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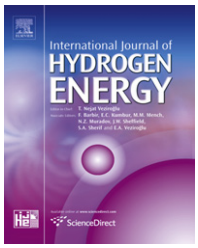

Bioenergy production from glycerol in hydrogen producing bioreactors (HPBs) and microbial fuel cells (MFCs)

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ABSTRACT

The supply of glycerol has increased substantially in recent years as a by-product of biodiesel production. To explore the value of glycerol for further application, the conversion of glycerol to bioenergy (hydrogen and electricity) was investigated using Hydrogen Producing Bioreactors (HPBs) and Microbial Fuel Cells (MFCs). Pure-glycerol and the glycerol from biodiesel waste stream were compared as the substrates for bioenergy production. In terms of hydrogen production, the yields of hydrogen and 1,3-propanediol at a pure-glycerol concentration of 3 g/L were 0.20 mol/mol glycerol and 0.46 mol/glycerol, respectively. With glucose as the co-metabolism substrate at the ratio of 3:1 (glycerol:glucose), the yields of hydrogen and 1,3-propanediol from glycerol significantly increased to 0.37 mol/mol glycerol and 0.65 mol/glycerol, respectively. The glycerol from biodiesel waste stream had good hydrogen yields (0.17–0.18 mol H_2/mole glycerol), which was comparable with the pure-glycerol. In terms of power generation in MFCs, pure-glycerol was examined at concentrations of 0.5–5 g/L with the highest power density of 4579 mW/m^3 obtained at a concentration of 2 g/L. The power densities from the biodiesel waste glycerol were 1614–2324 mW/m^3, which were likely caused by the adverse effects of impurities on electrode materials. An economic analysis indicates that with the annual waste stream of 70 million gallons of glycerol, the expected values generated from HPBs and MFCs were $311 and $98 million, respectively.

1. Introduction

Volatile energy prices and global warming concerns are the driving force for developing alternative fuels. Biodiesel has emerged as one of the promising alternative energy resources, with the production substantially increasing from 0.5 to 700 million gallons over last decade [1]. Biodiesel is produced through the transesterification of lipids (e.g. vegetable oil, animal fat) with short-chain aliphatic alcohols (e.g. methanol, ethanol) in the presence of a catalyst (e.g. NaOH or KOH) [2]. One of the main by-products of biodiesel is glycerol ($C_5H_8O_3$), with approximately 1 kg of glycerol being generated per 10 kg of biodiesel [3]. Glycerol is an important chemical widely used in industries such as pharmaceutical, food, and cosmetics [4]. However, with glycerol supply far exceeding its demand, the market price of glycerol has decreased substantially [5]. If excess glycerol cannot be utilized for producing valuable end products, it will be disposed as a waste product, which increases the cost of biodiesel production. In order to avoid glycerol disposal costs and explore value generation, recent industrial initiatives (e.g. Dow Chemical) have aimed to utilize excess glycerol for producing valuable chemicals including epichlorhydrin and propylene glycerol [6].

To explore the value of glycerol from biodiesel waste stream for further application, the conversion of glycerol to valuable bioenergy (e.g. hydrogen and electricity) could be an effective

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approach to degrade glycerol and generate energy. Two anaerobic technologies, "hydrogen producing bioreactors (HPBs)" and "microbial fuel cell (MFCs)" have been used to generate bioenergy through glycerol treatment. In HPB processes, the anaerobic fermentation of glycerol yields two valuable products (hydrogen and 1,3-Propanediol: $C_3H_8O_2$) [7]. Hydrogen is a clean energy resource and only produces water during combustion, while 1,3-Propanediol is a monomer used for production of plastics, textiles and upholstery [8]. Until now, the research on anaerobic fermentation of glycerol is limited and only focused on pure cultures under sterile conditions (e.g. *Klebsiella aerogenes*, *Enterobacter aerogenes*, *Clostridium butyricum* [7,9–11]), which are not suitable for the real-world industrial applications. Although a few studies used mixed cultures (e.g. activated sludge, heat treated soil) for hydrogen production, the hydrogen yields remain low (0.05–0.41 mol H_2/mole glycerol) [12,13] and must be improved to justify the application of anaerobic hydrogen production on a large scale. On the other hand, the MFC is a bioelectrochemical technology utilizing anaerobic bacteria to generate electricity from organic substances (e.g. carbohydrate, protein, and glycerol). The anaerobic bacteria growing on the anode surfaces produce electrons and protons through the degradation of organic substances. The electrons travel through the external circuit to the cathode surface, where they combine with oxygen and protons to form water. Different MFC configurations such as single chamber MFC (SCMFC) have been developed to reduce internal resistance and improve power densities [14,15]. However, MFC studies have typically utilized simple organic substances (e.g. volatile fatty acids, alcohols, and acetate) for electricity generation [14,16,17,18]. Although glycerol has been used as the organic substrate [19], no study has been conducted for optimizing the power generation from glycerol in continuous flow MFCs, and the performance of MFCs using glycerol from biodiesel waste stream has not been reported yet.

This study targeted the bioenergy production from glycerol using HPBs and MFCs. Three main tasks were undertaken. First, hydrogen production from pure-glycerol was studied in batch mode HPBs. The enhancement of hydrogen production from glycerol through co-metabolism with glucose was examined. Second, electricity generation from glycerol was investigated at different concentrations in a continuous mode SCMFC. Bioenergy production from glycerol in biodiesel waste streams was compared with pure-glycerol. Third, the economic value of bioenergy products (hydrogen, 1,3-propendiol, and electricity) from glycerol was estimated for the benefits of incorporating anaerobic bioenergy technologies into glycerol treatment processes.

