

Exploring necessity to pre-treat organic fraction of waste prior to use in an earthen MFC modified with bentonite

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ABSTRACT

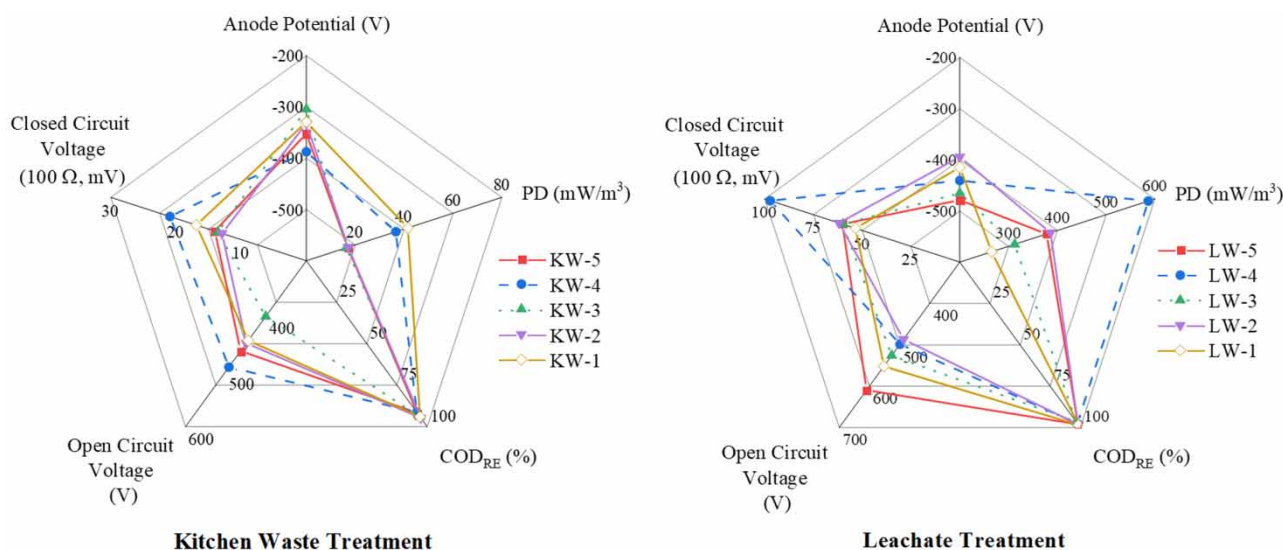
In this study, the addition of bentonite at different proportions as clay minerals and various thicknesses (4, 5, and 6 mm) of ceramic membranes were evaluated for proton and oxygen mass transfer coefficients. Bentonite (20% and 4 mm) was found to be optimum and was then employed to assess earthen microbial fuel cell (EMFC) performance for different substrates (kitchen waste (KW) slurry and leachate) under batch mode. Both substrates were added in different concentrations of chemical oxygen demand (COD), i.e., 18, 15.2, 12.5, 9.7, and 6.9 g/L to EMFCs. The EMFC achieved superior organic removals for leachate (>96%). Intriguingly, the volatile fatty acids (VFAs) generation and consumption were different for each substrate. Each system expressed affinity towards acetic acid, but limited VFAs (acetic and propionic) were generated by KW while leachate generated acetic, propionic, and butyric. The leachate concentration having COD of 15.2 g/L produced the highest power density of $586.5 \pm 38.8 \text{ mW/m}^3$, while for KW, only 41.5 mW/m^3 (6.9 g/L of COD for KW) was obtained. The study consolidates the need for an intermediate step to pre-treat the organic fraction of waste before its use for resource recovery. Bentonite was found as an effective clay mineral for manufacturing ceramic membranes.

Key words: ceramic membrane, kitchen waste, leachate, microbial fuel cell, volatile fatty acids

HIGHLIGHTS

- Bentonite (20%) at 4 mm thickness was found optimum for ceramic membrane.
- Organic removal was superior for leachate (>96%) than kitchen waste (KW).
- Highest PD of $586.5 \pm 38.8 \text{ mW/m}^3$ for leachate (LW-4) and 41.5 mW/m^3 for KW (KW-1).
- Resistance increased with KW concentration while decreased for leachate.
- Substrate inhibition and limitation show importance of substrate strength and type.

GRAPHICAL ABSTRACT



Performance of Earthen Microbial Fuel Cell

1. INTRODUCTION

The quantity of solid waste generated by communities across the world, is increasing at a steady pace, with an estimated annual generation of 3.4 billion tons by 2050 (Kaza *et al.* 2018). Almost 50% of this fraction is biodegradable, mainly kitchen waste (KW). This organic fraction can undergo different treatment techniques to obtain valuable products, such as (1) fermentation to obtain alcohols and hydrogen, (2) electricity through direct or liquefied waste (leachate) with the help of bioelectrochemical systems, (3) bio-oil and fuels by pyrolysis and hydrothermal liquefaction, etc. Among all these techniques, the bioelectrochemical system holds great potential, as it allows direct electricity generation, unlike others that require separation and purification. Nowadays, these systems are not only restricted to producing electricity, but tweaking the architecture may aid in the formation of various by-products, e.g., hydrogen, ethanol, methanol, acetate, etc. (Harnisch & Schröder 2010). The main factors governing the efficiency of valuable products generated are substrate type, operating conditions, electrode material, and system geometry and configuration. The focus of this paper is to evaluate the effect of substrate type, i.e., KW slurry and leachate, on electricity generation from microbial fuel cells (MFC). The MFC consists of cathode and anode chambers with individual electrodes separated by a cation exchange membrane (CEM). The electrons and protons generated from bio-oxidation of organics are respectively transferred by an external circuit and membrane to the cathode. Here they are reduced by an electron acceptor, oxygen (Gurjar & Behera 2020).

There are two strategies to use waste as a substrate. The first is to use waste either directly or after pre-treatment, while the other is to convert it into leachate with the help of intermediate steps. When fermented substrate comprising beef patty, potatoes, tomatoes, milk powder, biscuits, and water was fed to MFC. It reduced coulombic efficiency (CE) by 60% (Bridier *et al.* 2015). In another communication, a fungal mash-assisted hydrolysis of food waste from the cafeteria produced low power density (PD) in an air MFC Xin *et al.* (2018). El-Chakhtoura *et al.* (2014) carried out the degradation of the organic fraction of municipal solid waste (OFMSW) in an air-cathode MFCs. The study produced improved values of PD and CE due to the use of platinum as a catalyst. This improvement in the degradation of waste can be due to the presence of catalysts along with the use of manure as an inoculum. These studies show difficulties in utilizing complex organic substrate as a fuel source in the anode. The use of heterogeneous waste directly or after fermentation will require multiple metabolic pathways to allow complete catabolism of waste by electroactive bacteria (Jung & Regan 2007). Although the KW has high energy potential, its heterogeneity will suppress electricity yield.

These studies showcase the possibility of resource recovery from waste, but the experimental setting through which we can maximize recovery from KW is conflicting. Most of the studies conducted until now have been performed by waste

degradation in the anode, unlike a few studies which managed to separate the multiple metabolic pathways under different stages, i.e., two-stage treatment techniques (Cavdar *et al.* 2011; Li *et al.* 2013). The effluent from the first stage became the influent of the second stage and was then utilized to improve resource recovery. As suggested, the substrate used in an MFC must be easily consumed by exoelectrogens. Therefore, the simpler the compound, more will be the recovery of desired by-products. This deliberation suggests a requirement to have an intermediate step for waste homogenization into simple compounds, i.e., volatile fatty acids (VFAs). This task can be fulfilled by employing a leach bed reactor (LBR), which can be easily restricted to hydrolysis and acidogenesis to generate leachate. This leachate can be fed directly as a fuel in MFC with minimum limitations from the substrate for power generation. The leachate comprises hydrolyzed soluble products, which are easily consumed by the exoelectrogens. Cavdar *et al.* (2011) employed LBR to perform acidogenic fermentation. The generated leachate was subjected to air-cathode MFC at different dilutions (3 and 40%). Li *et al.* (2013) compared the performance of MFC treating acidic food waste leachate using different inoculums. Using specific inoculum instead of mixed culture will limit the non-electrogens pathway.

