



Harnessing of bioelectricity in microbial fuel cell (MFC) employing aerated cathode through anaerobic treatment of chemical wastewater using selectively enriched hydrogen producing mixed consortia

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ABSTRACT

The possibility of bioelectricity generation from anaerobic chemical wastewater treatment was evaluated in a microbial fuel cell (MFC) [dual-chambered; mediator less anode; aerated cathode; plain graphite electrodes] employing selectively enriched hydrogen producing (acidogenic) mixed culture. Performance of MFC was evaluated at two organic/substrate loading rates (OLR) (1.165 Kg COD/m³-day and 1.404 Kg COD/m³-day) in terms of bioelectricity production and wastewater treatment at ambient pressure and temperature under acidophilic microenvironment (pH 5.5) using non-coated plain graphite electrodes (mediatorless anode; air cathode). Experimental data demonstrated the feasibility of *in situ* bioelectricity generation along with wastewater treatment. The performance of MFC with respect to power generation and wastewater treatment was found to depend on the applied OLR. Maximum voltage of 716 mV (2.84 mA; OLR = 1.165 kg COD/m³-day) and 731 mV (2.97 mA; OLR=1.404 kg COD/m³-day) was observed at stable operating conditions. Substrate degradation rate (SDR) of 0.519 Kg COD/m³-day and 0.858 Kg COD/m³-day was observed at two OLRs studied. Maximum power yield (0.73 W/Kg COD_R and 0.49 W Kg/COD_R) and current density (339.87 mA/m² and 355.43 mA/m²) was observed at applied 50 Ω resistance. Fuel cell performance was evaluated employing polarization curve (100 Ω–30 KΩ), Coulombic efficiency (ε_{cb}) and cell potentials along with sustainable power yield at stable phase of fuel cell operation. Designed MFC configuration, adopted operating conditions and used parent inoculum showed positive response.

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1. Introduction

Recently, considerable interest is noticed in the research fraternity on green energy production utilizing renewable resource by sustainable means. Ethanol, bio-diesel, biohydrogen and bioelectricity production from waste materials are finding prominence in this direction [1–42]. Bioelectricity generation using microbial fuel cells (MFC) is one of the areas gaining importance due to its clean, efficient, and renewable nature [4,6,8,9,14–42]. MFC is a bio-electrochemical device which generates electrical energy through the potential developed by oxidation of organic matter in the presence of fermentative bacteria under mild reaction conditions (ambient temperature and pressure). The potential developed between the bacterial metabolic activity [reduction reaction generating electrons and protons (H⁺)] and electron acceptor conditions separated by a membrane leads to generation of bioelectricity. MFC design and configuration, characteristics of carbon source, nature and coating of electrodes, membrane electrode assembly, mediators, electrolytes used, nature of inoculum (biocat-

alyst) in the anode chamber, operating conditions such as loading rate, pH, temperature, retention time, etc. are considered to be important aspects which govern the overall efficiency of electricity generation.

In this direction, diverse configurations of MFC, different types of mediators (anode and cathode) and various types of substrates and anode inoculum were studied [1,2,4–9,14–42]. Different types of MFC configuration were developed for the efficient energy recovery from substrates [2,4–9,24–28]. Since microorganisms act as a catalyst in the transfer of electrons from the substrate to anode, selection of a high performing microbial consortium (either pure or mixed culture) is of crucial importance [29–31]. Various substrates including carbohydrates such as glucose and starch, fatty acids, amino acids and proteins have been exploited for electricity generation with various other carbon sources. Recently, MFC are deployed for simultaneous generation of electricity from treatment of waste. Exploiting wastewater as substrate to harness electricity is considered as sustainable approach and is presently in the early stages of research [4,5,8,9,23,25,26,36–42].

In this communication, we have made an attempt to study bioelectricity generation from anaerobic treatment of chemical wastewater using dual chambered MFC employing selectively enriched

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Nomenclature

COD	chemical oxygen demand (mg/l)	MB	methanogenic bacteria
BOD ₅	biochemical oxygen demand (5 days; mg/l)	ε _c	Coulombic efficiency (%)
OLR	organic loading rate (Kg COD/m ³ -day)	ε _{cb}	Coulombic efficiency in fed-batch mode (%)
SDR	substrate loading rate (Kg COD/m ³ -day)	<i>M</i>	molecular weight of O ₂ (32)
PEM	proton exchange membrane (Nafion 117)	<i>F</i>	Faraday's constant (96485 °C)
H ₂	bio hydrogen	<i>b</i>	number of electrons exchanged per mole of O ₂ (4)
AB	acidogenic bacteria	<i>v</i> _{An}	volume of liquid in the anode compartment (l)
SEM	scanning electron microscope	ΔCOD	change in COD over period of time (mg/l).
VFA	total volatile fatty acids (mg/l)	RDAP	relative decrease in anodic potential (RDAP) (%)
VSS	volatile suspended solids (mg/l)	<i>E</i> _{o,anodic}	initial anodic potential without resistance (mV)
MFC	microbial fuel cell	<i>E</i> _{anodic}	anodic potential at specific external resistance (mV)
ORP	oxidation–reduction potential (mV)		

hydrogen (H₂) producing mixed culture as anodic inoculum (biocatalyst). Aerated cathode (electron acceptor), mediatorless anode and non-coated graphite electrodes were considered in MFC design and the performance was evaluated at two organic loading rates (OLRs) with respect to power generation and substrate degradation employing acidophilic microenvironment (pH 5.5).

