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Development of Microbial Fuel Cell Using Distillery Spent Wash: Evaluation of Current Generation and COD Removal with Respect to pH

Vanita R. Nimje, Yogita P. Labrath and Vilas G. Gaikar

Department of Chemical Engineering,
Institute of Chemical Technology, Matunga, Mumbai, India

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Abstract: A single chamber microbial fuel cell (SCMFC) was operated with distillery spent wash (DSW) wastewater and microorganisms in cow-dung as inoculum source from pH 4 to 9. MFC signifies maximum current in the sequence of pH 6 (0.46 mA) > pH 7 (0.4 mA) > pH 8-9 (0.16-0.19 mA); whereas the chemical oxygen demand (COD) removed in order of pH 8-9 (80-81%) > pH 7 (79%) > pH 6 (68%). The losses in coulombic yield were due to alternating electron acceptors and air diffusion through the reactor. The polarization curve yielded the maximum current density of 84 mA/m^2 and maximum power density of 29 mW/m^2 at an external resistance of 820Ω (pH 6). The cyclic voltammetry (CV) demonstrated 3-electron transfer process with best electrochemical responses at pH 6 and 7. The MFC at desired operating conditions showed a positive response for bioelectricity generation.

Key words: Microbial fuel cell · Distillery spent wash · Cow dung inoculum · Bioelectricity · Cyclic voltammetry

INTRODUCTION

Production of ethyl alcohol in distilleries based on fermentation of cane sugar molasses constitutes a major industry in Asia and South America. Molasses is an important raw material for the fermentation industries because of its easy availability, low-cost and suitability for the fermentation process. Alcohol distilleries are also rated as one of the 17 most polluting industries [1]. The alcohol industry daily generates huge amount of wastewater; which is usually rich in organic materials, less toxic and easily amenable for microorganisms. The aqueous effluent stream coming out of the distillation unit as a dark brown highly organic effluent is known as spent wash. It is one of the most complex and troublesome, non-toxic, dark brown coloured effluents at high temperature, low pH and high ash content as well as high COD value of 25000-30000 mg/L [2]. Although, it does not contain toxic substances, discharge of raw spent wash into open land or nearby water bodies without any treatment brings about immediate discolouration and depletion of dissolved oxygen in the receiving water streams and its dark colour hinders photosynthesis by blocking sunlight and is therefore deleterious to aquatic life [1]. Adequate treatment is, therefore, imperative before the effluent is discharged.

Physico-chemical and biological treatments are normally employed to treat distillery spent wash (DSW). Screening and equalization followed by biomethanation is a typical treatment. Although, these methods are effective, they require high reagent dosages, high cost, formation of some hazardous by-products, generate excessive amount of sludge and required intensive energy consumption [3]. As an alternative, anaerobic biological treatment with microbes has been drawing attention.

Among biological wastewater treatment methods, microbial fuel cells (MFC) have attracted increasing interest in recent years because of their applications in bioremediation and energy recovery while treating high strength wastewater. MFCs are bioreactors that can degrade organic and inorganic matters presence in wastewater simultaneously achieving energy recovery in the form of electricity [4].

Corresponding Author: Vanita R. Nimje, Department of Chemical Engineering, Institute of Chemical Technology, Matunga, Mumbai, India-400019. Tel: +91-9833513305, Fax: +91-22-33611020, E-mail: vanita.nimje@gmail.com

MFC substrates have sufficient amount of growth promoters that can enhance growth of bio-electrochemically active bacteria during wastewater treatment. Recently, molasses and DSW are effectively used as substrate in MFC's. The studies performed by Mohanakrishna *et al*. delineate the bio-electrochemical treatment of distillery wastewater for electricity generation using acidophiles (pH 6) [5]. Chen *et al*. employed a novel anaerobic baffled stacking microbial fuel cell for effective utilization of molasses [6]. In another study, distillery wastewater was treated in single chamber and dual chambers MFC without additional nutrients and sewage as source of inoculums [7]. Whereas, Mohanakrishna *et al*. have reported results based on different COD concentrations of distillery wastewater at specified pH of 6 [5]. MFC research with molasses or DSW also requires considerable research inputs to optimize the experimental conditions pertaining to bioelectricity generation and COD removal under different pH conditions.