Further, the acceptability of MFCs can be broadened by making the CEM economical. It governs MFCs cost, and by replacing a popular polymer-based membrane such as Nafion, a low-cost alternative can be provided. The earthen membrane has many advantages, including natural availability, lower production cost, chemical, thermal and mechanical stability, along with a non-ion selective characteristic and reusability. Earthenware, as CEM, was incorporated in an MFC and performed at par with polymer membrane MFCs with an expensive cathode catalyst. Behera *et al.* (2010) incorporated earthen pot as an anode chamber in an MFC to treat synthetic wastewater. It had a total production cost of less than 1.0 USD for an anode chamber. The cation exchange capacity (CEC) and soil minerals such as alumina and silica add negative charged centers that drive proton transfer through the membrane. Ghadge *et al.* (2014) employed two clayware MFCs with red soil (CEC = 125 mmol/kg) and black soil (CEC = 20 mmol/kg) under batch mode. The study fed a synthetic substrate to generate a power density of 1.49 W/m³, which was 1.4 times the power density of MFC employing black soil. The X-ray fluorescence analysis suggested the presence of aluminum and silica in red soil as the prominent reason that led to a high CEC and power density. On these findings, Ghadge & Ghangrekar (2015) optimized the proportion of clay minerals to enhance the proton transfer of membranes made from natural soil in MFCs.

Therefore, an attempt has been made to compare the performance of an earthen MFC treating two varieties of the same waste and the development of various metabolic pathways in an anode chamber. The study aims to evaluate the addition of bentonite as a clay mineral in a locally available soil for different thicknesses.

2. MATERIAL AND METHODS

This section describes an overview of the whole experimental study, which includes membrane preparation, description of the EMFC, substrate preparation, and the various chemical and electrical analysis performed to assess the EMFC's performance. A process diagram of the study is presented in Appendix A. The EMFC was operated with two substrates, i.e., KW slurry and leachate. The slurry was prepared by the addition of KW in deionized water while the leachate was obtained from a leach bed reactor (LBR) operation. Both the substrates, after required dilution, were fed to the EMFC having an optimized earthen membrane. Before incorporating the membrane in the MFCs, the membranes were compared based on two mass transfer coefficients, i.e., oxygen and proton. After obtaining an optimized proportion of the clay mineral, the membranes were then placed in the EMFC as described in section 2.1.

2.1. Earthen membrane fabrication

The red soil (IIT-Bhubaneswar campus) was sieved through a 600 µm sieve and mixed thoroughly with bentonite in 10, 20, 30, 40, and 50% (w/w). The mix with the desired plasticity was then filled in the molds (7 cm × 9 cm) of various thicknesses, i.e., 3, 5, and 6 mm. The membranes were allowed to air dry for one week and were then baked in a muffle furnace at 670 °C for 30 min. The membrane characterization included the determination of cation exchange capacity (CEC), elemental composition, and mass transfer coefficients for oxygen and proton. The CEC was determined with the help of ASTM (2010). While the setup for determining mass transfer coefficient consists of two compartments (250 mL), joined with a rubber gasket. The earthen membrane is placed between the two compartments and later made watertight. Electrodes for measuring various parameters were placed in the measurement port provided at the top of each compartment. The membrane preparation and setup for obtaining coefficients are shown in Appendix B.

2.1.1. Proton mass transfer coefficient

The proton mass transfer coefficients (k_H , cm/s) of earthen plates of different thicknesses were calculated as suggested by Zhang *et al.* (2009). Both reactor chambers were filled with deionized water (pH ~7). HCl solution (0.1 N) was added to one chamber to adjust the pH to ~3.5. NaOH solution was then added to the other chamber to adjust the pH to ~9.0. The pH was continuously monitored for a total duration of 5 h at an interval of 15 min, using a pH meter (Orion Star A325, Thermo Fischer). The mass transfer coefficient was then calculated as:

$$k_H = -\frac{v}{2At} \ln\left(\frac{C_{1,0} + C_{2,0} - 2C_2}{C_{1,0} - C_{2,0}}\right) \quad (1)$$

where, v is the liquid volume of the anode chamber (250 mL), $C_{1,0}$ and $C_{2,0}$ = initial proton concentration in anode and cathode chamber, respectively; C_2 = proton concentration in cathode chamber at time t . The proton diffusion coefficient (D_H , cm²/s) was calculated as:

$$D_H = k_H L_{th} \quad (2)$$

where, L_{th} = average thickness of the earthen plate.

2.1.2. Oxygen mass transfer coefficient

The oxygen mass transfer coefficient for the earthen pot of different thicknesses was calculated as per the method described by Chae *et al.* (2008). Before measurement of k_o , the DO of anode chamber was made zero by purging N₂ gas. During k_o measurement, cathode chamber DO was maintained at saturated condition by a continuous supply of air. The DO was continuously monitored for a total duration of 5 h at an interval of 15 min, using a DO meter (Orion Star A123, Thermo Fischer). The value of k_o was calculated using:

$$k_o = -\frac{v}{At} \ln\left(\frac{C_{0c} - C_{0a}}{C_{0c}}\right) \quad (3)$$

where, v is the liquid volume of the anode chamber (250 mL), A is the earthen plate cross-sectional area, C_{0c} is the saturated DO concentration in the cathode chamber, and C_{0a} is the DO in the anode chamber at time t . The oxygen diffusion coefficient (D_o , cm²/s) was calculated as

$$D_o = k_o L_{th} \quad (4)$$

where, L_{th} = average thickness of the earthen plate.

2.1.3. Energy dispersive X-ray spectroscopy (EDX)

The soil samples were first oven-dried at 105 °C for 24 h and were then coated with gold employing ion sputtering (Q150R ES) before performing scanning electron microscopy with energy dispersive X-ray (SEM-EDX, 51-XXM 1004, Oxford Instruments).

2.2. Microbial fuel cell fabrication

A dual-chamber MFC having earthen membrane (EMFC) was fabricated by employing perspex glass sheets. Each chamber had an effective volume of 95 mL (4 × 4 × 7 cm). The anode and cathode chamber were separated by an earthen membrane (4 mm thick) manufactured by adding bentonite (20%) to laterite soil (w/w %). Stainless steel having a surface area of 37.5 cm² became the anode electrode, while the graphite plate (31 cm²) was utilized as a cathode. The wire used to connect the whole setup through a resistance (external) of 100 Ω was made up of copper. The schematic of the EMFC is shown in Appendix C.

2.3. Analytical methods

2.3.1. Leachate and KW slurry

The KW was obtained from the mess facility of the Indian Institute of Technology–Bhubaneswar. After sorting out inert compounds, viz., bones, plastic, and paper, the KW was used for the study. The KW was then subjected to acidogenic degradation in an LBR to generate leachate. The constituents of KW were cooked (70%) and pre-cooked food (30%). The former included rice, curries, and bread, while the latter consisted of fruits, vegetables, and peels. The various physico-chemical parameters for solid waste, i.e., moisture content (MC), volatile solids (VS), total solids (TS), pH, chemical oxygen demand (COD), and bulk density, were also determined. The MC, VS, TS, pH, and COD of waste were analyzed by [Standard Methods \(1998\)](#) and bulk density by [ASTM \(2004\)](#).

