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Bioelectrochemical Reactions at the Surface of Bioelectrodes for Biofuels Production

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Summary:

In most countries, the major energy source is fossil fuels. Fossil fuels are cost-effective and reliable. But it faces the issue of greenhouse gas (GHG) emissions. Therefore, new renewable energy sources must be developed. Utilizing bioelectrochemical synthesis for the production of methane is a promising process. This study aims to provide a method for determining the features of a complex bioelectrochemical system (reactor). The method attempted to explain relevant biochemical reactions at the electrodes and establish the reaction's thermodynamic properties as Gibbs free energy. A fundamental aspect of this method was determining the relationship between Gibbs free energy and the applied voltage in the bioelectrochemical system. The main principle of the model was based on McCarty and Heijnen's analysis of the reaction's stoichiometry. The relationship between stoichiometry, Gibbs free energy, and the applied voltage is specified using the Nernst equation. Relevant biochemical reactions were determined, and acetate, glucose, ammonium, and hydrogen were selected for parameter assessment. Considering the effects of temperature, pressure, and energy fraction on yield and stoichiometry, pressure has the most significant influence on the applied voltage. As the pressure rises, the needed voltage falls.

Furthermore, changes in the energy fraction could affect the stoichiometry of a reaction. In practice, this change will have little effect on the applied voltage if other variables are not taken into consideration. According to the research, if there are more accessible electrons, the reaction will be more spontaneous with less voltage.

In addition, a rise in temperature causes an increase in applied voltage, which can have a negative effect on the system. It can be shown that the temperature should be within an acceptable range that does not inhibit microbial population growth.

Glucose oxidation has the highest applied voltage value among the evaluated substrates in regards that it has a higher yield than the other relevant compounds. Furthermore, the findings show that the ammonium oxidation process requires the least amount of voltage.

Preface

This project aims to identify the relevant biochemical reactions and introduce a base model for predicting the applied voltage with regard to the yield. The scenario is a bioelectrochemical reactor where substrates are oxidized at the anode to produce methane at the cathode.

The project was extensive. It is expected that the reader of this thesis has a basic knowledge of microbiology and chemical thermodynamics. Nevertheless, we encountered some obstacles. There were limited literature resources on this topic. So, future work is necessary on the subject to further analyze the data and focus on developing thermodynamic models.

I wish to thank our supervisors, especially Professor Carlos Dinamarca, without whose cooperation I would not have been able to conduct this study. Finally, I thank the University of South-Eastern Norway for providing this opportunity to research this interesting topic.

Porsgrunn, 18/05/2022

Mosayeb Asarimendi

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List of abbreviations

GHG: Greenhouse Gas
PtG: Power to Gas
e^-eq Electron equivalents
MES: Microbial Electrosysnthesis
MFC: Microbial Fuel Cell
ΔG: Gibbs free energy
ΔG_s: Gibbs free energy for cell synthesis
ADM1: Anaerobic Digestion Model number 1
AQUASIM:
Y: Yield
Y_{DX}: Yield of C-mol biomass per mol electron donor
Y_{AX}:Yield of C-mol biomass per mol electron acceptor
Y_{QX}: Yield of C-mol biomass per kJ heat produced
Y_{GX}: Yield of C-mol biomass per kJ Gibbs free energy released
kJ: Kilo Joule.
TEEM: thermodynamic electron equivalents model
EET: extracellular electron transfer
COD: Chemical Oxygen Demand
EET: Extracellular Electron Transfer
EAM: Electroactive microorganism
BES: Bioelectrochemical systems
DET: Direct Electron Transfer
IET: Indirect Electron Transfer
RET: Reversed Electron Transport
R_e: Energy Reaction
R_a: Electron acceptor half-reaction
R_d: Electron donor half-reaction
R_s: Synthesis reaction
f_e: Fraction of electrons used for energy production
f_s: Fraction of electrons used for cell synthesis
ε: Energy transfer efficiency
μ: Growth rate
m_G: Maintenance
F: Faraday's constant
E_{cell}: Electromotive force of the cell (Volt)

1 Introduction

1.1 Power to Gas Technology

Most countries around the world rely on fossil fuels such as coal, oil, and natural gas as their primary energy source. Fossil fuels are a cheap and reliable energy source, relatively easy to exploit, and have a high energy density which has helped society's development in the last 150 years. However, it faces the challenge of greenhouse gas (GHG) emissions, which have resulted in a temperature rise of around 2°C, causing environmental concerns. As a result, it is vital to develop new energy systems via the diversification of renewable energy sources [1].

It is vital to develop new energy production technologies in order to enhance the percentage of green energy [2]. Renewable energy sources are becoming more essential components of power systems, and their share of total energy production will continue to increase [3]. One of the renewable energy sources is electricity, which can be generated practically everywhere on the planet and produces far fewer greenhouse gases and pollutants than fossil fuels [3-5]. The leading non-fossil energy in Norway is electricity, which is generated through hydropower (96%), wind (1.7%), and thermal energy (2.3%) [7]. Solar and wind energies have been developed in the last 20 years. However, wind and solar energy production can be very unpredictable, which can cause a lot of problems for power systems. A significant portion of the energy generated is lost due to the inability of expected production levels and power plants to produce electricity. Consequently, energy storage systems are required to address this issue [3].

According to a global energy roadmap, electricity will account for over half of total energy usage in 2050 [8]. To satisfy power consumption demands, installed capacity should be enhanced. This huge output of electricity from renewable sources will result in periods of overproduction. As a result, it is necessary to store this energy in an appropriate way. Several electricity storage technologies, such as batteries, hydropower plants, and hydrogen storage technologies, are available at different prices, run times, and storage capacities [9]. In this regard, the natural gas network has a large storage capacity, and thus converting electricity to gas would be an interesting technology to store excess electricity.

Two further non-fossil energy sources are biomass and waste-derived biofuels. The treatment of waste is a continuous process that is dominated by the oil industry, followed by natural gas and electricity. Biofuel has a significant potential to become the primary source of transport. Biogas generation through waste treatment has the potential to be a viable non-fossil energy source [7].

Power-to-gas (PtG) technology may significantly enhance total methane (CH_4) production from biogas by combining carbon dioxide (CO_2) in the biogas and converting it to CH_4 [10]. The primary feature of PtG technology is removing industrial-produced CO_2, which provides

a low-cost carbon capture solution. PtG creates an attractive possibility for many industries which enforced by government policies to cut their carbon emissions.
Not only can companies benefit from PtG technology in terms of reduced CO_2 emissions and waste creation, but they may also benefit from its use or sale of high-quality methane generated. Additionally, PtG technology enables more effective conversion of excess electricity to biogas and storage [11].
Electrochemical methods can be used to reduce industrial carbon dioxide to biogas. Due to its compressibility, generated biogas is an effective transport fuel, particularly in Nordic countries [12]. The purity of biogas (60% methane) as compared to natural gas (> 85 % methane) is the critical limiting factor for its use as a transportation fuel [13]. As a solution, the priority has shifted to innovative technologies that employ electrochemical methods to convert carbon dioxide to methane.
Methane generation was once seen as an undesirable byproduct of electrolytic hydrogen synthesis, and attempts were made to avoid methane production [14]. But, it was soon discovered that methane could be produced as the primary energy-rich product [15]. Afterward, many studies indicate that coupling microbial electrosynthesis systems (MESs) with anaerobic digestion (AD) is an effective methane production method [16]. Other advantages of this transition included enhancing methane production and biogas quality due to the potential of electrochemically converting carbon dioxide to methane [17].

1.2 Scope

The purpose of this study is to develop a technique or approach for identifying the characteristics of a bioelectrochemical system. The approach aimed to describe relevant biochemical reactions at the electrodes and determine the reaction's thermodynamic properties as Gibbs free energy. In this approach, the determination of the relationship between Gibbs free energy and applied voltage in the bioelectrochemical system is a vital step. Figure 1.1 represents the phases in creating a model's basis.

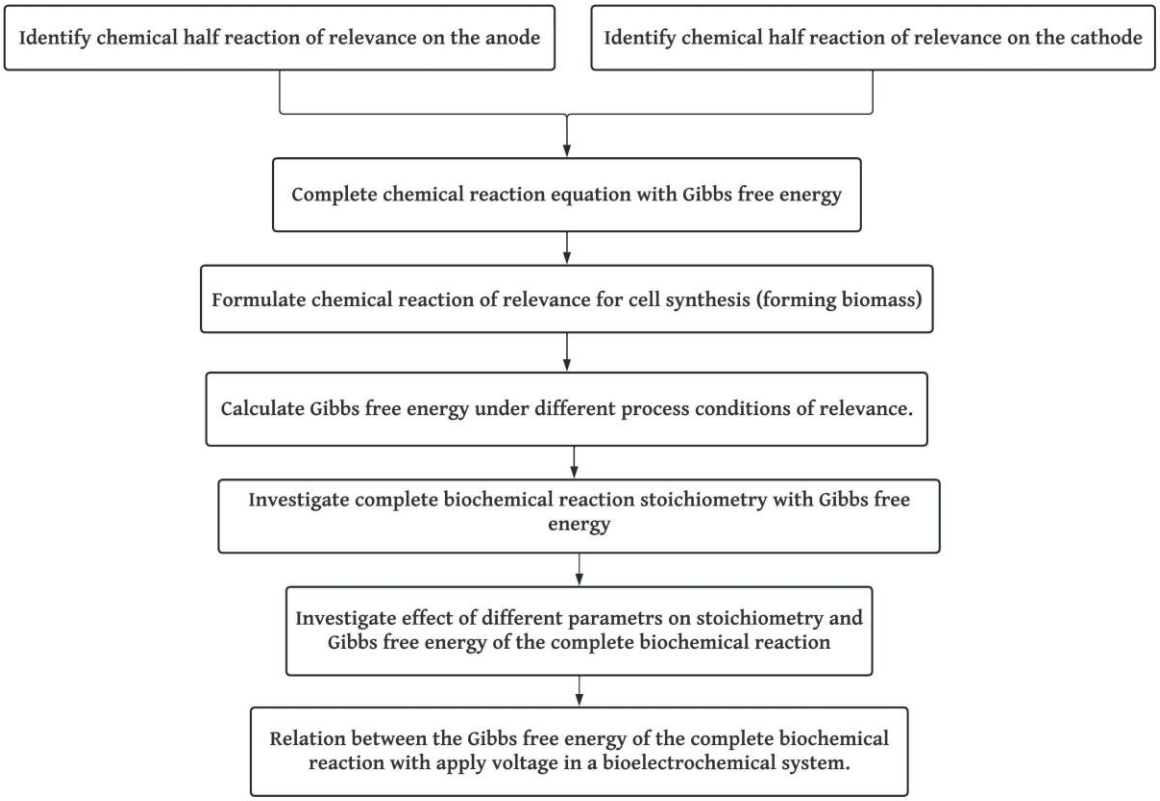

Figure 1.1: Procedure to identify steps of the proposed method.

Heijnen et al. [18] propose a method for analyzing chemotrophic microbial growth systems that is based on bioenergetic analysis. The major parameters are determined based on the Gibbs energy providing for redox reaction and the carbon and nitrogen sources for microbial development. Stoichiometric and kinetic parameter values that differ significantly from the expected values indicate the presence of a highly specialized microbial system. As a result, the generalized technique may be used to interpret stoichiometric and kinetic parameter values that describe microbial growth [18].

In the other research, Heijnen et al. studied the parameters for microbial growth [19]. This research investigates the parameters in terms of their general relationship to the Second Law of Thermodynamics. Nevertheless, it demonstrates that the Gibbs energy transfer is a quantity that satisfies the criteria for microbial growth. The Gibbs energy dissipation is discovered to be a function of the type of the C-source. This dissipation appears to be independent of the electron acceptor's composition[19].

Tijhuis et al. create a thermodynamic system that analysis microorganism maintenance. The factors for assessing the maintenance include a wide variety of organisms, mixed cultures, heterotrophic and autotrophic growth, growth under aerobic and anaerobic conditions, and a

wide temperature range (5–75°C). Results indicate that temperature is the only factor affecting maintenance, which has an activation energy of 69 kJ/mol [20].

To estimate microbial growth yield, Liu et al. proposed a novel correlation for Gibbs energy dissipation [21]. Despite its more straightforward structure, this correlation is as accurate as the complicated literature correlation. Furthermore, because of the relationship between biomass yield and Gibbs energy dissipation, an approximate estimate of the latter is not required to determine the yield [21].

Flores-Rodriguez et al. studied the effect of integrating the bioelectrochemical process with an anaerobic digestion process. Results indicate that an applied voltage of 1.0 V has higher biomethane generation (2 times), and COD removal is higher by 83.6%. Microbial activity was inhibited when the voltage was increased to 1.5 V [22].

1.3 Biological Systems

Biological processes remove biodegradable, soluble, organic, and nutrient chemicals and colloids from wastewater employing microorganisms. There are two types of biological activity-based methods: aerobic and anaerobic. The biological treatment of wastewater is not a well-defined process that integrates biology and biochemistry [23]. Biochemistry investigates the chemical reactions that occur in living organisms [24].

Mass balance is the most critical argument in biological treatment systems. A mass balance indicates the amount of substrate and other substances required to fulfill the energy, nutritional, and environmental requirements of microorganisms. Additionally, it specifies the quantity of products created [25].

The mass balance can be estimated using a balanced chemical equation. A balanced chemical reaction is predicated on the concept of stoichiometry and the molar ratio of reactant to products [25]. Numerous properties of microorganisms complicate the stoichiometry of biochemical reactions. Additionally, microorganisms play a role in the reaction as both catalysts and products. The microorganisms are responsible for the reaction's overall energy generation, and they capture a portion of that energy for cell synthesis and maintenance [25].

Microorganisms acquire energy by converting compounds through oxidation and reduction reactions. A biological reaction requires an electron donor, an electron acceptor, a carbon source, and a nitrogen source. An electron donor can be either organic or inorganic. Additionally, oxygen, iron (II), nitrate, sulfate, and carbon dioxide are the most often used electron acceptors in biological reactions. Carbon source is either derived from organic matter or from carbon dioxide. Numerous kinds of nitrogen may be utilized as a source of nitrogen. Ammonium is the most often used type of nitrogen in the conversion of energy to biomass. [25].

1.3.1 Chemical Reactions

Chemical reactions are also thermodynamically expressed by a change in free energy (G^0), known as the Gibbs free energy. The variation in energy based on the reaction is known as ΔG^0 (pH = 7, Temperature 25^0 C, Pressure 1 bar). The Gibbs free energy for reactants and products in half-reactions can be estimated with known standard Gibbs free energy. Half-reactions indicate the transfer of one mole of electron in oxidation-reduction reactions [26]. There are specific electron donors and electron acceptors in biological reactions. Appendix A shows organic and inorganic half-reactions as electron donors and the Gibbs free energy of relevance at standard conditions (p = 1 bar, Temperature = 25°, and pH = 7). Common electron acceptor half-reactions and the Gibbs free energy at standard conditions illustrates in Appendix A.

1.3.2 Metabolism

Metabolism refers to the accumulation of all chemical reactions in the cell. Catabolism and anabolism are the two processes that form metabolism. In the catabolism process, a substrate is oxidized to generate energy, and some of that energy is utilized to synthesis the cells in the anabolism process [25]. Figure 1.2 demonstrates the metabolism pathway.

