

# Bioelectricity generation from chemical wastewater treatment in mediatorless (anode) microbial fuel cell (MFC) using selectively enriched hydrogen producing mixed culture under acidophilic microenvironment

S. Venkata Mohan\*, G. Mohanakrishna, B. Purushotham Reddy, R. Saravanan, P.N. Sarma

*Bioengineering and Environmental Centre, Indian Institute of Chemical Technology, Hyderabad 500007, India*

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## Abstract

Bioelectricity generation from composite chemical wastewater treatment was evaluated in a dual chambered microbial fuel cell (MFC) [anode chamber (mediatorless; perforated plain graphite electrode); cathode chamber (50 mM potassium ferricyanide  $[K_3Fe(CN)_6]$  in phosphate buffer; pH 7.5; plain graphite electrode)] inoculated with selectively enriched hydrogen ( $H_2$ ) producing mixed culture under acidophilic microenvironment (pH 5.5). Anode chamber, which resembles anaerobic suspended contact reactor was fed with wastewater and operated in absence of artificial mediator at acidic environment to proliferate  $H_2$  producing bacteria. Experimental data showed the feasibility of producing bioelectricity from wastewater treatment, though power production was found to be dependent on the substrate loading rate. Maximum voltage of 271.5 mV (5.43 mA) and 304 mV (6.08 mA) was recorded at operating organic loading rates (OLR) of 1.165 kg COD/(m<sup>3</sup> day) and 1.404 kg COD/(m<sup>3</sup> day), respectively when measured at 50  $\Omega$  external resistors at stable operating conditions. COD removal efficiency of 35.4% (substrate degradation rate (SDR) of 0.412 kg COD/(m<sup>3</sup> day)) and 62.9% (SDR, 0.88 kg COD/(m<sup>3</sup> day)) was observed at OLRs 1.165 kg COD/(m<sup>3</sup> day) and 1.404 kg COD/(m<sup>3</sup> day), respectively. Maximum specific power production of 0.163 W/kg COD<sub>R</sub> (1.165 kg COD/(m<sup>3</sup> day); 50  $\Omega$ ) and 0.198 W/kg COD<sub>R</sub> (1.404 kg COD/(m<sup>3</sup> day); 100  $\Omega$ ) was observed during stable phase of fuel cell operation. Current density of 747.96 mA/m<sup>2</sup> (1.165 kg COD/(m<sup>3</sup> day)) and 862.85 mA/m<sup>2</sup> (1.404 kg COD/(m<sup>3</sup> day)) was documented at 10  $\Omega$ . Utilizing chemical wastewater for the production of renewable energy (bioelectricity) from anaerobic treatment is considered as a feasible, economical and sustainable process.

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**Keywords:** Bioelectricity; Microbial fuel cell (MFC); Chemical wastewater treatment; Acidophilic; Anaerobic; Biohydrogen; Mixed culture; Graphite

## 1. Introduction

Energy need has been increasing worldwide exponentially. At present global energy requirements are mostly dependent on the fossil fuels, which eventually lead to foreseeable depletion of limited fossil energy sources [1,2]. Combustion of fossil fuels also has serious negative affect on the environment due to CO<sub>2</sub> emission. Concerns about climate change, increased global demand for the finite oil and natural gas reserves and energy security, are intensifying the search for alternatives to fossil fuels [3]. Much attention is being paid on the usage of hydrogen ( $H_2$ ) as an alternative fuel throughout the world [3–6]. More recently, generation of electricity using microbial fuel cells (MFC) is seemingly gaining importance in the research

fraternity [3,7,8]. These two approaches of alternative fuel generation have numerous advantages—clean, efficient, renewable, and does not generate any toxic byproduct. Although, biohydrogen produced from anaerobic fermentation is considered as a viable alternative fuel and energy carrier of the future, storage, purification, low production rates and generation of energy (electricity) by using fuel cells are some of the inherent limitations. Alternatively, MFCs, emerge as the best option for the complete recovery and facilitates *in situ* conversion of energy (bioelectricity) [3].

MFC is a biochemical-catalyzed system which generates electrical energy through the oxidation of biodegradable organic matter in the presence of either fermentative bacteria or enzyme under mild reaction conditions (ambient temperature and pressure) [3]. The biocatalyst present in the anode chamber of fuel cell generates electrons ( $e^-$ ) and protons ( $H^+$ ) and the potential between the respiratory system and electron acceptor generates electricity. Thus, bacterial energy is directly converted to elec-

\* Corresponding author.

E-mail address: vmohan\_s@yahoo.com (S. Venkata Mohan).

trical energy and to close the cycle, protons migrate through a proton exchange membrane (PEM) from anode to cathode. Biofuel cells have characteristics similar to traditional power sources as well as to anaerobic reactors which can on the one hand be described by electrochemical parameters such as power density, electrical current output and cell voltage and on the other hand by biological parameters such as the substrate loading rate [7].

Rapid industrialization contributes for large quantity of wastewater and its treatment is highly imperative. Reducing the wastewater treatment cost and finding ways to produce useful products from wastewater is gaining importance recently in view of environmental sustainability. In anaerobic process, chemical energy is converted to H<sub>2</sub> and methane, which can be used as a fuel or to produce electricity, but in MFCs chemical energy present in the waste components is directly converted to electricity [4–10]. Exploiting wastewater as substrate to generate electricity is considered as sustainable and promising approach to meet the increasing energy needs and also as a substitute for fossil fuels. Technologies for electricity production especially using wastewaters as substrates employing MFC are in the early stages of research and development [6,8,11–19].

Since microorganisms act as a catalyst in the transfer of electrons from the substrate to the anode, the selection of a high-performing microbial culture (either pure or mixed) is of crucial importance in the MFCs [11,12]. MFC design, effective assembly of membrane electrode for reducing the proton transfer resistance, enhancing cost effective turbulence for effective interaction of substrate with bacterial colony on anode and suspended bacteria, reducing the potential loss at anode, providing adequate surface area for the bacterial growth, improving the cathode reaction, selection of bacterial consortium were the parameters on which electricity generation potential in MFC depends.

Utilizing chemical wastewater along with adopting the mixed anaerobic microflora for selectively enriching the bacteria is considered in this study. In this direction, the present work aims to study the feasibility of bioelectricity generation utilizing composite chemical wastewater as substrate through MFC employing selectively enriched H<sub>2</sub> producing mixed anaerobic consortia under acidophilic microenvironment. The performance of MFC (mediatorless anode) with respect to electricity generation and substrate removal efficiency from chemical wastewater treatment was evaluated at two organic loading rates (OLR).