2. Experimental

2.1. Hydrogen producing mixed inoculum

Mixed anaerobic culture acquired from anaerobic bioreactor (producing H₂ from chemical wastewater treatment) was used as parent inoculum in the anode chamber of MFC as biocatalyst. Prior to inoculation the parent culture was selectively enriched with acidogenic bacteria to facilitate effective H₂ production as described by Venkata Mohan and co-workers [10–13,43,44]. Microbial culture was washed thrice in saline buffer after separation (5000 rpm, 20 °C) and the resultant pellet was harvested in the designed synthetic wastewater (glucose – 4.5 g/l, NH₄Cl – 0.5 g/l, KH₂PO₄ – 0.25 g/l, K₂HPO₄ – 0.25 g/l, MgCl₂ – 0.3 g/l, CoCl₂ – 25 mg/l, ZnCl₂ – 11.5 mg/l, CuCl₂ – 10.5 mg/l, CaCl₂ – 5 mg/l, MnCl₂ – 15 mg/l; COD – 4.8 g/l) under anaerobic microenvironment (100 rpm; room temperature) in acidophilic conditions (pH 5.5). The resulting culture was subjected to pretreatment [heat-shock treatment (100 °C; 2 h), chemical treatment (2-bromoethane sulphonic acid sodium salt (0.2 g/l); 24 h) and acid treatment (pH 3 adjusted with *ortho*-phosphoric acid (88%); 24 h)] to inhibit the growth of methanogenic bacteria (MB) simultaneously enriching the H₂ producing microflora. The pretreated culture was inoculated in anodic chamber of MFC through feed.

2.2. Chemical wastewater

The chemical wastewater used as substrate in anode compartment of fuel cell was a composite one aggregated from various types of wastewaters from bulk drugs, chemical intermediates, dye and dye intermediates, pharmaceuticals, pesticides and various chemical process units. Characteristically, the wastewater [pH 7.82; ORP –75 mV; suspended solids –0.98 g/l; total dissolved solids (TDS) 25.5 g/l; total alkalinity 0.12 g/l; chlorides 7.71 g/l; COD 8.1 g/l; BOD₅ 2.48 g/l] can be considered as complex in nature due to its composite nature and low-biodegradability (BOD/COD ~ 0.3). The wastewater was evaluated for bioelectricity generation and treatment efficiency.

2.3. MFC design and configuration

Dual chambered MFC was fabricated using 'perplex' glass in the laboratory by providing sample ports, wire input points (top), inlet

and outlet ports, gas outlet points, etc (Fig. 1). Equal volumes (0.7 l) of anode and cathode compartments were separated by proton exchange membrane (PEM; Nafion 117, Sigma Aldrich). Arc punched PEM disc (50 mm diameter) subjected to sequentially pretreated in 30% H₂O₂, deionized water, 0.5 M H₂SO₄ and deionized water (for 1 h each) to increase porosity was fixed between clamps connecting both the chambers. Plain graphite plates (5 × 5 cm; 10 mm thickness) without any surface treatment and catalytic addition were used as electrodes for both cathode (surface areas: 75 cm²) and anode (perforated by providing nine uniform size holes of 10 mm diameter to increase the overall surface area to 83.56 cm²). Electrodes (soaked in deionized water; 24 h) were placed at a distance of 6 cm on either side of PEM. Copper wires were used as contact from electrodes and contact area was sealed with epoxy material. Cathode chamber was filled with 0.65 l of phosphate buffer (50 mM) and kept for aeration using fish aquarium pump by adjusting pH to 7.4.

2.4. MFC operation

Anode chamber was inoculated with the selectively enriched mixed microflora (2.0 g VSS/l) through designed synthetic wastewater (0.65 l). Immediately after voltage started to drop, fresh chemical wastewater (0.65 l) was loaded. Contents in anode chamber (anolyte) were continuously recirculated at a rate of 100 ml/min using peristaltic pump to facilitate homogeneous distribution and contact of the substrate with consortia. Before changing the feed, inoculum was allowed to settle down (30 min) and exhausted feed (0.6 l) was pumped out under anaerobic conditions. The settled inoculum (50 ml by volume) was used for subsequent experiments. MFC was operated in batch mode at room temperature (29 ± 2 °C) by maintaining anode chamber at acidophilic pH (5.5) under anaerobic conditions. Analyte pH was maintained at 5.5 ± 0.1 using *ortho*-phosphoric acid (88%) to sustain the functioning of acidogenic bacteria (AB) at the same time suppressing the methanogenic activity. After each feeding event, the anode chamber was sparged with oxygen free N₂ gas for 2 min to create anaerobic microenvironment in the cell. Feeding, decanting, and recirculation operations were performed employing peristaltic pumps (Micclins, India) controlled by preprogrammed electronic timer. The OLR of the influent wastewater as required was adjusted by diluting with tap water before feeding. Voltage was recorded once in every 3 h. After reaching stable performance, power output was monitored by measuring voltage using an external resistor (50 Ω) connected across the electrodes.

2.5. Analysis

Bio-electrochemical calculations were done based on the procedure outlined by Logan et al. [45]. Current (*I*) and potential (*V*)

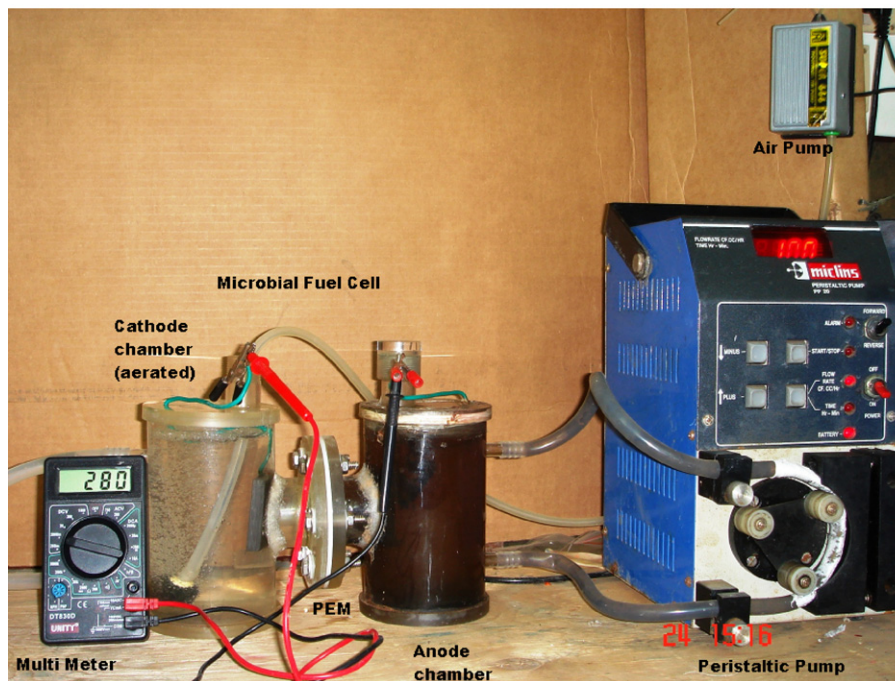
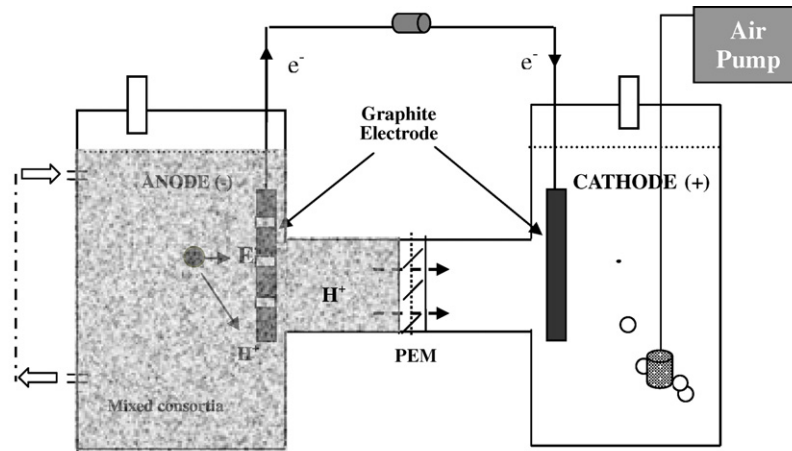