Due to unique metabolic assets of microorganisms, variety of microbes are used in MFCs either as pure culture or mixed consortia [8-14]. Apart from MFC's, Fan *et al*. have studied biohydrogen production by anaerobic microbes enriched from heat shocked cow dung compost [15]. It has been reported that bioremediation of spent wash are available in MFC's. However, production of electricity using combination of DSW as substrate and cow dung microbial consortia as inoculums source would be advantageous in MFC research.

Looking towards this, in the current work, a novel attempt has been made to generate electricity while treating DSW using cow dung as bacterial inoculum under different pH conditions for single chamber air cathode MFC. Inoculum was obtained as culture of anaerobic bacteria cultivated from cow-dung. This study shall provide detailed insights of DSW at various pH's for effective establishment of MFC technology.

MATERIALS AND METHODS

MFC Assembly: Air cathode single chamber MFC (SCMFC) reactor was constructed from inexpensive and readily available transparent polyacrylic plastic sheets. The reactor vessel was rectangular shape (length=11 cm, width=19 cm) with a capacity of 1.3 L. The reactor was covered with polyacrylic plastic sheet with sampling ports and fitted with rubber gasket for an airtight operation. The materials used for the anode and cathode were made from carbon cloth. The cathode was coated with 4 layers of PTFE adhesive mixed with carbon powder to provide diffusion layer and to prevent water loss on the air-facing side as described by Cheng *et al*. [16]. Cathode was placed on the side of the reactor as shown in Fig. 1.

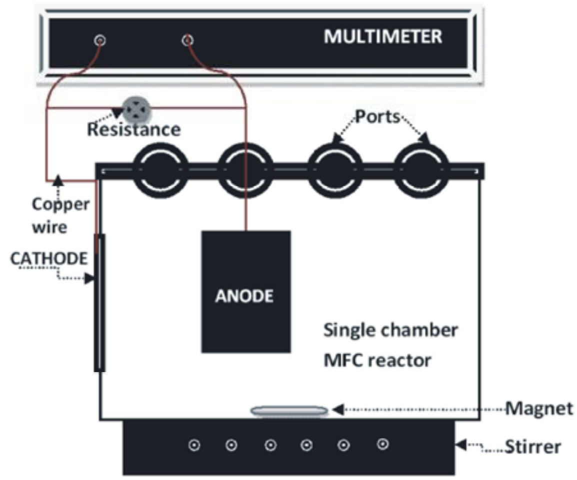

Fig. 1: Schematic diagram of single chamber microbial fuel cell.

The dimensions of the anode and cathode were 0.05 m x 0.05 m, with a projected area of 0.0025 m^2 for anode. Copper wire was used to connect the circuit and the fuel cell was placed under a constant load by connecting the anode and cathode to an external resistance of 330Ω. The reactor was magnetically stirred continuously. After the installation of electrodes, substrate and addition of inoculum, the total anode void volume was 1.3 L, this volume being used in the calculations of current density and power density. The experimental set-up is shown in Fig. 1.

Sample Collection and Analysis: DSW was supplied by Godavari Biorefineries Ltd., Nashik, India and stored at 4 °C. The DSW was characterized according to standard methods [17]. The DSW showed a pH of 4.5, dark brown colour, sweet odour and has total dissolved solids of 63200 mg/L. The chemical oxygen demand (COD) by potassium dichromate method was 14300 mg/L. Before use in MFC, the DSW was diluted to COD concentration of 3000 mg/L with deionized water.

Seed Culture (Inoculum): Locally available cow dung (5 g) and 100 mL DSW were collected in 6 conical flasks (250 mL) purged with nitrogen (pH adjusted to 4, 5, 6, 7, 8 and 9). Further anode electrodes (0.05 m x 0.05 m with a projected area of two sides 0.005 m^2) were individually inserted in each conical flask. These flasks were shaken for a period of one week on an orbital shaker (200 rpm, 32°C) to obtain a seed culture. The enriched inoculum was obtained; then inoculums size of 10 mL of seed culture was transferred to 100 mL of autoclaved DSW in an anode electrode. This enrichment procedure was repeated after every 2 days for a period of 8 days and the whole acclimatizing period was of 15 days. Viable biofilm formation was observed at pH 6 and 7 compared to pH 8 and 9 at which very thin slimy layer was observed. The anodes with enriched inoculums from these conical flasks were obtained for direct use in MFC experiments.

