“Nature provides a free lunch, but only if we control our appetites.”

- ***William Ruckelshaus****, former head of the Environmental Protection Agency*

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Electrifying biotechnology for the production of CO_2-based chemicals

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Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences: Environmental Technology

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Cover illustration

Pascale Wagener

Notation Index

AD	Anaerobic digestion
AEM	Anion exchange membrane
BES	Bioelectrochemical system
BPM	Bipolar membrane
CAPEX	Capital expenditures
CCS	Carbon capture and storage
CCTG	Combined cycle power plant
CCU	Carbon capture and utilization
CDW	Cell dry weight
CE	Coulombic efficiency
CEM	Cation exchange membrane
CHP	Combined heat and power
CNG	Compressed natural gas
-CoA	-Coenzyme A, *e.g.* acetyl-CoA
COD	Chemical oxygen demand
CV	Cyclic voltammetry
DM	Dry matter
DRM	Dry reforming of methane
EBU	Electrochemical biogas upgrading

ETS	Emission trading system
FM	Fresh material
HHV	Higher heating value
HOB	Hydrogen oxidizing bacteria
HX	Heat exchanger
Ir MMO	Iridium mixed metal oxide
LHV	Lower heating value
MCCA	Medium chain carboxylic acid
ME	Membrane electrolysis
MEC	Microbial electrolysis cell
MES	Microbial electrosynthesis
MFC	Microbial fuel cell
MMO	Mixed metal oxide
MOB	Methane oxidizing bacteria
MP	Microbial protein
OFMSW	Organic fraction of municipal solid waste
OPEX	Operational expenditures
PEM	Polymer electrolyte membrane
ppm	Parts per million
PV	Photovoltaics

P2G	Power-to-gas
SCCA	Short-chain carboxylic acid
SDR	Super-dry reforming of methane
SESR	Sorption-enhanced steam reforming
SHE	Standard hydrogen electrode
VFA	Volatile fatty acids
vs.	versus

F	Faraday's number (96 485.3 C mol^{-1})

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CHAPTER 1

General introduction

This chapter has partly been redrafted after:

De Vrieze, J.*, **Verbeeck, K.***, Pikaar, I., Boere, J., Wijk, A., Rabaey, K. & Verstraete, W. **(2019)**. The hydrogen gas bio-based economy and the production of renewable building block chemicals, food and energy. *Submitted to New Biotechnology*. *Equal contribution.

1. The current fossil carbon economy

Carbon (C) is one of the main building blocks of life on Earth. It is a vital element in the majority of things produced and consumed by man. Our civilization is literally built on carbon. The clothes we wear, the food we eat, the fuel we burn: carbon is involved in all our daily-use goods. Due to the prominent role of carbon atoms in the supply of energy, chemicals and materials, our global society is often referred to as a 'carbon economy'.

1.1. The human influence on the carbon cycle

The vast majority of carbon on Earth is bound to oxygen atoms, solidified in sedimentary rocks within the planet's crust and dissolved in the oceans. However, our industrial civilization relies on energy-rich hydrocarbons that have been formed over periods of millions of years through fossilization of ancient living biomass under intense temperature and pressure. These immense historical fossil carbon reserves form the starting point of our current carbon-based production of energy, materials and chemicals. At present, coal, oil and natural gas represent about 85 % of the world's primary energy demand (BP, 2018). Unfortunately, the life cycle of every reduced carbon atom ends up with the formation of carbon dioxide (CO_2), which is known to be the prime greenhouse gas causing global climate change (IPCC, 2013).

For millennia, natural processes for carbon capture could balance the release of CO_2 to the atmosphere, making the global carbon cycle to be in a dynamic equilibrium. Human interference, however, seriously disrupted the global carbon-energy cycle: the burning of fossil fuels, rapid changes in land use, and the decomposition of carbonates give rise to excessive CO_2 emissions that could not be compensated by the quite slow natural CO_2 capturing mechanisms (*i.e.*, dissolution in oceans and photosynthesis) (Archer, 2008). Fossil carbon, that has been stored for millions of years, is currently being mined, refined and oxidized at a time constant that is up to 10 000 000 000 times higher than the time constant at which CO_2 is cycled back to hydrocarbons (Martens et al., 2017). In geological terms, the combustion of fossil fuels can, thus, be seen as a relatively rapid flux of large amounts of carbon from the Earth's crust to the atmosphere. Over the last half-century, the Earth's natural CO_2 sinks were only able to absorb 54 % of the total anthropogenic CO_2 emissions, and, thus, the size of the atmospheric carbon pool is increasing slowly, but with a huge impact on our climate (Le Quéré et al., 2018).

In order to limit average global warming by 2°C with a 75 % probability, the cumulative worldwide CO_2 emissions in the period 2000-2050 needs to be limited to around 1100 gigatonnes of CO_2 (expressed as CO_2 equivalents) (Figure 1.1). Realizing that this is equivalent to burning just 1.9 - 3.3 % of the total fossil carbon resources (estimated at 9 000 to 16 000 Gt C), it is clear that the depletion of fossil fuel stocks is not yet the main argument for the transition to alternative and more sustainable sources of energy and carbon (McGlade & Ekins, 2015). To achieve the climate goals defined under the 2015 Paris climate agreement, improvements in energy efficiency and a massive deployment of low-carbon energy technologies are key to increase the independence from fossil resources and lower anthropogenic CO_2 emissions. Some scenarios even conclude that without a net removal of CO_2 from the atmosphere, it will be nearly impossible to achieve the rate of anthropogenic CO_2 reduction that is required to meet the two-degree scenario. It is expected that it will be necessary to store 120 - 160 Gton CO_2 *via* carbon capture and storage in the period to 2050 (Mac Dowell et al., 2017). Deployment of technologies for capture, storage and utilization of carbon will most likely become a vital part of the energy system of the future, in which low- carbon, available, and affordable renewable energy is massively employed to drive these processes.

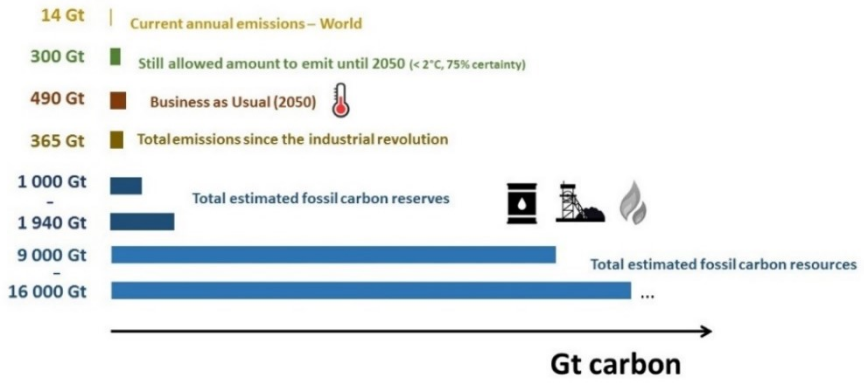


Figure 1.1 - Emission of greenhouse gases through human activity (expressed as carbon) *vs*. estimation of the amounts of fossil carbon reserves and resources. Reserves: recovery is possible with current technology and is economically viable. Resources: not mineable or exploitation is currently not economically viable.

1.2. The transition to a renewable energy system

As the unabated use of all known fossil fuel stocks is incompatible with the target to limit global warming to 2°C, we need to reduce our dependency on fossil carbon and move towards an economy based on alternative forms of energy and chemical building blocks. With photovoltaics (PV), wind, hydropower, tidal, bioenergy, and solar thermal energy systems we have the technology to harvest our natural resources at large scale and catalyze a transition towards a low carbon energy mix. In fact all these energy sources stem in some way or another from the constant flow of solar energy that strikes the surface of the Earth. The incoming solar energy averages 161 W m^{-2} (Overmann & Garcia-Pichel, 2013; Trenberth et al., 2009). At a rough average of 12 hours of daylight, this amounts up to 705 kWh m^{-2} $year^{-1}$, averaged over the entire planet. Considering that the total surface area of Earth is about 510 billion km^2, about 360 550 PWh is reaching the Earth's surface every year. In one hour, the sunlight energy striking the earth produces enough energy to meet the current global power needs for one full year (Zervos et al., 2010). The main challenge is to grasp the energy of about 1 % of the incoming solar photons and to use them to meet our energy needs.

Positive developments show that the renewable electricity transition is possible: the renewable power generating capacity is growing rapidly (around 9 % every year), costs are falling and governments are becoming leaders in renewable energy initiatives. A comparative study on the levelized cost of electricity (LCOE) of newly commissioned power plants reveals that both utility-scale PV and onshore wind farms are now competitive with new fossil fuel and nuclear power generating facilities in an increasing number of locations, even without any governmental support (Figure 1.2) (IRENA, 2018b). The challenge is to get to this renewable energy mix fast enough and to find strategies to overcome the intermittency of renewable energy sources (Trainer, 2017). As renewables are considered to contribute to the decarbonization of transport, heating/cooling and industry, electricity will have a much greater role to play than it does now. Achieving net zero emissions by all sectors require boosting the role of low-carbon renewable electricity up to a level at which Earth's natural processes for CO_2 capture could balance the unavoidable CO_2 emissions of human activity.

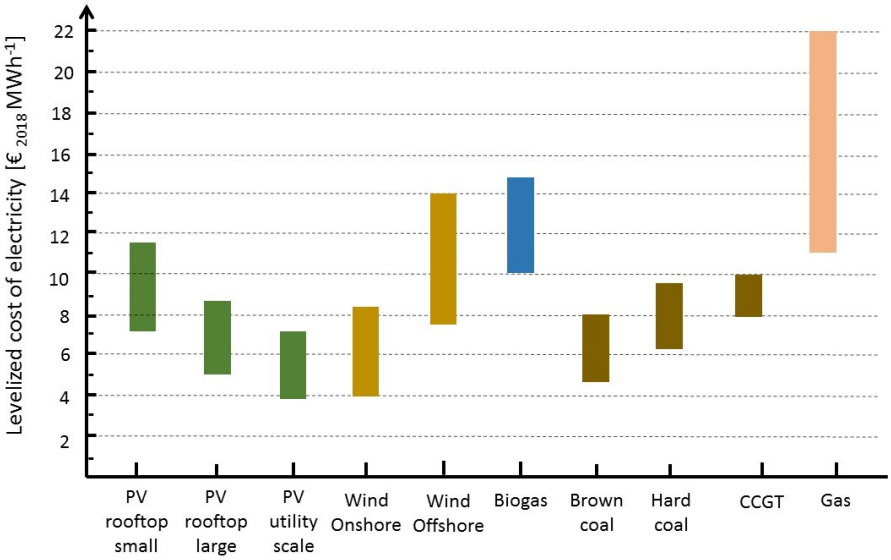


Figure 1.2- Range of levelized costs of electricity from newly built renewable and fossil power projects. Adapted from Fraunhofer (2018). PV: Photovoltaics. CCTG: Combined cycle power plant.

1.3. Our dependency on carbon compounds

Despite all decarbonization efforts, improvements in energy efficiency and a growing number of climate change mitigation policies, energy-related CO_2 emissions are still on the rise (2 % increase in 2018) (Le Quéré et al., 2018). Given the global increase in energy demand and the enormous power capacity renewables need to replace, it is reasonable to suggest that fossil fuels will continue to be important for decades, with some models even estimating that fossil carbon will still account for about 65 % of the global energy mix in 2100 (IRENA, 2018a). Even with the complete decarbonization of electricity generation through the widespread implementation of renewable energy technologies, considerable amounts of CO_2 from cement, steel and chemical industries will still be produced. Furthermore, the exceptional properties of carbon as a building block and energy carrying compound make it unrealistic to rapidly decarbonize the entire energy and materials cycle, for sure with current technological capabilities. At present day, the chemical industry represents about 6 % of the global primary energy demand (BP, 2018). Plastics and other petrochemical products account for roughly 12 % of the global oil demand, a share that is expected to increase as the result of an increasing demand for plastics, fertilizers and other petrochemical building blocks (IEA, 2018). As the role of the chemical sector in today's global energy system is set to increase significantly, a path to an alternative scenario for feedstock security and environmental degradation is set to be crucial.

2. The road to a CO_2-neutral world

2.1. Shortcutting the carbon cycle through biomass-conversion processes

The carbon cycle in which photosynthesis, fossilization, refining and combustion are key processes can be shortened by directly injecting fresh biomass into the loop, eliminating the rate-limiting fossilization step (Figure 1.3). Just like petrochemical refineries process fossil oil into chemical building blocks, so do biorefineries with renewable carbon from biomass (Cherubini, 2010). Integrated biorefinery facilities use multiple technologies and unit processes to separate and convert organic feedstocks into different classes of bio-based products (fuels, materials, bulk commodities and fine chemicals). The development of bio-based value chains from renewable raw materials has resulted in a broad range of fuels and building block chemicals that are at present being produced at commercial scale, such as ethanol and biodiesel, but also non-fuels, such as succinic acid, lactic acid, glycerol and sorbitol (Aslanzadeh et al., 2014; Cherubini, 2010). Nevertheless, the production of so-called 'first-generation' biofuels, produced from edible oil- and sugar-rich food crops is rather controversial, mainly due to their potential conflict with the agricultural food supply chain (Mohr & Raman, 2013). Advanced, or second-generation biofuels, produced from lignocellulosic energy (non-food) crops or agricultural by-products, are widely seen as a sustainable alternative, but a number of major technical and economic hurdles are still to be faced before advanced biofuels can be widely deployed on a fully commercial scale (Naik et al., 2010). The main challenge for all cellulosic-derived bioproducts is that cost competitiveness of these products remains behind their fossil or even conventional sugar, starch or vegetable oil-based analogues. Reductions in the costs of pretreatment and hydrolytic enzymes, and improvements in the conversion efficiency are necessary to improve the economics of these processes and justify their commercialization (Cheng & Timilsina, 2011). The major constraint is, however, the shortage in sustainably available biogenic waste and agricultural and forestry residues to meet the current fossil carbon demand (Oh et al., 2018; Searle & Malins, 2016). From a resource perspective, the biorefinery concept is, thus, challenged by a huge imbalance between the speed at which biomass is produced through photosynthesis and the speed our economic model consumes reduced carbon (Martens et al., 2017).

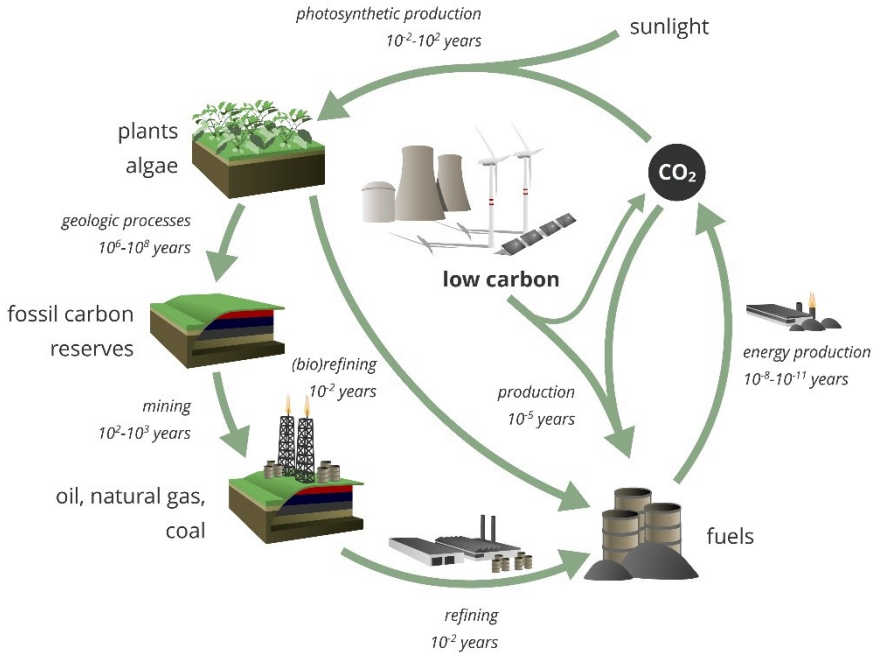


Figure 1.3 - Timing in the carbon-energy cycle. The net CO_2 accumulation in the atmosphere can be explained by an imbalance in flows: large amounts of fossil carbon emitted as CO_2 into the atmosphere that could not be cycled back by the relatively slow natural CO_2 reduction processes. Only *via* efficient and large-scale processes for catalytic conversion of CO_2 into fuels and chemicals the carbon cycle can be closed. Copied from Martens et al. (2017).

2.2. The carbon energy cycle at its shortest: CO_2 as feedstock

Only with efficient and large-scale (bio)catalytic processes in which CO_2 is converted into fuels and chemicals at the same rate CO_2 is formed, the slow photosynthesis-based CO_2 capture can be bypassed, thereby allowing to close the carbon loop. In order to fulfil the permanent demand for carbon-based compounds, CO_2 will have to be recycled in an artificial version of the natural carbon-energy cycle, a strategy defined as “carbon capture and utilization” (CCU) (Markewitz et al., 2012).

In the carbon cycle of a CO_2-neutral world, not the use of carbon, but its emission into the atmosphere is thus avoided (Martens et al., 2017). By replacing the fossil carbon used to

produce fuels and chemicals with carbon from CO_2 we can preserve our carbon-based standard of living without net emission of CO_2.

Compared to (bio)chemical refining of organic substrates, (bio)chemical catalysis for CO_2-based production has some distinct advantages, including no competition with edible feedstock, a low impact on land, water or biodiversity, low water and carbon footprint, and the possibility of renewable energy storage as chemical energy (Carus, 2014). Utilizing the concentrated carbon emissions from large point sources such as power plants and industrial production sites seems to be an obvious first step to contribute to a carbon-neutral economy.

Because CO_2 is a highly stable molecule, for any conversion of CO_2 to take place, a substantial energy input, optimized reaction conditions and active (bio)catalysts are required. The massive amount of energy to drive this infinite loop of rapid reduction and oxidation of carbon atoms in a closed artificial carbon cycle should come from sustainable sources with minimal CO_2 footprint, as fossil fuels will be excluded in the future energy mix. The external energy can be supplied as: (i) heat (thermocatalysis); (ii) electrons (electrocatalysis); (iii) photons (photochemical catalysis) or (iv) chemical energy carriers (chemocatalysis). A wide variety of CO_2 conversion technologies remain under development, with a diversity of end products from CO_2 being produced at lab scale (Aresta et al., 2014b). Although the first industrial demonstration projects have recently been commissioned, the development of technology for the required large-scale and cost-efficient conversion of CO_2 still imposes a considerable scientific challenge. In general, two approaches can be considered to achieve CO_2-based production: (i) the indirect pathway through synthesis gas as intermediate, and (ii) the direct reduction of CO_2 into fuels or chemicals, such as methane, methanol, formaldehyde, dimethyl ether and formic acid.

Synthesis gas (or syngas), a mixture of H_2 and CO, is a key platform chemical for C_1 chemistry and a crucial intermediate resource for the production of hydrogen, ammonia, methanol, and synthetic hydrocarbon fuels. In the present day chemical industry, syngas is produced from a wide range of carbonaceous feedstocks through steam reforming (mainly from natural gas and naphtha) and gasification (mainly from coal and biomass). Syngas can

be converted to an ever-increasing number of chemical compounds through methanol or Fischer–Tropsch (FT) synthesis (Cheng et al., 2017). Commercial technology for methanol production from syngas is already available in the petrochemical industry, making it a very attractive option for large-scale deployment of carbon recycling. Methanol is an important and versatile starting bulk chemical for the production of a broad variety of valuable chemicals, such as short carboxylic acids, light olefins, aromatics, and all kinds of hydrocarbons (Galadima & Muraza, 2015). Moreover, methanol has been recently used as a clean synthetic fuel, suitable for use in gasoline engines. The annual worldwide production of methanol is estimated to be around 70 million tons (2015).

The CO–H_2 mixture can also be transformed into alkanes *via* FT synthesis. Several industrial scale FT plants in Malaysia, Qatar and South Africa use iron or cobalt catalysts to convert coal and natural gas to a variety of synthetic petroleum products (Mahmoudi et al., 2017). For the syngas pipeline, it is of great importance to have a high degree of control over the H_2/CO ratio to be able to steer the synthesis towards the desired products.

The direct synthesis, on the other hand, eliminates the need for a two-stage process in two successive reactors by converting the reactants immediately. Some emerging technologies are discussed below, both for direct and indirect conversion (Figure 1.4).

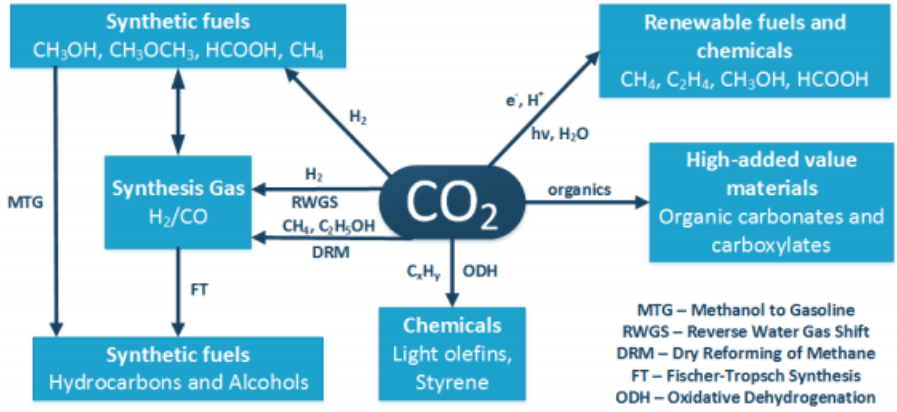


Figure 1.4 - Catalytic routes for CO_2 transformation into fuels and chemicals. Copied from Debek (2016).

2.2.1 (Thermo-) chemical catalysis

Production of synthesis gas

The most widely investigated chemical reduction processes for CO_2 conversion involve the rearrangement of chemical bonds in a hydrogen-bearing reducing agent, like CH_4 and H_2. The reaction of CO_2 and CH_4, known as the dry (CO_2) reforming of methane (DRM), produces syngas with a H_2/CO ratio of 1 [Eq. 1] (Arora & Prasad, 2016).

$$CO_2 + CH_4 \rightarrow 2\ H_2 + 2\ CO \qquad \Delta H° = 247\ kJ\ mol^{-1} \qquad [Eq.\ 1]$$

In order to obtain the desired H_2/CO ratio of 2 for methanol and Fischer-Tropsch synthesis, the H_2/CO ratio can be adjusted by (i) converting CO with H_2O to CO_2 and H_2 in an additional water-gas shift step or (ii) adding external H_2. DRM needs to be carried out at high temperatures (900–1200 K) in the presence of a catalyst, typically containing nickel. The major obstacle preventing the application of DRM on an industrial scale is the rapid deactivation of conventional reforming catalysts *via* deposition of solid carbon as well as sintering of active material (Pakhare & Spivey, 2014). Advanced catalyst design concepts could prevent deactivation by coke formation, but these catalysts are not yet implemented at commercial scale. Alternative reforming processes such as the super-dry reforming of methane offer the possibility to intensify the CO_2 utilization, as up to three times more CO_2 per kilogram of CH_4 can be converted [Eq. 2] (Buelens et al., 2016). Through chemical looping CO_2 capture and conversion, the reverse water-gas shift reaction is promoted and CO production from CH_4 and CO_2 is enhanced.

$$CH_4 + 3\ CO_2 \rightarrow 4\ CO + 2\ H_2O \qquad \Delta H° = 330\ kJ\ mol^{-1} \qquad [Eq.\ 2]$$

Hydrogenation of CO_2

The catalytic hydrogenation of CO_2 covers a large number of catalytic reactions, such as the production of methanol, methane, formic acid, or higher alcohols and carboxylic acids (Aresta et al., 2014a). Hydrogenation of CO_2 requires very selective catalysts in order to avoid the formation of undesired by-products. The selective hydrogenation of CO_2 with H_2 to methanol is commercially achieved by Carbon Recycling International, an Icelandic company that

produces approximately 5 million liters of methanol annually by using geothermal power to generate hydrogen *via* electrolysis (CRI, 2019). In addition, a number of methanation projects that aim the direct synthesis of methane from CO_2 and H_2 are initiated, with several pilot- and full-scale applications, of which the 6 MW E-Gas project of car manufacturer Audi is the largest (Rönsch et al., 2016).

2.2.2 Electrochemical conversion

Electrochemical reduction enables the direct transformation of CO_2 into value-added hydrogenated products, using electrons made available at an electrode. The major products obtained through electrochemical reduction include CO, formic acid, formaldehyde, methanol, methane, ethylene and ethanol. The catalyst, the electrode potential and the reaction conditions (electrolyte, pH, buffer strength, temperature, pressure, *etc.*) all determine the product (mix) that can be obtained (Qiao et al., 2014). A wide variety of CO_2 reduction electrocatalysts has been described, including metals, metal oxides (typically containing gold, silver, copper and cobalt), polymers, enzymes and organic molecules. At present, the catalytic activity, product selectivity, and catalytic stability do not yet reach the requirements for commercialization (Albo et al., 2015). Opposite, electrocatalysis for H_2O reduction to H_2 gas is becoming a mature technology for H_2 production, being close to commercialization at an industrial scale (Refhyne, 2018). Advanced electrolytic cells convert electric energy into chemical energy in H_2 with an energy efficiency of approximately 70%. Water electrolysis is envisaged as the key enabling technology to transfer renewable electricity into other energy sectors, like chemical industry (Schmidt et al., 2017).

2.2.3 Plasmatechnology

In recent years, there has been an increasing interest in the use of plasma technology for CO_2 conversion into value-added chemicals and fuels. Plasma, an ionized gas formed by introducing heat or electrical energy into a gas, allows thermodynamically difficult reactions, such as the dry reformation of methane or the hydrogenation of CO_2, to occur under mild operating conditions, even though these reactions typically require much more harsh reaction conditions (high temperature and high pressure) (Snoeckx & Bogaerts, 2017). The

energy efficiency of plasma-based CO_2 conversion is still relatively low, but nonetheless the use of plasma as a highly reactive and complex chemical cocktail for CO_2 reduction looks promising.

2.2.4 Biocatalysis

Although chemical processes are generally faster than biological conversions, the use of microorganisms as biocatalysts to convert CO_2 and H_2 (or CO) into organic products has several distinct advantages compared to chemo- or electrocatalytic conversion processes, including (i) higher specificity and/or selectivity, (ii) higher conversion efficiencies, (iii) lower energy costs (mild operating conditions), (iv) lower sensitivity to variations in gas composition and (v) less susceptible to poisoning by gas contaminants, *e.g.*, tars, sulphur compounds or chlorine (Liew et al., 2013; Rabaey & Rozendal, 2010; Seifert et al., 2013). Furthermore, microbial catalysts can facilitate complex CO_2 reductions that cannot be achieved by chemical (electro)catalysis, producing multi-carbon compounds like higher alcohols and medium-chain fatty acids of even C6-C8 length that are of industrial relevance. With cyanobacteria even complex commodity chemicals like 2,3-butanediol, 1,3-propanediol, ethylene, glycogen, lactate, 3-hydroxypropanoic acid, 3-hydroxybutanoic acid, 4-hydroxybutanoic acid, isoprene, and farnesene can be produced (Knoot et al., 2018). Products from bacteria and algae can also include dietary protein, polyunsaturated fatty acids, and pigments (Martínez-Francés & Escudero-Oñate, 2018).

Four emerging biotechnological platforms for CO_2 conversion are discussed in more detail in the next section. The biological conversion of CO_2 by natural photosynthesis using microalgae is not discussed, and the reader is referred elsewhere for a detailed overview of the status of this research field (Brennan & Owende, 2010; Schenk et al., 2008).

3. Biotechnologies for CO_2 conversion to chemical building blocks

Among the microorganisms capable of metabolizing CO_2 (or CO), acetogenic bacteria, hydrogenotrophic methanogens and hydrogen-oxidizing bacteria are the most relevant, as these naturally occurring microorganisms act as cheap, robust, and self-repairing catalyst in carbon recycling processes.

3.1. Anaerobic gas fermentation

Gas fermentation is an emerging platform for the production of value-added bulk chemicals from carbon-rich gaseous feedstocks (syngas, CO or CO_2/H_2) that relies on homoacetogenic organisms as biocatalysts in CO_2 conversion processes (Liew et al., 2016). Homoacetogens are anaerobic chemolithoautotrophic microorganisms that assimilate carbon *via* the Wood–Ljungdahl pathway, also called reductive acetyl-CoA pathway, in which carbon uptake occurs *via* two linear branches, the methyl-branch and the carbonyl-branch, both delivering precursors for the formation of acetyl-CoA**,** a precursor for enzymatic production of various organic end-products. The Wood-Ljungdahl pathway is able to assimilate both CO and CO_2, but the fact that CO acts as both energy and carbon source makes it thermodynamically more favorable to work with CO relative to CO_2, which requires H_2 as electron source for microbial carbon assimilation [Eq. 3 and 4] (Drake et al., 2008).

$$2\ CO_2 + 4\ H_2 \rightarrow 1\ CH_3COO^- + 1\ H^+ + 2\ H_2O \qquad \Delta G_0' = -\ 95\ kJ\ mol^{-1} \qquad [Eq.\ 3]$$

$$4\ CO + 2\ H_2O \rightarrow 1\ CH_3COO^- + 1\ H^+ + 2\ CO_2 \qquad \Delta G_0' = -\ 175\ kJ\ mol^{-1} \qquad [Eq.\ 4]$$

Carbon monoxide can enter directly into the carbonyl branch and be converted to acetyl-CoA by the acetyl-CoA synthase enzyme. If additional energy is required, CO will be oxidized to CO_2 in the methyl branch *via* a water-gas shift reaction, followed by its conversion to formate. If starting from CO_2, CO_2 is reduced to CO in the carbonyl-branch with electrons obtained from H_2 *via* hydrogenases (Ragsdale & Pierce, 2008).

Acetogenic bacteria like *Clostridium carboxidivorans, Clostridium ljungdahlii* and *Clostridium autoethanogenum* perform a biphasic fermentation under autotrophic conditions. During the first phase, or the acidogenic phase, carboxylic acids are produced (mainly acetic acid), mostly during exponential growth. During the second phase, or solventogenic phase, the produced acids are converted into solvents (mainly alcohols), mostly during stationary growth (Liew et al., 2013). By conversion of acids into the corresponding alcohols, the extracellular pH is increased and the acids are dissociated into the respective salts and protons, securing the survival of the cells for a longer time (Richter et al., 2013). All homoacetogens are able to produce acetic acid from acetyl-CoA and several strains can derive ethanol, butyric acid, butanol and 2,3-butanediol from this intermediate (Heijstra et al., 2017). These molecules are bulk chemicals that are used on a diverse array of industrial application. The product outcome can be steered towards the desired product, being either acids or alcohols, by controlling the process conditions.

At present, ethanol biosynthesis from CO-rich gases has been proven robust at scaled up operations, and is commercialized by the US-based company LanzaTech. With one commercial-scale ethanol plant commissioned at the Shougang Group's Jingtang Steel Mill in China (Hebei) and several full-scale production projects under development, syngas fermentation technology is more and more envisaged as a profitable carbon recycling operation for carbon-intensive heavy industries like steel mills, ferroalloy production plants and oil refineries (De Tissera et al., 2017). Key to the further development of this microbial production platform is the establishment of more efficient metabolic pathways from CO_2. Most studies on CO_2/H_2 fermentations report acetic acid as sole fermentation product, with only traces of other higher-value organics, such as formic, butyric or caproic acid (Bengelsdorf et al., 2013; Demler & Weuster-Botz, 2011). Hence, higher-value products should be targeted at high rates to evolve towards an economically feasible technology. To achieve this, research is warranted to overcome the energetic limitations, related to thermodynamic constraints, by optimizing gas composition and other operational parameters (Molitor et al., 2017).

3.2. Hydrogenotrophic biomethanation

The hydrogenotrophic methanogens are the main representatives in the archaeal domain that can use molecular hydrogen gas as electron donor to produce methane [Eq. 5] (Liu & Whitman, 2008; Zabranska & Pokorna, 2017).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad \Delta G_0' = -165 \text{ kJ mol}^{-1} \qquad \text{[Eq. 5]}$$

This group of chemoautotrophic biocatalysts presents us with the opportunity of producing an attractive renewable energy carrier with well-established facilities in terms of distribution (*e.g.*, the existing natural gas grid) and use (*e.g.*, road transportation, power, heat or chemical production) (Weiland, 2010). Hydrogenotrophic biomethanation is often discussed in the context of power-to-gas (P2G) technology. The P2G concept links the power grid with the gas grid by converting intermittent or off-peak power into methane through electrolytic H_2 production and subsequent CO_2/H_2 conversion *via* (bio)methanation (Bailera et al., 2017). This concept recently gained interest as a scalable option for long-term and large-capacity storage of surplus renewable electrical power within the existing natural gas distribution grid (Götz et al., 2016; Meylan et al., 2017). The P2G technology could address the issue of an existing electricity transmission infrastructure that is found inadequate in transmitting large volumes of renewable power from wind and solar farms to the end users. As the share of renewable energy sources in the electricity mix is increasing rapidly, the need for efficient power balancing technologies becomes more important (Ould Amrouche et al., 2016).

Large stationary point sources of CO_2 (such as power plants, (bio-)refineries, steel and cement industries) are often put forward as top candidates for methanation, but also relatively small biogas plants can suit the P2G process. In this conceptual idea, the biomethanation process is used as an alternative strategy for CO_2 removal from biogas (Angenent et al., 2018; Bassani et al., 2015). The P2G can be used as a biological biogas upgrading process unit that results in an increase in the total production of CH_4 from an organic feedstock (Angelidaki et al., 2018).

Since the first description of the P2G technology, major technological developments have been achieved that resulted in rapid scale-up and industrialization of various biomethanation concepts (*e.g.* Krajete, Electrochaea, MicrobEnergy) (Götz et al., 2016). Two configurations for the conversion of CO_2 from biogas with H_2 have been proposed: (1) direct H_2 injection into the anaerobic digester to stimulate the autochthonous hydrogenotrophic archaea (*in situ* biomethanation) (Agneessens et al., 2017; Luo & Angelidaki, 2013), and (2) H_2 and biogas injection in a separate anaerobic reactor containing a pure or mixed hydrogenotrophic culture (*ex situ* biomethantion) (Kougias et al., 2017; Martin et al., 2013). At present, volumetric methane production rates of both concepts are still low compared to industrially established biogas formation in anaerobic digestion plants (Geppert et al., 2016). As recently reviewed, *in situ* biomethanation is, at present, not considered a suitable option for industrial biomethanation, due to various operational challenges, such as pH control and thermodynamic limitations related to high H_2 partial pressures (Angelidaki et al., 2018; Angenent et al., 2018).

A critical aspect of any fermentation involving gases as a substrate is the ability of the gas to dissolve in the liquid phase. The key limitation for microbial CO_2 conversion is the hydrogen gas-to-liquid mass transfer that can become rate-limiting. This limitation is typically addressed by: (1) vigorous mixing, gas recirculation or fine-bubble gas distributors, as these will increase the volumetric mass transfer coefficient (k_La), and (2) enhancing the solubility of H_2 by increasing the partial pressure of H_2 (pH_2) by elevating the headspace pressure (Guiot et al., 2011; Kougias et al., 2017). A variety of fermenter configurations attempting to achieve a high volumetric mass transfer coefficient have been extensively reviewed in the literature: continuous stirred tank reactors, bubble (gas lift) columns, loop reactors, immobilized beds, and hollow fiber membrane columns are described to guarantee a high substrate availability (Asimakopoulos et al., 2018).

A strategy that has been proposed to overcome the energy-intensive gas-liquid mass transfer of H_2 is the use of a submerged cathode as sole source of reducing power, feeding the microorganisms directly with electrons through the integration of electro- and microbial-catalysis in a hybrid process. While in microbial gas fermentation organics are produced from

CO_2 and H_2 with the latter being produced in an external electrolyzer, MES uses a biocatalyzed cathode to *in situ* supply electrons to the fermentation broth at a theoretical electrode potential less negative than an abiotic cathode and without the pumping and mixing of the low-soluble and explosive H_2 gas at significant costs and safety risks. This strategy is referred to as microbial electrosynthesis (MES) (or electromethanogenesis if methane is intended as target compound) (Rabaey & Rozendal, 2010; Blasco-Gómez et al., 2017).

3.3. Microbial electrosynthesis: a direct route coupling electricity with bioproduction

MES occurs in so-called bioelectrochemical systems (BES), where microorganisms function as catalysts for electrode reactions. The observation that microorganisms can accept electrons from a solid-state electrode that is at low enough potential for CO_2 conversion has broadened the horizon of research concerning bioelectrochemistry (Zaybak et al., 2013). Proof of principle was demonstrated using several pure acetogenic cultures, as well as mixed anaerobic communities, which produced acetic acid from CO_2 with electrons drawn from an electrode without the addition of an electron shuttle (Nevin et al., 2011; Marshall et al., 2012). MES relies on the same microorganisms and pathways as acetogenic gas fermentation, but MES offers the possibility to directly link renewable energy with CO_2-based bioproduction in one integrated system.

Thus far, acetic acid is the sole product of MES that can be generated at elevated rates and selectivity. More recently, the production of longer chain carboxylic acids, such as butyric and caproic acid, and their corresponding alcohols (n-butanol, and n-hexanol) has also been demonstrated from CO_2 in MES using a mixed reactor microbiome (Ganigué et al., 2015; Jourdin et al., 2018; Vassilev et al., 2018). It is hypothesized that acetate is fermentatively elongated to medium-chain fatty acids using ethanol as electron donor *via* the reverse β-oxidation chain elongation pathway, a metabolic pathway typically present in anaerobic reactor microbiomes (*e.g. C. kluyveri*) (Seedorf et al., 2008). Alternatively, butyric acid can also be produced directly *via* acetyl-CoA without intermediate production of acetate and ethanol. It is generally considered that an acidic pH triggers the observed metabolic shift in the product spectrum, from acetate through acetogenesis toward solvents and more

valuable C4 and C6 carboxylates through solventogenesis and chain elongation (Vassilev et al., 2018).

How the microorganisms receive electrons from the cathode during MES is still not understood well (Rosenbaum et al., 2011; Tremblay & Zhang, 2015). For the different pure culture studies, a direct electron uptake mechanism was suggested based on the bacterial attachment on the electrode surface and the absence of detectable concentrations of molecular hydrogen in abiotic control experiments (Nevin et al., 2010, 2011). So far, all acetogens described in MES studies have been selected for their autotrophic growth on H_2. For this reason, it cannot be excluded that most of the electrons used for acetic acid production were indirectly derived from H_2 that is abiotically generated by the electrode and rapidly taken up by the biofilm. A shift in the onset potential of the H_2 evolution reaction to less negative potentials has regularly been reported for the acetogenic communities enriched on cathodes (Marshall et al., 2013; LaBelle et al., 2014; Patil et al., 2015). There is strong evidence that this enhanced catalysis can be explained by the sorption of enzymes, such as hydrogenases, released from the cells, catalyzing the production of H_2 at the cathode. The authors showed that a cell-free spent culture medium of the methanogen *Methanococcus maripaludis* catalyzed hydrogen production at a rate that is sufficient to explain its electron uptake rate through microbial electromethanogenesis (Deutzmann et al., 2015). A second possible explanation for the increased catalytic activity of biocathodes has been given by Jourdin and co-workers (2016b). In their experiments it was found that microorganisms present in the reactor microbiome can induce the precipitation of copper nanoparticles on the electrode surface, thereby increasing the catalytic activity of the electrode.

Since its first demonstration, the process has been intensively studied in terms of microbial catalyst selection (Nevin et al., 2011), electron transfer mechanism (Jourdin et al., 2016b; Marshall et al., 2012), electrode materials (Jourdin et al., 2016; Zhang et al., 2013), CO_2 supply (Bajracharya et al., 2016) and product outcome (Ganigué et al., 2015; Vassilev et al., 2018). Recently, *in situ* product recovery *via* electrochemical membrane extraction was proposed as a strategy to avoid product inhibition as a result of product accumulation and

acidification of the broth, without the need to switch to a continuous mode operation that dilutes the product (Gildemyn et al., 2015).

