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RESEARCH ARTICLE

Improved energy recovery from dark fermented cane molasses using microbial fuel cells

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Abstract A major limitation associated with fermentative hydrogen production is the low substrate conversion efficiency. This limitation can be overcome by integrating the process with a microbial fuel cell (MFC) which converts the residual energy of the substrate to electricity. Studies were carried out to check the feasibility of this integration. Biohydrogen was produced from the fermentation of cane molasses in both batch and continuous modes. A maximum yield of about 8.23 mol H_2/kg $COD_{removed}$ was observed in the batch process compared to 11.6 mol H_2/kg $COD_{removed}$ in the continuous process. The spent fermentation media was then used as a substrate in an MFC for electricity generation. The MFC parameters such as the initial anolyte pH, the substrate concentration and the effect of pre-treatment were studied and optimized to maximize coulombic efficiency. Reductions in COD and total carbohydrates were about 85% and 88% respectively. A power output of 3.02 W/m^3 was obtained with an anolyte pH of 7.5 using alkali pre-treated spent media. The results show that integrating a MFC with dark fermentation is a promising way to utilize the substrate energy.

Keywords dark fermentation, biohydrogen, microbial fuel cell, volatile fatty acid, anolyte

1 Introduction

With the continuing depletion of fossil fuels and increasing climate change effects, alternative renewable energy sources are receiving a great deal of attention. Biohydrogen is one of the most promising new energy sources [1]. Hydrogen can be produced by both biological and chemical processes. However, the biological production of hydrogen is preferred because it is completely sustainable, renewable and environmentally friendly [2]. Dark fermentative hydrogen production is among the best methods because light is not needed and very large amounts of hydrogen can be obtained [3].

Although, dark fermentation processes are well established, recovery of the energy in the spent media has not been maximized. The end products of this process are various carbon rich materials such as acetate, butyrate and ethanol. Many researchers have investigated ways to recovery energy from the spent media [4]. The methods that have shown the most potential are photo-fermentation and methanogenesis. However, the application of photo-fermentation is limited due to low conversion efficiencies, the requirement of light and expensive reactors [5–7]. Methanogenisis also has problems like slow biodegradation kinetics, the requirement of gas treatment and the need for post-treatment [8,9]. Electricity generation through biomethanation process from the organic wastes is a two stage process whereas that of MFC is a single step process [8,10]. Application of microbial fuel cell (MFC) for wastewater treatment could be an attractive alternative to reduce the cost of treatment and generate electricity. In the anode chamber of a single chambered MFC, electroactive bacteria (EAB) oxidize electron donors and transfer these electrons to the anode by various electron-transferring mechanisms. Electrons are conducted through a circuit to the passively air-fed cathode where oxygen is reduced to water by combing with electron and proton producing electrical current. In recent years it is emerging as one of the promising alternate technology for harvesting renewable energy through wastewater treatment in the form of direct electricity, for biohydrogen production, for to desalination of water and as BOD sensor [10]. This eliminates the problems associated with storage and the cost of conversion required in methanogenisis [11].

MFCs have several operational and functional advantages compared to energy genaration processes from the biomass. First, they generate power from biomass without

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combustion. Second, they can effectively utilize a wide range of organic complex wastes and can simultaneously be used to treat wastewater [12,13]. Guwy et al. compared the advantages and challenges associated with a dark fermentative hydrogen production process coupled with processes like photofermentation, methanogenesis or MFCs [14]. They concluded that MFC could be a potential alternate for bioenergy generation. However, more research is required to establish MFCs as a viable option for residual waste treatment. Hence the present paper investigates dark fermentation followed by a microbial fuel cell system for better energy recovery.

A few number of research experiments were carried out by integrating dark fermentative hydrogen production with MFC. Sharma and Li demonstrated the simultaneous production of hydrogen and electricity from glucose containing wastewater at different organic loading rates using a mixed microbial consortia [15]. A 2-L fermenter followed by a 100-mL single chambered MFC was used. Mohanakrishna et al. showed the conversion of volatile fatty acids (VFAs) from a batch biohydrogen reactor by MFC. The MFCs were found to be feasible for both electricity generation and higher chemical oxygen demand (COD) reduction (approx. 80%) [16]. For even better hydrogen recovery from the spent media, an MFC-MEC (microbial fuel cell-microbial electrolysis cell) system has been used [17]. Wang et al. reported a two-stage production in which, in the first stage, fermentation was done and in the second stage, both MEC and MFC were simultaneously used. The MEC system was used for hydrogen production and MFCs connected in series served as the power source for the whole process. This eliminated the need for an external power source. Nevertheless, little information is available on what pretreatment methods are needed for acid rich effluents to better utilize them in MFCs. The present study demonstrates the effect of pretreatment on the performance of an MFC.

Molasses is a widely used substrate for dark fermentation [17–19]. It is a by-product in the sugar processing industry with a high carbohydrate content (around 50%w/w). It is extensively used as a cheap carbon source in many industrial fermentation processes such as baker's yeast fermentation and amino acid and alcohol production. The presence of furan derivatives in molasses based distillery effluents usually inhibit the fermentation process. González et al. carried out experiments with white-rot fungus *Trametes* sp. I-62 on the detoxification of these furan derivatives. Further they also evaluated the efficacy of ligninolytic property of the fungus *Trametes* sp. They observed that the white-rot fungus *Trametes* sp. I-62 was found suitable in the treatment of recalcitrant distillery effluent [20].

Volatile fatty acids (VFAs)are the key intermediates produced by the dark fermentation of carbohydrate rich wastes like cane molasses, lignocellulosic agro-industrial wastes, sludge, and various other biodegradable organic wastes. Singhania et al. discussed the different ways of producing, separating and utilizing VFAs. VFA production via anaerobiosis can be utilized in biolipid, biohydrogen, methane, and bioplastic production as well as in MFCs and denitrification. The major barriers in the use of fermentation processes for VFA production are technical difficulties associated with their recovery from the fermentation broth and an increase in overall cost. There are various techniques available to recover organic acids from fermentation broths including electrodialysis, ion-exchange, adsorption, liquid–liquid extraction, pertraction, membrane based solvent extraction and extractive fermentation [21]. Recently, Poggi-Varaldo et al. compared the performance of two different types of inoculum (methanogenic and sulfate-reducing consortia) using extracts from spent media produced from dark fermentation processes. They found that sulfate reducing consortia as inoculum was more effective in terms of power generation [22].