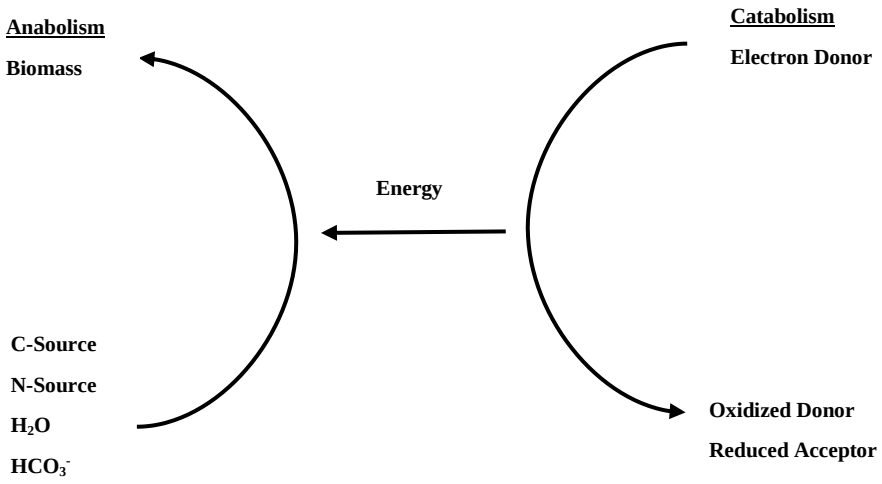

Figure 1.2: Pathway of microbial growth[19]

Velvizhi et al. [27] assessed the anaerobic and electro fermentation conversion of CO_2 to value-added products. The conversion of CO_2 to short-chain fatty acids, volatile fatty acids, and methane was studied in this research. The findings indicate that electro-fermentation may efficiently intensify biochemical reactions and significantly increase CO_2 conversion to organic substances [27].

Braga et al. [28]evaluated the parameters affecting anaerobic digestion biofuel production. Various factors need to be addressed in order to obtain reliable biofuel production. Maximizing

methane production requires consideration of the metabolic pathway, inoculum source, pH, temperature, nutrients, toxic substances, and reactor configuration [28].

Several substrates cause a significant threat to the environment. Tsigkou et al. studied the use of an anaerobic digester reactor to treat olive mill waste. The study aimed to develop a high-rate thermophilic anaerobic digester capable of extracting a substantial amount of organic matter. An anaerobic treatment unit that produces 9.51 $NLCH_4$/LFeed for olive mill wastewater valorization without co-digestion is considered promising.

Sivalingam et al. [29] investigated the moving bed biofilm process in activated sludge model. AQUASIM was used to simulate the process. The reduction of high ammonium concentrations was investigated using various aeration schemes. The simulation findings showed the lack of Anammox-related biological pathways. However, the main simulation outputs are biofilm thickness, fluctuation in substrate concentration, and biomass distribution, which are partly verified using experimental data [29].

Table 1.4 demonstrates cell synthesis half-reactions and the Gibbs free energy at standard condition (p = 1 bar, Temperature = 25°, and pH = 7).

Table 1.4: Cell synthesis half-reaction (Rc) with different N-sources. Adapted from [25].

N-Source	**Half-reaction (*Rc*)**
NH_4^+	$\frac{(n-c)}{d}\ CO_2 + \frac{c}{d}\ NH_4^+ + \frac{c}{d}\ HCO_3^- + H^+ + e^- = \frac{1}{d}C_nH_aO_bN_c + \frac{(2n-b+c)}{d}\ H_2O$ $where, d = (4n + a - 2b - 3c)$
NO_3^-	$\frac{n}{d}\ CO_2 + \frac{c}{d}\ NO_3^- + \frac{(4n+a-2b+6c)}{d} H^+ + e^- = \frac{1}{d}C_nH_aO_bN_c + \frac{(3c+2n-b)}{d}\ H_2O$ $where, d = (4n + a - 2b + 5c)$
NO_2^-	$\frac{n}{d}\ CO_2 + \frac{c}{d}\ NO_2^- + \frac{(4n+a-2b+4c)}{d} H^+ + e^- = \frac{1}{d}C_nH_aO_bN_c + \frac{(2c+2n-b)}{d}\ H_2O$ $where, d = (4n + a - 2b + 3c)$
N_2	$\frac{n}{d}\ CO_2 + \frac{c}{2d}\ N_2 + H^+ + e^- = \frac{1}{d}C_nH_aO_bN_c + \frac{(2n-b)}{d}\ H_2O$ $where, d = (4n + a - 2b)$

1.3.3 Bioelectrochemical Systems

Bioelectrochemical systems (BESs) are electrochemical cells in which microorganisms act as catalysts on both electrodes [30]. Microorganisms catalyze the reactions that occur at the electrodes. Electrons are transferred to the anode, or electrons are accepted for a reduction reaction from the cathode [16], [31]. BESs are either electron-producing microbial fuel cells

(MFCs) or electron-consuming microbial electrosynthesis (MESs). Although the anodic oxidation reactions are equivalent, the different cathode reactions result in MFCs producing electrical energy due to the overall thermodynamically favorable reaction and MECs providing extra energy to drive the favorable overall reaction [32]. Figure 1.3 shows theoretical cathodic potentials applied to drive the electrosynthesis of different compounds.

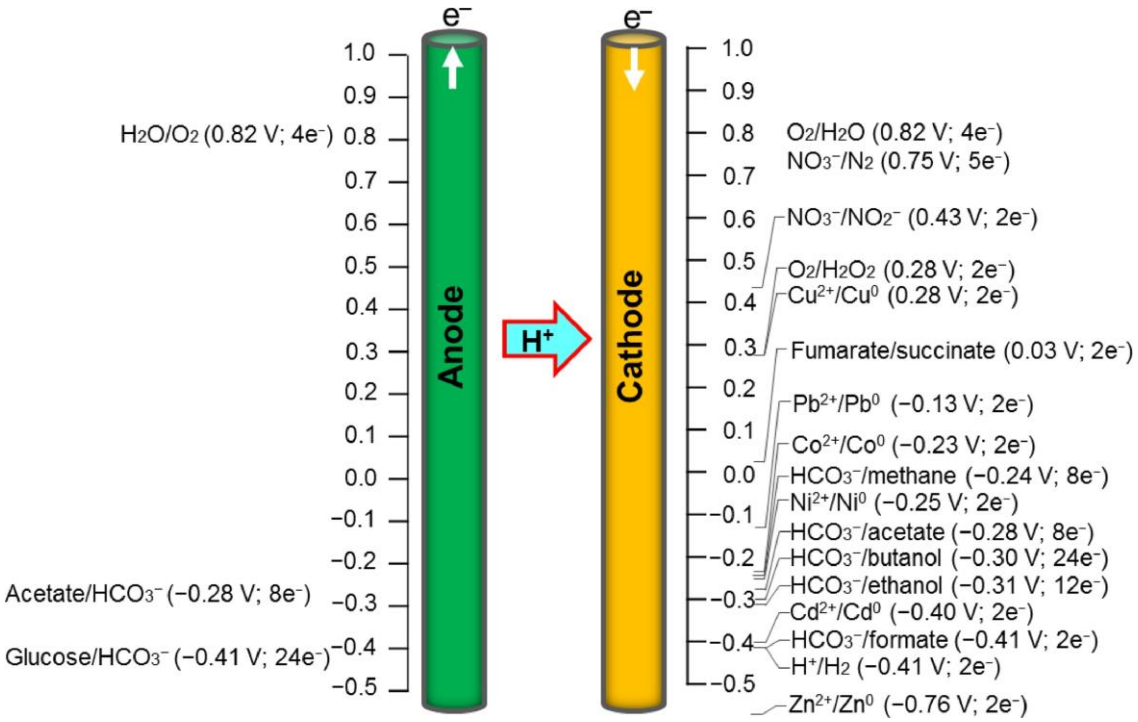

Figure 1.3: Theoretical cathodic potentials to drive the electrosynthesis of different compounds in bioelectrochemical systems [33] [34] [35].

1.3.4 Extracellular Electron Transfer (EET) mechanisms

Electroactive microorganisms (EAMs) can transfer electrons throughout intracellular and extracellular electron donors and acceptors [36]. Electroactivity is a term that relates to the ability and effectiveness of extracellular electron transfer (EET) during biofilm formation on the surface of electrode [37]. Exoelectrogens conduct electrons from an oxidation substrate to the anode. The current is then sent via a circuit to the cathode. The applied voltage is used to compensate the current for favorable reactions that occur. Electrotrophs then use electrons from cathodes to power the cell's metabolism [38]. Due to a lack of knowledge of the actual processes, the EET process has not been adequately described. These include the mechanisms by which electrons are transferred through the membranes of microbial cells and between the microbial surface and electrodes [39]. Direct electron transfer (DET) and indirect electron transfer (IET) are two different types of electron transfers that occur in a bioelectrochemical

system. DET relies mainly on c-type cytochromes[1] (c-Cyts) and conductive nanowires. The direct transmission of electrons between electrodes and cytochromes is one of the mechanisms used in DET [40], [41]. Another process is the generation of conductive pili or pilus-like structures to enhance DET distance and transmission efficiency[42].

In IET, some EAMs employ endogenous mediators, natural compounds, and artificial substances to carry electrons to the anode [43], [44]. The additional electron transfer mechanism in IET is via redox mediators or energy carriers like H_2 or enzymes such as hydrogenases, which act as intermediaries between the cathode and the microorganisms. [45].

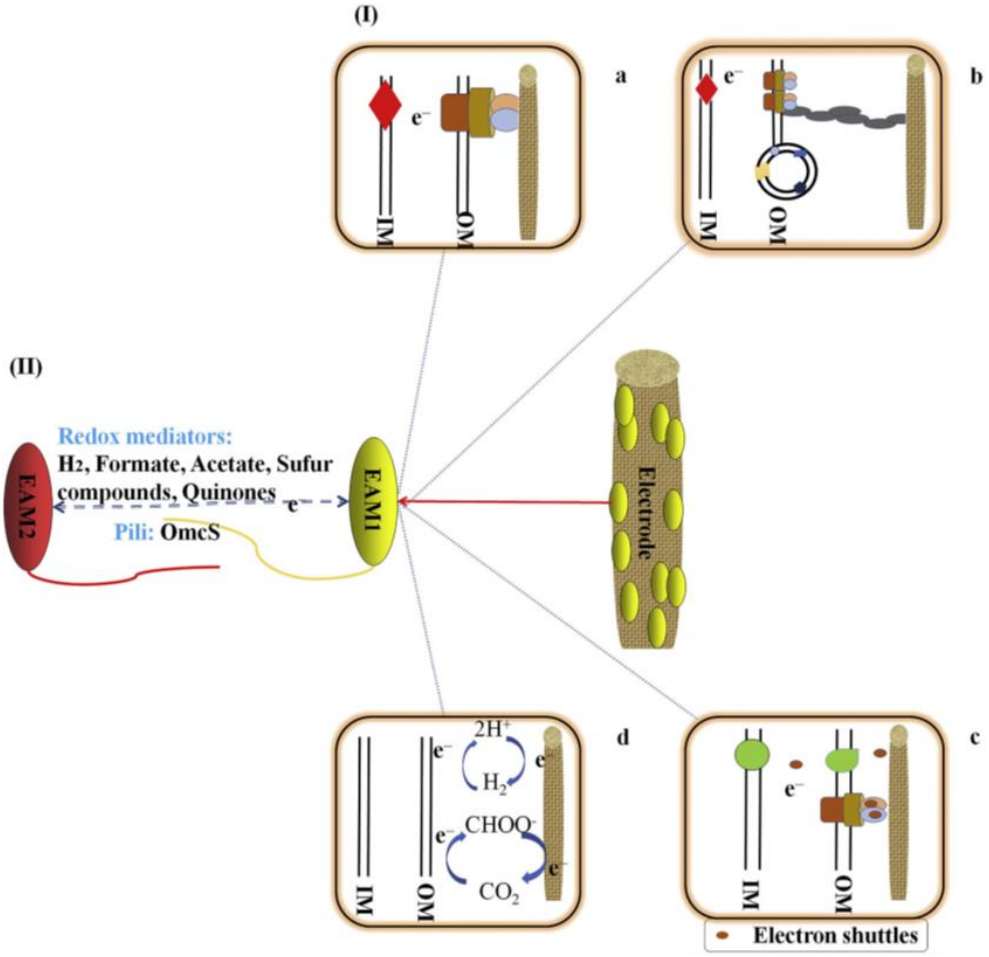

Fig 1.4: Extracellular electron transfer takes place via microorganisms and electrodes, metal ions, or species. (I) EET between microorganisms and electrodes or metal oxides occurs via four main pathways that involve (a) cytochrome proteins, (b) nanowires, (c) free-electron shuttles, and (d) redox mediators. (II) EET between species is mainly based on redox mediators (H_2, formate, acetate, sulfur compounds, quinones)[35].

[1] Any of a number of compounds consisting of heme bonded to a protein. Cytochromes function as electron transfer agents in many metabolic pathways, especially cellular respiration

1.3.4.1 EET pathway in Bacteria, Fungi, and Archea

Some important bacteria which have been used in MEC:

- Shewanella: Because of its metabolic capability and electron transport pathway diversity (DET and IET), Shewanella oneidensis MR-1 is a comprehensive organism for EET research[46].
- Geobacter: Geobacter species are commonly employed in EET research [47].

Some fungi, such as yeast, utilize DET or IET mechanisms to transfer electrons to extracellular electron acceptors. Yeast is straightforward to cultivate and adjusts to a wide range of environments. In fungus, electron transfer through a freely diffusing mediator occurs [48].

Despite superficial similarities between archaea and bacteria, archaea have different and more complicated processes to create energy for cell growth and maintenance. Methanogens, for instance, can take electrons from metals, live cells, or electrodes [49].

Methanogens utilize both DET and IET. In IET, Methanogens use H_2 and require a cathode potential of less than 590 mV compared to a standard hydrogen electrode (SHE)[50]. DET in methanogens may require interaction among a cathode and the microbial cell surface, which needs a high potential cathode (-400 mV to -500 mV relative to a SHE)[51].

1.3.5 Reaction at the surface of the cathode

The primary and probably most suitable and efficient pathway for cathodic EET is through DET (Figure 1.5(i)) [52]. Alternatively, cathodic EET can be achieved using H_2 (Figure 1.5 (ii)). In this pathway, hydrogen gas is created at the cathode and acts as an electron carrier to transfer electrons [53]. Aside from H_2, other possible electron-carriers include formate (Figure 1.5(iii)), which are very soluble and might be utilized to produce high-value chemical products such as long-chain fatty acids [54]. According to certain studies, enzymes like formate dehydrogenases catalyze the production of H_2 and formate to create methane with CO_2 reduction when adsorbed onto suitable redox-active regions of the electrode surface (Figure 1.5 (iii and iv)) [55]. Mediated electron transfer is another mechanism for cathodic EET (Figure 1.5 (v)). Everitt's salt ($K_2Fe(II)[Fe(II)(CN)_6]$), iron citrate, neutral red are examples of the mediators [56]–[58].

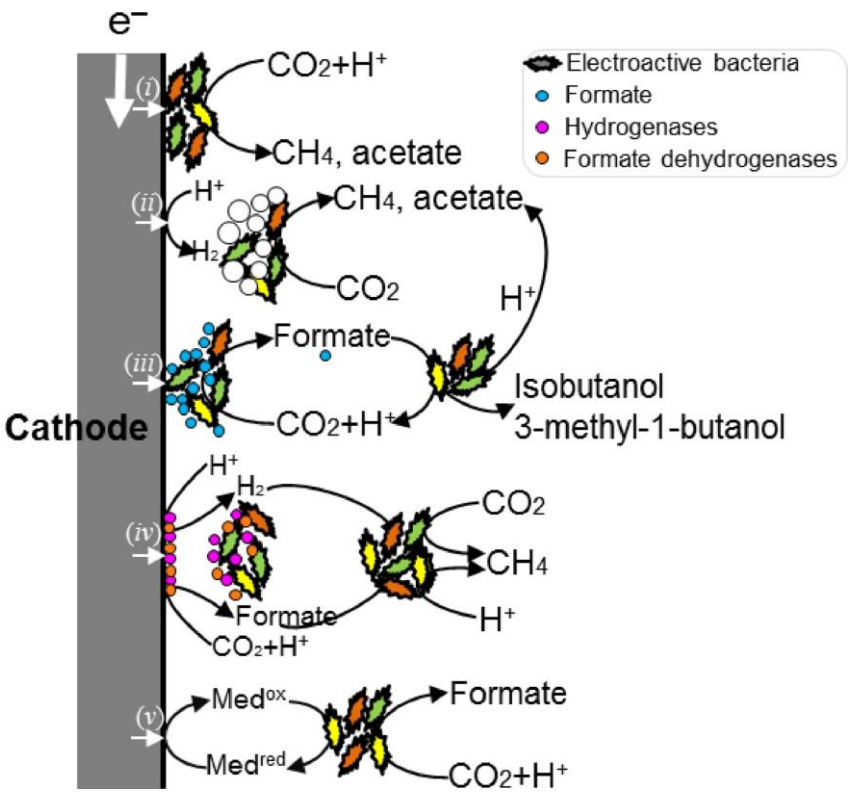

Figure 1.5: Mechanisms for electron transfer for electro-reduction of CO_2 to biofuel and value-added products: (i) Methane, acetate production via DET. (ii) H_2 as the electron carrier to deliver electrons for methane formation. (iii) Electrochemical CO_2 reduction to produce formate, which is then converted to higher valuable compounds. (iv) Hydrogenases and formate dehydrogenases discharge when adsorbed onto electrode surfaceand then is catalyzed to form H_2 and formate while transporting electrons for methane production. (v) Indirect electron transfer via mediators [35]

2 Material and Method

Establishing a base model for determining the yield of a biological process is a critical part of this study.Additionally, the applied voltage required to achieve the desired yield in a bioelectrochemical system is determined. Certain simplifications are considered while building the model:

- Assume the electron donor is the only substrate that exists in the reactor.
- Assume CO_2 is the only electron acceptor in the reaction.
- The concentration of the N-source is adequate for the reaction and neglects the effect of the N-source on the reaction.
- Resistance in the electrochemical system is neglected.