2. Materials and methods

2.1. Hydrogen producing bioreactor (HPB) setup

Glass bottles (volume: 250 mL) (Wheaton Scientific, Millville, NJ) were used as the batch mode HPBs in this study (Fig. 1). The reactors had two glass extensions capped by septa for gas and liquid sampling. A tube inserted into the headspace of the HPBs was connected to a glass syringe for biogas volume measurement with biogas pushing the syringe plunger against the atmospheric pressure. The reactors were filled to 100 mL

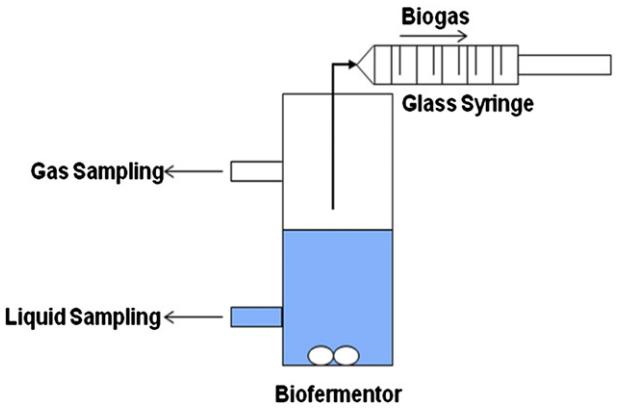

Fig. 1 – The experimental setup of batch mode HPBs.

with the mixture of glycerol and an inorganic medium to provide nutrients for bacteria to grow. The inorganic medium consisted of (per liter of water): 2.0 g NH_4HCO_3, 1.0 g KH_2PO4, 100 mg $MgSO_4 \cdot 7H_2O$, 10 mg NaCl, 10 mg $Na_2MoO_4 \cdot 2H_2O$, 10 mg $CaCl_2 \cdot 2H_2O$, 15 mg $MnSO_4 \cdot 7H_2O$, and 2.78 mg $FeCl_2$ [20].

The mixture solution was buffered with 0.05 M 2-(*N*-morpholino)ethanesulfonic acid monohydrate (MES; J.T. Baker, Phillipsburg, NJ) and pH adjusted to 5.5. The solution was then sparged with nitrogen for 5 min to remove oxygen. The reactors were constantly stirred at 300 rpm and operated at 30 °C. The soil collected from a blueberry farm was used as the inocula. Due to the effectiveness of heat shock treatment in inhibiting methanogens and selecting for hydrogen producing bacteria, the soil was heated shocked at 100 °C for 2 h before being added to the batch reactors [20]. The soil concentration in the batch reactors was kept at 10 g/L for all tests. All batch tests were conducted at least in duplicate.

Three HPB tests were conducted with pure-glycerol and glucose. In the first HPB tests, the pure-glycerol was examined at a concentration of 3 g/L. In the second HPB tests, the pure-glycerol (3 g/L) was co-metabolized with glucose (1 g/L). In the third HPB tests, the glucose (1 g/L) was examined in a control reactor and the hydrogen produced in this control reactor would be subtracted from the co-metabolized tests. The duration of all these batch mode tests was 96 h.

2.2. Microbial fuel cell (MFC) setup

The single chamber MFC (SCMFC) used in this study was constructed from a glass bottle with an operating volume of 100 mL (Fig. 2) (Wheaton Sci., CT). A graphite fiber brush (Panex 35, Gordon Brushes, CA) was used as anode due to its large surface area suitable for biofilm formation. The brush was placed inside the bottle. The length and diameter of the brush were 5 and 3.5 cm, respectively. A carbon cloth containing 0.35 mg/cm^2 Pt (E-Tek B1B, NJ) on its inner surface (facing to the media solution) was used as cathode and placed on the extension arm of the bottle with ambient oxygen as the electron acceptor. Four layers of polytetrafluroethane (PTFE) were coated on the outer surface of the cathode (facing air) to reduce water evaporation [21]. The surface area of the cathode was 6 cm^2. The distance between anode and cathode was 4 cm. The external resister ($R_{ext.}$) of 1000 Ω was used to connect

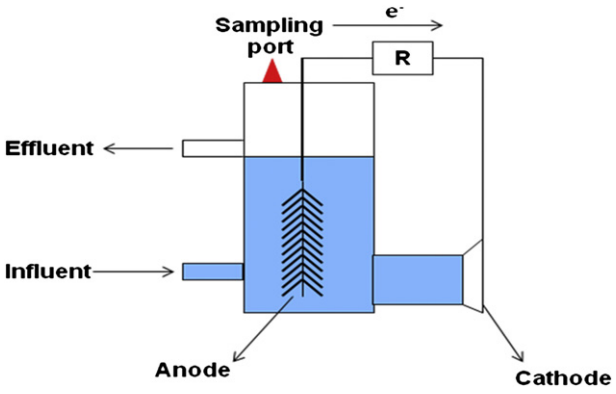

Fig. 2 – The experimental setup of continuous mode SCMFCs.

anode and cathode. The SCMFCs were operated in an incubator (Fischer Sci., PA) at a constant temperature of 30 °C.

Domestic wastewater collected from the University of Connecticut Wastewater Treatment Plant was used as the inoculum for the SCMFCs. The wastewater had a chemical oxygen demand (COD) concentration of 250–300 mg/L and a pH of 7–8. A growth media containing (per liter) NH_4Cl (0.31 g), $NaH_2PO_4 \cdot H_2O$ (4.97 g), $Na_2HPO4 \cdot H_2O$ (2.75 g), a mineral solution (12.5 mL) and a vitamin solution (12.5 mL) was added to the wastewater to provide sufficient nutrients for the growth of electrogenic bacteria in wastewater [22].

