INVESTIGATION THE EFFICIENCY OF INTEGRATION MICROBIAL ELECTROLYSIS CELL TO ANAEROBIC DIGESTER FOR BIOMETHANE PRODUCTION

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ABSTRACT The integration of Microbial electrolysis cell to anaerobic digestion has emerged as a promising solution for the upgrade of biomethane within the system. It facilitates the conversion of organic waste into biomethane without the need to CO_2 capture and separation downstream processes. Recent studies have showed that modifying the electrodes has a major effect on the microbial stages, specifically hydrolysis, acidogenesis's, which are key steps for the final stage methanogenesis. Understanding these stages in the MEC-AD system allows researchers to identify potential bottlenecks and optimize the conversion of organic matter into methane. In addition, the final stage, namely methanogenesis which is responsible for the biomethane production and upgrade, is highly affected the by the density of the methanogenic community and the diversity of the inoculum. This study investigated the effect of integrating unmodified, and modified electrodes of MEC to anaerobic digester on the two stages hydrolysis and acidogenesis, then the kinetic modelling of biomethane production with mixing two inoculums namely cow manure and effluent of a previous digester. Hybrid systems showed a higher hydrolysis efficiency especially modified systems, with a percentage of 39.4% by the 48th hour, followed by unmodified systems. The acidogenesis pathway results showed that the hybrid systems were dominated by the acetic acid pathway, which is favourable in the hybrid system, unlike the conventional digester, which was dominated by a different pathway. Mixing the original inoculum obtained from a previous AD with cow manure has enhanced and increased the competitiveness of the microbial community. Thus, it was positively reflected on the biomethane production potential and rate, with a value of 38ml/g COD and 1.2 ml/h, respectively.
Keywords: MEC-AD, Modified electrodes, fermentation stages

1 INTRODUCTION

The integration of Microbial electrolysis cell to anaerobic digestion has emerged as a promising solution for the upgrade of biomethane within the system. It facilitates the conversion of organic waste into biomethane without the need to CO_2 capture and separation downstream processes. Hydrolysis, which is the first stage is deemed to be a rate-limiting step in the fermentation process, hindering acidogenesis process (Appels et al., 2008). In addition, Acidogenesis, which is the 2nd stage of fermentation plays a role in the biomethane upgrade through the hydrogenotrophic and electroactive methanogenesis pathway, In the 2nd stage process, two main pathways are responsible for the production and utilization of hydrogen namely acetic acid and propionic acid pathways respectively (Wattiaux et al., 2019). Recent studies have showed that modifying the electrodes has a major effect on manipulating the microbial stages (Khatami et al., 2021), specifically hydrolysis, acidogenesis's, which are key steps for the final stage methanogenesis. Understanding these stages in the MEC-AD system allows researchers to identify potential bottlenecks and optimize the conversion of organic matter into methane. In addition, the final stage, namely methanogenesis which is responsible for the biomethane production and upgrade, is highly affected the by the density of the methanogenic community and the diversity of the inoculum. Multiple previous researchers have reported that mixing the inoculum can increase the selectivity, diversity and competitiveness of the microbial community, hence simulating new pathways(Rolfe et al., 2012). In addition, (T. Liu et al., 2017) reported on the importance of the initial community source on the fermentation process for biomethane production, the study concluded that the initial microbial community and composition played a crucial role on the 2nd and last stage of the process. This study investigated the effect of integrating unmodified, and modified electrodes of MEC to anaerobic digester on the two stages hydrolysis and acidogenesis, then the kinetic modelling of biomethane production with mixing two inoculums namely cow manure rich in methanogenic microbes and effluent of a previous digester which is rich in fermentative microbes.

2 MATERIALS AND METHODS

2.1 System Set-up and analysis

Three systems, namely conventional digester, hybrid system equipped with MWCNT-Modified electrodes (carbon felt anode and stainless-steel mesh cathode), hybrid system with unmodified electrodes (carbon felt anode and stainless-steel mesh cathode) were set-up for this experiment and monitored for 48h. Samples were collected at several time points 0, 2, 4, 8, 16, 24, 48h. Sample's COD was calculated and analyzed using HPLC with RI detector was used. Zorbax C18 column. System's biogas volume and composition were monitored.

2.2 Inoculum And Substrate

Original inoculum was collected from a previous anaerobic digester of POME from Simedarby. The samples were centrifuged at 8000rpm, supernatant was discharged, and precipitate was seeded to the system making up 10% of the total volume.

Cow manure was collected from UTM research center at farm fresh, the manure was mixed with food-waste and was given two weeks to adapt to the new environment.

2.3 Modified Gompertz Model

Then Modified Gompertz model was employed to study the methane yield by the system, using the Equation below:

$$M(t) = fd \cdot \exp\{-\exp\left[\frac{Rm.e}{fd}(\lambda - t) + 1\right] t > 0\} \quad (1) \qquad (1)$$

where M(t) - the accumulative CH4 yield at the time of t (mL/g COD); fd -the maximum CH4 potential (mL); λ - the lag-phase (d); Rm – the maximum CH4 production rate t - the digestion time (d); and e - the exponential e (2.71828).