Molasses was used as the sole substrate. The organism *Enterobacter cloacae* IIT-BT 08 was used for hydrogen production. The acid rich spent media was utilized for bioelectricity generation using MFCs. In the first phase, the effect of the substrate concentration (molasses) was evaluated. The optimized molasses concentration was supplemented with yeast extract and malt extract to evaluate the improvement in H_2 production in the batch process. This was followed by H_2 production in a continuous mode using immobilized whole cells. In the second phase, the effects of the initial pH and the substrate concentration of the spent media on the volumetric power density production were studied using mixed microbial consortia. The initial substrate concentration was varied from $3\ g \cdot COD \cdot L^{-1}$ to $15\ g \cdot COD \cdot L^{-1}$ after alkali pretreatment at the optimized pH. The suitability of mixing a buffer with the anolyte was also investigated.

2 Materials and methods

2.1 Microorganism and culture conditions

E. cloacae IIT-BT 08 (MTCC 5373), one of the most efficient hydrogen producing bacterium, was used. It is a Gram negative, rod-shaped and facultative anaerobic bacterium. The desired amount of inoculum was obtained by growing it overnight in a nutrient broth in an incubator shaker (New Brunswick Scientific, NJ, USA) at 200 $r \cdot min^{-1}$ at 37 °C. It was then used for dark fermentative hydrogen production.

2.2 Feedstock for dark fermentation

The cane molasses was procured from a sugar refinery. The fermentation media was supplemented with 1% (w/v) malt extract and 0.4% (w/v) yeast extract in order ensure adequate supplies of nitrogen, phosphorus and growth

factors for the bacteria. It has been reported that such supplementation makes the media more suitable for hydrogen production [23].

2.3 Hydrogen production set-up

2.3.1 Batch system

The batch experiments were carried out in a double jacketed reactor with a working volume of 500 mL. The production media consisted of malt extract, yeast extract and cane molasses with 10% (v/v) seed culture grown overnight as the inoculum. The agitation speed and temperature in the reactor were maintained at 200 rpm and 37°C respectively. The fermentation media was adjusted to an initial pH of 6.5 [23]. Anaerobic conditions were maintained by sparging nitrogen gas for a fixed amount of time after the inoculation. Samples were withdrawn from the reactor at regular intervals for the analysis of COD and total VFA. The gas mixture was allowed to pass through a 40% (w/v) KOH solution in order to absorb the carbon dioxide. The filtered gas was collected in a graduated water displacement system at ambient temperature and pressure. The batch experiment was continued until hydrogen production ceased.

2.3.2 Immobilized continuous system

The continuous experiments were carried out in a 500-mL glass double jacketed reactor. The bioreactor was packed with coconut coir as the carrier material to obtain a packing density of 50 $g \cdot L^{-1}$. After packing, the void volume of the reactor was 450 mL [24]. The bioreactor was thereafter kept undisturbed for 12 h to stabilize the cells. The production media was then passed through the reactor after stabilization until a maximum rate of gas production was obtained. The production media was fed into the reactor at different dilution rates in order to study the effect of substrate concentration on the biohydrogen production.

2.4 MFC configuration and operation

Eight identical cuboidal-type sMFCs single microbial fuel cells (sMFCs) were used for the experimental studies. A single-chamber MFC consists of an anode and cathode placed on the opposite sides of a 0.6-cm-thick acrylic (Plexiglas) cuboidal chamber. The empty anode chamber had a capacity of 1.2 L and the working anolyte volume was 1 L. The anode was made of carbon cloth (surface area 72 cm^2). The sMFC was operated in a fed-batch mode at a fixed external resistance of 100 Ω (unless indicated otherwise) and the media by replenished every 96±2 h.

For each sMFC, the membrane cathode assembly (MCA) was prepared using Nafion 117 (DuPont, Wilmington, DE). It was hot pressed with catalyst [MnO_2-NR 0.1 $mg \cdot cm^{-2}$ that has been ultrasonically mixed with carbon black Vulcan XC-72 3 $mg \cdot cm^{-2}$] by heating it to 125 °C at 1780 kPa for 3 min using a Moore Max Ton Hydraulic Press [25]. The MCA (surface area 36 cm^2) was then placed in the anode chamber. The inter-electrode distance was kept constant in all the experiments (~1.25 cm), with the anode being placed equidistances from the MCA. The anode chamber had two ports on the top; one for the terminal and the other for the reference electrode (Ag/AgCl, saturated KCl; + 197 mV, Equiptronics, India). The joints were sealed with epoxy sealant to maintain an anaerobic microenvironment in the anode compartment. Copper wire was used to connect the circuit.

2.5 Anodic mixed consortia and anolyte

An anaerobic mixed consortia obtained from the sludge of a septic tank in IIT Kharagpur was used as the parent inoculum (pH 7.2). The sludge was filtered through a 1-mm sieve, preheated at 100 °C for 15 min to suppress methanogens and then allowed to cool [21]. Prior to inoculation, the sludge was washed three times in saline buffer and enriched in glucose-based synthetic wastewater under an anaerobic microenvironment. The anodic compartments of the MFCs were then inoculated with the pretreated mixed microflora. The MFCs were first operated with sucrose-based synthetic water for a week for adaptation [26]. Once the biofilm formed (after about a week), spent dark fermentation media was fed into the MFCs without any dilution. The same media was fed for nine consecutive cycles (96 h/cycle) at ambient temperature (29±2 °C) and pressure. After each feeding, the MFC was sparged with N_2 for 2 min to maintain the anaerobic microenvironment. Prior to feeding, the pH of the wastewater was adjusted to the desired pH.

The feed for the MFC was prepared as follows: the spent dark fermentation media was initially centrifuged at 5000 rpm (Sigma Sigma 3K30), and the supernatant was collected. The media was diluted to 6 $g \cdot COD \cdot L^{-1}$ and then the pH of the spent media was varied between 5.5 and 8.5 in order to find the best anolyte pH for electrogenesis in the MFC. Fig. 1 depicts the photographs of the bioreactors used in the two-stage integrated process.

