university of groningen

Investigating the effects of applied electricity and ammonia concentration on the performance and biogas production of anaerobic bacteria.

A master thesis submitted for the degree MSc Energy and Environmental Sciences

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1. Abstract

Anaerobic digestion (AD) is a method which produces biogas through the treatment of bio-waste. However, there are limitations in the process such as low conversion to biogas and poor quality of biogas (high CO_2 content) which limits the application of biogas. Multiple factors play a role in the operation of the AD process. Ammonia is necessary for microorganism growth, but can inhibit methanogenesis in high quantities. The first part of this paper investigates the effect of NH_4Cl concentration on the methanogenic activity. Reactors with 10 g/L NH_4Cl performed the best for biogas production at 59 ml/day, while reactors with 4 g/L NH4Cl resulted in the best biogas quality, with 56.7 mol% CH_4, and 43.7 mol% CO_2.

Secondly, there have been recent studies which demonstrated the improvement of methanogenic activity in AD systems through application of a milli-voltage, through a microbial electrolysis cell (MEC) reactor set up. Two-chambered MECs reactors are difficult and expensive to upscale. Thus, the second part of this paper investigates the effects of a single chamber MECs on the performance and biogas production of anaerobic bacteria. The data suggests that the addition of a milli-voltage is advantageous in terms of the volume of biogas production. Batch MEC experiments with 1.34 V / 0.006 A performs the best both in terms of production rate and total gas production. The batch MECs reactor is then set up as a continuous system for further application.

2. Introduction

In the present, a rapid increase in the energy demand has exhausted fossil fuel reserves and increased worries concerning climate change. As a result, there has been a shift in the focus from a fossil fuel-based energy model, increasingly towards renewables. One of the main sources of energy is natural gas, which is a hydrocarbon gas mixture mainly made up of methane. Biogas is a promising green alternative to natural gas, mainly composed of CH_4 (50-80 mol%) and CO_2 (20-50 mol%) , and is made through a process called anaerobic digestion (AD) [1][2]. The process involves many different groups of bacteria which carry out the process in consecutive steps, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1.) [3], [4]. Firstly, complex organic matter such as protein or polysaccharides are broken down by microorganisms in an oxygen-free environment into smaller chemical components through hydrolysis and acidogenesis. Methanogenesis, the last step, is carried out by anaerobic methanogenic Archaea which convert acetate, H_2, and CO_2 into methane. This happens through three different pathways: Reaction 1) acetotrophic pathway, Reaction 2) hydrogenotrophic pathway, and Reaction 3) methylotrophic pathway [4], [5] . Multiple factors play a role in determining the composition of biogas including the carbon oxidation-reduction state of the organic material, operation conditions, and the type of AD process [1].

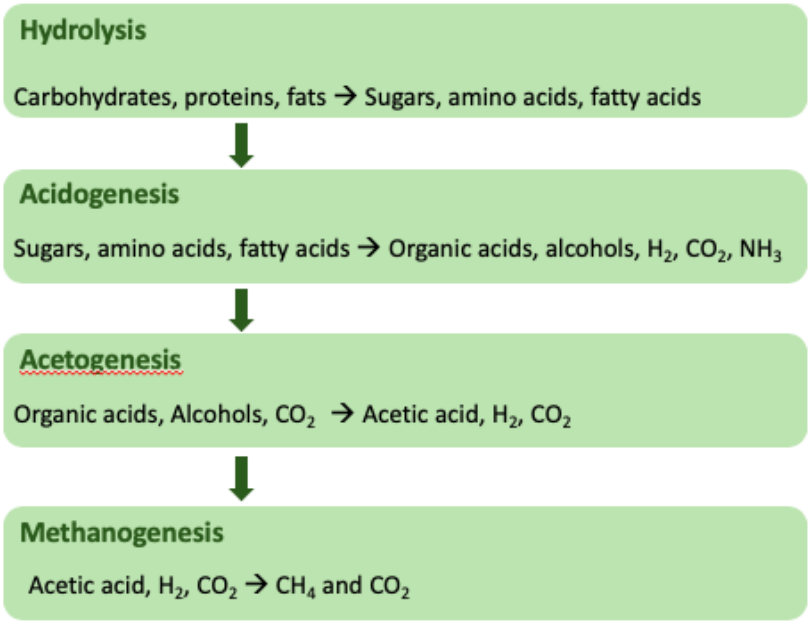


Figure 1. Schematic overview of the consecutive steps in the anaerobic digestion process. This involves hydrolysis of polymers to the formation of biogas through methanogenesis. [4][6]

1) $Acetotrophic\ pathway$ $\quad CH_3COOH \rightarrow CO_2 + CH_4$

2) $Hydrogenotrophic\ pathway$ $\quad CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

3) $Methylotrophic\ pathway$ $\quad CH_3OH + H_2 \rightarrow CH_4 + H_2O$ [6], [11]

Overview of the different methanogenic pathways. Reaction 1) Acetotrophic pathway, 2) Hydrogenotrophic pathway, and 3) Methylotrophic pathway.

AD can be used as a novel method for resource recovery through the combination of waste treatment with the production of a high value product [7]. Other than energy production, the process also has a wide range of environmental benefits as the process allows for odor reduction of waste, conservation of nutrients, and a reduction in greenhouse gas emissions [8]. There are many possibilities for different wastes to be used as the starting material, such as municipal sludge/wastewaters, animal manure, and industrial sludge, which allows for flexibility in the application of the method. However, a problem found when using complex substrates such as those in manure or municipal waste is that it contains traces of ammonia, produced through the degradation of nitrogenous matter [9]. Small amounts of ammonia are essential for the growth of microorganisms, but can cause inhibition at larger concentrations. The total ammonia nitrogen (TAN) is the value which depicts the total amount of nitrogen in the forms of NH_3 and NH_4^+ in water. Ammonium chloride is added as the TAN source, of which corresponds to 1/3 of the mass of NH_4Cl (NH_4^+: 18.039 u, Cl^-: 35.45 u) [10], [11]. A previous study showed that initial inhibition of AD reactors occurred at 30 g/L of NH_4Cl, and 50% inhibition was observed at 35 g/L of NH_4Cl [12]. The first part of this research paper is to investigate the effect of NH_4Cl concentrations lower than 30 g/L on the methanogenic activity of AD reactors. There are still many other problems surrounding the replacement of natural gas with biogas as conventional AD processes have low efficiency, with only 50-60% of the starting material

converted into CH_4 [13].Furthermore, the produced biogas typically contains a high fraction of CO_2 (20-50 mol%), resulting in a low calorific value [13], [2]. Other contaminants such as H_2S (0.01-0.4 mol%) and condensates of H_2O (0-12 mol%) causes corrosions of equipment within the gas grid, while high concentrations of O_2 (~2%) is an explosion hazard [14], [2]. Thus, the use of biogas is currently limited to on-site heat, steam, and electricity generation. For a larger application such as that of natural gas, biogas must reach high standards through cleaning and upgrading, in order to reach compositions of CH4 (95-99 mol%), CO_2 (1-5%), and no H2S [1], [15],[16]. There is a plethora of different upgrading methods of biogas of which may involve water/organic solvent/amine scrubbing, membrane technology, etc. [5]. However, these tends to be energy and resource intensive, and further produces waste which limits their use [17]. The ideal situation would be for the production process to be economically and environmentally viable, without compromising the quality of biogas. This would mean that the calorific value should be suitable for injection into the main gas grid with little to no energy requirement. However, methane production in traditional AD processes is limited by energy barriers, causing low electron transfer efficiency. A method to surpass these barriers could be through the use of bio-electrochemical systems (BES) [18].

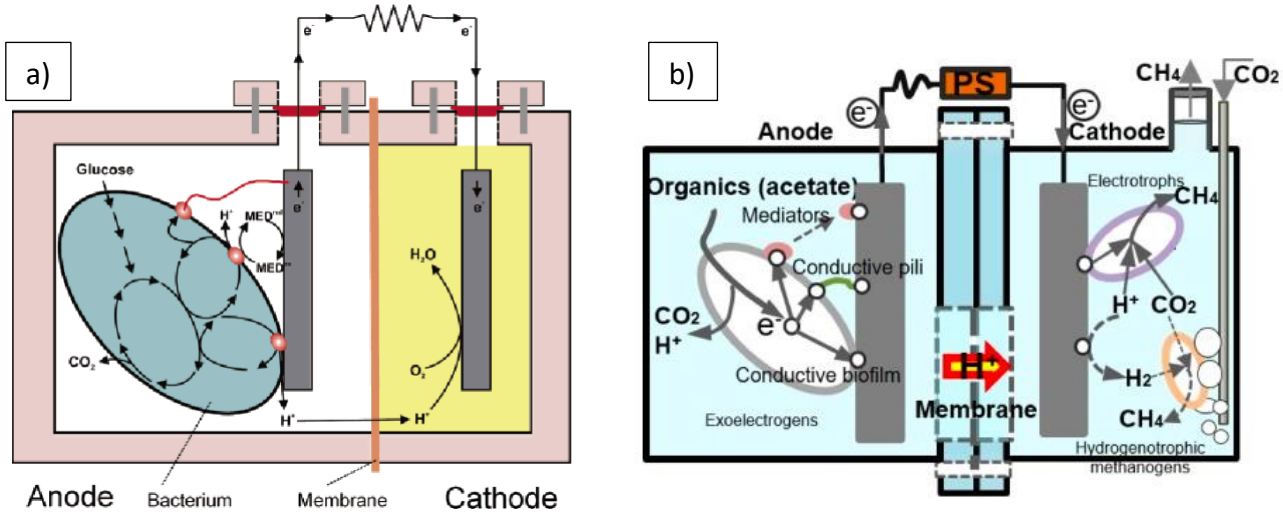


Figure 2. (a) Schematic diagram of the operating principles of a two-chamber microbial fuel cell (MFC). The Anodic chamber is under anaerobic conditions while the Cathodic chamber is under aerobic conditions. The bacterium in the anode compartment transfers electrons from an electron donor such as glucose to the cathode, while the protons travel through the proton exchange membrane (PEM)1. They then recombine with oxygen, forming water [18], [19]. Picture sourced from: [19]. (b) Schematic diagram of the operating principles of a two-chamber microbial electrolysis cell (MEC). Both chambers operate under anaerobic conditions.