2.3. Glycerol samples from biodiesel waste stream

Glycerol from biodiesel waste stream collected from the Biodiesel Production Research Laboratory at the University of Connecticut consisted of 52% methanol, 35% glycerol, and 6.4% potassium hydroxide with 6.6% unknown components (by weight) [23]. Two purification stages were performed to clean the glycerol for HPBs and MFCs. In the first purification stage (termed B1), phosphoric acid was added in a stoichiometric amount and the glycerol sample was centrifuged for 10 min at the speed of 8000 rpm to precipitate potassium phosphate. The purified products from B1 stage (termed B1-glycerol) consisted of 56% methanol, 37% glycerol and 7% unknown components. In the second purification stage (terms B2), the B1-glycerol was further evaporated in a fume hood for 48 h to remove methanol. The purified products from B2 stage (termed B2-glycerol) consisted of 80% glycerol, 8% methanol and 12% unknown components.

2.4. SCMFC operation

The SCMFC was acclimated in the batch mode with acetate as the organic substrate. A cycle of MFC operation normally lasted for 3–4 days and was completed when the voltage became lower than 20 mV, indicating that the substrate was consumed. After two cycles, the anode was colonized with electrogenic bacteria and the voltage production stabilized at 400 mV. The SCMFC was then operated in the continuous flow mode with influent fed into the SCMFC using a peristaltic pump (Carter Pump, NJ). The pure-glycerol was examined at concentrations of 0.5, 1, 2, 4, and 5 g/L at an optimal hydraulic retention time (HRT) of 13.1 h previously obtained [24]. The glycerol from biodiesel waste stream at two purification stages (B1-glycerol, B2-glycerol) was individually fed to MFC at a concentration of 2 g/L (HRT: 13.1 h). The tests were repeated at least 7 times for each concentration.

2.5. Polarization curve measurement

The power densities (P) of SCMFCs were determined using polarization curve measurement, in which a series of external resistors (R_{ext}) ranging from 15 to 1000 Ω were used and the corresponding voltages over the R_{ext} were recorded using a multimeter (RadioShack, TX) [24]. The power densities and current densities were calculated using the equations ($P = V^2/(k \times R)$, and $I = V/(k \times R)$), where V is the voltage across the external resistors, R is resistance of each external resistor, and k is the operating volume of the SCMFC.

2.6. Coulombic efficiency (CE)

Coulombic efficiency (CE, %) is a parameter to determine the fraction of total electric energy in wastewater obtained from an MFC. The CE was calculated as the ratio of coulombs generated to theoretical coulombs supplied (Eq. (1)):

$$CE(\%) = \left(\frac{M \times I}{F \times b \times q \times \Delta COD}\right) \times 100 \quad (1)$$

Where M is the molecular weight of oxygen, $F = 96{,}500$ is Faraday's constant, $b = 4$ is number of electrons exchanged per mole of oxygen and q is volumetric influent flow rate (mL/min).

2.7. Chemical analysis

The glycerol concentration was measured with a commercially available enzyme based kit (R-Biopharm AG, Germany). The glucose concentration was measured using phenol–sulfuric acid assay with a spectrophotometer (Cary 50, Varian, CA) [25]. The liquid products including 1,3-propanediol, acetic, butyric acid, methanol, ethanol, propanol, butanol and acetone were measured with a gas chromatograph (GC) (Agilent 6950, CA) equipped with a fused-silica capillary column (DB–FFAP, Agilent, CA) and flame ionization detector.

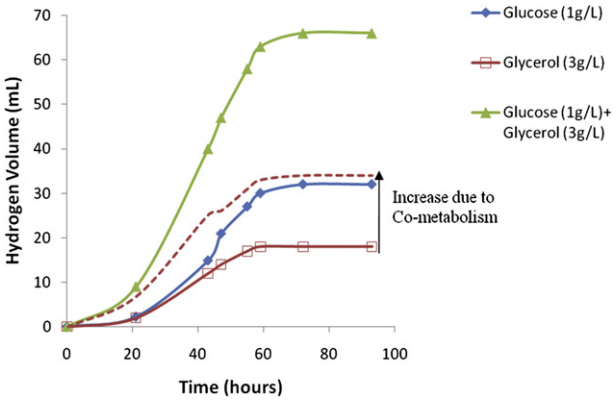

Fig. 3 – The cumulative hydrogen production from pure-glycerol (3 g/L) and pure-glycerol-glucose co-metabolism (3 g/L glycerol with 1 g/L glucose) in HPBs.

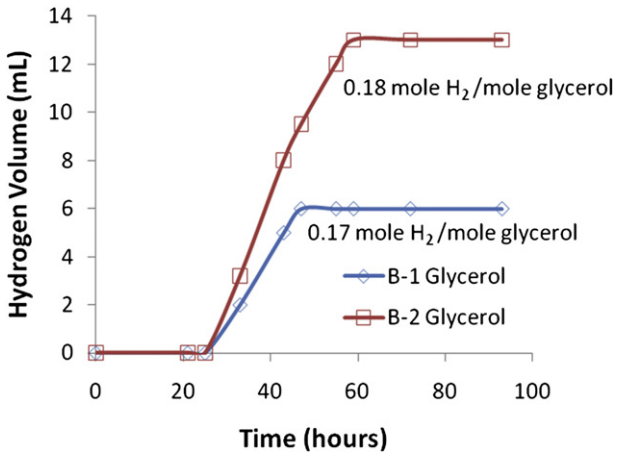

Fig. 4 – The cumulative hydrogen production from purified glycerol (B1 and B2) from biodiesel waste stream in HPBs.

All liquid samples were passed through a membrane filter with a pore size of 0.2 μm prior to analysis. The biogas generated in the HPBs was sampled with a 50 μL gas tight syringe (SGA, IL). The biogas constituents (H_2 and CO_2) were measured with a GC (Agilent 6950, CA) equipped with a packed column (60/80 Carboxen-1000, Supelco, PA) and thermal conductivity detector.