## 2. Experimental

### 2.1. Selective enrichment of anodic mixed consortia

Anaerobic mixed consortia producing molecular H<sub>2</sub> in a laboratory scale biofilm bioreactor was used as inoculum in the anode chamber of MFC for electricity generation [4,5]. The reactor was in operation in our laboratory for the past two years using chemical wastewater as substrate. After acquiring microbial inoculum from the bioreactor, it was centrifuged (5000 rpm,

20 °C) and washed thrice with saline buffer. The resultant pellet was enriched in the designed synthetic wastewater (NH<sub>4</sub>Cl, 0.5 g/l; KH<sub>2</sub>PO<sub>4</sub>, 0.25 g/l; K<sub>2</sub>HPO<sub>4</sub>, 0.25 g/l; MgCl<sub>2</sub>, 0.3 g/l; CoCl<sub>2</sub>, 25 mg/l; ZnCl<sub>2</sub>, 11.5 mg/l; CuCl<sub>2</sub>, 10.5 mg/l; CaCl<sub>2</sub>, 5 mg/l; MnCl<sub>2</sub>, 15 mg/l; glucose, 3 g/l; pH 5.5; COD, 3.4 g/l) by keeping the bottle closed with rubber septum (100 rpm; room temperature) under aseptic anaerobic microenvironment. Acidophilic pH (5.5) was maintained to sustain the activity of acidogenic bacteria (AB) and to inhibit the activities of the methanogenic bacteria (MB) in order to enhance hydrogen production [4–6]. Prior to inoculation, the consortia was subjected to pretreatment [heat-shock treatment (100 °C; 2 h) and acid treatment (pH 3 adjusted with *ortho*-phosphoric acid (88%); 24 h)] to inhibit the growth of MB at the same time to enrich the H<sub>2</sub> producing mixed microflora. The resulting selective enriched microflora was used as inoculum for startup of the MFC (anode chamber).

### 2.2. Chemical wastewater

Chemical wastewater was used as substrate for the bioelectricity generation. The composite/combined chemical wastewater was collected from a common effluent treatment plant (CETP) in Hyderabad, India where wastewater aggregated from bulk drugs, chemical intermediates, dye and dye intermediates, pharmaceuticals, pesticides and various chemical process units was being treated. The characteristics of wastewaters used in the experiments: pH 7.82; oxidation reduction potential (ORP) 750 mV; suspended solids 0.98 g/l; total dissolved solids 25.5 g/l; total alkalinity 0.12 g/l; chlorides 7.71 g/l; chemical oxygen demand (COD) 12.1 g/l; biological oxygen demand (BOD) 3.63 g/l. Characteristically, the wastewater is complex in nature due to its composite nature, low-biodegradability (BOD/COD ~0.3) and sulfate content.

### 2.3. MFC configuration

Dual chambered MFC was fabricated in the laboratory using ‘perplex’ glass using leak proof sealing. The schematic details of MFC along with photograph are depicted in Fig. 1. Total volume of anode and cathode compartments were 0.75 l each and the two chambers were separated by proton exchange membrane (PEM) (Nafion 117, Sigma–Aldrich). PEM disc was arch punched to 50 mm diameter from the Nafion 117 sheet and subjected to pretreatment (boiling in 30% H<sub>2</sub>O<sub>2</sub>, deionized water, 0.5 M H<sub>2</sub>SO<sub>4</sub> and deionized water sequentially each for 1 h) to increase the porosity and stored in deionized water prior to use. PEM was fixed between clamps in the hollow tube (50 mm diameter) connecting both the chambers. Plain graphite plates (5 cm × 5 cm; 10 mm thickness) without any coating were used as electrodes for both anode and cathode. Cathode have projected surface area of 70 cm<sup>2</sup> and anode was provided with nine uniform holes of 0.1 cm diameter to increase the overall surface area (83.56 cm<sup>2</sup>). Prior to use electrodes were soaked in deionized water for a period of 24 h. Electrodes were positioned at a distance of 6 cm on either side of PEM. Copper wires were used as contact with electrodes and the contact area was sealed

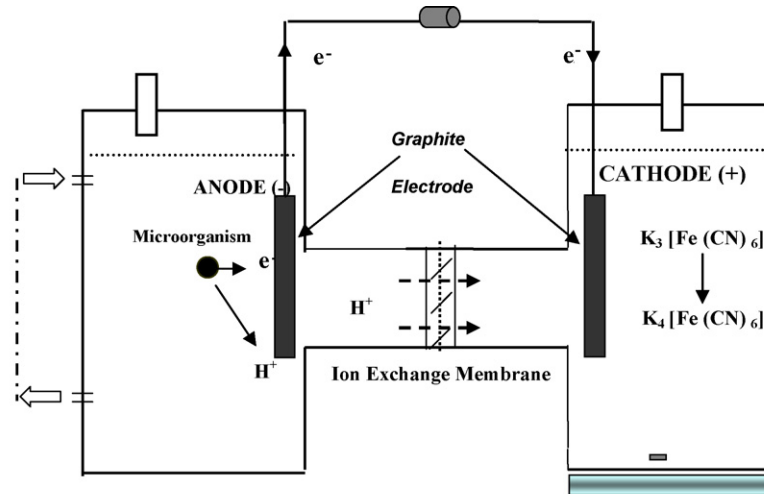


Fig. 1. Schematic details and photograph of dual chambered MFC used in the experiments.

carefully with ‘epoxy’ material. Each chamber was designed to have sample ports, wire point input provision (top), inlet and outlet ports. Anode chamber was sealed with washers to ensure anaerobic microenvironment.