To obtain the COD contribution of KW, 0.5 g of waste was added with HNO_3 (9 mL) and H_2O_2 (1 mL) with the help of an advanced microwave digestion system (Ethos Easy, Italy). After digestion, the same solution was then used to determine the COD concentration after necessary dilution. The pH was determined from the waste slurry prepared by the addition of water as per the required run. Subsequently, the leachate obtained from LBR was subjected to analysis for pH and COD ([Standard Methods 1998](#)). The substrate's pH was measured using a pH meter. For individual VFAs (acetic (AA), propionic (PA), butyric (BA), and valeric acid (VA)), the filtered leachate after sufficient dilution was used for component analysis using HPLC (reverse phase C18-column, Thermo Fischer, Germany). The mobile phase was a combination of acetonitrile (25%) and 0.02 M KH_2PO_4 and was allowed to flow at a 0.5 mL/min flow rate and 215 nm wavelength ([Xu et al. 2011](#)).

2.3.2. Electrical performance of MFC

Daily assessment of EMFC performance was done by a digital multimeter (Keysight 34972A, Keysight). The voltage under open circuit (OCV) and closed circuit (100 Ω , external resistance) along with anode and cathode potential were monitored against a reference electrode (Ag/AgCl, +197 mV vs. Standard Hydrogen Electrode, SHE). Polarization studies were carried out by varying resistance from 10,000 to 1 Ω . From the polarization curve, internal resistance was calculated by taking the slope of the linear part of the voltage vs. current curve. The MFC was subjected to cyclic voltammetry (CV), Tafel plots, and impedance studies (EIS) with the help of 'four' electrode system potentiostat (Compact Stat-plus B09135, IVIUM Technologies). The CV and Tafel studies were performed between ± 1.0 V. The EIS was conducted in the frequency range of 1,00,000 Hz–0.1 Hz. The Tafel plots were formed by plotting the potential against \ln (current density). A range of ± 0.5 V from the potential of lowest current density (CD) was selected to plot and obtain slopes for anode and cathode using best-fit analysis.

2.4. Experimental setting

The EMFC was subjected to two types of substrates at the same organic loading under batch mode operation for four cycles. The end of the cycle was marked on either the 11th day or when OCV drops below 100 mV, whichever is earlier. The EMFC was fed with an organic load of 6.9, 9.7, 12.5, 15.2, and 18.0 gCOD (abbreviated as 1, 2, 3, 4, and 5, respectively) by both KW and leachate (LW). The details of different systems are presented in [Table 1](#).

Table 1 | Details of EMFCs system treating substrate

Sl No.	Initial COD (g/L)	System ID	COD removal efficiency (%)	Power density (mW/m ³)
1	18.0 \pm 0.25	KW-5	91.66	16.9 \pm 3.5
2	15.2 \pm 0.35	KW-4	91.34	36.6 \pm 6.6
3	12.5 \pm 0.29	KW-3	94.01	16.3 \pm 1.31
4	09.7 \pm 0.31	KW-2	94.68	17.1 \pm 4.1
5	06.9 \pm 0.24	KW-1	94.01	41.5 \pm 7.1
6	18.0 \pm 0.45	LT-5	98.01	377.9 \pm 30.2
7	15.2 \pm 0.36	LT-4	97.64	586.5 \pm 38.8
8	12.5 \pm 0.31	LT-3	96.45	386.0 \pm 55.7
9	09.7 \pm 0.24	LT-2	98.03	312.5 \pm 41.2
10	06.9 \pm 0.34	LT-1	98.41	265.5 \pm 25.9

The COD of KW was found to be 29.5 ± 0.8 g/L. The leachate was obtained by carrying out acidogenesis of KW in a semi-continuous LBR. For LBR operations, first, the KW was grounded with an electric mixer and mixed with inoculum obtained from the sediment zone of a pond at the Indian Institute of Technology-Bhubaneswar. Before addition to the LBR, the inoculum was heat-treated in the oven for 15 min at 100°C to suppress methanogenesis. Finally, a 1 L mix having inoculum (20%, v/v), water (30%, v/v), and KW in the required quantity were added to the LBR. The mix was then allowed to acclimatize for three days. After the acclimatization period, the LBR followed a sequence of runs combining two operating parameters. The parameters were 10 gVS/L.d (organic loading rate) and pH of 5.74 with a leachate recirculation and replacement of 50% with distilled water.

Both the concentrated leachate and the KW slurry were stored at -4°C until required for dilution. The substrates were diluted in accordance with the required organic loading. Later, the EMFC was inoculated with the same inoculum used in LBR with respective substrates. At last, all the systems were allowed to self-buffer without any pH adjustment at any stage.

3. RESULTS AND DISCUSSION

3.1. Earthen membrane characterization

The membrane characterization included the determination of CEC, elemental composition, and mass transfer coefficients for oxygen and proton. The soil colloids will attract and hold positively charged ions to their surface. This phenomenon in soil science is known as cation exchange. This property is measured in terms of CEC, which is the sum of all the exchangeable cations per dry weight of soil at a given pH. The CEC analysis exhibited a higher exchange capacity for bentonite of 67.58 meq./100 g higher than soil (16.43 meq./100 g).

Further, the following elemental composition were obtained from EDX study for bentonite (Na-2.13%, Mg-2.22%, Al-19.28%, Si-50.69%, Cl-1.22%, K-1.54%, Ca-1.31%, Ti-2.57%, Fe-18.6%) and soil (Mg-0.72%, Al-24.16%, Si-49.6%, K-3.08%, Ti- 1.83%, Fe-20.61%). Both the materials show near about the same contribution of silica with a slightly higher percentage for bentonite, but the presence of well-distributed base cations allows an increase in the net negative charge on the bentonite particles having swelling potential. Also, the presence of silica improves water absorption capacity and prevents membrane fouling (Raychaudhuri & Behera 2020a).

The proton and oxygen mass transfer coefficients of the membranes with different bentonite proportions were determined. The oxygen transfer decreased with an increase in thickness from 4 to 6 mm. For mass oxygen transfer, a rise in bentonite proportion up to 30% led to a depreciation in mass transfer while it grew for 40% and 50% proportion. As the proportion of bentonite increased, the oxygen transfer expedited due to a reduction in the membrane thickness. This response occurs because of a reduction in membrane thickness governed by the high swelling potential of bentonite in the presence of moisture. The oxygen transfer for bentonite for different thicknesses is illustrated in Figure 1(a). On the other hand, the proton mass transfer increased with the addition of bentonite from 10% to 50% for a 4 mm thick membrane. The highest proton transfer achieved was 6.55×10^{-6} cm/s at 50% bentonite. Similarly, with an increase in membrane thickness (5 and 6 mm), the proton