The approach is initiated by determining the various percentages of energy consumed during cell synthesis, and then the yield is determined. The needed applied voltage is then computed using the Nernst equation and the Gibbs free energy obtained from the biological process.

2.1 Occurrence of reactions at the anode and cathode

Different compounds can be engaged in a chemical reaction depending on the relevant component presented in the previous part of this research. Various organic and inorganic components can serve as electron donors and electron acceptors. In Appendix A, Table A.1 lists several organic compounds that can serve as electron donors. Inorganic substrates that may act as electron donors are included in Table A.2. Substrate oxidation takes place at the anode. Acetate, glucose, ammonium, sulfate, and hydrogen are the main relevant substances that will be investigated in this study. Half- reaction of these compounds are as follow:

Acetate =	$CH_3COO^- + 3\,H_2O \rightarrow CO_2 + HCO_3^- + 8H^+ + 8e^-$	ΔG = - 27.40
Ammonium =	$2NH_4^+ \rightarrow N_2 + 8H^+ + 6e^-$	ΔG = - 26.70
Hydrogen =	$H_2 \rightarrow 2H^+ + 2e^-$	ΔG = - 39.87
Sulfate Ion =	$SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2H^+ + 2e^-$	ΔG = - 50.3

The electron acceptors that are relevant are listed in Table A.3. Reduction reactions take place on the cathode's surface. This research is intended to improve biomethane synthesis in order to minimize the effects of CO_2. Consequently, CO_2 is referred to as the electron acceptor in this study.

Carbon Dioxide	$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2\,H_2O$	ΔG = 23.53

Due to environmental conditions and procedures, any substrates may be involved in the reaction. For simplicity, we assumed that just one substrate serves as an electron donor, and only one component acts as an electron acceptor.

2.2 Identify biomass for cell synthesis

The elemental composition of the biomass depends on the environmental condition and average weight of the elements in the biomass. In this research, based on adaptation from Esener et al. [59], the biomass is expressed as:

$$X = CH_{1.8}O_{0.5}N_{0.2}$$

By considering NH_4^+ as the N-source, cell synthesis half-reaction can be as :

$$0.800\ CO_2 + 0.197NH_4^+ + HCO_3^- \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 1.691\ H_2O \qquad \text{(R2.1)}$$

2.3 Method for determining the stoichiometry of biochemical reactions

Numerous techniques may be employed to investigate a biological process from various perspectives. Rittmann and McCarty proposed a method for determining a reaction's stoichiometry. It is based on thermodynamic principles and is compatible with biological systems.

Additionally, Hiejnen created a methodology for predicting the reaction's stoichiometry based on the yield of the biological process. The stoichiometry of a reaction can be computed using energy, element, and charge conservation. The approach is applicable to chemotrophic processes and is based on thermodynamics' second law.

Identification of the electron donor and acceptor in biological processes allows for analyzing energy in biochemical reactions. It is influenced by the amount of energy released throughout the oxidation-reduction reaction cycle. Additionally, the critical steps for energy analysis include estimating the amount of energy necessary to convert the carbon source into biomass and computing the yield based on the balanced chemical equation [26]. The applied voltage can be determined with the use of stoichiometry, Faraday's law, and other principles and concepts.

2.3.1 Overall reaction Rittmann and McCarty (TEEM)

Energy production and cell synthesis are two fundamental processes that affect bacterial growth. Energy reaction (R_e) can be expressed with electron acceptor half-reaction (R_a) and electron donor half-reaction (R_d):

$$R_e = R_a - R_d \qquad (2.1)$$

The synthesis reaction (R_s) is demonstrated with the combination of the cell synthesis half-reaction (R_c) and electron donor half-reaction (R_d):

$$R_s = R_c - R_d \tag{2.2}$$

These two reactions can be expressed into a single stoichiometric reaction which shows biomass growth and substrate consumption.

$$R = f_e(R_a - R_d) + f_s(R_c - R_d) \rightarrow R = f_e R_a + f_s R_c - R_d \tag{2.3}$$

The fraction of electrons used for energy production (f_e) and cell synthesis (f_s) can be calculated and should be equal to 1.0:

$$f_e + f_s = 1 \tag{2.4}$$

TEEM is a model based on thermodynamic principles to estimate f_e and f_s. Microbial growth takes place in two steps. In the beginning, the energy reaction creates high-energy carries. And then, the energy carries are spent to drive cell synthesis. In the TEEM model, energy for maintenance is set to zero so that all energy is assumed to be used only for cell synthesis.

For calculating f_e and f_s following procedure can be considered:

$$\Delta G_p = \Delta G_{in} - \Delta G_c^0 \tag{2.5}$$

Where ΔG_{in} is Reduction free energy for Acetyl-CoA as intermediate (30.9 kJ/eeq), ΔG_c^0 is Gibbs free energy for electron donor half-reaction (kJ/e^-eq). In order to convert inorganic carbon to activated acetate, a substantial amount of energy is needed. Water provides electrons for reducing CO_2 to generate cellular organic matter in photosynthesis. In relation, we can calculate the energy consumed if we define ΔG_c^0 as equal to the value for the water-oxygen reaction (-78.72 kJ/e^-eq) [25]. So now it is possible to compute ΔG_s (Gibbs free energy for cell synthesis (kJ/e^- eq)):

$$\Delta G_s = \frac{\Delta G_p}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon} \tag{2.6}$$

Where ΔG_{pc} is Gibbs free energy for intermediate conversion to cells (kJ/eeq) = 3.33 kJ/gcells (Molecular weight Cells/pcells) = 3.33(24.8/20) = 4.13 kJ/e^-eq with ammonium as nitrogen source and cell formulation of $CH_{1.8}O_{0.5}N_{0.2}$.

ε is energy transfer efficiency and n = +1 if (ΔG_{in} - ΔG_c^0) > 0, otherwise n = -1. From equations 2.5 and 2.6 we can express equation 2.7:

$$A = -\frac{\Delta G_s}{\varepsilon \Delta G_e} = -\frac{\frac{(\Delta G_p)}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}}{\varepsilon(\Delta G_a - \Delta G_c^0)} \tag{2.7}$$

A is a Parameter that shows the relationship between Gibbs free energy and energy transfer efficiency. ΔG_e is Gibbs free energy for energy reaction (kJ/e^- eq), and ΔG_a is Gibbs free energy for half-reaction electron acceptor. As the final step f_s and f_e can be calculated:

$$f_s = \frac{1}{1+A} \text{ and } f_e = \frac{A}{1+A} \tag{2.8}$$

The most crucial parameter in the Rittmann and McCarty procedure is ε. Energy transfer efficiency varies according to process conditions, and this factor affects the entire biochemical reaction. According to experimental data, ε can be estimated to be 0.6 under anaerobic conditions such as those encountered in our investigation.

2.3.2 Overall reaction Heijnen:

Figure 1.1 shows the typical composition of components to form biomass. However, variation in N-source, C-source, electron donor, and electron acceptor can change the growth rate of the biomass. Indicator yield (Y) is introduced for the characterization of this variation. Yield is defined based on the C-mol of biomass per C-mol of organic substrate (mol inorganic substrate). Empirical data shows that the yield value (Y_{DX}) can vary between Y_{DX} = 0.01 – 0.80 [19]. Heijnen introduced macro-chemical equation of microbial growth:

$$-\frac{1}{Y_{DX}}(C-)mol\ electron\ donor-(\dots)N-source-\frac{1}{Y_{AX}}mol\ electron\ acceptor \quad + \\ 1\ C-mol\ biomass+\frac{1}{Y_{QX}}(heat)+\frac{1}{Y_{GX}}Gibbs\ Energy+(\dots)H_2O+(\dots)HCO_3^+ +(\dots)H^+ \tag{2.9}$$

Y_{AX} is the yield of C-mol biomass per mol electron acceptor, Y_{QX} is the yield of C-mol biomass per kJ heat produced, Y_{GX} is the yield of C-mol biomass per kJ Gibbs free energy released.

Heijnen assumed that the value of Y_{DX} is known in a specific condition, and he defined the stoichiometric coefficient of biomass as equal to 1. All other coefficients can be calculated based on the energy, element, and charge conservation. A macro-chemical equation with known stoichiometric coefficients provides general information about the biological process. Furthermore, it shows there is a relation between Y_{DX} and the amount of the electron acceptor (Y_{AX}), the amount of heat that must be removed (Y_{QX}), the amount of energy released (Y_{GX}) [19].

2.3.2.1 Method for predicting stoichiometry of biochemical reaction based on thermodynamics

Heijnen [19] made the assumption in the preceding section that the biomass yield is known. As a result, all unmeasured stoichiometric coefficients in equation 2.9 may be determined. Over the previous several decades, the value of Y_{DX} has been determined for a variety of microorganisms, electron donors, carbon sources, and electron acceptors. Heijnen proposed a set of requirements that any model must meet:

- The method should be acceptable to all chemotrophic reactions.
- The method must be based on the second law of thermodynamics.
- It is not necessary to have a complete understanding of metabolism.

- There are no methodological problems.

Battley introduced a method that satisfied all of the criteria [60]. This method depends on $1/Y_{GX}$, the amount of Gibbs energy required to produce 1 C-mol biomass. The Gibbs energy stoichiometric parameter $1/Y_{GX}$ has already been introduced in equation 2.9. Other stoichiometric coefficients can be calculated by knowing this parameter in equation 2.9, including biomass yield (Y_{DX}).

The growth rate determines the yield value due to the Gibbs energy that must be used for maintenance and biomass growth. This indicates that the Gibbs energy required to generate biomass should be divided into two parts: growth-related part and maintenance-related part.

$$\frac{1}{Y_{GX}} = \frac{1}{Y_{GX}^{m}} + \frac{m_G}{\mu} \qquad (2.10)$$

Where $\frac{1}{Y_{GX}^{m}}$ is the Gibbs energy needed to make 1 C-mol of biomass(kJ/C-mol X) and m_G is the Gibbs energy needed for maintenance (kJ/C-mol biomass h), and μ is the biomass growth rate (h^{-1}).

We need information about Y_{GX}^{m} and m_G to calculate Y_{GX} as a function of growth rate μ. Two simple empirical correlations have been discovered to estimate Y_{GX} and m_G [61]. An Arrhenius type of equation can be used to correlate empirical data for calculating m_G as a function of temperature. Furthermore, one can assume that the maintenance Gibbs energy for biomass is negligible in a high growth rate.

$$m_G = 4.5 \exp\left[-\frac{69{,}000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right] \qquad (2.11)$$

This relationship is valid for a wide range of microorganisms, different electron donors, aerobic and anaerobic conditions, and temperatures ranging from 5 to 75 °C. Temperature is the most critical factor. Other factors such as type of microorganism, electron donor, and acceptor have a negligible impact [61].

The empirical data of heterotrophic and autotrophic growth can be correlated as an equation 2.12 for calculating Y_{GX}.

For Heterotrophic growth/Autotrophic growth (-RET)

$$\frac{1}{Y_{GX}^{m}} = 200 + 18(6 - C)^{1.8} + \exp\left[((3.8 - \gamma)^2)^{0.16}(3.6 + 0.4C)\right] \qquad (2.12a)$$

For Autotrophic growth (+RET)

$$\frac{1}{Y_{GX}^{m}} = 3{,}500 \qquad (2.12b)$$

for autotrophic growth, it is important to determine electron donors for which reversed electron transport (RET) is necessary. Such electron donors (e.g., Fe^{2+}/Fe^{3+}) supply electrons with insufficient Gibbs energy to reduce the CO_2 source to biomass. Microorganisms that use such electron donors must first increase the Gibbs energy level of the electron donor via the

biochemical RET process. The Gibbs energy dissipation needed for the generation of 1 C-mol biomass is highly dependent on the C source used. It is also shown that the type of microorganism and electron acceptor have a negligible impact [61]. The impact of the C-source on Y_{GX}^{m} can be described as follows:

- Number of carbon atoms (C) (e.g., for CO_2, C = 1 and for glucose ($C_6H_{12}O_6$) C = 6).
- Degree of reduction (γ).

2.3.3 Cell Potential

The cell potential is the difference in potential between two half cells in an electrochemical cell. As a consequence of the chemical reaction, electrons can travel between electrodes. Furthermore, The availability of electrons causes the potential difference to flow from one cell to the other. The standard cell potential is equal to the difference between the voltages of the two electrodes that define the cell.

In terms of Gibbs free energy, If the overall reaction is thermodynamically favorable, potential will produce, and the Gibbs free energy of the reaction will be calculated as [62], [63]:

$$\Delta G_r = \Delta G_r^0 + RT \ln(\prod) \quad (2.13)$$

where "ΔG_r (J) " is the Gibbs free energy for the specific conditions," ΔG_r^0 (J)" is the Gibbs free energy under standard conditions usually defined as 298.15 K, 1 bar pressure, and 1 Mole concentration for all species, "R" is the universal gas constant (8.31447 $\frac{J}{Mole.K}$), " T " is the absolute temperature (K), and " $\prod$ " is the reaction quotient calculated as the activities of the products divided by those of the reactants (unitless).

It is simpler to assess the reaction in terms of the theoretical cell potential, E_{Cell} (V), defined as the potential difference between the cathode and anode[64].

$$E_{cell}Q = -\Delta G_r \quad (2.14)$$

Q = nF denotes the charge transferred, expressed in Coulomb (C), and determined by the number of electrons exchanged in the reaction, " n " represents the number of electrons per reaction (Mole), and " F " denotes Faraday's constant (9.64853 104 C/mol).

So we have:

$$E_{cell} = -\frac{\Delta G_r}{nF} \quad (2.15)$$

And in standard condition $\prod = 1$

$$E_{cell}^0 = -\frac{\Delta G_r^0}{nF} \quad (2.16)$$

Where E_{cell}^0 is the electromotive force of the cell(V) at standard condition. As a result, we can express the overall reaction in terms of potentials[64]:

By combining (2.13) to (2.16):

$$E_{cell} = E^0_{cell} - \frac{RT}{nF}\ln(\prod) \quad (2.17)$$

Equation (2.17) calculated the maximum range for cell voltage; the real potential obtained will be lower because of several potential losses. The concentration of reactants and products ($\prod$) is calculated based on the Rittman & McCarty method.

3 Results

This section discusses the outcomes of methane generation using acetate, glucose, ammonium, and hydrogen. The yields of numerous biological processes are estimated with TEEM method. The applied voltage for the different yields of the substrates stated above can be determined using the stoichiometry of the biochemical reaction and the Nernst equation.