#### 2.4. Operation

Fuel cell was operated in fed batch mode at room temperature ( $29 \pm 2^\circ\text{C}$ ). The cathode compartment was filled with 700 ml of potassium ferricyanide [50 mM  $K_3Fe(CN)_6$ ] in phosphate buffer [50 mM  $K_2HPO_4$ ] and the solution pH was adjusted to 7.5. During operation the catholyte solution was mixed continuously by stirring with magnetic beads. The anodic chamber prior to the startup was inoculated with the selectively enriched  $H_2$  producing mixed microflora (volatile suspended solids (VSS) 2.0 g/l) dissolved in designed synthetic wastewater (700 ml). Fresh wastewater (650 ml) was loaded (feeding, 15 min) once voltage started to decrease. Before changing the feed, inoculum was allowed to settle down (30 min, settling) and exhausted feed (650 ml) was pumped out (decanted, 15 min)

under anaerobic conditions. The settled inoculum (50 ml by volume) was used for subsequent experiments. Anolyte was continuously recirculated at a rate of 100 ml/min using peristaltic pump to achieve a homogeneous distribution of the substrate as well as consortia along the reactor. During operation, anode chamber was maintained under aseptic anaerobic microenvironment. After every feeding event, the anode chamber was sparged with  $N_2$  for 2 min to maintain anaerobic microenvironment. Prior to feeding feed pH was adjusted to  $5.5 \pm 0.1$  using concentrated *ortho*-phosphoric acid (88%) to sustain the survival of acidogenic bacteria (AB) at the same time suppressing the methanogenic activity. Feeding, decanting, and recirculation operations were carried out employing peristaltic pumps (Micclins, India) controlled by electronic timer (ETTS, Germany). The controller was programmed to operate sub-program and output dedicated to the operation of each controllable element. The performance of MFC was evaluated with chemical wastewater at two substrate loading rates (1.165 kg COD/( $m^3$  day) and 1.404 kg COD/( $m^3$  day)). Each cycle at OLR of 1.165 kg COD/( $m^3$  day) was for 5 days

and for OLR of 1.404 kg COD/(m<sup>3</sup> day) was for 7 days. OLR of the influent wastewater as required was adjusted by diluting with tap water before feeding. Constant COD removal and voltage outputs were considered as indicators for satisfactory formation of the biofilm and subsequently shifted to designated chemical wastewater. Voltage was recorded once in every 3 h. After reaching stable performance, power output was monitored by measuring voltage across an external resistor of 50 Ω connected across the anode and cathode. Polarization curve was generated by measuring current at variable external resistances (100–30,000 Ω) to evaluate the relationship between resistance and current.

## 2.5. Analysis

COD (closed refluxing method), alkalinity (total), volatile suspended solids (VSS), volatile fatty acids (VFA), pH/ORP and BOD<sub>5</sub> were determined according to the standard methods [20]. pH and oxidation–reduction potential (ORP) values were determined by a pH meter (Model 20, Denver Instruments Ltd.). H<sub>2</sub> gas generated in the anodic chamber was estimated using a microprocessor-based pre-calibrated H<sub>2</sub> sensor (electrochemical three electrode H<sub>2</sub> sensor, FMK satellite 4–20 mA version, ATMI GmbH Inc., Germany) [4–6,8]. The output signal displayed the % volume of H<sub>2</sub> in the head space of the fuel cell. H<sub>2</sub> gas monitoring was carried out under closed conditions to avoid external environmental contamination. The separation and quantitative determination of VFA was carried out by high-performance liquid chromatography (HPLC; Shimadzu LC10A) employing optimized conditions (UV–VIS detector; C18 column – reverse phase column – 250 mm × 4.6 mm and 5 μm particle size; flow rate, 0.5 ml/h; wave length, 210 nm; mobile phase, 40% of acetonitrile in 1mN H<sub>2</sub>SO<sub>4</sub> (pH 2.5–3.0); sample injection, 20 μl).

Bio-electrochemical calculations were done based on the procedure outlined by Logan et al. [12]. Current (*I*) and potential (*V*) measurements were recorded at every 3 h using digital multimeter (Metravi 901) by connecting 50 Ω in external circuit. For polarization, current generation was monitored at various external resistances (100–30,000 Ω) connected for few minutes and readings were noted after stabilization, the voltage was calculated using the formula  $V=IR$  from the current. Power (*W*) was calculated using  $P=IV$ , where *I* is in amperes and *V* is the voltage in mV. Power density (mW/m<sup>2</sup>) and current density (mA/m<sup>2</sup>) were calculated by dividing the obtained power and current with the surface area (m<sup>2</sup>) of anode. Specific power production (W/kg COD<sub>R</sub>) was obtained by dividing power generated with the substrate (COD<sub>R</sub>) removed.

Performance of fuel cell was also evaluated by estimating the substrate (COD) removal efficiency ( $\xi_{\text{COD}}$ ) during operation using Eq. (1). *C*<sub>SO</sub> represents the initial COD concentration (mg/l) in the feed and *C*<sub>S</sub> denotes COD concentration (mg/l) in the reactor outlet:

$$\xi_{\text{COD}} = \frac{C_{\text{SO}} - C_{\text{S}}}{C_{\text{SO}}} \times 100 \quad (1)$$

Organic loading rate (OLR, kg COD/(m<sup>3</sup> day)) was calculated using the following equation

$$\text{OLR} = \frac{C_{\text{SO}} \times \text{feed rate}}{\text{reactor volume}} \quad (2)$$

Substrate degradation rate (SDR, kg COD/(m<sup>3</sup> day)) was calculated to study the rate and pattern of COD removal during the cycle operation according to the Eq. (3), where,  $\xi_t$  represent substrate removal efficiency (%) at time '*t*':

$$\text{SDR} = \frac{\text{OLR} \times \xi_t}{100} \quad (3)$$

## 3. Results and discussion

### 3.1. Bioelectricity generation

After inoculating the anode chamber with selectively enriched H<sub>2</sub> producing mixed consortia, the fuel cell was operated with designed synthetic wastewater as feed to support the formation of biomass and subsequent adaptation to the new environment. During the operation, anolyte pH of the fuel cell was maintained at 5.5 to facilitate proliferation and functioning of H<sub>2</sub> producing microflora. Constant substrate (COD) removal efficiency and voltage output were considered as an indicator to assess the successful formation of the biomass. Consistent increase in voltage was observed with the exhaustion of time accounting for a maximum voltage output of 245 mV and substrate removal efficiency (69%) after 51 days of the startup. Subsequently, the MFC was shifted to chemical wastewater and the performance was evaluated at two OLRs 1.165 kg COD/(m<sup>3</sup> day) (120 h) and 1.404 kg COD/(m<sup>3</sup> day) (168 h) with respect to power generation and substrate (COD) removal efficiency.