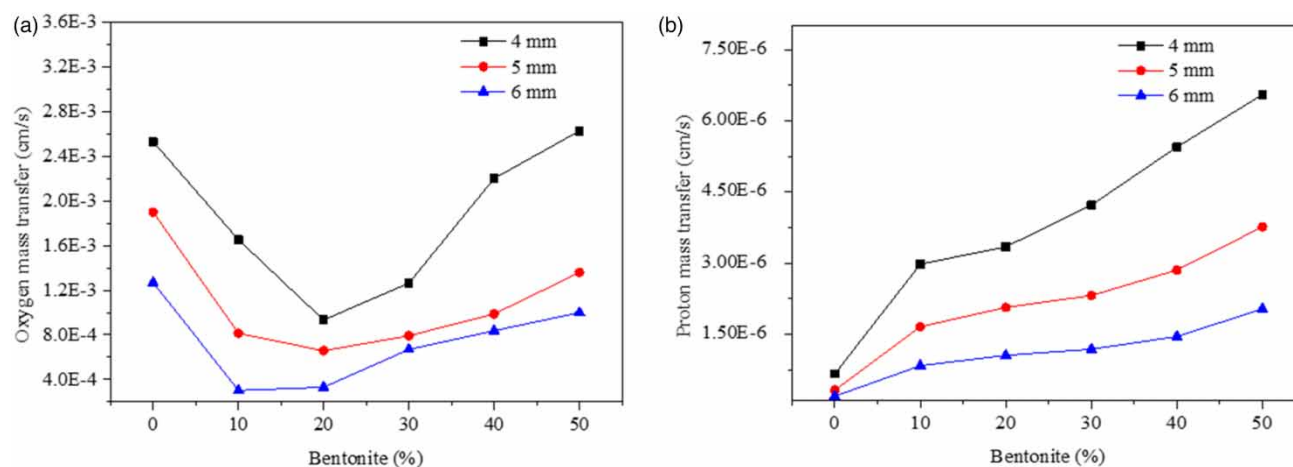


Figure 1 | (a) Oxygen and (b) proton mass transfer of varying membrane thickness modified with bentonite.

transfer reduced. Figure 1(b) shows proton transfer for bentonite for various thicknesses and proportions. It is observed that the membrane thickness plays a crucial role and acted as a barrier for both proton and oxygen transfer. However, this behavior is essential in decreasing oxygen transfer but hinders proton transfer, which is not desirable. Hence, a 20% bentonite proportion was selected to manufacture membrane at a thickness of 4 mm. The coefficients of mass transport of proton and oxygen for the optimized membrane were found to be respectively 3.34×10^{-6} cm/s and 9.33×10^{-4} cm/s.

3.2. Substrate characterization

The waste characteristics play a crucial role in a heterogeneous system carrying out anaerobic digestion (AD) in an LBR. The various factors, such as MC, TS, VS, bulk density, and particle size with inoculum, have a synergistic effect in degrading organic waste. The characteristics of the KW are as follows: pH (4.39 ± 0.10), MC ($76.37 \pm 4.17\%$), VS ($92.48 \pm 1.80\%$), TS ($23.63 \pm 4.17\%$), and bulk density ($1,069.92 \pm 26.10$ kg/m³) with a COD (30.1 ± 1.3 g/L). Additionally, the hydrolysis of waste is a rate-limiting factor in AD (Veeken *et al.* 2000). Several factors contribute to its slow rate, including moisture limitation, poor waste shredding, high bulk density, and lack of inoculum. The moisture acts as a medium to enhance contact between waste and microorganisms while supplying necessary growth components. The water carries hydrolyzed products from one phase to another, i.e., solid to liquid. In conjunction with LBR operation, this property ensures the transportation of solubilized products in the form of leachate. Nevertheless, leachate generation is also a function of waste's capacity to hold water, i.e., field capacity. Recirculating leachate or adding water to obtain a steady leachate generation rate can overwhelm this waste capacity.

Another critical property of waste is VS content; it is proportional to the biodegradability of waste and governs organic loading applied to the biological system.

On the other hand, high bulk density indicates more solid particles are in close contact with water, thus facilitating better contact. Therefore, more water to waste contact per unit volume will be present for waste with high bulk density compared to a waste having similar moisture content but low bulk density (Barlaz *et al.* 1990). The waste hydrolysis can be improved by reducing waste particle size. The smaller size leads to a higher specific surface area of waste, which provides better contact between microorganisms and shredded waste and accelerates the hydrolysis rate. A 10% increase in VFA generation was observed when OFMSW had a size of 0.4 cm to a waste having 2–3 cm particle size during AD (Dogan *et al.* 2009; Cavdar *et al.* 2011). Finally, the inoculum also assists in the waste hydrolysis. Its addition ascertains the presence of mixed consortia of microorganisms. However, operating parameters regulate the growth/inhibition of specific microorganisms in the treatment process to maximize VFA production.

3.3. Kitchen waste slurry treatment in EMFC

3.3.1. Organic removal

The KW slurry at different concentrations was allowed to degrade in the MFC. The KW is generally characterized as a set of complex compounds which causes slow waste degradation. Initially, the contribution of waste in terms of COD is less, but with time their disintegration takes place, i.e., hydrolysis, and contributes to the anolyte COD. From the graphs, the degradation process is observed to be lagging in the initial cycle (I) and accelerates with ensuing cycles (II, III, and IV). The COD conversion values were compared for the third day of first and last cycle for each system. The COD values were higher by 17.6, 36.6, and 40.3% for KW-1, 2, and 3, respectively, on the third day in last cycle than that of the first cycle. Conversely, the COD values for KW-4 and 5 reduced by 24.4 and 43.9% on the third day of the last cycle in comparison to the third day of the first cycle. As the system stabilizes in the subsequent cycles, the rate of waste hydrolysis increases along with the consumption of hydrolyzed products. The organics degradation with time is shown in Figure 2. Once the system matures, the degradation and consumption processes occur in tandem. This is visible from the EMFCs behavior in Cycle-IV, where the anolyte COD dropped steeply after 4–6 days from the start of the last cycle (IV).

The degradation of KW (4 and 5) should have been contributing more to the anolyte COD; however, the trend suggests otherwise as KW (2, and 3) showed higher contribution to the anolyte COD. This indicates that a low KW fraction in the slurry led to better degradation than higher fraction of KW. On the basis of the cumulative COD generated from the last cycle of each system treating KW, it can be observed that KW-2, and 3 contributed more COD, i.e., better degradation as compared to KW-4 and 5. Whereas, for KW-1 the KW content was the least. However, on comparing with the initial COD to be contributed and actual COD contribution, the system (KW-1) performs better than both KW-4 and KW-5. This behavior of contributing to low COD by systems (4 and 5) is attributed to substrate inhibition. The COD removal efficiencies were

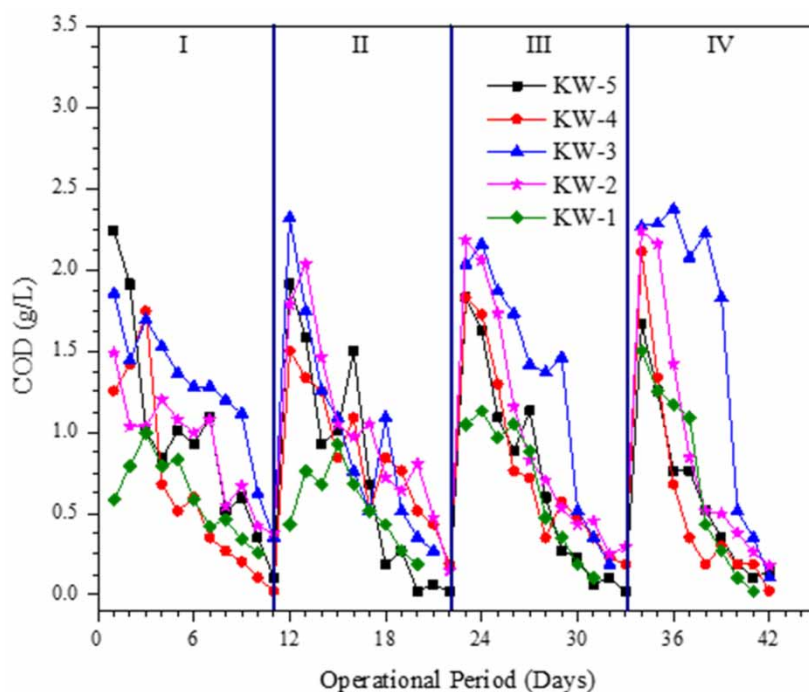


Figure 2 | Observed COD removal in systems treating kitchen waste.

above 90% for all the systems but were slightly above for KW-1, 2, and 3 (>94%). The removal obtained is an improvement as compared to other studies, which attained around 78% removal efficiencies (Tremouli *et al.* 2019; Yang *et al.* 2019).

