ENHANCED HYBRID SYSTEM OF MICROBIAL ELECTROLYSIS CELL AND ANAEROBIC DIGESTER (MEC-AD) PERFORMANCE THROUGH MODIFIED ELECTRODES WITH MULTI-WALL CARBON NANOTUBES

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ABSTRACT: The hybrid system of microbial electrolysis cell (MEC) and anaerobic digester (AD) has been a promising approach for sustainable energy production and waste treatment. The integration of MEC and into the AD digester offers multiple advantages over conventional AD systems. The study was conducted on the modification of carbon felt (CF) anode, and stainless-steel mesh (SSTM) cathode with multi-wall carbon nanotubes (MWCNT), to facilitate the biomethane production and upgrade within the hybrid system. The microbial attachment to the electrodes was analyzed, the substrate concentration, current density, and biogas composition and volume were monitored. The SEM imaging of the electrodes showed that the microbes followed a different growth behaviour in modified and unmodified electrodes. In addition, MWCNT modified SSTM showed a potential hydrogenotrophic growth selectivity, unlike unmodified SSTM, which had a more syntrophic microbial community. Stainless steel mesh-modified cathode showed the highest biogas and methane production with a value of 14.4 ml CH_4/g glucose. In addition, the carbon-felt modified electrodes showed a maximum substrate degradation value of 93% and a current density of 4.5 mA/m^2.

KEY WORDS: *MEC-AD hybrid system, Stainless steel mesh with MWCNT electrode, Carbon felt with MWCNT electrode, and Modified electrodes.*

1. INTRODUCTION

The hybrid system of microbial electrolysis cell and anaerobic digester has been a promising approach for sustainable energy production and waste treatment [1].The approach has leveraged the microbial communities' capabilities of both systems to produce electricity and biomethane with the enhanced breakdown of organic waste [2].

The integration of MEC and AD into digester offers multiple advantages over conventional systems, anaerobic digestors are responsible for breaking down complex substrates into fermentable sugars and acids, and finally converted to mainly biomethane and carbon dioxide, while MEC's is responsible for the production of hydrogen utilized for the upgrade of CO_2 to biomethane within the system [3]. To commercialize this hybrid system, researchers have turned to modifying commercially available, cheap carbon electrodes as a replacement for precious metal electrodes currently used in the hybrid system, like palladium and platinum electrodes [4]. Multiple modifications to carbon-electrodes are made to improve bacterial adhesion and conductivity specifically, and overall performance in general, like the modification of carbon fibre with self-supported N-doped C/Fe_3O_4-nanotube composite arrays [5], carbon black with humic-acid [3], carbon felt with carbon derived from mango wood biomass [6], preparing porous carbon cloth using Nickel (N-doped). While previous research on MEC-AD electrode's modification demonstrate promising results in terms of biogas enhancement, the research gap remains in finding modifications that not only enhances the biomethane production, but also the overall performance of the fermentation stages, in terms of substrate fermentation and utilization, along with methanogenic microbes' enrichment, and biomethane upgrade. Among the promising approaches in electrode's modification is the incorporation multiwall carbon nanotubes (MWCNT's).

MWCNT's exhibits an expectational electrical conductivity, speeding up the electron transfer efficiency within the biofilm [7]. In addition, the modification of electrodes with MWCNT's increases the electrode surface area, biocompatibility, hence increasing the colonization of microorganisms, facilitating an efficient fermentation process, and biomethane production [8]. The study focuses on the modification of carbon felt (CF) anode, and stainless-steel mesh (SSTM) cathode with MWCNT, to facilitate the biomethane production and upgrade within the hybrid system. The MWCNT growth on electrodes and microbial attachment were observed through scanning electrode microscopy (SEM), substrate degradation in terms of glucose utilization, current density, and biogas volume and composition was monitored.

2. MATERIALS AND METHODS

2.1. Electrodes Modification

Stainless steel mesh grade (304) with mesh size (1 mm), was used as cathode. Carbon felt research grade was used as the anode. The electrode's thickness, width, and length were fixed at 0.1, 4, 9 cm, respectively.

Carbon felt and stainless-steel mesh electrodes surface were washed with ethanol and acetone prior to modification. Carbon felt was modified using carbon ink prepared by mixing distilled water, MWCNT with a purity of (99%) and polyvinyl pyrrolidone (PVP) binding agent in the ratios of (1:2:0.4) (ml/mg/mg), respectively were mixed and vortexed for 1 min to insure material dispersion. Then, CF was submerged in the carbon ink and sonicated for 1 h to ensure ink dispersion within the fiber's strands, then the electrode was removed and dried in the oven at 170 °C for 20 min.