Bio-electrochemical systems (BES) are emerging novel configurations, utilizing bacteria as a catalyst for organic waste treatment and subsequent recovery of resources such as bioelectricity, hydrogen, or biogas. This consists mainly of two different categories [20]. Firstly, microbial fuel cells (MFC) utilizes electrochemically active bacteria called exo-electrogens in order to oxidize organic waste within the anodic chamber, subsequently releasing electrons and protons (see Figure 2. (a))[21]. The protons flow through the proton exchange membrane to the cathodic chamber, while the electrons flow via an external circuit from anode to cathode, creating an electrical current which can then be utilized [22]. The proton exchange membrane (PEM) regulates ionic flux between both chambers, which could otherwise affect the pH balance of the system, in turn affecting the performance of the

methanogens [23]. Finally, electrons in the cathodic chamber, which is kept under aerobic conditions, combines with oxygen and protons to form water, although many other electron acceptors can be used [19], [20], [22]. The achieved working cell potential of this system has been shown to be approximately 0.4V, but theoretically has a potential of up to 1.1 V under neutral pH conditions[19], [21].

A modified version of this, and the focus of this paper, is called a Microbial electrolysis cells (MEC) (see Figure 2. (b)). It operates fully under anaerobic conditions, and requires an applied voltage in order to produce biofuels such as H_2 gas or further synthesize more complex, high grade chemicals such as CH_4 from waste such as CO_2 [18], [20]. As a fuel, methane has an advantage over H_2 as it can be easily stored or transported as opposed to H_2, so biogas is prioritized as the desired product in this paper [21]. Similar to MFCs, MECs operate using electrochemically active microorganisms attached to the electrodes as biofilms in order to anaerobically digest the substrate in the anodic compartment. However, the voltage produced at the anode is insufficient to drive the formation of H_2 ($E_{cell} \cong$ -0.414 V, pH=7), or the production of biomethane through the reduction of carbon dioxide (theoretical $E_{cell} \cong$ 0.244 V, pH=7) [21], [24]. Applying a small voltage of around 0.2-0.8V, substantially lower than that of conventional water electrolysis (1.23 V), allows for a reduction in the thermodynamic barrier, and the process can be fine-tuned depending on the applied voltage [18].

$$Anode \quad 4) \quad C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+$$

$$Cathode \quad 5) \quad 8H^+ + 8e^- \rightarrow 4H_2$$

$$6) \; CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$$

Overview of the half reactions occurring at the anode and cathode. Reaction 4) Oxidation of acetate at the anode, and the formation of H_2 or CH_4 at the cathode. [21][25]

Recent studies have shown that a significant amount of methane can be produced through MECs (see *table 1*). This is achieved through different pathways, mainly from acetate via acetoclastic methanogenesis, followed by H_2 and CO_2 via hydrogenotrophic methanogenesis (see *figure 1.*). Furthermore, there is evidence in literature that suggests the possibility that methanogens are able to receive direct electron transfer from the cathode for methanogenesis, without the need for hydrogen evolution [26][27]. This could potentially make the process more tolerant to toxic compounds such as ammonia, which could otherwise limit the activity of methanogens [28].Furthermore, there is literature that suggests the possibility of reduced nitrogen compounds such as ammonium as a source of electrons [29]. It was shown that ammonium oxidation can be coupled to H_2 production in MECs at potentials between +550 to +150 mV (vs. standard hydrogen electrode), and further used to produce methane.

The production of methane in MECs possesses multiple advantages over conventional AD reactions, firstly being that it produces biogas with higher methane concentrations. Furthermore, the process occurs at ambient temperatures and only requires milli-voltages in order to drive the process. However, there are still many difficulties with two-chambered MEC systems, specifically with upgrading to large scale applications. Furthermore, membranes such as PEMs can be expensive and adds difficulty to the operation of the system. It also affects the rate of diffusion, causing accumulation of protons at the anode, affecting the microbial activity, and increases internal resistance of the system [30]. As a result, single chambered MECs will be explored in this paper (see *figure 3.*).

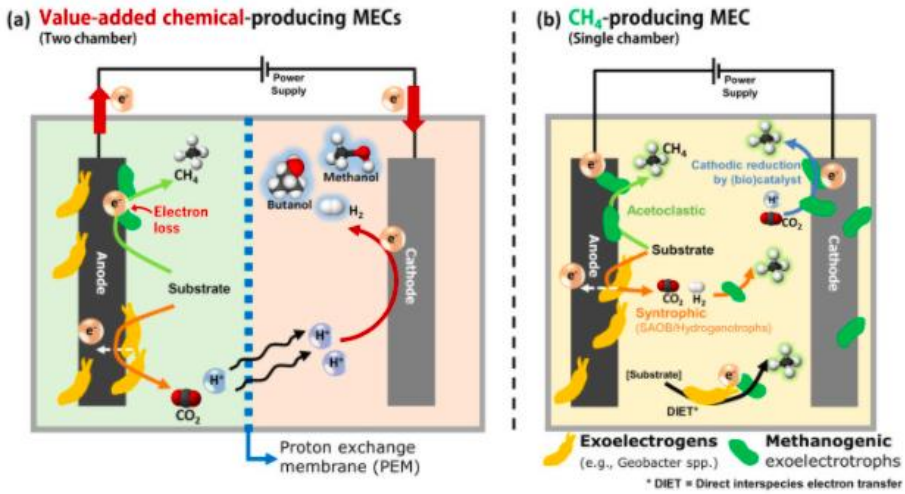


Figure 3. A schematic diagram of the operating principles of (a) two-chamber MECs reactor and (b) single-chamber MEC reactor. The main methanogens, along with the methanogenic pathways are depicted. [31]

The aim of this research paper is to investigate the effects of a microbial electrolysis system and ammonia concentration on the performance and biogas production of anaerobic bacteria. The research was split into 2 main experiments: 1) investigate the sensitivity of methanogens to different concentrations of ammonia in an AD set up in order to establish a baseline for future experiments with MECs; 2) investigate the feasibility of producing biogas using single chambered MECs in a continuous set up in order to increase the biogas production, quality, and improve the application prospects of this method. The second experiment was further divided into three main stages: A) set up batch reactors until the activity of the methanogens are stabilized and find a baseline for future reactions; B) apply electricity to the batch reactor in order to see the effect of a milli-voltage on the methanogenic activity; and C) set up a continuous microbial fuel cell system.

Table 1. Literature review on bio-electrochemical systems.

Reactor			Input voltage (V vs. SHE)	Substrate		CH4 yield (L/L.d)	Conclusion	Reference
Type	**Electrode**	**Operation**		**Type**	**Concentration (g/L)**			
Dual chamber	Anode: Graphitized carbon brush Cathode: Graphite drum/brush	Batch	+0.5	Organic matter in remnant waste water sludge	NG	-	Ammonium oxidation can be coupled to H2 production in microbial electrolysis cell	[29]
Single chamber			+0.55 to – 0.15			** Drum: 3 μmol $l^{-1}d^{-1}$ Brush: 8 μmol $l^{-1}d^{-1}$	-N2 can be oxidized to methane. -Brush cathode produced methane faster than drums	
Single chamber	Carbon cloth	Batch	−0.8	Acetate	1	0.093	Methane production was related to methanogen development within cathodic biofilm as stratification	[32]
Single chamber	Ni foam (spiral wound)	Batch	−1.3	Sodium acetate	1	0.17	optimal applied voltage of this system was about 0.95V	[33]
Single chamber	Stainless steel	Batch, Continuous (HRT: 4, 8, 12, 24)	−1.0	Sodium acetate	0.3	0.012	-Batch tests improved organic matter degradation and methane production rate. - Continuous tests more favorable at high HRTs. Anodic bacteria have a competitive advantage over acetoclastic methanogens.	[34]
Single chamber	Carbon fiber brush	Batch	-0.8	Dog food	15	0.775	hydrogenotrophic methanogenic pathway dominated.	[30]
Dual chamber						0.658	primarily acetoclastic methanogenic pathway	

*Notes: *NG: Not given, **= different units, SHE: standard hydrogen electrode, HRT: hydraulic retention time*

3. Materials and Methods

A. Normal pressure anaerobic digestion (NPAD) reactors

3.A.1. NPAD reactors: reactor set up

The NPAD reactors were set up in glass bottles with a volume of 500 ml, and the liquid phase was controlled to approximately 250.0 ml (see *figure 6*.). The bottle is securely sealed with a rubber stopper. The stopper is equipped with a gas valve connected to a needle positioned at the gas phase, and a valve for feeding and sampling, connected to a metal tube positioned to the top part of the liquid phase. The gas production was monitored with a water displacement bottle connected to the gas valve. The reactors were incubated in an incubator shaker (New Brunswick Scientific Co. inc. Incubator Shaker) at 36.5ºC, and a shaking speed of 150 rpm.

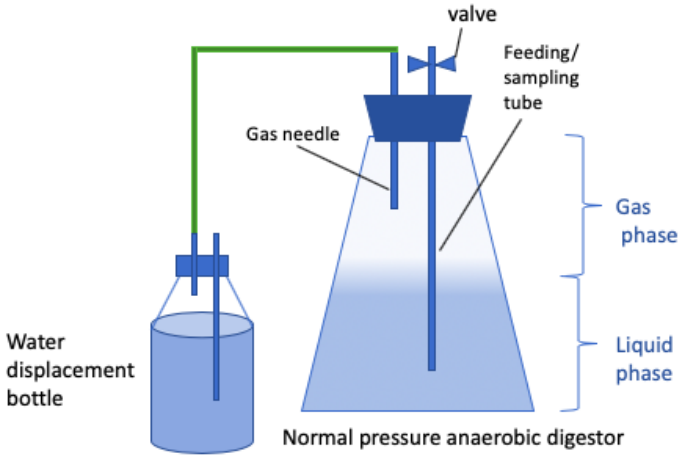


Figure 6. A schematic diagram of the set-up of NPAD reactors.