MES performance has improved in the past years, but the low production rates, low product titers and the high cost to fully drive the production process from electricity within a BES hamper its further development as a full scale reactor platform for bioproduction.

3.4. Microbial protein production using hydrogen-oxidizing bacteria

Since the 1960s, a growing awareness for the acute food needs of the world's expanding population has led to the development of alternative food and feed sources as potential additions to the conventional agriculture based food supply chains (Goldberg, 2013). Several attempts were made to bring to practice the production of high-quality protein from microorganisms, known as microbial protein (MP), or single cell protein (SCP), of which the Imperial Chemical Industries (ICI) were the first to commercialize a methanol-based MP product called Pruteen™ (Westlake, 1986). A combination of low prices for soybean and fishmeal, increased fossil fuel prices, the underdeveloped state of fermentation technology and a reduced digestibility due to high nucleic acid contents resulted in the discontinuation of the process in the late 1970s (Øverland et al., 2010).

In view of the still growing world population, a steep increase in the prices of fishmeal and an increased awareness of the enormous environmental costs of nitrogen pollution, MP is regaining momentum as a renewable alternative protein source that could be produced in confined reactors at high rate (Pikaar et al., 2018c). The high nitrogen uptake efficiency and the low environmental pressure of MP production on land and water use make MP a protein substitute with an environmental impact that is much lower than the current nitrogen inefficient feed and food chain (Cumberlege et al., 2016; Pikaar et al., 2018a). The application of natural gas-based MP has reached feasible industrial scale production, with products as Feedkind™ (by Calysta) and UniProtein™ (by UniBio), allowing to grow at high volumetric productivities (3 - 4 kg MP dry matter per m^3 reactor volume per hour).

An interesting alternative to protein production by natural gas-based MP is represented by hydrogen-oxidizing bacteria (also known Knallgas bacteria). Hydrogen-oxidizing bacteria (HOB) are aerobic, facultative autotrophic bacteria growing on H_2 (electron donor) and O_2 (electron acceptor) while fixing CO_2 into bacterial cellular biomass and assimilating nitrogen into high-quality protein (Matassa et al., 2015b). The conversion of molecular hydrogen into microbial biomass using HOB has recently gained renewed interest as an efficient strategy for up-cycling of mineral nitrogen and carbon dioxide recovered from used water streams into protein-rich feed and food substances (Matassa et al., 2016b). Hydrogen oxidizing bacteria are currently explored by the Belgium-based company Avecom on pilot scale with renewable-powered electrolysis for H_2 and O_2 production, CO_2 coming from biogas, and NH_3 stripped from wastewater.

4. Electrifying bioproduction and recovery

The integration of electrochemical and fermentation technologies gives the option to redox balance any substrate–product combination in a fermentative conversion. By interfacing an electric current with microbial metabolism reducing equivalents can be delivered by a cathode and a surplus of electrons can be withdrawn by an anode (Flynn et al., 2010; Zhou et al., 2013). Electrode reactions can be used solely to provide electrons (directly or *via* H_2) in unbalanced fermentations, but can also be designed specifically to recover the end-product, or control the pH in the fermentation broth (Andersen et al., 2015). The electricity-driven extraction of short- and medium-chain carboxylates from a fermenter through an anion exchange membrane into a clean and acid extraction medium was proposed as a recovery strategy for electro-fermentation and MES (Gildemyn et al., 2015). The principle to extract charged molecules from the 'reaction' medium through an ion selective membrane, driven by electrical current (referred to as membrane electrolysis) has also been described using a cation exchange membrane for the recovery of ammonium from an anaerobic digester with the aim to control ammonia toxicity (Desloover et al., 2015). In these electricity-driven extraction processes the electrocatalytic electron flux does not interact directly with the microbes, yet the extraction supports a digestion or fermentation by extracting inhibitory products and balancing the pH of the broth (Schröder et al., 2015).

5. Objectives and outline of this work

Transitioning towards a carbon-neutral world economy implies that most of the known fossil fuel resources have to remain untouched, and that renewable energy technologies like solar and wind have to secure our future energy demand. As fossil fuels are also employed as raw materials for the production of chemicals and materials, CO_2-neutrality will require the use of alternative renewable carbon sources for the production of our daily-use carbonaceous goods with renewable power to drive the carbon conversions in the future carbon economy. The main objective of this thesis was to **investigate the potential of methane and carbon dioxide as key building blocks for production in the future carbon-neutral world economy** (Figure 1.5).

Biogas produced through anaerobic digestion of low-grade biomass is considered as a CH_4 and CO_2 containing renewable gas mix, that can be used as feedstock for on-site production: methane for the production of carbon monoxide or feed protein, while CO_2 can be used to make more methane. These high-value production concepts have the potential to ensure a sound economic argument for biogas projects as they could bypass the inherently low value of methane as fuel. A first challenge is to critically assess the real economic potential of these alternative biogas utilization routes.

In **Chapter 2**, the valorization of renewable CH_4 through reforming of CO_2 was proposed as a promising step towards a biomethane-based production of chemicals or fuels. By coupling decentralized biomethane production facilities to large-scale chemical plants *via* the existing natural gas infrastructure anaerobic digesters could have the potential to become the drivers of a new “bio-industry” in which waste CO_2 is incorporated in chemicals and fuels. The assessment was based on technical modelling and detailed cost and revenue calculations for each step in the value chain (biogas production, upgrading, injection and valorization). The economic comparison of different utilization pathways was followed by an estimation of chemical production volumes and CO_2 emission reduction potentials based on current and future availability of biomethane.

To enable the linkage of decentralized biogas production to the natural gas grid, there is a need for small scale upgrading technologies so that smaller biogas plants can also be connected to the grid. In **Chapter 3**, membrane electrolysis was applied as a novel electricity-driven approach for upgrading biogas to biomethane. The effect of current density and biogas flow rate on the removal of CO_2 and H_2S *via* electrochemical membrane extraction was investigated. The electrolytic generation of hydroxide ions and protons at the cathode and anode, respectively, was tested as a way to efficiently scrub and strip CO_2 from synthetic biogas.

Chapter 4 expands the valorization options for biogas further by looking into microbial biomass production as an alternative protein source for animal feed. The economics of a manure digester up-cycling ammonia and biogas was assessed for both renewable methane and hydrogen as carbon-neutral energy sources for the production of protein-rich microbial cells, and recovered ammonia from the digestate as nitrogen source. Furthermore, the economic benefits of nutrient upgrading over dissipation were demonstrated.

This thesis furthermore looked at how electricity can directly drive microbial processes to capture CO_2 in added-value chemicals. Critical aspects to obtain a mature and scalable CO_2-based bioproduction technology are improvements in production rates and efficiencies, as well as decreased power inputs and production and recovery costs. **Chapter 5** evaluates the performance of a microbial electrosynthesis reactor system for integrated production and extraction of acetic acid, using an anion exchange membrane in the reactor configuration. Based on critical performance parameters the novel reactor system is compared with systems without extraction.

To further catalyze the establishment of a microbial CO_2-based production platform, the coupling of membrane electrolysis with a gas fermenter was evaluated in **Chapter 6**. The features of electrochemical water reduction on the fermentation broth were investigated.

A general discussion was provided in **Chapter 7**, together with some suggestions for future research.

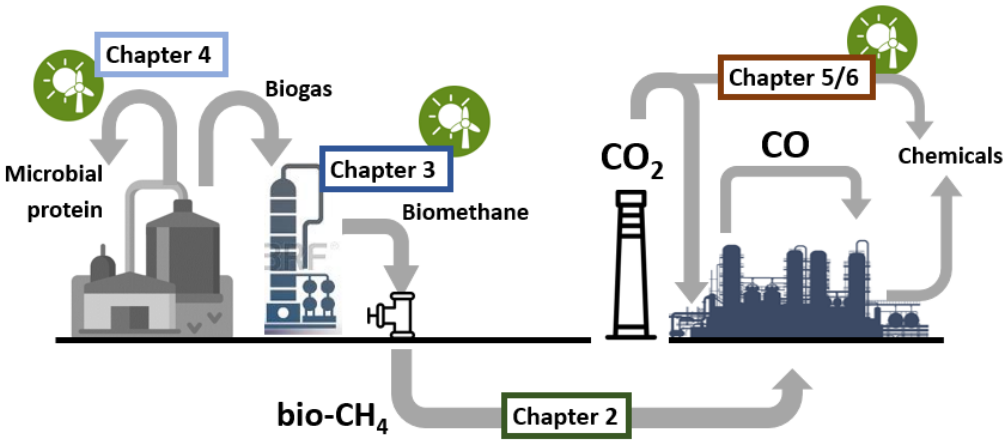


Figure 1.5 - Overview of the experimental chapters in this PhD thesis.

CHAPTER 2

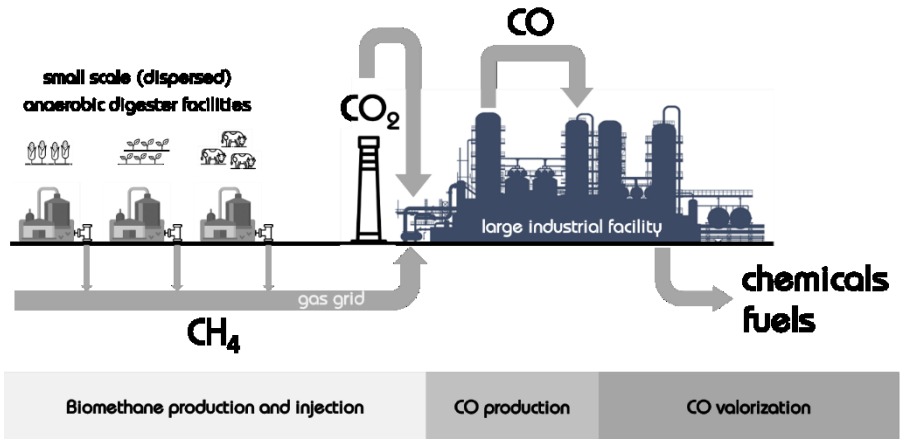
Upgrading the value of anaerobic digestion *via* chemical production from grid injected biomethane

This chapter has been redrafted after:

Verbeeck, Kristof, Buelens, L., Galvita, V., Marin, G., Van Geem, K., & Rabaey, K. **(2018)**. Upgrading the value of anaerobic digestion *via* chemical production from grid injected biomethane. Energy & Environmental Science, 11(7), 1788–1802.

Abstract

Anaerobic digestion can already at small scale effectively convert (waste) biomass to biogas. This biogas is typically combusted to generate electricity and heat, which is incentivized by regulatory support schemes. Because biogas can also be upgraded to biomethane and subsequently injected in the gas grid, the anaerobic digester can be considered a means to connect decentralized biomass production to a centralized gas grid. We currently estimate the level of required government support to realize a profitable investment in Europe at 20 – 50 € MWh_e^{-1} for valorization of an average biogas in a combined heat and power unit, and at 15 – 25 € MWh^{-1} for production of pipeline-quality biomethane, typically used as fuel. Here we explore both technically and economically an alternative scenario where biogas is upgraded to biomethane, injected into the existing gas grid, and used elsewhere to produce CO, syngas or H_2. Super-dry reforming of CH_4, a chemical looping approach using up to three CO_2 molecules per CH_4, allows an intensified production of CO as feedstock for synthesis of platform chemicals and fuels through CO_2 utilization. At present values and costs, this creates an economically positive case which can promote anaerobic digestion as an important driver for a new bio-industry. Through economic allocation of the environmental burdens of fossil gas use, syngas and CO production from biomethane could have the potential to offset the opportunity cost associated with the use of more expensive biomethane relative to cheaper natural gas, so that theoretically no subsidies are needed to compensate for the price difference. The approach studied avoids biomass transportation, contrary to present biorefineries, while effectively valorizing decentralized biomass feedstocks such as agricultural waste or energy crops.



Broader context

Biogas is a renewable and low-carbon source of CH_4, generally produced by anaerobic digestion of organic (waste) material (Giontoli et al., 2014). With an average annual biogas production growth rate of 13.7% since 1990, the installation of biogas production units has boomed in Europe during the past decades owing to legal financial incentives (Eurostat, 2015a; Kampman et al., 2016). Nevertheless, further expansion is limited by a lack of profitable valorization strategies. Indeed, biogas is often used for local heat or power production at a low overall energetic efficiency despite its potential as a C_1 feedstock (Pöschl et al., 2010). Alternatively, the valorization of renewable CH_4 through reforming can be discerned as a promising first step towards biomethane based production of chemicals or fuels (Nahar et al., 2017). Such a strategy allows a more widespread use of this renewable C_1 feedstock and hence facilitates its valorization by reaching out to new markets such as the chemical industry (Moghaddam et al., 2016), enabling the biomethane end-users to profile themselves as more sustainable. The challenge of transporting bio-based CH_4 from delocalized small scale producers to localized large scale industrial sites can be overcome by upgrading of biogas to biomethane and subsequent injection in the existing natural gas grid (Urban, 2013). Along with the emerging availability of renewable energy, a well-considered and more efficient use of bio-based chemical feedstocks has the potential to significantly decrease society's carbon footprint and fossil resource dependency.

1. Introduction

In the past decades, anaerobic digestion (AD) has become a well-established commercial technology for renewable energy production from (low-value) organic feedstocks such as manure, sewage sludge, the organic fraction of household waste, agricultural residues and energy crops (Appels et al., 2011). Biogas, a mixture of mainly CH_4 (40 to 75 vol.%) and CO_2 (25 to 60 vol.%), is envisaged as one of the key resources in achieving *e.g.* the European Union's 2020 and 2030 decarbonization and renewable energy targets (Council of the European Union, 2009; European Commission, 2011; Kampman et al., 2016). With 17 376 biogas installations in operation and a primary biogas energy production of 182 TWh, biogas represented about 7.7% of the EU's primary renewable energy mix and accounted for 51% of the global biogas production in 2015 (Eurostat, 2015a; IEA, 2015). At the same time, the European substrate potential from biomass and renewable wastes is estimated at 1500 TWh, or 32% of the EU natural gas demand in 2017, highlighting the potential for anaerobic digestion as a renewable energy platform (Eurostat, 2015b). Biogas is an all-round energy and carbon carrier that can be used in all downstream applications that are developed for natural gas: residential heating, power production, road transportation and the production of chemicals (Weiland, 2010).

Transportation of biogas from typical small scale producers (on average 160 kg raw biogas h^{-1}) to large scale end-users such as chemical or power plants (up to hundreds of tons methane h^{-1}) poses a considerable challenge. Therefore, over 90% of the biogas is used on site (after drying and H_2S scrubbing) to produce power (60.8 TWh_e) and heat (38.0 TWh_{heat}) in an on-site cogeneration unit (Kovács, 2016). The preference for this biogas valorization route mainly occurs because in most European countries biogas production is supported through heat and power associated subsidies (Kampman et al., 2016).

Alternatively, biogas can be upgraded to biomethane which can be injected into the natural gas grid and used as a natural gas substitute, or compressed and supplied to a gas fueling station (Bauer et al., 2013). Today, only a small fraction (ca. 11%) of the produced biogas energy in the EU is upgraded to natural gas quality (EBA, 2017; Kampman et al., 2016), as its production is more costly (IEA, 2015) and effective support schemes are lacking in many of the EU member states (Capodaglio et al., 2016). In countries where the economic attractiveness of biomethane projects is secured through sufficient financial incentives, biomethane production is emerging (Sorda et al., 2010). In Europe, there are currently over

460 upgrading plants, mostly feeding the pipeline-quality biomethane into the existing natural gas grid. To allow grid injection of upgraded biogas or the use as a vehicle fuel, the calorific value of biogas needs to be increased, which means that CO_2 is removed while increasing the share of CH_4, typically above 96%, so that it meets the national quality standards for gas grid access (Ryckebosch et al., 2011). The injection of biomethane into the gas grid allows the decoupling of biogas production and utilization, leading to a much higher overall energy efficiency for biogas use compared to on-site combustion, mainly due to the high flexibility through redistribution and storage of renewable methane within the natural gas infrastructure (Pöschl et al., 2010). It is estimated that at least a third of the biogas potential could more efficiently be used when converted to biomethane because heat recovery is often impossible in agricultural areas due to a lack of local heat sinks (Strauch et al., 2013). Moreover, the use of biomethane as a substitute for natural gas could be considered owing to their similar chemical properties.

In all aforementioned cases, the key fate of the biomass *via* the biogas intermediate is "only" local energy recovery, either electrical or thermal. As for the foreseeable future, it appears better to embed the carbon in biochemicals or biofuels because of several reasons: (i) As mentioned above, delocalized (small-scale) biogas plants typically produce heat and power with a low efficiency (Pöschl et al., 2010; Strauch et al., 2013). (ii) The use of renewable feedstocks is one of the 12 principles of green chemistry (Anastas & Warner, 1998). While the other 11 principles should obviously not be ignored, the chemical industry may pursuit a more sustainable production of chemicals and improve its public image by using renewable feedstocks (Gallezot, 2012). (iii) Renewable solutions for reducing CO_2 emissions in the transportation sector, *e.g.* through biomethane derived biofuels, are becoming ever more urgent (Santos, 2017). (iv) Biofuels are more easily stored and biochemicals have a longer product lifetime than biomethane, allowing a prolonged fixation of biomass derived carbon (Martens et al., 2017).

Today, natural gas is already employed as a feedstock for hydrogen and syngas production. Hydrogen is used for hydrocracking, hydrodesulphurization and ammonia production among others, while syngas is the dominant feedstock for methanol synthesis and its classical derivatives such as MTBE, formaldehyde and acetic acid (Boot-Handford et al., 2014; Goeppert et al., 2014; Shamsul et al., 2014). Besides these derivatives, a growing variety of bulk chemicals and fuels are increasingly being produced starting from methanol.

These emerging pathways for methanol conversion include the production of gasoline and olefins such as ethylene and propylene. These olefins are some of the most widely produced chemicals by the petrochemical industry and important building blocks of many polymers, essential in modern society (Boulamanti & Moya, 2017; Goeppert et al., 2014; Tian et al., 2015). Because the usage of fossil resources leads to an elevated atmospheric CO_2 concentration which in turn causes an uncertain climatic response (Keith, 2009), the industry is increasingly being pushed towards biomass as renewable resource in order to meet legislation and consumer's demands for sustainability. The key route explored today is the gasification of biomass to CO and H_2, where several issues can be identified: (i) Costly and energy intensive transportation of biomass or intermediates to a central processing plant is needed. (ii) Gasification of biomass leaves an ash-based residue, while anaerobic digestion results in a residue of stable organics that can be used for agricultural purposes. (iii) The CO gas typically contains impurities requiring further gas treatment. (iv) The approach does not use the existing infrastructure such as existing anaerobic digesters and the existing natural gas grid (Sørensen, 2005). The use of biomethane could solve these issues and provide an ideal substitute for fossil natural gas as a feedstock for the production of base chemicals through intermediates such as synthesis gas or methanol. An additional benefit of applying CH_4 as base chemical rather than merely a source of H_2 [Eq. 1] – as now practiced – is that its carbon atom can be fixed and utilized. Whereas H_2 production by electrolysis of H_2O using renewable energy such as solar, wind or hydro seems more environmentally benign for present H_2 needs (Lewis & Nocera, 2006; Symes & Cronin, 2013; Voiry et al., 2013), the carbon-containing CH_4 could be used as a valuable C_1 stream which can be combined with CO_2 to provide CO, a versatile platform molecule. An existing process for chemical valorization of biomethane is dry reforming of methane, a reforming process that converts CH_4 and CO_2 into syngas [Eq. 2].

$$CH_4 + 2H_2O \rightleftarrows 4H_2 + CO_2 \qquad \text{[Eq. 1]}$$

$$CH_4 + CO_2 \rightleftarrows 2CO + 2H_2 \qquad \text{[Eq. 2]}$$

$$CH_4 + 3CO_2 \rightleftarrows 4CO + 2H_2O \qquad \text{[Eq. 3]}$$

Recently, an approach for intensified CO_2 utilization termed super-dry reforming was developed in which CO becomes the target product of the reaction [Eq. 3] (Buelens et al.,

2016). The benefit of this strategy lies in the potential to combine CH_4 and CO_2 utilization at large facilities. The amount of CO_2 emitted from stationary point sources amounted to 13.4 Gt CO_2 on a total of 22.6 Gt CO_2 in 2002, ~ 99% of which was emitted by sources larger than 100 kt CO_2 $year^{-1}$ (IPCC, 2005). Unfortunately, no such data is available for recent years because there is no detailed global monitoring system for CO_2 emitters in place (Singer et al., 2014). Assuming a similar fraction of CO_2 emissions from large stationary point sources as in 2002, however, it can be estimated that 21 Gt CO_2 out of the 35 Gt CO_2 emitted in 2015 originated from large stationary point sources. Since a majority of these large sources would typically be located in or near industrial zones, the immediate valorization of CO_2 with CH_4 by CO production followed by the conversion of CO with renewable hydrogen into above mentioned chemicals/fuels appears as a tempting approach for carbon recycling. Indeed, the application of biomethane in such a pathway could address the challenge of renewable CH_4 and redundant CO_2 valorization simultaneously. The existing natural gas grid is the crucial link between the two, as it avoids costly road transportation of low-energy density biomass from producer to biorefinery.

When a novel conversion route is suggested, it is important to assess the technological feasibility and the economics of this route and compare it with that of existing 'state-of-the-art' approaches, since this will largely determine the success of its introduction into the market. To estimate which biogas application (electricity and heat generation or chemical feedstock) shows the highest market potential, a thorough economic evaluation was performed, accounting for costs and revenues related to the various utilization routes. This was done based on detailed technological process calculations. The scope of our study is illustrated in Figure 2.1. The aim was to assess the economic viability of three biogas applications in a European framework: (i) The conventional valorization of biogas, *i.e.* as a fuel for on-site combined heat and power (CHP) production. (ii) The upgrading of biogas to biomethane for residential use or power production. (iii) The upgrading of biogas to biomethane and its use as a feedstock for the production of hydrogen, syngas or carbon monoxide. This analysis includes cost contributions to biogas, biomethane and chemicals production as well as revenues of electricity, heat, biomethane and the products obtained through CH_4 valorization. Our results demonstrate the importance of financial incentives to operate a biogas or biomethane facility that sells the produced heat, power or biomethane to the receptive markets, but, importantly, our evaluation also shows the features and

economic potential of CO or syngas production starting from grid injected biomethane and large stationary CO_2 sources.

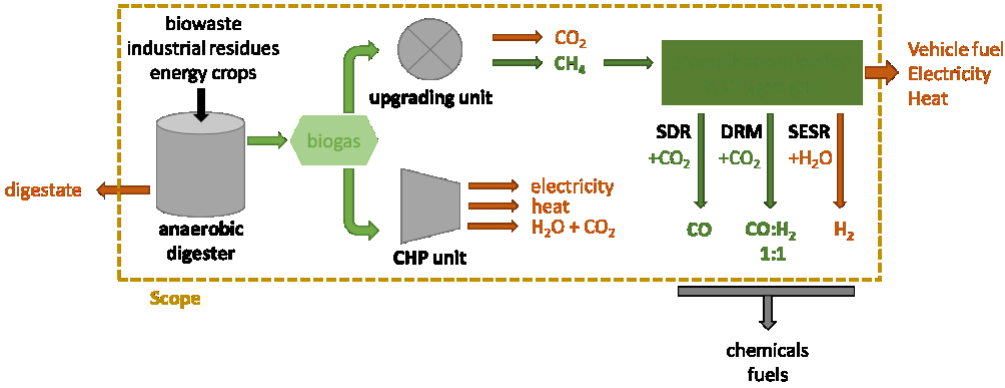


Figure 2.1 - Schematic representation of the scope of this work. Various types of biomass can be converted into biogas, which in turn can be used in a combined heat and power (CHP) unit to produce electricity and heat or upgraded to biomethane with subsequent injection in the CH_4 gas grid. Biomethane can be used as a feedstock to produce chemical building blocks CO, syngas or H_2 *via* super-dry reforming (SDR), dry reforming of methane (DRM) or sorption-enhanced steam reforming of methane (SESR).

2. Methodology and assumptions

The costs and revenues of both biogas and biomethane production (including feedstock, road transport of feedstock, AD, upgrading and injection to the gas grid) and the utilization of this biogas and biomethane through different routes were included in this analysis. Appropriate reference systems (electricity, heat and gas generation) were defined for each route considered. The main technical design data are listed in Table S 1.1 and S 1.2 (in Supporting Information) and characterize the whole supply chain, up to the quantity of produced electricity, biomethane or chemicals. Calculations are performed in order to identify the required electricity, biomethane and chemical prices as well as production support in order to reach break-even in each case. The present study also includes calculations of the potential replacement of fossil products on the market and the CO_2 mitigation potential through biomethane conversion to CO. The assessment is based on an extensive literature review and Aspen Plus simulations. While anaerobic digestion (AD) and biogas upgrading are both mature commercial technologies, super-dry reforming and sorption enhanced steam reforming are still in a research phase. It should, thus, be recognized that the production cost range for biogas and biomethane are well known, while

for the chemical looping processes the availability of information on full-scale implementation is more scarce. The data presented in this study are evaluated in order to identify general trends rather than case specific conclusions. Revenues are primarily derived from the sale of electricity, heat, biomethane, carbon monoxide, hydrogen or syngas. The methodology and main assumptions regarding biogas production costs, CHP production costs, biomethane production costs, costs for chemical production and revenues are summarized in the following sections.

2.1. Unit I: Biogas Production

2.1.1. Biogas production costs

The actual production cost of raw biogas depends on investment-, operation- and maintenance cost, which are influenced by highly varying costs for feedstock, capital, transportation, process energy input, labor, *etc*. It is, thus, difficult to provide accurate costs without detailed plant specifications. Therefore, the biogas under study is chosen to be originating from a large-scale digester, operated as a wet fermentation process under mesophilic (25-40°C) and completely mixed conditions, representing the 'average' biogas in terms of (substrate) origin, composition, flow rate, as well as production costs and revenues. As an approximation of the average substrate fed into an anaerobic digester, it is assumed that the 'feedstock mixture' consists of 6 different organic substrates (pig manure, sewage sludge, the organic fraction of municipal solid waste (OFMSW), agro-industrial waste, food waste and maize silage) that are mixed in a ratio so that every substrate represents $1/6^{th}$ of the total biogas production. Based on the biogas yields reported for these substrates (Weiland, 2010), manure represents about 46% of the input mass going into the digester, sewage sludge 16%, OFMSW and agro-industrial waste both 13%, food waste and maize silage both 6% (% on a fresh material basis). It is presumed that only maize silage has a feedstock cost (average cost of 30 € ton^{-1} fresh material) (Balussou et al., 2012), while most of the waste streams typically come in at very low or zero costs. For a large centralized biogas plant collecting substrates from surrounding farms, industry or cities, transport costs need to be taken into account. To ascertain the costs of raw material transportation, the average transport operating costs per ton-km was fixed at 0.14 € ton^{-1} km^{-1} (Schade et al., 2006), and a feedstock transport distance from its production site to the AD plant was set to be 20 km. For the minimal feedstock cost, a food waste digester with minimal transport costs was assumed (2 € ton^{-1}), while for the maximal feedstock cost an energy crop of 50 € ton^{-1} was used without addition of bio-waste. In order to create a correct view on the range of costs

that can be expected, data is provided for each individual feedstock as well (Figure S 1.1). The average total capital investment of equipment and construction is set to corresponds with an investment for biogas production of 4000 € Nm^{-3} h^{-1} installed biogas capacity (IRENA, 2013). Capital costs (set up from depreciation and interests) are calculated according to the annuity method. Depreciation is assumed to be linear over the economic lifetime of the investment (20 years). Interest costs have been calculated with a rate of 5%. OPEX costs were calculated based on a fixed percentage of the CAPEX (5, 7.5 and 10% of the investment sum on a yearly basis for the minimum, average and maximum OPEX cost, respectively). This simplification has been validated with OPEX values reported in literature (Budzianowski & Budzianowska, 2015; Hahn, 2011). Production costs used in this paper are presented in Table S 1.3, where the average, minimum and maximum production costs are represented. Raw biogas was assumed to have an average CH_4 content of 60% and an energy content of 6.5 kWh Nm^{-3} raw biogas (Table S 1.1).

2.1.2. Combined heat and power production from biogas

In this scenario biogas was utilized in an on-site CHP unit with an installed (slightly over-dimensioned) electrical capacity of 2.5 MW_e. Output electricity is sold to the power grid at the wholesale electricity market price. It is assumed that 50% of the produced CHP waste heat is used as process heat for the digester heating system or other on-site systems, and that 25% of the surplus waste heat is sold to local district heating networks or external users at a wholesale market price of 10 € MWh_{th}^{-1}. The greenhouse gas emissions from the CHP system were considered CO_2 neutral as they are of biogenic origin, so no emission taxes were taken into account. The cost contributions for the considered biogas-fed CHP unit are listed in Table S 1.4.

2.2. Unit II: Biomethane Production and Injection

2.2.1. Biomethane production costs

The final cost for biomethane production can be broken down into three major contributions: (i) biogas production cost, (ii) upgrading cost and (iii) cost for grid injection (including transportation from the upgrading unit to the grid connection point, gas pressurization, odorization and conditioning). Upgrading and injection costs are computed with the Biomethane Calculator, a software cost estimator tool developed by the Vienna University of Technology to address the costs from various upgrading technologies and operational conditions (biogas composition, biogas flow rate, required biomethane purity

and pressure, *etc*.) (Mitner et al., 2012). In this tool, specific upgrading costs, including both investment and operational expenditures for gas-permeation, pressure swing adsorption, pressurized water scrubbing, amine scrubbing, and specific costs related to the injection of biomethane are included. To calculate the investment costs for biogas production and upgrading, data on the specific investment at a biogas flowrate of 1000 $Nm^3\ h^{-1}$ (29.3 ton day^{-1}) was used. Biomethane was assumed to be purified to a CH_4 content of 97% and injected in the adjacent regional transportation gas grid (14 bar) (Table S 1.5). Conditioning by propane dosing was not included.

2.2.2. Energy prices

Sources of income are likely to include the revenues from selling electricity, heat and digestate as well as from the regulatory support for renewable heat and power production. If biomethane production is intended, revenues are made through the sale of methane and CO_2 (CO_2 fertilization in a greenhouse where feasible, 15 € ton^{-1} CO_2). The sale of CO_2 in the exhaust gas of the CHP was not included, as treatment costs are assumed to offset the revenues. In this study, it is assumed that digestate has no economic value nor cost (product value of organic fertilizers compensates for the costs incurred with the digestate treatment efforts) and that the income does not include financial incentives by means of policy instruments. Other advantages of biogas projects for operators of anaerobic digesters (nutrient management, waste treatment, *etc.*) are not taken into account. Savings through own heat or electricity production are not included. The average electricity market price in 2000–2017 was approximately 38 € MWh_e^{-1}, varying between 20 and 73 € MWh_e^{-1}. When sold to the natural gas market, revenues from biomethane injection are currently in between 10 and 20 € MWh^{-1}. A parametric analysis points out how changes of some variable cost (feedstock and upgrading technology) and revenues (electricity and biomethane) can influence the economics of a biogas project.

2.3. Unit III: Biomethane Valorization

2.3.1. Aspen Plus simulations

Three cases for biomethane valorization through chemicals were considered: (i) sorption-enhanced steam reforming of CH_4 for H_2 production (SESR), (i) dry reforming of CH_4 for syngas production (DRM) and (iii) super-dry reforming of CH_4 for CO production (SDR). The global reactions for these three cases have been presented in equations [1] - [3].

An estimation of the OPEX of these processes was made after performing Aspen Plus simulations. The details of these simulations are given in what follows. As physical property method, NRTL – an activity coefficient method for chemical systems at low pressure – was used. This method was selected based on the built-in method selection assistant. In the process scheme, the feed was preheated from room temperature in a first heat exchanger (HX1 in Figure S 1.2, shell and tube, countercurrent) using the heat of the product stream. The minimum temperature approach in HX1 was set to be 10°C, a typical minimum-temperature approach used in pinch analysis studies (Reyniers et al., 2017). In a second heat exchanger (HX2 in Figure S 1.2, heater), the hot feed stream was brought to reactor temperature (750°C) by combustion of biomethane. The reactor was operated at 750°C and 1.013 bar, its heat for the endothermic reaction being delivered by combustion of biomethane. The maximum possible conversion according to reaction stoichiometry was assumed for all three cases, *i.e.* reaction equations [1] - [3], were assumed to yield full conversion towards the product side. A large degree of complexity through implementation of equilibria, which would cause a more difficult comparison between cases due to the uncertainty of cost contributions, is avoided by making this assumption. Separation processes or costs were not considered in this study. Likewise, heat recovery from the flue gases of CH_4 combustion for heat supply to HX2 and the reactor was not considered, but could reduce energy consumption costs. Process energy consumption is assumed to be the sum of energy required in HX2 and in the reactor, and hence assumed equal to the required energy to be delivered by methane combustion. Methane as fuel is considered to be the same as the chemical feedstock, *i.e.* biomethane when considering a biomethane feedstock and natural gas when considering a natural gas feedstock. The combustion of methane is assumed to be complete, equation [4].

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \qquad \text{[Eq. 4]}$$

The feed and product flow rates used in the Aspen Plus simulation as well as the net required energy input for the three considered cases are summarized in Table S 1.2.

2.3.2. Cost calculations

The consumer price of natural gas (assumed 100% CH_4) for industrial end users was taken to be 440 € ton^{-1} (EU-28 average price in 2015, including network costs, taxes and levies) (Boulamanti & Moya, 2017; EC, 2016). The variability of the natural gas price in the EU case

was accounted for by performing the same calculations using the minimum and maximum price of natural gas in the EU between 2008 and 2015 (considering yearly average prices). These values were taken from a report of the European Commission (EC, 2016), as well as the different contributions (market price, additional costs for the distributor, network costs, taxes and levies, see Figure S 1.3). The values for these contributions are also used to estimate the price of biomethane for the end-user.

The base-case consumer price of biomethane (assumed 100% CH_4) was assumed 745 € ton^{-1}, based on a weighted average using different feedstocks for anaerobic digestion (paragraph 2.1.1.). The same calculations using cheap (540 € ton^{-1}) or expensive (1070 € ton^{-1}) feedstocks and biogas treatment (paragraph 2.1. and 2.2.) were performed to account for the variability of the biomethane production cost. The energy density of methane in biomethane or natural gas was taken to be 54.5 GJ ton^{-1}. The water feedstock cost for steam production in the SESR case was assumed to be negligible, while the base-case market price of H_2 and CO were assumed to be 1500 € ton^{-1} and 300 € ton^{-1}, estimated based on previously reported values within the EU (Boulamanti & Moya, 2017; Teuner et al., 2001).

When determining the CO_2 emission rate for each of the three cases, the CO_2 separated from the initial biogas (assumed 60 vol.% CH_4 and 40 vol.% CO_2) during its upgrading to biomethane was not taken into account (both for biomethane as feedstock and combustion fuel), since the biomethane producer and biomethane consumer are considered to be separated units. Moreover, this CO_2 originates from biomass, a renewable carbon source that captures CO_2 on a small timescale (Martens et al., 2017). To the contrary, CO_2 produced by combustion of biomethane in HX2 and the reactor (Figure S 1.2) was taken into account as CO_2 emission by the chemical production plant. Naturally, while the net CO_2 utilization in the SDR and DRM case was considered a negative CO_2 emission, the net production of CO_2 in the SESR H_2 production case was considered a positive emission. In cases with a net CO_2 emission rate, CO_2 produced as product or flue gas component was assumed to be released as a stack gas rather than purified and sold. An emission tax of 30 € ton^{-1} CO_2 was applied in the base-case scenario as a method for taking the social cost of carbon (SCC) emission into account, a cost that has been estimated ⁻o lie in a range as broad as 1-1500 US$ ton^{-1} CO_2 with a mean of peer-reviewed estimates around 40-45 US$ ton^{-1} CO_2 (IPCC, 2007). In cases with a net CO_2 consumption, the base-case cost of the CO_2 feedstock was assumed to be 25 € ton^{-1}, slightly higher than the typical low-end cost for CO_2 separation – realized through absorption,

adsorption or carbonation technology – from large point sources such as industrial flue gases (20-1000 US$ ton^{-1} CO_2) (Boot-Handford et al., 2014) but corresponding closely with the estimated cost of 29 US$ ton^{-1} CO_2 for CO_2 capture through CaO looping (Zhao et al., 2013). In locations where CO_2 is available on-site, such as CO_2 from steam reforming plants or from stack gases, this cost of CO_2 can be interpreted as the cost of its separation and purification. In locations where CO_2 is not available on-site, it corresponds with the market price of CO_2. Recycling of CO_2 from combustion flue gases (from HX2 and from the reactor) was not considered. When determining the CO_2 tax avoided in case of SDR and DRM, these flue gas emissions are subtracted from the CO_2 consumption in the reaction.

Process profitability is estimated considering feedstock cost, energy cost, product market price and CO_2 tax. The profit is calculated based on following assumptions and is expressed per ton CH_4, taking into account the different CH_4 input streams (both as reactant and combustion fuel). No feedstock costs other than those of the reactants and the fuel for heat production are considered, equation [5]. The feedstock cost is assumed to make up 76% of total OPEX, equation [6], while O&M and other contributions were each assumed to make up 12% of total OPEX (FCTO, 2015). The CAPEX contribution to the total cost was assumed to be 41 € ton^{-1} CH_4 based on several independent studies (Bressan & Davis, 2013; Compagnoni et al., 2017; Khojasteh Salkuyeh et al., 2017). More detailed information about CAPEX is given in Table S 1.6. The sum of OPEX, CAPEX and CO_2 tax then yields the total cost, equation [8]. The profit is calculated by subtracting the revenue from products and the total cost, equation [9].

$$Feedstock\ cost \approx feedstock\ cost\ for\ reaction\ and\ heat\ production \quad \left[€\ \boldsymbol{ton_{CH4}^{-1}}\right] \quad \text{[Eq. 5]}$$

$$OPEX \approx \frac{Feedstock\ cost}{0.76} \quad \left[€\ \boldsymbol{ton_{CH4}^{-1}}\right] \quad \text{[Eq. 6]}$$

$$CAPEX \approx 41.0 \pm 13.8 \quad \left[€\ \boldsymbol{ton_{CH4}^{-1}}\right] \quad \text{[Eq. 7]}$$

$$Total\ cost \approx CAPEX + OPEX + CO_2\ tax \quad \left[€\ \boldsymbol{ton_{CH4}^{-1}}\right] \quad \text{[Eq. 8]}$$

$$Profit = Revenue\ from\ products - Total\ cost \quad \left[€\ \boldsymbol{ton_{CH4}^{-1}}\right] \quad \text{[Eq. 9]}$$

2.3.3. Assumptions made in the overview table with fuels and chemicals

In calculating the annual potential production volumes of C, CO and CO_2 corresponding with fuels and chemicals, following assumptions were made. Coal, oil and natural gas were assumed to consist of 30-85wt.% carbon, 85wt.% carbon (H/C molar ratio ~ equal to 2) and 100% CH_4. The potential use of CO as a base molecule for the production of chemicals/fuels was considered according to following reactions, where the methanol to olefins process is written as MTO:

Fuels *via* Fischer-Tropsch synthesis	$n\ CO + 2n\ H_2 \rightarrow (CH_2)_n + n\ H_2O$	[Eq. 10]
Ethylene through MTO	$2CO + 4H_2 \rightarrow 2CH_3OH \rightarrow C_2H_4 + 2H_2O$	[Eq. 11]
Propylene through MTO	$3CO + 6H_2 \rightarrow 3CH_3OH \rightarrow C_3H_6 + 3H_2O$	[Eq. 12]
Ethanol through fermentation	$6CO + 3H_2O \xrightarrow{Clostridium\ ljungdahlii} CH_3CH_2OH$	[Eq. 13]
Methanol	$CO + 2H_2 \rightarrow CH_3OH$	[Eq. 14]
Formaldehyde	$CO + H_2 \rightarrow CH_2O$	[Eq. 15]
Acetic acid	$CO + CH_3OH \rightarrow CH_3COOH$	[Eq. 16]
Phosgene	$CO + Cl_2 \rightarrow COCl_2$	[Eq. 17]
Acetaldehyde (and other aldehydes)	$CO + H_2 + olefins \rightarrow aldehydes$	[Eq. 18]
Polycarbonate	Through phosgene or dimethyl carbonate	[Eq. 19]
Dimethyl carbonate	$2CH_3OH + 0.5O_2 + CO \rightarrow (CH_3O)_2CO + H_2O$	[Eq. 20]
Acids	$CO + alkene\ or\ alkyn\ or\ conjug.dienes \rightarrow acids$	[Eq. 21]
Glycolic acid	$CH_2O + CO + H_2O \rightarrow CH_2(OH)COOH$	[Eq. 22]
Lactic acid	$CH_3CHO + CO + H_2O \rightarrow CH_3CH(OH)COOH$	[Eq. 23]

The rate at which CO_2 is emitted by large stationary sources was based on a report published by IPCC stating that 13375 Mt CO_2 $year^{-1}$ was emitted by sources larger than 0.1 Mt $year^{-1}$ in 2002 (IPCC, 2005). The same report contains a distribution of global CO_2 emissions, indicating that these emissions constituted about 60% of the total CO_2 emissions

(Figure S 1.4). Hence, the rate of CO_2 emissions by large stationary sources in 2015 was estimated to be 60% of the 2015 global CO_2 emission rate (35000 Mt CO_2 $year^{-1}$) (Olivier et al., 2015), *i.e.* 21000 Mt CO_2 $year^{-1}$.