Data Acquisition and Calculations: The electrode output voltage was recorded against time in every 30 min. The anode and cathode were connected directly to a Philips True RMS Digital Multimeter (model- DM-441B). The electrode closed circuit was permanently connected to an external resistance of 330Ω. To obtain the polarization curves, variable external resistances of 6.8, 4.7, 2.7, 1, 0.82, 0.68, 0.47 and 0.33 kΩ were applied.

Potential (V) was monitored in order to estimate the current (I) as I=V/R. Power was normalized based on the cross-sectional area (projected area 0.005 m^2) of the anode in order to calculate the power density in mW/m^2 as $P=I*V/0.005\ m^2$. Based upon the polarization curve, the internal resistance of the MFC was calculated as [18]:

$$E_{cell}(V) = OCV\ (V) - I\ (A)^* R_{int}\ (\Omega) \quad (1)$$

where, E_{cell} is the potential of MFC in Volts, OCV is the open circuit voltage on Volts, I is the current in ampere, R_{int} is the internal resistance in ohms. Substrate removal efficiency (%) was calculated as [19]:

$$\xi = [(C_{so} - C_s)/C_{so}] \times 100 \quad (2)$$

Coulomb yield (C), expressed as current x time was calculated by integrating the current over the time from the start point to the end of the experimental cycle.

Electrochemical Analysis: All electrochemical experiments were conducted under potentiostatic control and at steady state potential for each cycle using a conventional three-electrode set-up using a potentiostat (ΩMetroohm, µAutolab IME663 Potentiostat/Galvanostat, Swiss made). Direct electrode reaction of bacterial cells was examined by *in situ* cyclic voltammetry in order to delineate the substrate oxidation reaction at the anode surface. A scan rate of 0.1 mV/s was used with a potential ranging from –750 to +750 mV. Voltammetry measurement was made with the anode as the working electrode, an Ag/AgCl as a reference electrode and a platinum electrode as the counter. Electrodes were arranged so that contact between any of the electrodes was avoided. Electrochemical analyses in the form of cyclic voltammetry at the middle of the cycle were conducted. All experiments were performed under strictly anoxic conditions.

Operation of MFC: Prior to its use in MFC experiments, DSW was autoclaved at 121°C for 15 min. Further, MFC's were operated in a fed-batch mode at different pH values ranging from 4 to 9. One MFC system was used for all the operated pH condition. The pH was adjusted with acid (0.2 M HCl) or base (0.2 M NaOH) with phosphate buffer (50 mM). Final COD concentration at the end of batch cycle was also estimated. All the experiments were conducted at a constant room temperature (30±2 °C) unless otherwise stated.

RESULTS AND DISCUSSION

Evaluation of Current Generation and Wastewater Treatment: The experimental results clearly showed the influence of pH on current generation for air cathode single chamber MFC system. The organic fraction present in wastewater starts metabolized in the presence of biocatalyst and generates electrons and protons through redox reactions, which results in the development of potential facilitating current generation. Improvement in current generation was observed with each injection of the feed solution at the beginning and decreased after reaching at its peak. The maximum current generation was found to be in the order of acidic > neutral > basic. Acidophilic pH of 6 evidenced relatively higher current generation (0.46 mA) compared to the corresponding neutral (0.4 mA) and alkaline conditions (0.19mA). The peak current increased with increasing pH from 4 to 6, reached the highest value at pH 6 and dramatically decreased at pH 8 to 9 (Fig. 2). An energy-neutral system with low current production at pH 4, 5, 8 and 9 might be due to the inefficiency or difficulty by the bacterial culture to metabolize the organic compounds present in wastewater or limited bacterial density might be another reason to accomplish energy rich MFC. It is known that the impact of pH on current generation is significant [20, 21]. In a double-chamber MFC, the highest current was observed at neutral pH between 6.5 and 8 [20, 21]. Gil *et al*. [21], suggested in their studies that, microbial activity diminished at sub-optimal pH values. Whereas, He *et al*. [22] have reported a basic pH (around 9) was