3.3.2. VFAs

VFAs are the primary products produced when complex compounds are broken down into simpler compounds during AD. These transformations of compounds will follow the hierarchy of first producing higher chain fatty acids and then smaller chains. This degradation pathway is evident from the behavior of EMFC systems with individual VFA components generated throughout the operational period (Novak & Carlson 1970; Cohen 1982; McInerney 1988).

For all systems treating KW, the VFA generation or consumption profiles for KW treatment is shown in Appendix D. The order of VFA contribution from systems with different KW concentrations is as follows KW-1 < 4 = 5 < 3 < 2. In addition, the fraction of simple and complex compounds will also govern VFA generation for individual systems. The cooked fraction of KW will degrade more easily than pre-cooked (raw) material having fibrous, lignin, and proteinaceous content. From VFA profiles of KW-4 and 5, the major contribution was from AA, followed by PA. The tendency of the biomass present will always be to break down easily degradable compounds, thereby making AA dominant among VFAs produced.

When KW fraction decreases, a drop in fraction of both simple and complex compound is observed. This change in fraction increases PA concentration as the biomass competes for the complex content. Further, this helps in improving KW degradation, which is neither obtained in systems with the least and highest KW fraction. The least KW concentration leads to substrate limitation, whereas the highest leads to the development of a microbial community, which eclipses the growth of electrogens by microbes aiding complex compound degradation (Morris *et al.* 2013; Cai *et al.* 2019). Overall, the KW degradation leads to the contribution of mainly AA and PA in the anolyte. The biomass favored AA over other VFAs. In response to other studies, using OFMSW produced only 0.8 g/L of VFAs (El-Chakhtoura *et al.* 2014) whereas all the systems produced VFA greater than 0.75 g/L with a maximum value being 1.8 g/L for KW-2.

3.4. Leachate treatment in EMFC

3.4.1. Organic removal

The leachate is an entirely different substrate to the KW slurry used in the MFCs. It comprises solubilized compounds that are readily consumable. As the content of leachate is solubilized, its concentration is high with respect to KW. Organics removal

is presented in Figure 3. The abundant substrate availability will lead to higher microbial growth with the possibility of encouraging different metabolic pathways of degradation. However, these compounds will facilitate exoelectrogens development in the anode, as they are desirable for their growth (Freguía *et al.* 2010). When the MFC was subjected to leachate, the COD consumption was gradual relative to the one obtained for KW.

In Cycle-I, the anolyte COD increased by 6–15% compared to the previous day's COD. After 4–5 days, this phenomenon was observed in the MFCs systems (LW-2, 3, and 4) treating leachate. This rise suggests that once readily consumable compounds reduce, the biomass then focuses on the complex fractions of the leachate and hence contributes again to anolyte COD (Freguía *et al.* 2010). When Cycle-I and IV are compared, the leachate consumption is rapid, which manifests biomass's maturity to consume substrates. During leachate treatment, the cycle length reduced drastically from the ones treating KW. The latter took almost 11 days for each cycle to complete, whereas, for the former, the duration reduced with each cycle to consume a major fraction of the leachate content. Finally, as the operational period nears the end of the cycle, there is a possibility that the complex fractions that are present in the leachate may be degrading and sustaining the MFC's performance in terms of OCV. All the systems achieved >96% organics removal.

3.4.2. VFAs

The leachate as a substrate consists of solubilized products obtained from hydrolysis and acidogenesis of the KW in an LBR. And hence for leachate treatment, the fractions of all the other VFAs are different from the ones obtained for KW. It means the formation of VFAs in the anode chamber while treating leachate is not limited by the degradation of KW compounds, as observed in KW treatment. The presence of complex fractions in leachate will be degraded when the readily consumable compounds are exhausted (Freguía *et al.* 2010). This disintegration will add some VFA content to the anolyte. The VFA generation or consumption profiles for leachate treatment are shown in Appendix E. From the study, the VFA consumption was gradual in the starting cycle (I). But as the operational period progressed, the consumption became rapid and led to a shortening of cycles. All the systems were subjected to leachate having three types of VFAs, viz., AA, PA, and BA. From VFA profiles of all systems, the perceived affinity of the microbial culture was towards AA. This inclination towards AA reiterates the necessity for carrying out phase transformation of KW in a separate stage. It will improve its overall treatment efficiency instead of treating KW slurry directly in a process to maximize electricity generation.

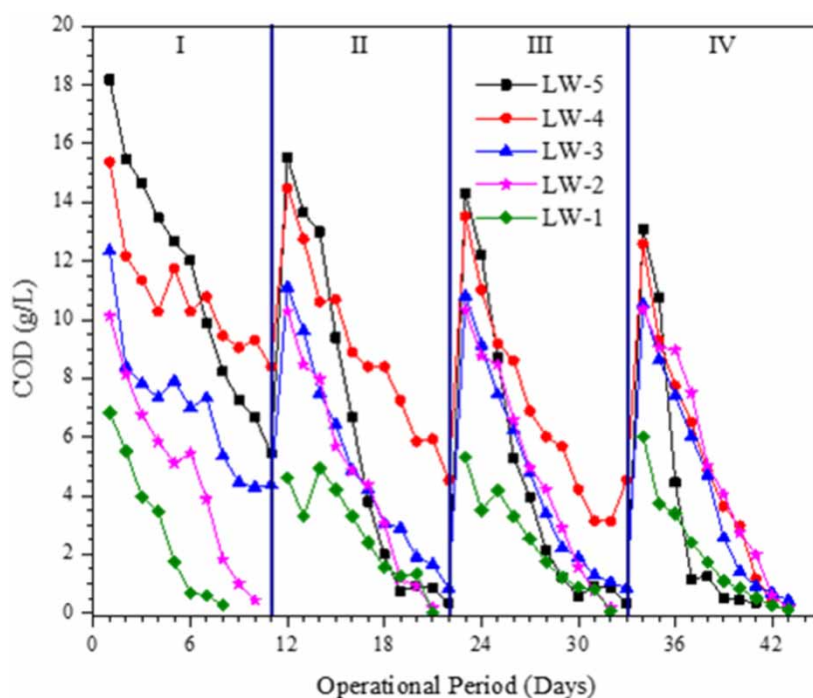


Figure 3 | Observed COD removal in systems treating leachate.

Despite having a low substrate (LW-1) for degradation, the system took a long time to consume all VFAs when compared with the highest substrate (LW-5) concentration. This distinguished behavior may be because of the different biomass concentrations of the system. A higher substrate allows the development of microbial culture, supporting multiple metabolic pathways present in the mixed culture. However, for low leachate concentration due to substrate limitations, the growth of exoelectrogens is encouraged. This growth prospect can be reaffirmed in a section discussing the electrical performances of the system.

3.5. pH

The system's pH acts as an indicator and plays an essential role in understanding the stage of the process. At the beginning of the cycles, the pH of both the KW slurry and leachate fell in the range of 5.0–5.5 and 5.5–6.0. The variation of pH for treating KW and leachate is illustrated in Figure 4. For treatment of KW slurry, the pH change was gradual, unlike leachate, which was rapid. In addition, during the cycle (I), the pH varied from 5.1 to 6.3; whereas, at the end of the cycle (IV), it reached 6.8. This response shows the biological processes started substrate degradation effectively within 24 h without any lag due to substrate change.