Stainless-steel mesh was modified using carbon ink prepared by mixing 95% ethanol and MWCNT with a ratio of 1:2 (ml/mg), respectively. Then, the mesh was submerged in the carbon ink and mixed at 100rpm for 1h, removed and dried at 170 ºC for 20min, the process was repeated until the mesh was completely coated.

2.2. Synthetic Substrate and Inoculation

The reactor was fed with modified growth medium was prepared by adding glucose 5 g/l; peptone 10 g/l; yeast extract 5 g/l; starch 1 g/l; sodium chloride 0.5 g/l; sodium acetate 0.5 g/l; cysteine hydrochloride 0.5 g/l [9]. The pH of the substrate was adjusted to 7 with every feeding.

The initial microbial source was collected from an anaerobic digester at the Sime Darby plantation at Carey Island, Selangor. The samples were kept in the chiller until further usage.

The effluent of previous anaerobic digester was centrifuged at 8000 rpm for 5 min. Then the supernatant was discharged, and precipitate was used as the seeding sludge for the systems.

2.3. Set-Up of MEC-AD System

Three systems were set up, two MEC-AD hybrid systems and one anaerobic digester. The first hybrid system was equipped with modified carbon felt and unmodified stainless-steel mesh (MEC-AD-CF), while the other hybrid system was equipped with unmodified carbon felt and modified stainless-steel mesh (MEC-AD-SSTM). The hybrid systems were connected to a power supply with an applied voltage of 0.9 V. All three systems' temperature were maintained at 37 °C, with an initial pH of 7. The biogas volume was monitored using water displacement method [10]. The water's pH was adjusted to 3 to avoid CO_2 solubilization. Fig .1 shows the MEC-AD hybrid system set-up.

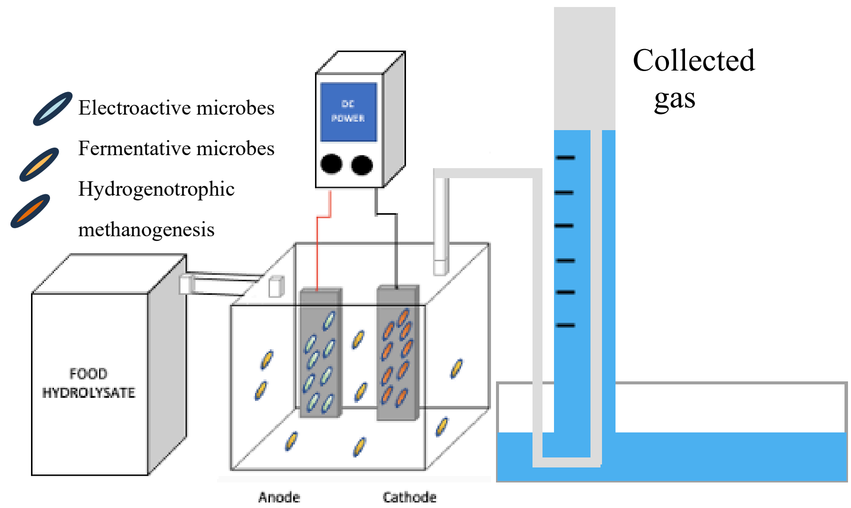

Fig. 1. MEC-AD hybrid system set-up.

2.4. Sample Preparation for Scanning Electrons Microscopy (SEM) Imaging

A piece with the dimension of 0.5X0.5cm of electrode was cut, washed with phosphate buffer, then fixed using 2.5% glutaraldehyde for 4 h. Then the samples were washed and dehydrated with ethanol (50%, 75% and 100%), respectively for 15 min. Then, they were analyzed using scanning electron microscopy (SEM).

2.5. Biogas and Sample Analysis

The biogas composition of all four systems was monitored using CH_4, H_2 and CO_2 multi-gas analyzer (PG810) daily. To measure the amount of reducing sugar consumed, 1 ml of reactors media was mixed with 1 ml of 3,5-Dinitrosalicylic acid (DNSA) solution, placed in boiling water for 15 min, and then was analyzed at 540 nm. The current of the hybrid systems were monitored using an ammeter connected to both ends of the anode and cathode.

3. RESULTS AND DISCUSSION

3.1. MWCNT Growth on Electrodes and Microbial Attachment

3.1.1. SEM Imaging Carbon Felt Electrodes

Microbes had different behavioral growth on modified and unmodified electrodes based on SEM's electron images as shown in Fig. 2 (A, D) of unmodified, and modified CF respectively. Fig. 2B and 2E show the general distribution, bacterial growth, and colonization of microbes on modified and unmodified electrodes, respectively. In contrast, Fig. 2C and 2F are closer images of microbial growth behavior of modified and unmodified electrodes, respectively. Based on Fig. 2B of unmodified CF, the microbial behavior was a big lumpy biofilm formation and growth in certain fiber regions, rather than a full coverage. On the other hand, the microbial growth on CF exhibited a different growth behavior in which the microbes thoroughly covered the fiber's surface and the MWCNT in between the fibers with the distribution of irregular individual colonies on different areas on the fibers as shown in Fig. 1E, the microbial density on the unmodified CF was much less than the modified CF.