3.A.2. NPAD: Experimental set up

Reactors were inoculated with anaerobic sludge collected from an anaerobic digester treating aerobic sludge from Garmerwolde wastewater treatment facility located in the Netherlands. Synthetic wastewater was prepared according to [35] as follows: $K_2HPO_4.3H_20$ 3.6 g/L, KH_2PO_4 2.8 g/L, $NaHCO_3$ 5 g/L, glucose 6.8 g/L, trace element solution 1.0 ml/L, 1.0 ml/L Wolfe's vitamin solution, and 1.0 ml/L of a pre-prepared Cysteine sulfide reducing agent (L-Cysteine·HCl·H_2O and $Na_2S·9H_2O$) dissolved in deionized water. Different concentrations of NH_4Cl were added in order to make synthetic wastewater with the following concentrations: 2 g/L, 4 g/L, 6 g/L, 8 g/L, 10 g/L, 12 g/L. Equal amounts (100 ml) of Synthetic wastewater and anaerobic sludge were added into each reactor. The experiment was duplicated for each variable (see *table 2*.). The NH_4Cl concentrations of the control reactors 1 & 2 were changed from 2 g/L to 24 g/L starting from day 26 by changing the feed buffer in order to investigate the effect of higher NH_4Cl concentrations.

Table 2. The reactors with their respective NH_4Cl concentration in g/L

Reactor number:	**1 , 2**		**3,4**	**5,6**	**7,8**	**9,10**	**11,12**
NH_4Cl concentration (g/L)	2	24	4	6	8	10	12
Total N	0.7	8	1.3	2	2.7	3.3	4

Gas samples of 10.0 ml were taken every other day through the gas valve for analysis using GC. Using a syringe, 15.0 ml liquid samples were taken daily from the reactors and the volume was replaced with a fresh feed of synthetic wastewater with their respective NH_4Cl concentrations. The hydraulic retention time (HRT) of the reactors are 13.33 days. The water displacement bottles were weighed daily in order to calculate the daily gas production.

3.A.3. Expected gas production

$$Feed\ \ 15\ ml\ /day$$

$$Glucose\ concentration{:}\ 6.8\frac{g}{L}$$

$$Mass\ of\ glucose\ in\ feed{:}\quad 6.8\frac{g}{L}\times 0.015\frac{L}{day}=0.102\frac{g}{day}$$

$$=\frac{0.102}{180.156}\frac{g}{\frac{g}{mol}}$$

$$=0.566\ mM\ of\ glucose\ in\ feed\ daily$$

$$C_6H_{12}O_6\rightarrow 3CO_2+3CH_4 \qquad \therefore 1\ mol\ glucose{:}\ 6\ mol\ biogas$$

$$1\ M\ gas=24\ dm^3$$

$$If\ 100\%\ conversion{:}\ 0.566\ mM\ glucose\ \times\ 6$$
$$\approx 3.4\ mM\ biogas\times\ 24000\ ml=81.6\ ml\ biogas/day$$

3.A.4. Physical and chemical analysis

The pH of the samples was analyzed using a digital pH meter (H160, Hach, Germany). The samples were then centrifuged for 10 minutes at 1000 rpm. The supernatant was then analyzed by titration using an auto-titrator (AT1000, Hach, Germany) with 0.1 M H_2SO_4 to the end points of pH 5.0 and 4.4. This gave the results for the volatile fatty acids content (VFAs) and total alkalinity (TA). The biogas compositions of the gas samples were analyzed using gas chromatography (C2V-200 Micro GC, Thermo Scientific). The machine was equipped with a thermal conductivity detector and a GCC200-U-BND cartridge. The injector and detector had a temperature of 120°C, while the column was at 60°C. The carrier gas used was helium.

B. Microbial electrolysis cell (MEC) reactors

3.B.1 MEC: Reactor set up and analysis

A potentiostat was aimed to be used to control the applied voltage in the electrical system. This was set-up according to instructions mentioned in https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0201353#sec002, and consists of a PSoC 5LP (part number CY8CKIT-059) Cypress Semiconductor. The PSoC was inserted into the computer through the USB programmer end (male end), and using the free PSoC Programmer from Cypress Semiconductor (found at :https://www.cypress.com/products/psoc-programming-solutions), the internal firmware was firstly upgraded. Then the necessary file for the potentiostat was uploaded into the device (found at: https://github.com/KyleLopin/PSoC-Potentiostat). With the device removed, wires for the working electrode and counter electrode were connected in the pins of their respective places. The female end of the device was then connected through a USB cable into the computer. The free program called Zadig (found at: https://zadig.akeo.ie) was used to install a generic USB driver which allows communication with the device. The graphical user interface for the potentiostat from Github (found at: https://github.com/KyleLopin/Potentiostat_GUI/releases) could then interact with the device. In practice, this did not work, thus, a power supply with a constant voltage mode was used as an alternative (Velleman PS3005D).

Phase 1: Establish base line through batch experiments

A baseline for the reactors were first established by setting up the batch reactors without electricity (see *figure 4*.). Two reactors were set up using the Eppendorf single use bioreactor BioBlu®. Metal needles were used to hold the carbon felt electrodes (~6.0 cm x 1.0 cm) within the reactors, and the open-ended hub was tightly sealed using a plastic cap in order to stop air flow into the reactor. The metal needles were connected using wires to the power source. However, during this phase no electricity was applied to the system. A sampling/feeding tube was connected to the outlet port, which is connected to a plastic tube positioned within the reactor at the top part of the liquid phase. A gas valve was used to connect Gas counters (Ritter MGC-1 V3.4 PMMA) to the gas port, which is positioned to take samples from the gas phase. The gas counters are connected to a raspberry pi programmed to measure and log the results in order to constantly monitor the gas production. The reactors were inoculated with approximately 115 ml of anaerobic sludge and 115 ml of synthetic wastewater as previously mentioned in section 3.A.2, making up a total volume of approximately 230 ml. No NH_4Cl was added to the synthetic wastewater. The reactors were kept in a water bath at 39^{o}C (Julabo D 7633, Germany), and stirred using an electric motor (XINDA motor Co., LTD DX-3420) set on low. Using a syringe, 10 ml of 30 g/L glucose solution was fed daily through the feeding/sampling valve. The gas counters were disconnected during feeding as to not count the gas flow due to the addition of the liquid to the system. No volume was removed until the gas production reached a stable rate. During the operation of phase 1, the reactor set ups were made sure to be entirely air tight, and the gas counters were calibrated in order to show the correct amount of gas production.

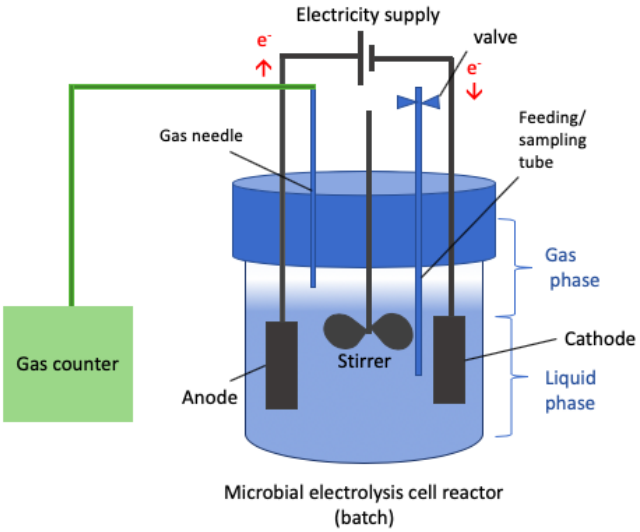


Figure 4. A schematic diagram of the set-up of batch MECs reactors. Electricity was not applied during *phase 1*. Electricity was supplied in later phases using an energy source (Velleman PS3005D).

Phase 2: Application of electricity to operate MEC reactors

The batch experiments were operated for a total of 45 days. Initially, the batch reactors were run for 24 days without applied electricity until it reached stable gas production. On day 24, a total of 100 ml of liquid was removed from the reactors, equivalent to the total amount of feed fed since the beginning of the experiment. A sample of 15 ml was kept for later analysis in -20°C. The gas counters were disconnected from the gas outlet and replaced with a syringe filled with N_2 gas. This ensured anaerobic conditions. When liquid was withdrawn from the reactors, N_2 gas is drawn into the headspace instead. After the liquid was removed, the gas counters were reinstalled. The reactors were left to run over night for one more day in order to remove the N_2 gas in the headspace. The next day (day 25) gas bags were connected to the gas counters in order to collect the gas produced from the reactors. Wires were used to connect the electrodes of reactor 1 to the power supply (Velleman PS3005D). The voltage was set to 10 V and the maximum current to 0.01 A. The voltage is set higher than needed as to by-pass the limitations of the power supply and operate the machine in constant current mode. Once turned on, the voltage is able to increase until it reaches the set value for the current. The power supply records a voltage of 1.34 V and a current at 0.006 A. These conditions were operated for 4 days. Reactor 2 is the control. It contains electrodes but does not have a power supply. The reactors were stirred using an electric motor as mentioned previously. Using a syringe, 10.0 ml samples were taken daily (aside from weekends) through the sampling valve from each reactor, and kept in -20°C conditions for later analysis. The volume was then replaced with 10.0 ml of 30 g/L glucose solution. The hydraulic retention time (HRT) of the reactors is 23 days, and an organic loading rate (OLR) of 1.3 g/L day. Gas samples of 10.0 ml were taken daily from the gas bag using a syringe and analyzed using GC as previously mentioned in 3.A.4. The gas bag was then emptied in order to collect new gas samples for the following day.

On day 28, the current on the power supply was shown as 0 A due to corrosion of the cathode, which interrupted the circuit. As a result, the needle was replaced with a metal tube. The opening was sealed with a rubber tube, tied at the end so as not to allow air into the reactor. The electrode material used was the same as previously mentioned. The voltage was set to 5 V and the current at 0.05 A. Once turned on, the power supply records a voltage of 2.21 V and 0.003 A. These conditions were operated for 3 days. On day 31, the power supply was set to a voltage of 0.6 V and a current at 0.05A. Once turned on, the power supply records a voltage of 0.6 V and a current at 0 A. The power supply does not seem to be sensitive enough to record microcurrents. These conditions were operated for 14 days. The GC results signified that the gas samples taken via the gas bags were being diluted. Thus, the gas bags were removed on day 38 and samples were taken from the overhead chamber instead. During this process, all ports were closed with clamps, and the volume was replaced with N_2 gas via the gas port.

