Cathode material as an influencing factor on beer wastewater treatment
and methane production in a novel integrated upflow microbial
electrolysis Cell (Upflow-MEC)

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ABSTRACT

As a reliable source for methane production, the major goal of our research was to design and testify a novel microbial electrolysis assisted upflow anaerobic (Upflow-MEC) reactor for beer wastewater treatment and simultaneous methane production. Three reactors with different cathode materials were constructed and the reactor with Ni cathode had a maximum COD removal of 85%, methane yield of 142.8 ml/gCOD, TOC removal of 83%, Carbohydrate removal of 97%, Protein removal of 62% and current production of 8.6 mA, under 0.8 V applied voltage at HRT of 24 h. It was an application-oriented, membrane-free, continuous reactor and shortening the reaction time and increasing organic content conversion to methane were the key developing targets.

Keywords: Beer wastewater; Up flow microbial electrolysis cell; Methane production; Nickel mesh; innovative coupled reactor

1. Introduction

Bioenergy is a renewable energy that plays an indispensable role in meeting the ever increasing energy demand. A multi-faceted approach is needed to alleviate the energy crisis. Bioenergy uses renewable resources to produce bio fuels, bioelectricity by microbial fuel cells (MFCs) [1], biohydrogen and methane by microbial electrolysis cells (MECs) [2]. MECs are electrochemical transducers that produce hydrogen by the synergising ability of electrogenic bacteria to oxidize organic matter with the hydrogen evolution reaction at the cathode, utilizing the anode as the electron acceptor [3]. Though these processes require the input of electrical energy, the specific energy consumption is lower than that typically associated with

hydrogen production through conventional water electrolysis processes [4]. It also has the additional advantage of simultaneously treating wide ranges of wastewater, offering a renewable and potentially autonomous energy technology for wastewater treatment [5]. This technology is relatively new and scale-up studies using single-chambered MECs have been performed in our previous studies [6-7]. MECs were constructed and operated to optimize various parameters like applied voltage, hydraulic retention time (HRT), sludge pre-treatment methods etc [6-9]. In this present study we have investigated the efficiency of lab scale, tubular, upflow MECs, utilizing beer wastewater as substrate for the production of methane. Tubular type MECs have been successfully used by various researchers and has proved to be compatible, reliable and highly efficient for hydrogen production [5, 10].

Though most studies have concentrated on hydrogen production from MECs, methane is also an energetic compound and is a product of anaerobic digestion. Though the energy content of methane is less than that of hydrogen, methane is also a robust and vital bio-energy source. Wang et al. [11] have sorted out vital methods to reduce the formation of methane in single chambered MEC by running the MEC for short cycles and under higher applied voltages. They have demonstrated that it is only possible to reduce methane production but elimination is very difficult. Single chambered MECs have been successfully designed and operated for methane production [12-14].

A wide range of wastewaters have been administered in MECs such as potato-processing and dairy manure wastewaters [15], domestic wastewater [16], swine wastewater [17], winery wastewater [18-19] and food-processing wastewater [20]. Beer industry wastewater is considered to be compatible for methane generation due to its food-derived nature and it is also rich in organic contents like carbohydrates,

protein and starch. It can be considered an apt source for anaerobic treatment as it has high COD and carbohydrate content [21]. China has more than 200,000 breweries and for the production of 1 m³ of beer, 10-20 m³ of water is consumed, of which 90% is discharged into sewer systems with high amounts of organic pollutants. Beer industry wastewater has been subjected to a number of treatment methods like anaerobic sequencing batch reactor (ASBR) [21], expanded granule sludge blanket reactor (EGSBR) [22], microbial fuel cell (MFC) [23-25], nanofiltration [26], upflow sludge blanket reactors (UASB) [27-28]. Despite the great potential of MECs in employing different types of wastewater, beer wastewater has not yet been used as a substrate in MECs to the best of our knowledge. So our work proves to be novel in the choice of wastewater for treatment in MEC.