2.8. *Scanning electron microscopy observation (SEM) of anode surface*

After 7 cycles of SCMFC operation, the graphite fiber brushes were taken from the SCMFCs and were fixed at 4 °C for 12 h in a solution containing 2.5% paraformaldehyde, 1.5% glutaraldehyde and 0.1 M cocadylate buffer solution. The brushes were washed three times with cocadylate buffer solution, and then dehydrated in a series of ethanol/water solution (the volume ratios of ethanol to water: 25%, 50%, 70%, 85%, 95%, and 100%) for 15 min [14]. Thereafter, the brushes were dried in an anaerobic chamber at 30 °C and sputtered with gold at 2.2 kV, 10 mA for 2 min before SEM observation (Model: Joel 6335F).

3. Results and discussion

3.1. *Enhanced hydrogen production from glycerol via co-metabolism with glucose in HPBs*

The effects of co-metabolism with glucose on hydrogen production were examined. In the process of co-metabolism, the hydrogenase released through metabolism of glucose was expected to facilitate hydrogen production from glycerol [26]. Two batch mode HPBs were seeded with pure-glycerol (3 g/L) and mixed glycerol/glucose (3 g/L glycerol and 1 g/L glucose), respectively. The glycerol concentration selected (3 g/L) was consistent with high organic strength media required for hydrogen production [27]. The glucose concentration (1 g/L) was chosen since it had high hydrogen yields and high levels of hydrogenase [28].

For the reactors with pure-glycerol (3 g/L), hydrogen production was observed after a lag period of 21 h (Fig. 3). The exponential phase for hydrogen production lasted 35 h and a total of 18 mL hydrogen gas were produced. The hydrogen production eventually ceased at the 59th hour after inoculation, with the yield of 0.20 mol H_2/mole glycerol. Previous study using glycerol showed that the hydrogen yields varied from 0.05 to 0.41 mol H_2/mole glycerol [12,13]. It has been also reported that hydrogen yields varied with different inoculum sources (e.g. pure cultures, sludge, and soil from different agricultural fields) [9,12]. The highest hydrogen yield of 0.41 mol was obtained using anaerobic digested sludge as inoculum [13]. Nonetheless, the hydrogen yields obtained in this study were comparable to maximum hydrogen yields reported using mixed inocula [12,13].

For the reactors with pure-glycerol (3 g/L) co-metabolized with glucose (1 g/L), the hydrogen yields from glycerol was 0.37 mol H_2/mole glycerol after subtracting the hydrogen yields from glucose in the "control" bioreactor (Fig. 3), which was much higher than that of pure-glycerol (0.20 mol H_2/mole glycerol). Because glucose is the easily biodegradable organic substrate for hydrogen production, the co-metabolism of glycerol with glucose may enhance the metabolic activity of hydrogenase and augment their capability to utilize glycerol for hydrogen production.

For reactors with the glycerol from biodiesel waste stream (3 g/L), the lag time for hydrogen production was 33 h (Fig. 4), which was much longer than that of pure-glycerol (21 h). The hydrogen producing bacteria may take longer time to acclimatize due to the presence of impurities (e.g. methanol) in biodiesel waste stream. Two steps of purification (B1 and B2) were conducted to remove impurities in glycerol from biodiesel waste stream. The batch mode HPB tests showed that the total hydrogen production in B1-glycerol and B2-glycerol was 6 and 13 mL, respectively. Hydrogen yields for these two purified glycerol samples were similar, with 0.17–0.18 mol H_2/mole glycerol and comparable to that of pure-glycerol (0.20 mol H_2/mole glycerol). This indicated that once the hydrogen producing bacteria acclimatized to the operational conditions, the impurities in glycerol had no apparent effect on hydrogen production.

Table 1 – The components of liquid fermentation products in batch mode HPBs.

Substrate (g/L)		Ethanol (g/L)	Acetate (g/L)	Butyrate (g/L)	1,3-Propanediol (g/L) (moles of 1,3-PD/mole glycerol)
Glycerol	Glucose				
3	0	0.01 ± 0.00	0.48 ± 0.04	0.01 ± 0.001	1.14 ± 0.05 (0.46 ± 0.02)
3	1	0	0.65 ± 0.01	0.24 ± 0.02	1.62 ± 0.06 (0.65 ± 0.02)
0	1	0.034 ± 0.00	0.39 ± 0.03	0.25 ± 0.02	0

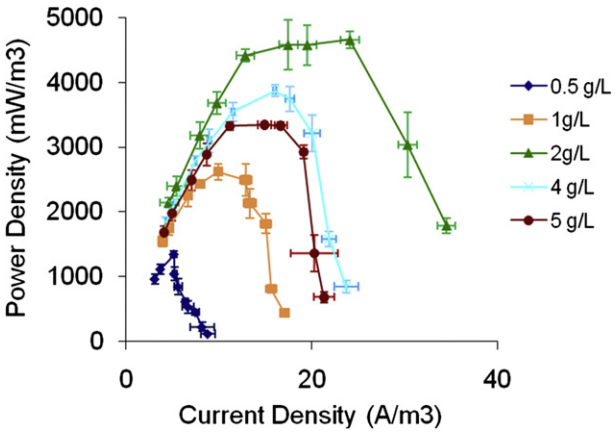

Fig. 5 – The variation of power density at different pure-glycerol concentrations in SCMFCs.

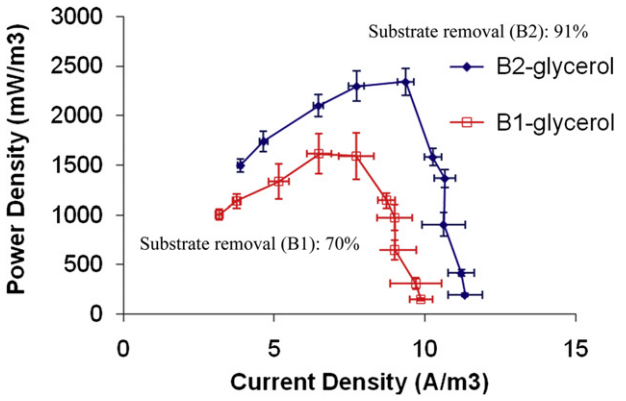

Fig. 6 – The power densities and current densities of SCMFCs utilizing the purified glycerol (B1 and B2) from biodiesel waste stream.