3.1 Calculating the yield based on TEEM

A critical factor for measuring the yield of a biochemical reaction is energy-transfer efficiency. In optimum conditions, the transfer efficiency for anaerobic and chemoautotrophic reactions considers $\varepsilon = 0.6$. Different factors can affect transfer efficiency. TEEM considers a portion of the donor is synthesized in the optimum conditions. The yield variation is measured by applying various transfer efficiency values to the model. Several substrates with transfer efficiency in the range of $\varepsilon = 0.1$ until $\varepsilon = 0.8$ are discussed. Also, CO_2 is chosen as the electron acceptor to study yield and methane production. Appendix A shows the overall reaction of different electron donors. For consistency, yield is calculated as C-mol biomass per mol substrate. Figure 3.1 illustrates the variation of the yield of substrates with changes in the energy transfer coefficient.

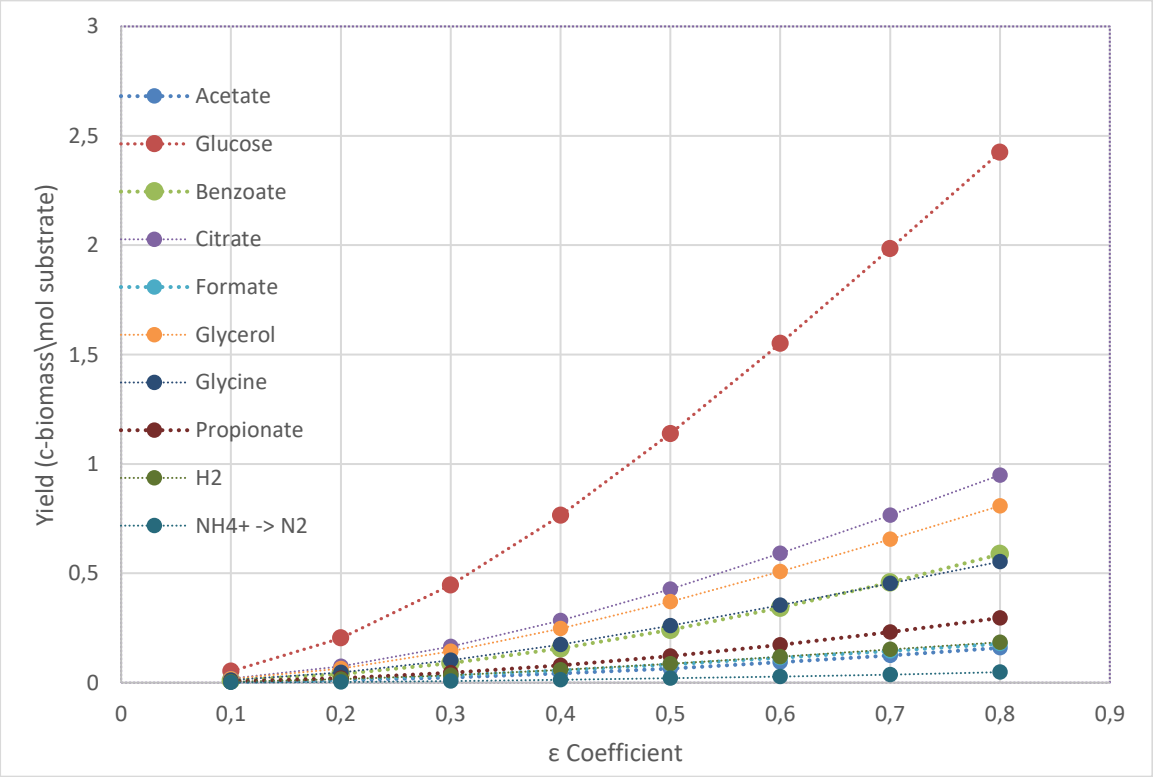

Figure 3.1. Yield (C-mol biomass per mol substrate) for different substrates (electron donor) and transfer efficiency.

As seen in Figure 3.1, enhancing the energy efficiency coefficient leads to an increase in the biomass yield of the reaction. Glucose has the most considerable biomass yield value of all substrates. At the optimum energy fraction for anaerobic reactions ε= 0.6, the highest Glucose yield value is around 1.55. It is shown that ammonium has the lowest yield value among all substrates with varying energy fractions.

3.2 Yield and cell potential of acetate for methane production based on TEEM

The overall reaction of acetate as the electron donor and CO_2 as the electron acceptor can be calculated as follow:

The electron donor's half-reaction (R_d) is written as:

$CH_3COO^- + 3H_2O \rightarrow CO_2 + HCO_3^- + 8H^+ + 8e^-$ $\Delta G° = -27.40$ kJ/e$^-$eq

The electron acceptor's half-reaction (R_a) is written as:

$CO_2 + 8H^+ + 8e^- \rightarrow CH_4(g) + 2H_2O$ $\Delta G° = 23.53$ kJ/e$^-$eq

Biomass cell synthesis reaction (R_c) can be expressed as:

$0.800\ CO_2 + 0.197NH_4^+ + HCO_3^- \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 1.691\ H_2O$

Energy reaction (R_e):

$R_e = R_a - R_d$

$R_e = 0.125\ CH_3COO^- + 0.125\ H_2O \rightarrow 0.125CH_4 + 0.125\ HCO_3^-$

Cell Synthesis Reaction (R_s):

$R_s = R_c - R_d$

$R_s = 0.125CH_3COO^- + 0.0655CO_2 + 0.0476NH_4^+ \rightarrow 0.2381CH_{1.8}O_{0.5}N_{0.2} + 0.0774HCO_3^- + 0.0298H_2O$

The fraction of electrons used for energy production (f_e) and cell synthesis (f_s) can be calculated as follow:

$\Delta G_p = \Delta G_{in} - \Delta G_c^0$

ΔG_{in} for Acetyl-CoA with a reduction free energy of 30.9 kJ/e$^-$eq has been selected as an appropriate intermediate. And ΔG_c^0 is equal to -78.72 kJ/ e$^-$eq.

$\Delta G_p = 30.9 - (-78.72) = 109.6$ kJ/ e$^-$eq

and ΔG_s can be calculated as:

$\Delta G_s = \frac{\Delta G_p}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon} = \frac{109.6}{0.6^1} + \frac{4.13}{0.6} = 56.48$ kJ/ e$^-$eq

And now, A can be calculated as:

$$A = -\frac{\Delta G_s}{\varepsilon \Delta G_e} = -\frac{\frac{(\Delta G_p)}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}}{\varepsilon(\Delta G_a - \Delta G_d)}$$

$$A = -\frac{56.48}{0.6(-3.87)} = 24.32$$

As we now $f_s = \frac{1}{1+A}$ and $f_e = \frac{A}{1+A}$, the fraction of energy for cell synthesis and energy of the reaction is calculated as:

$$f_s = \frac{1}{1+A} = \frac{1}{25.32} = 0.04$$

$$f_e = \frac{A}{1+A} = \frac{24.32}{25.32} = 0.96$$

For calculating the overall reaction base on equation 2.3:

$$R = f_e(R_a - R_d) + f_s(R_c - R_d) \rightarrow R = f_e R_a + f_s R_c - R_d$$

$$R = 0.96(R_a - R_d) + 0.04(R_c - R_d)$$

And the overall reaction is:

$$0.125\, CH_3COO^- + 0.0032 CO_2 + 0.0023 NH_4^+ + 0.1175 H_2O \rightarrow 0.0116\, CH_{1.8}O_{0.5}N_{0.2} + 0.1189 CH_4 + 0.1227 HCO_3^-$$

During the MES process, anode respiring bacteria in anodic biofilms degrade compounds into bicarbonate, protons, and electrons. The electrons are transmitted to the cathode and react with protons with subtle energy, and the added voltage is generated via the reference electrode [35]. In the case of acetate, the overall reaction can express as:

$$0.125\, CH_3COO^- + 0.0032 CO_2 + 0.0023 NH_4^+ + 0.1175 H_2O \rightarrow 0.0116\, CH_{1.8}O_{0.5}N_{0.2} + 0.1189 CH_4 + 0.1227 HCO_3^-$$

The yield of the reaction is equal to $Y = 0.093\ \frac{mole\ Cell}{mole\ substrate}$ and Gibbs free energy of the reaction is equal to $\Delta G_{an}^0 = -3.87 \frac{KJ}{e^-\ eq}$.

The anode reaction is expressed as follows:

$$CH_3COO^- + 3H_2O \rightarrow CO_2 + HCO_3^- + 8H^+ + 8e^- \qquad \text{(R3.1)}$$

According to (2.17), the theoretical anode potential (E_{an}) for acetate oxidation under standard biological conditions can be calculated as follows:

$$E_{an} = E_{an}^0 - \frac{RT}{8F} \ln \frac{[CO_2][HCO_3^-][H^+]}{[CH_3COO^-][H_2O]^3} \qquad \text{(3.1)}$$

Where " E_{an}^0 " is the standard electrode potential for acetate oxidation which calculate as:

$$E^0_{cell} = -\frac{\Delta G^0_r}{nF} = -\frac{-27.40\times1000\times8}{8\times96500} = 0.284\ V$$

Under standard biological conditions of pH = 7.0, T = 298.15 K, p = 1 bar, $[CH_3COO^-]$ = 0.0169 mol L^{-1} and $[HCO_3^-]$ = 0.005 mol L^{-1} [65] anode potential is[35]:

$$E_{an} = 0.284 - \frac{8.31\ \times 298.15}{8\ \times (9.65\ \times 10^4)} \ln \frac{[0.005]^{0.032}[10^{-7}]^8}{[0.0169]^{0.125}} = 0.284 - (-0.411)$$

$$E_{an} = -0.695\ V$$

Protons and electrons produced from the anodic oxidation of organic components will transfer to the cathodic compartment and react with carbon dioxide to produce methane. Methane electrosynthesis from CO_2 reduction can theoretically happen under standard biological conditions via direct electron transfer (DET) between electrotrophs and cathode at E_{cat} –0.244 V vs. SHE [66 - 67] or indirect electron transfer (IET) with abiotic hydrogen gas at –0.414 V vs. SHE [68 - 69].

Step *i*: $2H^+ + 2e^- \rightarrow H_2(g)$ (R3.2)

Step *ii*: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ (R3.3)

In overall reaction:

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4(g) + 2H_2O \quad \text{(R3.4)}$$

The theoretical cathode potential can be represented as follows in standard conditions:

$$E_{cat} = E^0_{cat} - \frac{RT}{8F} \ln \frac{PCH_4}{PCO_2[H^+]^8} \quad (3.2)$$

" P_{CH4} " and " P_{CO2} " are the partial pressures of methane and carbon dioxide, respectively, while E^0_{cat} is the standard electrode potential for methane evolution.

$$E^0_{cat} = -\frac{\Delta G^0}{nF} = -\frac{23.53\times1000\times8}{8\times96500} = -0.243$$

$$E_{cat} = -0.243 - \frac{8.31\ \times 298.15}{8\ \times (9.65\ \times 10^4)} \ln \frac{1^{0.1189}}{1^{0.0032} \times\ [10^{-7}]^8} = -0.243 - 0.413$$

$$E_{cat} = -0.656\ \text{V}$$

Thus, under standard conditions, the equilibrium voltage (E_{eq}) for carbon dioxide reduction can obtain:

$E_{eq} = E_{cat} – E_{an}$ = (-0.656) – (-0.695) = + 0.039

Theoretically, this shows that spontaneous electromethanogenesis of carbon dioxide can occur in an acetate-fed MES. However, The outcomes presented here are unlikely to be true in actual systems. Moreover, several assumptions have been made regarding the model's simplicity. If the other parameters take into account, the value will vary, and the necessary applied voltage may be raised.

3.3 The cell potential of the acetate for methane production based on Heijnen

The standard microchemical reaction equation for producing 1 C-mol biomass through acetate oxidation is as follows:

$$f\ CH_3COO^- + aCO_2 + bNH_4^+ + c\ H_2O \rightarrow CH_{1.8}O_{0.5}N_{0.2} + dCH_4 + eHCO_3^-$$

The degree of reduction for acetate is γ = 4 and C = 2. Equation 2.12a can be expressed as follow:

$$\frac{1}{Y_{GX}^m} = 200 + 18(6 - C)^{1.8} + \exp\left[((3.8 - \gamma)^2)^{0.16}(3.6 + 0.4C)\right]$$

$$\frac{1}{Y_{GX}^m} = 432.123$$

For maintenance, equation 2.11 (temperature 308.15) can be expressed as:

$$m_G = 4.5 \exp\left[-\frac{69{,}000}{R}\left(\frac{1}{308.15} - \frac{1}{298}\right)\right]$$

$$m_G = 11.105$$

Based on equation 2.10, Gibbs free energy of the reaction can be calculated. We assumed the rate of reaction is $\mu = 0.03\ h^{-1}$[61].

$$\frac{1}{Y_{GX}} = \frac{1}{Y_{GX}^m} + \frac{m_G}{\mu}$$

$$\frac{1}{Y_{GX}} = 802.291\ kJ$$

The conservation equations for C, H, O, and N, as well as the electric charge and Gibbs energy balance, can be written. The stoichiometry of a reaction can be determined by solving these equations:

C - balance: f + a + 1 + d + e = 0

H - balance: 3f + 4b + 2c + 1.8 + 4d + e = 0

O – balance : 2f + 2a + c + 0.5 + 3e = 0

N- balance : b + 0.2 = 0

Charge – balance : -f + b – e = 0

Gibbs energy – balance : (-369.41)f + (-394.359)a + (-79.37)b + (-237.18)c + (-67) + (-586.85)e +802.291 = 0

By solving these six equations and the six unknowns can be calculated. The stoichiometry of the reaction can be expressed as:

$$-89.375CH_3COO^- + 250\ .845CO_2 + 2733.676NH_4^+ - 1013.214\ H_2O \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 1092.085\ CH_4 + 13.641HCO_3^-$$

The cell potential can be determined:

$$E = E^\circ - \frac{RT}{8F}\ln\frac{[CH_{1.8}O_{0.5}N_{0.2}][CH_4]^{1092.085}[HCO_3^-]^{13.64}}{[CH_3COO^-]^{-89.375}[CO\_2]^{250.845}[NH_4^+]^{2733.676}[H_2O]^{1013.214}}$$

E = 1.596 V

3.4 Different substrate oxidation for methane production

Oxidation of acetate, glucose, ammonium, and hydrogen is evaluated for methane production at standard conditions. The standard condition has a pH of 7, a pressure of 1 bar, and a temperature of 298.15 °C. Variation in pressure and temperature parameters is used to assess the applied voltage.

3.4.1 Various applied voltages for acetate oxidation

Figure 3.2 shows the results of acetate oxidation at different temperatures. Applied voltage for methane production is plotted against yields.