3. Results

The average specific raw biogas production cost was estimated at 114 € ton^{-1} biogas for a feedstock mixture dominated by manure and agro-industrial waste (Table S 1.3). The minimum and maximum production cost (52 and 219 € ton^{-1} biogas, respectively) are mainly determined by the feedstock cost of the substrate mixture, and only to a minor extent by the CAPEX and OPEX (Figure 2.2). The impact of the feedstock on the overall biogas production cost is represented in Figure S 1.1.

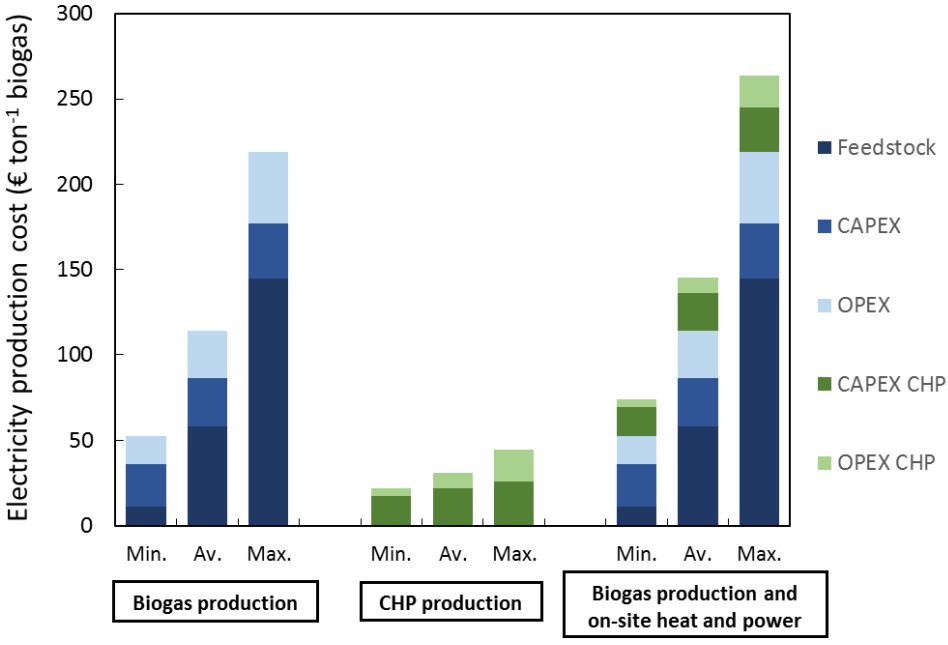


Figure 2.2 - Averaged specific biogas and combined heat and power production costs broken down into components for biogas production and combustion in a combined heat and power unit.

3.1. Electricity production from biogas

The production cost of electricity in a CHP unit is estimated at 145 € ton^{-1} biogas (Figure 2.2), including 31 € ton^{-1} as the average cost for the CHP unit (Table S 1.4). If expressed per MWh of electrical energy produced, a production cost of 62 € MWh_e^{-1} is calculated. The produced electricity should be sold to the grid for at least 80 € MWh_e^{-1} to reach break-even provided no financial support is present (at the average biogas production cost of 114 € ton^{-1} biogas) (Figure 2.3A). Only if extremely cheap biogas can be produced (at 52 € ton^{-1} biogas), break-even can be obtained in the range of the current electricity wholesale prices, indicating that most of the biogas plants combusting biogas for the production of electricity and heat depend on the implementation of instruments supporting CHP generation. Assuming that the AD operator receives 40 € per MWh electricity injected in the grid, a subsidy of 40 € MWh_e^{-1} will be needed to guarantee the economic viability (Figure 2.3B). As can be seen from the graphs, results are significantly influenced by whether average data or extremes are used as a reference.

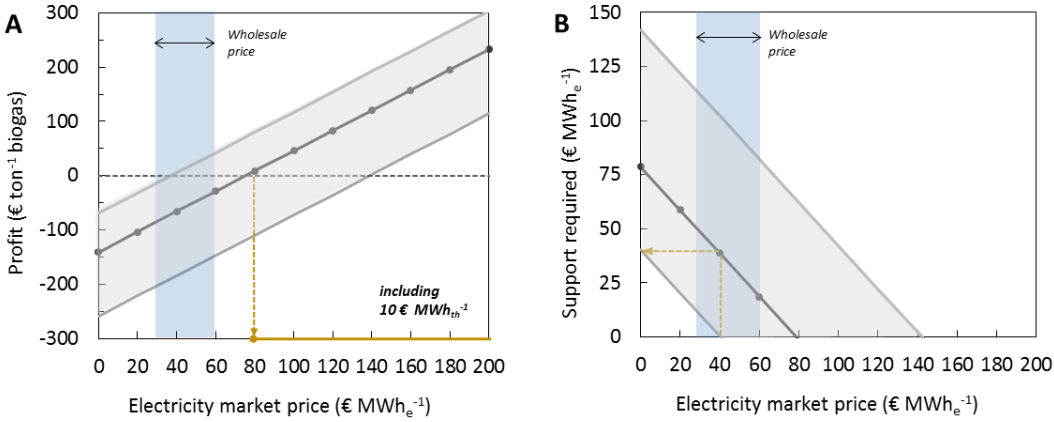


Figure 2.3 - Economic analysis for on-site CHP production from biogas. (A) Profit generated (in € ton^{-1} biogas) from selling biogas-derived electricity in function of the electricity market price (in € MWh_e^{-1}) for three base cases: extremely cheap, average and extremely expensive biogas, not including any financial incentive. The shaded vertical (blue) region represents the variation in current wholesale electricity price. A thermal energy price of 10 € MWh_e^{-1} and a fixed percentage of sold combined heat and power waste heat of 25% is set. (B) Required financial support (in the form of feed-in tariffs, certificates or other regulatory incentives) to match costs and revenues (break-even point). With the current retail prices of electricity, 20 to 50 € MWh_e^{-1} is needed to incentivize electricity production from biogas, if an average biogas production cost of 144 € ton^{-1} biogas is assumed.

3.2. Biomethane injection

The contribution of the different cost components (CO_2 removal, desulphurization, compression, odorization, gas transportation and injection in the gas transfer station) to the total biomethane production cost is represented in Figure 2.4. For the biomethane plant under study, average specific biogas upgrading costs are between 6.34 and 8.58 c€ Nm^{-3} raw biogas. Injection costs can be estimated at 3.38 c€ Nm^{-3} raw biogas for the specific set of operational conditions and represent the lowest cost component. Specific biogas upgrading and injection costs add on average 250 € ton^{-1} biomethane, leading to total costs of 574 € ton^{-1} biomethane (Figure 2.4). Deviations from this value for different upgrading technologies are small and also the variability in costs for the different injection steps is of minor importance in relation to the entire biomethane production cost (Figure S 1.5). Most of the variability can be attributed to the feedstock type since biogas production is the largest contributor to the total cost for biomethane grid injection. With an average European natural gas market price of 240 € ton^{-1} for the year 2000 – 2017, biomethane deployment options are far away from being economically viable on this market without applying any support scheme.

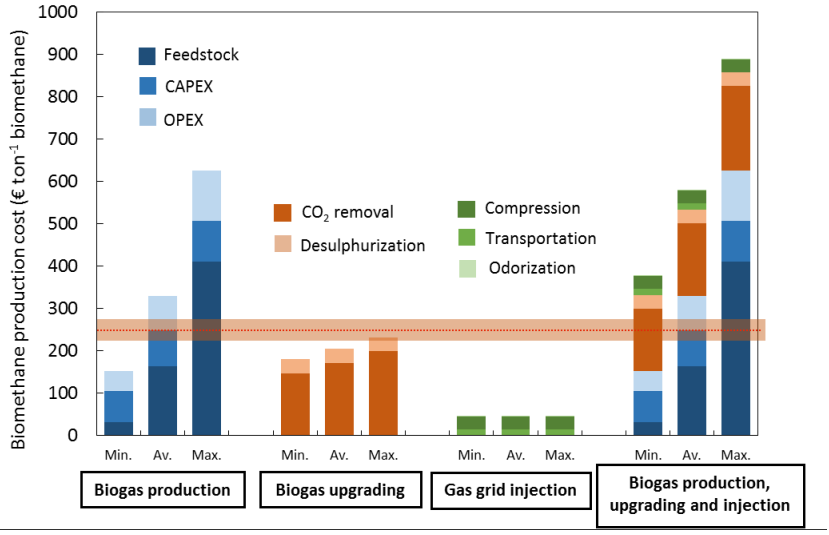


Figure 2.4 - Averaged specific biomethane production and injection costs broken down into components (biogas production, biogas upgrading and biomethane injection). An average natural gas wholesale price of 240 ± 20 € ton^{-1} for the period 2000 - 2017 was plotted as a red horizontal zone.

Currently, feed-in-tariffs for biomethane injection into the regional natural gas pipeline between 20 and 30 € MWh^{-1} are needed to make the production of biomethane economically comparable with the current (subsidized) generation of "green" electricity.

If biomethane production is evaluated, the results show that for the entire range of natural gas market prices no profit can be made, even when cheap biogas is used. Natural gas wholesale prices should triple (to around 45 € MWh^{-1}) to make it profitable to operate an upgrading facility without support (Figure 2.5).

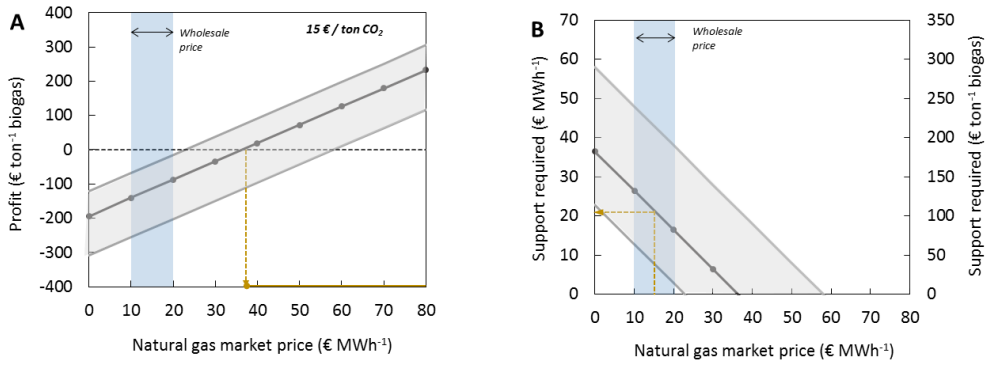


Figure 2.5 - Economic analysis for biomethane production and grid injection. (A) Profit generated (in € ton^{-1} biogas) by selling cheap, average and expensive biomethane as a function of the natural gas market price (in € MWh^{-1}), not including any financial incentive. The shaded vertical (blue) region represents the variation in current wholesale natural gas price. A CO_2 price of 15 € ton^{-1} is set. (B) Required financial support to match costs and revenues (break-even point). With the current retail prices of natural gas, 20 to 30 € MWh^{-1} (or 100 to 150 € ton^{-1} biogas) is needed to incentivize grid injection of upgraded biomethane, if an average biomethane production and injection cost of 574 € ton^{-1} biomethane is assumed.

3.3. Biomethane as chemical feedstock

The economic feasibility of biomethane chemical valorization towards H_2, syngas or CO is discussed in what follows. Figure S 1.6 in the supplemental information shows that the heat requirement for the CO production case is the highest, while the potential for heat recovery through a feed-effluent heat exchanger is also the highest. This causes a slight reduction in the energy demand gap between SDR and DRM/SESR when considering the net heat input. As expected, the required amount of reaction heat scales with process endothermicity: SDR > DRM > SESR. When considering the heat input per unit CO_2 converted, however, the

SDR process for CO production proves to be more efficient than the DRM and SESR case (Table S 1.2).

Evaluating the economic viability of the base-case scenario for SDR, DRM and SESR (paragraph 2.3.2.) reveals that only CO and syngas production through the SDR and DRM process yield a positive profit of 302 € ton^{-1} CH_4 and 75 € ton^{-1} CH_4, respectively, while the SESR process for H_2 production yields a significantly negative profit (Figure 2.6). Indeed, H_2 production starting from biomethane is not economically feasible under these assumptions contrary to CO and syngas production. If the CO_2 feed, required for the SDR process, can be obtained from local emission point sources, its conversion through CO production results in an avoided cost of 156 € ton^{-1} CH_4 by avoiding CO_2 emission taxes (considering a tax of 30 € ton^{-1} CO_2 and a CO_2 utilization ratio of 5.2 ton CO_2 ton^{-1} CH_4 (paragraph 2.3.2.)).

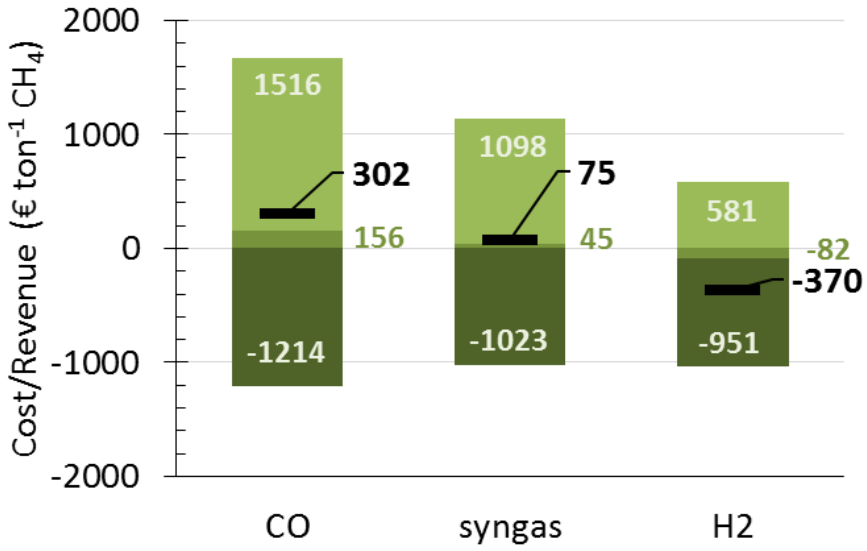


Figure 2.6 - Overview of process cost (dark green), product revenue (light green), CO_2 tax and profit (solid black line). Positive values for the CO_2 tax indicate the avoided cost by CO_2 utilization (and are not taken into account when calculating the profit), while negative values represent an additional cost. Profit is calculated by adding process cost, CO_2 tax (if negative) and product revenue.

In order to study the effect of different variables on the calculated profit a parametric analysis, always starting from the base-case scenario, was performed. Figure 2.7A shows the effect of CH_4 market price on the profit for the three CH_4 valorization cases. These results

suggest that only the SDR and DRM process can potentially be economically viable considering the current range of prices associated with biomethane, while the SESR process is not economically viable. When considering natural gas as a CH_4 feedstock, the SESR process is close to the break-even point but still economically unfavorable while both the DRM and SDR process seem to yield a positive profit. Because of the favorability of the SDR process over the other two cases, the SDR process is the focus of this work in what follows. The effect of a possible underestimation of the energy costs is studied through the heat duty (Figure 2.7B). It is observed that doubling the heat duty (from base-case 21 GJ ton^{-1} CH_4 to around 45 GJ ton^{-1} CH_4) leads to the break-even point when considering the average biomethane price. A further increase of the energy input would limit the economic viability of the process towards the cheaper end of biomethane feedstocks. The effect of the CO market price on the process profitability is presented in Figure 2.7C. For the cheapest end of biomethane resources, the break-even point is reached at market prices as low as 190 € ton^{-1} CO. Using the average biomethane source requires a market price around 240 € ton^{-1} CO to reach break-even. Considering the Calcor process, for which a CO production cost between 250-500 € ton^{-1} CO is reported (Teuner et al., 2001), the SDR process indeed seems economically favorable with estimated production cost between 190-320 € ton^{-1} CO depending on whether cheap or expensive biomethane is used. Figure 2.7D illustrates the effect of the CO_2 market price on the process economics. Starting from the base-case scenario, increasing the feedstock cost of CO_2 or the on-site cost of CO_2 separation to 65 € ton^{-1} CO_2 yields the break-even point when considering an average priced biomethane feedstock. Considering the cheaper end of biomethane feedstocks shifts this point towards 100 € ton^{-1} CO_2. A similar parametric analysis for all three cases was performed (Figure S 1.7).

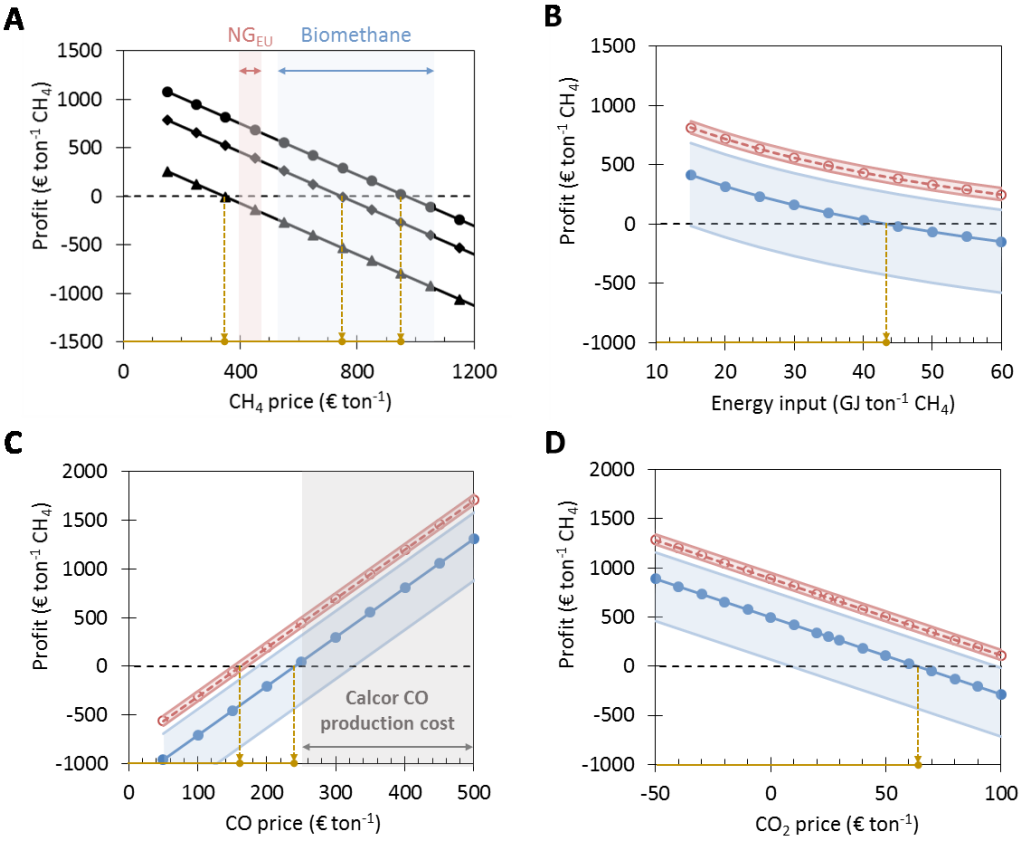


Figure 2.7 - Economic analysis for the production of chemicals from biomethane or natural gas. (A) Profit generated by producing H_2, CO/H_2 or CO as a function of CH_4 market price. —▲— H_2 production through sorption-enhanced steam reforming of methane; —◆— CO/H_2 production through dry reforming of methane; —●— CO production through super-dry reforming. The shaded vertical regions represent the variability in EU natural gas price (red) and biomethane price (blue). (B) Profit generated by producing CO as a function of energy input using a biomethane (—●—) or EU natural gas (--⊖--) feedstock. The shaded regions represent the variability in feedstock price of biomethane (blue) and natural gas in the EU (red). (C) Profit generated by producing CO as a function of CO market price using a biomethane (—●—) or EU natural gas (--⊖--) feedstock. The shaded regions represent the variability in feedstock price of biomethane (blue) and natural gas in the EU (red). The grey shaded region corresponds with the reported CO production cost range of the Calcor process. (D) Profit generated by producing CO as a function of CO_2 market price using a biomethane (—●—) or EU natural gas (--⊖--) feedstock. The shaded regions represent the variability in feedstock price of biomethane (blue) and natural gas in the EU (red).

Figure 2.8 shows a parametric analysis of the avoided cost of CO_2 taxes, which may be important *e.g.* when on-site CO_2 emissions from a different plant can be mitigated by implementation of the SDR or DRM process. In such a case, the CO_2 price as presented in Figure 2.7D would correspond with its separation and purification cost. Taking this avoided cost, ~150 € ton^{-1} CH_4 corresponding with 30 € ton^{-1} CO, into account for the SDR case moves the break-even point towards market prices of 160 € ton^{-1} CO and 210 € ton^{-1} CO for the cheap and average biomethane feedstock. Moreover, Figure 2.8 indicates how the economic favorability of the SDR and DRM process increases with increasing CO_2 tax rate, contrary to the SESR case.

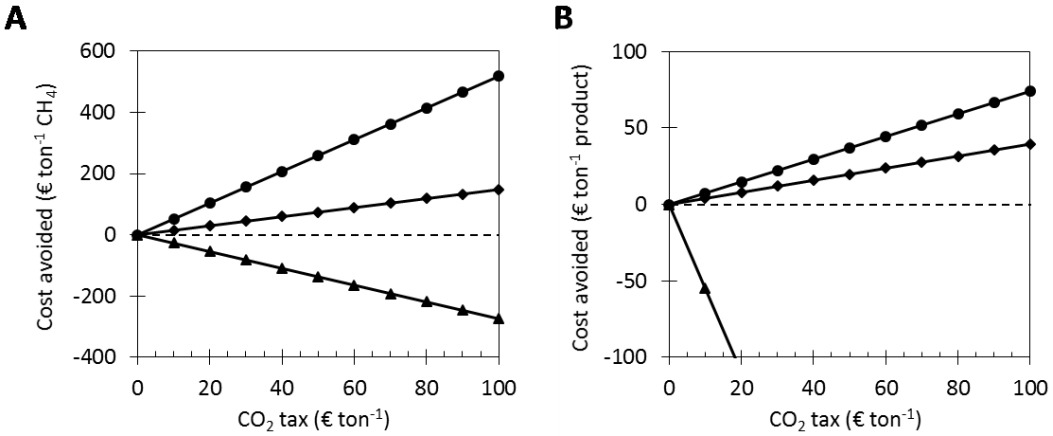


Figure 2.8 - Economic analysis of avoided costs when producing H_2, CO/H_2 or CO. —▲— H_2 production through sorption-enhanced steam reforming of methane; —◆— CO/H_2 production through dry reforming of methane; —●— CO production through super-dry reforming. (A) Effect of CO_2 tax on cost avoided (€ ton^{-1} CH_4). (B) Effect of CO_2 tax on cost avoided (€ ton^{-1} product).

Based on the results presented in Figure 2.7, the financial support required to reach the break-even point was estimated. A parametric analysis of the effect of CH_4 feedstock price and CO product price is given in Figure 2.9. Figure 2.9A and Figure 2.9C show that, as seen in Figure 2.7A, the minimum required financial support amounts to 130 € ton^{-1} CH_4 (25 € ton^{-1} CO_2 utilized) and 420 € ton^{-1} CH_4 (283 € ton^{-1} CO_2 utilized) for the SDR and DRM case when only expensive biomethane is available. For H_2 production through SESR, this amounts to 250-950 € ton^{-1} CH_4 depending on whether a cheap or expensive biomethane feedstock is applied. Since the latter process results in a net production of CO_2 rather than a net utilization, the

amount of support required cannot be given in terms of € ton^{-1} CO_2 utilized. Figure 2.9B and Figure 2.9D show that for a drop in CO market price from 300 € ton^{-1} to 200 € ton^{-1}, the required financial support for SDR becomes at least 200 and 630 € ton^{-1} CH_4 when using average and expensive biomethane, which roughly corresponds with 40 and 125 € ton^{-1} CO_2 utilized.

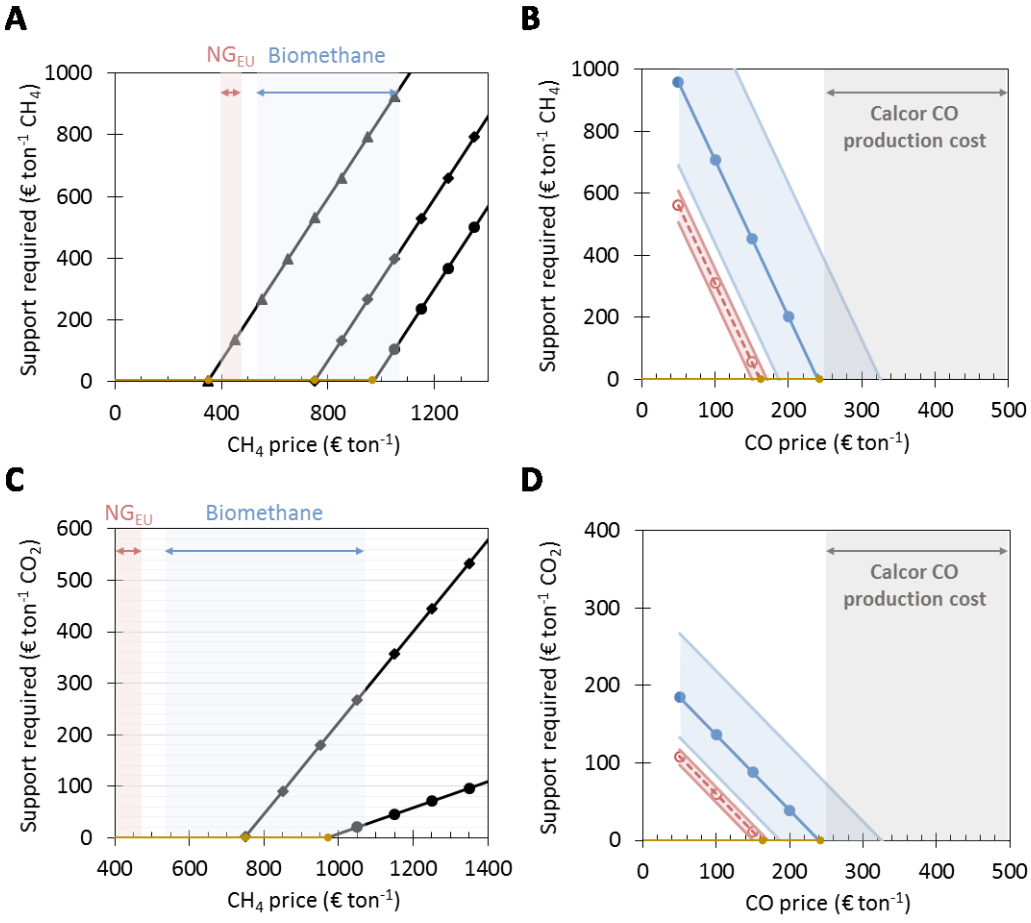


Figure 2.9 - Economic analysis for the production of chemicals from biomethane or natural gas. (A) Support required (€ ton^{-1} CH_4) for producing H_2, CO/H_2 or CO as a function of CH_4 market price. —▲— H_2 production through sorption-enhanced steam reforming of methane; —◆— CO/H_2 production through dry reforming of methane; —●— CO production through super-dry reforming. The shaded vertical regions represent the variability in EU natural gas price (red) and biomethane price (blue). (B) Support required (€ ton^{-1} CH_4) for producing CO as a function of CO market price using a biomethane (—●—) or EU natural gas (--⊖--) feedstock. The shaded regions represent the variability in feedstock price of biomethane (blue), natural gas in the EU (red) and CO production cost range of the Calcor process (grey). The grey shaded region in (C) corresponds with the reported CO production cost range of the Calcor process. (C) Support required (€ ton^{-1} CO_2) for producing CO/H_2 or CO as a function of CH_4 market price. H_2 production is not considered here since it is not a CO_2 utilization technology. —◆— CO/H_2 production through dry reforming of methane; —●— CO production through super-dry reforming. The shaded vertical regions represent the variability in EU natural gas price (red) and biomethane price (blue). (D). Support required (€ ton^{-1} CO_2) for producing CO as a function of CO market price using a biomethane (—●—) or EU natural gas (--⊖--) feedstock. The shaded regions represent the variability in feedstock price of biomethane (blue), natural gas in the EU (red) and CO production cost range of the Calcor process (grey).

4. Discussion

Energy

Today, many European biogas plants struggle for their economic existence. The lower feed-in tariffs, higher prices for raw material, and the current low energy and methane prices endanger the economic viability of biogas projects. The profitability of an investment in a biogas or biomethane project generally depends on the availability of a national support scheme (either as feed-in tariff, 'green' certificates, investment support or tax incentive), as well as the assurance that the project will be eligible to benefit from the legal incentive at the operational phase (thus, ruling out the regulatory risk for investors) (van Foreest, 2012). Contrarily, utility-scale solar PV and wind projects now provide electricity in the cost range of fossil fuel based technologies without any financial support (IRENA, 2015).

Biomethane

The level of required support per MWh produced energy to realize break-even is lower for upgrading and injection than for on-site heat and power production. It is therefore remarkable to observe that current support schemes in many EU member states tend to be limited to heat and power while the grid injection of renewable methane is most often neglected, although the technology for upgrading biogas to biomethane is mature, efficient and scalable. Once in the gas grid, biomethane use can be physically separated from the generating plant, offering more flexibility in utilization of this renewable energy carrier.

Although government support has helped the integration of renewables in the energy market, guaranteed legal support is typically costly and faces growing opposition from the tax-paying public, causing a shift towards a real marketing approach that stimulates consumer demand for renewable energy. Implementation of a blending obligation for biomethane, as is currently in place for biofuels to ensure at least a 10% share of renewable energy on the EU energy consumption for transport, seems to be feasible on the short term to make biomethane part of the decarbonization strategy for transport and (chemical) industry.

Towards chemical production

The use of biomethane can offer intrinsic competitiveness since it allows the production of products with a lower carbon footprint from a secure and local supply of renewable energy. Instead of looking at the (limited) cost reduction that can be achieved, however, focus should shift to the income side. Particularly for the fledgling biogas sector, the revenues from biogas could be increased by applying it to a chemical production process. Production of chemicals captures and utilizes both the electrons and the carbon of CH_4. The use of the existing natural gas grid for the transport of biomethane from the biogas plant to the chemical plant is crucial, allowing to make maximal use of the existing natural gas infrastructure. Biomethane production and chemical valorization should be spatially uncoupled since the decentralized conversion of CH_4 to CO at the biogas facility is unrealistic today. Instead, making the CO where it is needed and where CO_2 is available is preferred because of following reasons: (i) CO is a toxic gas that is always produced on-site rather than being transported from one site to another. (ii) The discrepancy between the scale of biogas production (on average 160 kg raw biogas h^{-1}) on the one hand and the methane consumption in a chemical plant (up to hundreds of tons methane h^{-1}) on the other hand makes it economically more challenging to turn an AD plant into a chemical plant (economies of scale play a crucial role in the chemical industry). (iii) In most cases the CO_2 for SDR or DRM is not available in agricultural areas. When a source of CO_2 is available, it may preferentially be used as fertilizer in greenhouses unless the scale is sufficient to warrant *in situ* chemical production (Jaffrin et al., 2003).

At the moment, the biomethane production cost usually lies substantially higher than the natural gas price, and, thus, it needs to be realized that from an economic perspective that – at current low natural gas prices – there is no real incentive in place to push the chemical industry to more expensive biomethane. Without subsidies and at the current allowance price of CO_2 emissions, the high opportunity cost of biomethane use ($\sim$300 € ton^{-1} CH_4 for the base-case) will, thus, prevent the market uptake of this 'green' raw material. Future customer expectations and an increased demand of carbon-neutral chemicals and fuels may increase the price tolerance of the industry in the future, while cost reductions in biogas or biomethane production may be possible through scale effects and technological advances. Nonetheless, at current low natural gas prices, chemical industry will need financial support to compensate for the price difference between green and fossil methane as the current savings on CO_2 emission taxes incurred with the switch to a more sustainable feedstock can

only partially offset the opportunity cost (saving of ~70 € ton^{-1} CH_4 at a CO_2 cost of 25 € ton^{-1} CO_2). However, it needs to be recognized that a fair comparison cannot be made, as the cost that industries pay for fossil-fuel derived carbon and energy does not reflect the cost that they would pay if the true cost of the damage caused by their consumption is internalized, for example, the local damage to our health related to having particles in the air, water pollution caused by mining for fossil carbon, or the global climate change related to the emission of CO_2. The fact that end-users do not face environmental damage as a negative externality makes every renewable alternative *de facto* economically uncompetitive. If the economic and environmental burden of natural gas for syngas production would be internalized, the biogas-to-chemicals route would result in a more rational alternative, able to offer immediate advantages in terms of greenhouse gas emissions and harmful local air pollutants. It has been estimated that the average direct costs to the healthcare system associated with natural gas-related air pollution are 300 € per ton CH_4 (Machol & Rizk, 2013). Other extern effects such as mining and drilling, land use changes, methane leaks from natural gas transmission and distribution pipelines and wastewater discharge are not included in this value as their valuation is more uncertain than that of local air pollutants and GHG emissions (McKain et al., 2015; Phillips & Goldberg, 2013). The full lifecycle environmental performance should reveal the actual reduction potential in terms of health and environmental damage from an increased use of biomethane, and should made clear to decision makers (IRENA, 2016).

The scale-up from biogas as a local energy source towards the upgrading to biomethane and injection in the gas grid implies the requirement of a monitoring system allowing to match biomethane production and demand, as is currently in place in the Netherlands, Germany and the UK. An effective and transparent mechanism for distribution of biomethane through the gas infrastructure is mass balancing and tracing with the help of guarantees of origin (Bowe, 2013). In mass balancing, rather than biomethane being physically transported from the producer to the consumption location, the energy seller feeds into the existing gas grid a certain unit of renewable energy, which is booked into a registry after verification of the origin and quality (caloric value and purity) of the biomethane. The end-user buying the biomethane subsequently marks the quantities in the register and receives a certificate confirming its origin and amount (administrative trade). To ensure proper trading and mass balancing for the biomethane which is transported *via* the natural gas grid, uniform and cross-border standards for biomethane composition will be necessary. While currently there

are several national standards in Europe for the injection of upgraded biogas into the gas grid, since 2006 actions are on-going to technically standardize gas grid injection specs. The challenge is to define standards which support the market implementation in such a way that they are attractive for the different stakeholders involved (biomethane producer, gas grid owner, end user, *etc.*) (Wellinger, 2013).

The European natural gas grid is a well interconnected infrastructure suitable to distribute biomethane, produced in decentralized and small-scale facilities, to densely populated areas or industrial clusters. It is likely that initially only larger scale digesters and associated upgrading facilities will be competitive (Persson et al., 2006). In time, technological development may allow connecting smaller installations either as greenfield sites or through retrofit.

Here, the biomethane feedstock cost for industry includes both the transmission and distribution costs as charged for natural gas. The direct injection of biomethane into the local low-pressure distribution network leads to lower costs than the use of natural gas though, as the latter typically has to be transported to the end-user at a higher pressure over significantly longer distances (Hoo et al., 2018). These cost savings could partially compensate for the higher feedstock price of biomethane.

CO, H_2 or syngas?

Our base-case scenarios show that the production of CO or syngas through respectively SDR and DRM could be economically viable without external financial support, which is not the case for H_2 production through SESR. The best case is the sole CO production from biomethane via SDR. For the production of CO or syngas, we expect that existing chemical plants would opt to use a "virtual" blend of natural gas and biomethane because of following reasons: (i) Biomethane blend-in provides industry with a means to decrease its carbon footprint, even though the currently available methane production via AD (around 23 Mt methane $year^{-1}$, based on the global biogas energy production in 2017 (Kummamuru, 2017)) is far from sufficient to achieve a full replacement of natural gas (around 2870 Mt natural gas $year^{-1}$ in 2015 (World Energy Council, 2016)). (ii) From an economic point of view, using a blend of natural gas and biomethane gives companies a degree of freedom in choosing the extra amount they are willing to pay for a share of biomethane in their feedstock.

Indeed, whereas the base case using average-priced biomethane suggests that CO can be produced through SDR with a profit around 60 € ton^{-1} CO corresponding with ~ 58 € ton^{-1} CO_2 consumed (as derived from Figure 2.7 taking into account productivity factors of 5.06 ton^{-1} CO ton^{-1} CH_4 and 5.19 ton CO_2 ton^{-1} CH_4, representing the amount of CO produced and CO_2 utilized per unit biomethane consumed as feedstock and heating fuel), the case changes significantly when the market price of CO decreases *e.g.* from 300 € ton^{-1} CO to 200 € ton^{-1} CO. In the latter case, external financial support up to 40 € ton^{-1} CO corresponding roughly with 38 € ton^{-1} CO_2 consumed is required to run break-even when making use of average-priced biomethane.

While the utilization of waste CO_2 in the base case scenario results in an avoided cost of 30 € ton^{-1} CO_2 when taking into account the social cost of carbon through a CO_2 taxation system, the case shifts closer to the break-even point with a loss of roughly 10 € ton^{-1} CO_2 when considering a market price of 200 € ton^{-1} CO instead of 300 € ton^{-1} CO.

What to produce from CO?

Table S 1.7 lists the most important chemicals and fuels that could be produced from biomethane through CO, where coal and natural gas are added for illustrating their share in fossil fuel resources. Three major groups of end-markets for biomethane can be distinguished based on the scale at which they are being produced: (i) raw chemicals and fuels produced on a Gt $year^{-1}$ basis, (ii) bulk chemicals produced on a 10-100 Mt $year^{-1}$ basis and (iii) other chemicals produced on a 100-1000 kt $year^{-1}$ basis. The amount of CO that would be necessary for producing these chemicals and fuels through the reaction listed in paragraph 2.3.3. is calculated and given in Table S 1.7. Similarly, the amount of CO_2 required for producing this CO through SDR is determined and the percentage of the estimated global biomethane production potential, necessary to meet the production volume of chemicals and fuels, is given as well as the percentage of CO_2 emissions from large point sources that could be valorized through CO via SDR. Indeed, these rudimentary calculations suggest that, considering only mass balances and market volumes, almost 50% of stationary CO_2 emissions from large point sources could be converted into fuels, assuming that a renewable source of H_2 would be available *e.g.* through electrolysis. At the same time, the amount of biomethane that would be needed is 2.2 times higher than the estimated global production potential, but could be combined with natural gas or methane from other renewable sources (*e.g.* through methanation reactions using renewable hydrogen). Moreover, it can be noted that globally

bulk chemicals could utilize ~ 5% of stationary CO_2 emissions from large point sources while requiring 26% of the estimated biomethane production potential. For other chemicals with production volumes smaller or equal to several Mt $year^{-1}$, contributions towards CO_2 emission reduction become negligible. Today, with a global biogas production corresponding to around 23 Mt biomethane $year^{-1}$ (Kummamuru, 2017), CO production for covering the global methanol demand could be achieved. Table S 1.7, thus, clearly illustrates the important role that fuels and bulk chemicals could play in reducing global CO_2 emissions (*ca.* 54% of stationary CO_2 sources can be valorized through these end-markets) as well as the need of vast amounts of methane for its valorization through CO (EBA, 2017).