Fig. 1. Experimental setup depicting MFC (aerated cathode) operating along with air supply, peristaltic pump, multi meter and electrode assembly.

measurements were recorded at every 3 h using auto range digital multimeter (Metravi 901) by connecting 50 Ω external resistor. Power (mW) was calculated using $P = IV$, where, I is current (mA) and V is voltage (mV). Power density (mW/m^2) and volumetric current density (mA/m^2) were calculated by relating power and current with the surface area (m^2) of the anode, respectively. Specific power yield ($\text{W}/\text{Kg COD}_R$) was obtained by relating power with the amount of substrate (COD) removed. Volumetric power (mW/m^3) and volumetric current (mA/m^3) were calculated based on the working volume of the anode. For polarization, current generation was monitored at various external resistances (100 Ω –30 K Ω) connected for a few minutes and voltage was calculated from current using formula $V = IR$. The anodic and cathodic potentials of MFC were measured against a saturated Ag/AgCl electrode using a pH meter. A variable resistance box was used to select an applied external resistance for current measurement. Appropriate amount of sample from anode chamber at regular time intervals (24 h) was collected with the help of a syringe and nitrogen gas was flushed to maintain anaerobic condition before closing the chamber during fuel cell operation. The COD (closed refluxing

method), alkalinity (total), volatile suspended solids (VSS), total volatile fatty acids (VFA), pH/oxidation–reduction potential (ORP) and biochemical oxygen demand (BOD_5) were determined according to the standard methods [46]. Acidogenic mixed consortium in the anodic chamber was subjected to scanning electron microscopy (SEM; Hitach S-3000N). Prior to imaging biomass samples were washed [saline water and 0.05 M phosphate buffer 204 (pH 7.2)] followed by dehydration in a series of graded alcohol (10–90%) and finally fixed in glutaraldehyde (2.5%; 24 h).

3. Results

3.1. Bioelectricity generation

Prior to inoculation, anodic chamber of MFC was operated in the absence of biocatalysts with chemical wastewater for a period of 10 days. During this phase of operation potential difference fluctuated between a narrow range of 60 and 85 mV. Subsequently, the fuel cell was inoculated with the selectively enriched mixed microflora and operated with synthetic wastewater at OLR of

1.15 Kg COD/m³-day to support the growth and adaption of inoculated consortia in the anode chamber. Once voltage started to drop, fresh synthetic feed was replaced in anode chamber. Maximum voltage output of 609 mV was observed after 60 days of the startup along with constant substrate (COD) removal efficiency of 69%. To presume stable conditions, constant substrate (COD) removal efficiency and voltage output were considered as factors to assess the successful adaptation of inoculated consortia. After achieving stable conditions, fuel cell fed with chemical wastewater and the performance was evaluated at two OLRs 1.165 Kg COD/m³-day (240 h; two cycles) and 1.404 kg COD/m³-day (168 h; one cycle) with respect to power generation and substrate (COD) removal efficiency. Adopted selectively enriched consortia showed good efficiency in bioelectricity generation utilizing chemical wastewater as substrate (Fig. 2). Immediately after feeding chemical wastewater (OLR 1.165 Kg COD/m³-day) a voltage of 606 mV was recorded. Stable voltage (716 mV) generation was recorded in the second feed cycle. Gradual improvement in the current generation was observed with time and substrate exhaustion. Maximum current of 2.84 mA (at 50 Ω) was recorded after 216 h (OLR 1.165 Kg COD/m³-day) of operation and thereafter a persistent drop in the current generation was observed. At higher OLR (1.404 Kg COD/m³-day) studied, initial voltage of 590 mV (1.9 mA; 50 Ω) was observed and with exhaustion of time fuel cell approached a maximum voltage of 731 mV (2.97 mA; 50 Ω) after 121 h of operation. During fuel cell operation H₂ generated in the head space of the anodic chamber was monitored. The fugitive H₂ gas stored in the head space of the fuel cell (anode) was only measured during operation. However, due to utilization of H₂ as proton, estimating of exact figures of H₂ production was not possible. Hence, accurate correlation between H₂ yield and electricity production may not be possible.