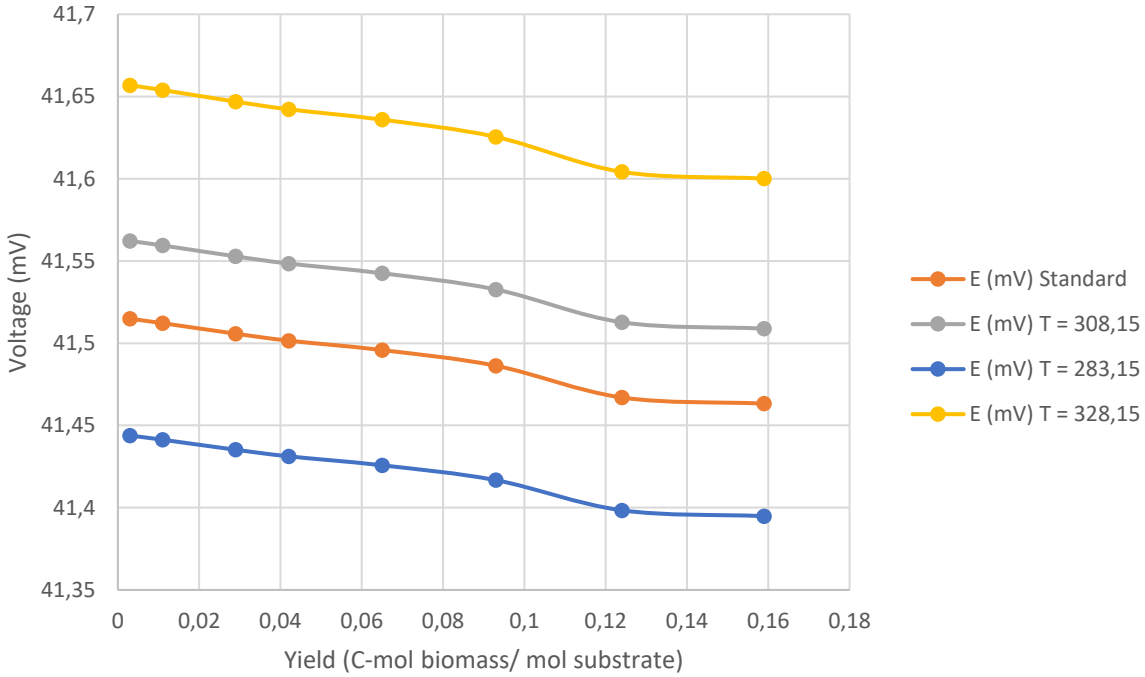

Figure 3.2: Applied voltage needed for different Yield of the acetate reaction at various temperatures

As illustrated in Figure 3.2, an increase in yield can lead to a decrease in the applied voltage for all different temperatures. The voltage needed for various yields is highest at 328.15 k. At 283.15 k, the lowest applied voltage required for acetate oxidation occurs. Furthermore, the degree of voltage variation decreased for yield between 0.124 and 0.159 C-mol biomass/mol substrate. Additionally, as the temperature increases, the required voltage increases.

Figure 3.3 illustrates applied voltage usage at different pressures. The lowest power usage occurs at P = 1.5 bar for different acetate oxidation yields. Additionally, when pressure rises, voltage usage drop. The lowest applied voltage is 41.30 mV at P = 1.5 bar.

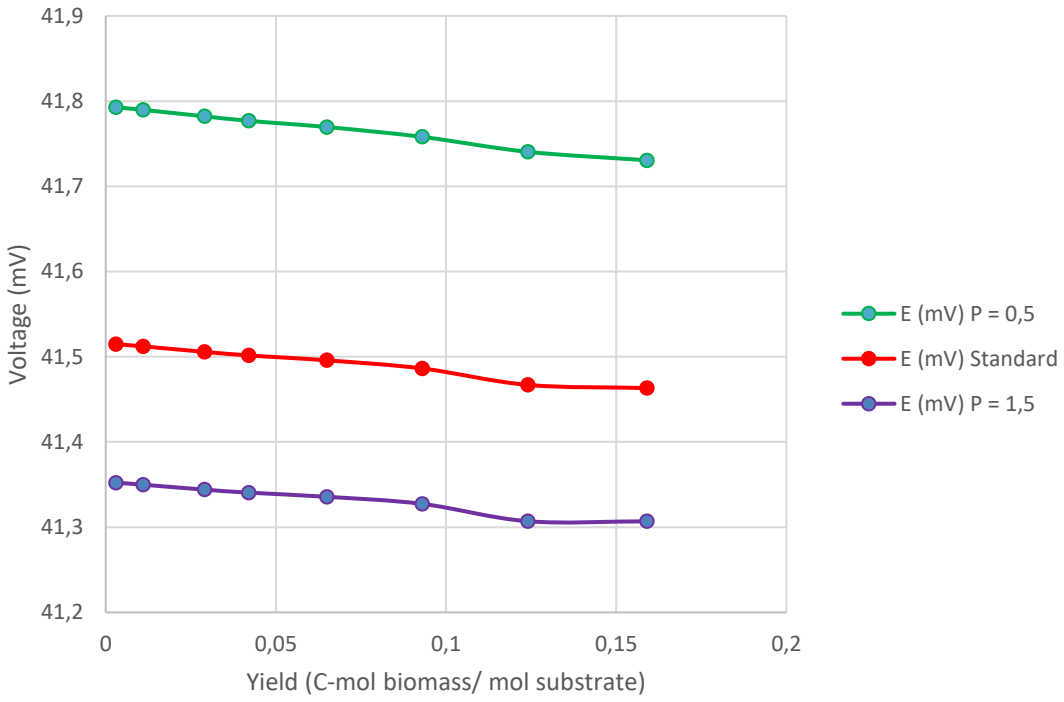

Figure 3.3: Applied voltage needed for different Yield of the acetate reaction at various pressure

3.4.2 Various applied voltages for glucose oxidation

Results of different yields for glucose oxidation are presented in Figure 3.4. The applied voltage is plotted against different yields at several temperatures.

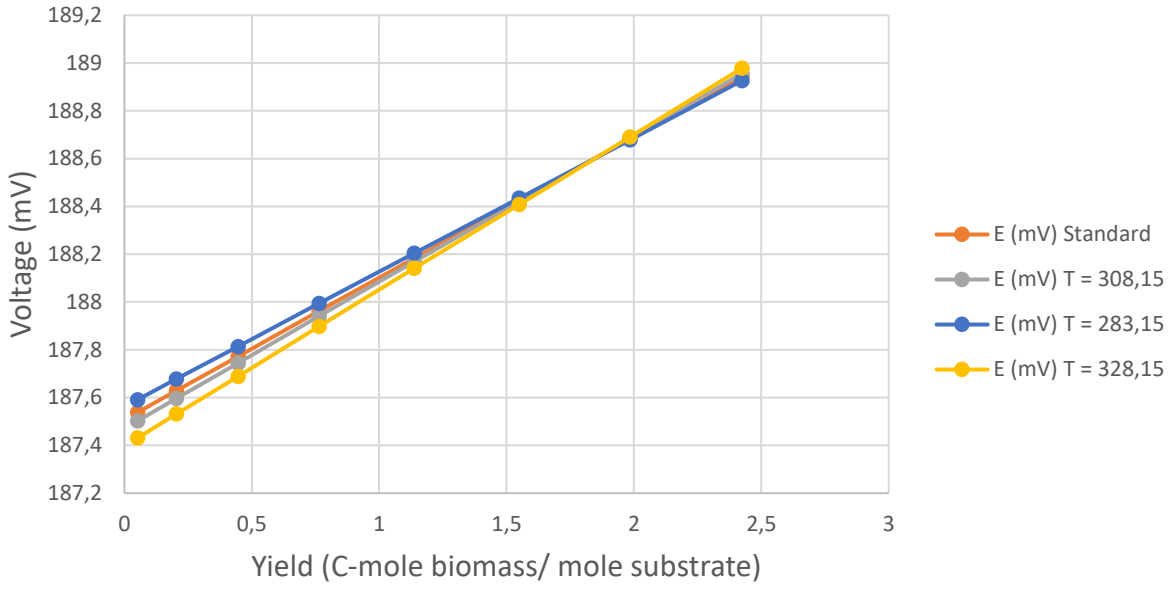

Figure 3.4: Applied voltage needed for different Yield of the glucose reaction at various temperatures

As seen in Figure 3.4, raising the yield increases the applied voltage. At temperature 328.15°k, the lowest voltage necessary yields 0.052. Additionally, at the lowest yield, the minimum applied voltage occurs at 328.15°k. Reversibly, at the highest yield, the applied voltage is maximum at 328.15°k. Voltage decreases as yield is at the highest value and reaches a minimum value at 283.15°k.

Figure 3.5 represents the necessary voltage along with yield while the pressure varies. As seen in Figure 3.5, raising the pressure decreases the necessary voltage.

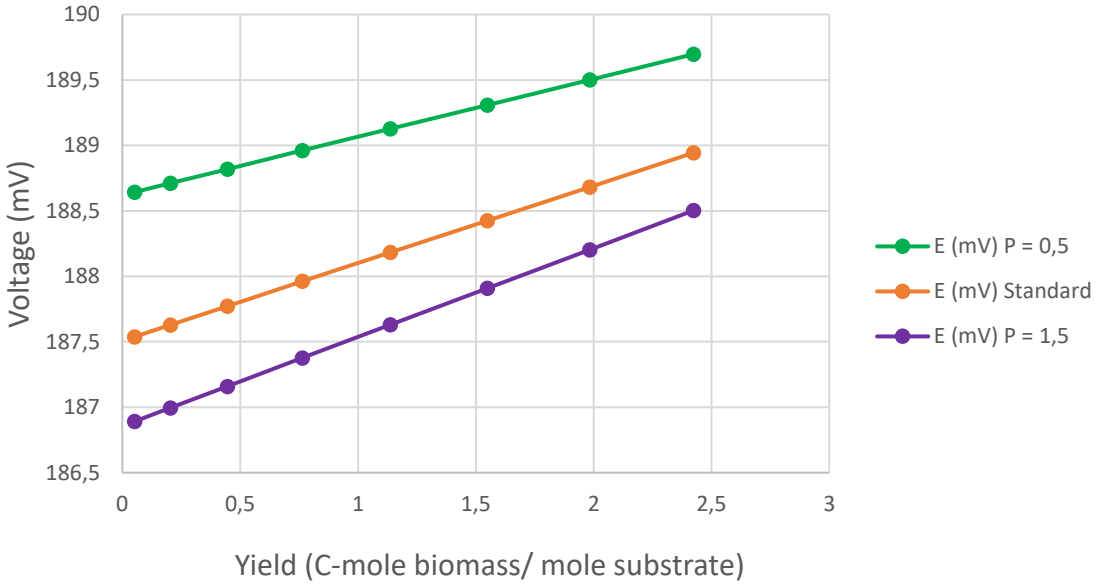

Figure 3.5: Applied voltage needed for different Yield of the glucose oxidation at various pressures

3.4.3 Various applied voltages for ammonium oxidation

Figure 3.6 shows the required voltage of ammonium oxidation at various temperatures. At the maximum temperature, 328.15 K, the lowest applied voltages occur. Additionally, the results indicate that the necessary voltages remain at the lowest value when the yields are increased at 328.15 K.

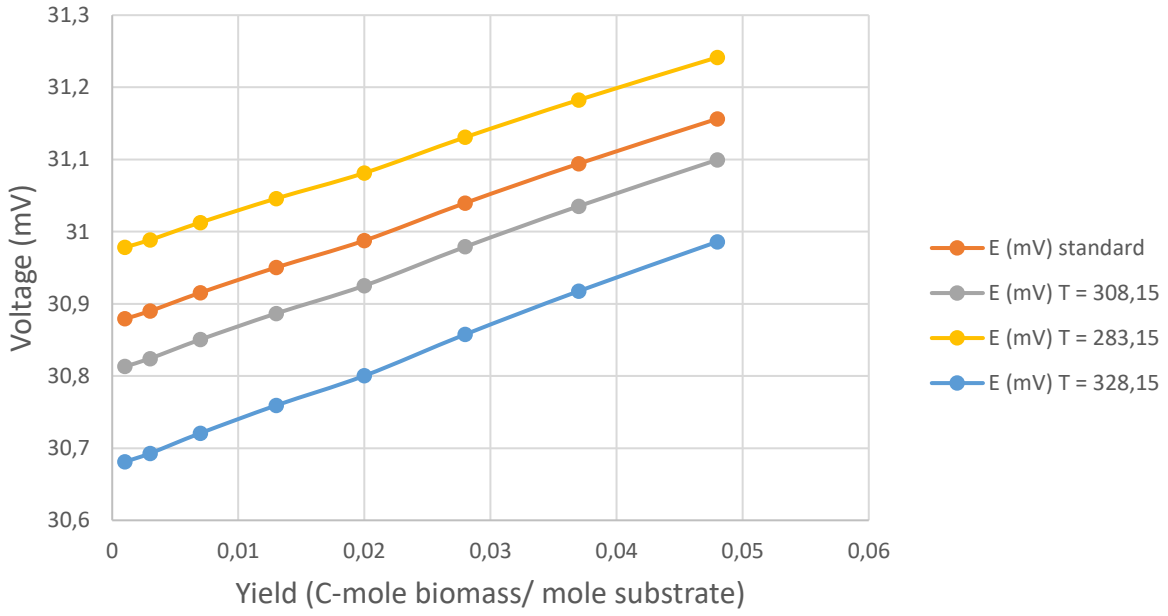

Figure 3.6: Applied voltage needed for different Yield of the ammonium reaction at various temperatures

Figure 3.7 shows the required voltage along with varied yields as the pressure changes. As demonstrated, increasing the pressure reduces the required voltage.

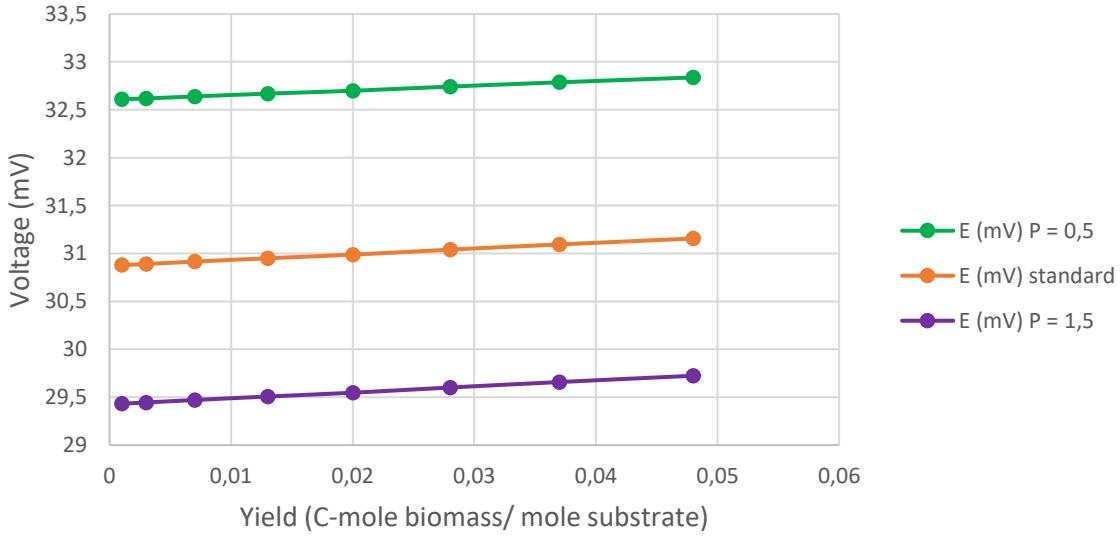

Figure 3.7: Applied voltage needed for different Yield of the ammonium reaction at various pressures

3.4.4 Various applied voltages for hydrogen oxidation

The applied voltage required for hydrogen oxidation is shown in Figure 3.8 for different temperatures. An increase in the yield will result in a rise in the applied voltage.