MECs have been limited to laboratories and they need to be scaled up for real world applications. One such challenge is the selection of cathode electrode material for better reduction. The effects of cathode materials on recovery products in BESs have been understood on their specific reductive potentials. Platinum catalysts have always been the choice of researchers, due to its very low over potential. Pt catalyzed electrodes (Pt coated graphite and carbon brushes, cloths, rods, plates, titanium electrodes etc) are commercially available. Pt coated titanium mesh electrodes were used by Rozendal et al.[29], Pt loaded carbon felt and gas-diffusion electrodes were employed by Tartakovsky et al.[30], whereas Call and Logan [31] and Selembo et al.[32], used carbon cloth with Pt catalyst. Due to the various disadvantages of Pt like its high cost, environmental impacts due to mining and poisoning by chemicals like sulphide, finding out an alternative for it became a major task. However, a cheap but efficient cathode could serve as electron donor to decrease oxidative-reductive potential (ORP), thus creating a more favourable environment for anaerobic

microorganisms. In the research to find out alternatives for expensive Pt catalyst, first row transition metals were useful due to their stability, availability, low cost, and low toxicity to living organisms. The most promising materials identified so far are nickel and stainless steel especially for their low overpotentials and stability [13, 33-35]. Our research study highlights the wastewater treatment and methane producing abilities of MEC reactors with insights into different cathode electrode materials like nickel, stainless steel and copper. It is worthy to note that COD removal related to methane production will be affected by various cathode materials.

2. Materials and Methods

2.1 Configuration and Operation of Upflow MEC

Three tubular, lab-scale, acrylic plastic, upflow-MEC reactors (S1, S2 and S3) were designed and operated. The working volume was 600 ml with a height of 35 cm and a diameter of 5 cm (7:1 ratio). Granular graphite was used as the anode in all reactors. Three types of cathode mesh materials (40 woven mesh), such as stainless steel (S1), nickel (S2) and copper (S3) were used to optimize the material which had a positive effect on methane production from the reactor and organic content removal from the effluent. The distance between the electrodes was 3 cm, and they were connected to data acquisition system over a 10 Ω resistance. The current was measured once in 10 min, and the applied external voltage was the 0.80 ± 0.01 V. The reactors were operated at 24 h hydraulic retention time (HRT), with acetate for one week until the current values got stabilized [9]. Artificial Beer Wastewater was the influent and it was prepared in the laboratory according to 1:4 ratio of fresh beer and PBS (Phosphate Buffer Solution, 50 mM), for 1 litre of influent, and in accordance with the characteristics of the real beer wastewater from Snow beer manufacturing industry in Harbin, China. The physico-chemical characters of the real-wastewater are

listed in Table 1. The PBS was added to increase the conductivity and maintain the pH of the influent. Before feeding, the influent was purged using high purity nitrogen gas (99.99%) for 20 min to remove oxygen. All the reactors were inoculated with 10 ml of municipal wastewater from older MECs in the lab that had been running for several months. This served as inoculum for anaerobic microbes. The reactors were run at room temperature of 30 °C. Reactors were equipped with an Ag/AgCl reference electrode (RE-5B; BASi) for measuring anode potentials.

2.2 Analytical Methods

Influent and effluent samples were collected after each HRT, and the determinations of Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), soluble carbohydrates and soluble proteins were performed. The values were calculated along with the standard error bars as previously described [2]. The measurement of volatile fatty acids (VFAs) were carried out by a gas chromatography (Agilent 7890, USA), at the beginning and end of each cycle according to standard methods as previously described [36]. Gas was collected in gas bags (Cali-5-Bond, Calibrated Instruments Inc.) attached to the top of the reactor and it was analyzed by gas chromatography (Agilent 7890, USA) with samples taken using a gastight syringe (250 mL, Hamilton Sample-lock Syringe), and its composition was calculated according to the methods previously prescribed [11]. Cyclic Voltammetry (CV) scans were performed on plain electrodes and biofilm covered electrodes at 0 V to -1 V and scan rate of 0.01 V/S.