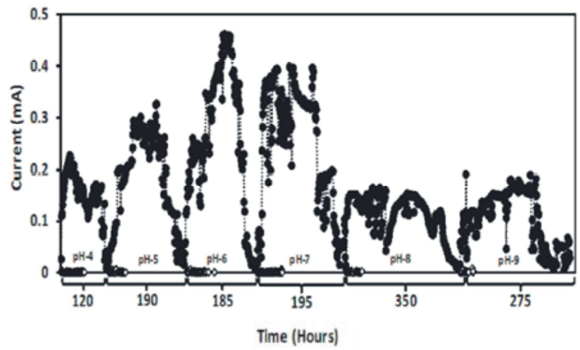

Fig. 2: Current generation profile for pH 4 to 9 from uninoculated (◇) and inoculated (•) culture.

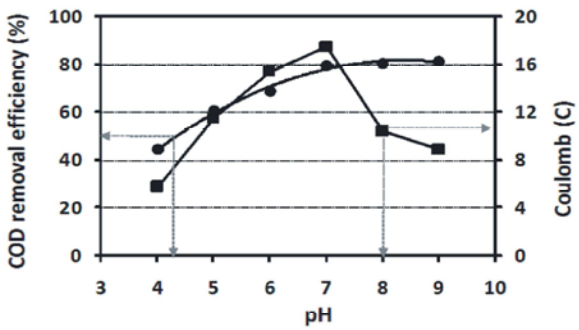

Fig. 3: COD removal efficiencies (•) and coulombic yield (■) for individual MFC from pH 4 to pH 9.

optimal in an air-cathode MFC using synthetic wastewater. In present work, the solution pH affected microbes in remarkably different ways. Particularly, the current output might get impacted by microbial growth and differentiation in planktonic cultures and biofilm formation which could be a probable explanation for these current differences. A very low current density at an acidic pH of 4 and basic pH also signifies that electron transfer from planktonic culture may required substantial microbial attachment to the electrode surface [23] which was not observed at these pHs. These differences in the current production could depend on the viable cell count and biofilm formation from acidic to basic PH [24]. This drop in current generation indicated that exoelectrogenic bacteria were affected by a pH lower than 5 and higher than 7. In the present study with no externally added mediators, the possible electron transfer pathway between the microbes and an electrode could include direct electron transfer (DET) where, the functionality of redox enzymes involved in DET is crucial [25]. At an acidic and alkaline pH, the functionality of ionic groups which are occupied on the active sites of the enzyme might change the enzyme activity and the reaction rate. This phenomenon likely affects electron discharge at acidic and alkaline pH [26]. Alternatively, higher dehydrogenase activity of these mixed culture at acidic to neutral pH compared to basic pH lead the release of electrons and protons through the biochemical pathway like glycolysis, TCA and Electron transport chain [25]. Our results are in accordance with those reported by Raghavulu *et al*. [27], where acidophilic (pH 5-6) and neutral MFC shows better efficiency of current generation corresponding to its basic pH. With acidic wastewater, the methanogenic activity of these microbes might get suppressed, which resulted in diversion of electrons towards anode for the purpose of electricity generation. This significant change in current generation with respect to pH may also be attributed to the effectiveness of extracellular electron transfer at acidic and neutral pHs [28]. The higher current generation was probably due to the effectiveness of intracellular electron carriers at an acidic and neutral pH, where higher dehydrogenase activity led to significant conversion of the substrate to the end product. The basic pH in turn produces protons in an insufficient concentration or quantity, which in turn led to a lower electron discharge or current [27].