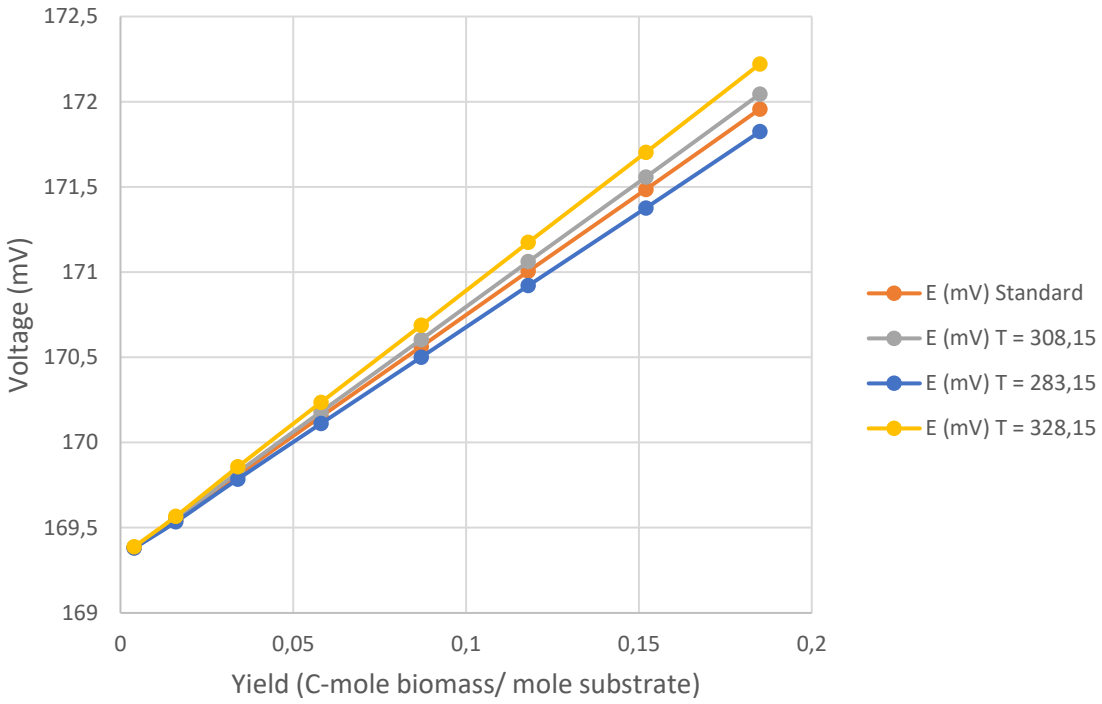

Figure 3.8: Applied voltage needed for different Yield of the hydrogen reaction at various temperatures

As seen in Figure 3.8, the required voltage has about the same value at different temperatures. The applied voltage reaches the maximum value for the temperature of 328.15 K by raising the yield. The findings also demonstrate that the lowest applied voltage occurs at 283.15 K.

The needed voltage and yield are shown in Figure 3.9 as the pressure varies. As illustrated, increasing the pressure results in a reduction in the required voltage.

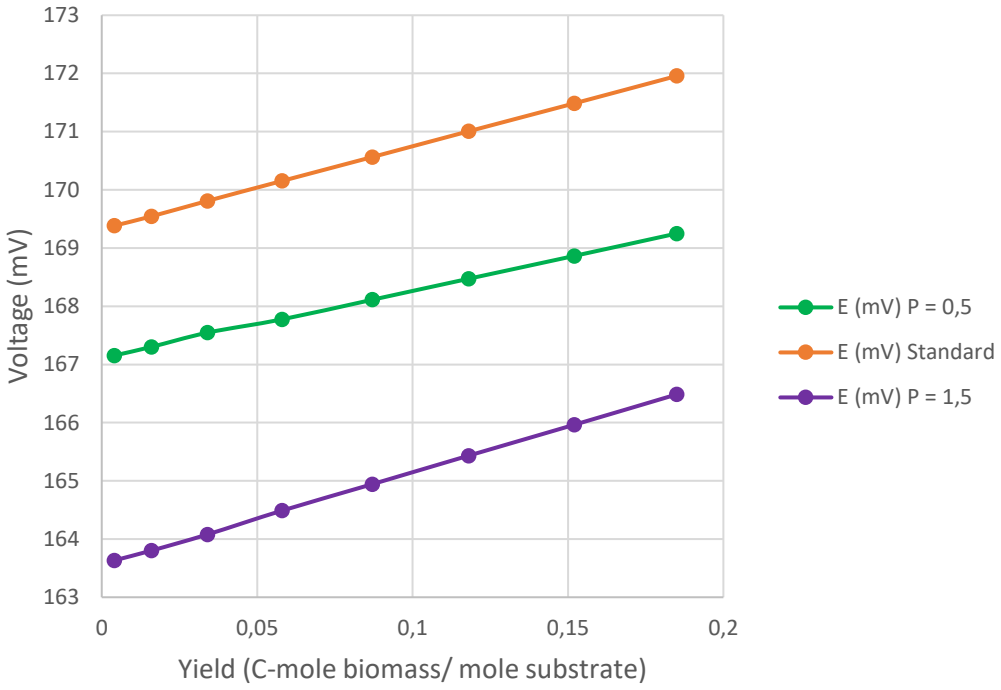

Figure 3.9: Applied voltage needed for the different yields of the hydrogen reaction at various pressures

4 Discussion

Bioelectrochemical systems are a novel technology that has the potential to significantly increase the amount of methane produced in anaerobic digestion (AD) reactors. Renewable energy could be used to further oxidize organic and inorganic waste-compounds in order to extract more energy. Investigating the biochemical reactions occurring on the surface of electrodes can facilitate understanding the methane production process. This study investigated critical biochemical reactions in a bioelectrochemical system as a basis. However, the most crucial task is establishing a method for the calculation of stoichiometries of microbial growth, linked to Gibbs free energy and voltage. Acetate, glucose, ammonium, and hydrogen oxidation are assessed for methane production. Among all of the relevant biochemical reactions, glucose oxidation has the highest applied voltage value of any of the reactions studied. The results also reveal that the ammonium oxidation reaction requires the least voltage.

This work identifies relevant biochemical reactions, and their Gibbs free energies are estimated. The stoichiometric calculation of biochemical reactions is developed based on the McCarty methodology (TEEM) and the Heijnen method. Since the reaction yield is the most crucial characteristic in biological reactions, it is determined by the stoichiometry of the reaction. The required voltage is calculated using the Nernst equation, Gibbs free energy, and stoichiometric coefficients. Finally, the relation between the yield and applied voltage in different conditions is established.
We hypothesized that just one component as the electron donor and one as the electron acceptor, and the ADM1 sequence is not seen. Moreover, we considered pH remained constant, and there were any external inhibitions. Also, potential loss, electrode materials, cathodic catalysts, electroactive biofilm, microbial biomass, extracellular electron transfer (EET) mechanisms, and reactor designs are disregarded.

These assumptions are considered to construct a basic mathematical approach that differs from the real system. one can create a model that closely reflects the actual system by developing the model and analyzing its various parameters.

As the results show, the applied voltage for the oxidation of acetate decrease as yields grow (Figure 3.2), but it increases as yields increase for the other substrates (Figures 3.4, 3.6, 3.8). The main difference between acetate and the other substrates is the amount of electron transfer. Compared to the other substrates, acetate has the maximum electron transport (eight electrons). It can be concluded that If there are more available electrons, the reaction can happen spontaneously, and less voltage is needed.

Acetate is the most often used substance in the production of methane. Once completely oxidized at the anode, acetate can transmit eight electrons across an electrochemical system. As the findings demonstrate, the applied voltage drops as the biomass yield increases. Additionally, the lowest voltage is needed at a temperature of 283.15 K (Figure 3.2). Also, it

is shown that as pressure increases, applied voltage decreases. It is possible to describe this as an increase in the fraction factor and yield causing the reaction to occur more spontaneously and providing more energy for the oxidation of acetate. Additionally, the results indicate that increasing the temperature inhibits the process. Also, decreasing the pressure can have a negligible effect on the reaction rate.

Glucose is also a common substrate that is employed in researches. Glucose oxidation provides four electrons in the half-reaction on the anode. As the results illustrate, the needed voltage increases in accordance to the yield. As can be observed (Figure 3.4), increasing the temperature has an inverse impact on the voltage required. Although the higher temperature requires a lower applied voltage at lower yields, the higher temperature shows a higher voltage when the yield increase. Additionally, it demonstrates that the reaction occurs at a lower temperature with less voltage when yields have a higher value. Consequently, the increased temperature serves as an inhibitor of the process, creating an unfavorable environment for microbial growth.

Also, increasing the pressure can positively affect the reaction rate of glucose oxidation. As the pressure goes up, the conditions become better for microorganisms to live and grow. Other research shows that an increase in pressure can help instant start-up of the methane production [35].

Ammonium can serve as both a source of nitrogen and an electron donor. As the half-reaction of ammonium is revealed, four electrons transfer through the electrodes. As the studies show, the needed voltage increases with the yield. As can be seen, raising the temperature has a negative impact on the voltage supply. The process requires the lowest achievable voltage, which occurs at the lowest temperature, providing a more suitable environment for bacterial growth.

The electrochemical process commonly produces hydrogen. The primary focus of our study is the utilization of hydrogen to create methane. Methane can be produced through hydrogen oxidation and carbon dioxide reduction. For every mol of hydrogen gas that is oxidized, two electrons will transfer in the electrochemical system. According to the data, an increase in temperature leads to a rise in voltage. Increased temperature can prevent the activation of the microorganisms that perform the processes. Consequently, the voltage must rise for the reaction to occur. In addition, increasing the pressure could boost the activation of microorganisms, and spontaneous reactions occur at lower voltages.

It is important to keep in mind that in a real MES-reactor, the only controlled parameter is the voltage at the cathode. The measured voltage is based on the reference electrode so that CO_2 can be turned into methane. The potential at the anode will fluctuate in real systems to achieve the desired cathode potential. This fluctuation is due to variations in the conductivity of the bulk liquid and changes in the concentration of anions and cations. The model has shown that the voltage will generally increase with increased biomass yields, while in practice, the voltage

is kept constant at the cathode. This implies that the electron flow will increase in the reactor if resistances are kept low or reasonably constant over time, and more methane will be produced as the system becomes more efficient.

This thesis builds the basis of a thermodynamic model for the study of MES for methane production. It presents a clear illustration of how biochemical reactions occur in an MES system. Additionally, it defines the relation between applied voltage and biochemical reaction yield. To establish a comprehensive model, more research must address the other factors.

5 Conclusion

Recent findings indicate that methane would be one of the primary energy sources. An effective way of making methane is to combine anaerobic digestion with microbial electrosynthesis systems (MES). Significant system parameters are substrates, electrode materials, cathodic catalysts, electroactive biofilm, microbial biomass, extracellular electron transfer (EET) processes, reactor designs, functions, and operational performance. To examine the effect of substrates on microbial biomass, a model based on the thermodynamic principle has been established. The model explores the energy fraction usage for biomass generation as its preliminary step. Next, stoichiometry and the Nernst equation determine the relationship between Gibbs free energy and applied voltage. According to the findings, pressure is the most crucial factor affecting the required voltage.

In developing the model, some assumptions were made, and the influence of other factors was disregarded. In comparison to temperature and pressure, yield variation has the least influence on the applied voltage. Moreover, variations in the energy fraction may alter the stoichiometry of a process. Without taking into account other factors, this adjustment will not substantially influence the applied voltage from a practical standpoint.

The amount of electron transmission is one of the major factors that separates the various substrates. It is possible to draw the conclusion that if the number of available electrons is increased, the reaction will be more spontaneous and will need a lower voltage.

In addition, an increase in temperature will lead to an increase in the applied voltage, which can have a negative impact on the system. It can be shown that the temperature should be within a reasonable range that does not inhibit the development of the microbial population. Additionally, a rise in pressure might be regarded as driving microorganisms to conduct the reaction more easily.

Among the assessed substrates that are the most significant components in this research, glucose oxidation has the greatest applied voltage value. Furthermore, the results indicate that the ammonium oxidation process needs the least amount of voltage.

6 Future work

- Investigate other parameters that affect the bioelectrochemical system, which is included: the electron transfer mechanism, electrode material, reactor configuration, potential losses, and mixed microbial culture.
- Complete the model by investigating the reactor base on autotrophic and heterotrophic bacteria, the feasibility of the different electron acceptors, electron transfer mechanism.
- Simulate the model based on the thermodynamic model and compare it to the experimental data
- Since the prediction of the biological processes is complex and has some difficulties to model mathematically, utilizing the experimental data and training the data in a black box can be a valuable technique to evaluate the results.

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Appendices

Appendix A: General half reactors.

Appendix A.1: Organic half-reactions and the Gibbs free energy according to [25].

#	Name	Half-reaction	ΔG° (kJ/e⁻eq)
1	Acetate	$\frac{1}{8}\,CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^- \rightarrow \frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O$	27.40
2	Alanine	$\frac{1}{6}CO_2 + \frac{1}{12}HCO_3^- + \frac{1}{12}NH_4^+ + \frac{11}{12}H^+ + e^- \rightarrow \frac{1}{12}CH_3CHNH_2COO^- + \frac{5}{12}H_2O$	31.37
3	Benzoate	$^1/_5\ CO_2 + {}^1/_{30}\ HCO_3^- + H^+ + e^- \rightarrow {}^1/_{30}\ C_6H_5COO^- + {}^{13}/_{30}\ H_2O$	27.34
4	Citrate	$^1/_6\ CO_2 + {}^1/_6\ HCO_3^- + H^+ + e^- \rightarrow {}^1/_{18}\ (COO^-)CH_2COH(COO^-)\ CH_2COO^- + {}^4/_9\ H_2O$	33.08
O-5	Ethanol	$^1/_6\ CO_2 + H^+ + e^- \rightarrow {}^1/_{12}\ CH_3CH_2OH + {}^1/_4\ H_2O$	31.18
O-6	Formate	$^1/_2\ HCO_3^- + H^+ + e^- \rightarrow {}^1/_2\ HCOO^- + {}^1/_2\ H_2O$	39.19
O-7	Glucose	$^1/_4\ CO_2 + H^+ + e^- \rightarrow {}^1/_{24}\ C_6H_{12}O_6 + {}^1/_4\ H_2O$	41.35
O-8	Glutamate	$^1/_6\ CO_2 + {}^1/_9\ HCO_3^- + {}^1/_{18}\ NH_4^+ + H^+ + e^- \rightarrow {}^1/_{18}\ COOHCH_2CH_2CHNH_2COO^- + {}^4/_9\ H_2O$	30.93
O-9	Glycerol	$^3/_{14}\ CO_2 + H^+ + e^- \rightarrow {}^1/_{14}\ CH_2OHCHOHCH_2OH + {}^4/_9\ H_2O$	38.88
O-10	Glycine	$^1/_6\ CO_2 + {}^1/_6\ HCO_3^- + {}^1/_6\ NH_4^+ + H^+ + e^- \rightarrow {}^1/_6\ CH_2NH_2COOH + {}^1/_2\ H_2O$	39.80
O-11	Lactate	$^1/_6\ CO_2 + {}^1/_{12}\ HCO_3^- + H^+ + e^- \rightarrow {}^1/_{12}\ CH_3CHOHCOO^- + {}^1/_3\ H_2O$	32.29
O-12	Methane		
O-13	Methanol	$^1/_6\ CO_2 + H^+ + e^- \rightarrow {}^1/_6\ CH_3OH + {}^1/_6\ H_2O$	36.84
O-14	Palmitate	$^{15}/_{19}\ CO_2 + {}^1/_{92}\ HCO_3^- + H^+ + e^- \rightarrow {}^1/_{92}\ CH_3(CH_2)_{14}COO^- + {}^{31}/_{92}\ H_2O$	27.26
O-15	Propionate	$^1/_7\ CO_2 + {}^1/_{14}\ HCO_3^- + H^+ + e^- \rightarrow {}^1/_{14}\ CH_3\ CH_2COO^- + {}^5/_{14}\ H_2O$	27.63
O-16	Pyruvate	$^1/_5\ CO_2 + {}^1/_{10}\ HCO_3^- + H^+ + e^- \rightarrow {}^1/_{10}\ CH_3\ COCOO^- + {}^2/_5\ H_2O$	35.09
O-17	Succinate	$^1/_7\ CO_2 + {}^1/_7\ HCO_3^- + H^+ + e^- \rightarrow {}^1/_{14}\ (CH_2)_2(COO^-)_2 + {}^3/_7\ H_2O$	29.09

Appendix A.2: Inorganic half-reactions and the standard Gibbs free energy according to [25]

#	Half-reaction	ΔG° (kJ/e⁻eq)
I-1	$^1/_8\ NO_3^- + {}^5/_4\ H^+ + e^- \rightarrow {}^1/_8\ NH_4^+ + {}^3/_8\ H_2O$	- 35.11

