding this relationship between operating factors such as pumping rate, external resistance, temperature, reactor configuration (dimension), etc. and performance (COD removal, desalination and bioenergy generation) will aid significantly in the advancement and application of this technology. Table 2 summarizes the relationship between the factors, and between operating factors and the output of the reactor based on this study.

### CONCLUSION

In this study, algae assisted biocathode MDC was used to simultaneously reduce COD concentration in real leachate and generate electricity under the varying condition of a FPR study, and this system also proved possible in desalination of saline water. PMDC proved 95% COD removal, a maximum power density of  $50.68 \text{ mWm}^{-2}$  (anodic volume) and an average desalination rate of 3.93 mg/L/h. The study considered a variation of operating conditions classified into three groups (FC A, B and C), such that, FC A varied external resistance, FC B pumping rate and FC C temperature, light intensity and dissolved oxygen under

**Table 2** | Parameter and factor relations from results

Parameter	Factors	Relations with performance	Condition/Mechanism of influence
Resistance	External resistance or load	COD removal (%) and rate (g COD/m <sup>3</sup> day). Electric current. Anodic/cathodic potential. Desalination rate	The external load determines the electric current density (mAcm <sup>-2</sup> ) of the system, which is related to the COD removal rate, as well as the anodic and cathodic potential. In addition, desalination rate is related to current density
Flow rate	Pumping rate	HRT. Current density. COD removal	Depending on the working volume of the reactor, the flow rate determines how long (in hours) a volume of wastewater and/or saline water will stay in the chamber (reactor) to be treated thus hydraulic retention time (HRT). The higher the HRT (low flow rate), the more effective the reactor and its processes are. Both continuous and batch flow modes have a distinct influence on the performance on the cell but the most significant factor is to increase HRT in order to enhance better mass transport inside the cell
Temperature	Temperature	DO concentration. Algae growth and adaptation. Desalination rate	As biologically base technology, the temperature is very influential since it directly affects the metabolism of microbes thereby reflecting in performance. Naturally occurring microorganisms (anaerobic; exoelectrogens) function properly in a certain range of environmental conditions. There are different species depending on the tolerance of the bacteria to temperature: (1) moderate or room-level temperatures (15–35 °C); (2) high temperatures (50–60 °C) tolerated by thermophiles; and (3) low temperatures (<15 °C) where psychrophilic can grow
Light	Light intensity (irradiance: W/m <sup>2</sup> )	DO concentration. Algae growth and adaptation. Energy generation	With the introduction of biocathodes in MDC, passive algae biocathodes for <i>in situ</i> oxygen generation has become very prominent. The availability of light has significant effects on the growth and performance of the photosynthetic microalgae (biocathode) as well as the concentration of oxygen (by-product). In the presence of passive algae biocathodes, the source is light is vital for good performance
Oxygen	Dissolved oxygen	DO concentration. Energy generation	Among the numerous electron acceptors applied in MDC, oxygen has proved to be a practical terminal electron acceptor due to its high reduction potential, oxygen reduction reaction (ORR) in the presence of transferred electrons and hydrogen ions at the surface of the cathode. The power density generated by the cell as well as desalination process is highly dependent on this phenomenon. Therefore, the higher the concentration of DO, the higher the reactor's performance
Anolyte and catholyte	Anolyte concentration and ionic species	Biofouling. Back diffusion. Microbial growth, tolerance and adaptation. Buffer capacity. Substrate concentration	Composition, concentration and transport of ionic species in electrolytes play a sensitive role in the performance of the cell. With desalination, ion-concentration gradient drives the process towards a balance, otherwise is back diffusion. Organic matter composition drives energy generation; in that, the more its concentration, the more electrons generated as by-products of their metabolism. On the contrary, the presence of certain ionic species (metal ions, Ca <sup>2+</sup> , Mg <sup>2+</sup> , etc.) is known to inhibit microbial growth and cause biofouling, which impedes performance. The electrolyte also influences internal resistance. Finally, buffer capacity could improve biological activity by compensating pH imbalances inside the biofilm (avoiding acidification of the biofilm)

(continued)

Table 2 | continued

Parameter	Factors	Relations with performance	Condition/Mechanism of influence
Electrogenic microorganisms	Start-up protocol	Microbial population. Microbial growth and adaptation. Microbial electrochemical performance	As the biological driving force of this technology, its tolerance and adaptation to environmental conditions are very crucial. Apart from pH imbalance and temperature fluctuation, which have been solved by recirculation and temperature control measures, the anolyte composition is one of the major factors impeding the growth and activities of microbes and performance in the end. Adapting skilful start-up protocol is one of the most effective solutions. From culturing stage to the start-up process, microbial species can adapt resistance to extreme conditions in order to enhance their performance in the reactor
Cell or reactor	Reactor configuration	Inter membrane distance. The vertical or horizontal length. Membrane surface area. Desalination rate. Power generation	It is always a priority to reduce internal resistance in the cell to boost general performance. With reactor configuration, it is important to consider: (1) Intermembrane distance (the distance between the IEMs) – can be used to decrease ohmic losses by reducing this distance (depending on the total volume of the reactor). (2) Vertical versus horizontal length (determines the surface area available for membrane exposure) – preferably, reactors should be longer vertically than horizontally. This phenomenon helps to increase the surface area of the membrane as it reduces the intermembrane distance to enhance general performance. (3) Membrane surface area (surface of the IEMs exposed in the desalination chamber) – generally, the desalination process is significantly enhanced when a larger surface of the membrane is exposed to the saline water

conditions flow and recirculation mode. Results proved the influence and relations between these factors and general performance. The study most importantly outlined the relationship between factors and performance. In summary, external resistance is as important as the internal resistance and a good balance can boost performance. Also, a suitable pumping rate range depending largely on the reactor's dimension as well as suitable growth conditions for microbes are essentials to enhance output. This is the baseline for further studies in this technology. PMDC can, therefore, under favourable operating conditions, be applied as a sustainable pre-treatment method to landfill leachate and to generate bioenergy for other processes. It has the potential to reduce pollutant concentrations in leachate into lower concentrations in order to make actual treatment (traditional/post) more efficient and less expensive.

We also propose some issues and areas that need further investigation in this area of PMDC application:

i. The use of saline-tolerant anaerobic microorganisms in the anode chamber.

- ii. The significance of pure and mixed cultures both in the bio-anodes and cathodes and their feasibility in real-life application.
- iii. The influence of algae on ion exchange membranes.
- iv. Standards for MDC reactor configuration with respect to inter-membrane distance, membrane surface area and vertical and horizontal dimensions.
- v. Storage of the electricity produced by the cell to monitor its capabilities over time.

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