2.6 Analytical methods

Biogas composition was determined by Gas chromatography (GC Agilent Technology 7890A U.S.A) with a thermal conductivity detector and a stainless steel packed Porapak Q (80/100) column. The operational temperatures of the injection port, the oven and the detector were 80, 150 and 200 °C, respectively. Nitrogen was used as the carrier gas for the determination of both the biogas and VFA at a flow rate of 20 $mL \cdot min^{-1}$. The VFA and alcohol compositions in the fermented broth were also determined by GC with a flame ionization detector and a 10% PEG-20 M and

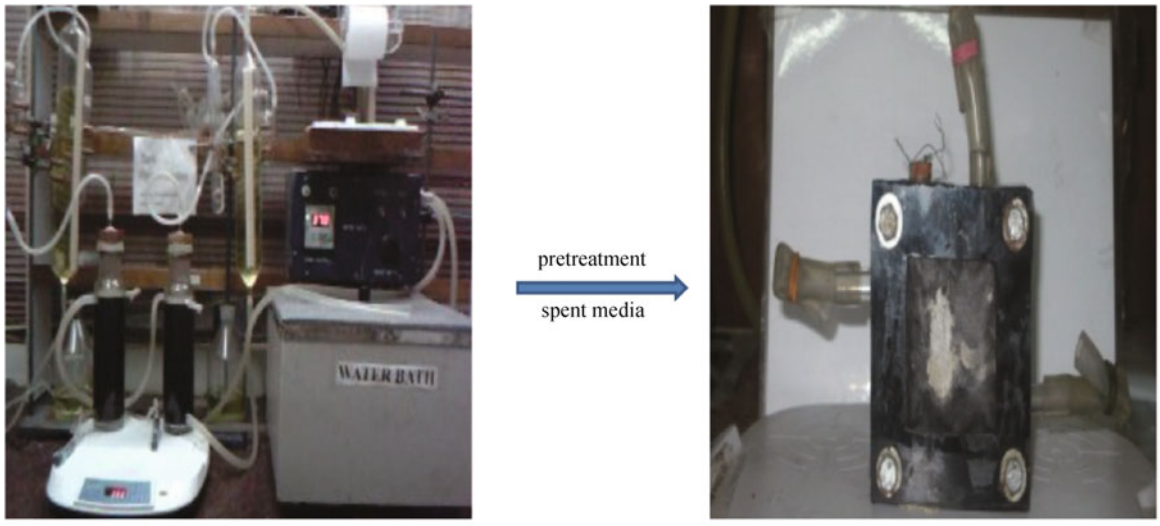

Fig. 1 Photographs of the experimental set-up used in the two-stage process: dark fermentation and single chambered MFC

2% H_3PO_4 (80/100 mesh) capillary column. The temperature of the injection port, detector and programmed column were 220, 240, and 130–175 °C respectively. A mixture of hydrogen and air at a flow rate of 30 mL·min^{-1} was used for the flame generation.

The heat of combustion of the molasses and spent media was determined with a Bomb calorimeter (Precision Electro Instrumentation India Private Limited, Kolkata, India). The media containing the molasses and the spent media were lyophilized and the solids obtained after lyophilization were used to determine the heat of combustion.

2.7 Theoretical considerations

The cumulative hydrogen production obtained in the dark fermentation (batch mode) was modeled by the modified Gompertz equation [27]:

$$H(t) = P \times \exp\left\{-\exp\left[\frac{R_m}{P}(\lambda - t) + 1\right]\right\}, \qquad (1)$$

where $H(t)$ represents the cumulative volume of hydrogen production (mL) as a function of time; P is the potential gas production (mL); R_m is the maximum rate of hydrogen production (mL·L^{-1}·h^{-1}); λ is the lag time (h); t is the incubation time (h); and the exp (1) = 2.718. The typical cumulative hydrogen production curve is nonlinearly modeled by Eq. (1). Parameters P, R_m and λ were estimated using the solver function in Matlab™ (Curve fitting toolbox ver. 1.1.7.).

The COD values of the samples were measured according to American Public Health Association (APHA) standard methods using a HACH COD estimation kit. Samples were added to the COD reagents (each vial contains a mixture of 0.25 mL of Solution 1 and 2.8 mL of Solution 2) [28] and were digested in a DRB200 Digital Reactor Block at 150 °C for 2 h. After cooling, the COD of the samples were estimated by using a DR 2800™ Portable Spectrophotometer.

MFC cell voltages were measured using a 16-channel data acquisition system (USB-6009, National Instruments, Texas, USA). The anode and cathode potentials were measured versus a saturated Ag/AgCl reference electrode. Voltages were recorded every 15 min (NI LabVIEW-based customized software, Core Technologies, India). Polarization curves were obtained by varying the external resistances from 90000 to 30 Ω. Power and current were calculated based on following Eq. (2):

$$P = V \times I, \qquad (2a)$$

$$i_d = V/R_{ext}V_{and}, \qquad (2b)$$

$$P_d = V^2/R_{ext}V_{and}, \qquad (2c)$$

where P is generated power, V is the measured cell voltage, R_{ext} denotes the external resistance, V_{and} is the volume of the anode chamber and I is the produced current. The volumetric current (i_d) and power density (P_d) were calculated by normalizing the current and power to the net liquid volume of the anode chamber. Provisions were made for the online observation of the polarization curves to show the variation of the power density and voltage with respect to the current. A variable-load resistor (range : 99 000–1 Ω) was used to generate the power density curves. Sustainable power calculations were also made after the MFCs reached a stable cell potential.

The internal resistances of the MFCs were calculated by the current interruption method [25]. Once the MFC produced a stable current output (I) and potential (V) in the closed circuit mode, the circuit was opened causing a steep initial rise in the potential (V_R) followed by a gradual increase. This steep rise is attributed to ohmic losses caused by the internal resistance (R_{int}) of the MFC and can be calculated as

$$R_{int} = \frac{V_R}{I}. \quad (3)$$

The coulombic efficiency (CE) can be calculated by dividing the total coulombs obtained from the cell by the theoretical coulombs that can be produced from the anolyte according to the equation [29].

$$CE = \frac{M\int_0^t I\,dt}{Fbv\Delta COD}, \quad (4)$$

where v is the volume of the MFC anode chamber, M is 32, the molecular weight of oxygen; F is Faraday's constant, 96485 C/mol; b is 4, the number of electrons transferred per mole of oxygen; and ΔCOD is the difference in COD in the influent and effluent [30].