I-2	$^1/_6\ NO_2^- + {}^4/_3\ H^+ + e^- \rightarrow {}^1/_6\ NH_4^+ + {}^1/_3\ H_2O$	- 32.93
I-3	$^1/_6\ N_2 + {}^4/_3\ H^+ + e^- \rightarrow {}^1/_3\ NH_4^+$	26.70
I-4	$Fe^{3+} + e^- \rightarrow Fe^{2+}$	- 74.27
I-5	$H^+ + e^- \rightarrow ½\ H_2$	39.87
I-6	$½\ NO_3^- + H^+ + e^- \rightarrow ½\ NO_2^- + ½\ H_2O$	- 41.65
I-7	$^1/_3\ NO_3^- + {}^4/_3\ H^+ + e^- \rightarrow {}^1/_3\ NO + {}^2/_3\ H_2O$	- 39.00
I-8	$^1/_4\ NO_3^- + {}^5/_4\ H^+ + e^- \rightarrow {}^1/_8\ N_2O + {}^5/_8\ H_2O$	- 57.54
I-9	$^7/_{24}\ NO_3^- + {}^{31}/_{24}\ H^+ + e^- \rightarrow {}^1/_6\ NO + {}^1/_{16}\ N_2O + {}^{31}/_{48}\ H_2O$	- 48.27
I-10	$^1/_5\ NO_3^- + {}^6/_5\ H^+ + e^- \rightarrow {}^1/_{10}\ N_2 + {}^3/_5\ H_2O$	- 72.20
I-11	$2H^+ + NO_2^- + e^- \rightarrow NO + H_2O$	- 33.72
I-12	$^1/_5\ NO_2^- + {}^4/_3\ H^+ + e^- \rightarrow {}^1/_6\ N_2 + {}^2/_3\ H_2O$	- 92.56
I-13	$H^+ + NO + e^- \rightarrow ½\ N_2O + ½\ H_2O$	- 115.83
I-14	$H^+ + ½\ N_2O + e^- \rightarrow ½\ N_2 + ½\ H_2O$	- 133.47
I-15	$^1/_8\ SO_4^{2-} + {}^{19}/_{16}\ H^+ + e^- \rightarrow {}^1/_{16}\ H_2S + {}^1/_{16}\ HS^- + {}^1/_2\ H_2O$	20.85
I-16	$^1/_6\ SO_3^{2-} + {}^5/_4\ H^+ + e^- \rightarrow {}^1/_{12}\ H_2S + {}^1/_{12}\ HS^- + {}^1/_2\ H_2O$	11.03
I-17	$^1/_2\ SO_4^{2-} + H^+ + e^- \rightarrow {}^1/_2\ SO_3^{2-} + {}^1/_2\ H_2O$	50.30
I-18	$^1/_6\ SO_4^{2-} + {}^4/_3\ H^+ + e^- \rightarrow {}^1/_6\ S + {}^2/_3\ H_2O$	19.15
I-19	$^1/_4\ SO_4^{2-} + {}^5/_4\ H^+ + e^- \rightarrow {}^1/_8\ S_2O_3^{2-} + {}^5/_8\ H_2O$	23.58
I-20	$¼\ O_2 + H^+ + e^- \rightarrow ½\ H_2O$	- 78.72

Appendix A.3: Common electron acceptor and the standard Gibbs free energy according to [25]

#	**Half-reaction**	**ΔG^o (kJ/e$^-$eq)**
I-20	$¼\ O_2 + H^+ + e^- \rightarrow ½\ H_2O$	- 78.72
I-4	$Fe^{3+} + e^- \rightarrow Fe^{2+}$	- 74.27
I-10	$^1/_5\ NO_3^- + {}^6/_5\ H^+ + e^- \rightarrow {}^1/_{10}\ N_2 + {}^3/_5\ H_2O$	- 72.20
I-15	$^1/_8\ SO_4^{2-} + {}^{19}/_{16}\ H^+ + e^- \rightarrow {}^1/_{16}\ H_2S + {}^1/_{16}\ HS^- + {}^1/_2\ H_2O$	20.85
O -12	$^1/_8\ CO_2 + H^+ + e^- \rightarrow {}^1/_8\ CH_4 + {}^1/_4\ H_2O$	23.53

Appendix B Overall reaction with varying ε

Table B.1: Overall Reactions of different electron donor and CO_2 as electron acceptor to Methane

Donor	Transfer Efficiency	Overall Reaction	Y (C- mol biomass/ mol substrate)	ΔG (kJ/e$^-$ eq)
Acetate	ε = 0.1	$0.125\ CH_3COO^- + 0.0001CO_2 + 0.0003NH_4^+ + 0.1241H_2O \rightarrow 0.0003\ CH_{1.8}O_{0.5}N_{0.2} + 0.1248CH_4 + 0.1249HCO_3^-$	0.003	-3.87
	ε = 0.2	$0.125\ CH_3COO^- + 0.0004CO_2 + 0.0003NH_4^+ + 0.1241H_2O \rightarrow 0.0013\ CH_{1.8}O_{0.5}N_{0.2} + 0.1243CH_4 + 0.1247HCO_3^-$	0.011	-3.87
	ε = 0.4	$0.125\ CH_3COO^- + 0.0015CO_2 + 0.0011NH_4^+ + 0.1216H_2O \rightarrow 0.0053\ CH_{1.8}O_{0.5}N_{0.2} + 0.1222CH_4 + 0.1239HCO_3^-$	0.042	-3.87
	ε = 0.5	$0.125\ CH_3COO^- + 0.0023CO_2 + 0.0016NH_4^+ + 0.1197H_2O \rightarrow 0.0082\ CH_{1.8}O_{0.5}N_{0.2} + 0.1207CH_4 + 0.1234HCO_3^-$	0.065	-3.87
	ε = 0.6	$0.125\ CH_3COO^- + 0.0032CO_2 + 0.0023NH_4^+ + 0.1175H_2O \rightarrow 0.0116\ CH_{1.8}O_{0.5}N_{0.2} + 0.1189CH_4 + 0.1227HCO_3^-$	0.093	-3.87
	ε = 0.7	$0.125\ CH_3COO^- + 0.0043CO_2 + 0.0031NH_4^+ + 0.1149H_2O \rightarrow 0.0117\ CH_{1.8}O_{0.5}N_{0.2} + 0.1186CH_4 + 0.1219HCO_3^-$	0.124	-3.87
	ε = 0.8	$0.125\ CH_3COO^- + 0.0055CO_2 + 0.004NH_4^+ + 0.1121H_2O \rightarrow 0.0199\ CH_{1.8}O_{0.5}N_{0.2} + 0.1146CH_4 + 0.121HCO_3^-$	0.159	-3.87
Glucose	ε = 0.1	$0.0417\ C_6H_{12}O_6 + 0.0004NH_4^+ + 0.0004HCO_3^- \rightarrow 0.0022\ CH_{1.8}O_{0.5}N_{0.2} + 0.1239CH_4 + 0.1244CO_2 + 0.0014H_2O$	0.052	-17.42
	ε = 0.2	$0.0417\ C_6H_{12}O_6 + 0.0017NH_4^+ + 0.0017HCO_3^- \rightarrow 0.0085\ CH_{1.8}O_{0.5}N_{0.2} + 0.1205CH_4 + 0.1227CO_2 + 0.0055H_2O$	0.204	-17.42

	ε = 0.3	$0.0417\ C_6H_{12}O_6 + 0.0037NH_4^+ + 0.0037HCO_3^- \rightarrow 0.0186\ CH_{1.8}O_{0.5}N_{0.2} + 0.1152CH_4 + 0.1199CO_2 + 0.0121H_2O$	0.446	-17.42
	ε = 0.4	$0.0417\ C_6H_{12}O_6 + 0.0064NH_4^+ + 0.0064HCO_3^- \rightarrow 0.0318\ CH_{1.8}O_{0.5}N_{0.2} + 0.1083CH_4 + 0.1163CO_2 + 0.0207H_2O$	0.764	-17.42
	ε = 0.5	$0.0417\ C_6H_{12}O_6 + 0.0095NH_4^+ + 0.0095HCO_3^- \rightarrow 0.0474\ CH_{1.8}O_{0.5}N_{0.2} + 0.1001CH_4 + 0.112CO_2 + 0.0308H_2O$	1.138	-17.42
	ε = 0.6	$0.0417\ C_6H_{12}O_6 + 0.0129NH_4^+ + 0.0129HCO_3^- \rightarrow 0.0646\ CH_{1.8}O_{0.5}N_{0.2} + 0.0911CH_4 + 0.1072CO_2 + 0.042H_2O$	1.55	-17.42
	ε = 0.7	$0.0417\ C_6H_{12}O_6 + 0.0165NH_4^+ + 0.0165HCO_3^- \rightarrow 0.0827\ CH_{1.8}O_{0.5}N_{0.2} + 0.0816CH_4 + 0.1023CO_2 + 0.0537H_2O$	1.984	-17.42
	ε = 0.8	$0.0417\ C_6H_{12}O_6 + 0.0202NH_4^+ + 0.0202HCO_3^- \rightarrow 0.101\ CH_{1.8}O_{0.5}N_{0.2} + 0.072CH_4 + 0.0972CO_2 + 0.0656H_2O$	2.424	-17.42
Benzoate	ε = 0.1	$0.0333C_6H_5COO^- + 0.0004NH_4^+ + 0.1831H_2O \rightarrow 0.0003\ CH_{1.8}O_{0.5}N_{0.2} + 0.1248CH_4 + 0.0749CO_2 + 0.0333HCO_3^-$	0.01	-3.81
	ε = 0.2	$0.0333C_6H_5COO^- + 0.0003NH_4^+ + 0.1825H_2O \rightarrow 0.0013\ CH_{1.8}O_{0.5}N_{0.2} + 0.1243CH_4 + 0.0746CO_2 + 0.0331HCO_3^-$	0.04	-3.81
	ε = 0.3	$0.0333C_6H_5COO^- + 0.0006NH_4^+ + 0.1814H_2O \rightarrow 0.003\ CH_{1.8}O_{0.5}N_{0.2} + 0.1234CH_4 + 0.0742CO_2 + 0.0327HCO_3^-$	0.089	-3.81
	ε = 0.4	$0.0333C_6H_5COO^- + 0.001NH_4^+ + 0.1799H_2O \rightarrow 0.0052\ CH_{1.8}O_{0.5}N_{0.2} + 0.1223CH_4 + 0.0736CO_2 + 0.0323HCO_3^-$	0.156	-3.81

	ε = 0.5	$0.0333C_6H_5COO^- + 0.0016NH_4^+ + 0.1781H_2O \rightarrow 0.008\ CH_{1.8}O_{0.5}N_{0.2} + 0.1208CH_4 + 0.0728CO_2 + 0.0317HCO_3^-$	0.241	-3.81
	ε = 0.6	$0.0333C_6H_5COO^- + 0.0023NH_4^+ + 0.1759H_2O \rightarrow 0.0114\ CH_{1.8}O_{0.5}N_{0.2} + 0.119CH_4 + 0.0719CO_2 + 0.0311HCO_3^-$	0.342	-3.81
	ε = 0.7	$0.0333C_6H_5COO^- + 0.0031NH_4^+ + 0.1734H_2O \rightarrow 0.0153\ CH_{1.8}O_{0.5}N_{0.2} + 0.117CH_4 + 0.0708CO_2 + 0.0303HCO_3^-$	0.458	-3.81
	ε = 0.8	$0.0333C_6H_5COO^- + 0.0039NH_4^+ + 0.1706H_2O \rightarrow 0.0196\ CH_{1.8}O_{0.5}N_{0.2} + 0.1147CH_4 + 0.0696CO_2 + 0.0294HCO_3^-$	0.587	-3.81
Citrate	ε = 0.1	$0.0556(COO^-)CH_2COH(COO^-)CH_2COO^- + 0.0004NH_4^+ + 0.1938H_2O \rightarrow 0.0011\ CH_{1.8}O_{0.5}N_{0.2} + 0.1244CH_4 + 0.0414CO_2 + 0.01665HCO_3^-$	0.019	-9.56
	ε = 0.2	$0.0556(COO^-)CH_2COH(COO^-)CH_2COO^- + 0.0008NH_4^+ + 0.1917H_2O \rightarrow 0.0042\ CH_{1.8}O_{0.5}N_{0.2} + 0.1228CH_4 + 0.0405CO_2 + 0.01658HCO_3^-$	0.075	-9.56
	ε = 0.3	$0.0556(COO^-)CH_2COH(COO^-)CH_2COO^- + 0.0018NH_4^+ + 0.1885H_2O \rightarrow 0.0092\ CH_{1.8}O_{0.5}N_{0.2} + 0.1202CH_4 + 0.0391CO_2 + 0.1648HCO_3^-$	0.165	-9.56
	ε = 0.4	$0.0556(COO^-)CH_2COH(COO^-)CH_2COO^- + 0.0032NH_4^+ + 0.1842H_2O \rightarrow 0.0158\ CH_{1.8}O_{0.5}N_{0.2} + 0.1167CH_4 + 0.0373CO_2 + 0.1635HCO_3^-$	0.284	-9.56
	ε = 0.5	$0.0556(COO^-)CH_2COH(COO^-)CH_2COO^- + 0.0048NH_4^+ + 0.179H_2O \rightarrow 0.0238\ CH_{1.8}O_{0.5}N_{0.2} + 0.1125CH_4 + 0.0351CO_2 + 0.1619HCO_3^-$	0.428	-9.56
	ε = 0.6	$0.0556(COO^-)CH_2COH(COO^-)CH_2COO^- + 0.0066NH_4^+ + 0.1731H_2O \rightarrow 0.0328\ CH_{1.8}O_{0.5}N_{0.2} + 0.1078CH_4 + 0.0326CO_2 + 0.1601HCO_3^-$	0.591	-9.56

	ε = 0.7	$0.0556(COO^-)CH_2COH(COO^-)CH_2COO^- + 0.0085NH_4^+ + 0.1668H_2O \rightarrow 0.0426\ CH_{1.8}O_{0.5}N_{0.2} + 0.1027CH_4 + 0.03CO_2 + 0.1582HCO_3^-$	0.776	-9.56
	ε = 0.8	$0.0556(COO^-)CH_2COH(COO^-)CH_2COO^- + 0.0105NH_4^+ + 0.1602H_2O \rightarrow 0.0527\ CH_{1.8}O_{0.5}N_{0.2} + 0.0973CH_4 + 0.0272CO_2 + 0.1561HCO_3^-$	0.949	-9.56
Formate	ε = 0.1	$0.5HCOO^- + 0.1255CO_2 + 0.0004NH_4^+ + 0.2488H_2O \rightarrow 0.0019\ CH_{1.8}O_{0.5}N_{0.2} + 0.124CH_4 + 0.4996HCO_3^-$	0.004	-15.68
	ε = 0.2	$0.5HCOO^- + 0.1271CO_2 + 0.0015NH_4^+ + 0.2451H_2O \rightarrow 0.0075\ CH_{1.8}O_{0.5}N_{0.2} + 0.1211CH_4 + 0.4985HCO_3^-$	0.015	-15.68
	ε = 0.3	$0.5HCOO^- + 0.1295CO_2 + 0.0033NH_4^+ + 0.2394H_2O \rightarrow 0.0163\ CH_{1.8}O_{0.5}N_{0.2} + 0.1164CH_4 + 0.4967HCO_3^-$	0.033	-15.68
	ε = 0.4	$0.5HCOO^- + 0.1327CO_2 + 0.0056NH_4^+ + 0.2318H_2O \rightarrow 0.028\ CH_{1.8}O_{0.5}N_{0.2} + 0.1103CH_4 + 0.4944HCO_3^-$	0.056	-15.68
	ε = 0.5	$0.5HCOO^- + 0.1365CO_2 + 0.0083NH_4^+ + 0.2229H_2O \rightarrow 0.0417\ CH_{1.8}O_{0.5}N_{0.2} + 0.1031CH_4 + 0.4917HCO_3^-$	0.083	-15.68
	ε = 0.6	$0.5HCOO^- + 0.1406CO_2 + 0.0114NH_4^+ + 0.2131H_2O \rightarrow 0.0568\ CH_{1.8}O_{0.5}N_{0.2} + 0.0952CH_4 + 0.4886HCO_3^-$	0.114	-15.68
	ε = 0.7	$0.5HCOO^- + 0.145CO_2 + 0.0145NH_4^+ + 0.2028H_2O \rightarrow 0.0726\ CH_{1.8}O_{0.5}N_{0.2} + 0.0869CH_4 + 0.4855HCO_3^-$	0.145	-15.68
	ε = 0.8	$0.5HCOO^- + 0.1494CO_2 + 0.0178NH_4^+ + 0.1923H_2O \rightarrow 0.0888\ CH_{1.8}O_{0.5}N_{0.2} + 0.0784CH_4 + 0.4822HCO_3^-$	0.178	-15.68