3 RESULTS AND DISCUSSION

3.1 Hydrolysis Efficiency

The Hydrolysis of macromolecules into the soluble matter is deemed a rate-limiting step inthe digestion process, limiting the activity of acidogenesis (Choi et al., 2021). The hydrolysis efficiency was measured for three reactors throughout 48h. Hybrid systems showed a substantial improvement in hydrolysis efficiency compared to conventional digesters. Unmodified electrode systems achieved an efficiency of 25% by the 8th hour, then remained constant towards the end of the cycle. While the modified electrode system'sefficiency was the highest on the first day, with a value of 17%, it gradually increased to 38% on the 16th hour. This could be attributed to the enrichment of hydrolytic enzymes onthe anode. Although Hydrolytic microbes are known to be very slow and perform incomplete degradation (Menzel et al., 2020). A study by Carrillo-Peña et al., (2022) reported that integrating the digester with MEC enriched hydrolytic microbes on the anode, and improved their performance, along with fermentative and VFA-consuming bacteria. AD showed the lowest yet the fastest increase in hydrolytic activity. The hydrolytic efficiency of the digester reached a maximum of 20%, then remained consistent towards the end of the cycle.

The findings are aligned with a similar study reported by Q. Huang et al., 2022 under the same voltage. Although the study's hydrolysis efficiency gradually increased withtime, our findings showed that hydrolytic enzyme activity increased and reached a point ofequilibrium for the three systems. To theoretically explain the difference and the behaviorof hydrolytic enzymes with time, hydrolytic organisms secrete extracellular enzymes in theliquid phase, thus, attacking the soluble compounds first, increasing hydrolysis efficiency. With the depletion of soluble compounds, extracellular enzymes attack solid compounds. Carrere et al., (2016), described that when solid-liquid phase is significant, hydrolysis activity is slower, which explains the drop in efficiency with time. In addition, a different source of inoculum offers different microbial consortia, hence different microbial performance, and behaviors.

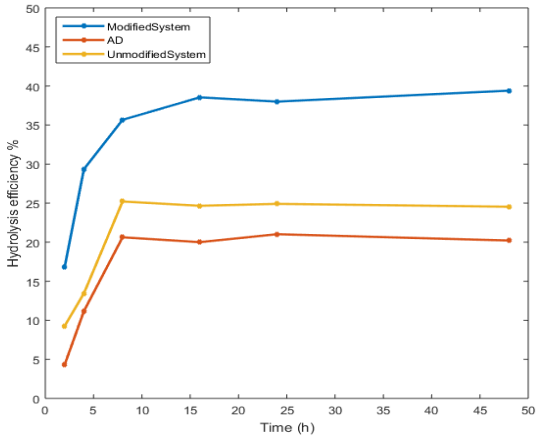

Figure 1: Hydrolysis efficiency of conventional digester, modified electrode system, and unmodified electrode system

3.2 Acidogenesis Pathway

The acidogenesis efficiency shows the performance of fermentative microbes in utilizing and converting the substrate to volatile fatty acids, mainly acetic acid, butyric acid, and propionic acid (Agnihotri et al., 2022). Often, the acidogenesis efficiency is affected by therate-limiting process of hydrolysis, which limits the activity of acidogenesis, hence slowingthe fermentation process (Cai et al., 2013). Thus, hydrolyzed food waste was fed to the systems to avoid process limitations. Initial and final samples collected from three systems were analyzed using RI-HPLC to determine the composition and quantity of volatile fatty acids and calculate each system's acidogenesis efficiency. However, due to unforeseen circumstances related low efficiency of the column used in separating certain volatile fattyacids, only acetic acid was spotted in the samples analyzed, as shown in the appendix. Hence, the acidogenesis efficiency calculated will need to be more accurate since differentmicrobial communities in each system might exhibit different behavior and follow differentmetabolic pathways in the production of VFA (Khatami et al., 2021). Nevertheless, the initial and the final VFA concentration, biomethane produced, and the pH value could be correlated to explain the performance of acidogenesis, along with other VFA-consuming microbes. The Initial and final VFA concentration, final pH, and Biomethane concentrationare tabulated in Table (1).

Table 1: Different analytical data on acidogenesis performance

System	VFA Initial concentration (mM)	VFA final concentration (mM)	Acetic acid COD (g/L)	pH	Final COD(g/L)	Biomethane (mL/g COD)
AD	14.5	45	2.8809	4.3	7	8.5
U-MEC	22.9	85.5	5.4417	4.5	7.4	13.8
M-MEC	90	106	6.8288	4.8	8.25	26.4

The anaerobic digester was referred to as AD. Systems equipped with unmodified electrodes were referred to as U-MEC, while systems equipped with modified electrodes were referred to as M-MEC. The available information on the concentration of acetic acidin terms of COD showed that more than 70% of the COD towards the end of the cycle wascomposed of acetic acid. Hence, the acetic acid pathway in U-MEC and M-MEC systems was the dominating pathway. Four hydrogen molecules are produced in the acetic acid pathway, as shown in the equation. In contrast, two molecules of hydrogen are consumed

in the propionic acid pathway, as shown in the equation (Wattiaux et al., 2019). Thus, the acetic acid pathway is favorable in the hybrid system since the CO_2 upgrade to biomethanerequires four molecules of hydrogen, following the hydrogenotrophic methanogenesis pathway, which is enriched in the hybrid system.