Further, the anolyte pH is a function of various components, i.e., if the major fraction present is VFAs, then the pH will be acidic. Thus, the pH moves towards neutrality/alkalinity as VFA consumption improves. Also, the pH can indicate the completion of treatment. For treatment of KW, the simultaneous consumption and degradation do not allow the pH to alter rapidly, whereas the consumption of soluble hydrolyzed product of leachate leads the pH to alkalinity at a faster pace.

Also, the lowering of pH can occur on the possibility of complex compounds of leachate being converted to VFAs. From close observations, such a counterbalance of reaction was not observed as compared to KW treatment. For KW, the simultaneous processes of hydrolysis and acidogenesis were being carried out. This did not allow the pH to change abruptly. Since the pH drop during the leachate treatment is negligible, then inferences of stunted or suppressed VFA formation by degrading the complex compounds can be drawn. However, their electrical performance remains superior to the systems treating KW. Moreover, the simultaneous substrate degradation in the KW slurry treatment confirms the performance of the EMFC will suffer and is evident from their electrical performance. In another observation, during Cycle-I, the initial drop in pH was not much. But for Cycles-III and IV, the initial drop in pH was significantly visible. This shows the biological processes started substrate degradation effectively within 24 h without any lag due to substrate change. In contrast, the other studies have regulated and maintained the anolyte at a pH of 7 (Cavdar *et al.* 2011; El-Chakhtoura *et al.* 2014; Bridier *et al.* 2015; Xin *et al.* 2018).

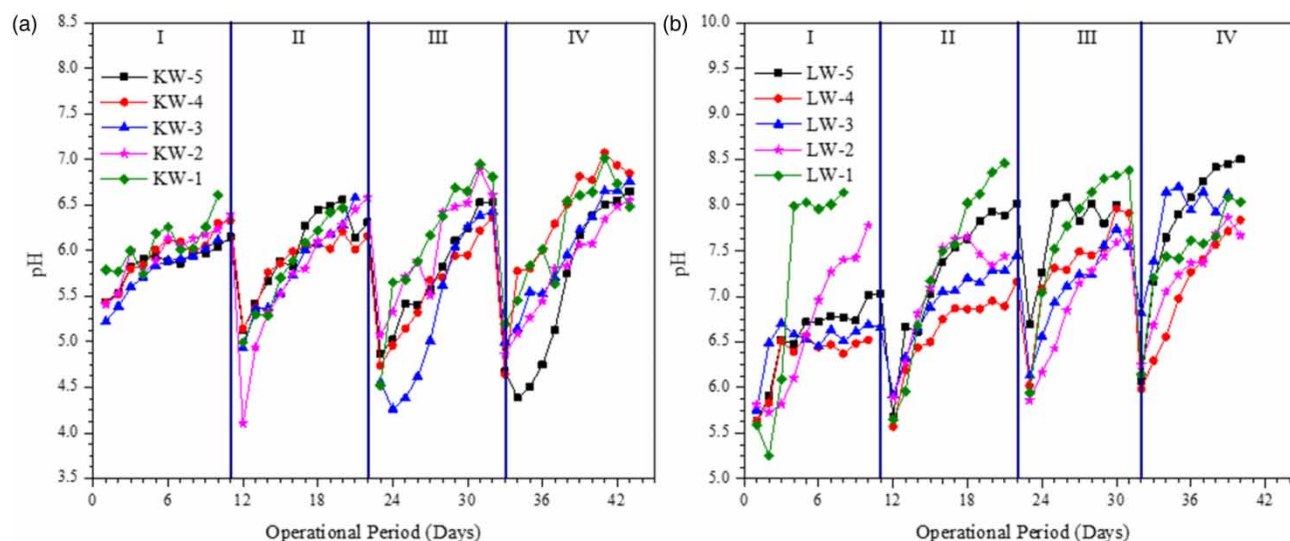


Figure 4 | Changes in pH while treating (a) kitchen waste and (b) leachate.

3.6. Electrical performance

3.6.1. Potentials

Overall, the OCV for systems treating KW was less than those treating leachate. Only for KW-5 and 4 the OCV values were 463.1 and 492.2 mV, respectively, and were higher than other KW concentrations. These values were equivalent to some systems treating leachate. The values for KW-3, 2, and 1 were 400.8, 449.1, and 444.31 mV, respectively. On the other hand, the maximum OCV generated was by LW-5 (609.4 mV); the other systems produced 498.4, 525.8, 486.7, and 551.7 mV respectively for LW-4, 3, 2, and 1. These values were majorly affected by the anode potential. The lower the anode potential of the system, the less it has the ability to carry out cell growth and maintenance, which were the characteristics of the systems treating leachate. The anode potential of the systems treating KW was observed to be on the higher side within a range of -320 to -390 mV, thereby suggesting enhanced potential for improved growth of cells and higher electricity generation (Aelterman *et al.* 2008). However, this not the case, as reduced electricity generation was observed. Despite the lower anode potential of the systems treating leachate, it led to decreased growth for exoelectrogens and a conducive atmosphere for the development of other metabolic pathways (Özakaya *et al.* 2013). The anode potential for systems treating KW reduces with a decrease in the substrate. This drop might suggest substrate limitation, but observing closely, the cathode potential for all the systems has remained nearly the same, suggesting substrate sufficiency and no substrate limitations.

3.6.2. Power density

The maximum PD from leachate was 20 folds more than that generated from treatment of KW slurry. The increase in substrate concentration assists PD improvement, and identical observations were made from EMFCs systems treating leachate. Interestingly, the EMFC subjected to the stepped-up organic load (LW-5) produced PD nearly 35% lower than the one subjected to LW-4. From the organic removal pattern and VFA consumption profiles, we can conclude that the decline in PD for the LW-5 system would only be due to the presence of other metabolic pathways that compromise exoelectrogens growth. This is due to the batch operation of the EMFC, which provides abundant substrate, unlike continuous mode (Rahimnejad *et al.* 2011).

For leachate, the maximum and minimum PD values were obtained as 586.5 ± 38.8 mW/m³ (LW-4) and 265.5 ± 25.9 (LW-1). For systems treating KW, the power generated was minimal to systems using leachate as substrate. Besides, the KW concentration in KW-1 and 4 did produce relatively higher PD when compared to KW-2 and 3. Increasing substrate concentration beyond a certain point can be considered to negatively affect the power generation capacity. Li *et al.* (2013) generated 195.4–445.6 mW/m³ from acidic food waste leachate despite using polymer membranes. In another communication, Xin *et al.* (2018) from the cafeteria waste produced PD of 173 mW/m³ in an air MFC. On the other hand, El-Chakhtoura *et al.* (2014) produced a PD of 123 ± 41 mW/m² with $24 \pm 5\%$ of CE with the help of platinum catalyst in an air-MFC treating OFMSW. These results show the suitability of leachate and the earthen membrane used in the EMFC.

3.6.3. Polarization studies

The polarization studies were conducted after completing four batch cycles and adding fresh substrate to the system. The study was conducted by jotting down voltage across the resistance (10 K Ω to 1 Ω). In the case of KW treatment, the internal resistance obtained from the polarization study suggests it was highest for the minimum substrate concentration. This phenomenon can be due to the waste complexity at low substrate concentration, except for leachate treatment. During leachate treatment, the resistance increased with a decrease in the substrate concentration. However, the highest internal resistance for systems treating leachate (LW-1, 850.1 Ω) was lower than the least resistance obtained for treating KW (KW-5, 1008.1 Ω). A similar organic load but different nature of the same substrate shows a stark difference in internal resistance. The lowest internal resistance was for LW-5, with a value of 369.5 Ω .