The conductivity of the influent was measured with a conductivity meter (Orion 5 star multimeter, 1219000, Thermo Fisher). The pH of the anolyte was measured with a pH meter (Orion 5 star multimeter, 1219000, Thermo-Fisher). The accuracy of the pH measurement was ±0.01 pH units. Estimation of the total sugar was done using the phenol sulfuric method described by Loewus [31]. All experiments were carried out in triplicate.

3 Results and discussion

3.1 Hydrogen production via dark fermentation in a batch system

The concentration of the carbon source plays a vital role in the fermentation process. It can affect the hydrogen production rate and yield, and most importantly the cost of the whole process [32]. A high carbon source concentration can promote the H_2 production rate and improve the substrate conversion efficiency; however, it can also modify the metabolic pathways and lead to the production of unwanted by-products [33]. The effect of the initial cane molasses concentration on the biohydrogen production was investigated at five different concentrations (5–25 g·COD·L^{-1} in increments of 5 g·COD·L^{-1}). The maximum H_2 production was obtained at an initial molasses concentration of 10 g·COD·L^{-1} (the total carbohydrate content was 5.5 g·L^{-1}) with a yield of 5.25 mol H_2/kg $COD_{removed}$ and a 55% reduction in total carbohydrates. Hydrogen production was increased from 5 g·COD·L^{-1} to 10 g·COD·L^{-1} and subsequently no increment in hydrogen production was observed with further increase in molasses concentration (15–25 g·COD·L^{-1}). Thus 10 g·COD·L^{-1} in the initial molasses was used as the concentration for subsequent production of hydrogen by *E. cloacae* IIT-BT 08. However, the cane molasses was supplemented with 1% (w/v) malt extract and 0.4% (w/v) yeast extract to supply adequate nitrogen, phosphorus and growth factors for the bacteria. After supplementation, the production media contained 22.4 g·COD·L^{-1} and the maximum cumulative H_2 production and yield were 2200 mL·L^{-1} and 8.23 mol H_2/kg $COD_{removed}$ respectively. The total reduction in COD and carbohydrates were 53% and 69% respectively.

The cumulative hydrogen production curve obtained from the modified Gompertz equation at the optimal media concentration (supplemented with yeast and malt extracts) is shown in Fig. 2. The kinetic parameters P, R_m and λ were obtained from the modified Gompertz equation and the values were 2200 mL, 164.47 mL·L^{-1}·h^{-1} and 4.7 h respectively. The regression coefficient was 0.998 which indicates a strong correlation between the experimental data and the fit. It has been reported that there is a lag time of more than 20 h for H_2 production when the concentration of molasses is higher (10 g·COD·L^{-1}) [33]. In the present study, the lag time (λ) was reduced to around 4.7 h which indicates that the organism is utilizing the complex substrate in an efficient manner.

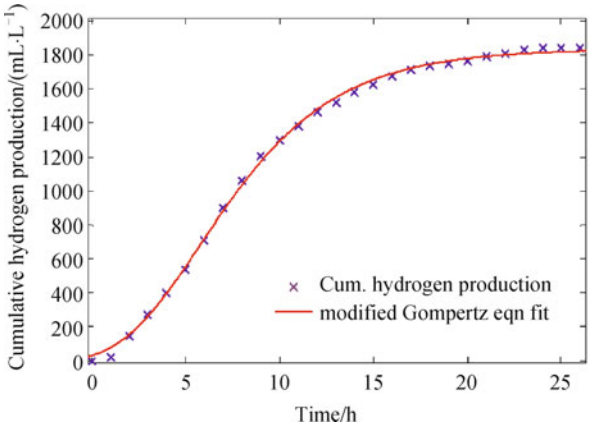

Fig. 2 Simulation of the cumulative hydrogen production using the modified Gompertz equation at optimal media composition

The hydrogen yield increased 1.5 fold when the production media was supplemented with yeast and malt extracts. The average VFA and ethanol concentrations in the spent media after the dark fermentation were 1180 mg·L^{-1} acetate, 1246 mg·L^{-1} butyrate, and 425 mg·L^{-1} ethanol. The carbohydrate concentration, COD and final pH of the spent media were 1.7 g·L^{-1}, 10.5 g·L^{-1} and 4.7 respectively. The spent media was dark-brown in color due

to the presence of melanoidins which are the result of the Maillard reaction between the sugars, carbohydrates and the proteins. This spent media was used as the anolyte in the sMFCs for the generation of bioelectricity after the necessary pre-treatment.

3.2 Biohydrogen production in continuous system

It has been established that a lack of nutrients enhances the adsorption of the microbial cells on the solid support matrix [34]. Coconut coir is suitable for the immobilization of whole-cells for two reasons: 1) It has a higher packing density as compared to other lignocellulosic packing materials like rice straw, wheat straw, etc. in the bioreactor. 2) The spongy porous surface texture of coconut fiber provides superior adsorption of the cells. Furthermore, it is inexpensive, environmentally friendly and there is an ample supply, making it a highly competitive and promising carrier material.

After stabilization of the immobilized bioreactor, the bioreactor was initially operated in a batch mode until the rate of hydrogen production was steady. This batch fermentation period lasted for about 10–12 h (data not shown). In addition to hydrogen, acetate and butyrate were the two major fermentative catabolites. Trace amount of ethanol production was also detected. The organic nitrogen in the media is responsible for the acetate-butyrate type of fermentation [35]. After the initial batch process, the bioreactor was operated in the continuous mode at different dilution rates (0.1 to 0.8 h^{-1}) with increments of 0.1 h^{-1}. Fig. 3 shows the rate of hydrogen production and the COD removal efficiency for the different dilution rates. The maximum rate of hydrogen production and yield were 1250 $mL \cdot L^{-1} \cdot h^{-1}$ and 11.6 mol H_2/kg $COD_{removed}$ respectively, with a COD reduction of about 47% at a dilution rate of 0.6 h^{-1}(Fig. 4). The hydrogen yield in the continuous process was 1.4 fold higher than that in the batch. This increase may be due to less accumulation of VFAs in the system.