The analysis of the liquid fermentation products showed that the majority of glycerol was fermented to 1,3-propanediol and acetate with smaller quantities of ethanol and butyrate (Table 1). The 1,3-propanediol is produced from glycerol via a reductive pathway (Eqs. (2) and (3)) [29].

$$C_3H_8O_3 \rightarrow C_3H_6O_2 + H_2O \quad (2)$$

$$C_3H_6O_2 + 2NADH \rightarrow C_3H_8O_2 + 2NAD^+ \quad (3)$$

A yield of 0.46 mol 1,3-propendiol/mole glycerol was obtained with glycerol as the sole substrate, and increased to 0.65 mol 1,3-propendiol/mole glycerol with glycerol being co-metabolized with glucose. The 1,3-propanediol is produced via reductive pathway rather than oxidative pathway for hydrogen production [29,30]. Therefore the high level of hydrogenase associated with oxidative pathway in co-metabolism could not explain this increase in 1,3-propanediol yields. However, co-metabolism might have resulted in a higher level of glycerol dehydrase responsible for the production of 1,3-propanediol. Due to the significance of 1,3-propanediol as a monomer in chemical industries (i.e. plastic, textile), the results reveal a great potential for producing valuable liquid fermentation products through anaerobic hydrogen production from glycerol.

3.2. *The effect of glycerol concentrations and purities on power density in MFCs*

Besides the production of hydrogen and 1,3-propanediol in HPB, glycerol was examined in continuous flow SCMFCs to determine its potential for electricity generation. The pure-glycerol was fed at five concentrations (0.5, 1, 2, 4, and 5 g/L). The SCMFCs were operated at the hydraulic retention time (HRT) of 13.1 h obtained in our previous study [24], which allowed sufficient contact time between anodic biofilms and organic substrates and resulted in high substrate degradation and electricity generation. The power densities increased from 1344 mW/m^3 at the glycerol concentration of 0.5 g/L to the highest level of 4579 mW/m^3 at the concentration of 2 g/L (Fig. 5). The increase in power density with glycerol concentrations was caused by the higher availability of biodegradable glycerol for electron generation in anode. However, further increase in glycerol concentrations (4–5 g/L) led to lower power densities, which decreased to 3647 mW/m^3 at 4 g/L and eventually dropped to 3345 mW/m^3 at 5 g/L. The possible reasons for the lower power generation at high glycerol concentrations were the inhibition of electrogenic bacteria activities and the accumulation of aqueous products in the anode. The liquid fermentation products (primarily ethanol and acetate) increased steadily with glycerol concentrations and reached at 0.70 g/L and 0.32 g/L, respectively, at the highest glycerol concentration of 5 g/L (Table 2). These aqueous products were significantly higher than those (0.25 g/L) obtained at glycerol concentration of 2 g/L. It had been found that high liquid product concentrations reduced the anaerobic bacterial activities [15]. The power densities obtained from glycerol were comparable to those obtained from anaerobically treated effluent consisting of ethanol, acetate and butyrate in SCMFCs [24]. Therefore, this study demonstrated the suitability of glycerol as a substrate for MFCs.

The liquid products in the MFCs were mainly ethanol and acetate (Table 2, 3), compared to acetate, butyrate and 1,3-propanediol in HPBs (Table 1). The 1,3-propanediol in

Table 2 – The comparison of liquid fermentation products at different pure-glycerol concentrations in continuous mode SCMFCs.

Glycerol (g/L)	Ethanol (mg/L)	Acetate (g/L)	Butyrate (g/L)
0.5	0	0.01 ± 0.00	0
1	0.06 ± 0.00	0	0
2	0.11 ± 0.01	0.14 ± 0.01	0.01 ± 0.00
4	0.62 ± 0.04	0.29 ± 0.00	0
5	0.71 ± 0.06	0.32 ± 0.03	0

Table 3 – The comparison of liquid fermentation products in continuous flow SCMFCs fed with the purified glycerol (B1 and B2) from biodiesel waste stream.

Sample	Methanol (mg/L)	Ethanol (mg/L)	Acetate (mg/L)
B1-glycerol	0.43 ± 0.04	0.04 ± 0.00	0.02 ± 0.00
B2-glycerol	0.02 ± 0.00	0.05 ± 0.00	0.06 ± 0.01