Phase 3: Making a continuous MEC reactor

On day 45, the batch experiments were stopped. Due to acidification of reactor 1, the contents of the reactor were discarded. Reactor 2 was set up as a continuous BES reactor (see *figure 5*.). The cathode was replaced with a metal tube, following the same procedure as previously mentioned in *Phase 2* to avoid rapid corrosion. Wires were used to connect the electrodes of reactor 2 to the power supply (Velleman PS3005D). The power supply was set to a voltage of 0.6 V and a current at 0.05 A. Once turned on, the power supply records a voltage of 0.6 V and a current at 0 A. A syringe filled with 120.0 ml of 13 g/L glucose solution was connected via a tube to the LA(O) port for feeding, while an empty syringe was connected via a tube to the sampling valve for sampling. Both syringes were placed within a syringe pump (INACCOM instruments, NE-1000X) set at 0.96 ml/hour in opposite directions. The HRT of the reactors is equal to that of the batch MEC reactors, at 20 days. The contents of the sampling syringe were emptied daily, of which 10.0 ml was taken for further analysis and kept under -20^{o}C conditions. The syringe for feeding was re-filled every 5 days. The reactor was stirred using an electric motor to ensure the reactor contents are homogenously mixed. Gas samples were taken from the headspace following the procedure as mentioned in *Phase 2*. For analysis, the samples kept under -20^{o}C were thawed and centrifuged at 8000 rpm for 20 minutes. The pH of supernatant was determined using a digital pH meter (H160, Hach, Germany). The VFAs in the supernatant of the samples were determined using high-performance liquid chromatography (Agilent Technologies 1200 series) which was equipped with a Bio-Rad Aminex HPX-87H 300 x 7.8 mm column at 60^{o}C and UV-detector at 210 nm. The eluent used was 5 mM H_2SO_4 at a rate of 0.5 mL/min.

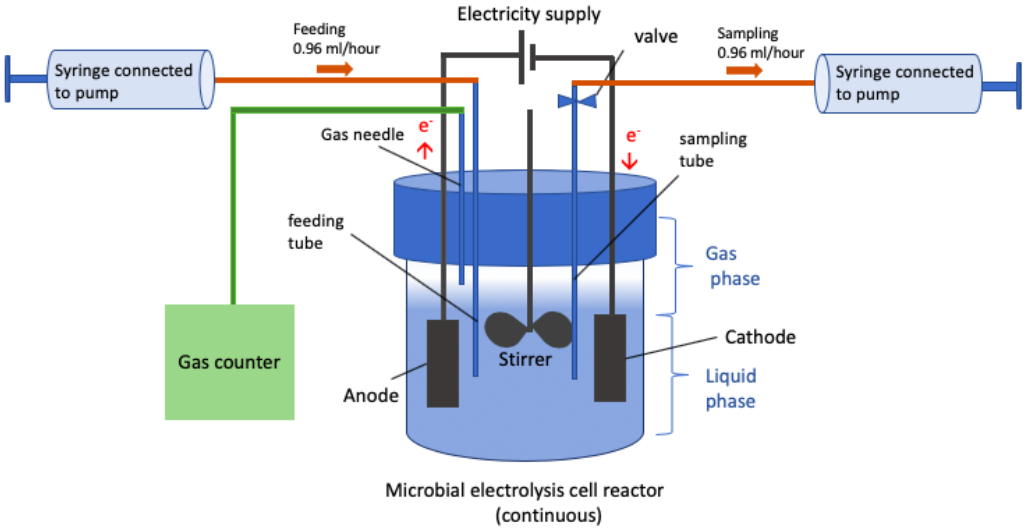


Figure 5. A schematic diagram of the set-up of a continuous MEC reactor.

3.A.2. Expected gas production of batch and continuous MECs reactors

Table 3. Calculations for the moles of glucose in feed for batch and continuous reactor

	Batch reactor	Continuous reactor
$Feed$:	$10\ ml\ /day$	$0.96\frac{ml}{hour} \times\ 24\ hours$ $= 23\ ml\ /day$
$Glucose\ concentration$:	$30\frac{g}{L}$	$13\frac{g}{L}$
$Mass\ of\ glucose\ in\ feed$:	$30\frac{g}{L} \times 0.010\frac{L}{day}$ $= 0.3\frac{g}{day}$	$13\frac{g}{L} \times 0.023\frac{L}{day}$ $= 0.299\frac{g}{day} \approx 0.3\frac{g}{day}$
$Moles\ of\ glucose\ in\ feed$:	$\frac{0.3}{180.156\frac{g}{mol}} = 1.7\ mM\ of\ glucose\ in\ feed\ daily$	

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4 \qquad \therefore 1\ mol\ glucose : 6\ mol\ biogas$$

$$1\ M\ gas = 24\ dm^3$$

$$If\ 100\%\ conversion: 1.7\ mM\ glucose\ \times\ 6$$
$$\approx 10\ mM\ biogas \times\ 24000\ ml = 240\ ml\ biogas\ per\ day$$

4. Results and discussion

4.A Normal pressure adiabatic (NPAD) reactors

4.A.1 NPAD reactors: Biogas production and quality

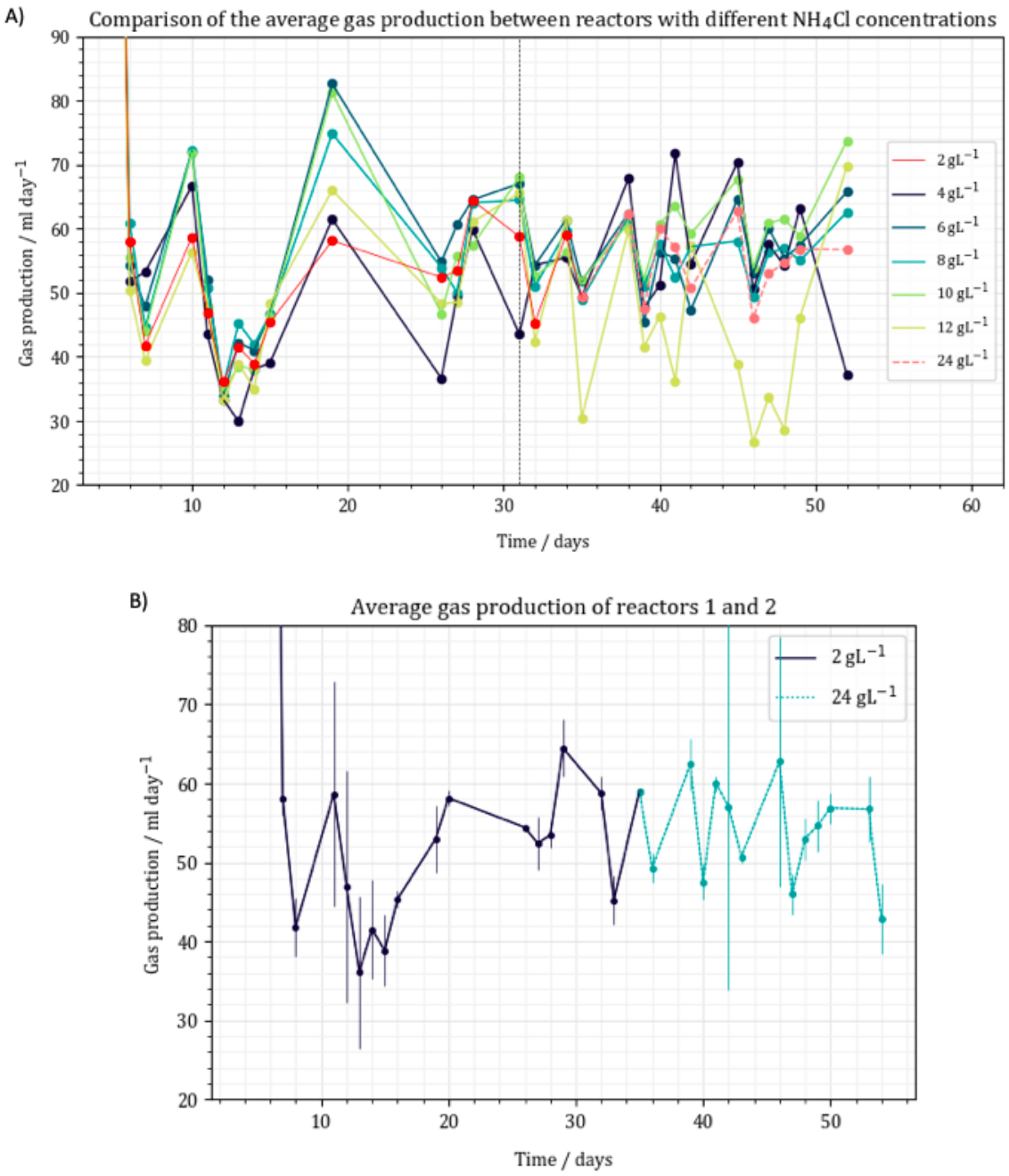


Figure 6. Daily gas production. A: Comparison of the daily average gas production of NPAD reactors with different NH_4Cl concentrations between 2-24 g/L. B: The daily average gas production of NPAD reactors 1&2 at concentrations 2 and 24 g/L.

Table 4. The total average gas production of reactors with different NH_4Cl concentrations after the stabilization period (first 30 days) and the

NH_4Cl concentration (g/L)	**Total average gas production (ml/day)**	**Standard deviation**	**yield (%)**
2	57	8	70
4	57	9	69
6	57	6	69
8	56	5	68
10	59	6	72
12	43	18	52
24	55	6	67

Looking at *figure 6. (A),* it can be seen that the gas production of all reactors follows a general trend and fluctuates around approximately 50 ml/day. The oscillation is largely due to absence during the weekends, where no feeding, sampling, or measurements were done. The increase in the biogas production is due to the continued conversion of organic carbon within the reactors to biogas during the weekend. This indicates that organic acid residues are present in the liquid phase. The largest fluctuations in gas production can be seen in the first 30 days, which is needed for stabilization of the reactors. This is signified by the vertical black dashed line. In the second half of the experiments after this line, it can be seen that the gas production is more stabilized.