The performance of fuel cell was evaluated by measuring voltage and current output during operation (Fig. 2). The experimental data documented the feasibility of bioelectricity

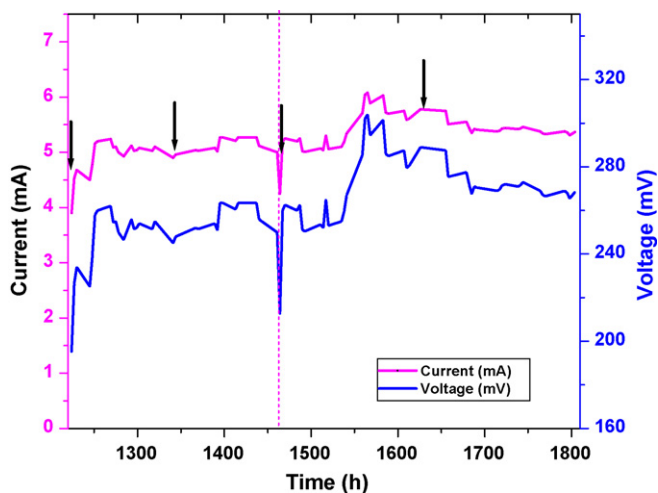


Fig. 2. Current and voltage generation measured at 50 Ω during MFC operation with chemical wastewater at two operating OLRs ('↓' indicates feed change event) [(a) 1.165 kg COD/(m<sup>3</sup> day) (COD<sub>feed</sub>, 6200 mg/l); (b) 1.404 kg COD/(m<sup>3</sup> day) (COD<sub>feed</sub>, 10,540 mg/l)].

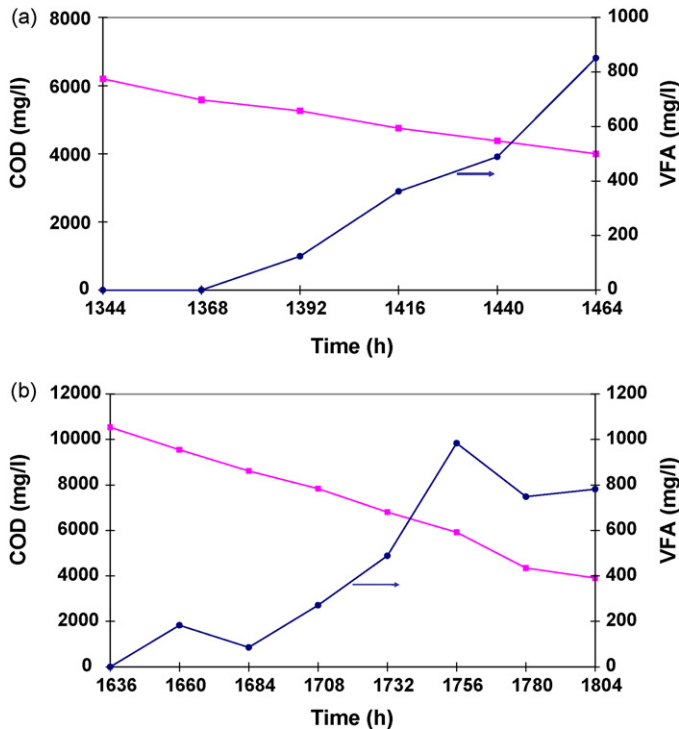


Fig. 3. COD concentration variation in conjugation with VFA production during MFC operation at two OLRs [(a) 1.165 kg COD/(m<sup>3</sup> day) (COD<sub>feed</sub>, 6200 mg/l); (b) 1.404 kg COD/(m<sup>3</sup> day) (COD<sub>feed</sub>, 10,540 mg/l)].

generation and substrate removal utilizing chemical wastewater as substrate under adopted operating conditions of MFC (Fig. 3). However, the performance and stabilization tendency with respect to power generation and substrate removal was found to depend on the applied substrate loading rate. At operating OLR of 1.165 kg COD/(m<sup>3</sup> day), during initial phase of fuel cell operation, voltage around 195 mV (3.9 mA; 50 Ω) was observed (Fig. 2). With the exhaustion of time, a steady increase in voltage was observed which reached a maximum of 271.5 mV after 219 h of operation. Current was measured by providing 50 Ω as external resistance. Current generation showed a maximum of 5.43 mA after 219 h of feeding the wastewater. In the case of higher OLR (1.404 kg COD/(m<sup>3</sup> day)), during initial operation phase, potential difference of 225 mV (4.50 mA; 50 Ω) was observed. Voltage increased steadily with time and reached a maximum of 304 mV after 78 h of operation and thereafter showed a decreasing trend. Maximum current generation of 6.08 mA (50 Ω) was observed after 78 h of feeding the wastewater. It is evident from the voltage profile, at lower substrate loading rate, that the fuel cell has taken relatively longer time to attain higher voltage potential (219 h), while at higher substrate loading rate, the system approached rapidly towards higher voltage (90 h). A steady increase in potential difference was observed with every additional feed and this might be attributed to the adaptation tendency of the inoculated microflora for new environment. Initially a marked drop in potential difference was observed with every fresh feeding event. Stable voltage generation was recorded after four transfers of feed. The system showed a

retention time 5 days (1.165 kg COD/(m<sup>3</sup> day)) and 7 days (1.404 kg COD/(m<sup>3</sup> day)) with respect to potential drop. With drop in voltage, a fresh wastewater was fed to the anodic chamber.

During fuel cell operation H<sub>2</sub> formed in the head space of the anodic chamber was continuously monitored. The H<sub>2</sub> concentration showed a gradual build-up with the proceeding of the fermentation period in both the substrate loading rates studied. Maximum H<sub>2</sub> production of 351.36 mmol H<sub>2</sub>/day and 358.56 mmol H<sub>2</sub>/day was observed at OLRs of 1.165 kg COD/(m<sup>3</sup> day) and 1.404 kg COD/(m<sup>3</sup> day), respectively during stable phase of operation (Fig. 4). However, due to utilization of H<sub>2</sub> in bioelectricity generation process as proton (passing along PEM to cathode), monitoring of exact figures of H<sub>2</sub> production was not possible. The fugitive H<sub>2</sub> gas stored in the head space of the fuel cell (anode) was only measured during operation. Hence, accurate correlation between H<sub>2</sub> yield and electricity production may not be realized.

Protons and electrons generated in the anode compartment were reduced (ferricyanide as ferrocyanide) in the cathode compartment. Absorbance of cathode electrolyte was monitored at 420 nm during the run. Initial absorbance was 2.3 and started to decrease throughout the run. The results showed that ferrocyanide functioned as electron acceptor in the cathode compartment enhancing the reduction process. Catholyte was changed once in 11 days due to the evaporation loss and reduction in ferricyanide concentration. The use of ferrocyanide in the cathode as electron acceptor might have enhanced the cathode reaction, which reduces the activation over potential [8].