2.3 Calculation

VFAs production was calculated as the sum of the equivalent COD value of measured acetic (HAc), propionic (HPr), n-butyric (n-HBu), iso-butyric (iso-HBu), n-valeric (n-HVa) and iso-valeric (n-HVa) acids. The COD conversion factors are 1.07 gCOD/g

HAc, 1.51 gCOD/g HPr, 1.82 gCOD/g HBU, and 2.04 gCOD/g HVA[37]. The Coulombic efficiency (CE %) was calculated on the basis of measured coulombs of current compared to the total coulombs from substrate removed (based on COD) [11]. The coulombs of current can be calculated by the equation $Q = \int i \cdot dt$, where i is the current of the external circuit. The total coulombs can be calculated by the equation $Q_t = (COD_{in} - COD_{out}) \cdot V \cdot F \cdot b / M_{O_2}$, where F represents the Faraday constant, 96485 C/mol; M_{O_2} is the molar mass of oxygen, 32 g/mol; b is the complete oxidation requirement of electron per mole oxygen and b is 4 mol-e⁻/mol; COD is measured from influent and effluent.

3. Results and Discussion

3.1 Effects of cathode materials on reactor performance

This experiment lasted for one month and the COD value of the influent wastewater varied from 1500-2000 mg/L. S2 reactors with Ni cathode showed a maximum COD removal value of 90% and maximum average of 85% at HRT=24 h, which was better than SS and Cu cathodes (Fig.1). SS cathode reactor (S1) documented a COD removal of 79%, whereas Cu cathode reactor (S3) had the minimum COD removal value of 69%. There was no marked difference in carbohydrate removal based on materials. But the TOC removal followed a similar trend with COD, with Ni cathode documenting a maximum of 83% removal efficiency (Fig.1). Moreover, the type of cathode material greatly affected the methane yield of the reactors. It is clearly illustrated in Fig.2 that a maximum methane yield of 143 ml/gCOD was observed in reactor S2, and a maximum methane production rate of 367 mL CH₄/L reactor/day was also measured in S2 reactor.

Stainless steel and Nickel cathodes are promising in MEC due to their relatively good catalytic activity, high corrosion stability, and low cost [38]. It was

interesting to notice that Ni performed well than SS, and the reason must be the high intrinsic electrocatalytic activity of Ni than SS. Ni is deposited on SS electrodes to increase its catalytic efficiency and prevent corrosion. The Ni-hydrides were responsible for accelerating the hydrogen evolution reaction (HER) for H₂ generation from the nickel cathode surface [39]. Among the Pt-free metals, Ni and its alloys have shown better electrocatalytic activity in HER [40]. It was pointed out that SS electrodes with a high Ni content have the best catalytic activation and are anti-corrosive[41]. So, this property might have contributed to better H₂ evolution from the Ni cathode which might have electrochemically induced high CH₄ production. A comparative study between the performance of SS cathodes and carbon cloth cathodes coated with Ni alloy for H₂ production in MEC reactors was conducted by Hu et al. [42]. The Ni alloy cathode reactors were reported to produce 17% more H₂ than the reactors which used SS cathodes. The COD removal rate and CH₄ production rate achieved in this study was better than the following pilot scale studies. Carrera et al. [5] designed a 4 L tubular MEC with Ni deposited carbon paper as cathode and operated with domestic wastewater. The COD removal efficiency was 80% and CH₄ production rate was 60 mL CH₄/L reactor/day. A maximum COD removal of 85% and CH₄ production rate was 130 mL CH₄/L reactor/day occurred in tubular MEC operated by Carrera et al.[10], with Ni deposited carbon paper electrode. Rader and Logan [43], operated a 2.7 L continuous flow MEC to estimate the biogas production from acetate and CH₄ production rate of 120 mL CH₄/L reactor/day was recorded in their reactors.