The expected trend is that with an increase in NH_4Cl concentration, the performance of the reactors will decrease. However, no significant trend could be observed in the first 30 days during the stabilization period, and often reactors with higher NH_4Cl concentrations were performing better. As a result, the concentration of NH_4Cl of the feed for the control reactor was significantly increased from 2 g/L to 24 g/L in order to observe a larger change. Looking at reactors 1 & 2 in *figure 1 (B)* after the concentration of the feed was changed to 24 g/L, it can be seen that the trend in the gas production does not significantly vary from previously and performs similarly as reactors with NH_4Cl concentrations between 6-10 g/L. The total average gas production does decrease slightly *(*See *table 4)*. However, due to the stabilization period it is hard to determine an accurate trend for the activity with 2 g/L, so an accurate comparison cannot be made. Furthermore, the reactors have a retention time of 20 days so it was not enough time to see the full effect of the large increase in NH_4Cl concentration.

Overall, reactors with 4 g/L of NH_4Cl can be seen to reach the highest amounts of gas production, but overall reactors with 10 g/L had the highest total average gas production (table 3.) at 59 ml/day, corresponding to 72% efficiency. Reactors with 12 g/L can be seen to perform the worst, and also has the lowest total average gas production at 43 ml/day, corresponding to 52% yield. However, reactors with 4 g/L and 12 g/L had the highest fluctuations, suggesting that they are the least stable. Reactors with concentrations in between these values do not follow a specific trend in the gas production, but their activity is more stable. Nitrogen is an important nutrient for microorganism's growth. The results suggest that AD systems can tolerate small concentrations of NH_4Cl and may even aid in the stability of the reactors. Concentrations of 2 g/L may not be sufficient for microorganism growth while 24 g/L may be too high, resulting in inhibition. Thus, the highest gas production was within this range, with

results showing that 10 g/L of NH_4Cl being the best for gas production. However, a small increase to 12 g/L of NH_4Cl can significantly affect the stability of the reactors and cause a ~20% decrease in the yield. More research is needed to see the full effects for larger concentrations such as 24 g/L.

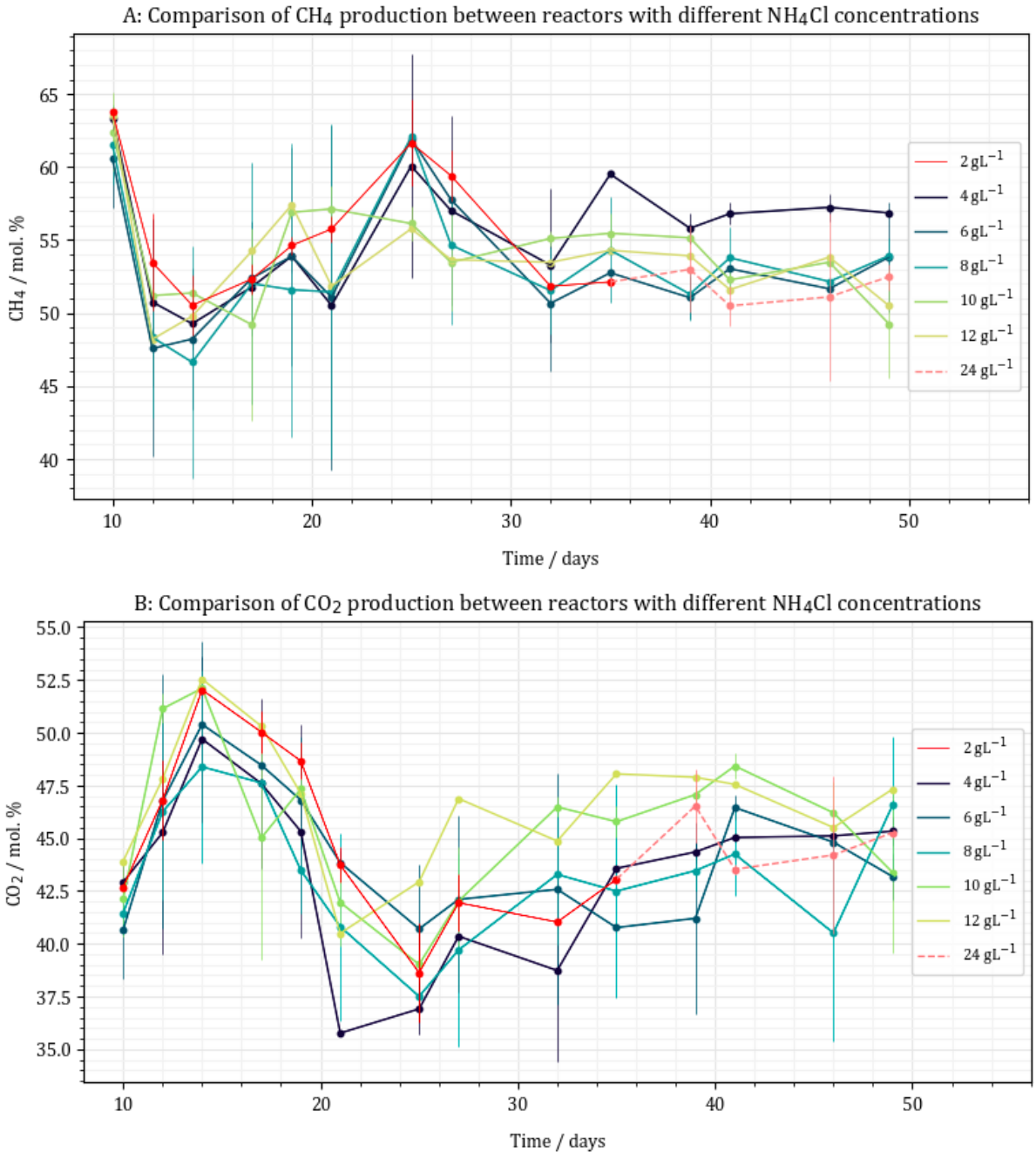


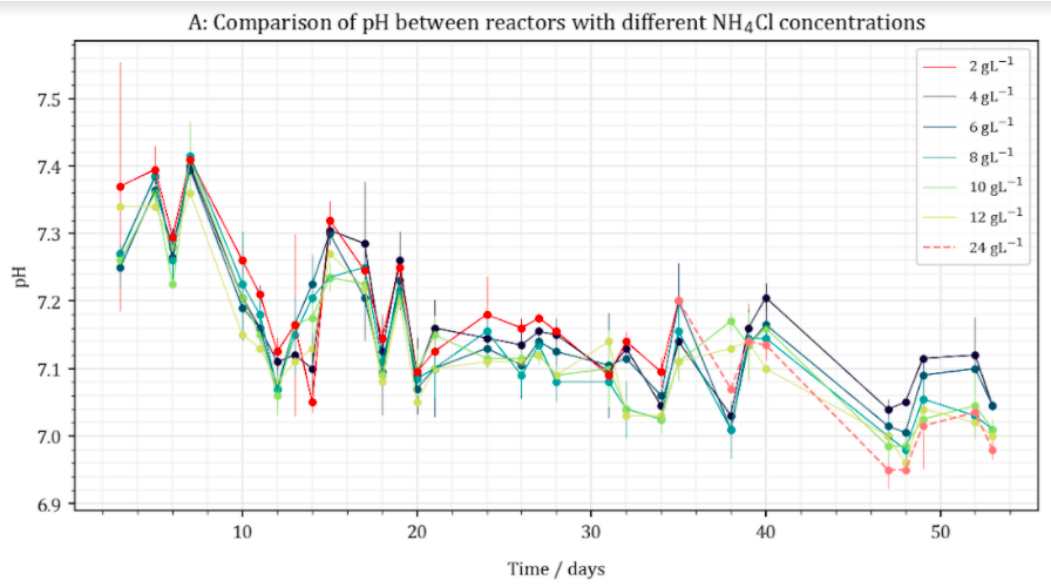
Figure. 7. Biogas quality. A: The average amount of methane (mol %) in the biogas sample from reactors of different NH_4Cl concentrations between 2-24 g/L. B: The average amount of carbon dioxide (mol %) in the biogas sample from reactors of different NH_4Cl concentrations between 2-24 g/L

Table 5. Average fractions of CH_4 and CO_2 in biogas produced from reactors with different NH_4Cl concentrations

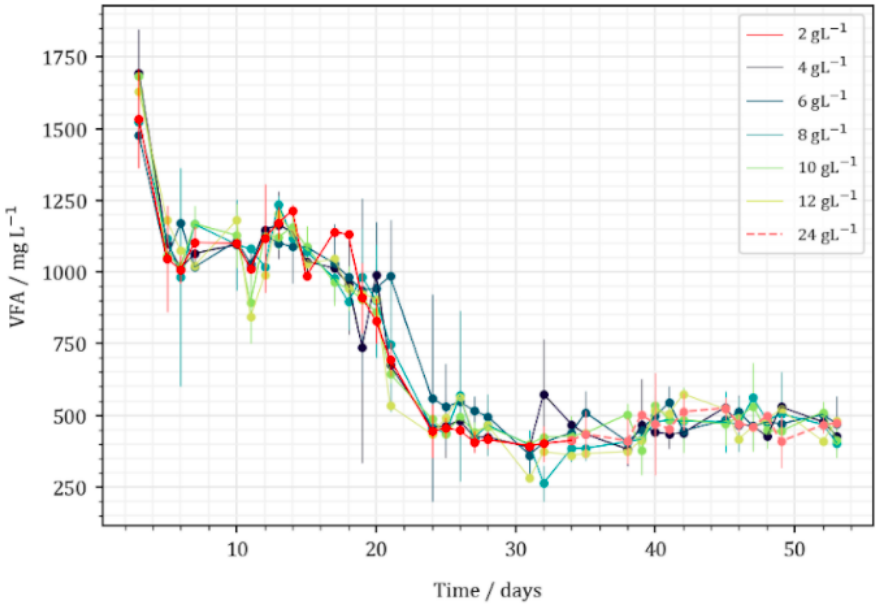
NH_4Cl (g/L)	CH_4 (mol %)	s.d	CO_2 (mol %)	s.d
4	56.6	1.38	43.7	2.51
6	52.2	1.23	43.2	2.17
8	52.8	1.33	43.4	2.00
10	53.5	2.40	46.2	1.67
12	52.9	1.53	46.9	1.34
24	51.8	0.92	43.9	1.90

Looking at *Figure 7.*, it can be seen that the largest fluctuations in biogas quality for both the CH_4 and CO_2 fractions can be seen in the first 30 days, which coincides with the stabilization period seen in the biogas production (*Figure 6.*). Looking at *Figure. 7 (a)*, it can be seen that reactors with 4 g/L NH_4Cl has the largest CH_4 fraction, with an average of 56.6 mol %, while the reactors with 24 g/L NH_4Cl has the lowest average, at 51.8 mol % (*Table 5*). Reactors with 10 & 12 g/L NH_4Cl have the highest CO_2 fraction at 46.2 and 46.9 mol %. The results confirm that with less NH_4Cl, there is an improvement in biogas quality, with an increase in CH_4 and a decrease in CO_2. However, there seems to be no obvious trend with NH_4Cl concentrations between 6-10 g/L. Reactors with 10 g/L NH_4Cl performs the best for biogas production, but is less advantageous in biogas quality as it has the second highest mol% of CO_2 following reactors with 12 g/L NH_4Cl.