3.2. Wastewater treatment

Performance of fuel cell with respect to wastewater treatment was also evaluated by estimating the substrate (COD) removal efficiency (ξ) during operation Eq. (1)

$$\xi = [(C_{S0} - C_S)/C_{S0}] \times 100 \quad (1)$$

where C_{S0} represents the initial COD concentration (mg/l) in the feed and C_S denotes COD concentration (mg/l) in the outlet. Organic loading rate (OLR- Kg COD/m³-day) was calculated using the Eq. (2)

$$\text{OLR} = [C_{S0} \times \text{Feed Rate}]/\text{Reactor Volume} \quad (2)$$

Substrate degradation rate (SDR-Kg COD/m³-day) was calculated to study the rate and pattern of COD removal during cycle operation according to the Eq. (3), ξ_t represent substrate removal efficiency (%) at time 't'.

$$\text{SDR} = [\text{OLR} \times \xi_t/100] \quad (3)$$

Experimental data depicted functioning of MFC as treatment unit by evidencing substrate removal apart from power generation (Fig. 3). COD removal efficiency of 44.19% was observed at operating OLR of 1.165 Kg COD/m³-day during stable phase of operation accounting for substrate degradation rate (SDR) of 0.519 Kg COD/m³-day. At higher OLR studied (1.404 Kg COD/m³-day), the fuel cell yielded higher substrate removal efficiency (61.11%) accounting for SDR of 0.858 Kg COD/m³-day. Comparatively higher substrate degradation was observed at higher OLR.

During stable operation phase of the fuel cell, substrate removal almost stopped after 96 h (OLRs 1.165 Kg COD/m³-day) and 144 h (1.404 Kg COD/m³-day) of the cycle operation. Specific power yield showed lower figures at higher loading rate [0.73 W/Kg COD_R [1.165 Kg COD/m³-day; 50 Ω]; 0.49 W/Kg COD_R [1.404 Kg COD/m³-day; 50 Ω]] (Fig. 4). On the contrary, volumetric power [3.08 W/m³ at 1.165 Kg COD/m³-day; 3.34 W/m³ COD_R at 1.404 Kg COD/m³-day; 50 Ω] and current density (339.87 mA/m² at 1.165 Kg COD/m³-day; 50 Ω and 355.43 mA/m² COD_R at 1.404 Kg COD/m³-day; 50 Ω) showed higher figures at higher substrate loading rates. The relatively lower specific power yield observed at higher OLR may be attributed to the loss in the electron transport process resulting in less power output. Utilization of substrate (COD) indicates effective functioning of selectively enriched mixed microflora in metabolizing the carbon source as electron donor. A gradual improvement in the current generation was observed with substrate exhaustion.

3.3. Polarization curve

Fuel cell operation at higher power density means operation at lower voltages (lower cell efficiency), causing instability in control as the system has a tendency to oscillate between lower and higher current densities. It is usual practice to operate the cell to the left side of the power density peak and at a high voltage or low current density. Polarization curve helps to find the cell design points.

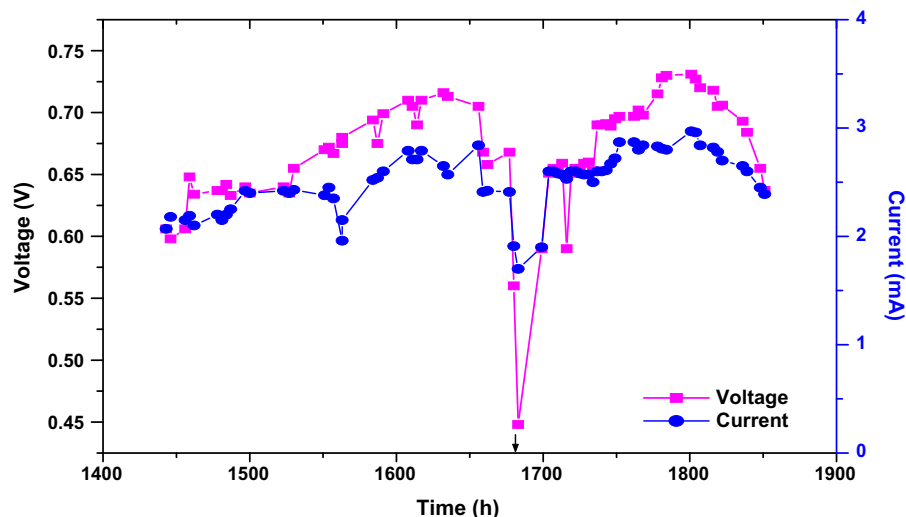


Fig. 2. Current and voltage generation during MFC operation with the function of time (measured at resistance 50 Ω) [↓ indicates OLR change from 1.165 Kg COD/m³-day to 1.404 Kg COD/m³-day].

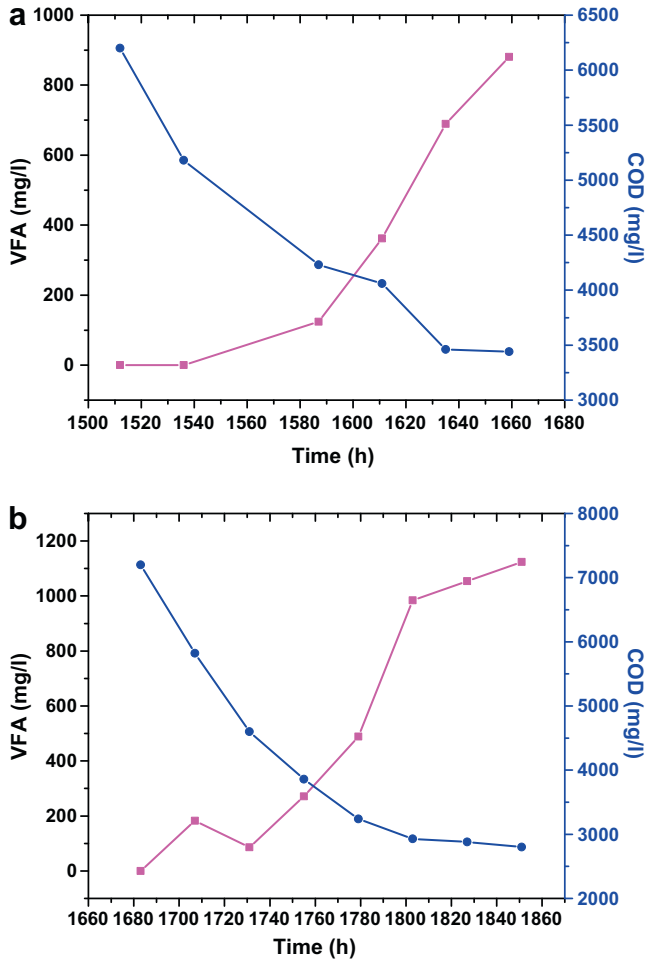


Fig. 3. Variation of COD and VFA during single feeding cycle of MFC operation [(a) 1.165 Kg COD/m³-day; (b) 1.404 Kg COD/m³-day].