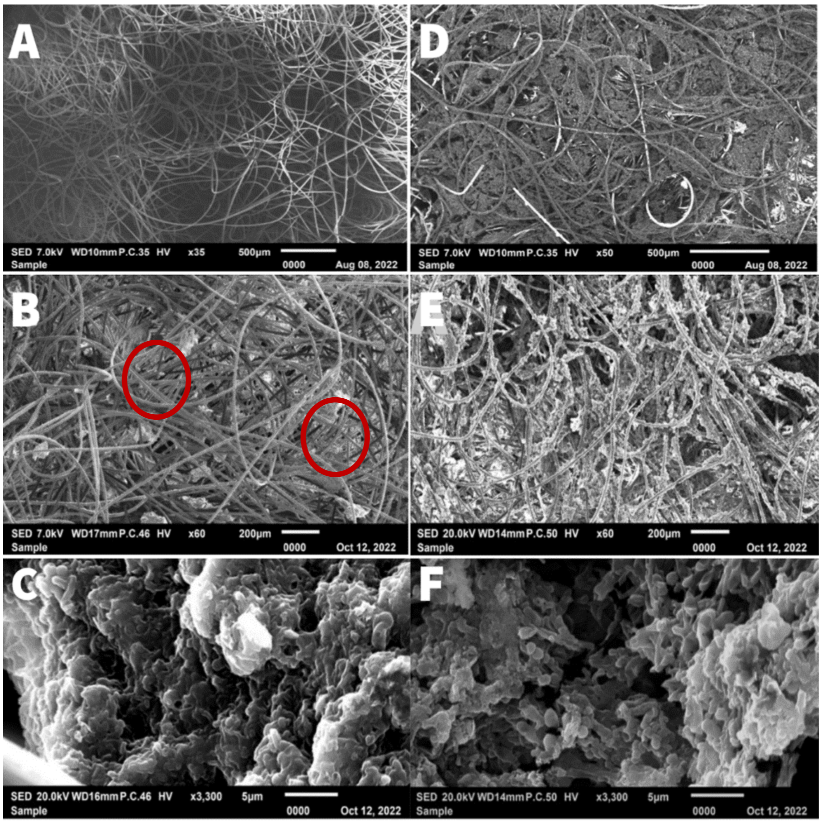

Fig. 2. SEM Images of Unmodified CF(A), Microbial growth on Unmodified CF (B, C), and Modified CF (D), Microbial growth on modified CF (E, F).

Fig. 2F of modified CF shows the direct growth of microbes on MWCNT-covered surface, offering a higher surface area for microbial growth. In addition, MWCNT, a conductive material, has also affected the electron transfer behavior of the microbes. From the same image, MWCNT facilitated the electron transfer directly from the microbe's surface to the electrode as can be seen in Fig. 2F of modified electrodes. To elaborate, a study by Kadier et al. reported that electrons generated from the oxidation of organic materials by a single microbe are directly transferred to the anode [11]. On the other hand, the microbial community growing on unmodified electrodes, as shown in Fig. 2C had a denser EPS secretion and formation, which could suggest that the method of electron transfer through conductive biofilm was dominated. Microbes in the unmodified electrodes secreted extracellular polymer matrix (EPS) to help them attach themselves to the electrode and facilitate the electron and substrate transfer. In the modified CF, EPS density was lesser compared to the unmodified electrodes since the microbes have a lesser secretion of substance in the reactor equipped with CNT. Fig. 3 shows an illustration of electron transfer mechanism in unmodified and modified electrodes. It explains the microbial behavior with and without modification. In the modified CF, the extracellular polymeric substance density was lesser compared to the unmodified electrodes and this has been reported previously by Andrea et al., owing to CNT, microbes have lesser secretion of substance in reactors equipped with CNT [12]. There is no direct explanation to the correlation between the amount of electron produced and transferred directly, or through EPS. However, electrons transferred directly from the surface of organism to the electrodes use less energy than electrons transferred through electron transfer chain, namely microbes and EPS [13].