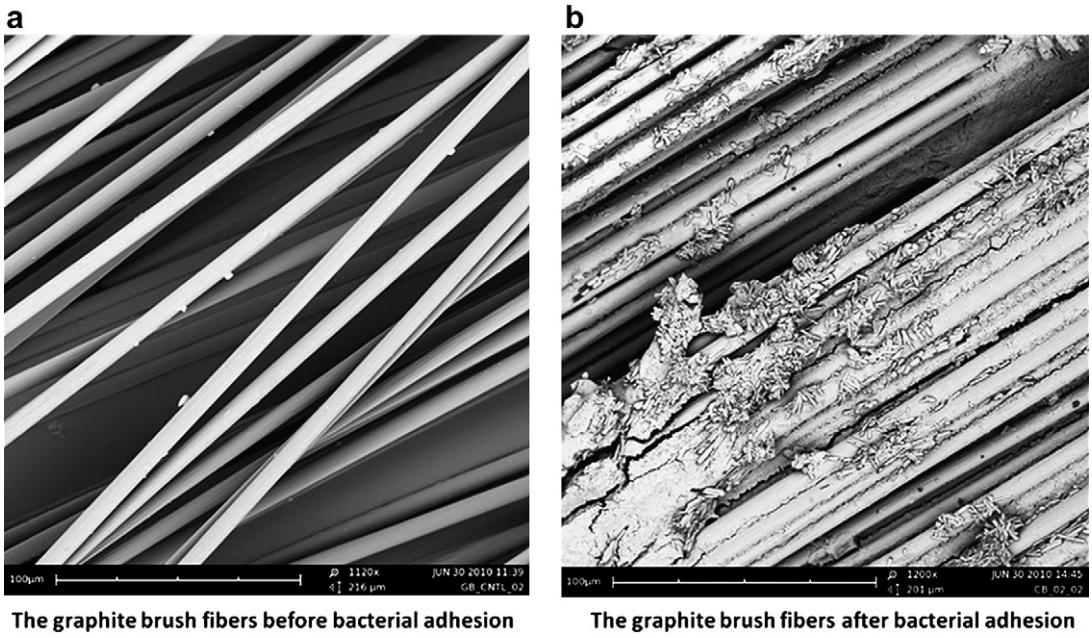

Fig. 7 – The SEM pictures of graphite fiber brush (as anode in SCMFCs) before bacterial adhesion (a) and after bacterial adhesion (b).

HPBs was produced through reductive pathways carried out by acidogenic and hydrogenic bacteria (Eqs. (2) and (3)). In contrast, electricity generation in MFCs was conducted via substrate oxidation and electron transfer to respiratory enzyme by NADH [31]. The lack of 1,3-propanediol production in MFC's may be attributed to the absence of a reductive pathway.

The electricity generation of glycerol from biodiesel waste stream was compared with pure-glycerol. Two stages of purification were conducted to clean the waste glycerol (B1-glycerol and B2-glycerol) suitable for MFCs. The power densities of 2340 and 1614 mW/m^3 were obtained for samples B2-glycerol and B1-glycerol, respectively (Fig. 6), which were lower than that (4579 mW/m^3) of pure-glycerol. These lower power densities could be attributed to the impurities present in vegetable oil used for biodiesel production. These impurities might be carried over in the glycerol products [32] and inhibited the metabolic activities of electrogenic bacteria in anode. Additionally, B2-glycerol had higher power density than B1-glycerol, which was the result of the lower methanol (8%) in B2 sample than in B1 sample (56%). It has been reported that methanol inhibited the electricity generation in MFCs through deactivation of catalysts coating on cathode [33,34]. The results showed the purification could substantially increase the power generation from glycerol in MFCs.

Because the electrogenic bacteria growing on anode surfaces act as a biocatalyst for substrate degradation, electron generation and transfer in anode [35,36], the morphology of anode surface (graphite brush) was observed using a scanning electron microscope (SEM) before and after biofilm formation (Fig. 7). A widespread biofilm growth was found on the anode with an interconnected tubular shaped cell structure. In the mediatorless MFCs, bacterial adhesion on anode surfaces is crucial since electron transfer from bacterial cells to anode surface depends on biofilms growing on anode surfaces. The inefficient bacterial adhesion to anode has been reported to be a major limiting factor in power generation [37]. In this study, the anodic biofilms were responsible for high power generation from glycerol.

3.3. *The effect of glycerol concentrations on glycerol removal in MFCs*

Both glycerol removal efficiency and CE values decreased with an increase in pure-glycerol concentration. The glycerol removal efficiency decreased linearly from 99% to 66% when glycerol concentrations increased from 0.5 g/L to 5 g/L (Fig. 8) (Eq. (4)).

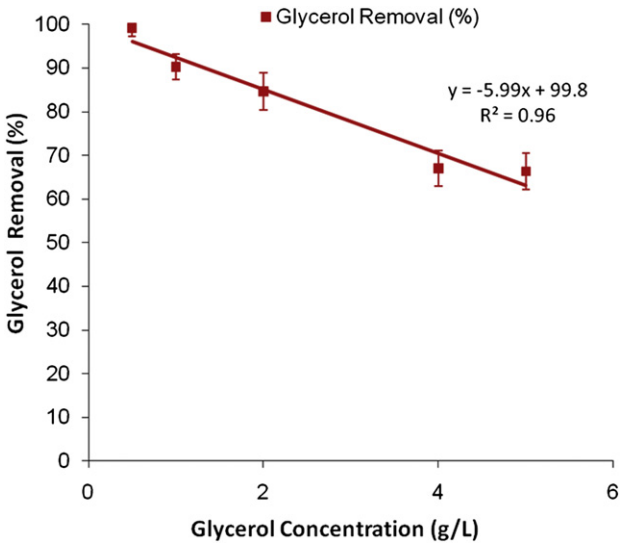

Fig. 8 – The glycerol removal efficiency (%) at different glycerol concentrations in SCMFCs.

Table 4 – The economic value of bioenergy products from glycerol treatment in HPBs and MFCs.

Products obtained	Production/Kg glycerol	Market value/Kg glycerol	Value based on total glycerol production of 70 million gallons
1,3-Propanediol	1.80 ± 0.06 kg	$1.68	$303 ± 10.8 million
Hydrogen	0.01 ± 0.00 kg	$3.00	$8.16 ± 0.07 million
			Total value in HPBs: $311.16 ± 10.8 million
Electricity	2.25 ± 0.20 kW h	$0.10	Total value in MFCs: $98.4 ± 0.85 million

$$\text{Glycerol Removal Efficiency} = -5.99 \times \text{Glycerol Concentration} + 99.8 \quad (4)$$

Low glycerol removal efficiency at high glycerol concentrations was caused by the presence of more glycerol not degraded at a given HRT of 13.1 h. Employing a longer HRT was expected to prolong the contact time between the anodic biofilms and organic substrates, and could improve glycerol removal efficiency. However, longer HRTs had been found to decrease power density [24], due to a lower availability of readily biodegradable substrates. Therefore, a tradeoff between power generation and glycerol removal should be considered for optimizing HRTs at MFCs.