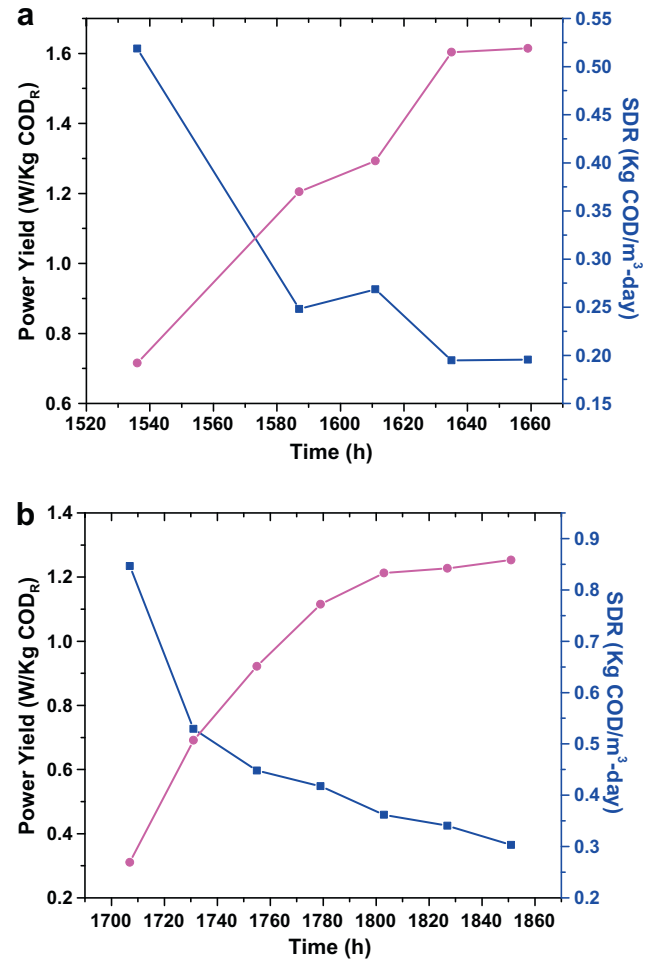


Fig. 4. Substrate degradation rate (SDR) and power yield with the function time (single cycle operation) [(a) 1.165 Kg COD/m³-day; (b) 1.404 Kg COD/m³-day].

Polarization curve was plotted with current density against potential and power density at different resistance (100 Ω–30 KΩ) to visualize the maximum power density and current density with respect to the OLR after attaining maximum voltage (Fig. 5).

Maximum current of 2.84 mA and 2.97 mA was observed at 50 Ω resistor for operating OLRs 1.165 Kg COD/m³-day and 1.404 Kg COD/m³-day OLRs, respectively. Power density curve documented a maximum value of 51.32 mW/m² at 300 Ω resistance at operating OLR of 1.165 Kg COD/m³-day. At higher loading rate (1.404 Kg COD/m³-day) maximum power density of 67.48 mW/m² was registered at 500 Ω resistance. Maximum current of 2.20 mA and 2.62 mA was observed at 100 Ω either after resistance up-shift or down-shift at OLRs 1.165 Kg COD/m³-day and 1.404 Kg COD/m³-day, respectively. At higher resistance (30 KΩ) current generation of 0.05 mA and 0.06 mA was observed at OLRs 1.165 Kg COD/m³-day and 1.404 Kg COD/m³-day, respectively. Comparatively rapid voltage stabilization was observed at higher resistances (30 KΩ). Effective electron discharge observed at lower resistances might be a probable reason for more potential drop and slow stabilization of the voltage at lower resistors. According to polarization concept, at OLR of 1.165 Kg COD/m³-day, maximum power density was observed at 300 Ω (power density-51.32 mW/m²) and the fuel cell could be operated effectively below 300 Ω resistances with stable performance and the corresponding power densities observed at 100 Ω and 200 Ω were 48.44 mW/m² and 45.74 mW/m², respectively. At operation OLR of 1.404 Kg COD/

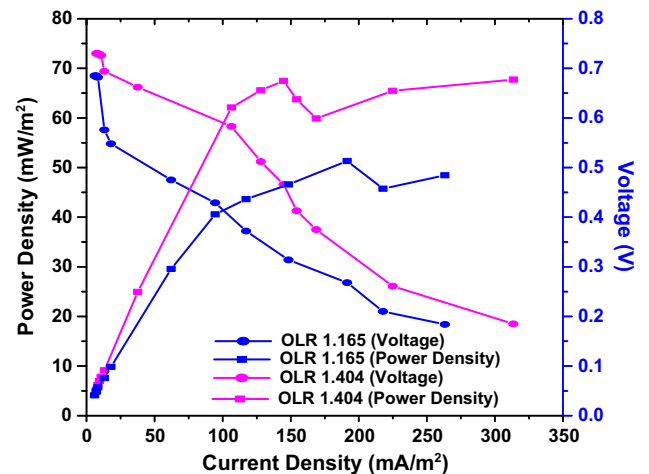


Fig. 5. Polarization curves measured at various resistances (100 Ω–30 KΩ) generated during stable performance of MFC operation at two OLRs studied.

m³-day, maximum power density (67.48 mW/m²) was observed at 500 Ω and the cell could be operated effectively below 500 Ω resistances and the corresponding power density observed at 100 Ω, 200 Ω, 300 Ω, and 400 Ω were 58.01 mW/m², 58.72 mW/m², 63.28 mW/m², and 63.76 mW/m², respectively.