Glycerol	ε = 0.1	$0.0714CH_2OHCHOHCH_2OH + 0.0004NH_4^+ + 0.0002HCO_3^- \rightarrow 0.0012\ CH_{1.8}O_{0.5}N_{0.2} + 0.1244CH_4 + 0.089CO_2 + 0.0365H_2O$	0.017	-15.13
	ε = 0.2	$0.0714CH_2OHCHOHCH_2OH + 0.0009NH_4^+ + 0.0009HCO_3^- \rightarrow 0.0047\ CH_{1.8}O_{0.5}N_{0.2} + 0.1225CH_4 + 0.088CO_2 + 0.0387H_2O$	0.065	-15.13
	ε = 0.3	$0.0714CH_2OHCHOHCH_2OH + 0.0021NH_4^+ + 0.0021HCO_3^- \rightarrow 0.0103\ CH_{1.8}O_{0.5}N_{0.2} + 0.1196CH_4 + 0.0865CO_2 + 0.0424H_2O$	0.144	-15.13
	ε = 0.4	$0.0714CH_2OHCHOHCH_2OH + 0.0035NH_4^+ + 0.0035HCO_3^- \rightarrow 0.0176\ CH_{1.8}O_{0.5}N_{0.2} + 0.1157CH_4 + 0.0844CO_2 + 0.0427H_2O$	0.247	-15.13
	ε = 0.5	$0.0714CH_2OHCHOHCH_2OH + 0.0053NH_4^+ + 0.0053HCO_3^- \rightarrow 0.0265\ CH_{1.8}O_{0.5}N_{0.2} + 0.1111CH_4 + 0.082CO_2 + 0.0529H_2O$	0.37	-15.13
	ε = 0.6	$0.0714CH_2OHCHOHCH_2OH + 0.0073NH_4^+ + 0.0073HCO_3^- \rightarrow 0.0363\ CH_{1.8}O_{0.5}N_{0.2} + 0.1059CH_4 + 0.0793CO_2 + 0.0593H_2O$	0.508	-15.13
	ε = 0.7	$0.0714CH_2OHCHOHCH_2OH + 0.0094NH_4^+ + 0.0094HCO_3^- \rightarrow 0.0469\ CH_{1.8}O_{0.5}N_{0.2} + 0.1004CH_4 + 0.0764CO_2 + 0.0662H_2O$	0.656	-15.13
	ε = 0.8	$0.0714CH_2OHCHOHCH_2OH + 0.0115NH_4^+ + 0.0115HCO_3^- \rightarrow 0.0577\ CH_{1.8}O_{0.5}N_{0.2} + 0.0947CH_4 + 0.0734CO_2 + 0.0732H_2O$	0.808	-15.13
Glycine	ε = 0.1	$0.1667CH_2NH_2COOH + 0.2487H_2O \rightarrow 0.002\ CH_{1.8}O_{0.5}N_{0.2} + 0.124CH_4 + 0.0411CO_2 + 0.1663NH_4^+ + 0.1663HCO_3^-$	0.012	-12.72
	ε = 0.2	$0.1667CH_2NH_2COOH + 0.245H_2O \rightarrow 0.0078\ CH_{1.8}O_{0.5}N_{0.2} + 0.1209CH_4 + 0.0395CO_2 + 0.1651NH_4^+ + 0.1651HCO_3^-$	0.047	-12.72

	ε = 0.3	$0.1667CH_2NH_2COOH + 0.239H_2O \rightarrow 0.017\ CH_{1.8}O_{0.5}N_{0.2} + 0.1161CH_4 + 0.037CO_2 + 0.1633NH_4^+ + 0.1633HCO_3^-$	0.102	-12.72
	ε = 0.4	$0.1667CH_2NH_2COOH + 0.2311H_2O \rightarrow 0.029\ CH_{1.8}O_{0.5}N_{0.2} + 0.1098\ CH_4 + 0.0337CO_2 + 0.1609NH_4^+ + 0.1609HCO_3^-$	0.174	-12.72
	ε = 0.5	$0.1667CH_2NH_2COOH + 0.2219H_2O \rightarrow 0.0433\ CH_{1.8}O_{0.5}N_{0.2} + 0.1023CH_4 + 0.0298CO_2 + 0.158NH_4^+ + 0.158HCO_3^-$	0.26	-12.72
	ε = 0.6	$0.1667CH_2NH_2COOH + 0.2217H_2O \rightarrow 0.059\ CH_{1.8}O_{0.5}N_{0.2} + 0.094CH_4 + 0.0255CO_2 + 0.1549NH_4^+ + 0.1549HCO_3^-$	0.354	-12.72
	ε = 0.7	$0.1667CH_2NH_2COOH + 0.2009H_2O \rightarrow 0.0755\ CH_{1.8}O_{0.5}N_{0.2} + 0.0854CH_4 + 0.0209CO_2 + 0.1516NH_4^+ + 0.1516HCO_3^-$	0.453	-12.72
	ε = 0.8	$0.1667CH_2NH_2COOH + 0.1901H_2O \rightarrow 0.0922\ CH_{1.8}O_{0.5}N_{0.2} + 0.0766CH_4 + 0.0163CO_2 + 0.1482NH_4^+ + 0.1482HCO_3^-$	0.553	-12.72
Propoinate	ε = 0.1	$0.0714CH_3CH_2COO^- + 0.0001NH_4^+ + 0.1069H_2O \rightarrow 0.0004\ CH_{1.8}O_{0.5}N_{0.2} + 0.1248CH_4 + 0.0178CO_2 + 0.0714HCO_3^-$	0.005	-4.1
	ε = 0.2	$0.0714CH_3CH_2COO^- + 0.0003NH_4^+ + 0.1062H_2O \rightarrow 0.0014\ CH_{1.8}O_{0.5}N_{0.2} + 0.1242CH_4 + 0.0175CO_2 + 0.0711HCO_3^-$	0.02	-4.1
	ε = 0.3	$0.0714CH_3CH_2COO^- + 0.0001NH_4^+ + 0.1069H_2O \rightarrow 0.0004\ CH_{1.8}O_{0.5}N_{0.2} + 0.1248CH_4 + 0.0178CO_2 + 0.0714HCO_3^-$	0.045	-4.1
	ε = 0.4	$0.0714CH_3CH_2COO^- + 0.0011NH_4^+ + 0.1035H_2O \rightarrow 0.0057\ CH_{1.8}O_{0.5}N_{0.2} + 0.122CH_4 + 0.0163CO_2 + 0.0703HCO_3^-$	0.079	-4.1

	ε = 0.5	$0.0714CH_3CH_2COO^- + 0.0017NH_4^+ + 0.1015H_2O \rightarrow 0.0087\ CH_{1.8}O_{0.5}N_{0.2} + 0.1204CH_4 + 0.0155CO_2 + 0.0697HCO_3^-$	0.122	-4.1
	ε = 0.6	$0.0714CH_3CH_2COO^- + 0.0025NH_4^+ + 0.0991H_2O \rightarrow 0.0124\ CH_{1.8}O_{0.5}N_{0.2} + 0.1185CH_4 + 0.0145CO_2 + 0.069HCO_3^-$	0.173	-4.1
	ε = 0.7	$0.0714CH_3CH_2COO^- + 0.0033NH_4^+ + 0.0964H_2O \rightarrow 0.0165\ CH_{1.8}O_{0.5}N_{0.2} + 0.1163CH_4 + 0.0133CO_2 + 0.0681HCO_3^-$	0.231	-4.1
	ε = 0.8	$0.0714CH_3CH_2COO^- + 0.0042NH_4^+ + 0.0934H_2O \rightarrow 0.0211\ CH_{1.8}O_{0.5}N_{0.2} + 0.1139CH_4 + 0.012CO_2 + 0.0672HCO_3^-$	0.296	-4.1
H_2	ε = 0.1	$0.5H_2 + 0.1255CO_2 + 0.0004\ NH_4^+ + 0.0004HCO_3^- \rightarrow 0.002CH_{1.8}O_{0.5}N_{0.2} + 0.124CH_4 + 0.2513H_2O$	0.004	-16.34
	ε = 0.2	$0.5H_2 + 0.1271CO_2 + 0.0016\ NH_4^+ + 0.0016HCO_3^- \rightarrow 0.0078CH_{1.8}O_{0.5}N_{0.2} + 0.1209CH_4 + 0.2551H_2O$	0.016	-16.34
	ε = 0.3	$0.5H_2 + 0.1297CO_2 + 0.0034\ NH_4^+ + 0.0034HCO_3^- \rightarrow 0.0171CH_{1.8}O_{0.5}N_{0.2} + 0.116CH_4 + 0.2611H_2O$	0.034	-16.34
	ε = 0.4	$0.5H_2 + 0.133CO_2 + 0.0058\ NH_4^+ + 0.0058HCO_3^- \rightarrow 0.0292CH_{1.8}O_{0.5}N_{0.2} + 0.1097CH_4 + 0.2691H_2O$	0.058	-16.34
	ε = 0.5	$0.5H_2 + 0.137CO_2 + 0.0087\ NH_4^+ + 0.0087HCO_3^- \rightarrow 0.0435CH_{1.8}O_{0.5}N_{0.2} + 0.1022CH_4 + 0.2782H_2O$	0.087	-16.34
	ε = 0.6	$0.5H_2 + 0.1413CO_2 + 0.0118\ NH_4^+ + 0.0118HCO_3^- \rightarrow 0.0592CH_{1.8}O_{0.5}N_{0.2} + 0.0939CH_4 + 0.2885H_2O$	0.118	-16.34

	ε = 0.7	$0.5H_2 + 0.1458CO_2 + 0.0152\,NH_4^+ + 0.0152HCO_3^- \rightarrow 0.0758CH_{1.8}O_{0.5}N_{0.2} + 0.0852CH_4 + 0.2993H_2O$	0.152	-16.34
	ε = 0.8	$0.5H_2 + 0.1505CO_2 + 0.0185\,NH_4^+ + 0.0185HCO_3^- \rightarrow 0.0926CH_{1.8}O_{0.5}N_{0.2} + 0.0764CH_4 + 0.3102H_2O$	0.185	-16.34
NH_4^+	ε = 0.1	$0.3334NH_4^+ + 0.1251CO_2 + 0.0001HCO_3^- \rightarrow 0.0003\,CH_{1.8}O_{0.5}N_{0.2} + 0.1249\,CH_4 + 0.3333H^+ + 0.1667\,N_2 + 0.2502H_2O$	0.001	- 3.17
	ε = 0.2	$0.3335NH_4^+ + 0.1253CO_2 + 0.0002HCO_3^- \rightarrow 0.0011\,CH_{1.8}O_{0.5}N_{0.2} + 0.1244\,CH_4 + 0.3333H^+ + 0.1667\,N_2 + 0.2507H_2O$	0.003	- 3.17
	ε = 0.3	$0.3338NH_4^+ + 0.1257CO_2 + 0.0005HCO_3^- \rightarrow 0.0024\,CH_{1.8}O_{0.5}N_{0.2} + 0.1237\,CH_4 + 0.3333H^+ + 0.1667\,N_2 + 0.2516H_2O$	0.007	- 3.17
	ε = 0.4	$0.3342NH_4^+ + 0.1262CO_2 + 0.0009HCO_3^- \rightarrow 0.0043\,CH_{1.8}O_{0.5}N_{0.2} + 0.1228\,CH_4 + 0.3333H^+ + 0.1667\,N_2 + 0.2528H_2O$	0.013	- 3.17
	ε = 0.5	$0.3346NH_4^+ + 0.1268CO_2 + 0.0013HCO_3^- \rightarrow 0.0066\,CH_{1.8}O_{0.5}N_{0.2} + 0.1215\,CH_4 + 0.3333H^+ + 0.1667\,N_2 + 0.2543H_2O$	0.02	- 3.17
	ε = 0.6	$0.3352NH_4^+ + 0.1276CO_2 + 0.0019HCO_3^- \rightarrow 0.0094\,CH_{1.8}O_{0.5}N_{0.2} + 0.1201\,CH_4 + 0.3333H^+ + 0.1667\,N_2 + 0.2561H_2O$	0.028	- 3.17
	ε = 0.7	$0.3358NH_4^+ + 0.1285CO_2 + 0.0025HCO_3^- \rightarrow 0.0126\,CH_{1.8}O_{0.5}N_{0.2} + 0.1184\,CH_4 + 0.3333H^+ + 0.1667\,N_2 + 0.2582H_2O$	0.037	- 3.17
	ε = 0.8	$0.3366NH_4^+ + 0.1294CO_2 + 0.0032HCO_3^- \rightarrow 0.0162\,CH_{1.8}O_{0.5}N_{0.2} + 0.1165\,CH_4 + 0.3333H^+ + 0.1667\,N_2 + 0.2506H_2O$	0.048	- 3.17

SO_3^{2-}	ε = 0.1	$0.5(SO_3)^{2-} + 0.1259CO_2 + 0.0007NH_4^+ + 0.0007HCO_3^- + 0.2479H_2O \rightarrow 0.0033CH_{1.8}O_{0.5}N_{0.2} + 0.1233CH_4 + 0.5\ (SO_4)^{2-}$	0.007	-0.05
	ε = 0.2	$0.5(SO_3)^{2-} + 0.1285CO_2 + 0.0026NH_4^+ + 0.0026HCO_3^- + 0.2417H_2O \rightarrow 0.0128CH_{1.8}O_{0.5}N_{0.2} + 0.1183CH_4 + 0.5\ (SO_4)^{2-}$	0.026	-0.05
	ε = 0.3	$0.5(SO_3)^{2-} + 0.1327CO_2 + 0.0056NH_4^+ + 0.0056HCO_3^- + 0.2318H_2O \rightarrow 0.0279CH_{1.8}O_{0.5}N_{0.2} + 0.1103CH_4 + 0.5\ (SO_4)^{2-}$	0.056	-0.05
	ε = 0.4	$0.5(SO_3)^{2-} + 0.1381CO_2 + 0.0096NH_4^+ + 0.0096HCO_3^- + 0.2189H_2O \rightarrow 0.0478CH_{1.8}O_{0.5}N_{0.2} + 0.0999CH_4 + 0.5\ (SO_4)^{2-}$	0.096	-0.05
	ε = 0.5	$0.5(SO_3)^{2-} + 0.1446CO_2 + 0.0142NH_4^+ + 0.0142HCO_3^- + 0.2037H_2O \rightarrow 0.0712CH_{1.8}O_{0.5}N_{0.2} + 0.0876CH_4 + 0.5\ (SO_4)^{2-}$	0.142	-0.05
	ε = 0.6	$0.5(SO_3)^{2-} + 0.1517CO_2 + 0.0194NH_4^+ + 0.0194HCO_3^- + 0.1869H_2O \rightarrow 0.097CH_{1.8}O_{0.5}N_{0.2} + 0.0741CH_4 + 0.5\ (SO_4)^{2-}$	0.194	-0.05
	ε = 0.7	$0.5(SO_3)^{2-} + 0.1591CO_2 + 0.0248NH_4^+ + 0.0248HCO_3^- + 0.1693H_2O \rightarrow 0.1242CH_{1.8}O_{0.5}N_{0.2} + 0.0598CH_4 + 0.5\ (SO_4)^{2-}$	0.248	-0.05
	ε = 0.8	$0.5(SO_3)^{2-} + 0.1667CO_2 + 0.0303NH_4^+ + 0.0303HCO_3^- + 0.1514H_2O \rightarrow 0.1517CH_{1.8}O_{0.5}N_{0.2} + 0.0454CH_4 + 0.5\ (SO_4)^{2-}$	0.303	-0.05