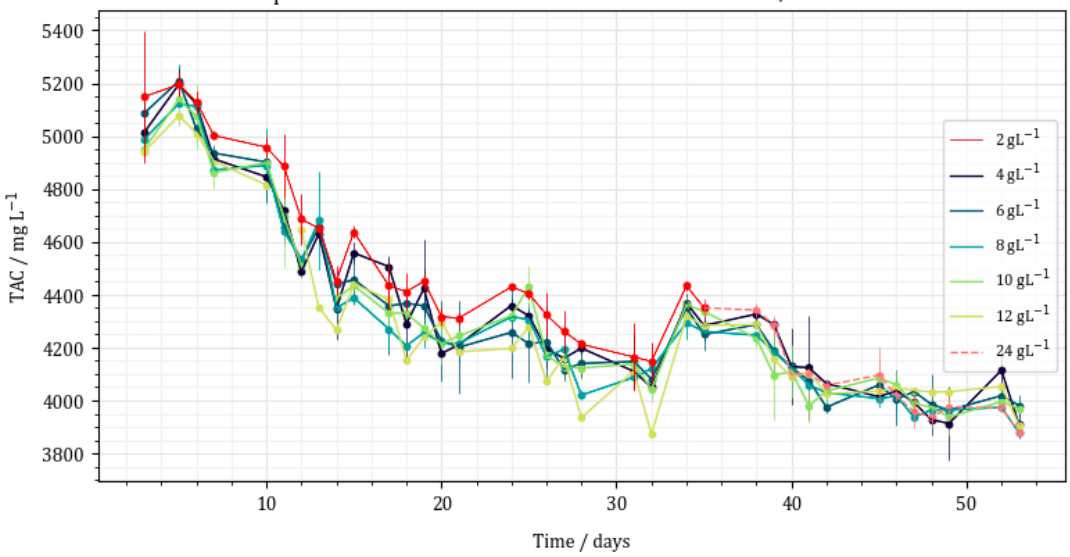
4.A.2 NPAD reactors: pH, alkalinity, and volatile fatty acids (VFAs)



B: Comparison of VFA between reactors with different NH_4Cl concentrations



C: Comparison of TAC between reactors with different NH_4Cl concentrations



D: Comparison of VFA/TAC between reactors with different NH_4Cl concentrations

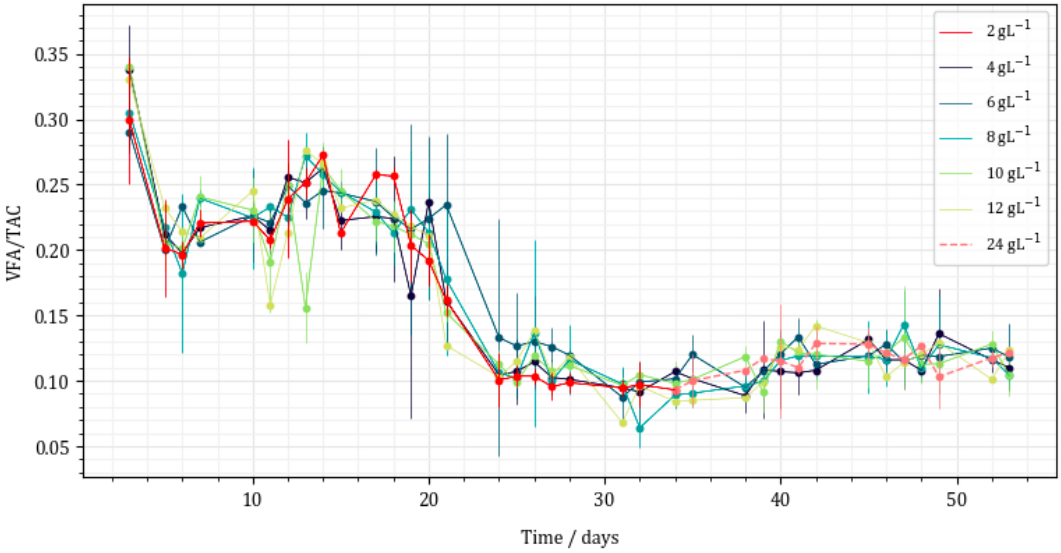


Figure 8. A: The average pH of reactors with different concentrations between 2-24 g/L.
B: The average VFAs concentration of reactors with different concentrations between 2-24 g/L.
C: The average TAC of reactors with different concentrations between 2-24 g/L.
D: The ratio VFA/TA of reactors with different concentrations between 2-24 g/L

The optimal pH range for the activity of methanogens is between 6.8-7.8 [36][37]. The pH of the reactors under the given conditions are within this range (*see figure 8.A*). However, the general trend shows that the reactor contents are acidified over time. This is trend is also seen in the behavior of the buffering capacity (TAC) (see *figure 8.C*). The TAC value corresponds to the buffer capacity of the system and is measured with respect to mg $CaCO_3$/L [38]. Once the buffering capacity decreases, the acidity of the VFAs cannot be as efficiently neutralized, resulting a continuous drop in the pH. As the TAC value continues to decrease, the pH is also expected to continue to decrease which is disadvantageous to the methanogenic activity for longer reactor operations. Comparing the pH of different reactors following the initial stabilization phase, it can be seen that with an increase in NH_4Cl concentration, the reactors become more acidic. However, the pH of the reactors is still above the lower limit of the optimal pH range (6.8), and thus in the range of optimal activity for methanogens.

Initially, the volatile fatty acids (VFAs) concentration of the reactors is high as a large amount of VFAs are present in the sludge, which limits the biogas production and coincides with the initial 30-day stabilization phase. This initial peak decreases as the methanogens metabolize VFAs into methane. There is no distinct trend between reactors with different NH_4Cl concentrations and VFAs concentration. The VFA/TAC ratio is an indicator for assessing the fermentation process. The values for a well operated digester range between 0.1-0.35. The VFA/TAC ratio of the different reactors throughout the experiment is within this range, indicating good fermentation activity and no significant problems.

B. Microbial electrolysis cell (MEC) reactors

4.B.1 MEC: batch reactors

gas production & quality

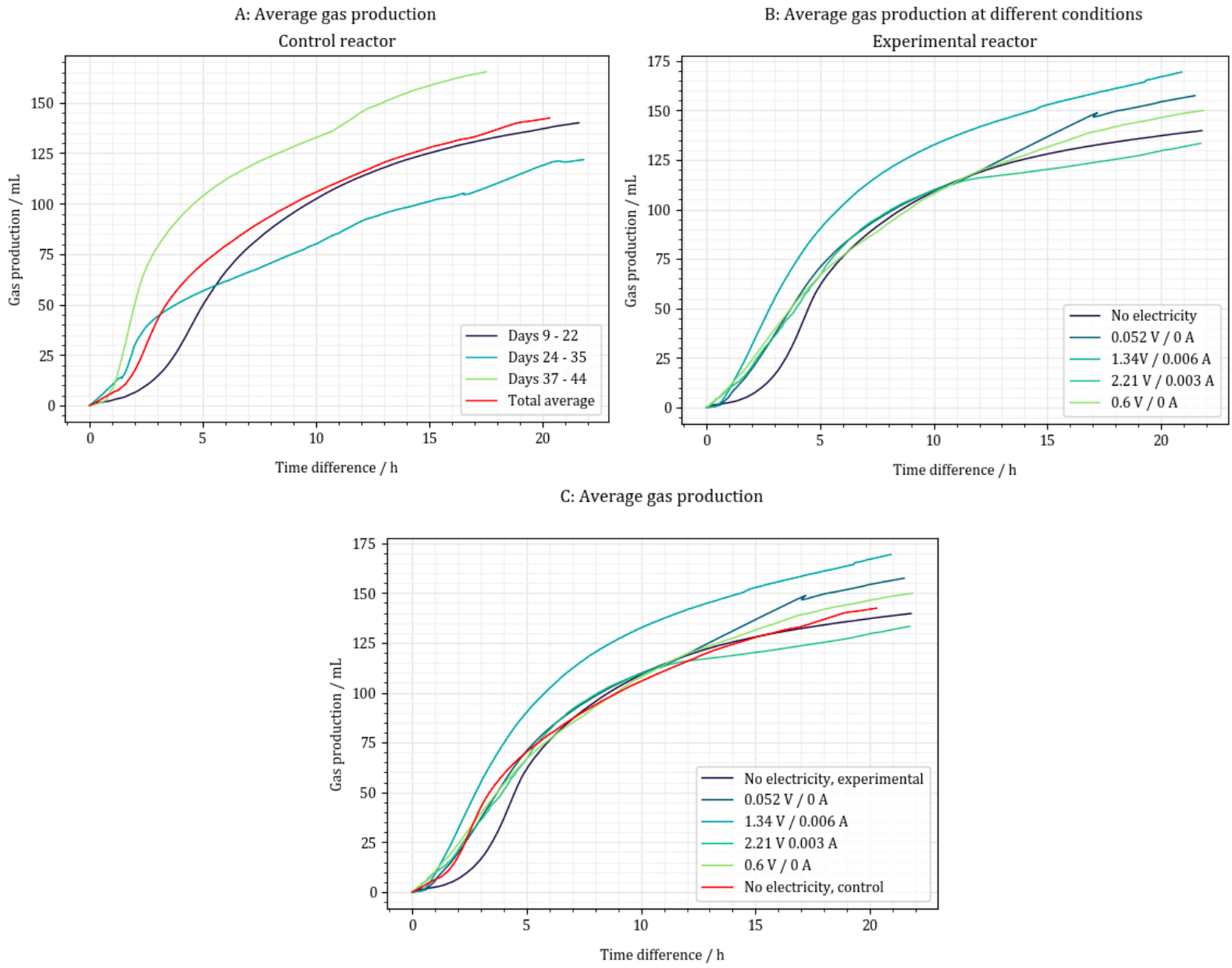


Figure 9. The average gas production of different reactors. A: the average gas production of the control reactor, split into three different time phases. B: the average gas production of the experimental reactor during operation with different applied potentials, and the resulting current. C: the results of the average gas production of the control and experimental reactors overlaid. The control reactor is portrayed as the total average, while the experimental reactor is shown during operation with different applied potentials, and the resulting current.