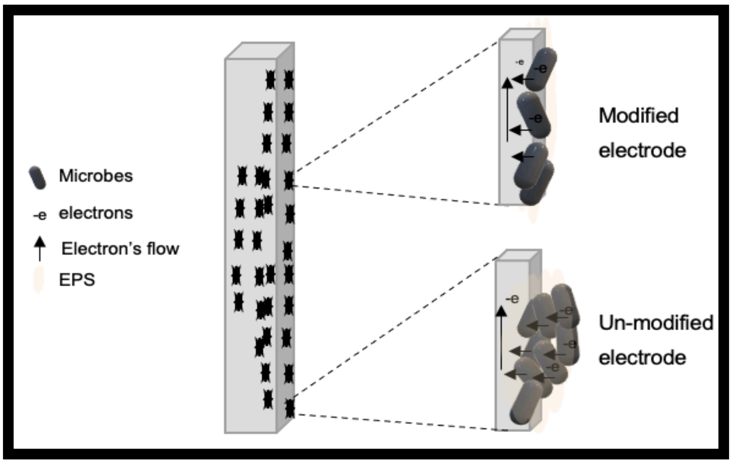

Fig. 3. Electron transfer mechanism in the unmodified and modified electrodes.

3.1.2. SEM Imaging of Stainless-Steel Mesh

Fig. 4(A, D) shows the images of unmodified and modified SSTM, respectively. The microbial growth on the modified and unmodified stainless-steel mesh had similar behavior to the microbial growth in the modified and unmodified CF electrodes. The unmodified electrodes had a cluster growth behavior, as shown in Fig. 4B. In the modified electrodes, microbes grew directly on the surface of the mesh and MWCNT, as shown in Fig. 4D. In Fig. 4C of the unmodified SSTM, and Fig. 4F of the modified SSTM, a different microbial community growth and distribution was observed. In the unmodified electrodes (Fig. 4C), a variety of different microbial shapes existed i.e., rod, long rods, cocci- and di-cocci-shaped microbes. In the modified SSTM (Fig. 4F), rod-shaped microbes were of significant population, followed by cocci and di-cocci-shaped microbes. Hydrogenotrophic methanogenesis has rod-long shapes, while acetolactic methanogenesis has cocci and di-cocci shapes. The same results were reported by studies conducted by Greesh & Pratishka and Sylvia et al. [14-15], where long-rod shapes microbes were identified as hydrogenotrophic, and cocci, di-cocci shaped microbes were identified as acetolactic methanogens. This is an evidence of the effect of MWCNT in enriching the methanogenic community, as reported previously by Andreia et al., the addition of CNT has accelerated the population of hydrogenotrophic methanogenesis culture in the digester [16].