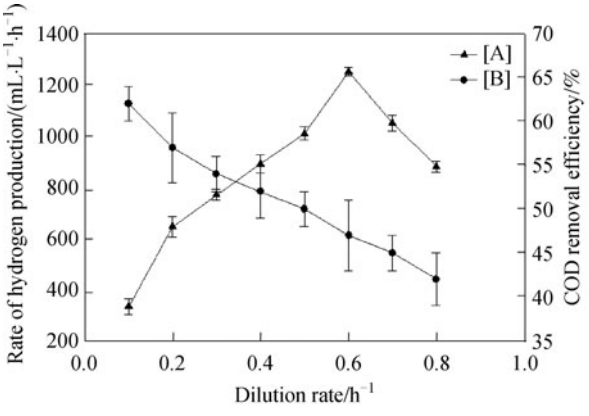

Fig. 3 Effect of dilution rate on: (a) the rate of hydrogen production and (b) COD removal efficiency

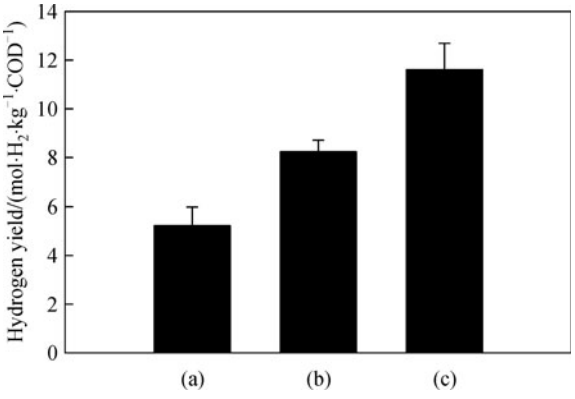

Fig. 4 Hydrogen yield for: (a) raw cane molasses in the batch process, (b) cane molasses with supplements (yeast and malt extracts) in the batch process and (c) cane molasses with supplements in the continuous process

3.3 Effect of the initial pH on the voltage generation during the MFC start-up phase

The spent molasses media is acidic so neutralization is necessary for biological treatment. High acidity or alkalinity of wastewater affects both wastewater treatment efficiency and the environment inside the reactor. The collected spent media was centrifuged and then pretreated with alkali solution (NaOH) to neutralize the acidity. The spent media was adjusted to different pH values to find the optimum pH for electrogenesis in the MFC. The initial anolyte pH was maintained at 5.5, 6.5, 7.5 or 8.5 with the reactors named MFC-1, MFC-2, MFC-3 and MFC-4 respectively.

All the MFCs were operated for nine cycles in the closed-circuit mode with an external resistance of 100 Ω. Initially the operating voltage increased due to the increase in the negative charge of the anode for the metabolism of the electrogen by donating electrons to the anode. After inoculation, during the 2th cycle, the current output of MFC-3 increased sharply and then stabilized at about 4.95 mA when it reached a stable operation (plateau). A much slower increase in current was observed for the MFCs with anolyte pH values of 6.5 and 8.5 and slightly lower currents of 3.4, 4.51 and 4.3 mA were obtained in MFC-1, MFC-2 and MFC-4 respectively after the second week operation.The performance of the MFCs was evaluated at different anolyte pH values after the fourth cycle and the results are in Table 1.

Polarization curves were obtained using an adjustable external resistance after the fourth cycle and after the sMFCs reached a steady-state maxima in their operating voltage. All the polarization results were recorded when the resistors were switched from higher loads to lower loads. It should be noted that there are no well-established methods for measuring the voltage drop in polarization curves in MFCs. So, the voltage drop was measured after the readings were stable, which took 10–15 min for each

Table 1 The effect of initial anolyte pH on single chambered MFCs with 6 g/L alkali treated anolyte

MFC	MFC-1, pH 5.5	MFC-2, pH 6.5	MFC-3, pH 7.5	MFC-4, pH 8.5
Max. open circuit potential/mV	627±5	804±11	858±9	822±7
Max. volumetric power density/($W \cdot m^{-3}$)	1.21±0.05	1.84±0.07	2.49±0.09	2.03±0.08
COD removal efficiency/%	43.73±2.4	70.4±3.5	80.2±1.1	78.1±0.9
Carbohydrate removal/(%, w/w)	56±1.5	60.4±1.1	67±0.8	62.6±1.5
Internal resistance/Ω	128.4±2.5	98.7±2.8	75.2±1.8	89.3±1.6

external load. As the resistance decreased, the stabilization period for the voltage also decreased. More rapid stabilization of the voltage was observed at lower resistances than for higher resistances. MFC-3 produced a maximum power density of 2.49 $W \cdot m^{-3}$ (at a current density of 5.26 $A \cdot m^{-3}$) and this was 51.4%, 26% and 18.4% higher that the maximums for MFC-1, MFC-2 and MFC-4 respectively. Therefore, it can be concluded that the anolyte pH affects the electron discharge of the electrogenic bacteria on the anode surface. The changes in the anode potentials during the polarization were different in each of the MFCs, which corroborates the conclusion that the microbial activities at the anodes played important role. The anode potential at pH 7.5 was more negative than the anode potential at pH 6.5, 7.5 or 8.5 see (Fig. 5(a)). This indicated that MFC-1, MFC-2 and MFC-4 had higher overpotentials than MFC-3 which may be due to a better formation of the biofilm on the anode surface at an initial anolyte pH of 7.5. This supports the results reported by Gil et al. [36] and He et al. [37]. Both studies observed that a low pH (pH of 5 and 6) resulted in lower electricity generation. Gil et al. reported that the highest current was observed between pH 7 and 8 [36]. Other researchers have also reported that an acidic pH in the anode chamber reduces the power production. Ren et al. reported that a significant decrease in power production occurred when the final pH, i.e., the pH in the anode compartment, dropped to 5.2 due to the acidic products of the fermentation and that power production quickly resumed when the pH was increased to 7.0 [38].