The maximum power density obtained for LW-5, 4, and 3 were 1,099.5, 1,123.49, and 946.99 mW/m³ and were higher than LW-1 and 2 having 740.8 and 714.6 mW/m³, respectively. For systems treating KW, the maximum PD was 283.8 and 257.0 mW/m³ for KW-4 and 1, respectively. The maximum PD for other systems were 153.1, 136.6, and 130.5 mW/m³ for KW-5, 3, and 2, respectively. All the systems treating KW had lower PD values with respect to leachate treatment. On weighing up maximum PD values produced from different substrates, the values for KW treatment were 75% lower than those generated from leachate.

In terms of CD, for KW, the maximum value was 129.5 mA/m² for KW-5. For leachate treatment, a similar pattern was spotted with the highest value of 373.97 mA/m² (LW-4) and a minimum of approximately 145 mA/m² for LW-1 and 2.

The drop in the internal resistance of the system suggests better substrate utilization aided by the enrichment of exoelectrogens on the electrode surface area and the presence of solubilized compounds. This phenomenon has been valid for the behavior of the systems treating leachate but has been surprisingly different for KW slurry. One plausible reason for this pattern can be the development of a complex community of microbes for degrading KW.

3.6.4. Cyclic voltammetry

Maximum oxidative and reductive currents were observed for systems using leachate as a substrate rather than KW. Among the systems treating leachate, it was highest for system LT-2 (4.24 mA), while for other systems, the decreasing order of current production was LT-3 > LT-5 > LT-4 > LT-1. Cyclic voltammetry response is shown in Figure 5. These results cannot predict a specific behavior. But for systems with higher substrate concentration, the current was quite high and vice versa. On the other hand, the oxidative current for all the systems treating KW was higher than 1 mA except for KW-2 (0.67 mA). The oxidative currents for different systems treating KW are 1.05, 1.48, 1.32, and 1.43 mA for KW-1, 3, 4, and 5, respectively. The oxidative current generated by LW-1, lowest among leachate treatment systems, was higher than the systems treating KW. This phenomenon shows the superiority of leachate over KW slurry as a substrate. Correspondingly, the reductive currents were more for leachate. The values for LT-1, 2, 3, 4, and 5 were -2.57 , -2.15 , -1.61 , -3.94 , and -1.34 mA, respectively. Whereas, those generated by KW-1, 2, 3, 4, and 5 were respectively -0.41 , -0.29 , -0.49 , -0.47 , and -0.63 . The lowest value generated by leachate utilizing systems was almost twice the maximum reductive current generated by systems treating KW.

According to Goud *et al.* (2011), the larger difference between oxidation and reduction currents led to the effective utilization of electrons (substrate) on working electrodes. On this premise, the maximum difference was for LW-2 but was similar to the values obtained for LW-4 and 3. Interestingly, the LW-1 and 5 had nearly the same difference. It depicts that despite LW-5 having the highest substrate values, it was not able to generate superior electrical performance. From the literature, the charge produced is proportional to organic loading. Therefore, the lesser charge generated from higher organic loading systems contributes to enhancing non-electrogenic pathways. Thus, a concentration neither very high to cause inhibition nor very little to cause limitation is desirable for maximizing electricity generation. For KW treatment, the reduction currents were almost equal and negligible. In contrast, the oxidation and reduction phenomenon stated a balance of redox reactions for leachate systems.

3.6.5. Impedance

Figure 6 illustrates the impedance results. Warburg element swelled with a decrease in KW slurry concentration from 171.1Ω to 443.2Ω for KW-5 to 3, respectively. Two separate behavioral responses to high and low KW concentrations are depicted.

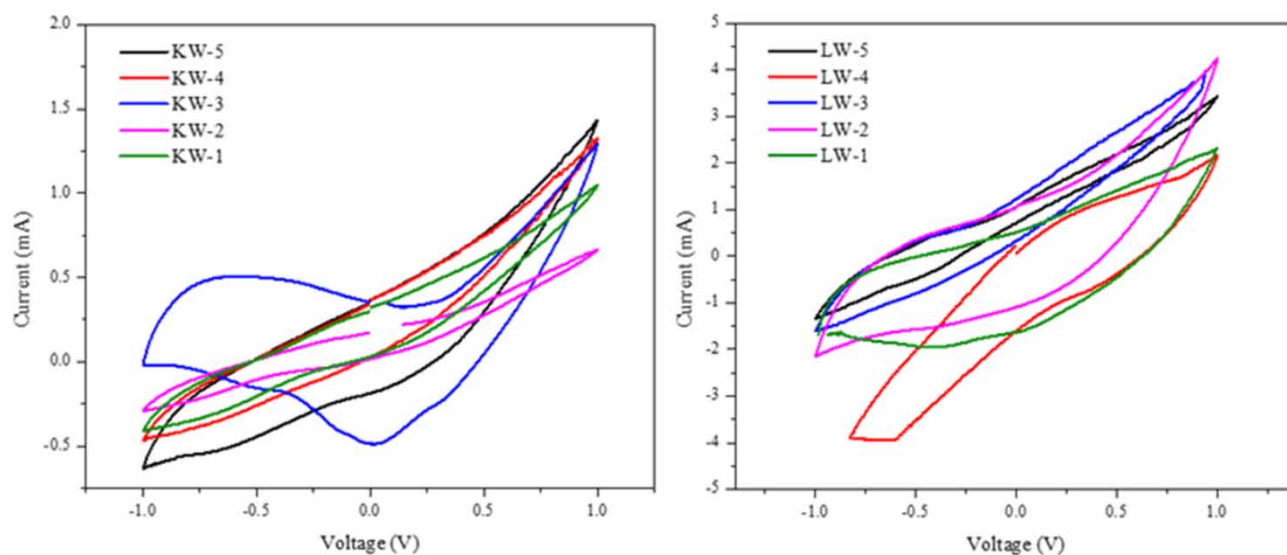


Figure 5 | Cyclic voltammetry of EMFCs treating (a) kitchen waste and (b) leachate.

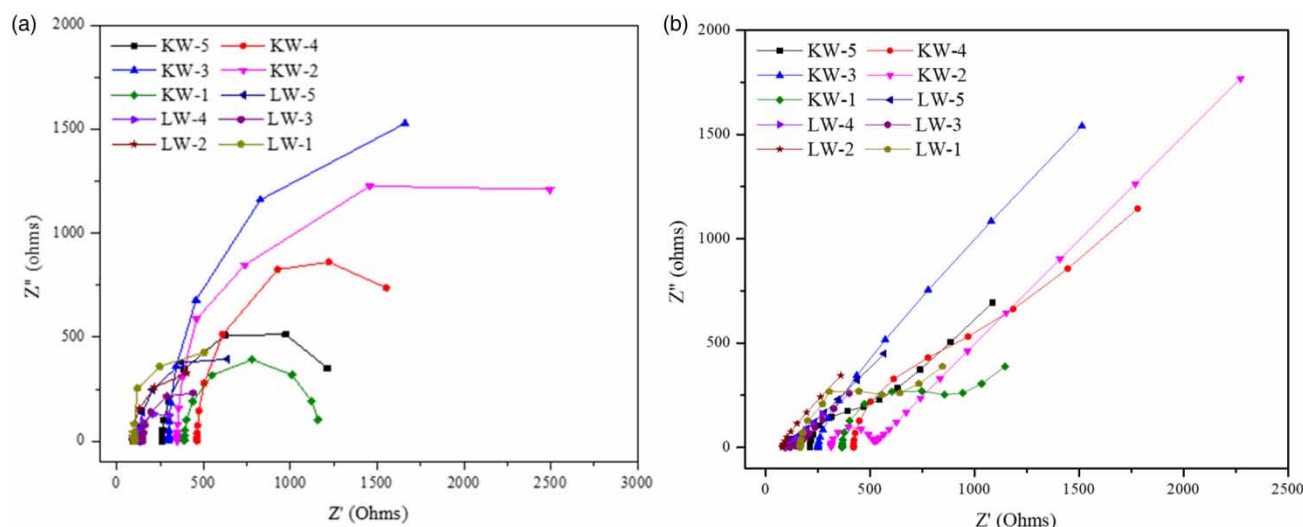


Figure 6 | Impedance studies for EMFCs treating kitchen waste and leachate (a) Randles circuit and (b) Randles and Warburg circuit.