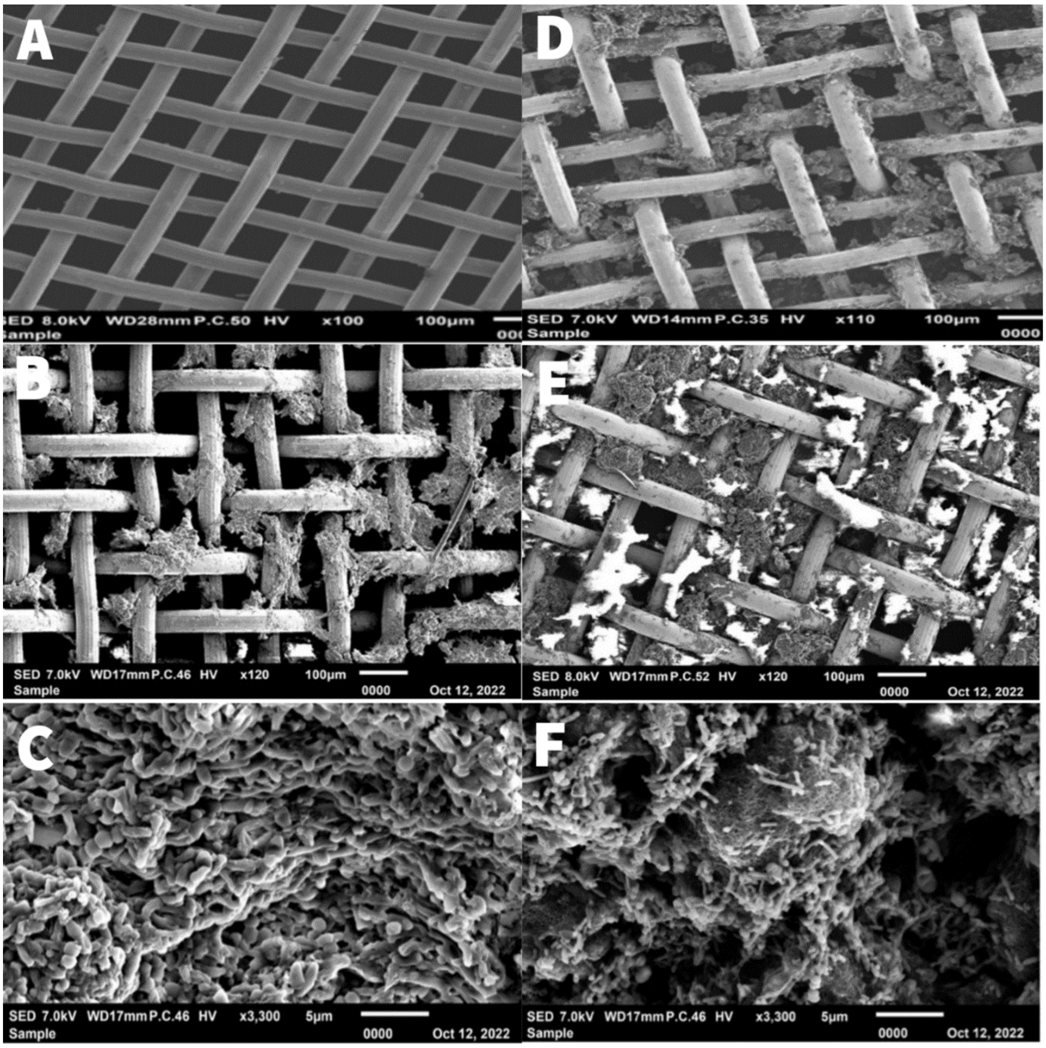

Fig. 4. SEM Imaging of Unmodified SSTM(A), Microbial growth on Unmodified SSTM (B, C), and Modified SSTM(D), Microbial growth on modified SSTM (E, F).

3.2. CURRENT DENSITY

The anode is responsible for the oxidation reaction of the substrate, producing acids, electrons, and hydrogen. The current density indicates the activity of electrogenic bacteria. A higher current density results in the more active and higher population of electrogenic microbes [17]. Fig. 5 presents the data on the current density of two different hybrid systems, equipped with the unmodified and modified CF electrodes. Based on the systems equipped with unmodified carbon felt anode, and modified stainless steel mesh cathode, it can be observed that no current was generated in the first few days. Starting from the sixth day, a small current volume was generated. The current volume increased up to day 10, and then a drop of 50% was observed on the following day. The increase in the current volume implies the growth and increase in the electroactive microbial community on the anode. This could be owing to the depletion of the substrate. The fluctuation in the current throughout the 20 days could also be owed to the microbes developing the extracellular polymer matrix on the electrodes [18].

On the other hand, reactors equipped with a modified carbon felt anode, and unmodified stainless steel mesh cathode showed a relatively high current density on the first cycle with a current density of 2.67 mA/m^2 compared to 0.0 mA/m^2 for reactors with unmodified carbon felt anode. A study by Ludovic et al. suggested that modifying porous electrodes with MWCNT increased carbon electrodes' biocompatibility and electrode's microbial density and thus generating the current density [19]. Coating with MWCNTs improves the electrochemical communication between the microbes and improves the conductivity of the materials [20]. Moreover, Mohita et al. reported that MWCNT modification reduces the inner resistance of the electrodes and increase the active surface area, which reduces the ohmic loss, hence improving the current density [21].

Besides, the increase in current density could be attributed to a novel type of microbe called Geobacter which are electroactive that coexists with the fermentable microbes[22]. Geobacter produces high current densities in the MFC and MEC systems [23]. They utilize VFAs like acetate using extracellular, insoluble Fe (III) and Mn (IV) oxides as terminal electron acceptors [24]. A similar study with the anode of graphite felt modified with MWCNT to treat landfill leachate showed high current density production of 4.2 mA/m^2 [25].

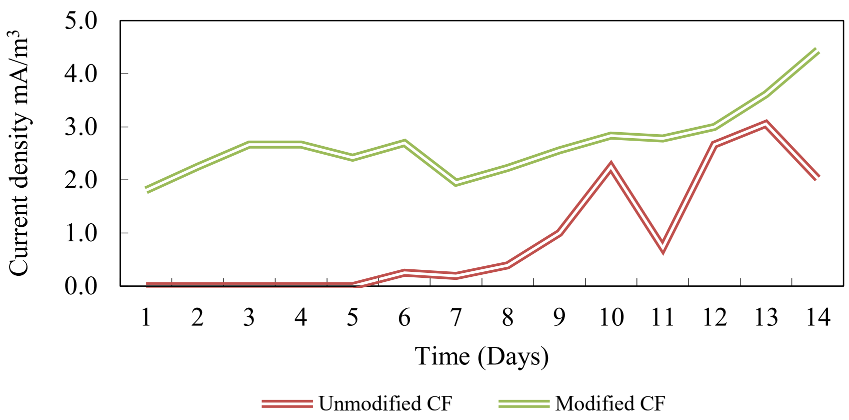

Fig. 5. Current density of system equipped with unmodified and modified CF.

