

Evaluation of fermentation effluent as substrate for single chamber MEC**T. R. Manikkandan*, Sonia K. Samson**Department of Chemical Engineering, Annamalai University, Annamalai Nagar,
Chidambaram, Tamilnadu-608 002, India***Corresponding author: E-Mail: trm_tech@yahoo.co.in****ABSTRACT**

Microbial consortium from ethanol plant was used as an inoculum for biofilm formation on electrode. A stable open circuit voltage of 0.32V was obtained after 5 days of operation, which then transferred to MEC as bioanode. By varying the applied voltage from 0.4V to 0.8V, 0.6V was found as the optimum for hydrogen production by single chamber MEC using fermentation effluent as feed. At the optimum substrate concentration of 15 g COD/L, maximum hydrogen yield of 121.59 mL H₂/g COD was obtained with an overall hydrogen recovery efficiency of 6.6%.

KEY WORDS: Biohydrogen, Effluent, MEC, HPLC.**1. INTRODUCTION**

The quest for an energy alternative has triggered research in the areas of solar energy, tidal energy, wind energy, biofuels, etc. With the wide options for alternative energies available, hydrogen shows a promising demand for future energy needs, which is endorsed by the inclination of the world energy market towards hydrogen (Sinha, 2011). Though elemental or monoatomic hydrogen is the most abundant element in the universe, molecular hydrogen is hard to find. Various methods are adopted to produce molecular hydrogen including thermochemical processes, electrolytic processes, direct solar water splitting processes and biological processes. However, these processes are expensive, energy intensive, require high operating temperatures and are detrimental to the environment (Rollin, 2015; Nath, 2004).

For the hydrogen-based economy to become a reality, hydrogen production must be cost effective, and this can be achieved only by using waste materials or biomass as a source of energy. Biological hydrogen production is the only option for sustainable hydrogen production because of its low energy requirements and environmental friendliness but has yet to reach a scale large enough to replace a significant portion of hydrogen supply (Brentner, 2010). Microbial electrolysis cell (MEC) or microbial electrohydrogenesis systems are modified form of microbial fuel cell (MFC), which convert both fermentable and non-fermentable organic substrates to hydrogen (Cheng, 2011). Electrochemically active microbes oxidize the organic substrate at the anode and hydrogen is liberated at the cathode. This research focuses on the possibility of using fermentation effluent as feed for hydrogen production in a single chamber MEC.

2. MATERIALS AND METHODS

Inoculum: Effluent from ethanol distillation plant before treatment, was collected from EID Parry, Cuddalore and was used as an inoculum for the microbial electrolysis cell.

Fermentation effluent: Effluent from the batch fermentation of *B. licheniformis* utilizing maize stalk hydrolysate as substrate was collected and utilized as feed for MEC.

MEC construction: The reactor was made of Plexiglas with an empty volume of 50 mL. Anode and cathode were placed in the same chamber, 2cm apart. To avoid short-circuiting, a J-cloth has been put between the electrodes. A gas collection tube was attached to the top of the cathode with provisions for sample collection. The sampling port on the top of anodic chamber was also sealed using butyl rubber stoppers.

The anode was ammonium treated (treated with ammonium solution for 1 hr followed by desiccation for 20 minutes) carbon cloth (2 cm X 2 cm, without wetproofing) and cathode was 30% wet proofed carbon cloth (2 cm X 2 cm) with 0.35 mg/cm² Pt catalyst. A graphite rod current collector (2-mm diameter, Sigma-Aldrich) was used to connect the anode and cathode electrodes and the outside circuit. A reference electrode (Ag/AgCl) was used to measure the voltage of the electrodes. A constant power (0.5-1.1 V) was applied using a regulated DC power supply (Aplab, L3220) when the system is in MEC mode.

Operation: Ammonium treated anodes were inoculated using ethanol plant waste water along with 5g/L of glucose. Anaerobic condition was maintained by sparging nitrogen gas for 5 minutes. The potential of the electrodes was measured daily using an Ag/AgCl reference electrode, and once it reached a steady state, the electrodes were aseptically removed and placed in MEC as anode. In experiments where the effect of substrate concentration was tested, the substrates were diluted by adding the required amount of distilled water. Initially, the MECs were operated in microbial fuel cell (MFC) mode with anaerobic anode and aerobic cathode. Once the MFC showed a reproducible voltage for three cycles, it was changed to MEC mode by making the cathode anaerobic and supplying an external voltage to the circuit by connecting the negative pole of the power supply to the cathode through a 10Ω resistor and

the positive pole to the anode. Hydrogen recovery and efficiencies of the system were evaluated as described by Logan (Logan, 2008; Lide, 1995).