Based on this study, we propose that incentives/subsidies should focus on not just promoting AD itself, but also the upgrading of biogas to biomethane and the utilization of CO_2 in order to increase the scale and efficiency of biomass and CO_2 valorization through chemicals. The results presented in this work also suggest that the conversion of CO_2 and biomethane to syngas or CO may already be economically viable without legal financial incentives, but upscaling processes for CO production from CO_2 is one of the major challenges to be addressed in future decades if mankind is to close the carbon loop.

5. Conclusions

Contrary to the production of heat and power from biogas and biomethane, the production of chemicals from biomethane could upgrade the value of anaerobic digestion in such a way that reliance on a legal support scheme is no longer a prerequisite to guarantee a cost-neutral investment. The production of CO or syngas from gas grid injected biomethane through (super-)dry reforming was found to be economically viable alternatives for the energetic valorization of biogas, and seems to be more economic than H_2 production. The reforming of waste CO_2 – generated by large-scale stationary producers and separated through techniques such as scrubbing, physical adsorption or calcium oxide looping – with biomethane enables industry to lower its carbon footprint in a cost-effective way while delivering a value-added product. Avoiding CO_2 taxes by converting on-site CO_2 to CO can partially compensate for the higher feedstock price of biomethane compared to natural gas.

Based on this study, following steps should be taken to realize the proposed biogas valorization strategy: (i) Investment in biogas upgrading to biomethane in order to provide an additional pathway/opportunity for the chemical industry to profile itself as striving towards sustainability; (ii) Upscaling and commercializing methods for CO production from renewable resources (such as biomethane), which would significantly decrease the carbon footprint of the chemical industry; (iii) Scenario calculations including feedstock and upgrading cost development, product revenues, energy supply and technological learning curves in order to evaluate future competitiveness of biomethane with natural gas.

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CHAPTER 3

Membrane electrolysis-assisted CO_2 and H_2S extraction as innovative pretreatment method for biological biogas upgrading

This chapter has been redrafted after:

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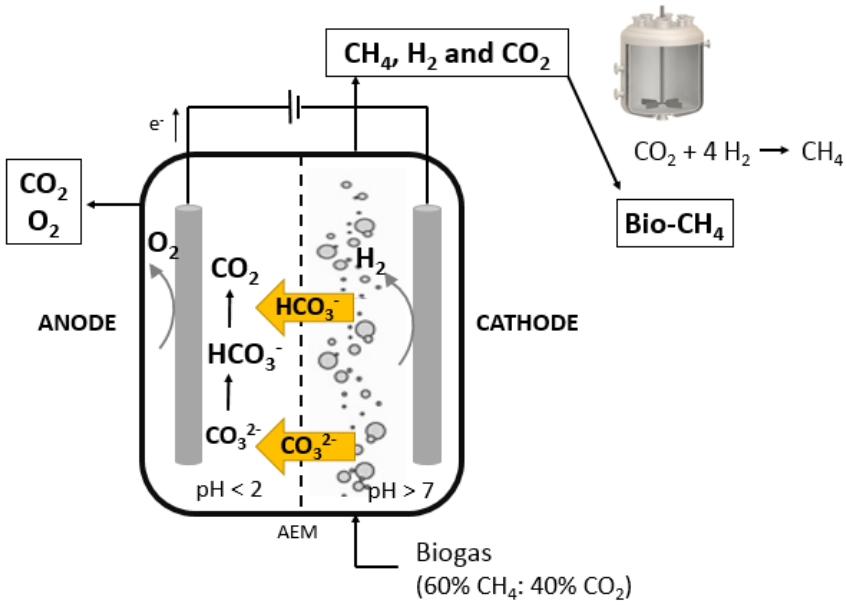
Abstract

Turning raw biogas into biomethane as energy carrier requires the selective removal of CO_2 in a biogas upgrading process or a total conversion of CO_2 to CH_4 which is generally energy intensive. During membrane electrolysis, electrical energy can be used to simultaneously remove CO_2 (and H_2S) and produce H_2 as side product. Biogas is, thus, scrubbed with catholyte and the captured HCO_3^- and HS^- migrate towards the anode. Simultaneously, cathodic H_2 mixes with residual biogas in a ratio that can be fine-tuned. We obtained in one step an ideal 4:1 H_2:CO_2 ratio in the reactor off gas. Subsequently the gas could be further upgraded *via* chemoautotrophic microbial conversion of CO_2 to CH_4. Biomethanation delivered biomethane with 98.9 ± 0.9 % purity. The electrochemically-assisted scrubbing and stripping of CO_2 and H_2S resulted in high CO_2 removal efficiencies (up to 100%), without addition of chemicals. The system was flexible depending on temporarily available power. Electrochemical biogas upgrading (EBU) can be envisaged as a scalable and decentralized storage of excess or off-peak renewable power, making better use of the power input used to drive a biological CO_2 conversion.



1. Introduction

Biogas is a mixture of mainly methane (CH_4) and carbon dioxide (CO_2), containing impurities, such as NH_3, H_2S, water vapour and siloxanes. It is produced from organic substrates through anaerobic digestion, and represents a reliable and versatile renewable energy carrier that is mainly valorized as a fuel for on-site heat and power generation. Alternatively, biogas can be upgraded to natural gas quality, injected into the natural gas grid and used in all the downstream applications of fossil methane (Ryckebosch et al., 2011). Raw biogas typically contains 40 - 75 % CH_4 and 25 - 60 % CO_2, but to work as an efficient renewable energy carrier that can be stored and distributed within the existing natural gas grid, its CH_4 content typically needs to be increased to more than 97 % CH_4, meeting the (local) quality standards for grid injection (Pöschl et al., 2010). At industrial scale, the transformation of raw biogas into pipeline-quality 'biomethane' is nowadays performed by various physical-chemical techniques to separate CO_2 from CH_4 (*e.g.*, water scrubbing, amine scrubbing, pressure swing adsorption, membrane technology or variants) (Bauer et al., 2013; Niesner et al., 2013). Recently, the chemoautotrophic microbial conversion of CO_2 to CH_4 was proposed as an innovative and sustainable method for biogas upgrading using renewable power (Luo & Angelidaki, 2012; Martin et al., 2013), particularly in the context of peak power shaving (Götz et al., 2016). It is increasingly considered as a form of intermittent energy storage, whereby through electrolytic H_2 is used to convert CO_2 into additional CH_4. The reduction of CO_2 to CH_4 relies on the action of hydrogenotrophic methanogens capable of using H_2 gas as sole electron donor for autotrophic CH_4 production ($CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$) (Agneessens et al., 2017). For a complete (theoretical) removal of CO_2 from biogas, H_2 needs to be supplied at a stoichiometric ratio of 4:1 (H_2:CO_2), implying a demand of 8 moles of electrons for every mole of CO_2 that needs to be converted. The H_2 gas can be supplied either *via* bubbling through the anaerobic digester itself (Luo et al., 2012), in a separate biomethanation reactor (Martin et al., 2013; Strevett et al., 1995) or *via in situ* electron or H_2 supply at a negatively poised electrode in a so-called bioelectrochemical system (Cheng et al., 2009; Villano et al., 2010; Xu et al., 2014). The low CH_4 production rates and efficiencies of this 'electromethanogenesis' route, compared to other CH_4-producing technologies, currently hamper its feasibility as a high-rate biogas upgrading technology (Geppert et al., 2016). Furthermore, the low conductivity of microbial growth media will limit the maximum current consumption (and thus the rate of upgrading), as cell potentials will increase rapidly due to high ohmic losses (Clauwaert et al., 2008). In this perspective, methanation of CO_2 and H_2 in a separate bioreactor seems to be the most straightforward way of scaling up biological

biogas upgrading today. A challenge is the production of H_2 *via* water electrolysis, which makes methanation energy intensive (> 7.2 kWh_e Nm^{-3} raw biogas) compared to conventional upgrading techniques (0.2 – 0.3 kWh_e Nm^{-3} raw biogas), but on the other hand it enables the use of renewable power to drive a biogas upgrading process without addition of chemicals, while storing renewable energy in a gaseous energy carrier (Muñoz et al., 2015).

Here we propose a combined electrochemical process, achieving simultaneous CO_2-H_2S scrubbing and H_2 production with the aim to lower the power input of biogas upgrading through a microbial conversion of CO_2 to CH_4 (Figure 3.1). The Electrochemical Biogas Upgrading (EBU) process proposed in this study uses an electrolysis cell equipped with an anion exchange membrane (AEM) to achieve cathodic CO_2 and H_2S capture, coupled to the active transport of (bi)carbonate and HS^- ions through the AEM [Eq. 1]. Water reduction at the cathode ($2\ H_2O + 2\ e^- \rightarrow H_2 + 2\ OH^-$) adds hydrogen to the biogas stream, while the slightly alkaline electrolyte enables CO_2 and H_2S removal from the biogas stream that is being sparged through the cathode chamber. The gas leaving the cathode chamber is a mixture of H_2, residual CO_2 and CH_4, and can be further upgraded to biomethane in an external biomethanation reactor where the H_2 and CO_2 are converted into autotrophically produced CH_4 [Eq. 2]. Additional H_2 could be supplied to this biomethanation reactor when power costs are low. Since an electrochemical cell inherently maintains cell electroneutrality (*i.e.*, the cell charge balance), negatively charged (bi)carbonate and HS^- ions will be transported across the AEM to compensate the electron flow from anode to cathode. The applied potential, thus, drives the transport of the charged HCO_3^- and HS^- over the membrane. In the anode compartment this HCO_3^- is converted to CO_2 due to the low pH obtained through water electrolysis ($2\ H_2O \rightarrow O_2 + 4\ e^- + 4\ H^+$), while it is expected that HS^- is oxidized to elemental sulfur or more oxidized S-species. This CO_2 removal strategy can be classified as a hybrid form of physical-chemical CO_2 scrubbing/stripping with H_2-mediated conversion of CO_2 to CH_4 as post-treatment to scavenge the produced H_2 and the residual CO_2, although other product outcomes based on CO_2-H_2 can also be envisaged.

The electrochemical system will have two major functions: production of hydrogen gas and extraction of (bi)carbonate across a membrane by providing electricity. In this way, the relative methane content will be increased and H_2 will be added to the gas mixture as an energy source.

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^- \rightleftarrows 2H^+ + CO_3^{2-} \quad \text{[Eq. 1]}$$

$$CO_2 + 4H_2 \rightleftarrows CH_4 + 2H_2O \quad \text{[Eq. 2]}$$

We hypothesized that this approach makes better use of the power input as, theoretically, 9 moles of CO_2 can be removed per 8 moles of electrons supplied, while conventional biomethanation can only remove 1 mole CO_2 per 8 moles of electrons. This work presents a two-step pipeline for CO_2-to-CH_4 based biogas upgrading, identifying the key operational parameters such as current density, pH and CO_2 loading rate on the electricity-driven extraction.

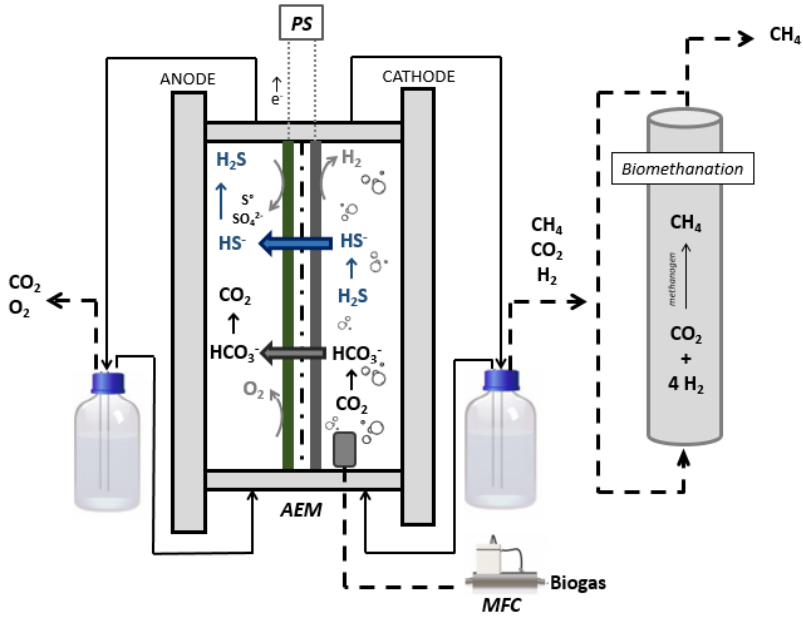


Figure 3.1 - Schematic reactor setup for electrochemical extraction of CO_2 (as HCO_3^- and/or CO_3^{2-}) and H_2S (as HS^- and/or S^{2-}) from a synthetic biogas stream (CH_4:CO_2:H_2S – 60:39:1 %). In the proposed pipeline, cathode off-gases could subsequently be upgraded further to biomethane in an external biomethanation reactor. Full black lines show liquid streams, dashed lines gas streams (– –), and dotted lines electrical connections (·····). PS: power supply. AEM: anion exchange membrane. MFC: mass flow controller.

2. Experimental Procedures

2.1. Electrochemical reactor setup

The experimental setup comprised a two-chambered EC, constructed from two identical Perspex frames and plates, and separated by an AEM (Type II, Fujifilm Manufacturing, The Netherlands). The membrane was soaked in 5% NaCl for at least 24 hours prior to operation. Both electrode chambers had a working volume of 0.2 L (internal dimensions: 5 × 20 × 2 cm^3). The anode was a dimensionally stable iridium mixed metal oxide (Ir MMO) coated titanium-electrode mesh (TiO_2/IrO_2, 0.35/0.65, Magneto Special Anodes, The Netherlands). A stainless steel wire mesh of 5 × 20 cm^2 exposed working area and 564 µm mesh width (Solana, Belgium) served as cathode. The membrane and electrodes had a projected surface area of 100 cm^2 (corresponding to 50 m^2 m^{-3} chamber). To limit the ohmic resistance, both electrodes were placed close to the membrane (± 1 cm between anode and cathode). Spacer material (turbulence promoter mesh, ElectroCell, Denmark) was used to prevent contact between the surface of the electrodes and the membrane.

2.2. Electrochemical reactor operation

Anolyte and catholyte were circulated from a 0.5 L Schott bottle to their respective compartment by a peristaltic pump at a recirculation rate of 4.8 L hr^{-1}. The recirculation bottles allowed compensation for water transport through the membrane, and ensured good gas-liquid separation. In both electrode chambers, the total working liquid volume was 0.5 L, including the liquid in the recirculation bottle, the electrochemical cell and tubing. The anolyte consisted initially of a 100 mM sodium sulphate solution, corrected to pH 2 with sulfuric acid. The absorption medium (referred to as catholyte) consisted of a 100 mM HEPES buffer solution with 100 mM sodium sulphate to ensure high electrolyte conductivity (20.6 ± 0.5 mS cm^{-1}). The solution was adjusted to pH 7 with NaOH. During experimental operation, the pH of both electrolytes was not controlled. All experiments were performed in a temperature controlled room of 20 ± 1°C. A range of biogas flow rates to the cathode and applied current densities were tested to assess EC performance for CO_2 and H_2S removal. Synthetic biogas with 60 % CH_4 and 40 % CO_2 or 60% CH_4, 39 % CO_2 and 1 % H_2S (Linde Gas Benelux) was used to simulate real biogas. A mass flow controller (Bronkhorst, The Netherlands) was used to inject the desired biogas flow rate at the bottom of the cathode compartment. A ceramic sparger was used to produce fine biogas bubbles. Calibrated semi-

open gas columns or in-house constructed gas meters with oil displacement were used to measure the flow rate of both cathode and anode off-gas.

2.3. Electrochemical experiments

The EC was controlled galvanostatically by a power source (DC Lab Power Supply, Velleman, Belgium). The cell was operated as a two-electrode setup in water electrolysis mode. The presumed anode and cathode reactions were the oxidation ($2\ H_2O \rightarrow 4\ H^+ + O_2 + 4\ e^-$) and reduction of water ($2\ H_2O + 2\ e^- \rightarrow H_2 + 2\ OH^-$), respectively. The applied current was reported as current density, and was defined as the set current divided by the exposed surface area of the membrane (100 cm^2). Flux characterization was performed at eight different fixed current densities (20 - 400 A m^{-2}), under three different volumetric biogas flow rates (25 ± 1, 50 ± 2 and 100 ± 2 L biogas d^{-1}). For each biogas flow rate, open circuit experiments were executed for 2 days to investigate diffusion-driven CO_2 transport through the membrane. Closed circuit extraction experiments were performed at least 3 hours in steady state conditions, and were executed in triplicate reactor runs. Gas samples were taken from the gas leaving the recirculation bottles. Liquid samples were taken from sampling ports in the recirculation loop of the reactor.

2.4. Biomethanation batch experiments

Batch biomethanation tests were set up to determine the potential of a pre-enriched anaerobic microbial inoculum to produce CH_4 from the cathode-off gas mixtures ($H_2/CO_2/CH_4$) originating from the EBU process. The original inoculum was obtained from anaerobic sludge harvested from a lab-scale mesophilic anaerobic digester that was fed for several months with waste activated sludge from a sewage treatment plant. The sludge was diluted 5 times in anaerobic modified *Methanobacterium* medium (1 L Scott bottle with 0.2 L medium), and the headspace was repeatedly (at least 2 times per week) flushed with H_2:CO_2 (70:30 %, v/v) at 0.7 - 0.9 bar overpressure for 2 months.

The enrichment experiments were conducted in serum bottles (120 mL) filled with 30 mL anaerobic growth medium. The modified *Methanobacterium* medium did not contain any organic compounds to enrich for autotrophic methane formation. The composition of the growth medium is provided in Supporting Information (Table S 2.1). Each serum flask was inoculated with 10 mL of the pre-enriched methanogenic culture. The inoculum biomass

accounted for 2 g VS L^{-1}. All experiments were performed in triplicate. At day 0, the headspace was flushed for 15 min with the $CH_4/CO_2/H_2$ mixture coming from the EBU reactor. All incubations were done at 37°C on a rotary shaker maintained at 100 rpm. The upgrading tests were performed for two different gas compositions: 4:1 and 2:1 ratio of H_2:CO_2. The headspace gas composition and pressure were monitored on a daily basis for 4 days.

2.5. Chemical analyses

Conductivity and pH were measured using a Consort EC and pH electrode respectively (Consort, Turnhout, Belgium). Gas samples were analyzed for the presence of O_2, N_2, CH_4, H_2 and CO_2 by a Compact GC (Analyser Solutions, Breda, The Netherlands), as described in Supplemental Information (Appendix 2: Analytical techniques). Measurements of the bicarbonate concentration in anode and cathode were made by adding 1 mL of electrolyte to a vacuum tube containing 1 mL 1 M H_2SO_4. After intensive mixing (200 rpm, 1 min), the headspace CO_2 was determined using the Compact GC. Using a standard curve, bicarbonate concentration could be determined from the vacuum tube CO_2 content. The total gas pressure in the serum bottles was measured using a UMS-Tensiometer (Infield 7). Liquid samples were immediately preserved in a Sulfide Antioxidant Buffer solution prior to analysis following Keller-Lehmann et al (Keller-Lehmann et al., 2006). Sulfide, sulfite (SO_2^{3-}) and thiosulfate ($S_2O_2^{3-}$) concentrations were measured by ion chromatography (IC), using an IC930 compact Metrohm IC system (Metrohm, Switzerland). The eluent consists of 3.5 mM Na_2CO_3 and 3 mM $NaHCO_3$ at a flow rate of 0.8 mL min^{-1}. A 0.1 M NaOH solution is used to produce a pH gradient needed for $S_2O_2^{3-}$ detection in the IC system. To measure the polysulfide and elemental sulfur (S^o) concentrations, all sulfur species were oxidized to SO_4^{2-} with excess H_2O_2 (Dutta et al., 2010). The difference in sulfur equivalent between the sulfate after H_2O_2 oxidation and other species measured before H_2O_2 oxidation (*i.e.* sulfide, sulfate, thiosulfite and sulfite) was regarded as the sum of polysulfides and elemental sulfur.

2.6. Calculations and data representation

The CO_2 and H_2S flux, CO_2 and H_2S removal efficiency, current efficiency for HCO_3^- and HS^- extraction, H_2:CO_2 ratio after EC treatment and power input were calculated to assess EBU performance. The methane production rate and gas composition were used to evaluate the biomethanation of the residual CO_2 in the EBU off-gas. Gas volumes are reported at standard temperature and pressure conditions (273 K; 101325 Pa). The CO_2 flux is defined as the

volume of CO_2 that crosses the AEM as CO_2, HCO_3^- or CO_3^{2-} per m² of membrane surface per day (L CO_2 m^{-2} d^{-1}). The membrane flux was calculated based on a CO_2 mass balance over the membrane and cathode chamber. The CO_2 removal efficiency (L CO_2 removed L^{-1} CO_2 supplied) is measured comparing the cathode off-gas CO_2 concentration with the CO_2 content of the synthetic biogas (40 %). Current efficiency refers to the molar fraction of bicarbonate transferred across the membrane per mole of electrons supplied at the cathode (mole CO_2 extracted / mole e^- supplied). Off-gas composition (in particular the H_2:CO_2 ratio, v/v) is used to evaluate the ability of the electrolyzer to generate a stoichiometric 4:1 gas mixture. Power input is reported both as kWh per Nm³ CO_2 extracted and Nm³ raw biogas treated, and reflects the electrical input to drive the extraction. Formulas used to calculate flux, efficiencies and power input are given in the Supplemental Information (Appendix 2: Performance parameters: calculations).

3. Results and discussion

3.1. High CO_2 fluxes enable high-rate biogas upgrading

Synthetic biogas was injected into the cathode compartment of the EC to determine the operational parameters that drive the electricity-driven extraction of CO_2 across the membrane. The CO_2 flux characterization was performed at various CO_2 loading rates (1000, 2000 and 4000 L CO_2 m^{-2} membrane d^{-1}), at current densities from 0 to 400 A m^{-2} projected membrane surface. By direct injection of biogas in the cathode chamber of the EC, CO_2 is absorbed in the (slightly) alkaline catholyte, extracted across the AEM towards the anode and stripped in the low pH anolyte. Hydroxyl ions generated at the cathode prevent a pH decrease in this cathode compartment as a result of the dissolution of CO_2, while the anodic proton production is used to keep the anode acidic, resulting in a continuous driving force for membrane extraction. The added, high-molecular-weight buffer stabilizes the catholyte pH.

In open circuit conditions (no current), the diffusional (concentration-driven) flux of CO_2 across the membrane was 13 ± 2 L CO_2 m^{-2} d^{-1}, accounting for only 5 ± 1 % of the flux at the lowest current density tested, 20 A m^{-2} (Figure 3.2). No effect of the biogas flow rate on this diffusional CO_2 flux was observed. In the absence of current, only 0.3 – 1.5 % of the incoming CO_2 was removed from the biogas (Figure 3.3), indicating that passive transport of CO_2 gas, dissolved CO_2, carbonic acid and the deprotonated species (HCO_3^- and CO_3^{2-}) through the membrane was limited, and that electromigration was the dominant process for CO_2 removal.

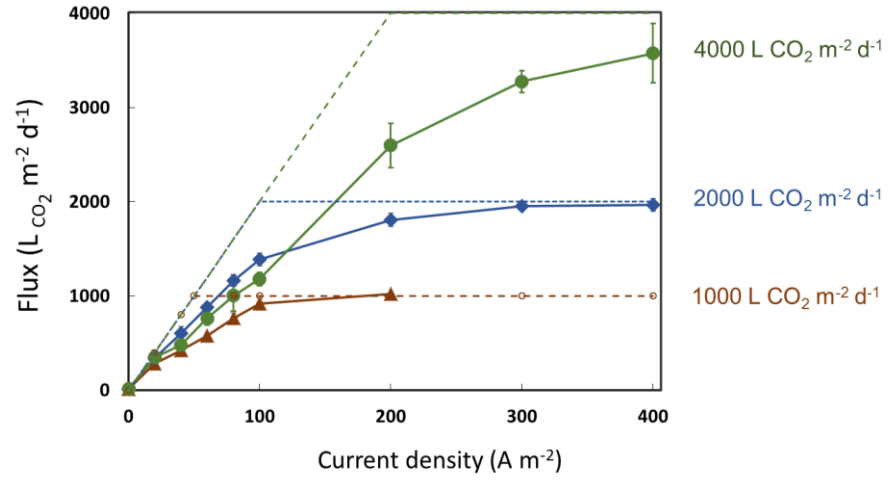


Figure 3.2 - Effect of the biogas loading rate and applied current density on the CO_2 flux across the anion exchange membrane (in L CO_2 m^{-2} d^{-1}). The dotted lines indicate 100% current efficiency for HCO_3^- transfer to the anode in function of the applied current density. Symbols: triangle, CO_2 loading rate of 1000 L CO_2 m^{-2} d^{-1}; diamond, 2000 L CO_2 m^{-2} d^{-1} and circle, 4000 L CO_2 m^{-2} d^{-1} . The flux characterisation indicates that the extraction rate of CO_2 strongly correlated with both applied current and biogas flow rate, and that the non-linearity in the flux profile is caused by CO_2 limitations and CO_2 scrubbing/extraction losses. Averages of three samples over steady state are reported with standard deviation.

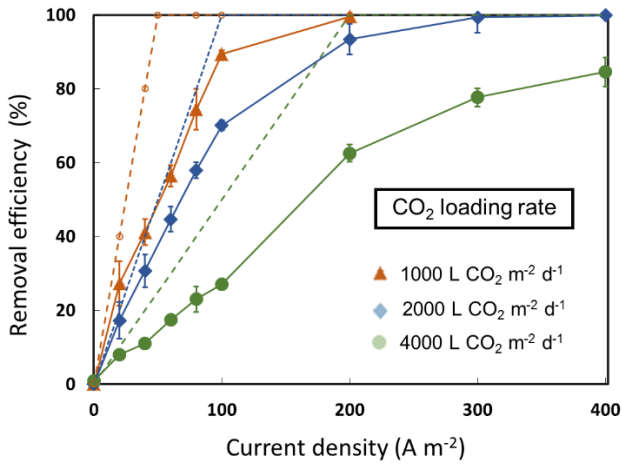


Figure 3.3 - The removal efficiency for CO_2 in function of the applied current density at different CO_2 loading rates (1000, 2000 and 4000 L CO_2 m^{-2} d^{-1}). The dotted lines represent the removal at 100% current efficiency for HCO_3^- transfer to the anode for the different CO_2 loading rates (coded for each biogas flow rate). The CO_2 removal indicates that a complete removal of CO_2 from the biogas stream is possible, but at much higher current densities than at maximum extraction rate. Averages of three samples over steady state are reported with standard deviation.

At a loading rate of 2000 L CO_2 m^{-2} d^{-1}, the flux of CO_2 was 1387 ± 67 L m^{-2} d^{-1} at 100 A m^{-2} (Figure 3.2), corresponding to a current efficiency (CE) of 64 ± 3 % (Figure 3.4). It is important to stress that current efficiency is not the key parameter: a low current efficiency can be beneficial to achieve optimal H_2/CO_2 ratios. At 400 A m^{-2} and a loading rate of 4000 L CO_2 m^{-2} d^{-1}, a maximum CO_2 flux of 3577 ± 314 L m^{-2} d^{-1} was achieved, corresponding to a removal of 85 ± 4 % of the incoming CO_2 (Figure 3.3). The CO_2 flux was maximized at high applied current density and high CO_2 loading rate. For any set CO_2 loading rate, the CO_2 flux increased with increasing current density, with the applied CO_2 loading rate as the maximum flux rate. Since the maximum theoretical CO_2 flux across the membrane is limited by the electron flow rate from anode to cathode, the electromigrational flux of (bi)carbonate ions could only be increased by increasing the applied current, assuming that both the loading and dissolution rate of CO_2 are not limiting the extraction. For current densities of 20 - 100 A m^{-2}, the CO_2 flux increased linearly with the current density, whereas above 100 A m^{-2} fluxes levelled off to the respective maximum flux of CO_2 (Figure 3.3). The clear decrease in flux at lower CO_2 loading rates is mainly due to carbonate depletion in the catholyte at higher currents or lower biogas sparging rates. A high dissolved HCO_3^- concentration, thus, needs to be maintained in the catholyte. As full CO_2 removal from the biogas is typically not the aim, this situation should generally be the case.

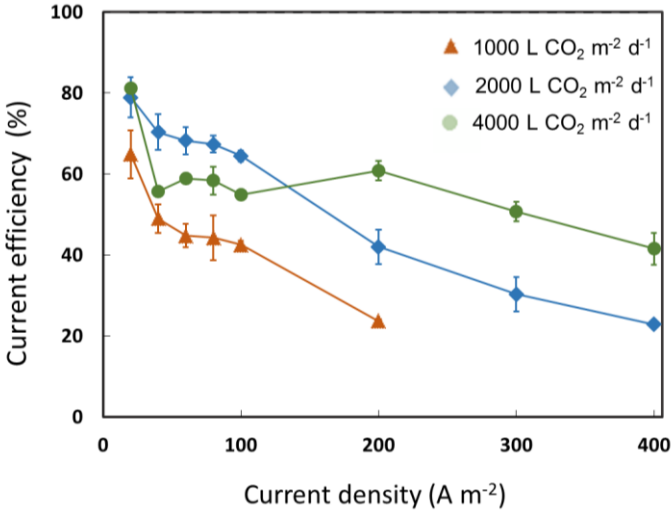


Figure 3.4 - Influence of the applied current density on the current efficiency (CE) for HCO_3^- transfer to the anode chamber at different fixed CO_2 loading rates. The dotted line represent 100 % charge balancing by the HCO_3^- ion. At current densities under 100 A m^{-2}, CEs up to 80 % were observed. If no CO_2 limitation occurs, high CE can be achieved, even at high current densities. Averages of three samples over steady state are reported with standard deviation.

The removal efficiency for CO_2 increased with increasing current density, whereas higher biogas loading rates implied lower removal (Figure 3.3). The complete removal of CO_2 from the biogas (RE = 100 ± 2 %) was achieved at 200 A m^{-2}, 1000 L CO_2 m^{-2} d^{-1} and 300 A m^{-2}, 2000 L CO_2 m^{-2} d^{-1}. For the highest loading rate tested, 4000 L CO_2 m^{-2} d^{-1}, complete removal of CO_2 could not be achieved for current densities of 20 - 400 A m^{-2}.

The CE for CO_2 extraction decreased with increasing applied current densities and decreasing biogas loading rates (Figure 3.4), with the highest CE, 81 ± 5 %, observed at 20 A m^{-2}, 4000 L CO_2 m^{-2} d^{-1}, and the lowest CE, 23 ± 1 %, observed at 400 A m^{-2}, 2000 L CO_2 m^{-2} d^{-1}. The CE increase at higher CO_2 feeding rates corresponds to higher molecular availability of the target anion (HCO_3^-) in the bulk, thereby avoiding CO_2 transport limitations. The higher molar concentration of HCO_3^- at higher CO_2 feeding rates is due to the combined effect of a lower cathode pH (neutral) and a higher gas-liquid CO_2 mass transfer. At high applied currents, the current use for CO_2 extraction was limited by this CO_2 transfer, as confirmed experimentally by low concentrations of carbonate species in the catholyte (data not shown). It was demonstrated that by increasing the biogas flow and, thus, buffer load, the pH increase in the cathode due to the hydroxyl production could be lowered (Figure S 2.1), which eventually resulted in a higher relative contribution of HCO_3^- to the charge balance. The cathode pH increased from 7.0 ± 0.2 at open circuit conditions to 12.4 ± 0.1 and 10.5 ± 0.5 at 400 A m^{-2} and 2000 and 4000 L CO_2 m^{-2} d^{-1}, respectively, showing that the buffer capacity of HEPES is not able to stabilize pH. The pH increase results in a 10^3 - 10^5 fold increase in the concentration of hydroxyl ions that then compete with (bi)carbonate transport, thus reducing the CE of HCO_3^- (OH^- has a higher ion mobility than carbonate species: 20.64 x 10^8 versus 7.18 x 10^8 m^2 s^{-1} V^{-1}). Furthermore, the proportion of bicarbonate ions in the total dissolved inorganic carbon decreases at higher pH. The distribution of carbonate species at a given pH can be derived from the Henderson-Hasselbach equation. At a cathode pH of 12.4, CO_3^{2-} accounts for 99 % of the total dissolved carbonate, whereas at pH 10.5 the degree of dissociation was only 60 % (40 % HCO_3^-). As CO_3^{2-} is a bivalent anion, the theoretical CO_2 flux carried by CO_3^{2-} is only half of the maximum flux carried by HCO_3^- (assuming equal ion transport numbers for both species). The CE decrease at higher current density is an apparent effect of this, as it is mostly linked to the higher relative contribution of CO_3^{2-} in the charge balance. It should be recognized that at high pH the current carried by carbonate species was drastically underestimated when represented by the CE for HCO_3^- extraction. It is also likely that the participation of protons in balancing the electron flux (backflux over AEM) increased

at higher current density, due to a decreasing pH of the anolyte (Andersen et al., 2014). Our observation that the membrane flux rate is closely linked with the relative molar concentration of the different species in the broth has been described in previous work involving membrane electrolysis (Andersen et al., 2014; Luther et al., 2015).

Considering only the membrane transport of carbonate species, a neutral cathode pH seems to be more favourable than a high pH, but since the extraction is highly dependent on the CO_2 mass transfer from biogas to catholyte, the kinetics of CO_2 dissolution, hydration and dissociation are crucial to take into account as well. The CO_2 hydration reaction, yielding carbonic acid, is kinetically relatively slow (slower than the ionization of carbonic acid), and proceeds much faster at higher pH, as CO_2 reacts directly with OH^- to form bicarbonate (hydroxylation reaction: $CO_{2,aq} + OH^- = HCO_3^-$) (Soli & Byrne, 2002). The absorption rate of CO_2 is strongly affected by the pH of the solution, since the Henry constant is a function of pH (affecting the solubility of CO_2 in solution) (Chen, 2012). Additionally, the mass transfer coefficient plays a major role in the absorption rate of CO_2 (Eckert et al., 2016), highlighting the need for the design of effective scrubbers (bubble column, sieve tray column, packed bed column) (Budzianowski et al., 2017). The mass transfer coefficient was not optimized in this study, but it is expected that significant improvements can be obtained by increasing the retention time and decreasing the bubble size of the biogas (higher gas–liquid interfacial area) (Vázquez et al., 2000). Another way to enhance the CO_2 water absorption rate is to increase the CO_2 partial pressure and, hence, solubility in the water, by increasing the operating pressure in the electrochemical cell. To obtain a full understanding of the reactor performance at set conditions, and the influence of varying conditions (biogas composition, flow rate, current, pressure, cathode pH, anode pH, *etc.*) on the (non-linear) reactor behavior, a mass transfer model including CO_2 chemistry and electrochemical reactions could give more insight in the process.

3.2. H_2:CO_2 ratio can be optimized by selection of an appropriate current density and CO_2 loading rate.

During fixed current operation, CO_2 is removed while H_2 is added to the biogas. The process described here is, thus, not relying on complete CO_2 removal, as it will downstream be converted to the product of interest, *i.e.* biomethane. The composition and flow rate of the biogas after electrolysis is highly dependent on the operational conditions. For experiments with a CE higher than 50 %, a net decrease in the biogas flow rate (out versus in) was observed, since more than one mole of CO_2 is removed per mole of H_2 gas formed (requiring 2 moles electrons). At higher currents (often CE < 50 %), a net increase in the biogas flow rate was achieved, due to the high H_2 production rate. The relative composition of off-gas mixture for the different biogas flow rates is shown in Figure S 2.2. At 400 A m^{-2} and 4000 L CO_2^{-2} d^{-1}, the CO_2 content was reduced to 5.2 %, while 33.8 % of the gas mixture was H_2, leading to a H_2:CO_2 ratio of 7:1. Biomethanation of this H_2-CO_2 mixture will allow to convert all CO_2 into CH_4, but as there is a stoichiometric excess of reducing equivalents, the final biomethane will still contain residual H_2. Since H_2 concentrations in most European natural gas grids can vary between 0.1 and 6 %, 100 % conversion is not necessary (Persson et al., 2006), but evidently excess H_2 comes at a cost. Alternatively, additional CO_2-rich biogas can be added to the biomethanation reactor. Theoretically, pure CH_4 can only be produced if the EC step generates a gas mixture with a H_2:CO_2 ratio close to 4:1, the stoichiometric ratio needed to convert all CO_2 into CH_4 (Agneessens et al., 2017). A ratio of 3.5:1 was achieved at 1000 L CO_2 m^{-2} d^{-1}, 80 A m^{-2} and 4000 L CO_2 m^{-2} d^{-1} and 300 A m^{-2} (Figure 3.5). At lower current densities the H_2 production and CO_2 removal rate were not high enough to obtain the ideal H_2:CO_2 ratio, as there was an excess of CO_2 relative to H_2. At higher current densities more H_2 was available than needed to convert all CO_2 to CH_4. In the EC system, biogas flow rate and current density can be adjusted to control the H_2:CO_2 ratio to optimize the overall upgrading capacity.

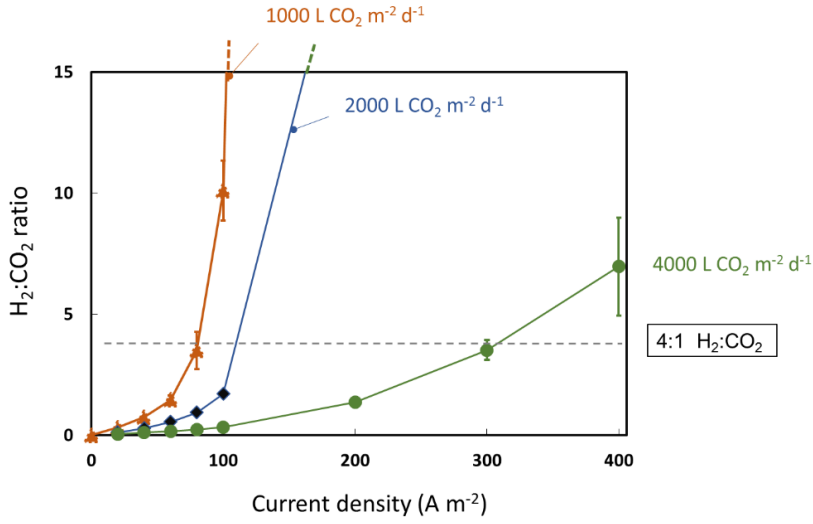


Figure 3.5 - The H_2:CO_2 ratio of the biogas leaving the electrochemical cell at different fixed current densities and CO_2 loading rates: an ideal 4:1 H_2:CO_2 ratio can be achieved by the appropriate selection of current density. The dotted line represent the stoichiometric ratio for biomethanation. Averages of three samples over steady state are reported with standard deviation.

3.3. CO_2 stripping at the anode results in stable operation.

The operational stability and reproducibility of the current-driven extraction of CO_2 was evaluated over an experimental period of 48 hr (100 A m^{-2}, 2000 L CO_2 m^{-2} d^{-1}). The experiment exhibited stable performance, since CO_2 was absorbed in the catholyte, extracted through the AEM and stripped from the anolyte proportionally with incoming CO_2. The bulk pH in the cathode compartment was stable throughout the experimental run (10.0 ± 0.1), enabling constant hydroxyl ion and carbonate species distribution and concentration (Figure S 2.3). The extraction of a gaseous compound through the liquid phase has the advantage that its extraction does not suffer from end-product accumulation in the acid anode chamber, as described for extraction of carboxylates (Andersen et al., 2014). Anodic (bi)carbonate measurements showed that no (bi)carbonate accumulation was observed, thereby enabling extraction at a constant efficiency. The protonation of (bi)carbonate in the anolyte (pH 1.5 ± 0.5) and the rapid dehydration of H_2CO_3 resulted in efficient stripping of CO_2 from the anolyte. The off gas had a 55.0 ± 5.0 % CO_2 content, the remainder was 44.8 ± 5.0 % O_2 and 0.2 ± 0.1 % H_2. Since only a small portion of the aqueous CO_2 exists as H_2CO_3 (< 0.3 %), little CO_2 is expected to pass back across the AEM. Both absorption and stripping processes are, thus,

driven by pH, which is steered by the reduction and oxidation of water in the absorption and stripping solutions. The extraction drives the hydration and ionization of CO_2 by continuous removal of the dissociation products (Le Chatelier's principle).