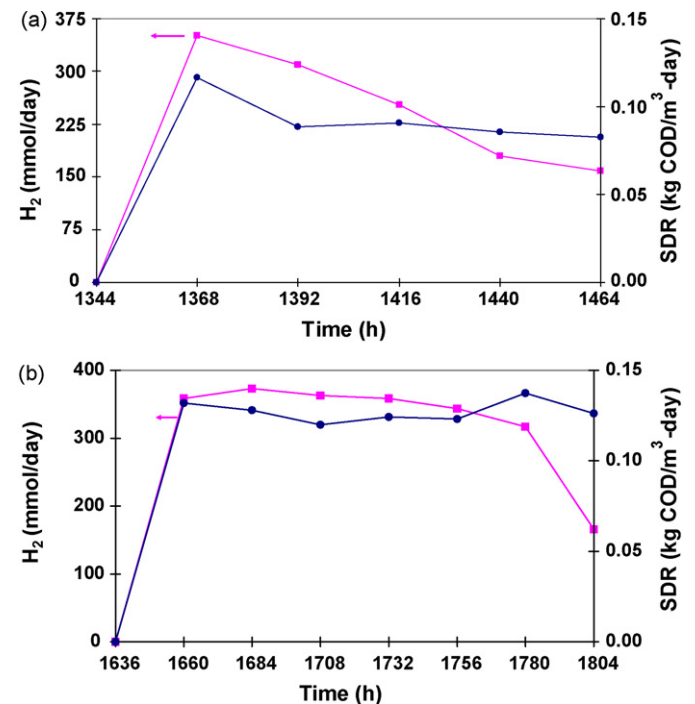


Fig. 4. Hydrogen production in conjugation with SDR during MFC operation at two OLRs [(a) 1.165 kg COD/(m<sup>3</sup> day) (COD<sub>feed</sub>, 6200 mg/l); (b) 1.404 kg COD/(m<sup>3</sup> day) (COD<sub>feed</sub>, 10,540 mg/l)].

### 3.2. Substrate degradation

The anodic chamber resembles anaerobic suspended contact reactor normally used in wastewater treatment was monitored for substrate (COD) removal efficiency during fuel cell operation (Fig. 3). Cumulative substrate degradation rate ( $SDR_C$ ) and specific substrate degradation rate ( $SDR_S$ ) with the function of time was also calculated during fuel cell operation. Cumulative SDR correspond to the overall substrate degradation rate occurred up to that point of time from the time of substrate loading (Fig. 4). Specific SDR specifies the amount of substrate degradation occurred over a period of time to enumerate the substrate degradation pattern during specific period of interest. During the stable phase of operation (4th cycle; OLR, 1.165 kg COD/(m<sup>3</sup> day)), COD removal efficiency of 35.4% accounting for SDR of 0.412 kg COD/(m<sup>3</sup> day) was observed at the end of the cycle period. At higher OLR (1.404 kg COD/(m<sup>3</sup> day)) studied, a COD removal efficiency of 62.9% was observed accounting for SDR of 0.88 kg COD/(m<sup>3</sup> day).  $SDR_S$  variation showed some interesting observations. With lower OLR studies, the substrate degradation evidenced initially depression (48 h) followed by a upswing (72 h) followed by a gradual fall (96–120 h). In the case of higher OLR, the substrate removal tendency showed a steady pattern from 24 to 120 h followed by a sharp raise (144 h) and an immediate suppression. Comparatively higher substrate degradation efficiency was evidenced at higher loading rate.

It is evident from the experimental data that, the MFC facilitated both substrate removal and power generation. With time and substrate exhaustion, a gradual improvement in the current generation was observed. The decrease in COD concentration indicated effective functioning of selectively enriched mixed microflora in metabolizing the carbon source as electron donors. During stable operation of fuel cell, substrate removal almost stopped after 90 h of the cycle operation. Specific power production with respect to substrate removal was calculated and plotted against current density as shown in Fig. 5. The relatively higher

current density (747.96 mA/m<sup>2</sup> [1.165 kg COD/(m<sup>3</sup> day); 10 Ω]; 862.85 mA/m<sup>2</sup> [1.404 kg COD/(m<sup>3</sup> day); 10 Ω]) and volumetric power production (1.664 W/m<sup>3</sup> [1.165 kg COD/(m<sup>3</sup> day); 50 Ω]; 2.02 W/m<sup>3</sup> COD<sub>R</sub> [1.404 kg COD/(m<sup>3</sup> day); 100 Ω]) at was recorded at higher loading rate studied. On contrary, specific power production showed relatively lower figures at higher loading rate studied (0.163 W/kg COD<sub>R</sub> [1.165 kg COD/(m<sup>3</sup> day); 50 Ω]; 0.198 W/kg COD<sub>R</sub> [1.404 kg COD/(m<sup>3</sup> day); 100 Ω]). A good correlation ( $R^2$ ) of 0.9976 and 0.9898 was observed between specific power production and current density at studied OLRs 1.165 kg COD/(m<sup>3</sup> day) and 1.404 kg COD/(m<sup>3</sup> day), respectively. The relatively lower specific power production observed at higher substrate loading rate studied may be attributed to the loss in electron transport which invariably contributed to less specific power production than required. Performance stabilization with respect to both substrate removal and power generation was observed after 4th cycle of feeding.

### 3.3. Polarization curve

Polarization curve helps to determine fuel cell function with respect to resistance and cell design point. Operation at higher power density will mean operation at lower cell efficiency. Operation at peak power density can cause instability in control because the system will have a tendency to oscillate between lower and higher current densities at peak. Operation at the higher power densities will mean operation at lower cell voltages or lower cell efficiency. 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. To obtain the polarization curve, the current density was calculated and plotted against potential and power density at different resistances (100 Ω to 30 kΩ) to visualize the maximum power density and current density with respect to the OLR once the maximum voltage was attained (Fig. 5). Power density curve showed a maximum power density of 129.40 mW/m<sup>2</sup> at 50 Ω resistance at operating OLR of 1.165 kg COD/(m<sup>3</sup> day). At higher loading rate (1.404 kg COD/(m<sup>3</sup> day)) maximum power density of 157.25 mW/m<sup>2</sup> was registered at 100 Ω resistance. Current generation showed decreasing trend with increase in the resistance which is in concurrence with literature reported earlier [8,21,22]. Current generation with different resistance was observed once the maximum voltage was attained. Maximum current of 2.92 mA and 3.11 mA was observed at 100 Ω either after resistance up-shift or down-shift at OLRs 1.165 kg COD/(m<sup>3</sup> day) and 1.404 kg COD/(m<sup>3</sup> day), respectively. This behavior indicated that of a typical fuel cell. At higher resistance (30,000 Ω) current generation of 0.04 mA at OLRs 1.165 kg COD/(m<sup>3</sup> day) and 1.404 kg COD/(m<sup>3</sup> day), respectively. Drop in the voltage was comparatively higher at lower resistance, which indicated more potential drop. Voltage stabilization was comparatively rapid at higher resistances (30,000 Ω) compared to lower resistances (100 Ω) studied. Relatively effective electron discharge observed at lower resistances may be probable reason for more potential drop and slow stabilization of the voltage at lower resistors. Oxidation of substrates by microbes was observed to be more at lower resistance than at higher resistance, where