Further, COD removal efficiency and coulombic output as a function of pH was evaluated at the end of the batch cycle as shown in Fig. 3. Moreover, COD removal efficiency was also correlated with the time taken to complete a batch cycle and substrate removal. The cycle time registered a marked variation as the function of pH from 4 to 7 with low cycle time (120 -195 h) while, alkaline MFC showed the high cycle time (350-275 h) (Fig. 2). Effective COD removal efficiency and lower current observed at basic pH may be attributed to the slower electron discharge by methanogenic bacteria which promoted complete metabolism of the substrate, hence resulted in long batch cycles. Instead of current generation at alkaline pH, utilization of electrons for biomass formation and metabolism possibly led to COD reduction and hence long operation time. In concurrence with Lu *et al*. [28], the high COD removal efficiency at higher pH were attributed to the long operation period, which in turn also extended the time for oxygen diffusion into the system, consequently lowering the coulombic yield. In accordance with Raghavulu *et al*. [27], higher dehydrogenase activity at basic pH depicted substrate reduction to the end products instead of causing potential generation leading to lower electron discharge/current, presumably resulted in longer cycle times.

The obtained experimental data showed that the COD removal efficiency increased as a function of increasing pH; the COD removal efficiencies were 44, 60, 68, 79, 80 and 81% for pH values of 4, 5, 6, 7, 8 and 9, respectively. The coulombic output on the other hand showed significantly different trend and increased from a pH of 4 to 7 with further decreased at pH 8 and pH 9 (Fig. 3). A minimum COD removal was obtained at pH of 4, whereas, the maximum removal obtained for a pH of 9. It can be inferred that the planktonic organisms at basic pH were utilizing the highest substrate in terms of COD while achieving the lowest coulombic output [12, 13]. The change in COD is not only caused by electricity generating bacteria, but also by other alternative electron sinks including aerobic respiration and biomass formation. Generally, decrease of coulombic output could account by utilization of alternate electron acceptors by the bacteria, either those present in the wastewater or those diffusing through the MFC. Possibility of utilization of alternate electron acceptors present in the wastewater could not be ignored and consumption of electrons by mechanisms other than cathode reaction could be an alternative means which might have decreased coulombic output [29]. It is believable that under the conditions of limited electron disposal through the circuit with a high resistance, the electrons are consumed at the anode to reduce other electron acceptors such as sulfate and nitrate present in wastewater [29]. As there were no proton exchange membranes between the anode chambers and carbon cloth cathodes, a large amount of the electrons might be lost to the aerobic respiration sustained by oxygen diffusion through the air cathode and the sampling ports. However, this takes an important part of investigations in the field of MFC.

Polarization Behaviour: The polarization and power density curves of the MFC's were compared during the middle of the cycle with respect to various pH conditions. The polarization curve demonstrates an unequal performance from an acidic environment to a basic environment. The variations of potentials, current density and power density are depicted in Fig. 4 and the values are presented in Table 1. Polarization data were generated