This experimental run also served to check if mass was conserved in the system (Figure S 2.3). Therefore, the gas flow rate and composition of both anode and cathode off-gases were measured. No measurable CH_4 transfer across the membrane was detected, indicating no significant CH_4 slip towards the anode. A mass balance over the cathode chamber also confirmed that no CH_4 was lost through tubing, sampling ports and connectors. Taking all experiments with synthetic biogas into account, the average CO_2 mass balance under closed current conditions was 88 ± 3 %. The small gap in the balance was likely due to CO_2 loss in the anode chamber and measurement errors (flow rates, concentrations).

The transport of water due to electro-osmosis, the motion of water through a membrane as a consequence of current-driven ion transport, was observed and showed (linear) current dependency. At 400 A m^{-2}, a water flux of 2.5 L m^{-2} d^{-1} was observed, indicating a considerable water loss from the cathode solution. For the purpose of the process, the excess anode effluent could just be sent to the cathode, balancing everything out without impacting the system in a major way (the cathode pH would benefit somewhat from a lowering in many cases).

The required cell potentials were stable over time. The cell potential was dependent on the applied current density, and varied from 2.51 to 6.47 V (Figure S 2.4). The power input needed to drive the CO_2 flux was determined by the voltage input together with the extraction efficiency, and ranged from 3.73 to 17.38 kWh per Nm^3 CO_2 removed from the biogas. The power input increased with increasing applied current (increasing voltage) and decreasing CO_2 loading (decreasing extraction efficiency). It has to be mentioned that both the flux and voltage input could be optimized to reduce the power input, which relates more to the design and operation of the electrolysis cell, including selection of electrolytes, electrodes, membrane, mixing and biogas injection strategies. The cell potential can be reduced substantially when switching to highly conductive electrolytes (Schröder & Harnisch, 2010). The use of biocatalyzed electrodes (so-called bio-anodes and bio-cathodes) has often been described as a way to reduce overpotentials of electrode reactions. The use of a bio-anode to provide the electrons for electrochemical bicarbonate extraction indeed showed a

low operating cell voltage (1.2 V) (Jin et al., 2017), but in terms of biogas upgrading rates and scalability the pure electrochemical extraction as described here currently seems to be closer to practical implementation.

Optimizing the system requires a compromise between power input (operational cost) and CO_2 removal (upgrading). The ideal working point should be defined *via* an in-depth energy assessment and further process development. Our study already revealed that achieving low residual CO_2 concentrations in the off-gas requires a proportionally higher applied current, which in turn leads to an increase in the power input for upgrading. However, in the combined EC - biomethanation approach, no 100% removal of CO_2 is needed, thereby avoiding the high energy losses associated with removal of residual CO_2. It should be recognized that in a fully realized system higher current densities than achievable in the presently used setup are preferable. However, the cell potential (and thus the power input) is expected to increase further at these current densities, unless the aforementioned interventions can alleviate this.

3.4. Simultaneous H_2S removal from biogas

At a fixed current density of 100 A m^{-2}, 100 ± 1 % of the incoming H_2S was scrubbed from the biogas (at a biogas feeding of 25 L d^{-1}, corresponding to a H_2S loading rate of 25 L H_2S m^{-2} d^{-1}). When the H_2S loading rate was increased to 100 L H_2S m^{-2} d^{-1}, the H_2S removal efficiency was 98 ± 1 % at an applied current density of 300 A m^{-2}, corresponding to a residual H_2S content of 160 – 180 ppmv after EBU. To meet the gas quality standards, the H_2S concentration must be limited to values < 5 mg H_2S Nm^{-3} (or 3.6 ppmv) (Díaz et al., 2011). It should however be recognized that typical concentrations of H_2S in biogas are of approximately 1000 ppmv rather than the 10000 ppmv tested (Rasi et al., 2011), indicating that desulphurization *via* EBU (without post-treatment) should be feasible. Moreover, the passage through the biomethanation reactor should further decrease H_2S through scrubbing and consumption of H_2S by methanogens, avoiding the addition of an external sulfur source (Zhang & Maekawa, 1996). Although all known methanogens can use sulfide as the sole sulfur source for growth (Liu et al., 2012), it needs to be confirmed that hydrogenotrophic methanogens are able to metabolize H_2S to a level that meets the injection specifications for biomethane.

The HS^- concentration in the absorption solution remained stable and low during closed circuit operation (0.4 – 3.2 mg HS^- L^{-1}), indicating that the extraction is continuously removing sulfide from the scrubbing liquid. Current use for extraction of sulfide was in between 1.5 – 7 %. At 100 A m^{-2} and a H_2S loading of 25 L m^{-2} d^{-1}, 100 ± 1 % of the sulfide that crossed the AEM was converted, indicating rapid oxidation of sulfide species in the acid anolyte. When the biogas flow rate was increased (to 100 L H_2S m^{-2} d^{-1}), the sulfide conversion efficiency in the anode dropped to 70 %, while 30 % of the sulfide coming over the membrane was stripped off in the acid anolyte, leaving the anode chamber as H_2S (anolyte pH < pK_a (H_2S/HS^-) = 6.9) (S- mass balance in Supplemental Information, Figure S 2.5). Quantification of the sulfide oxidation products *via* IC showed that sulfate was the main oxidized sulfur specie and that no elemental sulfur or polysulfide was formed. The indirect oxygen mediated oxidation of sulfide appears to be the most plausible mechanism because the low pH of the anolyte results in a fast stripping of the extracted sulfide, obstructing the direct anodic oxidation. Overall, the H_2S removal rates showed that even in acid conditions sulfide can be converted. Residual H_2S in the CO_2/O_2 rich anode off-gas (2700 ppmv at maximum) could be catalytically reacted with the O_2 downstream to obtain a likely pure elemental sulfur, but due to the complexity of this catalytic desulphurization system a small adsorption vessel will probably be the preferred strategy to avoid release of toxic and odorous H_2S to the atmosphere. To avoid additional off-gas treatment it would be highly beneficial to achieve a full oxidation of stripped H_2S in the anode chamber, and, thus, one needs to look at a well-engineered electrochemical cell that is able to enable a longer contact time between electrochemically produced O_2 and the H_2S.

3.5. Biomethanation of EBU off-gases

Abiotic (uninoculated but with $CH_4/CO_2/H_2$ headspace) and biotic (inoculated but no $CH_4/CO_2/H_2$ headspace) control experiments were conducted for one week, but did not result in production of methane or the removal of CO_2 from the headspace. The off-gas from two different EBU runs, one delivering a gas mixture with a non-ideal ratio of 2:1 H_2:CO_2 (at 100 A m^{-2} and 2000 L m^{-2} d^{-1}) and one with a stoichiometric ratio of 4:1 (at 60 A m^{-2} and 1000 L m^{-2} d^{-1}) were added as headspace in inoculated serum flasks. In all flasks, the concentration of H_2 and CO_2 decreased, and additional CH_4 was produced (Figure 3.6). Starting with a 4:1 ratio resulted in an ultimate CH_4 content of 98.9 ± 0.9 %, and all H_2 was consumed after 3 days. The residual amount of CO_2 accounted for 1.1 ± 0.9 %, showing good removal properties. This gas quality complies with the tightest standards for grid injection without the

need for additional CO_2 removal. If started from a 2:1 ratio, no complete CO_2 conversion took place (still 7.1 ± 1.6 % CO_2 after 3 days), as H_2 was the limiting reagent in the biomethanation process. These observations match with a reported optimum H_2/CO_2 ratio between 3.67 and 4.15 (Agneessens et al., 2017; Rachbauer et al., 2016).

The successful bioprocess development of biological methanation will largely depend on the (volumetric) H_2 conversion rate/CH_4 productivity of a pure or enriched culture of hydrogenotrophs, since this will determine the biogas upgrading capacity of the bioreactor. As H_2 transfer from the gas to the liquid is known to be the rate limiting step in the biomethanation process (Jud et al., 1997), H_2 gas-liquid mass transfer is the most important criterion when selecting or designing the bioreactor. Different reactor types, including stirred tank, bubble column, packed bed, trickle bed and hollow fiber membrane bioreactors, were evaluated in lab scale set ups both in the mesophilic and thermophilic range (Rittmann et al., 2015). At thermophilic conditions, volumetric methane productivities reached values in between 78 and 689 $Nm^3\ CH_4\ ^{-3}$ reactor d^{-1} when a surplus of the gaseous substrates, H_2 and CO_2, is provided (according to Monod kinetics). While aiming at high reactor conversion/production rates, the envisaged gas composition/quality is still the main criterion when operating a biogas upgrading technology, because grid injection standards (often >97%) needs be achieved. Low final methane concentrations in the produced gas are intrinsically linked to high gas retention times and lower volumetric methane production, so biomethanation rates in biological biogas upgrading reactors will always be lower than under excess gas atmosphere. A biogas upgrading capacity up to 3.7 Nm^3 biogas per m^3 reactor volume per day can be achieved in a trickle-bed bioreactor if a methane content higher than 96 % is targeted (Rachbauer et al., 2016).

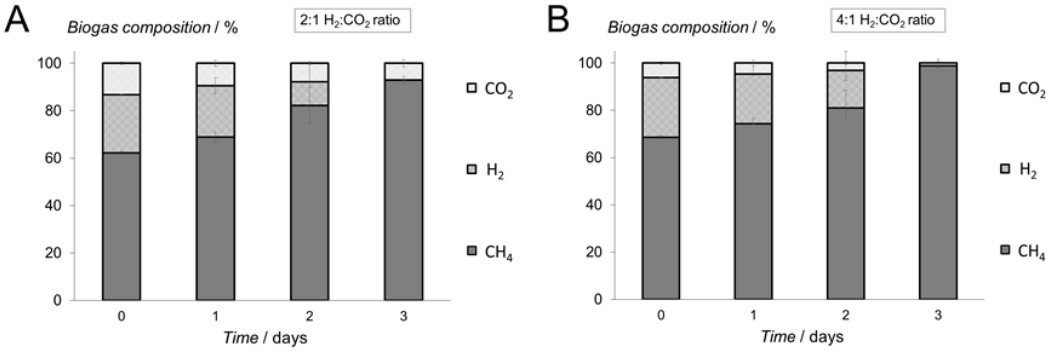


Figure 3.6 - Gas composition of the headspace over time for batch biomethanation tests with off-gases from EBU at 100 A m^{-2} and a biogas flow rate of 50 L_{biogas} d^{-1} (A) and at 60 A m^{-2} and 25 L_{biogas} d^{-1} (B) (n=3).

3.6. Energy requirements

In this study, H_2 was produced at an energy input of 6.5 to 15.5 kWh Nm^{-3} H_2, which is higher than the energy requirement of the current best water electrolysis processes (~4.5 kWh Nm^{-3} H_2) (Badwal et al., 2014). Considering that the produced H_2 has an energy content of 3.54 kWh Nm^{-3} (higher heating value) (Midilli et al., 2005), our EBU process has an energy efficiency towards H_2 of 23 – 54 %. Assuming that all H_2 is used for conversion of residual CO_2 to CH_4 in the biomethanation step, 2.7 kWh Nm^{-3} H_2 can be recovered in the form of CH_4 (assuming 10.8 kWh Nm^{-3} CH_4), leading to a net electrical input for CO_2 removal and conversion in between 2.0 and 11.4 kWh Nm^{-3} CO_2. The net energy requirement for biogas upgrading (from 60 % to 97 % CH_4) *via* the proposed approach is thus 0.8 – 4.6 kWh Nm^{-3} raw biogas. With a specific power demand of 0.2 – 0.3 kWh Nm^{-3} biogas (Bauer et al., 2013), water scrubbing and pressure swing adsorption require a lower electricity demand compared to the EBU process in combination with H_2 - mediated conversion of residual CO_2. However, compared with the net power demand of 3.6 kWh Nm^{-3} raw biogas for upgrading *via* methanation of all CO_2 into CH_4, our electrochemical extraction can reduce the energy input related to complete biological upgrading up to a factor 4.5 (Geppert et al., 2016). Moreover, techniques such as scrubbing only partially remove CO_2, still requiring additional technology for further biogas polishing. The approach also removed H_2S. It could be considered to use water scrubbing as first line bulk CO_2 removal process, followed by EBU based fine-tuning of the concentration and further production of CH_4 through biomethanation. Next to CO_2 removal, H_2 production and biogas compression, electrolysis generates an O_2-CO_2 gas mixture

at the anode. The O_2 can be used, for example, as a highly concentrated O_2 source (20 – 60 %) for on-site biological waste water treatment of the liquid fraction of the digestate, while the CO_2 can be sold to local greenhouses.

The economics of this approach is briefly demonstrated for a digester generating 1000 Nm^3 raw biogas hr^{-1} (60 vol. % CH_4) and a natural gas grid accepting biomethane with a CH_4 content of 97 %. Biogas upgrading, thus, needs to remove 370 Nm^3 CO_2 hr^{-1}. Assuming a CO_2 removal rate of 20 Nm^3 m^{-2} d^{-1} at 1000 A m^{-2} current density, 444 m^2 membrane surface area and a reactor of 11 m^3 (assuming 40 m^2 membrane electrode assembly per cubic meter reactor (Desloover et al., 2012a)) will be needed. If the EC can be operated at 3 V, upgrading *via* EBU can be realized at an electricity cost of 0.13 € Nm^{-3} raw biogas (assuming 0.10 € kWh_e^{-1}), not including the OPEX/CAPEX and methane revenues from methanation. With an upgrading cost for water scrubbing of 0.08 – 0.14 € Nm^{-3} raw biogas (Petersson & Wellinger, 2009) and an electricity cost for methanation with electrolytic H_2 of at least 0.72 € Nm^{-3} raw biogas, the EBU process is in between.

Further process optimization and testing with real biogas is required before a detailed process cost calculation can be made, including investment and operational cost. Nonetheless, EBU appears to be a promising technology for biogas upgrading *via* sequential electrochemical and biological CO_2 removal. The advantage of the two-step upgrading pipeline considered here over biological upgrading of the full CO_2 load is largely due to the lower power consumption, since electrons are used for both (bi)carbonate flux and CO_2 removal *via* H_2-mediated biomethanation. The injection of biomethane produced with electrolytic H_2 as sole energy source can be put forward as a scalable and decentralized upgrading strategy while storing excess or off-peak renewable power in methane molecules using CO_2 as carbon source.

4. Conclusions

In this work, we have proposed and demonstrated a two-step processing pipeline for upgrading biogas into pipeline-quality biomethane: (i) Membrane Electrolysis, the removal of CO_2 and H_2S *via* (alkaline) water scrubbing and the electrolytic extraction of (bi)carbonate and (bi)sulfide ions across an anion exchange membrane, and (ii) Biomethanation: a microbial conversion of CO_2 to CH_4 using electrolytic hydrogen gas as electron source and hydrogenotrophic methanogens as biocatalyst. The electrochemical extraction of (bi)carbonate ions across an anion exchange membrane was evaluated under a range of biogas flow rates and applied currents. This study has shown that membrane electrolysis can be used to remove 100% of the incoming CO_2, but has much more potential when coupled to a methanation reactor. Electrochemical biogas upgrading has been used to generate the ideal 4:1 H_2:CO_2 ratio for further biological upgrading into a high-purity biomethane (>97% CH_4), significantly reducing the power associated with upgrading *via* reduction of CO_2 into CH_4. Electrochemical extraction appears to be a promising pre-treatment process for biogas upgrading through biological methanation and should be further investigated for application in the power-to-gas context. With increased removal rates and decreased energy input this technology has the potential to become a sustainable alternative for biogas upgrading that is fully electricity driven and moves away from chemical and heat intensive upgrading processes.

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CHAPTER 4

Up-cycling recovered resources from anaerobic digestion through microbial protein production

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Abstract

Anaerobic digesters produce biogas, a mixture of predominantly CH_4 and CO_2, which is typically incinerated to recover electrical and/or thermal energy. In a context of circular economy, the CH_4 and CO_2 could be used as chemical feedstock in combination with ammonium from the digestate. Their combination into microbial protein, as animal feed, could help in optimizing the world's nitrogen cycle and improve the overall nitrogen efficiency of the current agro- feed/food chain. In this concept, renewable CH_4 and H_2 can serve as carbon-neutral energy sources for the production of protein-rich microbial cells, while ammonia is assimilated during production of the microbial biomass. As nutrients, carbon and energy are set free during the digestion process, we discuss anaerobic digestion as the prime candidate technology to liberate the gaseous materials for sustainable high-quality microbial protein production. We show that a practical case digester handling liquid piggery manure, of which the energy content is supplemented for 30 % with co-substrates, provides sufficient biogas to allow the subsequent microbial protein as feed production for about 37% of the number of pigs from which the manure was derived. The case of producing biomethane and upgrading the CO_2 to microbial protein by means of hydrogen oxidizing bacteria was also examined but found less attractive at the current production prices of hydrogen. There are additional advantages of the combination of anaerobic digestion and aerobic incorporation of the N, such as decreased water use, nitrogen pollution and GHG emissions. Overall, producing microbial protein on the farm from available methane and ammonia liberated by anaerobic digesters treating manure appears economically and technically feasible within the current range of market prices existing for high quality protein.

1. Introduction

Anaerobic digestion (AD) is a mature and energy-efficient technology, able to convert a broad variety of organic (waste) streams into biogas, a renewable source of methane (CH_4) and digestate, a nutrient-rich organic residue (Appels et al., 2011). The AD process has successfully been put forward as the first commercial 'waste-to-energy' bioreactor technology dealing with low-value carbon-rich waste streams, like manure, and is often envisaged as one of the key low-carbon technologies in the decarbonized energy mix of the future (Kampman et al., 2016). Today, 70 % of the more than 17,000 AD plants in the European Union are running on agricultural streams, with in many cases manure as the primary feedstock and often a second substrate, *e.g.*, grass or corn (typical on-farm feedstock), or various off-site feedstock, such as slaughterhouse waste, fats, organic household waste, to increase the biogas production and operational stability of the process (EBA, 2017). Biogas is typically valorized (and incentivized) through the production of electricity in a combined heat and power unit, but recently a study pointed out that the inherently low value of methane as energy carrier can be bypassed if the methane is considered as a renewable C_1 feedstock for the production of bio-based chemicals from CO_2 and grid-injected biomethane (Verbeeck et al., 2018). The conceptual idea to couple anaerobic digesters to centralized chemical industries *via* the existing natural gas grid, valorizing renewable methane as a green carbon source in production processes has opened new utilization options for the biogas industry, potentially even without being reliant on legal support schemes to guarantee a profitable investment (Verbeeck et al., 2018).

In addition to the conversion of biomass to biogas, anaerobic digesters are excellent liberators of ammonia and phosphates from the complex feedstock. Manure represents an exquisite mining resource, with typical concentrations ranging between 2.1 – 6.7 g N L^{-1} and 0.2 – 1.6 g P L^{-1} in piggery waste (Pintucci et al., 2017). At a yearly mass flow of 1.3 – 1.8 billion tons of livestock manure in the EU alone (Foged et al., 2012), manure represents one of the largest secondary flow of nutrients through agricultural supply chains. Historically, digestate produced from the process has been applied to land as an organic fertilizer or soil conditioner, enabling local nutrient cycling. The application to agricultural land is today often limited, due to legislative restrictions on nutrient application of digestate for agricultural purposes in areas with nutrient surpluses (Coppens et al., 2016). Due to a growing awareness of the economic and environmental costs incurred with the inefficient use of mineral fertilizers in current agricultural plant and meat production, technologies to recover nitrogen

and phosphorus from used water have gained more attention in recent years, preventing excessive losses of phosphates and reactive nitrogen species (NH_4^+, NO_2^-, NO_3^-) into our biosphere (Verstraete et al., 2016). Approaches such as ammonia stripping (Pedizzi et al., 2017), electrochemical ammonium extraction (Desloover et al., 2012a; Desloover et al., 2015) and struvite precipitation (Le Corre et al., 2009) are some of the key systems to directly refine and recover nutrients from anaerobic digestate, and produce a marketable product. However, the fertilizer products typically derived from digestate (like $(NH_4)_2SO_4$, NH_4OH and struvite) achieve, at present, a market value not higher than 20% of their intrinsic value, because they are endowed with an irregular composition, limited supply quantities and a poor physical condition. Revenues can only slightly compensate the investment and running costs incurred with the transportation, treatment or upgrading efforts (De Vrieze et al., 2019). Today, ammonia-nitrogen in digestate streams is, thus, mainly destroyed through biological nitrogen removal processes (nitrification-denitrification or partial nitritation-annamox), rather than recovered and reused (Matassa et al., 2015b). To ensure more secure and sustainable markets for recovered nutrients, with a lower dependence on land application, novel and higher-value products need to be created. The integration of technologies to upgrade low-value raw recovered nutrients to high-value end-products will be a key feature of next-generation AD installations.

Recently, innovative approaches implementing bacteria to produce microbial protein (MP), also known as single cell protein (SCP), within an AD context have been proposed (Matassa et al., 2015b). This MP is a more resource-efficient and high-rate protein that is put forward as a viable alternative for the conventional agricultural-based protein production chain, which is rather inefficient when it comes to the use of reactive nitrogen, and which causes serious environmental damages (Galloway et al., 2014; Steffen et al., 2015). Interestingly, MP can be aerobically produced from renewable raw materials, like NH_3, CH_4, CO_2 and H_2, generating a sustainable protein-rich biomass that can be used as a fertilizer, feed, or food additive (Pikaar et al., 2018a). Anaerobic digesters are providers of the most important building blocks for MP biosynthesis: carbon, energy (chemical or electrical) and NH_3 are available at considerably large amounts. The idea to utilize biogas as source of CH_4 for MP production by methane-oxidizing (methanotrophic) bacteria (MOB) has gained renewed interest (Pieja et al., 2017; Steinberg et al., 2017), mainly due the pressing need to find new business models for AD biorefinery concepts, and the successful market entry of two natural gas based MP production facilities using MOB (UniBio A/S and Calysta Inc) (Ritala

et al., 2017). Methanotrophs grow on methane as their sole carbon and energy source, directly converting methane into bacterial biomass, while assimilating mineral nitrogen (*i.e.*, ammonium) into high quality protein. The end products of this MP production technology have been approved as protein-rich feed additive, having an amino acid profile close to high-quality animal protein (Øverland et al., 2010). As an alternative to MOB, autotrophic hydrogen-oxidizing bacteria have recently received attention as potential production strains, due to their unique metabolic ability to fix CO_2 into new cellular material, using H_2 and O_2 as electron donor and electron acceptor, respectively. The HOB can contain up to 75 % crude protein (12 % N) based on cell dry weight (CDW), which is much higher than the 50, 46 and 15 % protein content in yeast, soybean and wheat grain, respectively (Matassa et al., 2016b). The fact that HOB can be grown on recovered CO_2, electrolytically produced H_2 and O_2, and recovered NH_3 can potentially create effective niches for novel application in the context of resource recycling and upgrade, mainly because HOB can exploit the potential of renewable energy generation to capture CO_2 from point sources (Pikaar et al., 2017). Carbon feedstocks under consideration for MP production in an AD context include CH_4 from biogas, CO_2 collected from the process of upgrading biogas to biomethane, or the CO_2 emissions coming from the biogas combustion in an on-site cogeneration unit. The concept that through solar power, coupled to electrolytic H_2 production, reactive nitrogen in the form of ammonia present in anaerobic digestate can be upgraded to valuable feed protein, thereby shortcutting current protein production processes, opens new options for anaerobic digestion as important driver of an entirely new decentralized economy for sustainable on-site feed production.

The main challenge in this context is the selection of the most cost-effective MP production pipeline. We determined to which extent different scenarios for on-site up-cycling of biogas carbon and recovered mineral nitrogen to microbial protein are economically suitable to be implemented in combination with existing or new AD facilities. To evaluate which MP application could potentially find effective niches for useful application in the AD process, the actual economic performance was calculated, accounting for costs and revenues related to the various production approaches. A model agricultural biogas plant was used as the basis of the calculations. Operational expenditure (OPEX), capital expenditure (CAPEX), potential savings and the revenues from the marketing of the resulting products were determined for the integration of two different MP production routes in a model European AD facility: (1) MOB cultivation on biogas methane and (2) HOB cultivation

on H_2 with CO_2 from biogas upgrading or CO_2 in the flue gases from biogas combustion (Figure 4.1). Our evaluation presents the features and economic potential of MP production through valorization of the different building block chemicals available at a digester facility, and could enable the selection of the most appropriate technology for decentralized carbon and nutrient recovery from organic feedstocks through MP.

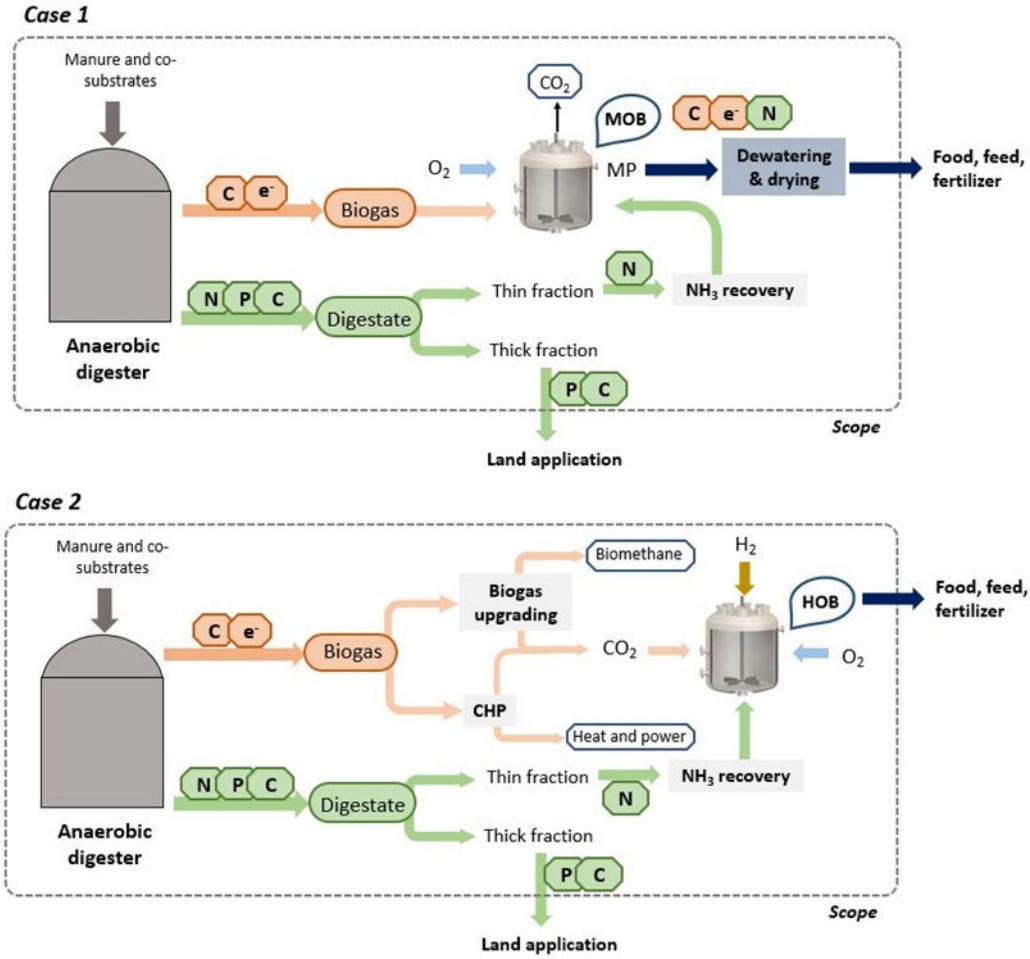

Figure 4.1 - Schematic representation of the two microbial protein production approaches in an anaerobic digestion context. The coloured arrows represent the flows of carbon (C), nitrogen (N), phosphorus (P) and energy (e^-) between the different unit technologies (anaerobic digestion, biogas upgrading, biogas combustion in a combined heat and power unit, and microbial protein *via* methane-oxidizing and hydrogen-oxidizing bacteria).

2. Methodology and assumptions

Two different scenarios were designed for the recovery of carbon and nitrogen from biogas and digestate, respectively. Each case has been studied for a model agricultural AD plant with a nominal raw biogas flow of 500 Nm^3 per hour, 60 vol.% CH_4, and a digester N-load of 36.7 kg TKN-N per hour (5.1 kg TKN-N ton^{-1} fresh material) *i.e.*:

- Protein production based on the methane in the biogas by MOB *(CASE 1).*
- Protein production based on the CO_2 from biogas upgrading to biomethane or from biogas combustion by extra energy input in the form of hydrogen gas and using HOB *(CASE 2).*

Each scenario contained a different combination of processes, depending on the carbon, energy and nutrient source for MP production and the integration within the AD facility. Performance was evaluated based on an extensive literature review and steady-state mass balancing of the different unit operations to determine biogas production, nitrogen release, ammonia recovery efficiency and MP production potential for each case. The costs of the input materials as well as capital and operational costs were estimated based on available data in literature. The economic viability of each scenario was assessed in terms of protein benefits and input costs for MP production. To account for the variability in cost estimations that can be found in the literature, the minimum and maximum costs are calculated as well (Table S 3.2). The methodology and main assumptions regarding costs and revenues are summarized in the following sections.

2.1. Input side: Raw materials and costs

Biogas

We assumed a model mesophilic farm-based digester fed with an agricultural feedstock mixture dominated by pig manure (70 % of the total fresh material input, wet weight). The manure was collected from several pig breeding facilities and processed in a central AD installation together with three co-substrates available in close proximity of the digester: crop residues (representing 10 w.% of the fresh material going into the digester), food waste (10 w.%) and maize silage (10 w.%) were selected as co-substrates to increase the biogas yield. Based on the biogas yield of the different feedstocks, the total COD converted to biogas was assumed to be 40 % from the manure and the remainder from the added waste. The

substrate mixture has a weighted average biogas yield of 42 m³ methane per ton fresh material.

As a reference technology for nutrient recovery from the raw digestate, a centrifugal separation into a liquid and solid fraction was selected, after which the solid fraction, rich in slowly digestible organic matter and organically bound nutrients, was used for composting (and thus land use), while the nitrogen rich aqueous phase was subjected to gas stripping and subsequent absorption in a sulfuric acid scrubbing solution to form ammonium sulfate (Vaneeckhaute et al., 2017). The main technical design data are listed in Table S 3.1 (in Supporting Information) and characterize the digester's supply chain, from the feedstock up to the quantity of biogas produced and ammonia-nitrogen liberated.

The average total capital investment of equipment and construction is set to correspond with an investment for biogas production of 4000 € Nm^{-3} h^{-1} installed biogas capacity (IRENA, 2013). OPEX costs for the digester were calculated based on a fixed percentage of the CAPEX (7.5 % of the investment sum on a yearly basis), including electricity and chemicals consumption, maintenance and labor. The feedstock mixture is assumed to have a fixed cost of 5.28 € ton^{-1} fresh material, transportation included (pig manure and crop residues were assumed to have no cost). The average specific raw biogas production cost for the agricultural digester under study was estimated at 115 € ton^{-1} biogas or 326 € ton^{-1} methane (Table S 3.2). Estimations of minimum and maximum costs are included in Table S 3.2.

Ammonia

The overall cost to recover 1 ton NH_3-N by means of conventional air stripping/absorption ranges from 1000 to 3000 € ton^{-1} NH_3-N with ammonium sulfate as the recovered product (Menkveld & Broeders, 2018). Considering the high N-concentration in liquid fraction of the digested manure (> 4 g L^{-1}) an average recovery cost of 1500 € ton^{-1} NH_3-N was assumed as base case, with 1000 and 3000 € ton^{-1} NH_3-N for the extreme cases. For this scale of stripping installation the capital investment is assumed to make up 23 % of the total NH_3-N recovery costs. The percentage of N present in the feedstock that ends up in the liquid fraction was assumed to be 80 %. The removal efficiency for NH_3-N *via* stripping was set at 90 %.

CO_2

Since CO_2 is inherently linked to the biogas production, we did not allocate costs to the CO_2. Upgrading and injection as well as CHP costs are allocated to the production of biomethane or power.

Hydrogen gas

At present, the costs for hydrogen production by means of PEM electrolysis including CAPEX and OPEX are estimated at 4400 € ton^{-1} H_2 (based on an electricity price of 44 € MWh^{-1}). Future predicted levelized costs for hydrogen production was set at 2600 € ton^{-1} H_2 (Ayers et al., 2010). It is forecasted that renewable power costs will reduce to 30 € MWh^{-1} by 2020-2025, and even down to 10 € MWh^{-1} by 2030-2040 (Fraunhofer, 2015), bringing down the electricity cost to < 2000 € ton^{-1} H_2.

Oxygen gas

The oxygen needed for MOB cultivation would have to be produced in an additional process (for example *via* cryogenic separation or pressure swing adsorption). The mean cost for generation of industrial grade oxygen is estimated at 30 € ton^{-1} O_2 (Allam, 2009). For the HOB case, oxygen is co-produced along with hydrogen in the electrolysis process. Given the fact that oxygen is not a limiting raw material in the thus produced quantities in relation to hydrogen gas (~ 8 kg O_2 per kg H_2 produced during electrolysis of water), the cost for O_2 is covered by the cost for H_2. An oxygen requirement for MOB and HOB production of 2.50 and 2.05 ton O_2 per ton MP is taken into account, respectively.

2.2. Microbial protein production and drying: Opex and Capex

The total capital investment of equipment and construction is set to corresponds with an investment of 5000 € m^{-3} installed reactor capacity (Loh et al., 2002). A depreciation period of 20 years with an interest rate of 5 % was assumed. A volumetric production rate for MOB and HOB of, respectively, 0.48 and 0.31 kg protein per m³ of reactor per hour was used as the basis of the required reactor volume [Eq.1].

$$Required\ reactor\ volume = \frac{Microbial\ protein\ production\ rate\ (ton\ per\ h)}{Volumetric\ production\ rate\ (ton\ per\ m^3\ per\ h)} \qquad \text{[Eq. 1]}$$

As a full conversion of the substrates is targeted, MP production rates are set at 20 % of the maximum rates reported in literature (that typically are obtained at high substrate loading rates that do not aim to achieve 100 % conversion).

The OPEX contribution to the total cost was set at a fixed sum of 200 € ton^{-1} MP, including utilities, labor and supervision, overhead, maintenance, *etc.* Raw material costs other than CO_2, CH_4, O_2, H_2 and NH_3 (like phosphorus, trace elements, micro nutrients and pH control chemicals) are included in the OPEX. Separation, sterilization and drying costs were set at 160 € ton^{-1} MP, based on the calculations performed in a recent study of Pikaar et al (2018b). This is the sum of the energy costs related to water removal by centrifugation (leaving a product with around 25% DM content) and spray-drying with integrated fluidized bed technology up to a dry solids content in the final product of 100 %. Assumptions made for the extreme cases are listed in Table S 3.2.

2.3. Output side: protein and revenues

The assumed yields, protein content and stoichiometry of MOB and HOB cultivation are listed in Table S 3.3 and S 3.4, and form the basis of the MP production taking into account the amounts of recovered feedstocks for bacterial growth. MOB and HOB biomass were assumed to consist of 12 wt.% nitrogen. An average market value of 1750 € ton^{-1} protein of the thus produced microbial biomass will be taken into account in this study as its protein and amino acid composition is comparable to that of fishmeal (which is worth in between 1500 and 2300 € ton^{-1} protein). Moreover, it is known that under stress conditions, especially under oxygen or nutrient limiting conditions, the microbial cells are able to accumulate polyhydroxybutyrate (PHB), a biopolymer used as energy storage by bacteria (Khosravi-Darani et al., 2013). This PHB is of special value for enhanced feeds as PHB are regarded as prebiotic feed additive and microbial control agent when used in the diet of different aquaculture species (De Schryver et al., 2010). This product could bring additional nutritional value to the produced microbial cells, but this added value is not taken into account in the economic evaluation.

Process profitability is estimated considering OPEX, CAPEX, feedstock cost and protein market price. The profit is expressed per ton biogas and per ton MP (expressed as 100 % protein crude content), taking into account the different input streams. To account for variability and uncertainty regarding cost estimations a parametric analysis was performed, providing details on the impact of operational cost and revenues on the economy of the facility under study.

3. Results and discussion

The key concern to push towards nutrient recovery rather than removal from digestate is the economic viability of the proposed recovery scenario. This viability is determined by the total production cost of the product and the market value of the final product(s). Estimations of the costs and revenues associated with the different options for biogas and ammonia upgrading to microbial protein are presented here.

3.1. Biomethane as feedstock for microbial protein production by MOB

The base case production cost of microbial protein obtained from MOB cultivation is estimated at 1544 € per ton crude protein (expressed in 100% dry weight), with 920 € ton protein^{-1} as the minimum and 2531 € ton protein^{-1} as the maximum production costs calculated. A cost breakdown analysis of the total MP production cost is represented in Figure 4.2. Costs associated with the production/recovery of the building blocks for MOB growth represent 71 % of the total base case MP production cost, with 46% for biogas methane, 20% for recovered ammonia and 5% for O_2, while 19 % can be attributed to CAPEX and OPEX of the MP production unit (293 € ton MP^{-1}) and 10 % to dewatering and drying of the wet product (160 € ton MP^{-1}). Considering a market price for feed proteins that typically ranges between 1000 € ton^{-1} protein for soybean meal (as the reference vegetable protein for livestock, expressed as protein active substance) and 2000 € ton^{-1} protein for fishmeal (as the reference high-quality animal protein, expressed as protein active substance), MP can be produced from recovered resources at competitive prices. At present, much still depends on factors relating to the quality demands posed on both the input raw materials (degree of refining) and final product (purity of the product), as well as the downstream processing that is required. The amino acid profile and overall nutritive value of a bacterial meal obtained from MOB growth appeared to be comparable to fishmeal and overall better than soybean meal (Øverland et al., 2010), it is likely that the produced microbial protein has a market value higher than or at least equal to fishmeal. Market values of protein sources are variable and highly depend on the macroeconomic variables, such as the global demand for livestock protein and the natural gas price for Haber-Bosch ammonia synthesis. As both the global protein demand and pristine ammonia price are expected to increase in the near future (FAO, 2017), MP can become a cost competitive route to produce a substitute for soy and fishmeal for animal feed. Figure 4.3 shows the impact of a change in MP market price on the profitability of this pathway considering the average MP production cost as well as the

minimum and maximum values. The base case, using average-priced methane and ammonia, suggests that at a protein market price of 1750 € ton^{-1} MP can be produced through the CH_4:NH_3 route with a profit around 200 € ton^{-1} MP, corresponding with ~ 33 € ton^{-1} biogas. Taking into account the savings from the avoidance of the treatment of the mineral nitrogen present in digestate makes this case much stronger. As the dissipation of reactive nitrogen back to the atmosphere as N_2 by means of nitrification–denitrification comes at a cost of about 3 – 4 € per kg NH_3-N (Van Hulle et al., 2010) and 200 kg NH_3-N is assimilated during MOB cultivation, some 600 – 800 € per ton MP can be saved on reactive N removal, as a result of the reduced need for nitrogen removal in the digestate. If MP production is evaluated in the context of nutrient up-cycling from digestate, almost for the entire range of protein market prices profit can be made (Figure 4.3B). The economic viability of an AD facility that turns its self-produced methane with recovered ammonia into proteins, thus, seems to be guaranteed, at present costs and revenues, without any legal support. The MP revenues can turn a manure processing facility in a cost neutral (or even profit gaining) installation. With an avoided net cost of 10.95 – 31.61 € per ton manure processed (De Vrieze et al., 2019) (equal to about 548 – 1581 € ton^{-1} protein produced), MP production seems to be a prime candidate technology to offset the costs associated with manure processing. The reason for this economically justified implementation of MP production technology is twofold. First, MP production could strongly increase the value chain of recovered nitrogen from around 1 € kg^{-1} N for $(NH_4)_2SO_4$ up to 16.7 € kg^{-1} N for microbial protein. Second, MP production bypasses the low inherent value of methane when energetically valorized on-site (in a CHP unit) or off-site (as biomethane in a power plant or car engine), generating more value per ton biogas. As discussed in our previous study, most biogas projects that produce and sell heat and power can only be economically viable with effective and long-term financial incentives, compensating for the high production costs of biogas/biomethane compared to their market value (Verbeeck et al., 2018). For the manure digester under study, governments should give a subsidy of at least 40 € per MWh produced electrical power (equal to 145 € per ton biogas) to realize break-even operation, considering an electricity wholesale price of 40 € MWh_e^{-1}. Protein production by using methanotrophic bacteria growing on biogas methane would, thus, offer a new business case for AD plants, without dependency on financial incentives from governments. Our results clearly indicate that through upgrading of low-value methane and ammonia to protein-rich microbial biomass, the economic potential of the otherwise often unprofitable exploitation of an AD plant can be strengthened.