The pH was also estimated to play a minor role in cathode activity. The influent pH was maintained at neutral by PBS solution. We measured the pH of the effluents almost every day and the S2 effluent maintained a value of 7.1-7.3,

throughout the experiment, whereas S1 and S3 showed values between 5.9 and 6.5. The results hinted that a higher efficiency of proton consumption was by Ni cathode than the other two cathodes. An accumulation of protons would be caused by slow proton consumption, leading to low efficiency in long term MECs [44]. When HER reaction occurs on a metal surface in a media with $\text{pH} > 5$, it results in the formation of metal hydrides. They combine with water and electrons to produce H_2 and hydroxyl (OH^-) ions [45]. These hydroxyl ions must have contributed to the alkalinity of the effluent as more hydroxyl ions may be produced in Ni than SS and Cu, due to its better catalytic activity. This increase in pH, might have influenced the better methane production in S2, to a certain extent. The conductivity of our influent (beer and PBS) was about 5.76 mS/cm. This shows that the addition of PBS has brought about a great difference in the conductivity between the real and artificial beer wastewater (1.8 mS/cm and 5.76 mS/cm) respectively.

The VFA contents in both the influent and the effluents were analyzed, and their production rates were determined. VFAs are produced during acidification process of anaerobic digestion and continued to be produced through acetogenesis. The VFA content in the reactor should be less, as their over production and accumulation will lead to the inhibition of methanogens, and will bring down methanogenesis. Therefore, low amount of VFAs production is advisable for continued digestion and to avoid reactor acidification [46-47]. The S3 effluent documented a highest HAc (acetic acid) production of 3200 mgCOD/L, from 150 mgCOD/L in the influent, whereas S1 and S2 showed increases of 2150 mgCOD/L and 1270 mgCOD/L respectively. HPro (Propionic acid) production also reached a maximum value of 345 mgCOD/L in S3, whereas it was 208 mgCOD/L in S1 and 179 mgCOD/L in S2 effluents. Influent HPro was only 110 mgCOD/L. S3 effluent had a

28 and 3 folds increase in HAc and HPro productions respectively from the influent. This condition might have led to the decrease in the effluent pH in S3&S1 than S2. Interestingly, we observed that the concentration of ethanol (a product of fermentation in anaerobic digestion) was more in S2 effluent than the influent and the other two effluents. A concentration of 362 mg/L of ethanol was detected in the influent, and it rose to 758 mg/L in S2. S1 and S3 effluents documented a negligible increase till 410 mg/L and 400 mg/L respectively. This has led to the increase in the pH of the S2 effluent making it appropriate for methanogenesis. Wang et al. [11] have also suggested that an acetic acid concentration level till 2000 mg/L did not have an inhibitory effect on methanogenesis. So, considering this level into account, we can also give a discussion that the level of acetic acid in S1 and S3 appear to be a considerable threat for methanogens survival. These above mentioned studies justify the results of our study, and provide the reasons for the better performance of Ni than SS and Cu cathodes.

3.2 Cathode selection by Cyclic Voltammetry and Current generation

The CE production of the reactors was very low, the highest being 15% in S2 reactor with a COD removal was 90% and current of 8.6 mA. This clearly shows that a major part of the oxidized organic content has been converted to methane gas (90.6% CH₄) by methanogens, than being utilized by exoelectrogens. Strategies to achieve a high CE can be accomplished by increasing the anode size to the reactor volume ratio, limiting the growth of methanogens in anode and optimizing the organic loading rate. The ratio of the anode to the reactor volume was only 1:10 in this reactor as the anode occupied only 10% of the reactor volume, so increasing its size may yield better CE. Similar low CE values have been recorded in lab-scale MEC reactors. Van Eerten-Jansen et al [48], used a 560 mL volume MEC which recorded a CE of

23%. They have reported that the presence of methanogens in anode was a reason for low CE. Carrera et al [49], operated an MEC with a volume of 855 mL, and anode size of 50 cm². The CE was 25%, and they have suggested that increasing the anode size to the reactor volume ratio will bring about a great increase in CE.