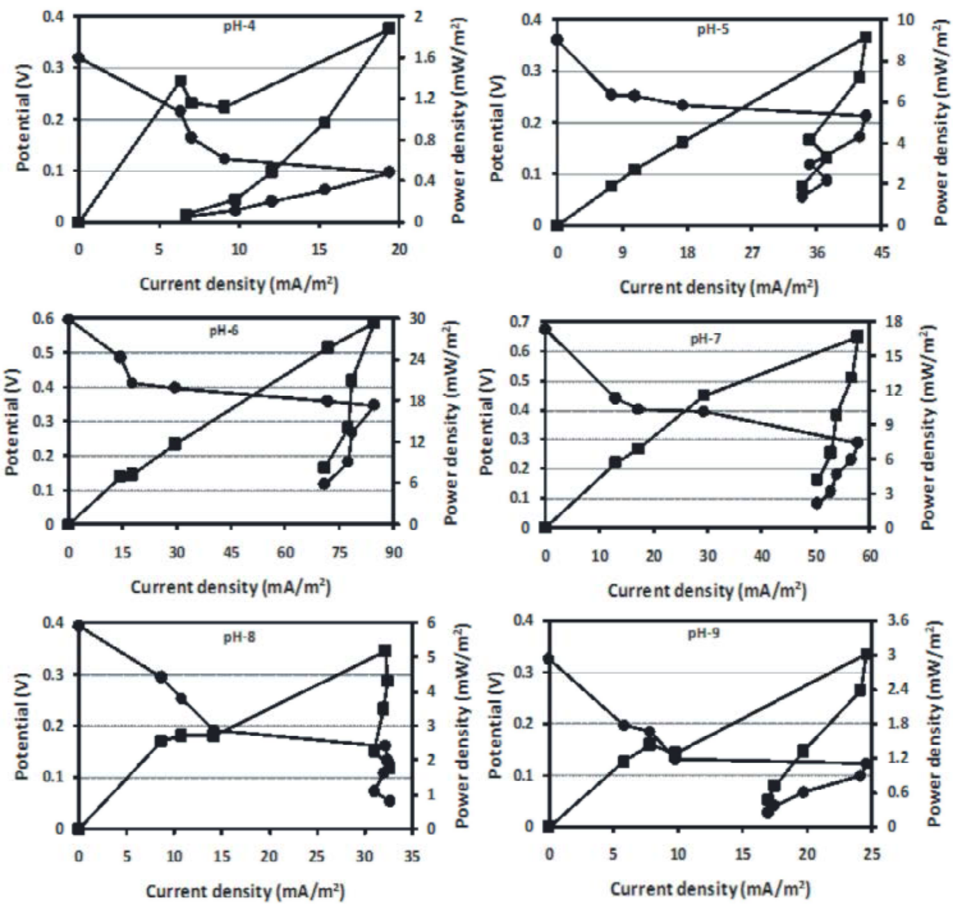

Fig. 4: Polarization behaviour of MFCs as a function of pH from 4 to 9 showing potential (•), current density and power density (<).

Table 1: Open circuit potential (OCP), maximum current density, maximum power density, internal resistances recorded against measured external resistances for pH 4 to 9.

pH	Maximum OCP (V)	External resistance (Ω)	Internal resistance (Ω)	Maximum current density (mA/m^2)	Maximum power density (mW/m^2)
4	0.319	1000	2288	19.4	1.9
5	0.361	1000	686	42.8	9.2
6	0.598	820	675	84.6	29.4
7	0.674	1000	1332	57.8	16.7
8	0.394	1000	1447	32.2	5.2
9	0.326	1000	1650	24.6	3.03

by plotting current density against potential and power density was measured at the various external resistances (6.8, 4.7, 2.7, 1, 0.82, 0.68, 0.47, 0.33 kΩ). To measure ohmic resistance, the MFC was operated with a 6.8 kΩ external resistance first. After system stabilization, the current was interrupted by a relay. Rapid stabilization of OCP was observed at higher external resistance and a relatively high potential drop and slow stabilization of the voltage was observed at lower external resistance, which might be due to effective electron discharge at lower external resistance. During the start-up of polarization curve, activation losses can be clearly observed in order to initialize the electron transfer from bacteria towards the electrode which results in a fast voltage decay or activation over potential at higher external resistance for all the pH studied. During a period of resistance change, the voltage recorded at a particular resistance is inflated. At very low and very high pH of 4 and 9, the overshoot shape is produced when the voltage inflation started to slow down. For pH 5, 6 and 7, the ohmic losses seems to be less corresponding to pH 4, 8 and 9, that can be seen from low internal resistance. In addition, a limited mass transfer of substrate towards the anode was observed at pH 5, 6 and 7, whereas, a steep decrease of the cell voltage near the maximum current densities during the polarization curve was observed for pH 7 and 8, respectively. Fig. 4 also shows the variation in power performance with respect to pH. The point at which maximum power density was obtained on the polarization curve is generally described as the cell design point of that particular MFC. High performance of fuel cell with respect to maximum power density output can be obtained on the right side of the cell design point [27]. Cell design point of comparatively all pH's were obtained at 820 -1000 Ω corresponding to maximum power density at acidophilic pH of 6 (maximum current density: 84.6 mA/m^2 and maximum power density: 29.4 mW/m^2 at 820Ω). The polarization curves were linear in the ohmic polarization region, where the power density reached its maximum value. When this pH was 4, 8 and 9, the power output was detrimentally affected as shown by power curves produced with very high internal resistance, which restricted the power output by causing a significant decrease in the operating potential due to ohmic limitations [30]. The emergence of overshoot can also be observed at pH 4 and pH 9 which concomitantly decreases the power output [31]. The MFC operated at pH 4, 8 and 9 was devoid of biofilm formation which results in an increased internal resistance, which deteriorated the performance and reproduced the overshoot (pH-4 and 9). In accordance with Winfield *et al*. [31], healthy anodophilic microbial community reduced the internal resistance and eliminate any power overshoot. The fuel cell exhibited a relatively effective electron discharge at pH 5, 6 and 7 where internal resistance was almost equal or less than external resistance. For all studied pHs, the current generation was decreasing with increasing external resistance which is consistent with literature and indicated typical fuel cell behaviour. Evidently, the change in microenvironment pH 4, 8 and 9 were incompatible for mixed culture to operate in and the electron demand could not be maintained for better fuel cell operation [31].