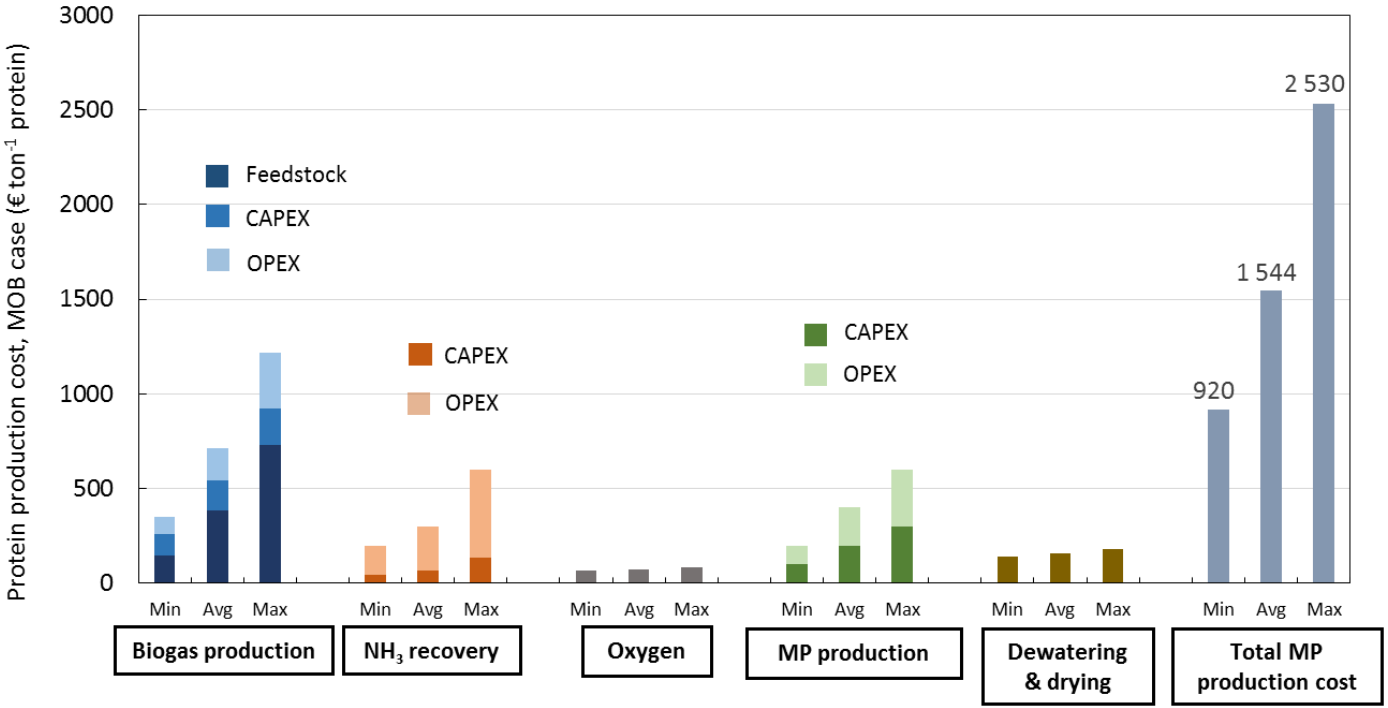

Figure 4.2 - Averaged, minimum and maximum protein production costs using methane-oxidizing bacteria, broken down into the components biogas production, ammonia recovery, oxygen production, dewatering & drying and the total microbial protein production.

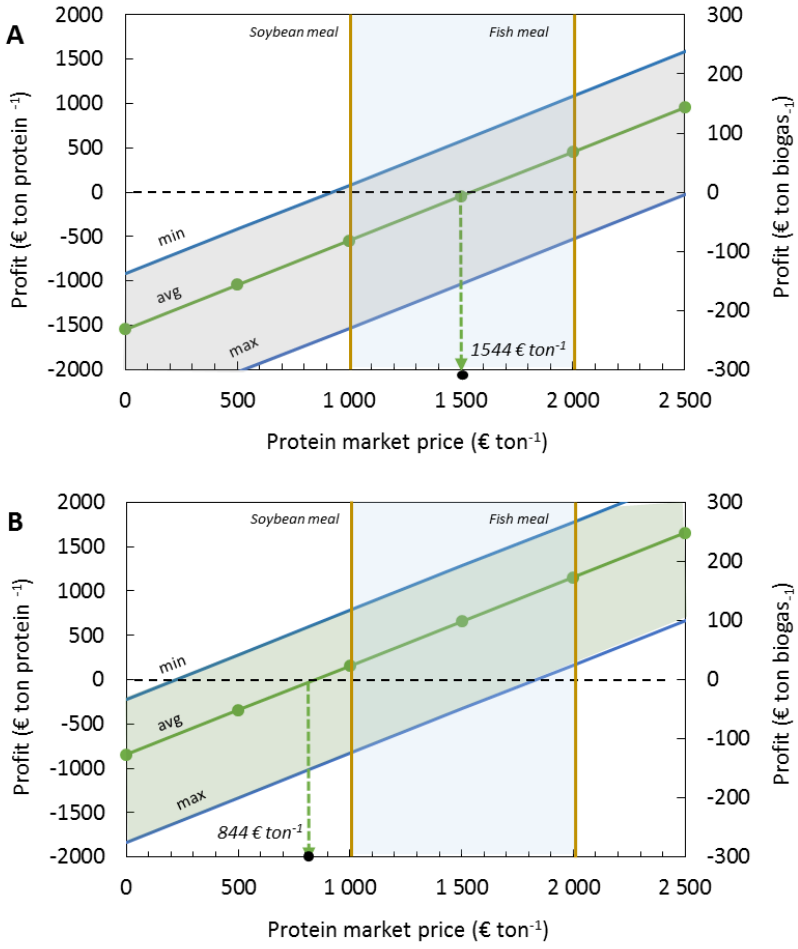

Figure 4.3 Economic analysis for microbial protein (MP) production with CH_4 as sole carbon and energy source. Profit generated (in € ton^{-1} MP) as a function of the protein market price, not including any financial incentive, for the estimated microbial protein production cost (minimum, average and maximum) without (A) and with avoided costs (B) for nitrogen removal from digestate. The shaded vertical (blue) region represents the variation in current wholesale agro-based protein price

The economic evaluation does not consider other key benefits of MP production, such as a decreased water consumption, a lower land occupation and decreased nitrogen pollution and greenhouse gas emissions. Some of the key global impacts were recently discussed by Pikaar and co-workers (2018b). The same trends were observed in a study that evaluated the

environmental impact of FeedKind™ protein, a MP produced from natural gas. The report shows that the water foot print of MP is about 20 - 140 times lower than fishmeal and soybean meal, respectively, and land use is > 100 times lower compared to soy proteins (Cumberlege et al., 2016). Including the externalized environmental costs of the current agro-production system in the price of protein would result in an allocation of resources that is more efficient for all of society as the MP route is a more rational alternative, able to offer immediate advantages in terms of water and land use (Matassa et al., 2016a).

As raw materials represent 66 % of the total cost, the major cost decrease can theoretically be achieved at the level of the digester and the ammonia recovery unit. However, both technologies are already very mature, and the cost decreases that could be expected are limited and more related to scale effects, rather than technological advances. In fact biogas represents, at present, already a relatively inexpensive source of renewable methane for on-site production, as the consumer price of natural gas for industrial end-users is around 440 € ton^{-1} (EU-28 average price in 2015) (EC, 2016), compared to 326 € ton^{-1} calculated for the base case in this study. This is mainly due to the high transmission and distribution costs of natural gas (see Chapter 2). In contrast, realizing that the gate cost for pristine ammonia is approximately 575 € ton^{-1} NH_3-N (Schnitkey, 2018), the use of recovered nitrogen is, at present, 2 – 6 times more expensive compared to Haber-Bosch derived NH_3 (see section 4.2.1). As 1 ton proteins can be produced at a cost of 1359 € ton^{-1} protein with freshly synthesized reactive nitrogen, nutrient recovery costs, together with avoided removal costs, will be decisive to guarantee the economics of future MP production pipelines. It needs to be recognized that the costs of nitrogen removal *via* stripping/absorption from highly ammonia-loaded used water streams (> 4 g L^{-1}) are in our base case estimated a factor 2 lower than conventional nitrogen dissipation *via* nitrification-denitrification. Above 2 g NH_3 - N L^{-1}, commercial stripping installations are able to recover NH_3 at a cost down to 1000 – 3000 € ton N^{-1} (Menkveld & Broeders, 2018), while treatment costs of the nitrification-denitrification process are estimated at 3400 - 4000 € ton^{-1} NH_3-N (van Eekert et al., 2012; Van Hulle et al., 2010). Considering that stripping could remove up to 90 % of the NH_3 in the liquid fraction, the nitrogen input at the wastewater treatment facility is drastically reduced, and a substantial reduction in costs at these facilities can be achieved. Furthermore, the release of free ammonia by the digester microbiome is so intensive that already in some lab-scale AD reactors an ammonia stripping unit is directly coupled to the digester as a side loop process to avoid inhibition of the methanogens, due to free NH_3 toxicity. Next to resource

recovery, ammonia stripping could, thus, also allow higher biogas production rates (Pedizzi et al., 2017; Siegrist et al., 2005).

Partial self-supply of feed on farm scale

Assuming that the full methane flow of 5.16 ton CH_4 per day is converted to microbial biomass at a biomass yield of 0.76 g CDW g^{-1} CH_4 (60 % crude protein content) (Matassa et al., 2015b), this accounts up to a daily protein production potential of 2.4 ton (or 3.9 ton if expressed as cell dry weight). If the microbial biomass is used as additional feed source, and considering that the total protein demand for 1 pig is approximately 45 kg (NRM, 2017), yearly, about 19 500 pigs can be raised with the proteins produced from the carbon and nitrogen contained in manure and liberated by anaerobic digestion. Based on an average cycle time of 166 days, a farm of about 8864 pigs can be supplied with the MP from the resources generated at the digester that is treating manure from about 24 000 pigs (assuming a daily manure production of 5 kg fresh material per pig per day). The use of on-site generated methane to produce bacterial biomass, thus, offers the farmer the opportunity of partial self-supply of feed (37 % in this specific case), replacing crop-based protein in animal feed by MP.

As the yield of soybean is on average 3.11 tons DM per hectare per year (Langemeier & Lunik, 2015), an estimated land footprint of 612 hectares would be required to produce the same amount that can be produced *via* MP in a very compact engineered bioreactor environment, *i.e.*, 204 m^3 for the case under study. Assuming a bioreactor height of 30 meters, this comes down to a reactor footprint of just 6.8 m^2. Besides having a much higher efficiency in land and nutrient use, MP do use water very efficiently, up to 99 % reduction in water footprint compared to agricultural based production (Cumberlege et al., 2016). Implementing a circular approach at digester scale, with the basic components recovered from waste and upgraded into new valuable microbial biomass rich in proteins, thus, offers the opportunity to process manure in a cost-efficient way, still generating a product that generates profit.

Full conversion of recovered ammonia from manure requires co-digestion with energy-rich substrates

For a complete valorization of the ammonia-nitrogen recovered on-site, methane should be available at a CH_4:N ratio of 11 kg CH_4 per kg N [(0.76 ton CDW ton^{-1} CH_4 x 0.12 ton N ton^{-1} CDW) $^{-1}$]. Considering that for manure the methane yield relative to available nitrogen is limited, *i.e.*, typically only in the range of 12 to 18 Nm^3 methane per ton FM, while nitrogen content can reach > 6 g N L^{-1}, the CH_4:N ratio of manure is too low to allow for a full valorization of the nitrogen present in the digestate. For the digester under study, the defined substrate mixture has a N content of 5.1 kg TKN-N per ton FM. Accounting for a 75 % conversion efficiency of Kjeldahl-N to NH_4^+-N, a NH_4^+ recovery in the liquid digestate of 80 % and a 90 % NH_3 stripping efficiency, 2.75 kg NH_4^+-N per ton wet substrate (or 54 % of the incoming N load) is extracted from the biomass and, thus, made available for MP production. For the optimal CH_4:N ratio of 11, this requires a substrate mixture with a methane yield of at least 42 Nm^3 CH_4 ton^{-1} FM, highlighting the need to amend manure with co-substrates to improve the biogas production and obtain a CH_4:N ratio sufficient for MP production with complete N valorization. For manure, maximum MP production without co-substrate addition is only possible if an additional electron donor is supplied, either by dosing fossil methane from the natural gas grid or by supply of hydrogen gas to achieve nitrogen assimilation *via* the HOB pathway. The amount of co-substrate that needs to be mixed with manure is determined by the N content and methane yield of the different substrates. For example, when readily available high strength organic waste streams, like fats or greases with a methane yield up to 800 Nm^3 per ton FM, are used as co-substrate (Weiland, 2010), 6 weight % would suffice to achieve the optimal C:N ratio. Opposite digesters that are limited in nitrogen will need to blend in high N feedstocks or purchase Haber-Bosch NH_3.

Resource mining from manure: potential to be import free

Coupling renewable methane generation with the full-scale production of MP using pure or mixed cultures of methane-oxidizing bacteria might be the most straightforward approach for MP production in the context of nitrogen and carbon valorization from anaerobic digestion, since MOB cultivation on fossil methane is already well established with several industrial demonstration plants in operation (*e.g.*, Feedkind™ by Calysta and UniProtein™ by UniBio A/S). The large amounts of renewable carbon and recovered nitrogen make manure digesters prime candidate facilities to shortcut the current unbalanced nitrogen cycle. Considering that livestock manure accounts for a nitrogen flow through the EU

economy of about 6 – 9 Mton per year (Foged et al., 2012), nitrogen upgrading from anaerobic digestate through MP production processes could produce some 27 – 40 Mton of microbial biomass, representing 16.2 – 24.0 Mton crude protein. Currently, the EU imports 20 Mton soybean per year (equal to approximately 9 Mton crude protein) (Schreuder & De Visser, 2015). This means that if we could upgrade 38 – 56 % of the nitrogen from livestock manure to protein, the EU can already be import free, highlighting MP are the prime candidate alternative protein source, surpassing soy and animal meat proteins.

3.2. CO_2 as carbon feedstock for microbial protein production using HOB

For the H_2-CO_2 route, the profitability of the biogas utilization scenarios, *i.e.*, power generation or biomethane injection, are not influenced by the production of MP, and CO_2 is envisaged as an unavoidable product of biogas upgrading/combustion that is fully allocated to the production cost of green electricity or methane (no CO_2 cost was taken into account for MP production). Although CO_2 fixating HOB could yield a potential revenue of ~ 160 € per ton protein in carbon credits (at a carbon allowance price of 50 € ton^{-1} CO_2), no savings are taken into account as CO_2 emissions from a biogas plant are considered CO_2 neutral due to their biogenic origin. The HOB fermenter can be considered as a biogas upgrading unit itself, due to its capacity to fix CO_2 from the biogas. This would eliminate the need for additional technologies, making the biomethane production cheaper. However, these savings are not considered in this assessment.

CO_2 from upgrading biogas to biomethane

With the daily flow of 9.6 ton CO_2 in the tail gas stream from the upgrading unit, about 2.9 ton crude protein DM can be produced (or 3.9 ton of dry microbial based biomass with a crude protein content of 75 %), provided that H_2 is supplied at the required feeding ratio. Production costs of protein by HOB are estimated based on the costs to produce hydrogen gas (and oxygen gas) *via* water electrolysis, recover NH_3 *via* ammonia stripping, operate the fermenter and dewater/dry the final product. The total base case production cost of 1 ton HOB biomass is estimated at 2289 € ton^{-1} (expressed as dry crude protein). Minimum and maximum costs are estimated at 1589 and 3781 € ton MP^{-1}, respectively, under the assumptions for extremes made (Table S 3.2). The cost breakdown clearly indicates that hydrogen gas production will be cost decisive (Figure 4.4). The hydrogen production costs by means of water electrolysis comprises about 67 % of the total production costs for H_2-based

microbial MP. This estimated base case MP production cost was based on a predicted levelized cost of hydrogen of 2.4 € per kg through water electrolysis using renewable energy at a unit price of 44 € per MWh. As recent bids for electricity produced with large scale-solar photovoltaics have reached prices as low as 30 $ per MWh generated (Haegel et al., 2017), it is not unthinkable that these costs will further decrease down to < 2 € per kg H_2. Considering a mean avoided cost of 3.5 € kg^{-1} N when implementing ammonia recovery instead of nitrogen removal *via* nitrification-denitrification, each ton MP produced saves about 560 € on wastewater treatment costs, making the economics look differently (breakeven point at 1729 € ton^{-1} MP, Figure 4.5B).

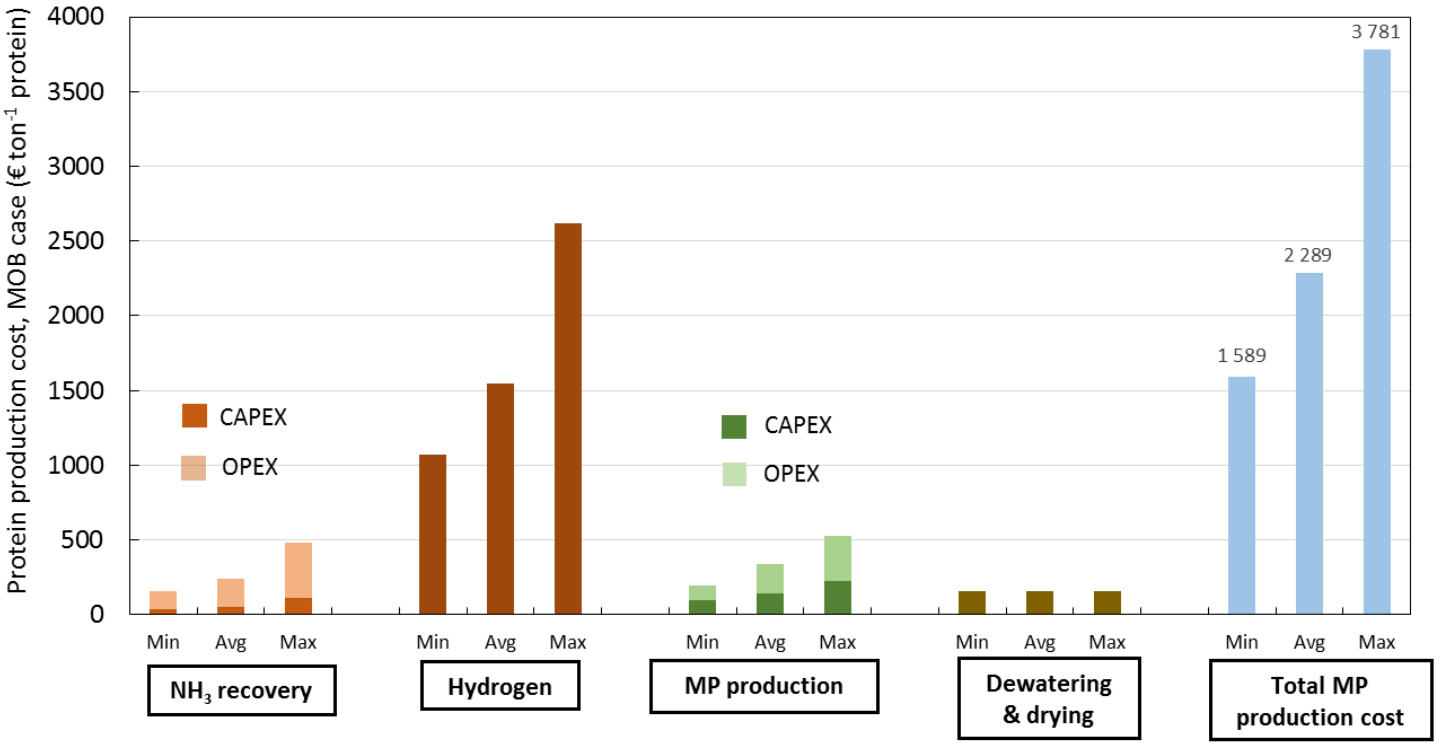

Figure 4.4 - Averaged, minimum and maximum protein production costs using hydrogen-oxidizing bacteria, broken down into the components ammonia recovery, hydrogen production *via* water electrolysis dewatering & drying and the total microbial protein production.

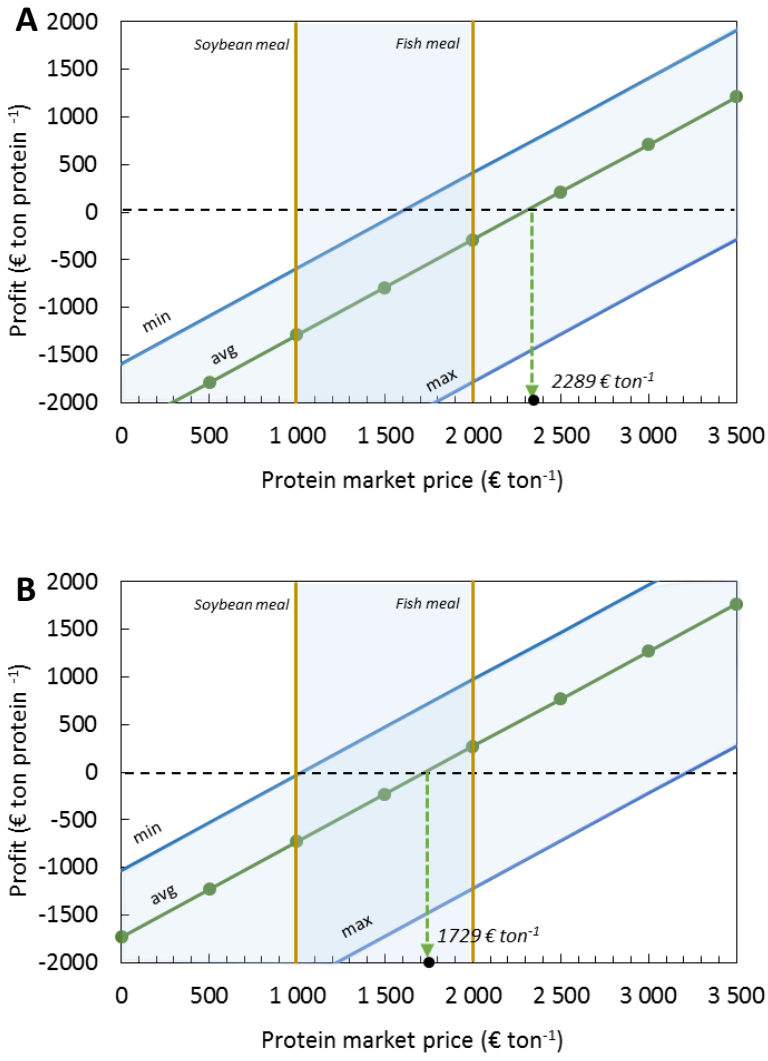

Figure 4.5 - Economic analysis for microbial protein (MP) production with H_2 as energy donor. Profit generated (in € ton^{-1} MP) as a function of the protein market price, not including any financial incentive, for the estimated MP production cost (minimum, average and maximum) without (A) and with avoided costs (B) for nitrogen removal from digestate. The shaded vertical (blue) region represents the variation in current wholesale agro-based protein price

Per kg protein produced, cells assimilate about 0.16 kg NH_3-N, leading to a gross daily uptake of 463 kg N, equal to 97.5 % of the nitrogen that could be extracted from the liquid digestate *via* stripping. The C:N of the feedstock mixture is, thus, sufficient for a full conversion of CO_2-C and NH_3-N.

Current practice for N-recovery is mainly air or steam stripping, which is energy intensive, *i.e.*, 3.9 to 28.2 kWh kg N^{-1} depending on the scale of the plant (Gulyas et al., 2014), and requires caustic and acid dosage for stripping and scrubbing, respectively. Recently, a proof of concept for NH_3 extraction from urine through electrochemical stripping was put forward as an energy-efficient way to produce a gas mix that was used for microbial protein production by HOB at less than 10 kWh kg^{-1} N when H_2 energy is considered. This process, that can be fully driven by renewable power, brings the 4 key building blocks for growth of HOB from 1 process: H_2 and NH_3 from the cathode and O_2 and CO_2 (originating from the urea hydrolysis product HCO_3^-) from the anode (Christiaens et al., 2017). Moreover, *via* the introduction of a membrane to assist the electrochemical stripping, the risks for cross-over of micro-organisms and trace contaminants into the nitrogen product flow was minimized (Christiaens et al., 2019).

CO_2 from CHP unit

For the biogas plant under study, combustion of the daily biogas flow generates 23.7 ton of CO_2. Without limitations on the availability of the other building blocks for HOB growth about 7.2 ton protein per day can be produced fixing the CO_2 in the combustion gases and assimilating about 1.15 ton NH_3-N per day. With this production capacity some 26 000 pigs can be fed daily. However, realizing that nitrogen is the limiting factor in this scenario, *i.e.*, only 475 kg recovered NH_3-N available, a maximum of 3.0 ton protein can be produced daily with the nutrients available on-site. Additional imports of nitrogen of the order of 675 kg per day are, thus, necessary if all available carbon on site is targeted for MP production.

The overall viability of the biogas plant was evaluated for this case as well, taking into account costs and revenues associated with CHP production. Total cost following this CHP-MP route is estimated at 605 € ton^{-1} biogas. Revenues from selling both electricity at 40 € MWh_e^{-1} and protein-rich biomass at 1750 € ton^{-1} MP are around 500 € ton^{-1} biogas, while avoided costs for N removal are about 109 € ton^{-1} biogas. Revenues and savings from MP could, thus, compensate the financial losses from CHP production, enabling a cost-efficient

treatment of manure and organic waste through anaerobic digestion and MP production. In conclusion, the MP production *via* the NH_3-H_2 route is only economically viable when production costs are assumed to be minimal and savings through nitrogen upgrading are taken into account. Further technological advances to bring down the cost might offer perspectives to increase the cost competitiveness.

3.3. Future perspectives

Complementary hydrogen and methane platforms

More than being self-excluding, the methane and the hydrogen gas platforms can be seen as complementary, depending on the availability of each resource on-site and the value/cost of renewable energy. The MP production from a mixture of methane and hydrogen opens the potential to consider a system that can valorize all gaseous carbon available at a biogas plant. This would imply the collaboration of two aerobic populations, MOB and HOB, in one engineered bioreactor environment. For the case in the study, 5.3 ton MP per day can be produced from the total carbon flow if an additional 460 kg N per day is purchased.

The fact that MOB are well-studied microorganisms that have been implemented in full scale production reactors is a strong asset of this technology platform. When compared with hydrogen-oxidizing bacteria, methanotrophs offer the benefit that they can be set to work directly on renewable methane without the need for additional energy input. However, relative to HOB, they possess a lower biomass yield, lower growth rates and lower protein levels (Matassa et al., 2016b).

There is even a potential for MP production from waste organics, such as carboxylic acids that are generated upon fast anaerobic treatment of organic streams, like slaughterhouse wastewater, although this entails that more attention will be needed for avoiding waste materials crossing over into the product. Emerging as microbial protein are the purple non-sulfur bacteria that require infrared light and an organic substrate to grow (Hülsen et al., 2014), although these come with the evident drawback of needing a photo-bioreactor. Recently, the use of protein-rich biomass as slow-release organic nitrogen fertilizer has been put forward as a novel outcome of MP. Key benefit of producing fertilizer over the MP based production of human food and animal feed lies in the fact that process conditions for non-food applications are less strict in terms of hygienization, sterilization, composition, and dry

solid content of the final product (Pikaar et al., 2018a). In this perspective, one could look into the option to directly grow MP in the (liquid) digestate, taking up residual carbon and mineral nitrogen from the medium. Realizing that stripping and assimilation are both not 100% efficient there is still an amount of NH_3-N that ends up in wastewater treatment plant. To be able to operate in a full recovery mode (without polishing in a nitrification-denitrification step), the production of MP for fertilizer applications through the assimilation of the residual reactive nitrogen is an interesting approach.

What is needed to drive implementation of MP at biogas plants?

The strong incentives decarbonization and renewable energy targets drive the valorization of biogas as a local and renewable energy source, either for on-site CHP production, or *via* injection of upgraded biomethane in the natural gas grid. As long as these 'green' feed-in premiums generate positive business cases for biogas projects, it will be hard to convince AD owners to valorize the methane in a different way, and particularly to consider making major capital investments. However, there is a second carbon feedstock available at the facility that is, at present, in many cases not valorized: CO_2. Either the CO_2 produced by upgrading of biogas to biomethane, or the CO_2 emissions from the combustion of biogas can be exploited as carbon feedstock for protein production using H_2-oxidizing bacteria.

It remains questionable whether farmers are willing to up-cycle carbon and nutrients into edible MP products, and replace a part of their crop-based animal feed protein demand by self-produced MP. A successful and widespread adoption of the MP biotech platform at biogas facilities is, even under a proven economic profitable plant operation taken into account the revenue from the avoidance of the treatment of the mineral nitrogen, prone to cultural factors in farm management, a lacking official legal recognition and the widespread public acceptance of microbial derived products as feed and food additive. Labels that clearly indicate to consumers that meats are produced with a lower environmental footprint could assist in market uptake, similar to labels such as "organic". It could even be considered that legislators put a cap on acceptable GHG and mineral nutrient emissions per unit meat protein to stimulate alternative sourcing. In this way, the high externalized environmental costs of the current conventional agricultural based supply routes for animal-based proteins would be made clear to the public, playing in favor of establishing a mindset more open to acceptance of alternative protein sources with a lower environmental impact. However, the market entrance of MP as main protein additive in livestock production and aquaculture is

probably less a concern compared to the direct consumption as human food as the product quality and taste of the meat will not be affected, and consumers are not directly in touch with the microbial-based product.

Obviously, safety and quality of the edible MP products must be guaranteed in order to allow a successful adoption of microbial-based products, for sure when produced from carbon and nutrients recovered from organic waste such as livestock manure. In this light, it is essential to sterilize the MP product and to provide safety barriers between the waste stream and the final product to avoid cross-over of potential opportunistic pathogens or harmful contaminants to the final product (*e.g*. membranes).

4. Conclusions

To ensure that both products of anaerobic digestion, *i.e*., biogas and digestate, are utilized to their full potential as renewable sources of raw materials, new valorization pipelines need to be implemented into the current AD process schemes. At present, products deriving from digestate achieve a low market value and recovery costs cannot be offset by the revenues. Nutrient recovery processes like ammonia stripping or struvite production, however, might represent the starting point of an entire new biorefinery concept in which microorganisms grow on renewable carbon sources and recovered reactive nitrogen while producing protein-rich microbial biomass (known as microbial proteins). The already well established methane-oxidizing bacteria represent a promising technology to upgrade low value methane and nitrogen to a product than can be used as an alternative high-quality food/feed protein source, surpassing the conventional agro-based protein generation. The technology for microbial protein production in the framework of an anaerobic digester facility by means of the NH_3-CH_4 route is of micro-economic interest, as this pipeline offers a better return on investment than burning biogas and the use of digestate products for land application. For the NH_3-H_2 case, calculations show that this route is of interest if the protein value equals the value of high-quality agro-based proteins like fishmeal and if the avoided costs for N removal are taken into consideration. As hydrogen production costs are expected to decrease further the process will be of higher economic relevance in the future and will, thus, enable maximal utilization of carbon processed through anaerobic digesters. Overall this study presents an interesting approach to partially shortcut the nitrogen cycle at the scale of a digester facility by direct introduction of MP as feed for animals.

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CHAPTER 5

The type of ion selective membrane determines stability and production levels of microbial electrosynthesis

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This chapter has been redrafted after:

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Abstract

Microbial electrosynthesis (MES) can enable electricity-driven bioproduction from CO_2. Several membrane types such as anion exchange, cation exchange, and bipolar membranes (AEM/CEM/BPM) can be used to separate the anodic oxidation from the biocathodic reduction. The impact of the membrane type on MES has not yet been studied. Therefore we compared the three membranes for MES of acetic acid. The reactor with AEM enabled *in situ* recovery of the acetic acid. This extraction led to a 32% higher production rate and efficiency compared to the systems that did not include product recovery, as product inhibition was likely occurring. Besides H^+/OH^-, mainly HCO_3^- contributed to charge balancing. Due to water displacement across the membrane, the product concentration in the AEM reactor (9 g L^{-1}) did not exceed the concentration in the CEM reactor (10.5 g L^{-1}). Overall this comparison shows that the membrane type in MES can be critical towards a stable and efficient process.

1. Introduction

Microbial electrosynthesis (MES) is a recently developed approach to microbially produce chemicals from organic substrates or CO_2 using electricity as driver. The process can take place at the cathode of a bioelectrochemical system (BES). It combines the production of (bio)chemicals without the use of fossil fuels as carbon source, with concurrent capture of greenhouse gases, in an electricity-driven CO_2-reduction process (Rabaey & Rozendal, 2010). Unlike photosynthetic processes, a direct conversion of CO_2 to organic chemicals is achieved, generally *via* a homoacetogenic metabolism, potentially at high sunlight-to-product efficiency if the power is derived from photovoltaics. Acetic acid is currently the most common product obtained in MES (Patil et al., 2015). This product can be produced at high rate *via* the autotrophic Wood-Ljungdahl pathway and is used, *inter alia*, as precursor for the production of polymers and solvents (Marshall et al., 2013).

MES research thus far has focused on several fundamental and technological aspects such as biocatalyst selection (Nevin et al., 2011), electrode materials (Jourdin et al., 2016a; Zhang et al., 2013) and reactor configuration (Gildemyn et al., 2015; Molenaar et al., 2016). Different reactor configurations have been proposed to improve MES, raising the question which configuration results in the best performance. A central element in the reactor setup that has not yet been studied is the membrane type. A cation exchange membrane (CEM), an anion exchange membrane (AEM) or a bipolar membrane (BPM) could be used, but also systems without membranes have been proposed for MES (Giddings et al., 2015). The CEM and AEM allow transport of cations and anions, respectively, across the membrane, to enable charge balancing. A BPM allows water dissociation within the dual membrane structure upon applying an electric field, resulting in the supply of protons to the catholyte and hydroxides to the anolyte. Hence, a different pH is conserved at either side of the membrane (Xu, 2005). These different membrane types have been previously compared for their performance in microbial electrolysis cells (MECs) (Cheng & Logan, 2007; Rozendal et al., 2008; Sleutels et al., 2009). In these MECs, the oxidation of organics at the anode by electroactive microorganisms is combined with abiotic production of H_2 at the cathode. For this process, AEMs have been shown to be better than CEMs in terms of electrochemical performance and in limiting the formation of pH gradients (Rozendal et al., 2008). The power input, usually regarded as the most important factor for determining the performance of such MEC systems, is negatively affected by a pH gradient over the membrane and therefore a membrane limiting the formation of a pH gradient is beneficial (Harnisch & Schröder, 2009).

The ohmic potential drop across the membrane also affects the power input. Lower transport resistance of ions in the AEM results in a lower ohmic potential drop (Sleutels et al., 2009). These pioneering studies highlighted the need to optimize the membrane: part of the charge is balanced by the back diffusion of cations, both for AEMs and BPMs (Harnisch & Schröder, 2009).

Despite their generally better performance, AEMs have not been widely used in BESs. Most MES studies make use of a reactor with CEM and only recently the use of an AEM for MES has been tested (Gildemyn et al., 2015). In a three-compartment setup with two membranes, the AEM, placed in between the cathode and extraction compartment (middle chamber), allows product extraction, avoiding product accumulation and subsequent product inhibition, and the CEM, placed in between the extraction an anode compartment, blocks anions to avoid unwanted oxidations at the anode, such as Cl_2 formation. In this reactor, a high acetic acid concentration of 13.5 g L^{-1} was obtained in the extraction compartment, at a current density of 5 A m^{-2}, corresponding to a coulombic efficiency (CE) of 61 % (Gildemyn et al., 2015). In this study the catholyte pH remained stable, as opposed to most MES studies with CEMs where a drop in pH is observed due to the anodic proton flux, subsequently leading to product diversification (Ganigué et al., 2015). BPMs have not yet been used for MES, and the effectiveness of particularly OH^- balancing in such systems is unknown. To avoid O_2 crossover and anodic Cl^- oxidation, the use of membrane(s) as separation barrier between anode and cathode appears preferred over setups without membrane (Harnisch & Schröder, 2009).

Given the limited knowledge on how the membrane would influence the MES process, both *via* pH control as *via* a Chatelier's principle based redirection of reactions upon extraction, we performed a direct comparison of the performance of reactors for MES of acetic acid with these three membrane types. The AEM was used in a three-compartment setup, with a CEM as barrier next to the anode to avoid unwanted oxidations, while a two-compartment setup was used to evaluate the use of a single membrane, either a CEM or BPM. The effect of the membrane type on MES bioproduction parameters and ion transport for charge balancing was evaluated.

2. Materials and methods

2.1. Reactor setup and operation

The experimental setup included three bioelectrochemical cells, constructed from Perspex plates and frames (Figure 5.1). All reactor compartments had a working volume of 0.2 L (internal dimensions: 5 × 20 × 2 cm^3). The three MES cells were constructed with identical materials, but differed by the membrane(s) used to separate the cathode compartment from the rest of the electrochemical cell. Depending on this membrane type, the cells were constructed with either two or three reactor compartments. The two-compartment configurations had a CEM (Fumasep FKB, Fumatech, Germany), or a BPM (Fumatech BPM, Fumasep, Germany) as separation barrier between the anode and cathode. The three-chambered reactor was evaluated using the same materials and experimental procedures as previously described (Gildemyn et al., 2015). The overall reactor setup is shown in the Supporting information (Appendix 4: Experimental setup for the reactor with in situ extraction). An AEM (Fumatech FAB, Fumasep, Germany) separated the catholyte and extraction compartment, while a CEM (Fumatech FKB, Fumasep, Germany) was used between the extraction compartment and the anolyte.

In all three reactors, acid/base-pretreated carbon felt was used as cathode material and a stainless steel frame placed in contact with the edges of the felt was used as current collector. The anode material was a dimensionally stable titanium-coated TiO_2/IrO_2 (35/65 %) mesh (Magneto Special Anodes BV, The Netherlands) and was placed in close contact to the membrane. All electrodes and membranes had a projected surface area of 0.01 m^2.

The homoacetogenic growth medium (catholyte) and all other electrolytes were prepared as previously described (Gildemyn et al., 2015). All compartments were operated in batch mode at a recirculation rate of approximately 60 mL min^{-1} to ensure sufficient mixing. To ensure sufficient CO_2 available for MES of acetic acid and to maintain anaerobic conditions at the cathode, the cathodic chambers were continuously purged with a N_2:CO_2 (90:10 %) gas flow. A mass flow controller (Bronkhorst, The Netherlands) or manual control valve (OMA-1, Dwyer, UK) was used to keep the gas flow rate at 30 ± 10 L d^{-1} in all reactors. The flow rates of N_2:CO_2 were monitored three times per week by water displacement measurements prior to sampling. The effluent gas from the cathode chamber was sent through the anodic or, if applicable, the extraction chamber to strip O_2 produced at the anode.