3.3. SUBSTRATE DEGRADATION

The anode is responsible for the substrate degradation. The substrate degradation rate was monitored in terms of glucose consumption. Fig. 6 illustrates glucose degradation of two different hybrid systems, equipped with unmodified and modified CF electrodes. AD reactors showed no significant substrate degradation in the first five days. The degradation value was lower than 55% throughout the analysis. The increase in degradation rate for the digester was faster in the first four days compared to the hybrid system with unmodified CF electrodes. However, the substrate consumption was higher in the hybrid system and increased throughout the analysis with microbial adaptation to the anode. The unmodified CF systems achieved a high percentage of 83% towards the end of the analysis. It can be attributed to the larger surface area for microbial growth hence, a faster substrate consumption. In addition, this could be attributed to the enhancement of performance by degradative and oxidative microbes by carbon felt anode. A study by Luo and co-workers suggested that carbon felt anodes with an applied voltage above 0.5 V highly enhance degradative microbes in MEC-AD hybrid systems, along with the oxidative microbes [26].

However, the modified CF systems showed the best substrate degradation performance throughout the analysis, maintaining a value over 80% and achieving a maximum percentage of 92.55%. In addition to the enrichment effects of carbon felt, MWCNT modification has a wider porous surface area with high biocompatibility for oxidative and degradative microbes to grow. The increase in substrate removal efficiency can also be attributed to the carbohydrate's bioconversion through the favorable redox potential between the electrodes, hence enrichment of functional degradative microbes [27]. The results align with a similar study by Mansoorian on the treatment of landfill leachate using MEC showed that the substrate degradation of systems equipped with MWCNT modified CF had a high substrate degradation value of 97%, compared to the control with a value of 72% only [25].

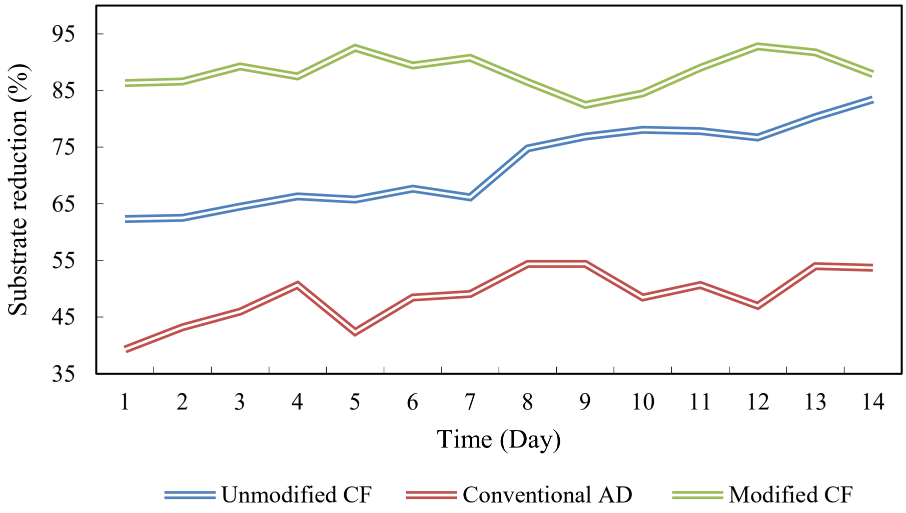

Fig. 6. Glucose reduction in semi-batch systems of unmodified, modified CF, and conventional AD fed with 50 ml/day substrate.

3.4. BIOGAS AND COMPOSITION

The cathode is responsible for biomethane production and upgrade within the system. Fig. 7 displays CH_4 and CO_2 volume. From the biogas production rate, the hybrid system with modified SSTM has substantially outperformed systems with the unmodified SSTM and conventional digester. In the modified reactor, the biomethane production substantially increased on the sixth day onwards, achieving a value of 287 CH_4/g glucose while only producing 12 ml CO_2/g glucose. On the other hand, unmodified reactors gradually increased biomethane throughout the cycle, outperforming conventional digesters with a cumulative biomethane value of 57.7 ml/g glucose and 26.8 CO_2 ml/g glucose. The digester had the lowest biomethane production of 37 mL/g glucose, yet the highest cumulative CO_2 with a value of 41 mL/g glucose. It was reported previously that conventional digesters' biomethane only accounts for 50-60%, and the remaining is CO_2 [28] compared to integrated systems. Integrating electrodes into the system gives a higher surface area for microbial growth. Hence, a higher volume of the substrate is available for faster consumption. Modifying the SSTM cathode with MWNT has increased the surface area and biocompatibility of the mesh, as observed in the SEM images. In addition, MWCNT and conductive materials have been reported previously to improve direct interspecies electron transfer (DIET) reactions between fermentative and methanogenic microbes [29].