Acetic acid production pathway:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2. \quad (2)$$

Propionic acid production pathway:

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (3)$$

Hydrogenotrophic methanogenesis:

$$CO_2 + 4\,H_2 \rightarrow CH_4 + 2H_2O \quad (4)$$

This result aligns with a previous study by Al-Sulaimi et al., (2022) on MEC-AD systemspre-acclimated with carbon-based material as acetic acid being the dominant pathway. However, this does not apply on the conventional digester, which might have beendominated by propionic or butyric acid's pathways.

Although M-MEC systems had the highest initial and final concentration of VFA's,followed by U-MEC, and AD, the accumulated VFA towards the end at hour 48 was 16 mM, compared to U-MEC with a value of 62.6 mM and 30.5 mM for AD. This could be attributed to the VFA's degrading microbes, namely Geobacter sulfurreducens, which oxidize VFAs, producing CO_2 and electrons (Fauque & Barton, 2012). To further support the statement, the previously reported results from sections (4.7.1 and 4.7.2) substrate degradation and current generation, where systems equipped with modified carbon felt anodes had the highest substrate degradation rates and current density compared tounmodified systems.Referring to the biomethane-produced values in table 4.5, M-MEC outperformed U-MEC and AD by two and three folds respectively of biomethane per g COD consumed,which means that the two stages prior to methanogenesis were efficient in fermenting the substrate for methanogenesis consumption, namely acidogenesis and acetogenesis as they are highly interconnected to methanogenesis(Detman et al., 2021). In addition, the biogas production from U-MEC and M-MEC systems did not cease after 48h. However, the accumulated VFA were higher in these systems, unlike AD, in which the biogas production ceased at hour 32, which means methanogenesis activity was inhibited, which might have occurred due to a pH drop with a value of 4.3. This also proves that a pathway other than acetic acid dominated the conventional digester.

3.3 Modified Gompertz Model of Biomethane Production from Different Inoculum

It has been reported that cattle manure has a high density and diversity of methanogenesis (Hwang et al., 2014). Enhancing the original inoculum, which is rich in fermentative and degradative microbes, with cow manure rich in the methanogenic community offers the essential microbial consortia for high performance. Hence, in this section, different inoculation to the MEC-AD modified electrode system, fed with hydrolyzed food waste, was run on three different cycles. In the first cycle, the system was inoculated with 10% of the original inoculum, namely, sludge from an anaerobic digester of POME. The second cycle was inoculated with 10% cow manure fed with food waste for one month. The third cycle was inoculated with 10% of a mixture of the previous two cycles. The kinetic studies of these cycles were fitted into the modified Gompertz model.

Table 2: Dynamically fitted parameters according to Modified Gompertz model

Inoculum	Fd ml/g COD	Rm(mL/h)	λ (h)	$R_{squared}$
Original	29.1	0.8754	11.42	0.9922
Cow-manure	31.24	0.825	12.61	0.991
Mixed inoculum	38.68	1.2	11.95	0.9923

The Biomethane production on the span of 72h of three systems was plotted in figure (2). Data collected from the model fitting were tabulated in Table (2). The coefficient of determination and R2 values for the modified Gompertz model was about 0.99 for all regression, showing a strong correlation between the experimental data and the fitted curve.

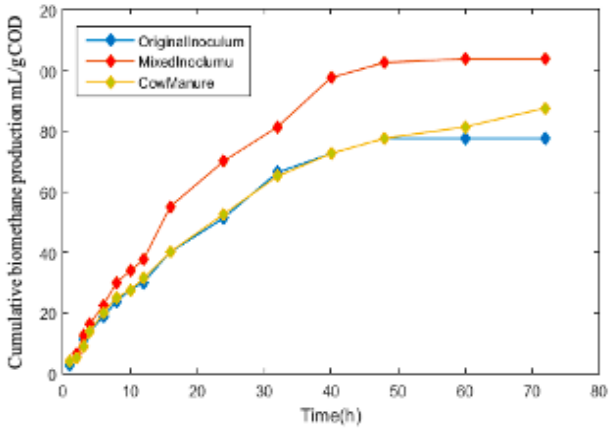

Figure 2: Cumulative biomethane production under different inoculation: Original inoculum, Cow-manure, and mixed of the previous inoculums

4 Conclusion

Modified electrodes have outperformed over the unmodified systems and conventional digesters regarding hydrolysis efficiency. Both hybrid systems were dominated by the acetic acid pathway, which is favourable for the upgrade of carbon dioxide to biomethane in the final digestion stage. Lastly, fitting the biomethane data from three different inoculations to the modified Gompertz model has shown that mixing the inoculum showed the best biomethane production rate and potential.

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