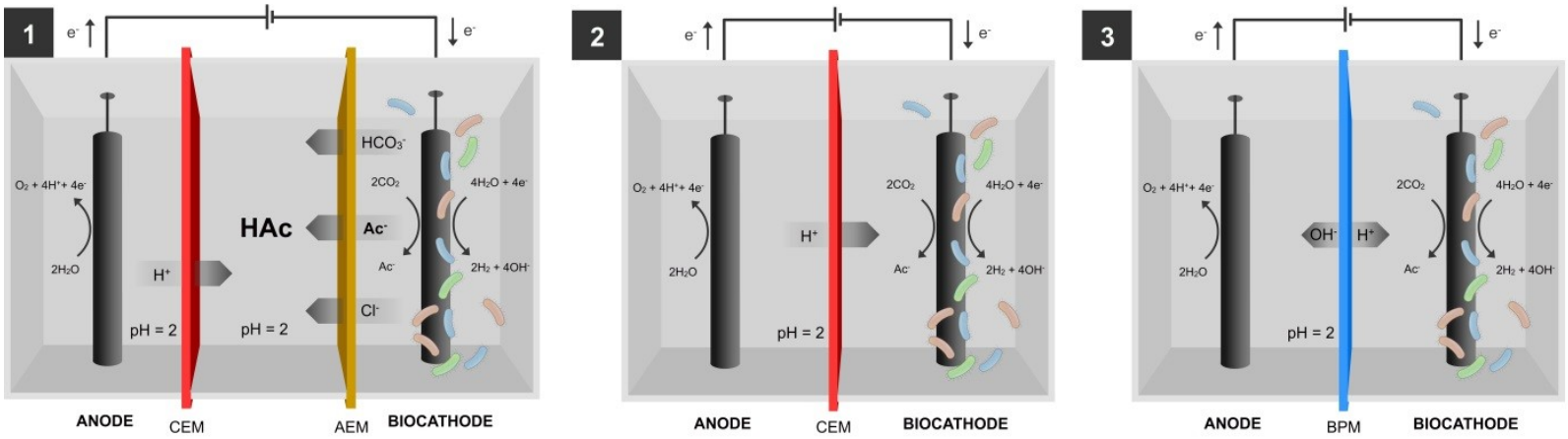

Figure 5.1 - Three different reactor systems for microbial electrosynthesis of acetic acid from CO_2 were operated. 1) Reactor with anion exchange membrane (AEM): setup that enables simultaneous bioproduction and extraction of acetic acid. An AEM separates the cathode and middle compartment and a cation exchange membrane (CEM) separates the anode and middle compartment. The middle compartment serves as the extraction compartment for recovery of acetate as acetic acid. 2) Reactor with CEM: conventional setup with CEM separating anode and cathode compartment. 3) Reactor with bipolar membrane (BPM): setup with BPM separating anode and cathode compartment.

The reactors were inoculated up to a final cell density of 3.0×10^6 viable cells mL^{-1} catholyte with a pre-enriched autotrophic acetate-producing mixed microbial community, dominated by *Clostridiales*, that produced no methane. The detailed procedure of the enrichment strategy as well as the microbial composition of the homoacetogenic inoculum can be found in Patil et al. (2015). Antibiotics were added weekly in the anodic and, if applicable, extraction compartment as a precaution to avoid microbial contamination and the associated consumption of carboxylic acids as described in Gildemyn et al. (2015). The experiments were conducted under anaerobic conditions, at room temperature (21 ± 2 °C).

Gas and liquid samples were taken three times per week from each reactor compartment for monitoring gas composition, short-chain carboxylic acids (SCCAs), alcohols, anions, cations, pH, conductivity and bicarbonate. All liquid removed during sampling was replaced with an equal amount of sterile anoxic stock solution. If pH dropped below 6, the pH of the catholyte was corrected with 1 M anoxic NaOH to ~ pH 7. The experiment consisted of one cycle of 43 days for all reactors. For abiotic control experiments with the reactor setup (current but no bacteria), as well as control experiments without current with the enriched culture, we refer to Gildemyn et al. (2015) and Patil et al. (2015). In none of these control experiments production of organic products or biomass was detected.

2.2. Electrochemical operation

The reactors were operated as three-electrode setups using the cathode as working electrode. A reference electrode (Ag/AgCl, 3 M KCl, + 0.21 V *vs.* SHE, BASi, France) was placed in the cathode compartment. The reactor was polarized for 24 hr at − 0.1 A m^{-2} before inoculation of the reactor. After polarization and inoculation, a fixed current density of − 5 A m^{-2} (corresponding to a reduction current of − 50 mA) was applied to the cathode using a potentiostat (VSP, BioLogic, France). Over the whole experimental period a total of 1.9 moles electrons was supplied as cathodic current, corresponding to a total charge of 1.84×10^5 Coulomb. Coulombic efficiencies for production were calculated as the ratio of electrons recovered in products and the total electrons supplied as current. The charge efficiency for extraction was calculated as the ratio of the charge transported as a specific ion through a membrane and the total electrical charge.

The electron transfer processes involved in the biocatalytic production of acetic acid were not studied. The reactor systems were considered as black boxes and only the productivity and efficiency were compared. For a detailed electrochemical analysis of the cathodic process with the same acetogenic culture and electrode material, we refer to Gildemyn et al. (2015).

2.3. Chemical analysis

Conductivity and pH were measured using a Consort EC and pH electrode respectively (Consort, Turnhout, Belgium). Total SCCA, alcohol, and inorganic anion concentrations were measured using ion chromatography as described by Gildemyn et al. (2015). Concentrations reported are total SCCA concentrations, unless mentioned differently. In the context of this article, carboxylic acids are reported in the acid form, even though these are fully or partially dissociated in reality. When specifically describing the charged species crossing the AEM, the anionic form will be used. Concentrations of undissociated acids were calculated based on the total SCCA concentration, medium pH, and the pKa of the acid. Sodium, potassium, magnesium and calcium were determined on a 761 Compact Ion Chromatograph (Metrohm, Switzerland) using a conductivity detector. The device was equipped with a Metrosep C6 – 250/4 column and a Metrosep C4 Guard/4.0 guard column. The eluent was 1.7 mM HNO_3 and 1.7 mM dipicolinic acid at a flow rate of 1.2 mL min^{-1}. Gas samples were analyzed for the presence of O_2, H_2 and CH_4 by gas chromatography as previously described (De Vrieze et al., 2013). Estimations of the bicarbonate concentration were made by adding 1 mL of catholyte to a vacuum tube containing 1 mL 1 M H_2SO_4. After intensive mixing, the headspace CO_2 was determined using Compact GC. Using a standard curve, bicarbonate concentrations could be determined from vacuum tube CO_2 contents. Ammonium was measured according to the standard Nessler method (Greenberg et al., 1992). Samples were filtered and diluted 20 times in demineralized water to fit in the working range of the method (0.1-5 mg NH_4^+-N L^{-1}). The color change from the Nessler reagent was colorimetrically monitored at a wavelength of 425 nm over a 1 cm path length using a UV-VIS spectrophotometer (Biochrom WPA Biowave, UK). The cell count for reactor inoculation was determined by viability staining method using flow cytometry (Van Nevel et al., 2013).

3. Results and discussion

3.1. Membrane type affects acetic acid production efficiency

To distinguish the effect of biocathodic production and membrane extraction of acetic acid separately, MES of acetic acid was directly compared in the three reactor configurations (Figure 5.1). Acetic acid production started soon after inoculation in all three reactors. In the reactor with CEM, production started on day 3, while the lag-phase was about 2 days longer for the reactor with BPM and AEM (extraction) (Figure 5.2). Acetic acid was the main SCCA produced in all three reactors (≥ 95 %). As H_2 was measured in the cathode off-gas, it is assumed that acetic acid production proceeded *via* indirect electron transfer and the Wood-Ljungdahl pathway (Drake et al., 2006).

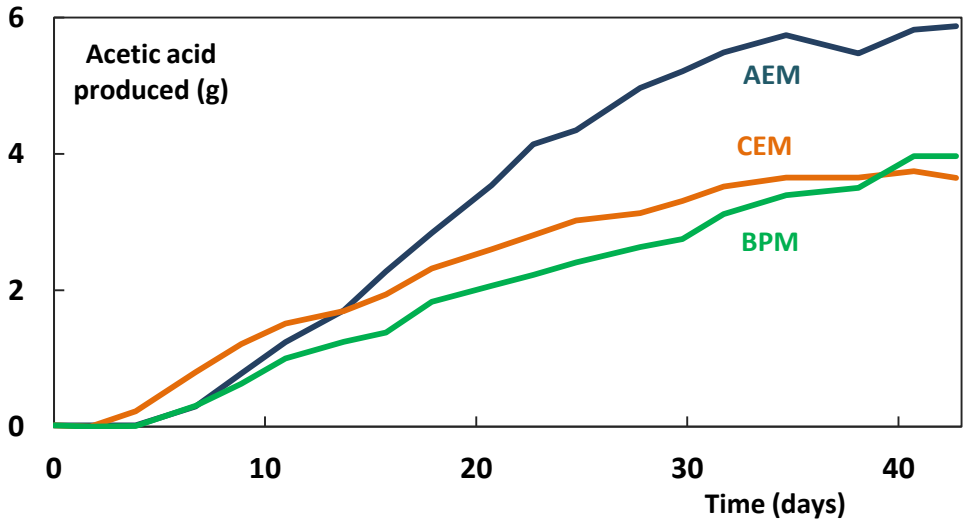

Figure 5.2 - The total mass of acetic acid produced was higher for the reactor with anion exchange membrane (AEM, blue line) compared to the two reactors without extraction: a reactor with cation exchange membrane (CEM, orange line) and bipolar membrane (BPM, green line).

From day 9 on all three reactors produced acetic acid at a relatively stable rate, which resulted in a total acetic acid production of 4.96 g, 2.44 g and 2.77 g by day 35 for the reactor with AEM, CEM, and BPM, respectively (Figure 5.2). This corresponds to a CE of 57 %, 28 %, and 32 % during stable operation, respectively (Table 5.1). After day 35, the production rates and efficiencies decreased for all three reactors. The acetic acid production resulted in

increased concentrations in the middle compartment of the reactor with AEM (extraction), up to 9 g L^{-1}, whereas the concentration in the catholyte remained below 1.5 g L^{-1}. In the catholyte of the reactor with CEM the concentration increased to 10.5 g L^{-1} and in the reactor with BPM to 8.3 g L^{-1} (Appendix 4: Acetic acid concentration per compartment). These concentrations do not reflect the true efficiency of the systems because of water displacement between the compartments. The electron balance for production can be further closed with the unused abiotically produced H_2, the presence of other SCCA, and other losses such as biomass production (Appendix 4: Carboxylic acid content per compartment, Appendix 4: Electron balance for MES). Biomass production was not closely monitored in this experiment. Methane was not detected in the gas effluent. Overall the behavior of the reactors was similar to the reactor with AEM previously described by Gildemyn et al. (2015). The lower productivity of the culture in this study (overall CE of 41 % *vs*. 61 % in previous study) could potentially be attributed to a loss of productivity due to the large number of transfers in serum flasks between the start of the two studies.

In contrast to the previous study with the configuration with AEM (Gildemyn et al., 2015), the AEM reactor in this study showed increased concentrations of formic acid in the middle compartment (Appendix 4: Carboxylic acid content per compartment). In the reactors with CEM and BPM, formic acid concentrations remained below 30 mg L^{-1}. Due to extraction, the formic acid concentration in the middle compartment of the reactor with AEM increased to 1.2 g L^{-1}. Production of formic acid in the reactor with AEM stopped after 10 days. Formic acid is an intermediate of the Wood-Ljungdahl pathway. It is often measured in autotrophic fermentations, and has been reported as side-product of MES as well (Nevin et al., 2011; Peters et al., 1999).

The productivity of the reactor with AEM was at least 32 % higher than for the reactors without extraction (Table 5.1). The pH of the catholyte in the reactor with AEM remained stable and high throughout the experiment (8.16 ± 0.15), while the pH dropped in the reactors with CEM and BPM, which likely negatively affected the culture (Figure 5.3, Table 5.1). A low pH has an inhibitory effect on homoacetogenic acetic acid production due to end-product inhibition (Menzel & Gottschalk, 1985). The drop to pH 5.4 on day 11, for example, was associated with concentrations of undissociated acetic acid of 740 mg L^{-1} and 470 mg L^{-1} for the CEM and BPM reactor, respectively (Appendix 4: Undissociated acetic acid concentrations). For the CEM reactor this also corresponded to the maximal undissociated

acetic acid concentration throughout the experiment. The maximal undissociated acetic acid concentration in the BPM reactor (1800 mg L^{-1}; 30 mM), calculated based on total SCCA concentrations and pH, was obtained on day 28 of the study when the pH dropped to 5.13. This concentration of undissociated acetic acid corresponds to the levels reported by LaBelle et al. (2014), at which no further acetic acid production was observed, corresponding to a peak in H_2 production as homoacetogenic metabolism was inhibited. In the catholyte of the reactor with AEM, the concentration of undissociated acetic acid remained low and never exceeded 1 mg L^{-1}, due to the combined effect of the higher pH and the extraction. The drops in pH in the CEM and BPM reactor were compensated by dosage of 1 M NaOH in the reactor after each sampling. Production of acetic acid was maintained, but at lower levels than the reactor with AEM, and at the cost of base addition. A total of 46 and 69 mL 1 M NaOH was added in the catholyte of the CEM and BPM reactor, respectively, over the total duration of the experimental run. Product diversification to ethanol or longer chain carboxylic acids was not observed, while this was the case in other studies were the pH was not controlled (Ganigué et al., 2015). Also for H_2/CO_2 gas fermentations, pH control is an important strategy to avoid decreased productivities due to undissociated acetic acid buildup. This has resulted in acetic acid concentrations as high as 44 g L^{-1} at pH 7 in pressurized reactors (Demler & Weuster-Botz, 2011). In this perspective, the reactor with AEM seems more promising to obtain high-rate production and higher product concentrations without dosage of chemicals (NaOH), or the need for a continuous mode operation, which dilutes the end-product.

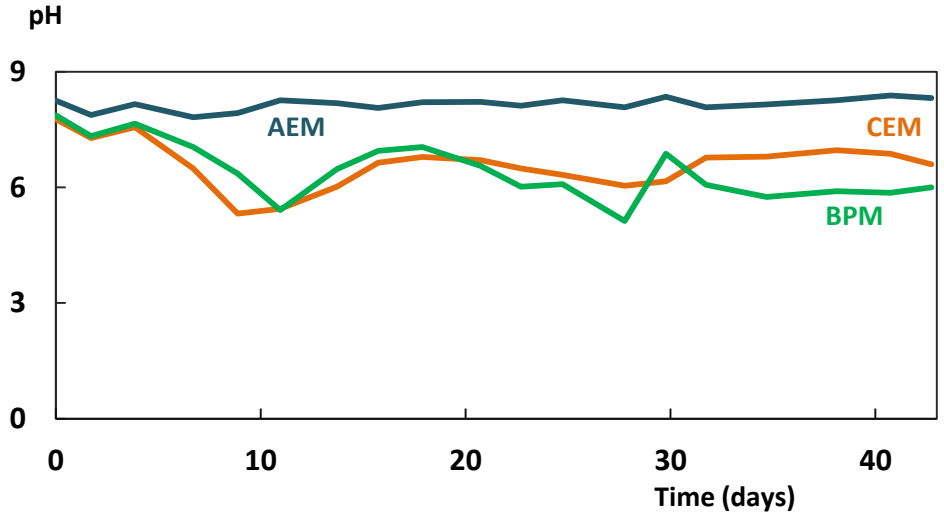

Figure 5.3 - The catholyte pH in the reactor with anion exchange membrane (AEM, blue line) remained stable while drops in catholyte pH were observed for the reactor with cation exchange membrane (CEM, orange line) and bipolar membrane (BPM, green line).

Table 5.1 - Critical performance parameters for the three reactor types: Coulombic efficiency for acetic acid production (overall and for the stable operation period day 9 - 35), production rates, addition of base per mass of acetic acid produced and product recovery (as extraction efficiency). AEM: anion exchange membrane; CEM: cation exchange membrane; BPM: bipolar membrane; stdev: standard deviation; na: not applicable.

Parameter	AEM	CEM	BPM
Overall CE for acetic acid (%)	40.9	25.4	27.7
CE stable operation (%)	57.2	28.1	31.9
Production rate (g m^{-2} d^{-1})	13.8	8.5	9.3
Production rate stable operation (g m^{-2} d^{-1})	19.2	9.5	10.7
pH catholyte (average ± stdev)	8.16 ± 0.15	6.58 ± 0.62	6.44 ± 0.75
NaOH addition (g g^{-1} acetic acid)	0	0.50	0.69
Power input stable operation (kWh kg^{-1} produced)	26.5	44.3	30.7
Extraction efficiency (%)	97.5	na	na

3.2. *In situ* extraction, an intrinsic property of a reactor with AEM

Acetic acid was produced in the three reactor systems, but only the reactor with AEM had the intrinsic ability to extract the produced acetic acid, as acetate, in a separate extraction compartment (Gildemyn et al., 2015). The main product of MES, acetic acid, is present as the negatively charged species, acetate, in the cathode compartment. This ion can therefore contribute to charge balancing, when it crosses the AEM. The low pH of the extraction compartment (1.76 ± 0.12) resulted in protonation of the acetate. At 100 % efficiency for acetic acid production and extraction, a maximum charge balancing efficiency by acetate of 12.5 % can be obtained, as 8 electrons are required for the production of 1 acetate molecule. In this study, acetate transport across the AEM accounted for 5.0 % of the charge balancing, indicating that the charge efficiency for extraction is limited by the efficiency for production. During the experimental run, 97.5 % of the produced acetic acid was extracted. As a consequence of this efficient extraction, the acetic acid concentration in the catholyte remained below 1.5 g L^{-1} for most of the experimental run (Appendix 4: Carboxylic acid content per compartment). Combined with the stable and relatively high catholyte pH, product inhibition did not occur.

3.3. Technology development: perspectives for the use of an AEM

Production rates and power input are crucial parameters for MES technology development. The highest rates were obtained in the reactor with AEM, during stable operation (19.22 g m^{-2} d^{-1}, Table 5.1), which is 21 % lower than our previous study with the same inoculum and reactor type (Gildemyn et al., 2015). Patil et al. (2015) obtained similar production rates with the same inoculum, but in a different reactor type (glass reactor with CEM). Due to the variability of reactor designs, operational conditions and measured variables, a comparison with other MES studies is not straightforward (Patil et al., 2015c). The acetic acid production rates decreased for all three reactor types by the end of the 43-day production cycle, after day 35. Methane and ethanol were not produced and no increase in O_2 was measured compared to the beginning of the experimental period. A limitation of nutrient availability might have caused the decreased productivity even though after sampling an equal volume of fresh catholyte was added in the reactors. Despite these additions, the concentration of NH_4^+ had decreased to 10 mg L^{-1} for the reactor with AEM, and < 1 mg L^{-1} for the reactor with CEM and BPM at the end of the experimental period (data not shown). Growth media for homoacetogens usually contain 100 to 130 mg L^{-1} NH_4^+ to not limit growth (Drake et al., 2006). Further research is required to monitor the nutrient availability and examine the possibility of addition of more concentrated medium after sampling during batch mode operation.

Maintaining high productivities at a low power input is critical for the process economics. The power input for the reactor with AEM was 26.5 kWh kg^{-1} acetic acid produced (Table 5.1), which is higher compared to our previous report (Gildemyn et al., 2015). This is partially due to the lower production rate, but also results from the higher operating cell potential in this experiment (4.2 ± 0.2 V) related to the quite broad middle compartment. A similar conclusion can be drawn for the CEM reactor (44.3 kWh kg^{-1} for 3.5 ± 0.2 V) and BPM reactor (30.1 kWh kg^{-1} for 2.7 ± 0.05 V). A higher operating cell potential can be expected for the reactor with extraction due to the presence of two membranes, increasing the distance between the electrodes and adding membrane resistance. However, due to the higher production rate, the reactor with AEM produced acetic acid at the lowest power input/cost. The higher operating voltage for the CEM compared to the BPM reactor is counterintuitive. Systems with BPMs typically require a larger power input due to the water dissociation reaction on the membrane (Harnisch et al., 2008) and the conductivity of the electrolytes was higher for the CEM reactor (Figure 5.4). Ohmic losses in the reactor with CEM, that were not

measured in this study (*e.g.* membrane resistance), could have caused the higher required power input for the CEM reactor (Clauwaert et al., 2008).

3.4. Ion balances: crucial role of HCO_3^-

Electrochemical systems require charge balancing, which is steered by the use of ion selective membranes. In total 1.9 moles of electrons were transported as current as a result of water electrolysis in each reactor of this study. To make ion balances, Cl^-, SO_4^{2-}, PO_4^{3-}, Na^+ and K^+ -transport was taken into account, besides transport of H^+, OH^- and HCO_3^-. This last ion, HCO_3^-, acts as a buffer in the catholyte. At low pH, the carbonate system equilibrium shifts towards CO_2 and, therefore, the impact of HCO_3^- on charge balancing is difficult to quantify.

Different charge balances were expected for each reactor type. In principle, AEMs allow anions to cross, CEMs cations, and BPMs induce water splitting. Studies with MECs already showed that in practice, ions with the opposite charge can also cross the membrane, and back-diffusion of H^+ or OH^- ions can take place (Harnisch & Schröder, 2009). In all three reactors, the conductivity of the catholyte stabilized by the end of the experiment, implying that mainly H^+/OH^- and HCO_3^- were responsible for charge balancing, rather than other ions (Figure 5.4) (Sleutels et al., 2009). The increase in conductivity in the CEM and BPM reactor between day 10 and 30 is mainly due to NaOH dosing to counter the pH decrease.

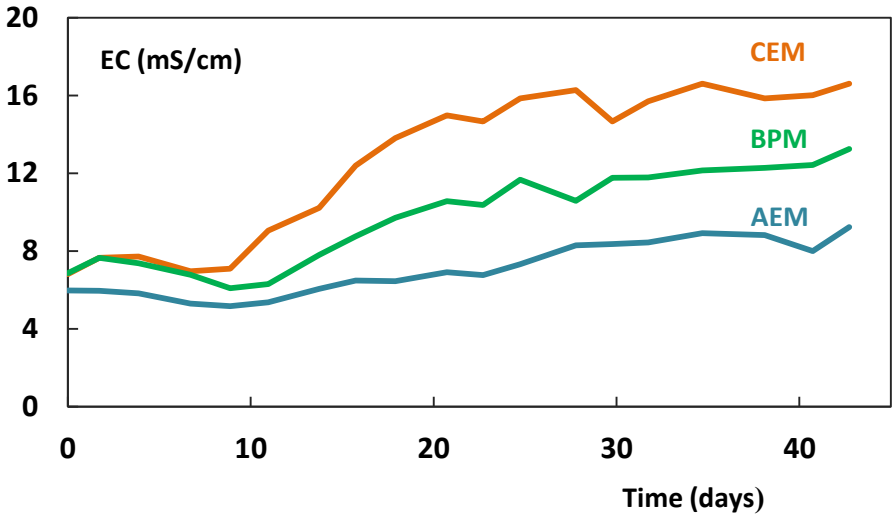

Figure 5.4 - Conductivity (EC, mS cm^{-1}) of the catholyte of the three reactor types (anion exchange membrane (AEM, blue line); cation exchange membrane (CEM, orange line) and bipolar membrane (BPM, green line)) stabilized over time, indicating that H^+/OH^- and buffer species were mainly involved in charge balancing.

In the reactor with AEM, acetate transport from the cathode to the middle compartment accounted for only 5.0 % of the charge balancing: 95.5 mmoles acetate was extracted, for a total of 1.9 moles electrons as current (data not shown). Other anion salts balanced only 1.6 % of the total charge during the 43 day-experiment. Cl^- was preferably transported in the first hours of the test, despite the higher molar concentration of HCO_3^-, which is related to the lower electrical mobility of this last ion compared to Cl^- (Block & Spiegler, 1963). The immediate transport of Cl^- from the medium might have caused the slower startup and formic acid production, as the medium composition differed from growth medium during preculturing. The concentration of Cl^- remained below 2.4 mM from day 2 on, and most likely HCO_3^- (> 50 mM) played a major role in charge balancing between the cathode and middle compartment for most of the experimental period (data not shown). The molar concentration of HCO_3^- in the catholyte was more than 30,000 times higher than the OH^- concentration. The concentration of HCO_3^- remained relatively stable in the catholyte despite the continuous CO_2 sparging and high pH (8.16 ± 0.15), which indicates that HCO_3^- was transported through the membrane. In preliminary experiments (data not shown) an increased CO_2 concentration in the off gas of the extraction compartment compared to the catholyte off-gas had been measured as a result of CO_2 stripping. The total amount of protons

in the middle compartment increased over time, which also supports the hypothesis that HCO_3^- was preferably transported compared to OH^- (Appendix 4: Reactor charge balances).

In the CEM setup, the results indicate that H^+ transport was responsible for most of the charge balancing. Na^+ transport could account for a maximum of 1.7 % of the charge balancing, based on measurements of the anolyte Na^+-concentration, while based on pH measurements H^+ transport was responsible for 98 % of the charge balancing (data not shown). Taking into account measurement errors, this charge balance can be considered closed (Appendix 4: Reactor charge balances). Especially in the second half of the experimental run, the high concentration of H^+ (~100 mM) in the anode compared to other cations (Na^+ below 40 mM; data not shown), combined with a higher mobility for H^+, likely resulted in preferential transport of H^+ (Okada et al., 1998). The role of HCO_3^- in the charge balance is unclear in this case. Transport of this anion over the CEM should in principle be limited. However, there is also dissolved CO_2 present in the cathode which can diffuse. The concentration of HCO_3^- in the catholyte remained relatively stable over time, and probably there was a balance between losses through stripping when the catholyte pH decreased and additional dissolving when the pH increased. Diffusion of HCO_3^- towards the anolyte, followed by stripping, was not measured.

In the reactor with BPM, non-ideal ion cross-over was minimal, with Na^+ transport accounting for 0.8 % of the charge balancing. Transport of salt ions was mainly concentration-driven, with minimal amounts of SO_4^{2-} and Na^+ moving towards the cathode and Cl^- towards the anode. Water dissociation on the BPM was thus the dominant process. The increase of the H^+ concentration in the anolyte due to water dissociation in the BPM corresponded to only 1.0 % of the charge generated, showing that OH^- production at the BPM was efficient (Appendix 4: Reactor charge balances). Regarding HCO_3^- transport, the conclusions made for the CEM reactor are also valid for the BPM reactor. Both in the CEM and BPM reactor, the H^+ transport/generation to the cathode compartment contributed to the catholyte acidification, augmenting the acidifying effect of acetic acid production *via* MES.

Overall, the use of HCO_3^- as buffer *via* CO_2 sparging is an advantageous strategy. As acetate can only account for a maximum of 12.5 % of charge balancing, the presence of another anion at elevated concentration is required to sustain the fixed current. HCO_3^- transport allows efficient charge balancing in the reactor with AEM while the catholyte pH

can be kept at a physiological pH. For long term reactor experiments the use of other buffers, such as $HPO_4^{2-}/H_2PO_4^-$, in combination with an AEM would not be feasible. This would require a continuous addition of salts, which ultimately leads to more complex wastewater treatment (Lefebvre & Moletta, 2006). Also for MFCs, CO_2/HCO_3^- has been shown to be an efficient buffer, by limiting the pH imbalance between the anolyte and catholyte, and by increasing the conductivity of the electrolytes without the addition of phosphate salts (Fornero et al., 2010). Usage of CO_2/HCO_3^- as buffer and for charge balancing, besides carbon source, does imply that the CO_2 conversion efficiency can never reach 100 %. To obtain a high conversion rate, which is a prerequisite for an economically feasible MES process, a surplus of CO_2 would in any case be needed to drive reaction thermodynamics. Through the HCO_3^- transport over the AEM and consequent stripping of CO_2, purification of this gas is in fact also obtained, which might lead to additional applications and increased sequestration efficiencies.

3.5. Critical evaluation of the AEM reactor technology

The reactor with AEM offers several advantages for MES. The *in situ* extraction of acetic acid enhanced the performance of MES up to 32 % in comparison with the reactors without product recovery. This performance enhancement was obtained through the combined effect of product recovery and *in situ* pH control. Certain aspects of the technology will however need to be improved. First, water displacement through the AEM decreased the product concentration in the extraction compartment. For downstream processing of the acetic acid stream, high concentrations are favorable (Andersen et al., 2016). Water displacement took place at an average rate of 0.5 L m^{-2} d^{-1}. Water displacement across membranes is driven by the salinity gradient and intrinsic membrane properties (Nagarale et al., 2006). The salinity gradient between the cathode and extraction compartment in the three-compartment setup was the main driver for water displacement. The 4-fold concentrated salt solution used as medium in the extraction compartment is intended to favor extraction of acetate over other salts but also contributes to water displacement. Most of the salt anions were however extracted from the catholyte to the extraction compartment at the start of the test, during the lag-phase for acetate production. The use of electrolytes with a similar salinity should be tested. It can be expected that the water flux does not proportionally increase with current density, hence at higher densities the impact of this water flux should be lessened (Indusekhar & Krishnaswamy, 1985). The impact of water transport on larger scale reactor systems will require further research. In the proposed

configuration, HCO_3^- transport over the AEM is furthermore highly important for charge balancing. The relatively low mobility of this ion compared to for example Cl^-, correlates to a higher electro-osmotic water transport (Block & Spiegler, 1963).

Second, the pH equilibrium in the cathode compartment is a fragile equilibrium. At higher current densities or in the absence of the buffering effect of CO_2 sparging, an increase in pH can be expected (Gildemyn et al., 2015). Higher CO_2 sparging rates can be considered, but would not contribute to CO_2 sequestration. Alternatively, the anolyte stream could be used to control the pH, as acid is continuously generated through water electrolysis. Salt should be added to the anolyte in the case that the conductivity would drop, but also the impact of this salt addition on the composition of the catholyte should be evaluated. For example, the use of Na_2SO_4 as anolyte would result in the dosing of SO_4^{2-} in the catholyte, which could result in the presence of sulfate reducing bacteria in mixed culture systems. This could lead to competition for H_2 as energy carrier, or consumption of acetic acid as substrate for heterotrophs.

Third, and as a general remark for MES, the energy input for acetic acid production, here 26.5 kWh kg^{-1} which is the lowest input of the three reactor systems, needs to be drastically reduced. Currently, acetic acid production *via* the Monsanto process requires 4 kWh kg^{-1}, when taking into account the intermediate methanol production step. This energy is usually obtained from fossil fuel resources (Ecoinvent, 2007). This process however results in a 98 % pure acetic acid stream, while here, the energy input was calculated for a 1 % acetic acid stream. An increase in conductivity of all media would be beneficial to decrease the cell voltage. Due to the configuration with two membranes, the AEM reactor would however always require a substantial energy input. The use of renewable electricity would be essential to achieve a sustainable process.

Overall, for the study of fundamental aspects of MES, such as electron transfer processes, the simple CEM or BPM design is more advantageous. Product inhibition for these studies can be avoided by: i) selecting a low surface-to-volume ratio; ii) running a continuous reactor; or iii) pH control. The integrated extraction by membrane electrolysis will possibly be an important achievement in the complete bioproduction pipeline enabling a zero-chemical-input process.

4. Conclusions

The MES process in a reactor system with *in situ* product extraction resulted in 32 % higher production rates and efficiencies and the recovery of a separated product. Stable operation without chemical dosing for pH stabilization is an advantage of this AEM extraction cell compared to operation with a CEM of BPM. Besides acetate, the charge balance showed that HCO_3^- was mainly extracted in the AEM reactor. H^+/OH^- was responsible for >98 % of the charge balancing in the CEM and BPM reactor. With increased rates and decreased energy input, this AEM reactor technology can become an integrated CO_2-based production pipeline.

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CHAPTER 6

Membrane electrolysis assisted gas fermentation for enhanced acetic acid production

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Abstract

Gas fermentation has rapidly emerged as a commercial technology for the production of low-carbon fuels and chemicals from (industrial) CO and/or CO_2-rich feedstock gas. Recent advances in using CO_2 and H_2 for acetic acid production demonstrated that high productivity and substrate utilization are achievable. However, the costly constant addition of base and the energy-intensive nature of conventional recovery options (*e.g.* distillation) need to be overcome to drive organic acid production forward. Recently, membrane electrolysis has been presented as a technology that enables for the direct extraction of carboxylates across an anion exchange membrane into a clean and low pH concentrate stream. Continuous *in situ* extraction of acetate directly from the catholyte of a microbial electrosynthesis reactor showed that membrane electrolysis allows pure product recovery while improving productivity. Here we demonstrate that the system can be further enhanced through additional input of electrolytic hydrogen, produced at higher energetic efficiency while improving the overall extraction efficiency. A gas-lift reactor was used to investigate the hydrogen uptake efficiency at high hydrogen loading rates. During stable operation acetate transport across the membrane accounted for 31% of the charge balancing, indicating that the use of external H_2 can lead to a more efficient use of the extraction across the membrane. By coupling membrane electrolysis with the gas fermentation reactor the pH decrease associated with H_2/CO_2 fermentations could be prevented, resulting in a stable and zero-chemical input process (except for the CO_2). This now enables us to produce more than 0.6 M of acetic acid, a more attractive starting point towards further processing.

1. Introduction

In recent years, microbial electrosynthesis (MES) has emerged as a promising bioreactor technology for the production of multi-carbon compounds from CO_2 and renewable electricity (Logan & Rabaey, 2012; Rabaey & Rozendal, 2010). This electricity-driven CO_2-conversion process uses the cathode of a so-called bio-electrochemical system to supply the reducing equivalents (in the form of electrons and/or H_2) for reducing CO_2 in the Wood-Ljungdahl pathway (May et al., 2016). Thus far acetic acid has been the main natural end-product of acetogenic metabolism in MES (Bajracharya et al., 2016; Chen et al., 2016; Jourdin et al., 2016a; Jourdin et al., 2015; Marshall et al., 2012; Nevin et al., 2011; Nevin et al., 2010; Patil et al., 2015; Song et al., 2018), but recent reports have demonstrated the production of higher-value organics like isopropanol (C3) (Batlle-Vilanova et al., 2017), butyric acid (C4) (Arends et al., 2017) and caproic acid (Vassilev et al., 2018) from CO_2 feed. Since its first description in 2010 (Nevin et al., 2010), considerable advancements in MES performance have been achieved, but today production rates, energy efficiencies and product titers are far too low to push MES forward as an industrial relevant platform for CO_2-based bioproduction (Desloover et al., 2012b). Since production rates are ultimately limited by the applied current, it is essential to engineer MES systems that have the ability to deal with high electron supply rates at a high conversion efficiency and low power input (Gildemyn, 2016).

Gildemyn and co-workers have already demonstrated the advantages of using membrane electrolysis (ME) for MES. This approach can uniquely couple the production and recovery of acetic acid through *in situ* product extraction across an anion exchange membrane (AEM) using nothing but an electrical current (Andersen et al., 2014; Gildemyn et al., 2015). The use of an AEM for MES can simultaneously separate, concentrate and acidify the product as a single organic acid in a solid-free extraction liquid, while enhancing performance through the combined effect of product recovery and *in situ* pH control (Gildemyn et al., 2017c). To date, the integrated MES approach for production and extraction is limited in terms of: (i) production rate; (ii) efficiency for electrons used for acetic acid recovery; and (iii) energy input requirements for acetic acid production. At best 40% of the electrons ended up in residual H_2 during MES experiments at 5 A m^{-2} applied current density, indicating that the transfer of reducing power to the homoacetogenic culture needs optimization (Gildemyn et al., 2015; Patil et al., 2015). At 100% efficiency for production and extraction, acetate transport can at most account for 12.5% of the charge balance, as 8 moles electrons are required per mole of acetic acid produced, while extraction of the monovalent acetate ion

(theoretically) only requires one electron. Since the extraction efficiency is limited by the production rate, acetate experimentally accounts for only 5-8 % of the charge balancing (Gildemyn et al., 2017c; Gildemyn et al., 2015). Most of the charge is thus balanced by other anions, mainly HCO_3^-. It should be recognized that the full extraction capacity of the reactor can only be utilized if additional acetic acid is produced with externally supplied reducing equivalents (as hydrogen gas). We thus proposed an improved design where acetate production from an external H_2 source is linked to an extraction reactor providing only 12.5% of the total load of reducing equivalents, aimed at enhancing extraction efficiency at a lower power input. An alternative embodiment for this would be the extraction of acetate from an organic side stream in combination with additional acetate production using the cathodic hydrogen.

Considering the aforementioned aspects, the focus of the present study was to investigate the impact of additional H_2 injection in an external fermenter on: (i) the current efficiency for acetate extraction; (ii) the final acetic acid concentration in the extraction liquid; (iii) the acetic acid production rate of the integrated MES-extraction approach; and (iv) the energy input for acetic acid production. Operation of the MES reactor was modified by coupling it to a bubble-column fermenter and adding externally produced H_2 to the reactor system to increase both H_2 retention time in the aqueous medium and productivity. Accordingly, this work reports on the development of a platform for CO_2 conversion based on existing gas fermentation technology coupled to membrane electrolysis as a tool for product recovery and pH control. Use of CO_2 as a raw material for large scale bioproduction will require proper integration of autotrophic biotechnology to fully exploit the intrinsic power of CO_2-based bioproduction.

2. Materials and methods

2.1. Reactor setup and operation

The experimental setup included a three-chambered electrochemical cell, a two-chambered water electrolyzer and a custom-made glass bubble-column reactor (Figure 6.1). The three-chambered reactor consisted of three identical Perspex frames with a working volume of 0.2 L per chamber (20 × 5 × 2 cm inner dimensions). The anode compartment contained a 50 mM Na_2SO_4 solution as electrolyte (adjusted to pH 2 with sulfuric acid) and a 20 × 5 cm MMO-coated titanium mesh electrode (Magneto Special Anodes BV, The Netherlands). The cathode compartment contained a modified homoacetogenic medium (pH 7.7) as described by Gildemyn et al. (2015) and a carbon felt electrode (100 cm^2 projected surface area, thickness of 3.18 mm, Alfa Aesar, Germany) with a stainless steel frame current collector. The initial volume of the catholyte was 1 L with the bubble-column reactor positioned in the recirculation loop op the cathode chamber. The electrolyte in the extraction compartment consisted of a 4-fold concentrated salt solution containing the same salts as the catholyte, adjusted to pH 2 with H_2SO_4. The initial working volume of the anolyte and extraction medium was 0.35 L, including an external recirculation flask. The anode and extraction compartments were separated by a cation exchange membrane (Fumatech FKB, Fumasep, Germany), while an anion exchange membrane (Fumatech FAB, Fumasep, Germany) was placed in between the cathode and extraction chambers. All compartments were operated in batch mode during the entire experimental period (86 days) and recirculated at approximately 50 mL min^{-1}. A N_2/CO_2 mixture (90/10%, v/v) was continuously bubbled into the cathode compartment at a flow rate of 28.5 ± 12.4 L d^{-1}. The reactor was operated as a three-electrode setup using the cathode as working electrode and placement of a reference electrode (Ag/AgCl, 3 M KCl, + 210 mV vs. SHE, BASi) in the catholyte. A fixed reductive current of − 50 mA (corresponding to a current density of − 5 A m^{-2}) was used to facilitate electrosynthesis by means of potentiostatic control (VSP, BioLogic, France).