3.4. Cell potentials and sustainable power

The anode and cathode potentials along with cell potentials were measured across various resistances (100 Ω–30 KΩ). Variation in potentials of the cathode, anode, and cell against the external resistance are presented in Fig 6. The cathode potential was

observed to be varied in narrow range around 274 mV at lower OLR (1.165 Kg COD/m³-day) and 296 mV at higher OLR (1.404 Kg COD/m³-day). The cathode potential was almost constant at external resistance, showing that the current was limited by the anode [46]. But the anode potential varied according to the external resistance applied from 34 mV to 412 mV in case of lower OLR

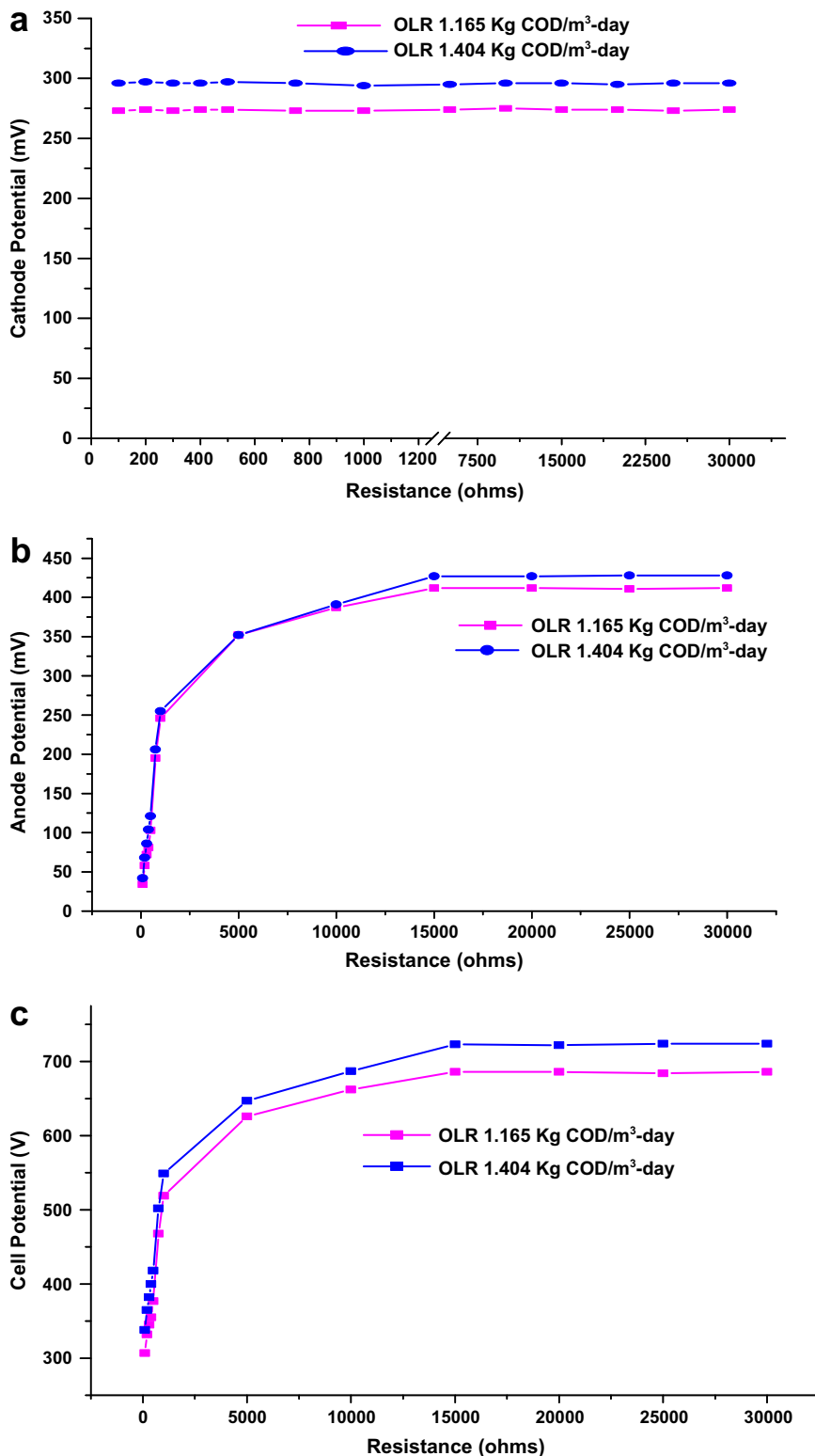


Fig. 6. Effect of external resistance on the (a) cathode; (b) anode; (c) cell potential during MFC operation.

(1.165 Kg COD/m³-day) and 42 mV to 428 mV in case of higher OLR (1.404 Kg COD/m³-day). The cell potential decreased significantly when resistance applied was less than 5 K Ω in applied higher and lower OLRs.

Power generated by fuel cell can be computed as the product of the cell potential and the current in the external circuitry [47]. The fuel cell and the external circuit will be in steady state if the power generated by the MFC equals the power consumption for an extended time at steady state conditions and the power production is sustainable. According to Menicucci and co-workers at steady state power production is considered to be sustainable [47]. Because many steady states are possible, it is important to define conditions for which the sustainable current reaches a maximum and compute the maximum sustainable power that can be generated by the fuel cell. Decreasing resistance was measured to evaluate the sustainable power, current and cell potentials by changing external resistance stepwise in equal time intervals (Fig. 7). Sustainable power calculations were made considering the initial anodic potential ($E_{o,anodic}$) and anodic potentials (E_{anodic}) at each applied external resistance. Relative decrease in anodic potential (RDAP) was calculated as shown in Eq. (5) [47] after the MFC reached a stable cell potential at respective experimental conditions.

$$RDAP(\%) = [(E_{o,anodic} - E_{anodic})/E_{o,anodic}] \times 100 \quad (5)$$

The relative decrease in anode potential (RDAP) was used to evaluate maximum sustainable power. The variation of percent deviation of anodic potential with respect to applied external resistance is shown in Fig. 7. The linear fit at high external resistances represented a region in which the external resistance controls the power,

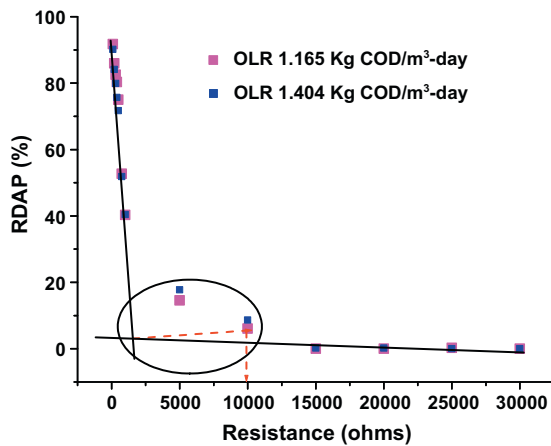


Fig. 7. Variation of percent deviation of anodic potential with respect to applied external resistance and evaluating sustainable power.