Electrochemical Activity Analysis: Electrochemical activity as a function of pH change during MFC operation was evaluated by cyclic voltammetry, an electrochemical-analytical technique used for the characterization of electrochemical systems that also helps to elucidate the electrochemical reactions taking place on/at the electrode surface. CV also measures the potential difference across the interface and the redox activities of the compounds involved in the biochemical system, both in solution as well as compounds attached to the bacteria. During the maximum current output under various pH conditions, voltammograms were recorded *in situ* in the MFC, which showed noticeable variation in the electron transfer processes and current generation. Fig. 5 shows the analysis of the CV profiles (vs. Ag/AgCl), which indicates noteworthy variations in the electron discharge and energy generation patterns as a function of the operated pH. The voltammogram shows a three-step change in the

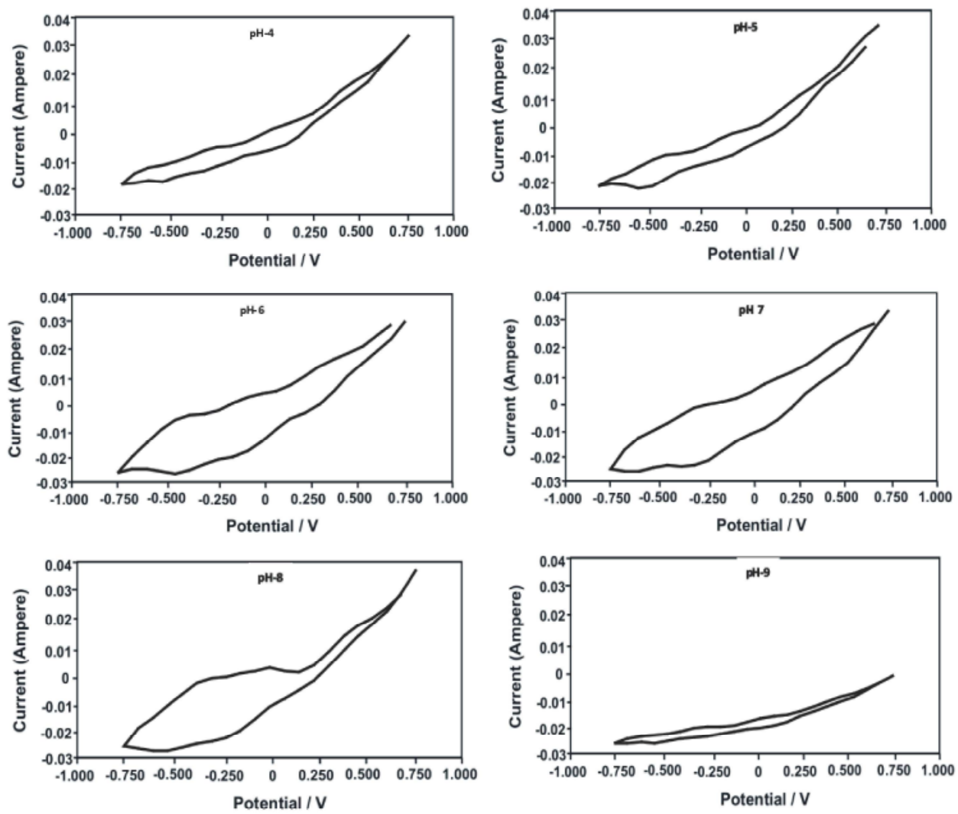

Fig. 5: Cyclic voltammetry behaviour for oxidation phenomenon from pH 4-9.