Moreover, Andreia et al. reported that CNT increases the population and selectivity of the hydrogenotrophic and electroactive methanogenesis community [16]. Unlike acetolactic methanogenesis, which consumes acetate to produce CH_4 and CO_2, hydrogenotrophic methanogenesis produces methane through the consumption of H_2 and CO_2 in the production of biomethane, thus, reducing the CO_2 concentration while increasing the biomethane volume.

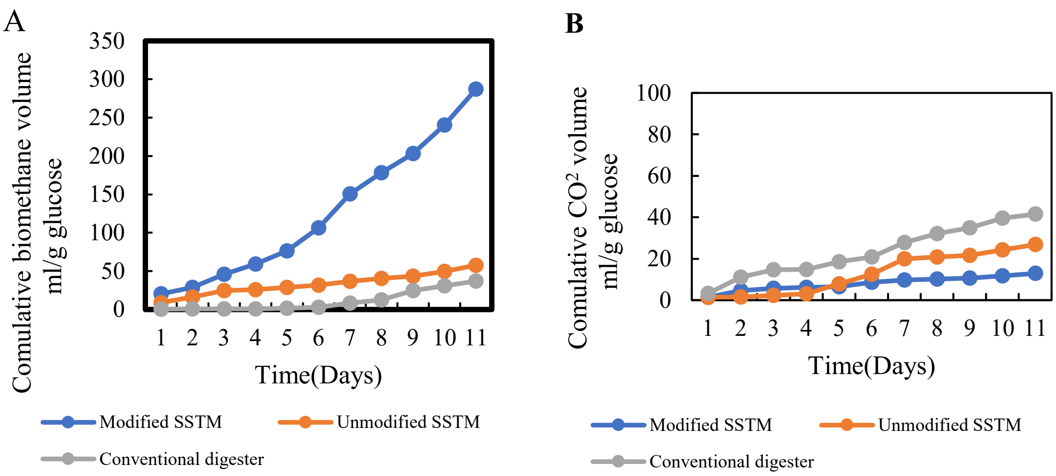

Fig. 7. Cumulative biomethane(A) and CO_2 production(B) of conventional digester, Modified system, and unmodified system.

4. CONCLUSION

The electrode's modification with MWCNT on the carbon felt and stainless-steel mesh highly improved the microbial attachment and behavior. High current density and substrate degradation indicates the elevated performance of fermentative microbes. In addition, the increase in biomethane and decrease in CO_2 values compared to the conventional AD digester and unmodified systems shows that the biomethane upgrade within the system was successful.

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REFERENCES

[1] Shreenag M, Lourdu AJ. (2015) Bio-hydrogen production in microbial electrolysis cell using wastewater from sugar industry. *International Journal of Engineering Science Resources Technology*, 4(4).

[2] Zakaria BS, Lin L, Chuang T, Dhar BR. (2020) *An overview of complementary microbial electrochemical technologies for advancing anaerobic digestion*. 5(11).

[3] Huang G, Fu G, He C, Yu C, Pan X. (2019) Ferroferric oxide loads humic acid doped anode accelerate electron transfer process in anodic chamber of bioelectrochemical system. *Journal of Electroanalytical Chemistry*, 851:113464.

[4] Hathaichanok S, Sabine S, Marianne H, Melissa M, Abdelaziz AJ. (2020) Enhanced methane producing microbial electrolysis cells for wastewater treatment using poly(neutral red) and chitosan modified electrodes. *Sustainable Energy Fuels*, 4(8): 4238–4248.

[5] Wang Y, Chuang C, Liu S, Zhou LZ, Fengyi G. (2021) Hierarchical N-doped C/Fe3O4 nanotube composite arrays grown on the carbon fiber cloth as a bioanode for high-performance bioelectrochemical system. *Chemical Engineering Journal*, 406: 126832.

[6] Cai S, Zhou Q, Mo CH. (2020) Spraying carbon powder derived from mango wood biomass as high-performance anode in bio-electrochemical system. *Bioresources Technoogyl*, 300: 122623.

[7] Wie HH, Tsai HY, Huang C. (2017) Characteristics of Carbon Nanotubes/Graphene Coatings on Stainless Steel Meshes Used as Electrodes for Air-Cathode Microbial Fuel Cells. *J Nanomater*.

[8] Yonghyung P, Hyunwoo C, Booki M, Hong SK. (2017) Response of microbial community structure to pre-acclimation strategies in microbial fuel cells for domestic wastewater treatment. *Bioresour Technol*, 233: 176–183.

[9] Aziz AHA, Engliman NS, Mansour FM, Abdul PM, Arisht S, Jamali N, Tiang MF. (2022) Synergistic enhancement of biohydrogen production by supplementing with green synthesized magnetic iron nanoparticles using thermophilic mixed bacteria culture. *Int J Hydrogen Energy*, 47(96): 40683–40695.