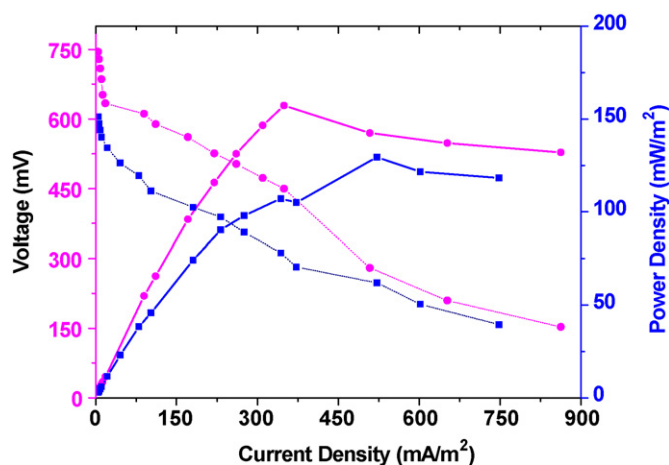


Fig. 5. Polarization curves measured at various resistances (30,000–100 Ω) generated during stable performance of MFC operation [(●) 1.165 kg COD/(m<sup>3</sup> day) (COD<sub>feed</sub>, 6200 mg/l); (■) 1.404 kg COD/(m<sup>3</sup> day) (COD<sub>feed</sub>, 10,540 mg/l); solid line, power density; dotted line, voltage].

microbes donate electrons to the anode electrode as the electrons are discharged in closed circuit [21]. At lower resistances the electrons move more easily through the circuit than at higher resistance, oxidizing electron carriers of the microbes in the anode. Higher fuel oxidation by the microbes is expected with high ratio of oxidized electron carriers of the culture at a low resistance and the reactor can be operated at low resistance to remove organic fraction at a higher rate. According to the concept of polarization, in the present experiment at OLR of 1.165 kg COD/(m<sup>3</sup> day), maximum power density (129.40 mW/m<sup>2</sup>) was observed at 50 Ω and the fuel cell can be operated effectively below 50 Ω resistances with stable performance and the corresponding power densities observed at 25 Ω and 10 Ω were 121.60 mW/m<sup>2</sup> and 118.18 mW/m<sup>2</sup>, respectively. At operation OLR of 1.404 kg COD/(m<sup>3</sup> day), the maximum power density was observed at 100 Ω (power density, 157.25 mW/m<sup>2</sup>) and the cell can be operated effectively below 100 Ω resistances and the corresponding power density observed at 50 Ω, 25 Ω and 10 Ω were 142.41 mW/m<sup>2</sup>, 136.97 mW/m<sup>2</sup> and 132.02 mW/m<sup>2</sup>, respectively.

### 3.4. Bioprocess monitoring

Volatile fatty acids (VFA; represented as the total of all acids generated during acidogenic fermentation step) concentration and composition along with pH were also monitored during the fuel cell operation to evaluate the bioprocess mechanism during bioelectricity production. VFA production was always associated with conversion of organic fraction to acid intermediates in the anaerobic microenvironment with the help of specific group of bacteria. Generally acidic intermediates enumerate changes in the metabolic pathway of the ongoing anaerobic process. A gradual raise in VFA concentration from zero was observed during MFC operation at both the substrate loading rates studied (Fig. 3). Stabilization in VFA production was not observed till the end of the cycle period. At OLR 1.165 kg COD/(m<sup>3</sup> day), a gradually raise in VFA concentration was observed and reached a maximum 851 mg/l at the end of the cycle period (120 h). By linear regression of curve (best fit option) has resulted in VFA production of 170.29 mg VFA/(l day) ( $R^2$ , 0.9159) for 5 days of operation. However, at OLR 1.404 kg COD/(m<sup>3</sup> day) and gradual raise in VFA (951 mg/l) concentration was noticed up to 120 h of cycle period and thereafter a marked fall in concentration was observed prior to stabilization at the end of cycle period at 782 mg/l. By considering a linear regression of single curve (best fit) has shown VFA production of 133.07 mg VFA/(l day) ( $R^2$ , 0.9159; 120 h). Due to batch mode operation of fuel cell, VFA accumulation in system was not observed after replacement of feed.

The relatively lower concentration of VFA production observed at higher OLR studied in association with good substrate removal can be explained based on the composition of VFA/soluble metabolites. Anode chamber outlet samples during the course of fuel cell operation were also analyzed for the composition of VFA in terms of acetate acid, butyric acid, propionic acid and ethanol by chromatography to have understanding of the soluble metabolic distribution. The distribution of soluble

metabolites formed during H<sub>2</sub> production was often crucial in assessing the efficiency of H<sub>2</sub>-producing cultures [4,5,22,23]. It was observed that high H<sub>2</sub> yields were associated with a mixture of acetate and butyrate fermentation products, and low H<sub>2</sub> yields were with propionate and reduced end products (alcohols, lactic acid) [4,5,24]. Chromatography data showed the presence of acetic acid and butyric acid in the outlet samples. At higher substrate loading rate lower concentration of propionic acid was also observed. Comparatively the mixture composes of higher concentration of acetic acid than butyric acid and propionic acid irrespective of the substrate loading conditions. Acetic acid was a major soluble metabolite formed during bioelectricity generation in anodic chamber with chemical wastewater as substrate. This observation correlates well with report pertaining to H<sub>2</sub> production using composite chemical wastewater [5]. Soluble metabolites/VFA composition suggests dominance of acid-forming metabolic flow associated with acidogenesis process instead of solventogenesis, which was considered as optimum environment for effective H<sub>2</sub> generation [5]. The susceptibility to acetic acid formation in the anaerobic fermentation suggests that mixed acid fermentation persisted in anodic chamber where pyruvate and CoA in the fermentation pathway is converted to acetyl-CoA. This type of fermentation was typically found in H<sub>2</sub> evolving bacteria like Enterobacteriaceae and related bacteria [25]. No ethanol production was detected, which confirmed the survival of H<sub>2</sub> producing species rather than H<sub>2</sub> consuming species. The discrepancy in VFA values associated with substrate degradation at two loading rates observed in this study may be attributed to soluble metabolic composition, which gives an idea of actual pathway of the metabolic process is taking place. Presence of propionic acid as soluble intermediate in outlet samples of fuel cell at higher loading rate indicates variability in metabolic pathway.