The Coulombic efficiency (CE) values (0.5–2%) in this continuous flow MFC system with glycerol as the substrate were comparable to those (1.7%) obtained in continuous flow MFCs with acetate as the substrate [38]. The CE value of 2% was found at a glycerol concentration of 0.5 g/L, and dropped to 0.5% at highest glycerol concentration of 5 g/L. The higher substrate concentration had been known to increase substrate oxidation reaction rate rather than electron generation reaction rate in MFCs, which decreased the CE values [17]. The CE values of continuous flow MFCs were normally lower than those in batch mode [24,34,37,38]. For MFCs with glycerol as the substrate, glycerol was first degraded to acetate, butyrate and ethanol, and then utilized by electrogenic bacteria (Table 2). These degradation processes divert the electrons from electricity production and result in low CE values. In order to improve CE values of glycerol in continuous flow MFCs, novel MFC configurations should be developed.

3.4. *Evaluation of the economic value of bioenergy products from glycerol*

Two anaerobic treatment processes (HPBs and MFCs) could efficiently convert glycerol to clean energy (hydrogen and electricity) and valuable liquid product (e.g. 1,3-propanediol). With 70×10^6 gallons of waste glycerol being generated from biodiesel production processes annually, there is a promising future to harvest energy stored in glycerol. Based on the lab-scale results in this study (hydrogen yield in HPBs: 0.37 mol/mol glycerol, 1,3-propanediol yield in HPBs: 0.65 mol/mol glycerol, power density in MFCs: 2.25 W/Kg glycerol), the economic values of bioenergy products and liquid products were evaluated by normalizing the amount of glycerol treated and incorporating the market values of products. The evaluation indicated that the use of HPB to treat purified glycerol would produce hydrogen with a value of $8.16 million and 1,3-propandiol of $303 million, so that the total value generation will be $311 million (Table 4). The use of MFCs to treat glycerol would result in electric power with a value of $98.4 million. Although 1,3-propanediol is produced from glycerol through reductive pathway in HPB processes, no such pathway is present for electrogenic bacteria in MFC process. Therefore, 1,3-propanediol was not observed in the liquid effluent from MFCs. It should be noted that even though HPB is a better process than MFCs in terms of total value generation from glycerol, MFCs exceeds in the bioenergy generation.

The results of this lab-scale study revealed the feasibility of bioenergy (hydrogen and electricity) production in the anaerobic treatment of glycerol from biodiesel waste stream. HPBs and MFCs are still in early stages and much effort has been invested to develop new configurations and optimize operational conditions for high bioenergy production [14,21,37]. In addition, purification processes (e.g. vacuum distillation, Ion Exclusion [39–41]) should be employed to produce pure-grade glycerol for efficient energy production in HPBs and MFCs.

4. Conclusion

Effectively harvesting glycerol from biodiesel waste stream and converting it to bioenergy has a great potential to reduce the treatment cost of biodiesel wastes and generate clean energy. The conversion of glycerol to hydrogen and electricity was extensively investigated in the lab-scale HPBs and MFCs. The enhancement of bioenergy production by the co-metabolism with glucose was examined, the effects of two purification stages of glycerol on bioenergy production were compared, and the economic values of bioenergy and liquid fermentation products were estimated. Three major conclusions were drawn.

First, the co-metabolism of pure-glycerol with glucose increased the hydrogen yields to 0.37 mol/mol glycerol, and increased the 1,3-propanediol yields to 0.65 mol/mol glycerol. The hydrogen yields of glycerol from biodiesel waste stream were 0.17–0.18 mol/mol glycerol.

Second, the power density in MFCs increased from 1344 mW/m^3 to 4579 mW/m^3 at the pure-glycerol concentrations of 0.5–2 g/L, but decreased to 3345 mW/m^3 when the concentration further increased to 5 g/L. The power densities of glycerol from biodiesel waste streams (1614 and 2314 mW/m^3) were lower than those of pure-glycerol, due to the adverse effects of impurities (e.g. methanol) on electrode materials.

Third, the total economic values of converting glycerol to bioenergy and 1,3-propanediol in HPB and MFCs were $311 million and $98 million, respectively. The majority of economic value (90%) of the HPB was from the production of 1,3-propanediol through anaerobic fermentation, while the MFC is a better alternative for power generation.