MFCs in a close-circuit mode will be in steady state if the power generated by the MFC equals the power consumption for an extended time, and at steady-state conditions, the power production is sustainable. Because many steady states are possible, it is important to define the conditions at which a sustainable current reaches a maximum in an MFC [39].

The variation of relative decrease in the anode potential (RDAP) with applied external resistance is shown in Fig. 5 (b). The linear fit at high external resistance represents the region in which the external resistance controls the power whereas the linear fit at low external resistances represents the region in which the power is limited by kinetics, mass transfer, or internal resistance [40]. When the external resistance was high, the RDAP increased linearly with decreasing external resistance because the electron deliv-

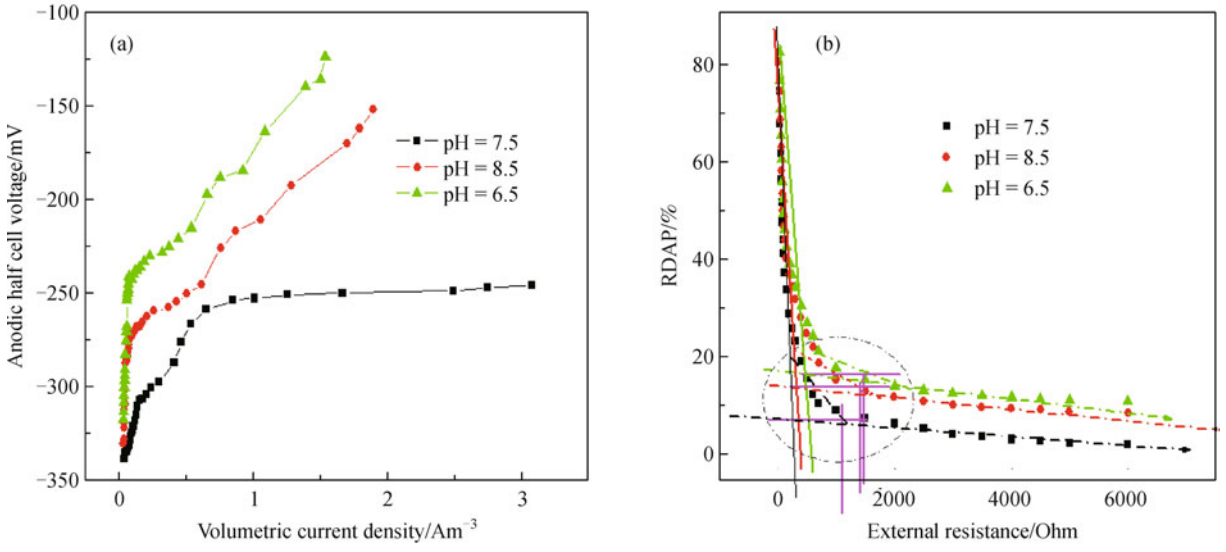

Fig. 5 (a) Polarization curves for anodic half-cell voltages versus current density at different external loads and power densities with initial anolyte pH values of 7.5, 8.5 and 6.5. (b) Percent relative decrease in the anode potential (RDAP) observed during MFC operation with respect to applied external resistance and depicting sustainable power

ery to the cathode was limited by the external resistance. At low applied external resistance, the electron delivery to the cathode was limited by kinetic and/or mass transfer (or the internal resistance) and the RDAP increased linearly with decreasing external resistance.

The RDAP analysis showed a lower sustainable resistance for MFC-3 (1.1 kΩ) than those for MFC-2 (1.4 kΩ) and MFC-4 (1.5 kΩ). This indicates that slightly alkaline condition result in a decrease in overpotential and an increase in system sustainability The corresponding sustainable power densities of the three MFCs were 0.39; 0.54 and 0.34 for MFC-2, MFC-3 and MFC-4 respectively. These results show that the value of the initial pH plays a crucial role for the anodic current production in electroactive biofilms. Yuan et al. reported that a highly electrochemically active biofilm was achieved under slightly alkaline condtions [41]. More bacteria attach to the anode at an alkaline pH which contributes to higher electroactive moieties which may be the reason for the higher power outputs at lower pH conditions [41].

The addition of NaOH to acidic spent media increased the salt concentration, which enhanced the overall performance of the system by reducing the internal resistance and increasing the maximum power production. However, in this study, even with the increase in conductivity due to the addition of NaOH, the volumetric power density did not increase much for MFC-4. This may be due to the presence of a stringent anodic microenvironment. The decrease in volumetric power production may be attributed to the high salinity (conductivity of 29.8 $mS \cdot cm^{-1}$ at pH 8.5) which created a hypertonic solution. Since the performance of the MFC is growth dependent, the amount of salt that can be added to the anode chamber is limited by the tolerance of the microorganism to the ionic strength of the anolyte. It has also been reported that methanogenesis is more predominant than electrogenesis under alkaline conditions [42].

3.4 Bioelectrochemical treatment of the spent media

The performance of the four sMFCs in terms of COD removal at different operating cycles was evaluated. After 16 days of operation, the average COD removal efficiencies from the fifth cycle were 43.5%, 70.4%, 80.2% and 78% for MFC-1, MFC-2, MFC-3 and MFC-4 respectively. The average COD removal efficiency for each of the MFCs was 70%–80%, which demonstrates an effective treatment efficiency for this system.

The coulombic efficiency of each MFC was calculated based on COD removal. CE is an indicator which is used to evaluate the capacity of an MFC for converting the energy present in the organic matter into electric energy. This term gives the number of electrons recovered as current versus the number initially present in the organic matter. The average CE of MFC-3 was 2.98%. The COD removal and power generation decreased as the feed pH decreased and they were lowest for a feed pH of 5.5. Average CEs of 3.6%, 2.89% and 2.75% were observed for MFC-1, MFC-2 and MFC-4 respectively after after 14th cycle (3 weeks). A pH of 7.5 inside the reactor might be favorable for the anaerobic degradation of the wastewater, and this slightly alkaline pH is favorable for the treatment of this VFA rich complex spent media.