[10] Selvankumar T, Sudakar C, Govindajaru M, Aroulmoji M. (2017) Process optimization of biogas energy production from cow dung with alkali pre-treated coffee pulp. *Biotechnology*, 7(4).

[11] Kadier A, Simayi Y, Abdeshahian N, Azman NF, Chandrasekhar K, Kalil MS. (2016) A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production. *Alexandria Engineering Journal*, 55(1): 427–443.

[12] Andrea S, Gilberto M, Ricardo S, Afons S, Ana C. (2017) Carbon nanotubes accelerate methane production in pure cultures of methanogens and in a syntrophic coculture," *Environmental Microbiology*, 19(7): 2727–2739

[13] Kracke I, Vassilev J, Krömer O. (2015) Microbial electron transport and energy conservation. The foundation for optimizing bioelectrochemical systems. *Front Microbiology*, 6.

[14] Greesh B, Pratishka P. (2015) Isolation and Identification of Methanogenic Bacteria From Isolation and Identification of Methanogenic Bacteria From Cowdung. International Journal of Current Research.

[15] Sylvia O, Duncan M, Ayub NG, Ndungwa M, Ingrid NW. (2016) Isolation and characterization of methanogenic bacteria from brewery wastewater in Kenya. *African Journal Biotechnology*, 15(47): 2687–2697.

[16] Andreia S, Gilberto M, Manuell M, Ricardo S, Stams A. (2017) Carbon nanotubes accelerate methane production in pure cultures of methanogens and in a syntrophic coculture. *Environmental Microbiology*, 19(7): 2727–2739.

[17] Carrillo P, Escapa A, Hijosa V, Paniagua G, Díez AR, Mateos R. (2022) Bioelectrochemical enhancement of methane production from exhausted vine shoot fermentation broth by integration of MEC with anaerobic digestion. *Biomass Conversion Biorefinery*, 1: 1–10.

[18] Salar G, Obata O, Kurt K, Chandran K, Greenman J, Ieropoulos IA. (2020) Impact of inoculum type on the microbial community and power performance of urine-fed microbial fuel cells. *Microorganisms*, 8(12): 1–16.

[19] Ludovic J, Stefano F, Jun C, Gordon GW, Jurg K. (2014) A novel carbon nanotube modified scaffold as an efficient biocathode material for improved microbial electrosynthesis. *J Mater Chem A Mater*, 2(32): 13093–13102.

[20] Aryal N, Ammam S, Patil A, Pant D. (2017) An overview of cathode materials for microbial electrosynthesis of chemicals from carbon dioxide. *Green Chemistry*, 19(24): 5748–5760

[21] Mohita S, Suman B, Sylvia G, Sunil P. (2014) A critical revisit of the key parameters used to describe microbial electrochemical systems. *Electrochimica Acta*, 140: 191–208.

[22] David W, Eric M, Dawn H, Zimu Z. (2019) The Archaellum of Methanospirillum hungatei Is Electrically Conductive. *mBio*, 10(2).

[23] Malvankar M, Tuominen D, Lovley DR. (2012) Biofilm conductivity is a decisive variable for high-current-density Geobacter sulfurreducens microbial fuel cells. *Energy Environmental Science*, 5(2): 5790–5797.

[24] Derek L, Tian Z, Nikhil M, Pravin S, Kelly F, Muktak A. (2011) Geobacter: The Microbe Electric's Physiology, Ecology, and Practical Applications. *Adv Microb Physiol*, 59: 1–100.

[25] Mansoorian A, Mahvi R, Nabizadeh M, Alimohammadi S, Nazmara S, Yaghmaeian K. (2020) Evaluating the performance of coupled MFC-MEC with graphite felt/MWCNTs polyscale electrode in landfill leachate treatment, and bioelectricity and biogas production. *Journal of Environmental Health Science and Engineering,* 18(2): 1067–1082.

[26] Luo L, Xu S, Jin Y, Han R, Liu H, Lü F. (2018) Evaluation of methanogenic microbial electrolysis cells under closed/open circuit operations. *Environmental Technology*, 39(6): 739–748.

[27] Lei Z, Ting W, Zi W, Xi J, Xu Z, Feng X, Jong L. (2021) The underlying mechanism of enhanced methane production using microbial electrolysis cell assisted anaerobic digestion (MEC-AD) of proteins. *Water Resources*, 201:117325.

[28] Choi KS, Kondaveeti S, Min B. (2017) Bioelectrochemical methane (CH4) production in anaerobic digestion at different supplemental voltages. *Bioresources Technology*, 245: 826–832.

[29] Baek J, Kim J, Lee C. (2018) Role and Potential of Direct Interspecies Electron Transfer in Anaerobic Digestion. *Energies. 11: 107.*