Analytical methods:

Chemical Oxygen Demand (COD): COD determination was made photometrically using cell tests (Spectroquant NOVA 60). The samples taken in COD vials along with mercury sulphite and sulphuric were digested for 2 hrs at 148°C in Spectroquant, Thermoreactor, TR320. The samples were then cooled, and the readings were taken against a blank.

Gas chromatography (GC): Gas collected in the conical flask was taken out using a syringe at regular time intervals for analysis using gas chromatograph (AIMIL-NUCON 5765, Mumbai, India), equipped with a thermal conductivity detector. Nitrogen gas at a flow rate of 30 mL/min was used as a carrier gas and the temperatures of injector, oven and column were set at 150°C, 80°C and 200°C respectively.

High-Performance Liquid Chromatography (HPLC): The simple sugars and metabolites were analysed using a High-Performance Liquid Chromatography (Shimadzu, Japan). Simple sugars were analysed using C18 column and 4µm Hydro RP column was used for the detection of organic acids.

3. RESULTS AND DISCUSSIONS

Biofilm formation: Biofilms were developed on separate carbon cloths (2 X 2 cm) without wet proofing. A constant open circuit voltage of 0.32 V was obtained after 5 days of operation. The electrodes were then transferred to the anodic chamber of MFCs.

Characterization of Fermentation Effluent: The fermentation effluent of *B.licheniformis* after 48 hrs of growth on maize stalk hydrolysate was analysed. pH of the fermentation effluent was found to be 4.89. The effluent had a total COD of 21.0 g COD/L, which constitutes of glucose, xylose, acetic acid, butyric acid, propionic acid and ethanol. Xylose and glucose were the major sugars present with concentrations of 5.25 g COD/L and 2.93 g COD/L respectively. Acetic acid was the predominant intermediate with a concentration of 2.49 g COD/L followed by ethanol (1.34 g COD/L), butyric acid (0.89 g COD/L), propionic acid (0.26 g COD/L), and formic acid (0.06 g COD/L).

Hydrogen production in single chamber MEC: Hydrogen production in single chamber MEC utilizing fermentation effluent of *B. licheniformis* was attempted with bioanode at a fixed substrate concentration of 10 g COD/L over different applied voltages (0.4V to 0.8V) and the results are illustrated in Figure.1 and 2. The maximum hydrogen yield and production rate of 110.27 mL H₂/g COD and 0.210 L H₂/L/d were achieved at applied voltages of 0.6 V and 0.8 V respectively as illustrated in Figure.1. Cathodic hydrogen recovery and overall hydrogen recovery reached the maximum of 30.6% and 7.9% respectively at applied voltage 0.6V.

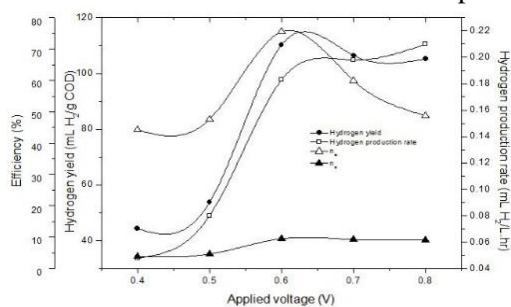


Figure.1. Hydrogen production and efficiencies in single chamber MEC

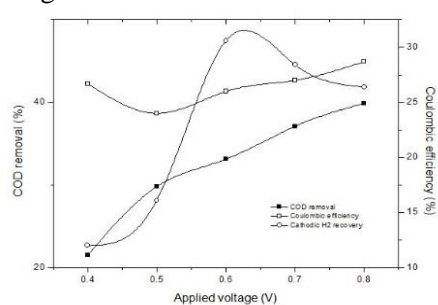


Figure.2. COD removal and hydrogen recovery in single chamber MEC

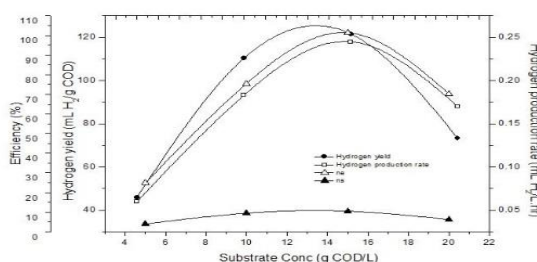
COD removal and coulombic efficiency increased from 21.5% and 26.7% at 0.4 V to 39.9% and 28.7% at 0.8 V respectively. Though the COD removal is slightly low, substrate conversion efficiency is high (9.5%). With electrical, substrate and overall efficiencies of 7.5%, 9.5%, and 8.4% respectively (at an applied potential 0.6 V), fermentation effluent can be considered as a suitable substrate for hydrogen production in single chamber MEC.