A lower CE was observed for MFC-4 at pH 8 than for MFC-3 due to less current generation. This might be attributed to the ineffective extracellular electron transfer in this microenvironment (pH 8.5) where electrogenic bacterial growth was partially suppressed due to the high salt concentration. Another possible reason for low CE in MFC-4 might be the presence of more methanogens than exoelectrogens at alkaline pH. High amounts of carbohydrates were found in the anolyte after the dark fermentation which plays an important role in the metabolic activity of the anaerobic bacteria during bioelectricity generation.

The concentration of carbohydrates in the feed influence bioelectricity generation. Therefore, the variations in the carbohydrate concentration was monitored during the experiments to evaluate their role on both bioelectricity production and substrate degradation. The average VFA removal in the last four cycles was 99%, 96%, 92% and 85% for MFC-1, MFC-2, MFC-3 and MFC-4 respectively. However, it is difficult to determine to what extent the VFAs were consumed by the electrogenic microorganisms. In general, in an MFC, an anaerobic microbial consortia can act on complex carbohydrates to convert them into simple carbohydrates and then into VFAs followed by conversion into carbon dioxide, electricity and methane. The average carbohydrate removal in the last four cycles (5–9th) was 93%, 90%, 86% and 81% for MFC-1, MFC-2, MFC-3 and MFC-4 respectively. These results suggest that electrogenic microorganisms prefer VFAs as the electron donor over complex carbon sources like carbohydrates.

3.5 Effect of the initial substrate concentration (spent media) on the MFC

The effect of the initial substrate concentration on bioelectricity generation was studied using an initial anolyte pH of 7.5. The initial concentration of spent media was varied from 2–10 $g \cdot COD \cdot L^{-1}$. The highest volumetric power production of 2.49 W m^{-3} was observed for a concentration of 6 $g \cdot COD \cdot L^{-1}$(Fig. 6). The maximum volumetric power density obtained from 2, 4, 6, 8 and 10 $g \cdot COD \cdot L^{-1}$ were 2.33, 2.41, 2.49, 2.25 and 1.43 $W \cdot m^{-3}$ respectively. The improvement power density was only 6.4%, when the initial concentration of the substrate was increased from 2–6 $g \cdot COD \cdot L^{-1}$. A gradual decrease in current was observed for concentration above 6 $g \cdot COD \cdot L^{-1}$. The highest spent media concentration (10 $g \cdot COD \cdot L^{-1}$) resulted in the lowest volumetric current generation of 1.43 $W \cdot m^{-3}$. It has been reported that high substrate concentration reduces electricity production due

to substrate inhibition and it has been demonstrated that current generation is proportional to substrate concentration only up to a certain limit (250 mg·L^{-1} in a relatively small volume MFC) [43,44].

The detrimental effect of salinity in association with the higher substrate concentration might be the reason for poor performance of the system. One way to measure total dissolved salts (TDS) is to find out electrical conductivity (EC) in a solution. EC is measured by passing an electric current between two metal plates (electrodes) in the water sample and measuring how readily current flows (i.e. conducted) between the plates. The more dissolved salt in the water, the stronger the current flow and the higher the EC. Measurements of EC can be used to give an estimate of TDS. Measurements of EC can be used to give an estimate of TDS. In spite of the high conductivity, the power density (28.5 mS·cm^{-1}) decreased 42.5% when the spent media concentration was 6 g·COD·L^{-1}. This decrease in MFC performance might be due to the negative effect of the salt on the growth of the microorganism [45].

Dark fermentative H_2 production is associated with VFA production. The acetate and butyrate accounted for 70%-80% of the total VFA concentration and the rest was mostly ethanol. As the initial concentration of spent media increased, the butyrate and acetate concentration increased As a consequence, the salinity of the solution increased during neutralization with the alkali which may affect electrogenesis in anode. The COD removal efficiencies, volumetric power densities and anolyte conductivities at different concentrations of spent media are shown in Fig. 6. The average CE of the MFCs for 4, 6, 8 and 10 g·COD·L^{-1} were 7.05, 3.63, 2.96, 2.61 and 3.69 respectively. Significant COD removal efficiency was observed at the lower substrate concentrations and substrate removal was poor for 10 g·COD·L^{-1}. This might be due to the impaired microbial metabolism at higher substrate concentrations and a relatively high saline microenvironment. It is known that the CE is inversely proportional to COD removal when the current generation is constant which is very much evident from these results.

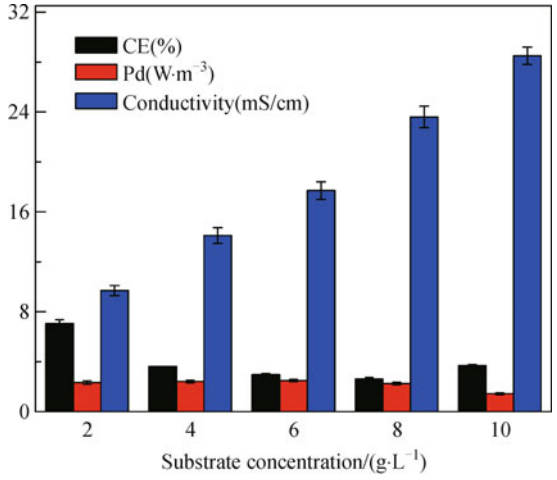

Fig. 6 Coulombic efficiency (CE), volumetric power density (P_d) and anolyte conductivity at different concentrations of alkali pre-treated spent media

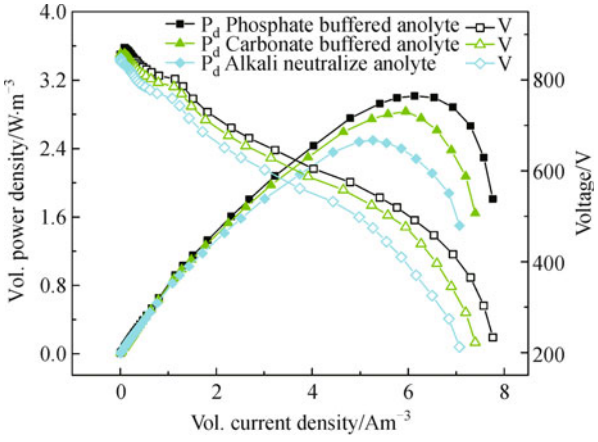

Fig. 7 Polarization of MFCs using different buffer supplemented anolytes

3.6 Effect of buffer supplementation

The effect of the addition of a buffer to the anolyte on power generation was also investigated. In most of the cases, the acid rich dark fermentative effluent was neutralized with alkali. Low concentrations (10 mmol·L^{-1}) of phosphate and carbonate buffers were added to the anolyte to maintain the initial pH at 7.5 and the effect on the power generation was studied. The power density of the MFCs were highest for the phosphate buffer followed by the carbonate buffer and then the alkali neutralized anolyte and the results are shown in Fig. 7. The corresponding polarization curves of the MFCs were obtained by varying the external resistance from 90 kΩ to 30 Ω.