To evaluate the electrochemical activities of various biocathodes based on three materials, the cathode materials were subjected to Cyclic Voltammetry studies before and after use in the MEC reactors. This was performed to choose the best cathode material based on the lowest over potential and highest current generation. The cyclic voltammograms were obtained in a potential region of 0 to -1.0 V at a scan rate of 0.01 V/S. Figure 3A, shows the CV results of plain electrodes before use in the MEC. No valuable over potential was detected in the electrodes, except a highest of -0.2 V, by Cu electrode, whereas a high current generation of 1.0 mA, was detected in both SS and Ni electrodes. The used cathodes were taken from the reactors after the cycles have stopped and were analyzed along with the biofilm (Fig.3B). The Cu electrode shows an over potential of -0.22 to -0.23 V, which was much higher than the over potential of -0.7 V, depicted by Ni electrode. These results indicate that Ni proved to be good in the consumption of a lower voltage for proton generation and was better in current production. This aspect was supported by Selembo et al. [32], where they have suggested that electrodes which produced the lowest overpotentials can be selected as cathodes for MEC reactors. The overpotential value obtained in this experiment for Ni (-0.7 V), was lower than the value for Pt electrodes (-0.5 V). By polarizing the Ni electrodes at potentials more than -1.2 V, their hydrogen evolution capacity will increase [50].

The current generation in Ni was also higher at 3.3 mA, than 4.0 mA in Cu. Cheng and Logan [51], have recommended that the material which generates the

steepest slope for current production at a given voltage can be selected as cathode for reactors as it can produce more current than the other materials. Figure 4, illustrates the current generation profile in all the three reactors. The better performance of the Ni reactor is clearly indicated in the graph, with a maximum current of 8.6mA. This result is in accordance to the above stated discussion.

It is very reasonable to know that functional bacteria will attach to electrode in BESs, which lead to varieties of products (H_2 , CH_4 , CO_2) [8, 11] and consequent change of electron recovery on cathode [52-53]. Nickel is a relatively abundant metal in the natural environments, and most microbes including methanogens have active, high-affinity, nickel-uptake hydrogenases. The Ni could activate some hydrogenotrophic methanogens to reduce CO_2 with H_2 to CH_4 . Therefore, in their natural habitats, methanogens have to continuously cope with the problem of nickel famine [54-56]. So, such bacteria might have well grown on the Ni electrode, which might have led to quick oxidation of organics and better COD removal in S2 reactor. Detailed microbiological studies have to be carried out to discuss the vital role played by microorganisms.

Conclusions

Compared with Stainless steel and Copper meshes, Nickel mesh cathode remarkably affected the reactor performance by showing promising results in COD with a maximum value of 85%, TOC removal efficiency of 83%. Though carbohydrate removal was not significantly affected by the cathode material, methane yield raised to a maximum of 143 ml/gCOD and methane production rate to 367 mL CH_4 /L reactor/day in S2 reactor, with a current generation of 8.6 mA. Cyclic voltammograms of the cathode materials showed significant peaks, which indicated the low over

potential and high current generation of Nickel. The CE values were relatively less and we suggest that increasing the electrode size and surface area may bring about a rise in energy efficiency. In depth microbial community analysis is also needed to further support the role of microbes in this study and take this research a step ahead to reality.

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Table 1 Physico-chemical parameters of the real beer wastewater

Parameter	Value
Ammonia	52-60 mg/L
Biological Oxygen Demand (BOD)	1285-1540 mg/L
Conductivity	1.8 mS/cm
Chemical Oxygen Demand (COD)	2106-2250 mg/L
pH	6.6-6.8
Suspended Solids (SS)	36-450 mg/L

Figure 1: COD, Carbohydrate and TOC removal profiles in reactors with different cathode materials

Figure 2 Methane generation with respect to cathode materials

Figure 3 Cyclic voltammograms for cathode in reactor

(A): Plain cathode (B): Biocathode

Figure 4 Current generation profile in the reactors