Effect of substrate concentration on single chamber MEC: To study the effect of substrate concentration on the hydrogen production potential of single chamber MEC, concentration of substrate was varied in the range of 5 to 20 g COD/L at a constant applied potential of 0.6 V (Table.1). With increased substrate concentration, hydrogen yield increased from 45.98 mL H₂/g COD at 5 g COD/L to 121.59 mL H₂/g COD at 15 g COD/L. Further increase in substrate concentration to 20 g COD/L decreased the hydrogen yield to 73.43 mL H₂/g COD (Figure.3). Maximum electrical and substrate conversion efficiencies of 101.3% and 10.5% were obtained for substrate concentration 5 g COD/L, which then decreased to 70.3% and 6.3% respectively at substrate concentration 20g COD/L.

Table.1. Effect of substrate concentration on hydrogen production and efficiencies in single chamber MEC

Substrate Conc (g COD/L)	Hydrogen Yield (mL H ₂ /g COD)	Hydrogen production rate (L H ₂ /L/d)	COD removal (%)	Coulombic efficiency, C _E (%)
20	73.43	0.170	23.15	18.6
15	121.59	0.245	26.87	21.3
10	110.27	0.183	33.1	26.0
5	45.98	0.060	52.2	32.9

Cathodic H ₂ recovery, r _{Cat} (%)	Overall H ₂ recovery, r _{H₂} (%)	Electrical efficiency, η _E (%)	Substrate conversion efficiency, η _S (%)	η _{E+S} (%)
21.4	4.0	70.3	6.3	5.8
30.8	6.6	101.3	10.5	9.5
22.9	6.0	75.5	9.5	8.4
7.5	2.5	24.8	4.0	3.4

**Figure.3. Effect of substrate concentration on hydrogen production and efficiencies in single chamber MEC**

COD removal and coulombic efficiency decreased with increase in substrate concentration. Chookaew (2014) also reported an increase in coulombic efficiency with increased concentration of fermentation effluent as substrate at an applied voltage of 0.6 V. Highest COD removal and coulombic efficiency were 52.2% and 32.9% for the substrate concentration 5 g COD/L. With increase in substrate concentration from 5 to 15 g COD/L, the cathodic hydrogen recovery increased 4.1 times. The maximum overall hydrogen recovery was 6.6% at substrate concentration 15 g COD/L, which decreased to 2.5% at substrate concentration of 5 g COD/L.

4. CONCLUSION

Composition of fermentation effluent used as feed in MEC were found to be as follows: glucose (2.93 g COD/L), xylose (5.25 g COD/L), acetic acid (2.49 g COD/L), ethanol (1.34 g COD/L), butyric acid (0.89 g COD/L), propionic acid (0.26 g COD/L), and formic acid (0.06 g COD/L). At the optimum applied voltage of 0.6V, maximum hydrogen yield and production rate of 110.27 mL H₂/g COD and 0.210 L H₂/L/d were achieved in single chamber MEC with bioanode.

REFERENCES

- Brentner L.B, Peccia J and Zimmerman J.B, Challenges in developing biohydrogen as a sustainable energy source, Implications for a research agenda, *Environ. Sci. Technol.*, 44 (7), 2010, 2243–2254.
- Cheng S and Logan B.E, High hydrogen production rate of microbial electrolysis cell (MEC) with reduced electrode spacing, *Bioresour. Technol.*, 102 (3), 2011, 3571–3574.
- Chookaew T, Prasertsan P and Ren Z.J, Two-stage conversion of crude glycerol to energy using dark fermentation linked with microbial fuel cell or microbial electrolysis cell, *N. Biotechnol.*, 31 (2), 2014, 179-184.
- Lide D.R and Haynes W.M.M, Eds., *CRC Handbook of Chemistry and Physics*, 76th ed. Boca Raton: CRC Press, Inc, 1995.
- Logan B.E, Call D, Cheng S, Hamelers H.V.M, Sleutels T.H.J.A, Jeremiasse A.W and Rozendal R.A, Microbial electrolysis cells for high yield hydrogen gas production from organic matter, *Environ. Sci. Technol.*, 42 (23), 2008, 8630-8640.
- Nath M.K and Das D, Improvement of fermentative hydrogen production, Various approaches, *Appl. Microbiol. Biotechnol.*, 65 (5), 2004, 520-529.
- Rollin J.A, Martin del Campo J, Myung S, Sun F, You C, Bakovic A, Castro R, Chandrayan S.K, Wu C.H, Adams M.W.W, Senger R.S and Zhang Y.H.P, High-yield hydrogen production from biomass by *in vitro* metabolic engineering, Mixed sugars coutilization and kinetic modeling, *Proc. Natl. Acad. Sci.*, 112 (16), 2015, 4964-4969.
- Sinha P and Pandey A, An evaluative report and challenges for fermentative biohydrogen production, *Int. J. Hydrogen Energy*, 36 (13), 2011, 7460–7478.