Table 1

Details of MFC operation (aerated cathode) with respect to output parameters during wastewater treatment

S. No.	Wastewater	Cathode ^a	OLR (Kg COD/m ³ -day)	pH transition	VFA buildup (mg/l)	Voltage (mV)	Current (mA)	COD reduction (%)	SDR (Kg COD/m ³ -day)	Specific power production (W/Kg COD _R)	Current density (mA/m ²)	Reference
1	Chemical	Aerated	1.165	5.5–7.5	+881	716	2.84	44.19	0.519	0.156	263.28	This work
			1.404	5.5–6.2	+1124	731	2.97	61.11	0.858	0.128	313.55	
2	Designed synthetic	Aerated	0.625	6.0–5.11	+374	572	1.68	74.15	0.464	0.44	190.28	[9]
3	Designed synthetic	Aerated	0.517	5.5–4.6	+546	350	1.44	62.5	0.323	0.168	172.33	[23]
			0.574	5.5–6.8	–10	423	1.66	53.3	0.306	0.274	198.65	
			0.646	5.5–6.8	+106	339	1.41	59.5	0.384	0.167	168.74	
			1.033	5.5–6.8	–70	332	1.38	60	0.419	0.167	165.15	

^a All MFC's were operated with same mixed culture in dual chambered configuration; OLR – organic loading rate; HRT – hydraulic retention time; VFA – total volatile fatty acids ('+' indicates VFA generation; '-' indicates VFA consumption); SDR – substrate degradation rate; anode microenvironment was uniform except for pH range.

while, the linear fit at low external resistances represented a region in which the power is limited by kinetics, mass transfer, or internal resistance [31,32,47]. When external resistance is high, the RDAP increases linearly with decreasing external resistance because the electron delivery to the cathode is limited by external resistance. However, when a low external resistance is applied, the electron delivery to the cathode is limited by kinetic and/or mass transfer (or internal resistance) effect. The RDAP increases linearly with decreased external resistance, with different slopes, for external resistance limited or internal resistance limited conditions. The conditions where external and internal resistance limitations are equal must be somewhere between these two lines, which is presented as a dotted line. When both lines intersect, a horizontal line from the intersection was drawn to estimate the external resistor that allows us to measure sustainable power. Fig. 7 shows the evaluated sustainable power and sustainable power yield corresponding to external resistance. From the graphical procedure we found an external resistor value at 10 K Ω for the experimental variations studied with the MFC that corresponds to a sustainable power generation. The power yield at this external resistance was observed to be more in the case of lower OLR [0.023 W/Kg COD_R] than higher OLR [0.017 W/Kg COD_R].

3.5. Coulombic efficiency

Coulombic efficiency (ϵ_c) is defined as the ratio of total coulombs actually transferred to the anode from the substrate, to maximum possible coulombs if all substrate removal produces current [45]. Coulombic efficiency (ϵ_{cb}) in fed-batch mode over a period of time (t_b) is calculated as shown below [45,48,49]

$$\epsilon_{cb} = \frac{M \int_0^{t_b} Idt}{Fbv_{An}\Delta COD} \quad (4)$$

where M is the molecular weight of oxygen (32), F is Faraday's constant (96485 C), b represents number of electrons exchanged per mole of oxygen (4), v_{An} is the volume of liquid in the anode compartment (0.65 l), and ΔCOD depicts change in COD over a period of time (t_b). Maximum ϵ_{cb} of 7.35% and 5.77% was observed at OLRs of 1.165 Kg COD/m³-day and 1.404 Kg COD/m³-day, respectively. The studied fuel cell was able to convert 0.519 Kg COD/m³-day to 0.858 Kg COD/m³-day to electricity depending on the substrate loading rate. Upon variation of the OLRs and the external resistance, changes in the overall power output and the power yield (substrate to current conversion efficiency) were obtained.

4. Discussion

It is evident from the experimental data, that fuel cell with plain graphite electrode (without coating) inoculated with selectively enriched H₂ producing mixed consortia showed *in situ*

bioelectricity generation along with the treatment of chemical wastewater. Table 1 illustrates consolidated experimental data pertaining to present study in comparison with work reported with same MFC configuration and mixed consortia operated with different types of wastewaters and operating pH (5.5/6.0) [9,23]. It was evident from Table, that the performance of MFC, with respect to electricity generation was dependent on the nature of wastewater used as substrate. Comparatively higher current outputs and substrate degradation were observed in the case of chemical wastewater studied. The current observed with chemical wastewater was around 76% higher than the maximum achieved with synthetic wastewater. Thirty percent higher voltage was observed with chemical wastewater. However, specific power yield was slightly lower than synthetic wastewater. This might be attributed to the higher SDR (85% higher) observed with chemical wastewater system leading to loss in electron transfer mechanism. A sharp increase in internal resistance from 0.105 K Ω to 0.199 K Ω was observed with increase in OLR from 1.165 Kg COD/m³-day to 1.404 Kg COD/m³-day. Oxygen as electron acceptor has good redox potential which also substitutes toxic chemical in cathode chamber resulting in water as final product in the reduction reaction.