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REFERENCES

[1] National Biodiesel Board, www.biodiesel.org.
[2] Zhang Y, Dube MA, McLean DD, Kates M. Biodiesel production from waste cooking oil: 1. Process design and technological assessment. Bioresour Technol 2003;89:1–16.
[3] Sabourin-Provost G, Hallenbeck PC. High yield conversion of a crude glycerol fraction from biodiesel production to hydrogen by photofermentation. Bioresour Technol 2009;100:3513–7.
[4] Johnson DT, Taconi KA. The glycerin glut: options for the value added conversion of crude glycerol resulting from biodiesel production. Env Prog 2007;26:338–48.
[5] Yazdani SS, Gonzalez R. Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. Curr Opin Biotechnol 2007;18:213–9.
[6] Dow Chemical Company, www.dow.com.
[7] Biebl H, Zeng AP, Menzel K, Deckwer WD. Fermentation of glycerol to 1,3-propanediol and 2,3-butanediol by *Klebsiella pneumoniae*. Appl Microbiol Biotechnol 1998;50:24–9.
[8] Kraus GA. Synthetic methods for the preparation of 1,3-propanediol. Clean 2008;36:648–51.
[9] Gonzalez-Pajuelo M, Andrade JC, Vasconcelos I. Production of 1,3-propanediol by *Clostridium butyricum* VPI 3266 using a synthetic medium and raw glycerol. J Ind Microbiol Biotechnol 2004;31:442–6.
[10] Ito T, Nakashimada Y, Senba K, Matsui T, Nishio N. Hydrogen and ethanol production from glycerol – containing wastes discharched after biodiesel manufacturing process. J Biosci Bioeng 2005;100:260–5.
[11] Murarka A, Dharmadi Y, Yazdani SS, Gonzalez R. Fermentative utilization of glycerol by *Escherichia coli* and its implications for the production of fuels and chemicals. Appl Environ Microbiol 2008;74:1124–35.
[12] Selembo PA, Perez JM, Lloyd WA, Logan BE. Enhanced hydrogen and 1,3-propanediol production from glycerol by fermentation using mixed cultures. Biotechnol Bioeng 2009; 104:1098–106.
[13] Seifert K, Waligorska M, Wojtowski M, Laniecki M. Hydrogen generation from glycerol in batch fermentation process. Int J Hydrogen Energy 2009;34:3671–8.
[14] Liu H, Logan BE. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environ Sci Technol 2004;38:4040–6.
[15] Tartakovsky B, Manuel MF, Neburchilov V, Wang H, Guiot SR. Biocatalyzed hydrogen production in a continuous flow microbial fuel cell with a gas phase cathode. J Power Sources 2008;182:291–7.
[16] Chen CY, Yang P. Performance of an air-breathing direct methanol fuel cell. J Power Sources 2003;123:37–42.
[17] Sharma Y, Li B. The variation of power generation with organic substrates in single-chamber microbial fuel cells (SCMFC). Bioresour Technol 2010;101:1844–50.
[18] Niessen J, Schroder U, Scholz F. Exploiting complex carbohydrates for microbial electricity generation–a bacterial fuel cell operating on starch. Electrochem Commun 2004;6:955–8.
[19] Fedorovich V, Varfolomeev SD, Sizov A, Goryanin I. Multi-electrode microbial fuel cell with horizontal liquid flow. Water Sci Technol 2009;60:347–55.
[20] Van Ginkel S, Sung S, Lay JJ. Biohydrogen production as a function of pH and substrate concentration. Environ Sci Technol 2001;35:4726–30.
[21] Cheng S, Liu H, Logan BE. Increased power and Coulombic efficiency of single-chamber microbial fuel cells through an improved cathode structure. Electrochem Comm 2006;8: 489–94.
[22] Lovley DR, Phillips EJP. Novel mode of microbial energy metabolism: organism carbon oxidation coupled to dissimilatory reduction of iron and manganese. Appl Environ Microbiol 1988;54:1472–80.
[23] Unker SU, Boucher MB, Hawley KR, Stuart JD, Parnas RS. Investigation into the relationship between the gravity vector and the flow vector to improve performance in two-phase continuous flow biodiesel reactor. Bioresour Technol 2010;101:7389–96.
[24] Sharma Y, Li B. Optimizing energy harvest in wastewater treatment by combining anaerobic hydrogen producing biofermentor (HPB) and microbial fuel cell (MFC). Int J Hydrogen Energy 2010;35:3789–97.
[25] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem 1956;28:351–6.
[26] Vasconcelos I, Girbal L, Soucaille P. Regulation of carbon and electron flow in *Clostridium acetobutylicum* grown in chemostat culture at neutral pH on mixtures of glucose and glycerol. J Bacteriol 1994;176:1443–50.
[27] Logan BE, Oh S, Van Ginkel S, Kim IS. Biological hydrogen production measured in batch anaerobic respirometers. Environ Sci Technol 2002;36:2530–5.
[28] Sharma Y, Li B. Optimizing hydrogen production from organic wastewater treatment in batch reactors through experimental and kinetic analysis. Int J Hydrogen Energy 2009;34:6171–80.
[29] Willke T, Vorlop K. Biotransformation of glycerol into 1,3-propanediol. Eur J Lipid Sci 2008;110:831–40.
[30] Slininger PJ, Bothast RJ, Smiley KL. Production of 3-hydroxypropionaldehyde from glycerol. Appl Environ Microbiol 1983;46:62–7.
[31] Logan BE, Regan JM. Feature article: microbial fuel cells – challenges and applications. Environ Sci Technol 2006;40: 5172–80.
[32] Thompson JC, He BB. Characterization of crude glycerol from biodiesel production from multiple feedstocks. Appl Eng Agric 2006;22:261–5.
[33] Heinzel A, Barragan VM. A review of the state-of-the-art of the methanol crossover in direct methanol fuel cells. J Power Sources 1999;84:70–4.
[34] Schröder U, Niessen J, Scholz F. A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude. Angew Chem 2003;42:2880–3.
[35] Bond DR, Lovely DR. Electricity production by *Geobacter sulfurreducens* attached to electrodes. Appl Environ Microbiol 2003;69:1548–55.
[36] Aelterman P, Freguia S, Keller J, Verstraete W, Rabaey K. The anode potential regulates bacterial activity in microbial fuel cells. Appl Microbiol Biotechnol 2008;78:409–18.
[37] Logan BE, Cheng S, Watson V, Estadt G. Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. Environ Sci Technol 2007;41: 3341–6.

[38] Fan Y, Hu H, Liu H. Enhanced coulombic efficiency and power density of air-cathode microbial fuel cells with an improved cell configuration. J Power Sources 2007;171:348–54.
[39] Pachauri N, He B. Value-added utilization of crude glycerol from biodiesel production: a survey of current research activities. Presented at the ASABE Annual International Meeting. Portland, OR; July 9-12-2006.
[40] Asher D, Simpson D. Glycerol purification by ion exclusion. J Phys Chem 1956;60:518–21.
[41] Yong KC, Ooi TL, Dzulkefly K, Wan Yunus WMZ, Hazimah AH. Refining of crude glycerine recovered from glycerol residue by simple vacuum distillation. J Oil Palm Res 2001;13:39–44.