oxidation peaks, however, indicating the occurrence of a three-electron transfer process at three different peak potentials. Oxidation peaks at the potential of -0.45 V, -0.15 V and +0.35 V (vs. Ag/ AgCl) were observed at all pH values. At pH 5, 6 and 7, these peak potentials are clearly observed in the forward scan with the maximum current of 0.02 mA recorded for pH 7 followed by 0.016 mA for pH 6 and 0.01 mA for pH 5. Contrary to this, voltammograms obtained at pH 4, 8 and 9 were devoid of some of these peaks or the peak intensity was very low with irregular peak patterns in the forward scan. Additionally, it can also be inferred that DET through biofilm-driven catalysis [32] or the functionality of active ionic groups on the bacterial enzymes were probably responsible for the higher oxidation peaks that occurred at pH 5, 6 and 7, respectively. Additionally, electrogenic bacteria could also lead to more electrochemical activity leading to electricity generation towards anode [33]. Substrate to energy conversion via metabolic activity can be governed by an oxidation peak which is accompanied by electron discharge and thus provides a good evidence for the presence of an electrochemical activity and provides information regarding the metabolic activity occurring in the system [34]. Moreover, the CV results are also in good agreement with current profiles generated in Fig. 2 and suggest that stabilization of the MFC performance for DSW could be achieved under acidic (pH 5-6) to neutral conditions.

CONCLUSION

A single chamber MFC with readily available materials was fabricated in this study. The performance was tested at various operating conditions of pH for electricity generation using DSW as the substrate, cow dung as inoculum. The feasibility of current generation and wastewater treatment was evaluated and the factors that affected the power output were also discussed. The pH effect of wastewater was correlated with microbial electrochemical activity, maximum current and power production. The pH of 6-7 and resistance of 820-1000 Ω was found to be suitable for the maximum current and power density generation. Cyclic voltammetry was employed to investigate electrochemical responses which demonstrate a three-electron transfer mechanism in the MFC. The low coulombic output of MFC was proposed to the loss of electrons for various biological activities other than electricity generation. The study of electricity production combined with distillery wastewater as substrate would approach the application and the performance of MFC in the future.

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Persian Abstract

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چکیده

یک پیل سوختی میکروبی تک محفظه (SCMFC) با استفاده از پساب حاصل از صنایع تقطیر الکل (DSW) و میکروارگانیزمهای موجود در فضولات گاو بعنوان مایه تلقیح در pH ۴-۹ راه اندازی شد. حداکثر جریان در MFC به ۰/۱۹ mA (۰/۱۶-۰/۱۹) pH ۸-۹ به دست آمد، در حالیکه مواد آلی پساب (COD) به ترتیب (۶۸٪) pH ۶ < (٪ترتیب (۰/۴۶ mA) pH ۶ < (۰/۴ mA) pH ۷ < (۷۹) pH ۷ < (۸۰-۸۱٪) pH ۸-۹ کاهش یافت. افت بازده کولومبیک ناشی از نوسانات سیستم دریافت کننده الکترون و نفوذ هوا به راکتور می باشد. از منحنی پلاریزاسیون حداکثر دانسیته جریان ۸۴ mA/m^2 و دانسیته توان ۲۹ mW/m^2 با مقاومت درونی Ω ۸۲۰ (۶ pH) حاصل شد. ولتامتری چرخه‌ای (CV) فرآیند انتقال ۳ الکترونی، بهترین پاسخ شیمیایی در ۷ و ۶ pH را نشان می-دهد. در شرایط عملیاتی مطلوب MFC پاسخ مثبتی را برای تولید بیوالکتریسیته نشان داده است.