The fuel cell configuration, mixed inoculum and the operating conditions adopted in this study showed the feasibility of harnessing power from chemical wastewater treatment. The idea of using the acidogenic mixed inoculum and the adopted acidophilic conditions is to directly generate H₂ and convert energy to power in a single defined system. The acidogenic mixed culture facilitates H₂ production in the anode chamber which is the major electron donor for electricity generation [8,9,23]. *In situ* production of H₂ and converting to electricity is advantageous compared to process of biohydrogen production in separate reactor followed by purification and storage prior to conversion. Generally, anaerobic cultures cannot produce H₂ as terminal product due to manifestation of methane formation through MB in association with consumption of H₂ [50–52]. Effective ways to enhance H₂ yield from the anaerobic culture is to restrict or terminate the methanogenesis process thereby allowing H₂ as terminal end product in the metabolic flow [10–13]. The applied pre-treatment strategies on parent inoculum helped to eliminate non-spore forming MB from inoculum and facilitated enrichment of spore forming acidogenic group. This led to the production of H⁺/H₂ associated with acid generation (VFA production). Stabilization in VFA generation was observed after 1635 h and 1803 h of operation at the studied OLRs 1.165 Kg COD/m³-day and 1.404 Kg COD/m³-day, respectively (Fig. 3). In anode chamber, the pH values showed increasing trend consistently with the fuel cell operation (data not shown).

Operation of MFC at acidophilic conditions (pH 5.5) helps to limit the MB activity and maximize biological H₂ production. Prior to feeding, wastewater was adjusted to pH 5.5 which facilitated effective functioning of AB at the same time inhibiting MB. This created a susceptible microenvironment for H⁺/H₂ evolution through acidogenic process. Optimum pH for the growth of MB was reported to be between 6.0 and 7.5, while AB functioned well below 6 pH [8–13,23,36,43,44,53–58]. By maintaining acidophilic pH around 5.5 compared to a near neutral pH the conversion efficiency (of H₂ production) was found to increase. The pH range of 5.5–6 was considered to be ideal to avoid both methanogenesis and solventogenesis [43,59,60] and could be considered optimum pH range for effective H₂ generation. SEM ($\times 2.5$ K and $\times 5.0$ K magnification; Fig. 8) image visualized morphologically similar group of bacteria on anode surface of the fuel cell. Most of the bacteria appeared in clusters with almost similar morphology (rod shape; approximately 20 μ m length). Morphological similarity observed might be attributed to the selective enrichment procedure applied on inoculum before inoculation and the adopted operating conditions. Moreover, it was reported that extent of biofilm growth on

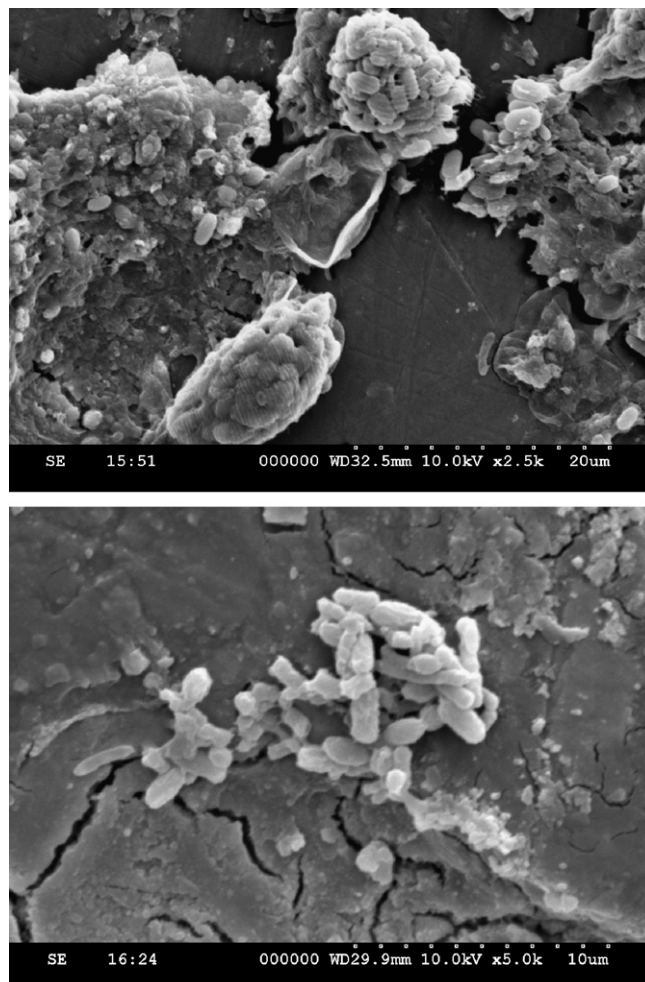


Fig. 8. SEM images of bioelectricity producing mixed consortia acquired from anode surface [$\times 2.5$ K and $\times 5.0$ K].

anode surface has significant influence on power generation potential in fuel cell operation [32]. Usage of mixed culture suits well for practical application in MFC as it does not require special care as in case of pure culture and could be considered as feasible methodology for practicable, cost-effective and promising approach to achieve bioelectricity generation at large scale.

Mixed acidogenic inoculum not only generated electricity but also functioned in wastewater treatment (COD reduction) by metabolizing the carbon source as electron donor. The substrate degradation observed in the anode chamber enumerated the functioning of MFC as alternative wastewater treatment unit in addition to renewable energy generation. Fuel cells anodic chamber represents a suspended growth anaerobic reactor configuration normally used in wastewater treatment. Less COD removal efficiency observed in fuel cell operation compared to the anaerobic treatment units might be attributed to the adopted acidogenic microenvironment (acidophilic condition; pH 5.5) rather than methanogenic microenvironment (pH 7). This condition has helped to generate H⁺/H₂ (acidogenic process) rather than CH₄ (methanogenic process) as metabolic by-product. The amount of proton (H⁺) production governed the resulting potential difference generated during the fuel cell operation.

5. Conclusion

Experimental data demonstrated the feasibility of power generation from anaerobic chemical wastewater treatment using dual

chamber MFC operated with aerated cathode and mediator less anode employing selectively enriched H_2 producing mixed consortia under acidophilic microenvironment. Fuel cell fabricated with plain graphite electrodes showed in situ bioelectricity generation along with the chemical wastewater treatment. Designed MFC configuration (aerated cathode), operating conditions (acidophilic) adopted and parent inoculum (selectively enriched acidogenic mixed culture) showed feasibility of power generation from chemical wastewater treatment. The performance and stabilization tendency of the fuel cell with respect to power generation was found to be dependent on the substrate loading rate applied. Power generation using chemical wastewater with low cost electrodes and mixed culture was considered as cost-effective and environmentally sustainable process which can reduce the cost of the existing effluent treatment plants by providing additional energy a part from treatment in a sustainable way.

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