The maximum volumetric power density increased 17.27% (from 2.49 to 3.01 W·m^{-3}) when phosphate buffer was added to the anolyte. The power density for the carbonate buffer was 5% lower than that for the phosphate buffer. This may be attributed to the buffering action in the anolyte which inhibit the pH splitting between anode and cathodic half , as a result it reduces overpotential of the MFC [40]. However, a carbonate buffer would be preferred to a phosphates buffer when the MFC is to be used for wastewater treatment. If the effluent is discharged without removal of the phosphates, it may lead to eutrophication of the water body. The cost of phosphate removal is high and it is difficult to scale-up. In comparison to phosphate buffer, the proton transfer rate of bicarbonate is about 34% higher due to the high mass transfer coefficient of bicarbonate in water (1.34×10^{-5} vs. 1.0×10^{-5} cm^2·s^{-1} at 30°C). The higher diffusion rates of the bicarbonate protons results in a smaller internal resistance and a larger

Table 2 Comparative study on dark fermentation integrated with MFC

Integrated system	Reactor type	Reactor volume /L	Feedstock	H_2 yield	COD removal efficiency	Power density	Energy recovery	References
Dark fermentation	Tubular reactor	2.0	Glucose based synthetic wastewater	2.85 mol H_2/mol glucose	97%	–	559 $J \cdot L^{-1}$	[15]
MFC	Single chamber	0.1		–				
Dark fermentation	Sequencing batch biofilm reactor	1.4	Vegetable wastewater	2.46 mmol $H_2 \cdot h^{-1}$	–	–	–	[16]
MFC	Single chamber	0.55		–		111.76 $mW \cdot m^{-2}$		
Dark fermentation	Double jacketed tubular reactor	0.5	Molasses	8.23 mol H_2 kg^{-1} $\cdot COD_{removed}$ $\cdot d^{-1}$	85%	–	19.8%	Present study
MFC	Single chamber	1.0		–		3.02 $W \cdot m^{-3}$	2.2%	

power density [24]. The internal resistances in the MFCs were estimated to be 56.7, 59.5 and 67.9 Ω for the phosphate buffer, the carbonate buffer and the pre-treated alkali respectively. For an initial pH of 7.5 with 6 $g \cdot COD \cdot L^{-1}$ substrate concentration, maximum COD and carbohydrate removals of (85.4% and 83.5%) and (89.25% and 87.6%) were obtained in MFC using phosphate and carbonate buffered anolytes respectively.

3.7 Energy analysis of the integrated process

Dark fermentative hydrogen production: Heat of combustion of hydrogen = 12.64 $kJ \cdot L^{-1}$. Heating value of cane molasses = 14 kJ/g solids. Total solids obtained from one liter of the media = 10 g. Total heating value of the fermentation media used in dark fermentation = 140 kJ. Volumetric yield of H_2 from batch fermentation under suitable conditions = 2.2 $L \cdot L^{-1}$. Total heating value of H_2 evolved from batch fermentation = 2.2 $L \cdot L^{-1}$ × 12.64 $kJ \cdot L^{-1}$ = 27.8 $kJ \cdot L^{-1}$. Energy recovery from feed = (Total heating value of H_2) × 100%/heat value of feed = 27.8 × 100%/140 = 19.8%.

Bioelectricity from single chambered MFC: Heat of combustion of spent media = 9.2 $kJ \cdot g^{-1}$. Power output (Watt) in MFC was calculated as $P = I \times V$ (5) and energy production (Joule) can be expressed as $E = P \times t$ (6). Since 1000 mL contain COD = 6000 mg and average COD removal = 85.4% (after 96 h), then COD utilized = 5124 mg. Heat of combustion of spent media = 9.2 $kJ \cdot g^{-1}$. So, heat of combustion of 5124 mg COD = (9.2 × 5.124) = 47141 J. According to Eq. (6) , 3.01 $W \cdot m^{-3}$ × 0.001 m^3 × 96 h × 3600 $s \cdot h^{-1}$ = 1040.2 J. Energy efficiency for unit MFC-1 per a batch cycle = (1040.2/47141) × 100% = 2.2%. For a substrate concentration of 6 $g \cdot COD \cdot L^{-1}$ with 1 L of liquid in the anode compartment (representing 47.141 kJ available energy), the average energy production in terms of electricity in the MFC (per batch cycle) is 1040.2 J. Hence, an energy recovery of 2.2% as electricity was achieved using an MFC unit. These results are compared with other work from the literature in Table 2.

These results show that the pre-treated acid rich spent molasses media is a suitable substrate for bioelectricity generation. The overall COD removal was 92.5% with 53% COD removal during dark fermentation. A carbohydrate reduction of 96.5% was achieved through the integrated process.

4 Conclusions

The suitability of using an MFC with the spent media from a dark fermentative hydrogen production process for additional bioenergy recovery was investigated. The substrate concentration plays an important role in the biohydrogen production in both batch and continuous processes. The study demonstrated that microbial fuel cells can be utilized for both the treatment of spent media and the generation of bioelectricity. A slightly alkaline anodic pH in the MFC is favorable for higher power generation and better organic matter removal. The addition of carbonate buffer to the spent media is even more effective than alkali pretreatment. Overall the COD and total carbohydrate reduction were improved significantly in the two stage system which is essential for the disposal of waste. The two stage process thus improves energy recovery from wastewater and reduces environmental pollution.

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