Looking at the average cumulative gas production in *figure 9*, it can be seen that there is a general trend where the biggest increase in the gas production occurs within the first 10 hours after feeding, and then leveling off. The average gas production of the control group, can be seen in *figure 9.A*. Overall, the rate and total gas production increases from days 9-22 to days 37-44. However, the graph for days 24-35 does not follow this trend, performing the most poorly. The graph for this period is also the least uniform, indicating that the results may not be reliable. This may be due to interruption in the stirring of the reactor, which effects the digestion and activity of the methanogens, or problems with the gas counter.

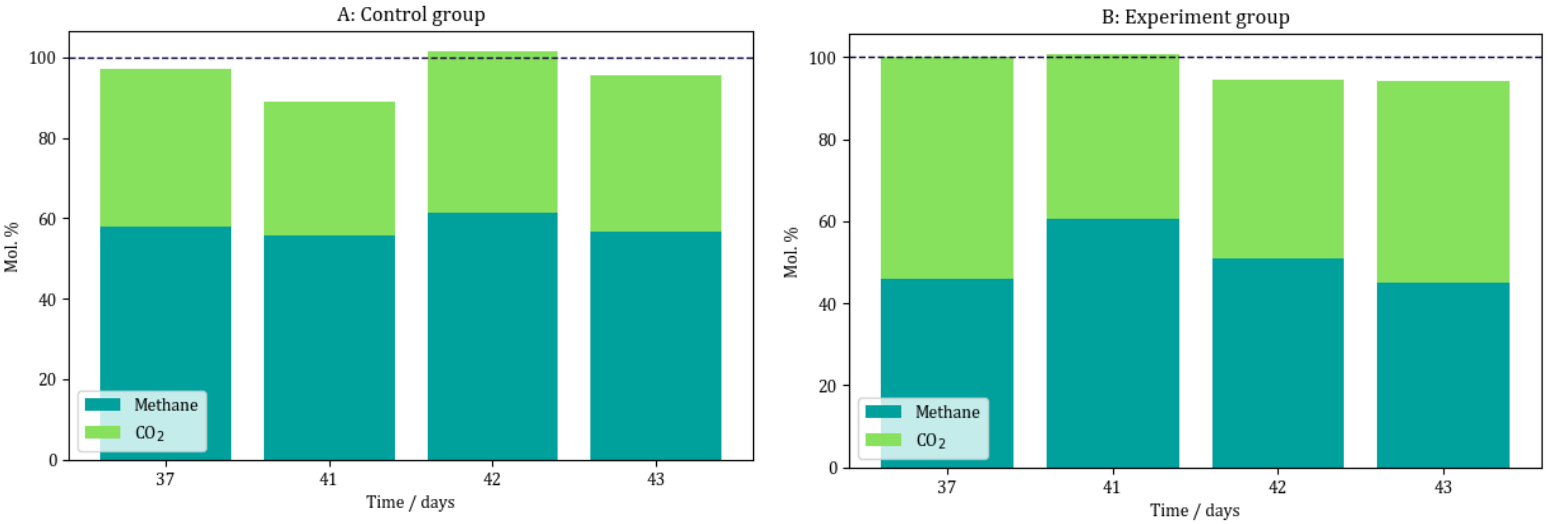


Figure 5. The biogas quality analysis results following GC technique of A: the control reactor B: the experimental reactor.

The average gas production of the experimental group, can be seen in *figure 9.B*. The addition of electricity increases the rate of gas production compared to the initial runs without electricity. However, as it was shown in the control group, the rate of gas production should increase with time, so this cannot be entirely attributed to the addition of electricity. Comparing the experimental runs with electricity, it can be seen that that with 1.34 V / 0.006 A performs the best both in terms of production rate and total gas production. However, the voltage exceeds the theoretical value at which water electrolysis takes place (1.23 V). In practice, higher voltages (1.8 V) are needed but some of the increase in gas production may be due to the production of oxygen and hydrogen [39]. No reference electrode was used in the set up so it is difficult to determine the real voltage of the system compared to the reference voltage. Furthermore, due to problems with the gas bags, the gas samples during this time were not reliable so information on the gas composition is not possible. Hydrogen can be used in the hydrogenotrophic and methylotrophic pathway, as a result, further gas production may have been encouraged due to the increase in hydrogen. However, oxygen limits the methanogenic activity. The following experimental run was with 2.21 V / 0.003 A, which initially performed at a rate similar to that of runs with conditions of 0.052 V / 0 A and 0.6V / 0 A, but the production rate slowed after approximately 5 hours, resulting in the lowest gas production. The voltage in this experiment was highest, which may disrupt the activity of the methanogens. Furthermore, if oxygen was produced in the previous experiment, this may further limit methanogenic activity. The experimental runs with 0.6 V / 0 A and 0.052 V/ 0 A behaved similarly due to the similarity in the conditions. The total gas production for 0.052 V/ 0 A was

slightly higher, which may indicate that smaller milli-voltages may be more advantageous to methanogenic activity, although more research must be done in order to confirm this. Compared to the average gas production of reactor 2 with no electricity, it can be seen that MECs with 1.34 V / 0.006 A overall performed the best both in terms of production rate and total gas production, while the remaining MECs have a slower production rate compared to that of reactor 2. However, the total gas production of 0.6 V / 0 A and 0.052V/ 0 A was higher than that of reactor 2, so the addition of a milli-voltage may be advantageous in terms of the volume of biogas production.

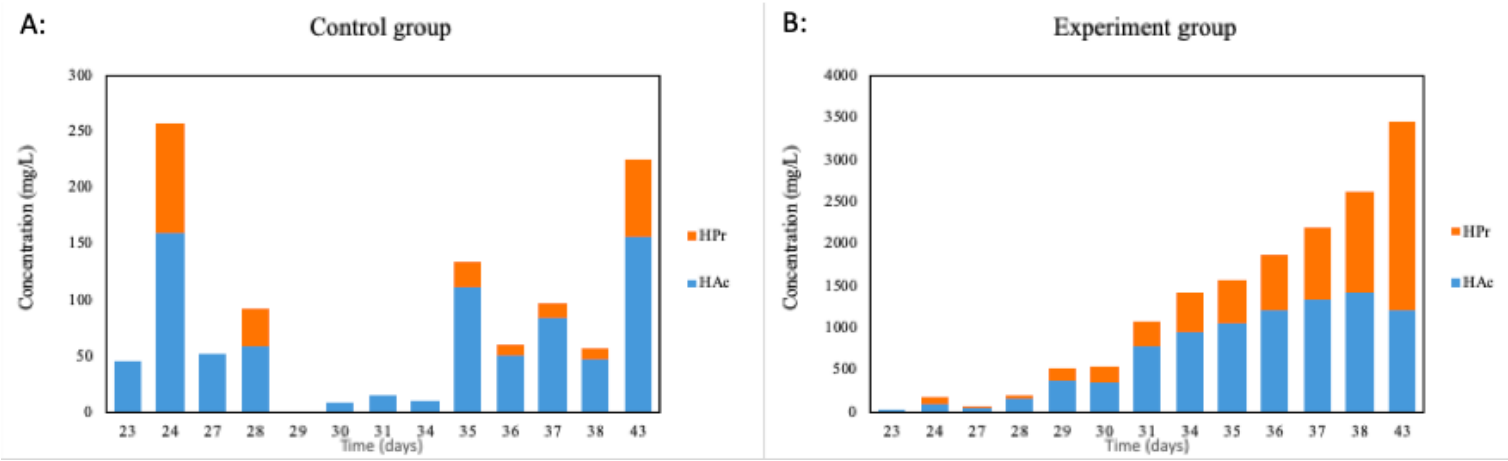


Figure 10. The VFAs analysis results of A: The control group B: The experimental group. HPr: propionic acid, HAc: Acetic acid

Looking at the VFAs analysis of the control group (*figure 10.A*) it can be seen that there are higher amounts of VFAs up until 28 days, which may coincide with the initial 30-day stabilization phase. After that, the amount of VFAs is low until it starts to increase once again after day 35. However, the concentration of VFAs is in the typical range for a digester between 50 and 300 mg/L, which signifies that the digester is operating well. However, looking at the experimental group, it can be seen that there is an increase in the accumulation of VFAs above the optimal range which signifies that the reactor is not well balanced. Firstly, there is an accumulation of acetic acid above the range from day 28, meaning that methanogenesis does not optimally occur. The accumulation of acetic acid inhibits methanogenesis, which then also inhibits fatty acid oxidation, resulting in the further accumulation of propionic acid [40]. This can also be seen in the acidification of the reactor. The results suggest that the addition of electricity increases the conversion of glucose to acetic acid resulting in a high VFAs concentration in the experimental group. However, the accumulation in acetic acid and low methane content in the biogas analysis suggests that the conversion of acetic acid to biogas is not efficient. During the reaction of the MECs reactor, the needle holding the electrode degraded and remained in the reactor for the remainder of the experiment. It has been shown in recent studies that heavy metals inhibit digestion at a soluble concentration of greater than 0.5 mg/L [41]. However, the needle is too small to have had a significant effect on the methanogenic activity of this reactor.