VFA values correlate well with specific power production and SDR<sub>S</sub> at lower OLR studied, VFA concentration showed a gradual increase with time where as the specific power production changes according to the corresponding SDR<sub>C</sub> values (Fig. 6). At higher OLR, VFA either with SDR<sub>S</sub> or specific power production does not exhibited good correlation. The SDR<sub>S</sub> and specific power production are found to be inversely related in both the OLRs studied. The accumulation of high concentration of VFA during the end of cycle period lowers the voltage production. Relatively increased potential difference was observed at lower VFA concentrations. However, VFA concentration cannot be considered to directly influence potential difference created in the given system as the voltage generation depends on number of other factors such as mass transfer, temperature and internal resistance.

Variation in pH values observed during fuel cell operation was in good agreement with the corresponding VFA variation at both the OLRs studied (Fig. 7). VFA and pH were integral expressions of the acid–base conditions of anaerobic process, as well as intrinsic index of the balance between microbial groups in the anaerobic system. The pH values showed consistently decreasing trend with the operation in anode chamber. Outlet pH range was found to be same for both the OLRs studied. During operation, AB out beats MB resulting in VFA accumulation

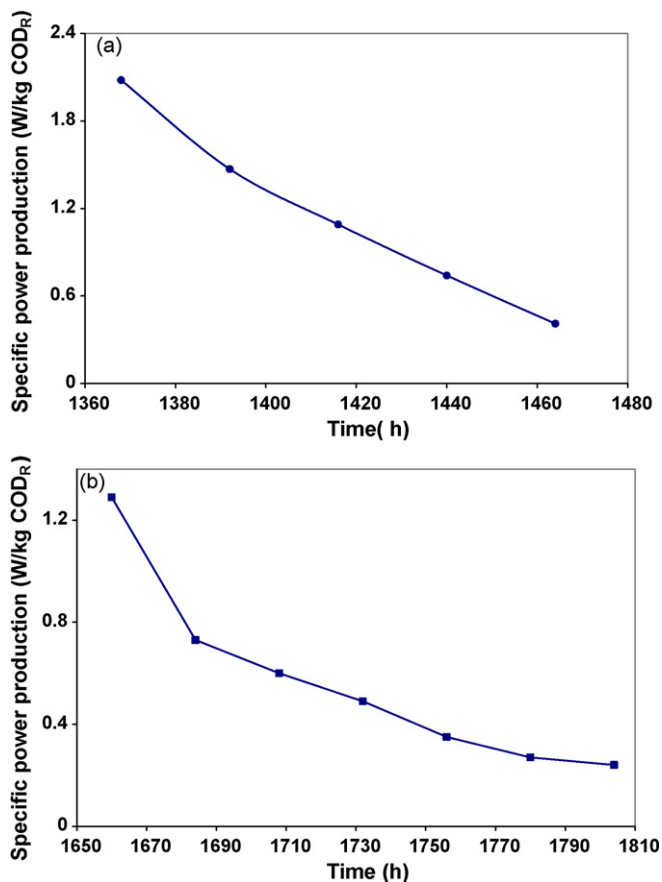


Fig. 6. Variation in specific power production during MFC operation [(a) 1.165 kg COD/(m<sup>3</sup> day) (COD<sub>feed</sub>, 6200 mg/l); (b) 1.404 kg COD/(m<sup>3</sup> day) (COD<sub>feed</sub>, 10,540 mg/l)].

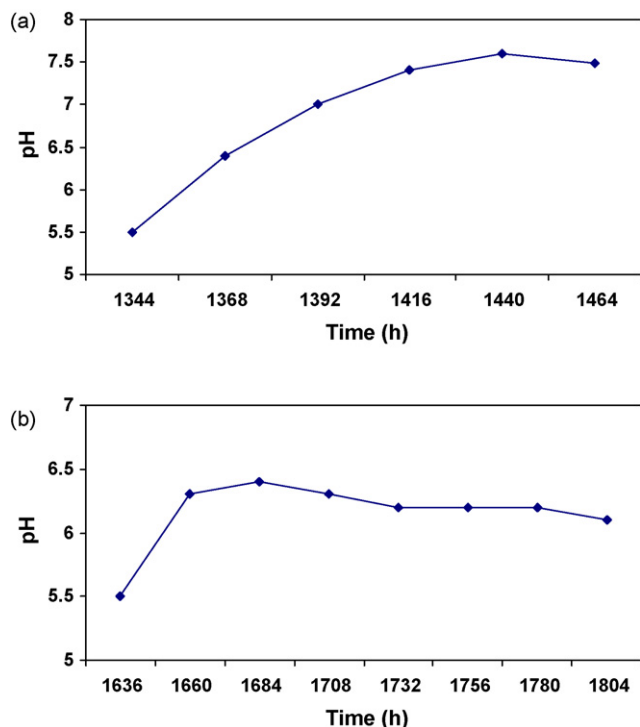


Fig. 7. pH transition during MFC operation [(a) 1.165 kg COD/(m<sup>3</sup> day); (b) 1.404 kg COD/(m<sup>3</sup> day)].

during fuel cell operation. Methane production was not observed during the fuel cell operation at both the OLRs studied. This may be due to the inhibition of MB due to the prevailing acidophilic microenvironment.