Contrastingly, for high concentrations, the readily available substrate was available, and thus the diffusion resistance was better as the system grew mature in handling the complex waste with subsequent cycles. However, the value surprisingly dropped for KW (1) to $189\ \Omega$. It can be attributed to the limited presence of substrate aided by faster aging of biofilm, thereby handling both the simple and complex substances efficiently. A similar trend was observed when treating leachate, the diffusion resistance grew from $40.2\ \Omega$ to $200.2\ \Omega$ for LW-4 to 1. However, the system subjected to the highest LW concentration should have had the lowest resistance with the general trend. But maybe due to the substrate inhibition, the resistance was $112.3\ \Omega$.

The charge transfer resistance was higher for the system treating KW when compared to leachate. It depicts the transfer of electrons between solution and electrode. The restriction in electron transfer is evident in KW slurry due to the requirement of multiple AD steps, whereas the leachate comprise of consumable compounds. While treating leachate, it was highest for LW-5, with the lowest being for LW-4, and took an upward trend with the reduction in substrate concentration. Correspondingly, the trend ceased to follow at KW-1.

Similarly, the ohmic resistance was high for KW. On comparing them within the respective group, there was not much difference. The capacitance value was minimum for all the runs, and better charge separation took place between the electrode–electrolyte interface with leachate treatment.

3.6.6. Tafel plot

Tafel plots are used to understand electrode kinetics and system losses. From the plots, the slope for anode (β_a) and cathode (β_c), CD at equilibrium (i_0), and polarization resistance (R_p) are determined. These kinetic parameters are calculated by best-fit analysis within a chosen potential range that minimizes mass transport limitations. The major factors whose behavior is represented through Tafel plots are substrate type, electrode material and surface area, and CEM. In this study, the other conditions, such as membrane and electrode material, are constant, while the only change is the substrate type and concentration. Therefore, the results and the commentary will shed light on the effect of substrate on the EMFC. The Tafel plots for KW and leachate systems are shown in Figure 7. First, the CD depicts the rate of reaction. A higher value of CD will require low activation energy. During KW treatment, the CD of $13.87\ \text{mA/m}^2$ was highest for KW-5, i.e., highest substrate concentration. Whereas the CD for all other systems (KW-2, 3, 4) was nearly the same, approximately $3.99\ \text{mA/m}^2$, except for KW-1, which had a value of $5.59\ \text{mA/m}^2$. Similarly, for leachate treatment, the CD trend increased with substrate concentration from 13.16 to $36.19\ \text{mA/m}^2$ for LW-1 to LW-4, respectively, while it reduced drastically for LW-5 to $16.46\ \text{mA/m}^2$. The CD value of LW-5 was just 20% higher than LW-1.

Other kinetic parameters are the slopes of the Tafel plot, which indicates the bio-electro catalytic reaction. Raghavulu *et al.* (2012) indicated that lower Tafel slope indicates a high bio-electro catalytic reaction. On this basis, the oxidative Tafel slope

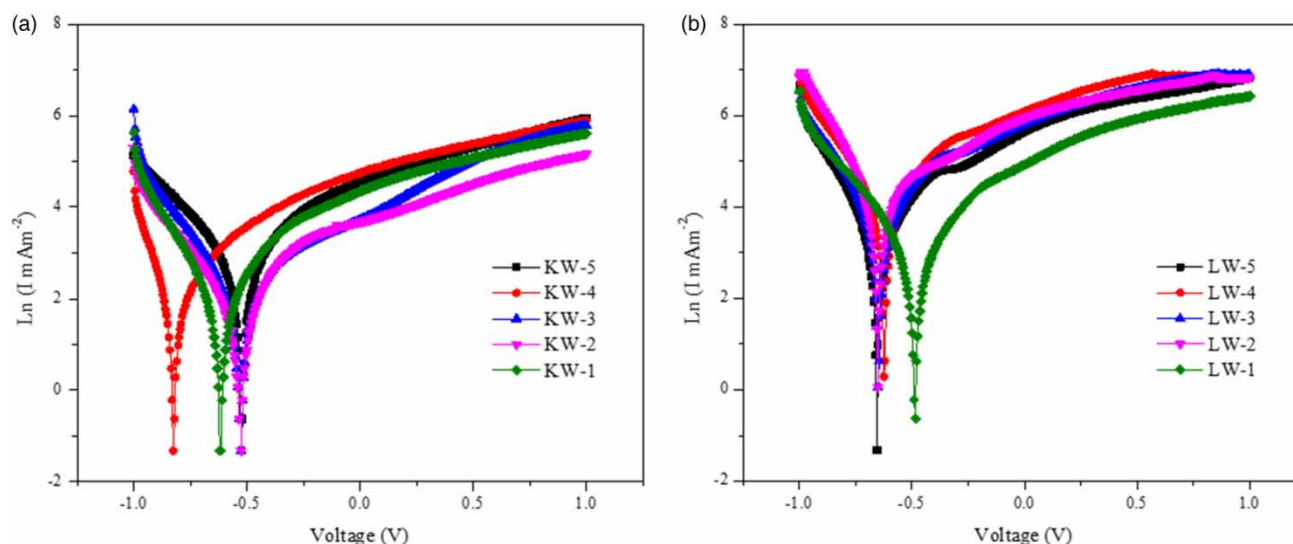


Figure 7 | Tafel plots for EMFCs treating (a) kitchen waste and (b) leachate.

had high values for substrate treating KW while comparatively low for leachate. Further, due to substrate limitation, the values of the slope increased considerably. However, there was an increment noticed in the values for maximum substrate concentration, thereby defying the trend. Similar patterns were observed for reductive Tafel slopes also. Interestingly, either the substrate limitation or substrate inhibition influenced slope. Therefore, not only the selection of substrates becomes essential, but their concentration also drives the reaction rate.

4. CONCLUSIONS

From the results, bentonite (20%) was an optimum proportion at 4 mm thickness for manufacturing ceramic membranes. The coefficients of mass transport of proton and oxygen for membrane were found to be respectively 3.34×10^{-6} cm/s and 9.33×10^{-4} cm/s. Both the mass transfer coefficients decreased with an increase in thickness from 4 to 6 mm. The proton transfer was proportional to bentonite fraction; whereas, for oxygen it initially decreased until 20% and then gradually increased until it reached 50%. This behavior may be attributed to the high swelling potential of bentonite.

In other portion of the study, the EMFC managed to treat both the substrates, i.e., above 90% organic removal. But system treating leachate showed superior reduction (>96%). The type of VFA generated and consumption patterns were quite different for both substrates. Each system expressed affinity towards AA. Nevertheless, the electrical performance was distinct because of the diverse nature of the substrate. The system (LW-4) produced the highest PD of 586.5 ± 38.8 mW/m³, while for KW, a mere 41.5 mW/m³ was generated (KW-1). Additionally, the trends were inconsistent, thereby indicating either substrate inhibition or limitation to be the reason for abrupt behavior. This portrays not only the substrate form but concentration also matters and have clout over the electrical performance. These extreme low and high concentration conditions are responsible for adding resistance to the system. The former is insufficient to overcome the system's overpotential while the latter encourages non-electrogenic pathways for degradation. These facts were reiterated from higher Tafel slopes for KW treating systems than leachate systems. Although the parameters studied support the trends and observation, for future studies, the biomass type and growth could provide essential insights into the functioning of MFCs.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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