4.B.2 MEC: continuous reactor

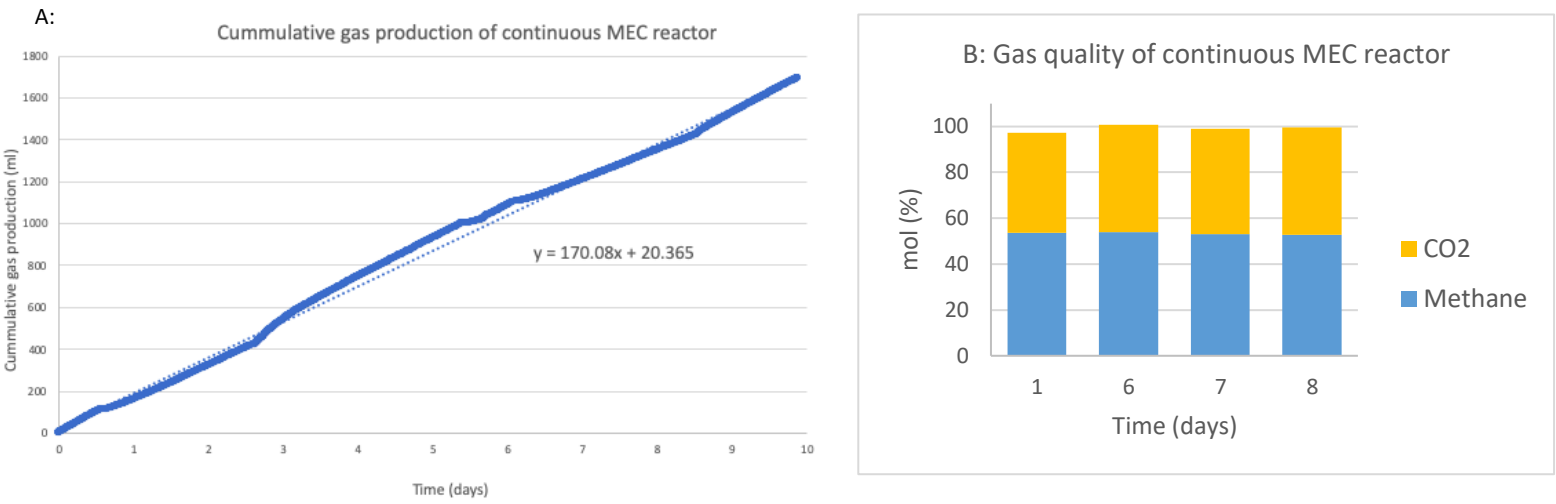


Figure 11. The cumulative biogas production (A) and quality (B) of the continuous MEC reactor

The daily gas production of the continuous MEC reactor is approximately 170 ml/day, which corresponds to 71% yield. The activity is comparable to that of the batch MECs with the condition of 1.34 V / 0.006 A, which is highest out of all MEC batch conditions. The average fraction of methane in the biogas samples is ~52 %, while that of CO_2 is at ~46 %. This is comparable to that of the batch reactors, although slightly lower than that of the one without electricity.

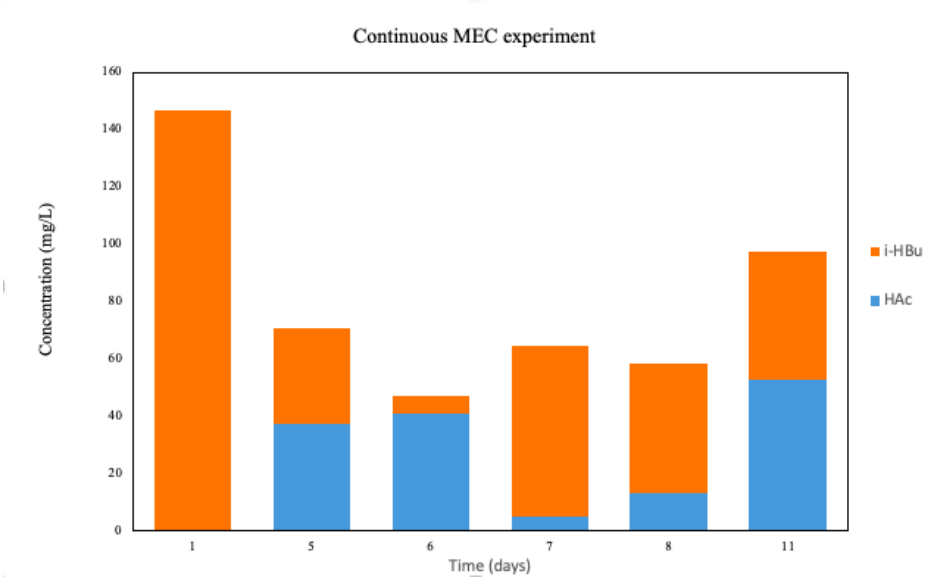


Figure 12. The VFAs analysis results of the continuous MEC reactor. I-Hbu: Isobutyric acid, HAc: Acetic acid

The VFAs results of the continuous MEC reactor is much lower than that of the batch MEC reactor, with most of the acetic and all of the propionic acid removed which indicates that the activity is sufficiently high to consume all the substrates. However, the reactor also has some accumulation of isobutyric acid, indicating that the performance is not optimal.

5. Improvement and future prospects

A. Normal pressure adiabatic (NPAD) reactors

A large factor in the fluctuation of the NPAD results was due to absence during the weekend where no feeding/sampling and analysis was done. Future improvements could be done by setting up the NPAD reactors as continuous systems so that feeding and sampling are taken automatically. This also eliminates a large fraction of human error, making the results more reliable. Furthermore, the results indicate that the reactors have a stabilization period of approximately 30 days. As a result, reactors should be run for longer in order to first stabilize the reactors, then collect a significant amount of data in order to observe a reliable trend. This experiment can then be combined with MEC reactors, as literature suggests that the application of a milli-voltage can aid in increasing the tolerance of the system to toxic compounds such as ammonia.

B. Microbial electrolysis cell (MEC) reactors

Throughout the operation of the experiment, there were multiple problems with the set up and equipment which have an effect on the operation of the reaction, and accuracy of the results. Firstly, there are a limited number of accurate GC results due to a fault in the gas bags used for collecting the gas samples. Gas samples taken from the overhead compartment of the reactors gave more accurate results, so this technique should be used in future experiments. Secondly, the motor system for stirring was unreliable, causing interruptions in the mixing of reactor contents. Mixing is crucial as it ensures even distribution of the substrate throughout the reactor, which enables microorganisms to have good nutrient supply. As a result, the interruptions in mixing may have caused microorganisms to not be sufficiently in contact with microorganisms, having an effect on their activity. Furthermore, the motor system was interchanged with a new system later on into the experiment which does not allow for accurate set up of the mixing rate at low rpms. As a result, the stirring rate could be too high, disrupting microbial aggregates especially important on the carbon felt electrodes, resulting in less efficient degradation of the substrate. As a result, future experiments should ensure a fully functional stirring system, with an accurate stirring set up in order to ensure stirring at optimum speeds.

The energy source for the MEC reactors used was the Velleman PS3005D
power supply. However, in practice this device did not allow for precise set up and monitoring of microcurrents. For future experiments, a potentiostat should be used which would allow for more accuracy and control, especially at such microcurrents, which has been shown in previous papers to be more beneficial to the growth and activity of the bacteria. The voltage should be kept to below 1V in order to avoid water hydrolysis which produces oxygen. This interrupts the anaerobic environment and limits the activity of methanogens, so should be avoided. Furthermore, the biofilm formation can be monitored using cyclic voltammetry and open circuit voltage (OCV), which could be insightful information for monitoring the activity of the bacteria.

For analysis, liquid samples should be taken for pH/VFA/TAC analysis before application of electricity in order to have reference values, allowing for comparison of the activity before and after application of electricity. Furthermore, liquid samples should be analyzed directly after sampling in order to ensure accurate results.

The data suggests that MECs systems operate well with glucose as the starting material. Future research may potentially use food waste as the substrate in order to give the system a larger variety and more complicated starting materials. This will aid in the applicability of the system. Furthermore, experiments should be repeated with a larger number of reactors and run for longer periods of time in order to increase the accuracy of the results. The continuous MECs reactor should also be repeated with control reactors for comparison.

C. General analysis

Further types of analysis can aid in monitoring the evolution of the reactors. The microbial community can be investigated using High-throughput 16s-rDNA gene sequencing and analysis. This can be useful to identify differences in the culture and see the effects of different conditions (NH_4Cl concentration, voltages) on the growth and selectivity of different microbial cultures. Furthermore, gas samples should be analyzed for hydrogen and oxygen in order to more accurately monitor biogas quality and bacterial activity, giving information on which anaerobic pathways are active and whether there is contamination within the reactors.

6. Conclusion

The research conducted in this paper suggests that AD systems can tolerate NH_4Cl concentrations of up to 12 g/Land may even aid in the stability of the reactors, but can be toxic in larger concentrations. Reactors with 10 g/L NH_4Cl performed the best for biogas production with 59 ml/day, but is less advantageous in biogas quality as it has the second highest mol% of CO_2 following reactors with 12 g/L NH_4Cl.However, a small increase to 12 g/L of NH_4Cl can significantly affect the stability of the reactors and cause a ~20% decrease in the yield. Reactors with 4 g/L NH_4Cl resulted in the best biogas quality, with 56.7 mol% CH_4, and 43.7 mol% CO_2. More research is needed to see the full effects for larger concentrations such as 24 g/L. Research may be more accurate if a continuous reactor is used, removing human error.

Batch MEC experiments with 1.34 V / 0.006 A performs the best both in terms of production rate and total gas production. Reactors with 0.6 V / 0 A and 0.052V/ 0 A applied voltage performed second best, and the gas production was higher than that of the control. The data suggests that the addition of a milli voltage is advantageous in terms of the volume of biogas production. However, the biogas quality slightly decreases and overtime the reactor acidifies which is detrimental to the methanogenic activity. The activity of the continuous MEC reactor at 0.6 V / 0A has better behavior in terms of biogas yield and production rate compared to the batch MEC reactors with and without electricity. The biogas quality is comparable to those of batch experiments and no acidification is observed so far. Due to time constraints the operation of the continuous MEC reactor was stopped early, but further research is needed to draw conclusive results on the reactor operation. Furthermore, a continuous system without voltage should be set up as a control in order to accurately compare the two systems.

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