Typical anaerobic cultures could not produce H<sub>2</sub> as it is an intermediate for methane formation, which was rapidly consumed by methane-producing bacteria in the population. One of the effective ways to enhance H<sub>2</sub> production from the anaerobic culture is to restrict or terminate the methanogenic process so that H<sub>2</sub> may be an end product in the metabolic flow. The adopted selectively enriched H<sub>2</sub> producing mixed consortia also helped in restricting the survival of MB during fuel cell operation. VFA formation and persistent acidogenic metabolism encountered in the anode chamber resulted in congenial microenvironment for proliferation of H<sub>2</sub> producing AB and inhibitory for MB [4–6,8]. The adopted operating conditions (acidophilic conditions) helped to limit the MB activity. Prior to feeding, wastewater was adjusted to pH 5.5 which facilitated growth of AB at the same time inhibiting the function of MB bacteria which creates susceptible environment for molecular H<sub>2</sub> evolution in the anaerobic environment. It is reported that, optimum pH for the growth of MB was between 6.0 and 7.5, while AB functions well below 6 pH [4–8,26–30]. Also by maintaining the operating pH around 5.5 (optimum being pH 5.5–6.0) compared to a near neutral pH the conversion efficiency (of H<sub>2</sub> production) has increased [4–8,27,31,32]. The pH range of 5.5–6 was considered to be ideal to avoid both methanogenesis and solventogenesis [33,34] and could be considered optimum pH range for effective H<sub>2</sub> generation.

Table 1 illustrates consolidated experimental data pertaining to present study in comparison with work reported with same MFC operated with different wastewaters, diverse cathode environment (aerated/potassium ferrocyanide) and operating pH (5.5/6.0) [6,8]. It is evident from Table 1, that the performance of MFC, with respect to electricity generation is dependent on the nature of wastewater used as substrate, nature of cathode and operating pH. Maximum current density (862.85 mA/m<sup>2</sup>) and specific power production (0.198 W/kg COD<sub>R</sub>) was observed with composite chemical wastewater at higher OLR (1.404 kg COD/(m<sup>3</sup> day)) using potassium ferricyanide catholyte at operating pH of 5.5. Incidentally, higher substrate degradation ( $\xi_{\text{COD}}$  62.9%; SDR 0.88 kg COD/(m<sup>3</sup> day)) was registered during this operation. Comparatively higher power generation was observed with potassium ferricyanide catholyte compared to aerated catholyte with designed synthetic wastewater as substrate in spite of similar substrate degradation efficiency operated at pH 6.0 [4]. Similarly potassium ferrocyanide catholyte responded to higher specific power productions compared to aerated catholyte when composite chemical wastewater (1.165 kg COD/(m<sup>3</sup> day)) and designed synthetic wastewater (1.033 kg COD/(m<sup>3</sup> day)) are used, respectively at same operating pH (5.5). When comparison was made in terms of operating pH, the system operated at pH 6.0 with OLR of 0.625 kg COD/(m<sup>3</sup> day) resulted in higher specific power production [8] compared to MFC operated at pH 5.5 with OLR of 0.646 kg COD/(m<sup>3</sup> day) [6]. This may be attributed to the fact that, substrate limiting condition prevailing

Table 1  
Details of MFC operation with respect to output parameters

S. no.	Wastewater	Cathode	OLR (kg COD/(m <sup>3</sup> day))	pH transition	VFA buildup (mg/l)	Voltage (mV)	Current (mA)	COD reduction (%)	SDR (kg COD/(m <sup>2</sup> day))	Specific power production (W/kg COD <sub>R</sub> )	Current density (mA/m <sup>2</sup> )	Reference
1	Composite chemical	Potassium ferricyanide	1.165, 1.404	5.5–7.4, 5.5–6.1	+851, +782	271.5, 304.0	5.43, 6.08	35.4, 62.9	0.412, 0.880	0.163, 0.198	747.96, 862.85	This work
2	Designed synthetic	Potassium ferricyanide	0.753	6.0–4.9	+553	586	2.37	74.20	0.559	0.635	222.59	[8]
3	Designed synthetic	Aerated	0.625	6.0–5.11	+374	572	1.68	74.15	0.464	0.440	190.28	
		Aerated	0.517, 0.574, 0.646, 1.033	5.5–4.6, 5.5–6.8, 5.5–6.8, 5.5–6.8	+546, –10, +106, –70	350, 423, 332, 339	1.44, 1.66, 1.41, 1.38	62.5, 53.3, 59.5, 60.0	0.323, 0.306, 0.384, 0.419	0.168, 0.274, 0.167, 0.167	172.33, 198.65, 168.74, 165.15	[6]

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 expect for pH range.

due to acidophilic microenvironment compared to 5.5 pH than 6.0 pH.

It is evident from the experimental data, that fuel cell with graphite electrode (without coating) inoculated with selectively enriched H<sub>2</sub> producing anaerobic mixed consortia showed *in situ* bioelectricity generation along with the treatment of chemical wastewater within a short period of time. Selectively enriched microflora adopted to the electron transfer phenomena with in a short period irrespective of the substrate loading conditions. Using H<sub>2</sub> producing mixed inoculum has facilitated direct generation of H<sub>2</sub> from carbon fermentation, which will intern convert into power *in situ* by bioelectrochemical system. Successful application of mixed inoculum also provides a feasible alternative for power generation by directly producing power through hydrogen generation along with wastewater treatment in a single system. Using mixed microbial culture is considered to be a practicable, cost-effective and promising approach to achieve bioelectricity generation at large scale. The procedure was cost effective and environmentally sound and sustainable due to utilization of wastewater as substrate for *in situ* power generation along with wastewater treatment, utilizing low cost and non-coated electrodes and mediatorless anode. Normal graphite electrode was used instead of platinum catalyst and considerable power generation with chemical wastewater could impart a cost effective MFC construction for treating wastewater.

#### 4. Conclusion

Experimental data demonstrated the feasibility of dual chambered microbial fuel cell (MFC) in bioelectricity generation from chemical wastewater treatment without using mediator in anode chamber. Designed MFC configuration, adopted operating conditions and used selectively enriched mixed inoculum showed feasibility of power generation from chemical wastewater treatment along with wastewater treatment. Performance and stabilization tendency with respect to power generation was found to be dependent on the applied substrate loading rate. Substrate degradation was observed in anode chamber of fuel cell enumerates the functioning of an alternative anaerobic wastewater treatment unit in addition to renewable energy generation. Operating anodic chamber of fuel cell at acidophilic conditions (pH 5.5) helped to sustain the activity of acidogenic bacteria along with inhibiting methanogenic bacterial activity. Utilizing wastewater (renewable energy) for the production of renewable energy (bioelectricity) from anaerobic treatment is a feasible, economical and sustainable alternative. Major advantages of energy produced from wastewater are the absence of environmental emissions, simultaneous recovery of energy and wastewater treatment.

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