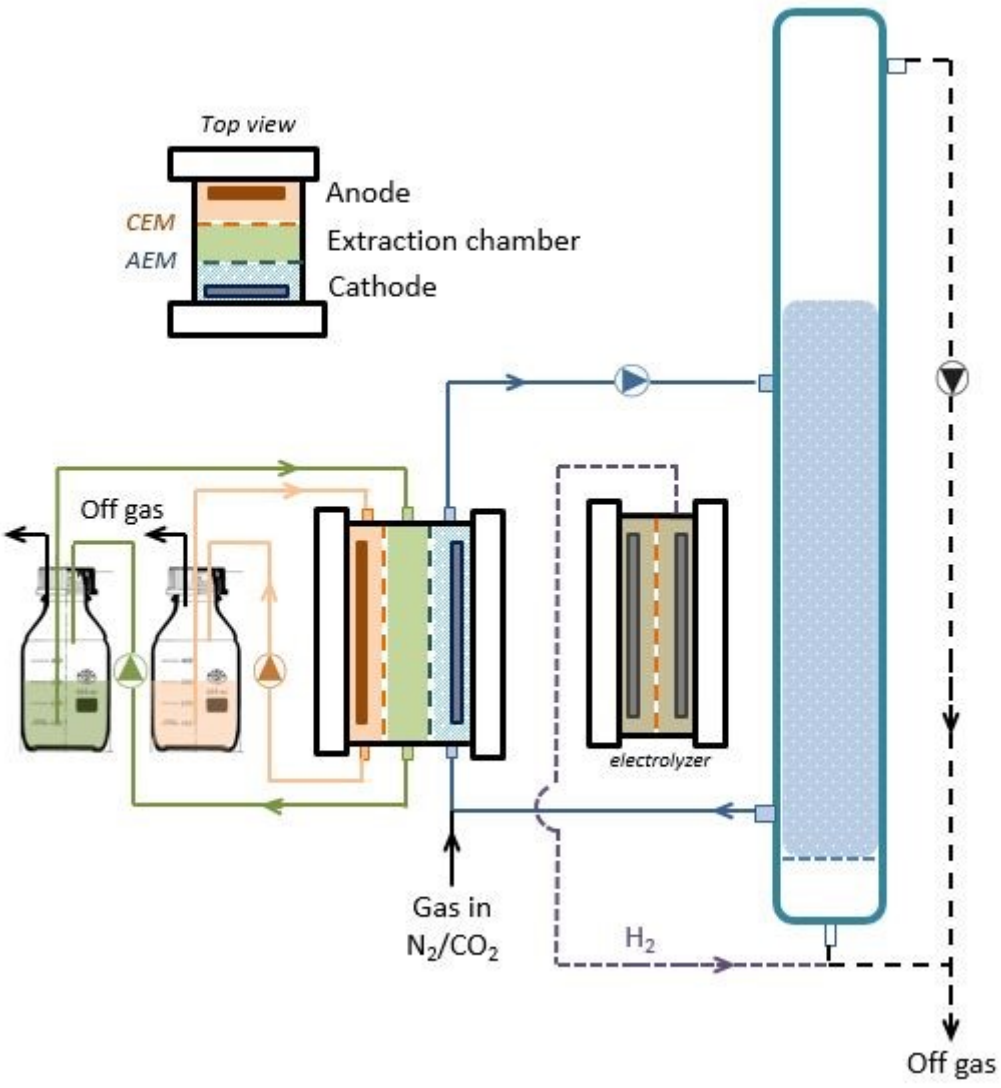

Figure 6.1 - Bubble column reactor setup for simultaneous biological production and extraction of acetic acid from CO_2 and electrical current through a hybrid microbial electrosynthesis-gas fermentation approach. An external fermenter is fed with additional hydrogen from a water electrolyzer to increase acetate production rates. Full black lines show liquid streams, dotted lines gas streams.

An additional two-chambered electrochemical cell was constructed using identical materials except that a stainless steel mesh was used as cathode material and a 0.5 M Na_2SO_4 solution was used as electrolyte in both reactor chambers. Both electrolytes were

recirculated over a buffer vessel at high speed (~100 mL min^{-1}) to ensure proper mixing. The cell was operated as a water electrolyzer (with CEM) at a fixed current density of − 35 A m^{-2} (0.35 A), producing H_2 gas that was sparged into the bubble column through anaerobic tubing.

The bubble-column was a cylindrical reactor with a volume of 2 L (1 m height, 5 cm internal diameter) and an integrated sintered glass to allow fine bubble dispersion. Connection to the MES cell was established through glass nipples on the side of the column. An ATEX gas pump (KNF Verder, Belgium) was installed to intensively recirculate the headspace gas through the fermentation medium (~ 15 L min^{-1}). The same experimental procedures as described in Gildemyn et al. (2015) were used. Any liquid removed during sampling as well as liquid lost *via* electro-osmosis was replaced with an equal amount of sterile anaerobic stock solution. The experiments were conducted under anaerobic conditions, at room temperature (21 ± 2 °C). The reactor setup (electrochemical cell + column) was inoculated at the start of the experiment up to a final cell density of ~ 10^5 viable cells mL^{-1} fermentation broth with a pre-enriched autotrophic acetate-producing mixed microbial community that was used in previous MES experiments (Gildemyn et al., 2017c; Patil et al., 2015). Gas and liquid samples were taken three times per week from each reactor compartment for monitoring gas composition, VFAs, alcohols, anions, cations, pH, conductivity and bicarbonate. Water transfer was estimated based on the volume changes in the different recirculation vessels. The flow rate of N_2/CO_2 was monitored by water displacement measurements prior to sampling. For abiotic control experiments (current but no bacteria as well as bacteria but no current) we refer to Gildemyn et al. (2015) and Patil et al. (2015) since these studies showed that in both control experiments no production of organic products or biomass was detected.

2.2. Analytical procedures

Conductivity and pH were determined according to standard methods. VFAs, alcohols and inorganic anions were measured using ion chromatography as described in Gildemyn et al. (2015). Sodium, ammonium, potassium, magnesium and calcium were determined on a 761 Compact Ion Chromatograph (Metrohm, Switzerland) using a conductivity detector. The device was equipped with a Metrosep C6 – 250/4 column and a Metrosep C4 Guard/4.0 guard column. The eluent was 1.7 mM HNO_3, 1.7 mM dipicolinic acid. Gas samples were analyzed

for the presence of N_2, O_2, H_2 and CH_4 by gas chromatography using a Compact GC (Global Analyser Solutions, Breda, The Netherlands) equipped with a thermal conductivity detector.

2.3. Data representation

The calculations for the volumetric acetic acid production rate [based on the fermentation broth volume (g L^{-1} d^{-1})], electron recovery and energy efficiency are based on the methods described in Patil et al. (2015). The calculation of electron recovery in unutilized H_2 is based on the residual H_2 concentration in the off-gas from the bubble column reactor. The extraction efficiency is defined as the ratio of extraction rate to production rate (of the whole system, electrolyzer + MES), while the charge balancing efficiency is defined as the ratio of the charge transported as a specific ion through a membrane and the total electrical charge of the extraction cell. Only the power input for electrochemical reactions (water splitting) is taken into account for specific energy input calculations. Gas recirculation was excluded from this calculation as the experimental power consumption to obtain a certain gas-liquid mass transfer rate is not representative for the absorbed power values in industrial scale reactors, that typically use compression rather than gas recirculation to obtain high gas conversion efficiencies.

In an abiotic test preceding the inoculation, the hydrogen production rate of both the MES cell (operated at a fixed current density of – 5 A m^{-2}) and the electrolyzer (operated at a fixed current density of – 35 A m^{-2}) was quantified. With a combined hydrogen gas flow rate of 3.5 ± 0.2 L day^{-1} leaving the reactor, the electron balance could be closed for 86.4 ± 0.1%, indicating some loss through tubing, connectors and sampling ports (provided 100% current efficiency for H_2 production).

3. Results and discussion

3.1. Additional hydrogen injection enhances acetic acid productivity

Production of acetic acid by the microbial community in a galvanostatic operated MES reactor started 10 days after inoculation. The longer lag-phase in this study (3 – 5 days in our previous studies) could potentially be attributed to the lower initial biomass concentration and the larger reactor volume. Once acetogenic activity started, gas recirculation was activated to improve the H_2 mass transfer to the fermentation broth. A cathode potential of -1.21 ± 0.07 V vs. SHE was recorded during the experiment. Carbon fixation *via* homoacetogenesis allowed for a sustained increase in the concentration of acetic acid throughout the test. Acetic acid gradually accumulated in the extraction chamber, reaching 37.0 g L^{-1} (617 mM) on day 86 (Figure 6.2A). This is the highest titer of acetic acid reported so far for MES from CO_2 feed. From day 56, acetate concentration in the catholyte remained fairly constant (4.1 ± 0.6 g L^{-1}), while the concentration in the anolyte rose to reach 13.7 g L^{-1} by the end of the cycle. For the whole 86 days operation, the average acetic acid production rate was 0.76 g acetate L^{-1} d^{-1}. Higher carbon fixation rates (1.48 g L^{-1} d^{-1}) were observed during stable operation (from day 37 to 65), whereas a maximum value of 3.54 g L^{-1} d^{-1} can be reported. These results confirm that an 8 times higher H_2 feeding rate and a higher H_2 retention time (~ 1 h by continuous recirculation of the H_2 headspace through the fermentation medium) resulted in 2.6 to 4.1 times higher acetic acid concentration and 2.1 to 2.7 times higher volumetric productivity compared to our previous studies (Gildemyn et al., 2015).

Acetic acid accounted for 99.8% of all organic compounds present at the end of the experiment (as carbon, sum of products in all reactor compartments). Other carboxylates such as formate, propionate and butyrate were present but only in low concentrations (< 50 mg L^{-1}). Just as in our previous work, product diversification to alcohols was not observed and methane was not consistently detected in the off-gas. The batch cycle resulted in a total acetic acid production of 42.9 g acetic acid by day 86 (Figure 6.2B), resulting in an overall electron recovery in acetic acid of 21% (Figure 6.2C). When only taking into account the stable operation period, the coulombic efficiency (CE) was 41%. CE increased throughout the test, probably due to a higher biomass density in the fermentation broth, and reached a plateau from day 75 (Figure 6.2C). Unutilized H_2 in the reactor off-gas resulted in an overall electron recovery in H_2 of 45%. The electron balance can be further closed with the presence of other

products (short-chain carboxylic acids and methane; < 1%), losses of H_2 gas through tubing, connectors and stoppers (13.6% based on abiotic quantification), and biomass production.

The concentrations in the different reactor compartments did not reflect the real productivity of the system because water displacement between the compartments caused a change in the volumes throughout the reactor run. An average water flow across the AEM of 0.75 L m^{-2} d^{-1} was observed (from cathode to extraction compartment), which was 11 times higher than the flow across the CEM (0.07 L m^{-2} d^{-1}). The water flux through the AEM is diluting the acetic acid stream, limiting the product titer in the extraction liquid so that a final concentration of 37 g L^{-1} (in 1.20 L) instead of 108 g L^{-1} (in 0.35 L) was achieved. It was observed that the water flux showed a linear dependency on current in the range of current densities tested (data not shown), and seems to be related to the hydration shell of the anions crossing the membrane (electro-osmosis) (Giorno et al., 2016; Lakshminarayanaiah, 1969).

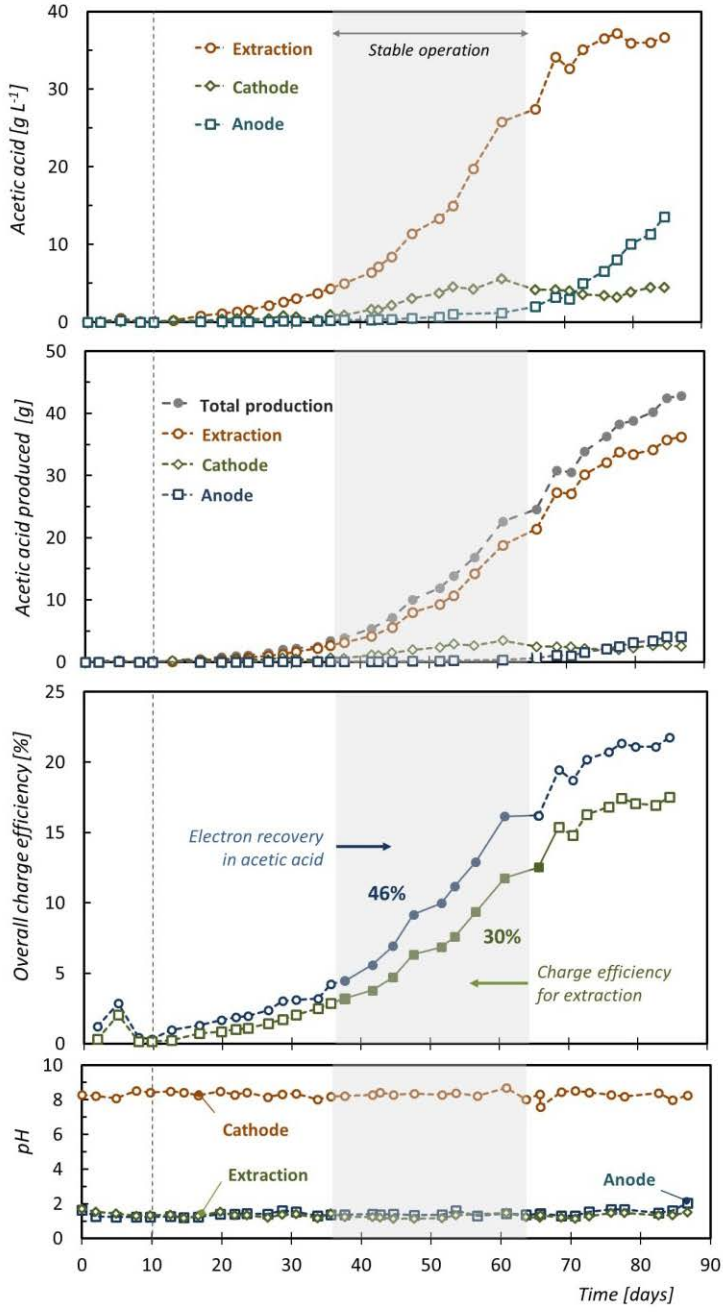

Figure 6.2 - (A) Acetic acid concentration in the catholyte, middle compartment and anolyte. (B) Total mass of acetic acid produced, extracted across the anion exchange membrane (AEM) and present in the extraction liquid. (C) Overall charge efficiency for production and extraction. (D) pH in the different reactor chambers**.** Dotted vertical lines represent the start of gas recirculation.

MES offers the intrinsic advantage to directly supply bacteria with electrons, however there is more and more evidence that production *via* MES is mainly driven by an indirect electron flow from the cathode to the acetogens, occurring *via* abiotically or biologically induced H_2 production (Jourdin et al., 2016b; Patil et al., 2015). The crucial role of H_2 in the conversion of CO_2 to organics is thus creating the need for a MES reactor design that can work at high current density and consume high H_2 fluxes. As discussed in previous reports, MES reactor design (often H-type, cylindrical or plate and frame type reactors) is not optimized for *in situ* H_2 conversion, leading to high losses of residual H_2. Efforts to increase CEs have been focusing on 3D electrodes that supply H_2 in the whole cathode chamber (Jourdin et al., 2016a; Song et al., 2018), but the scalability of these systems is questionable and channeling issues may arise when microbial growth completely fills the electrode pores (Klasson et al., 1991). Due to the fact that electrosynthesis is limited to the surface (and close surroundings) of the cathode reactor, scalability of this 2D system is more challenging compared to 3D gas fermentation systems. For the first time coupling MES to the gas fermentation platform is demonstrated as a strategy to achieve higher electron supply rates for CO_2-based bioproduction.

As a CO_2-based bioproduction platform MES is still far behind H_2/CO_2 or syngas based fermentation in terms of production rates, energy efficiencies, scalability and maturity, so integration within the gas fermentation platform could push MES forward as an elegant way to control/steer fermentation and achieve *in situ* product recovery (see further). The coupling of MES to a bubble column reactor is also a promising strategy to increase the H_2 retention in the reactor, thereby increasing the H_2 conversion efficiency. However, more optimization will be needed to boost production and achieve high H_2 uptake efficiencies typically obtained in optimized gas fermentation reactors (El-Gammal et al., 2017; Steger et al., 2017). The continuous supply of N_2:CO_2 gas mixture resulted in a relatively low H_2 partial pressure of 0.07 ± 0.03 bar, limiting the driving force for H_2 mass transfer from gas to liquid. The low gas-to-liquid mass transfer of H_2 has been identified previously as the rate-limiting factor in gas fermentation processes (De Tissera et al., 2017). It could be expected that production rates will increase when H_2 is not flushed out permanently, but accumulates in the headspace in a pressurized reactor system, increasing the pH_2. The use of pure CO_2 (limiting the dilution of H_2/CO_2 with N_2) or intermittent sparging of CO_2 (for example pH or $[CO_2]_{dissolved}$ controlled sparging of CO_2) could be exploited as gas feeding strategies to increase pH_2. Efforts to increase the volumetric mass transfer coefficient (k_La) mainly focus on increasing the

interfacial surface area for mass transfer *via* mixing, microbubble sparging, or the use of packing material (Orgill et al., 2013). Bioreactor designs such as bubble, immobilized cell and trickle bed columns are proposed as low-cost reactor platforms for gas fermentation (De Tissera et al., 2017). Although the design and operation of gas fermentation reactors has reached industrial scale, energy efficient recovery of the water-soluble products from the aqueous broth still presents an engineering challenge. For the first time interlinking of different autotrophic bioprocesses is proposed as a way to overcome the limitations of separate technologies. Furthermore, additional value could be created by coupling different production platforms to upgrade the low-value products typically produced in MES as well as gas fermentations and produce higher value chemicals. The further conversion of acetic acid to caproic and caprylic acid through chain elongation has been proposed as an efficient way to increase the product value of primary fermentation products (Gildemyn & Rabaey, 2016).

3.2. Membrane electrolysis as a tool to assist gas fermentation: *in situ* product recovery and pH control

As the result of charge balancing, an electrochemical reactor with an AEM has the intrinsic ability to extract *in situ* the produced acetic acid as the negatively charged acetate ion, into the acidic extraction compartment (termed membrane electrolysis, ME) (Andersen et al., 2014; Gildemyn et al., 2015). Since acetate synthesis from CO_2 requires 8 electrons per mole acetate (at 100% current efficiency), and since the electricity-driven extraction of one mole of acetate theoretically requires only one electron, the current use for acetate extraction (limited to a maximum of 12.5%) can only be improved by linking an external H_2 source to the MES reactor (increasing the theoretical production rate and, thus, the membrane availability for extraction). Acetate transport from the cathode to the extraction compartment accounted for 17.5% of the charge balancing through the AEM (Figure 6.2C), while in a MES cell without external H_2 injection, acetate accounted for only 8.1 ± 0.8% of the charge passing through the AEM (Gildemyn et al., 2015). This increase in charge balancing efficiency clearly indicates that through external H_2 supply, a more efficient use of the intrinsic extraction capacity of the reactor can be achieved, but also that the charge efficiency for extraction is limited by the efficiency for production.

During stable operation, a charge balancing efficiency for acetate production of 31% was achieved. Calculated on a mass balance, 94% of the produced acetic acid was extracted and

85% of the product was present in the extraction solution (Figure 6.2B). Diffusion of uncharged acetic acid molecules through the CEM resulted in an acetic acid loss of 9% towards the anode compartment. As in industrial scale reactors the extraction chamber will be operated with a continuous acetic acid purge and the anolyte in batch, the transport of acetic acid towards the anode should stabilize from the point that the acetic acid concentrations in both chambers are equalized. From day 75, the extraction efficiency was 100%, as no product build-up in the catholyte took place. Membrane electrolysis can avoid product build-up in the fermentation broth, and, thus, allows a batch mode operation without the occurrence of product inhibition or product diversification.

Separation of the fermentation product from the broth in a cost- and energy- efficient recovery process is envisaged as a crucial feature for scaling up gas fermentation processes to commercial-scale production plants. Distillation has been the traditional recovery technology for low boiling point fermentation products (Liew et al., 2013), but its energy-intensive nature has led to the development of alternative and potentially less expensive separation techniques (Ezeji & Li, 2010), of which membrane electrolysis is of particular interest for charged metabolites that have the tendency to lower broth pH. ME stabilized the pH of the fermentation medium throughout the operation at a pH value of 8.30 ± 0.19 (Figure 6.2D), while typically a pH drop in the broth of MES and gas fermentation reactors is observed unless a chemical pH control mechanism is applied (Arends et al., 2017; Liew et al., 2013). pH control is an effective strategy to achieve long-term stable acetate production and high product concentrations (De Tissera et al., 2017; Drake et al., 2006), but the addition of large amounts of base is costly and adds salts to the broth (Gildemyn et al., 2017c). Base (to prevent product inhibition) and acid dosage (to acidify the product stream) are fully replaced by OH^- and H^+ production at the cathode and anode of the ME reactor, respectively, highlighting that integration of ME in gas fermentation technology enables operation of a bioproduction reactor without addition of chemicals. This confirms earlier observations that an AEM can stabilize BES operation (Gildemyn et al., 2017c).

The results suggest that the *in situ* extraction of the acetic acid produced in a gas fermenter can enhance productivity through the combined effect of product removal and *in situ* pH control. The ME technology would be more efficient as 'secondary' microbial electrochemical technology (MET), assisting H_2/CO_2 fermentation, rather than as an electrosynthesis approach itself. In this way a larger fraction of the intrinsic extraction

capacity of the system can be used, and the power input of this cell can be lowered as only part of the reducing equivalents will be supplied by this reactor. As secondary MET, the extraction through ME supports H_2/CO_2 fermentation by: (i) extracting the produced acetic acid (avoiding product build-up and inhibition); (ii) balancing the pH of the fermentation broth (avoiding caustic addition); and (iii) providing additional reducing equivalents in the form of *in situ* electrochemically produced hydrogen (generating high pH_2 close to the electrode surface) (Figure 6.3). Periodic (ON-OFF) extraction could be exploited as a way to make fully use of the capabilities of ME during gas fermentation as it allows to recover the product more efficiently at higher product concentrations in the broth. It could, thus, be implemented as a recovery approach that intermittently extracts the product when pH stabilization is needed, or when acetic acid accumulates above a set concentration. Fine-tuning of this ON-OFF strategy could results in an optimized energy investment and reduction of water displacement across the membrane. By lowering the current density applied to the three-chambered reactor (and increasing the availability of acetate ions at the membrane surface) the electro-osmotic water transport per kilogram product can be reduced (compared a system where all H_2 is produced *in situ*). HCO_3^- transport over the AEM is of high importance for charge balancing and is a major contributor of the (electro-osmotic) water transport (Gildemyn et al., 2017c). Intermittent extraction could result in short periods of very efficient extraction with limited water drag.

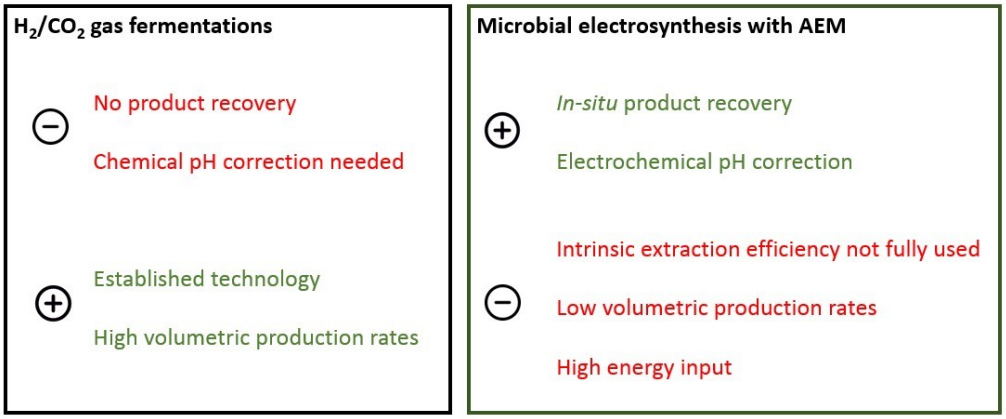

Figure 6.3 - Benefits and drawbacks of gas fermentation and microbial electrosynthesis as CO_2-based production platforms. The coupling of both technologies results in a hybrid reactor configuration that combines the product recovery and pH stabilizing ability with the maturity and productivity of gas fermentation.

3.3. Membrane electrolysis assisted gas fermentation as future scenario to reduce power input

Increasing productivity at a lower power input is crucial for the economics of both MES and H_2/CO_2 fermentation processes. The power input for the system presented here required 15 kWh kg^{-1} for the production of 3.7% acetic acid (only taking into account the electricity input of the electrochemical cells), which is 19 – 43 % lower compared to the energy input in MES-extraction reactors without additional H_2 (max. 1.35% acetic acid), but still, undeniably, too high to compete with current production standards (98% acetic acid production *via* methanol carbonylation at 4 kWh kg^{-1}) (Ecoinvent, 2007). The power consumption per kilogram product can be decreased by: (i) increasing the H_2 conversion efficiency (getting more product with the same power input), or (ii) reducing the cell voltage of the system (getting the same amount of product at a lower power input). For an industrial process it is critically important to operate a production process at high volumetric production rates, so for MES this means that current densities will need to drastically increase. It is, however, highly debatable whether H_2 can be produced at high energy efficiencies in a MES cell at high current densities when using the conventional (rather unconductive) bacterial growth media as electrolyte, while alkaline or polymer electrolyte membrane electrolyzers are optimized for efficient H_2 generation. Due to the fact that biological compatibility needs to be guaranteed during electrolyte selection, the low conductivity will make these systems not competitive with abiotic electrolyzers considering only H_2 production. During stable reactor operation, cell voltages of 3.91 ± 0.10 V were recorded for the MES cell (5 A m^{-2}), while conventional water electrolyzers are operated under current densities ranging from 1000 to 3000 A m^{-2} and stable cell voltages of 1.7 – 1.9 V (Zeng & Zhang, 2010). With an energy efficiency ranging from 65 to 82%, current industrial PEM electrolyzers are much more efficient in producing H_2 than MES systems currently do (35% energy efficiency at only 5 A m^{-2}).

Projecting forward to a fully realized system, the power input of acetic acid production *via* the ME-assisted gas fermentation pipeline should be calculated based on realistic rather than experimental and non-optimized cell voltages. The economics of the proposed concept is briefly demonstrated for a 10 000 L gas fermentation reactor. At 2000 A m^{-2} and 1.8 V, the power cost of water electrolysis is calculated at 4.3 kWh per Nm^3 of H_2 produced, which corresponds to € 4.8 per kg H_2 (at an energy cost of € 0.1 per kWh). Considering a gas

fermentation reactor fixing CO_2 into acetic acid at a volumetric productivity of 148 g L^{-1} reactor d^{-1} (experimentally achieved by Kantzow and co-workers (2015)) and 90% electron recovery, H_2 gas should be supplied at a flow rate of 106 Nm^3 per hour. If coupled to continuous extraction in a ME unit operated at 1000 A m^{-2}, 42 m^2 membrane surface is needed to allow for a stable broth concentration. At 5 V and a charge balancing efficiency by acetate of 65%, 17% of the total H_2 load is produced in the ME cell and ME is able to recover 1.48 ton acetic acid per day (extraction rate is set equal to production rate). Based on these assumptions a power input for acetic acid production and extraction of 9.56 kWh per kg acetic acid is calculated, of which 36% can be attributed to the electrochemical extraction. Assuming 40 m^2 membrane electrode assembly per cubic meter reactor (Desloover et al., 2015), a ME setup of 1.06 m^2 would be sufficient to control fermentation.

Potentially the power input can be further decreased if the system is operated with an intermittent rather than continuous extraction (for example 10% of the time ON, 90% OFF), since a higher molecular availability for flux results in a higher charge efficiency for carboxylate extraction and, thus, a potentially lower current use by the ME system. Assuming a charge balancing efficiency by acetate of 80% when acetate concentration in the broth is 20 g L^{-1}, the power input can be lowered to 8.76 kWh per kg product. The decrease in energy input demonstrates that the ME extraction would fit ideally with a high concentration fermentation to obtain an effective and cost-optimized ME step. To lower the energy input per kilogram acetic acid extracted, it is clear that a maximal use of the ‘expensive’ charge for target ion recovery in the ME cell should be targeted. Furthermore, off-gases from industrial processes, such as steel production and reformed biogas, as well as syngas from biomass gasification can serve as substrate gas in the flexible/hybrid MES-gas fermentation approach.

4. Conclusions

Since its first demonstration, MES has been intensively studied in terms of microbiology (Nevin et al., 2011), electron transfer (Jourdin et al., 2016b; Marshall et al., 2012), electrode materials (Jourdin et al., 2016; Zhang et al., 2013), CO_2 supply (Bajracharya et al., 2016), media modification (Ammam et al., 2016) and product outcome (Ganigué et al., 2015; Vassilev et al., 2018), but engineering of the system has only been studied in terms of product recovery using an integrated approach for acetic acid extraction over an anion exchange membrane. This study presents a reactor setup that allows operation of MES reactors at higher current densities, thereby increasing the availability of reducing equivalents and, thus, increasing the (theoretical) production rates (provided that the kinetics of the acetogens are not the rate-limiting factor). Coupling electricity-driven product extraction to an external H_2/CO_2 gas fermentation column allows for recovery of the pure product in an acid and clean extraction liquid while simultaneously stabilizing the pH in the fermentation broth. The external hydrogen injection allows acetic acid production from CO_2 at a lower power input and cost, offering opportunities for the scalability of autotrophic acetic acid production through ME-assisted gas fermentation.

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CHAPTER 7

Discussion and outlook

In today's carbon-based economy, fossil carbon is transformed into a plethora of chemicals, materials and fuels. Typical cracker products of naphtha and natural gas, like ethylene (C2), propylene (C3), butadiene (C4) and benzene (C6), represent an essential part of our current chemical production. The energy to drive these energy intensive transformations largely comes from the same source as the carbon, *i.e.* from coal, oil and gas. This carbon-energy-chemical relationship has resulted in an atmospheric CO_2 concentration that has exceeded 410 ppm, a level that has never been reached in the last 15 million years (Tripati et al., 2009). The transition from a fossil carbon-based to a CO_2 -neutral world economy can only be achieved if this relationship is broken in a sustainable way, using renewable raw materials and CO_2-free energy to drive the carbon conversions of the future carbon-chemical cycle. In the electrifying world of the 21st century, the utilization potential of renewable energy could enable the use of CO_2 as the ultimate renewable raw material in industrial biotechnological processes for production of a variety of bio-based building block chemicals.

This thesis looked at methane and carbon dioxide as gaseous C_1 feedstocks for (bio)production and investigated how electricity can drive the transformations of these compounds into value-added chemicals. The main research outcomes are:

Chapter 2

- Subsidizing the combustion of biogas produced through anaerobic digestion of biomass is expensive as the power that is produced is much more expensive than the market price. The level of required government support to operate break-even is currently estimated at 20 – 50 € MWh_e^{-1} for valorization of an averagely priced biogas in a combined heat and power unit.
- A techno-economic analysis shows that production of carbon monoxide or synthesis gas from grid-injected biomethane through chemical reforming could be an economically viable alternative valorization route that potentially does not need subsidies if the environmental and economic costs of fossil methane are benchmarked to those of biomethane, and internalized accordingly .
- Super-dry reforming of CH_4, an advanced chemical looping approach that uses up to three CO_2 molecules per CH_4, allows for an intensified production of CO, enabling industry to lower their carbon footprint in a cost-effective way, still generating a feedstock for the synthesis of platform chemicals and fuels.

- Upgrading biogas to biomethane and subsequently injecting it into the existing natural gas grid enables the transportation of renewable methane from decentralized small-scale producers to localized large-scale industrial sites, making maximal use of existing infrastructure.
- An estimation of chemical production volumes and CO_2 emission reduction potential showed that globally bulk chemicals could utilize roughly 5% of stationary CO_2 emissions from large point sources, while requiring 26% of the estimated biomethane production potential.

Chapter 3

- Membrane electrolysis allows the use of renewable electricity as driver for electrochemical CO_2 - H_2S removal and biomethanation of residual CO_2 with electrolytically produced H_2.
- Water electrolysis induces CO_2 and H_2S scrubbing in the cathode of an electrochemical cell while driving the dissociated anions across an ion exchange membrane into an acid stripping medium.
- Electrochemical scrubbing of CO_2 can generate gas mixtures with an ideal 4:1 H_2:CO_2 ratio for biomethanation, while H_2S absorption in the alkaline catholyte removes up to 98% of the incoming H_2S.
- The results demonstrate that CO_2 from biogas can be removed *via* electrochemical means at energy inputs of 0.8 – 4.6 kWh Nm^{-1} raw biogas, considering that the hydrogen generated is used for biomethanation. Critical for further development will be the ability to reduce this energy requirement as the energy efficiency towards H_2 of the set-up tested is only 23 –54%.

Chapter 4

- Producing microbial protein locally from available renewable methane/CO_2 and ammonia-nitrogen from digestate appears as an interesting approach to partially shortcut the nitrogen cycle at the scale of a manure digester facility by direct introduction of this protein-rich biomass as feed for animals.
- Upgrading valuable recovered nutrients and used carbon into microbial protein seems to be of interest to offset the costs associated with manure treatment, as microbial

protein production bypasses the low value of recovered ammonia when sold as $(NH_4)_2SO_4$ or NH_4OH.

- At average costs, MP obtained from biogas-CH_4 and recovered NH_3 can be produced at 1544 € ton^{-1} crude protein, while MP from H_2 requires a production cost of 2289 € ton^{-1} crude protein. When taking into account the revenue from the avoidance of the treatment of ammonia present in the digestate (600 – 800 € per ton DM protein when considering conventional dissipation *via* nitrification - denitrification), it should be possible to produce a NH_3-CH_4 based MP substitute for soy protein for animal feed on site at competitive prices.

Chapter 5

- Continuous *in-situ* extraction of acetic acid directly from the catholyte of a microbial electrosynthesis reactor allows pure product recovery and an increased reactor performance compared to systems without product recovery.
- The performance improvement is the result of a stable pH environment in the reactor and a low end-product concentration, both preventing a process that is hindered by the accumulation of undissociated acetic acid.

Chapter 6

- The integration of a membrane-assisted extraction unit within the gas fermentation platform allows for the recovery of the pure product in an acid and clean extraction liquid while simultaneously stabilizing the pH in the fermentation broth without base addition.
- By injection of additional hydrogen gas (produced *via* water electrolysis) the charge balancing efficiency by acetate can be increased, thus, making more efficiently use of the intrinsic ability to extract anions.

With an emphasis on economic competiveness and technical feasibility, this chapter will discuss the feedstocks of a CO_2-neutral chemical industry: renewable electricity, biomass and CO_2. Some of the global changes that can drive the shift towards renewable chemicals will be discussed, with a focus on how microbial biotechnology can play a role. In order to provide a comprehensive assessment of the impact of biotech production platforms on the global carbon economy, we considered the economic competiveness and scalability of the technology as key factors that will determine the actual adoption rate.

1. Renewable electricity systems: viability and feasibility of a 100 % renewable power mix

At present day, the chemical industry represents about 6 % of the global primary energy demand (BP, 2018). That is still a relatively small portion compared to the much larger volumes of primary energy destined to supply the world with energy for transportation, heating/cooling, power, *etc.*. Today, electricity represents about 40% of the global consumption of primary energy sources, but its share is expected to become increasingly larger, approaching 50% by 2035 (Birol, 2017). Electricity is more and more envisaged as an excellent, and increasingly sustainable energy vector, projected to contribute to a deep decarbonization of the complete energy sector (including transportation, residential and industry) (Delarue et al., 2011). The presence of carbon atoms in materials and chemicals is usually vital, and thus its irreplaceable nature will always result in CO_2 production at the end of the product's life cycle. The chemical industry has at present no large-scale alternative for fossil carbon, and thus the chemical sector is by far the most challenging one to decarbonize. Electrification is starting to become one of the most viable options to produce more sustainable chemicals (Schiffer & Manthiram, 2017), however, deep decarbonization of industrial production by renewable power is still in the stage of a conceptual idea and new CO_2-based value-chains need to be developed. By taking advantage of the rapidly growing renewable energy production, the chemical industry can shift towards an electricity-driven synthesis of all the carbon-based building blocks and platform chemicals our economy requires. Using CO_2 as a raw material for production is challenging as any transformation of this oxidized form of carbon requires a large input of electrons. A massive amount of sustainable carbon and electrons needs to be delivered to chemical industry to produce carbon-based building blocks and platform chemicals our economy requires. In this thesis several concepts to achieve carbon-neutral production have been presented, all based on renewable electricity to drive the conversion, so this chapter starts with a brief discussion of the technical feasibility and economic viability of renewable energy systems.

1.1. Economic viability

Reductions in total installed costs are driving a rapid fall in the levelized cost of electricity (LCOE) for renewable power technologies. The global weighted average LCOE of utility-scale solar PV has fallen 73% since 2010, and continued technical innovations suggest that costs will fall further in the future. Offshore wind, solar PV and hydroelectricity are already either in the range of current fossil fuel generation or even lower and the International Renewable Energy Agency (IRENA) expects that all renewable power generation technologies that are now in commercial use will fall within the fossil fuel-fired electricity generation cost range by 2020, with most of them at the lower end or even undercutting fossil fuel options (IRENA, 2018b). The low cost of renewables is borne out in recent auctions throughout the world (Dubai, Mexico, Peru, Chile, and Saudi Arabia), where, extremely low bids on solar PV and wind power have resulted in LCOEs well below 30 € per MWh, providing valuable signals about future electricity cost reduction trends by 2020 and beyond (Kruger et al., 2018). Even in less sunny locations as Northwest Europe, forecasts using learning curve models to estimate future developments, estimated that for ground-mounted PV installations the LCOE will range between 21.6 and 39.4 € per MWh (Fraunhofer, 2018).

It needs to be recognized that the LCOE metric is only a coarse measure, since it overlooks the temporal fluctuation of electricity and the natural intermittency of renewables. The integration of renewable energy technology is not simply about adding renewable energy to the fossil mix. Integration studies typically consider total system costs in models that accommodate wind and solar into the power system by comprising a high degree of spatial and temporal variability and uncertainty in power production and demand (Ueckerdt et al., 2013). These studies repeatedly show that 100% renewables-based systems are possible with system costs (including curtailment, storage and some grid costs) that are comparable and in many cases even lower than fossil-fuel-based systems. On a global average the renewable electricity is expected to decline to 52 € per MWh by 2050, compared to 70 € per MWh in 2015 (Figure 7.1) (Ram et al., 2017). In the United States, recent ultra-low bids have been seen for systems that include storage due to come online in 2023 (a median PV-plus-battery price of 36 US$ per MWh and a median wind-plus-storage price of 21 US$ per MWh for projects to be commissioned in the coming years) (EIA, 2018).

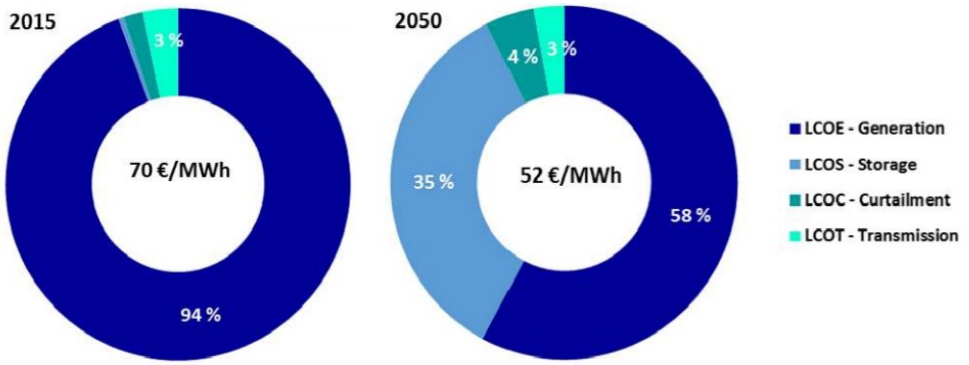

Figure 7.1 - 100% renewable electricity generation is more cost effective than out current fossil-dominated energy mix. Copied from Ram et al. (2017).

1.2. Technological feasibility

Recent modelling studies have shown that energy systems with very high shares of renewable energy are achievable with respect to (i) primary energy demand projections, (ii) balancing short-term variability and limitations of geography against the demand for energy, (iii) extreme events, (iv) transmission and distribution, and (v) technological maturity and scalability (Brown et al., 2018; Diesendorf & Elliston, 2018). Model predications indicate that by 2050 solar PV can account for 69%, with wind energy accounting for 18%, hydropower for 8% and bioenergy for 2% of the total global electricity mix, respectively (Ram et al., 2017) (Figure 7.2). Gas generation is only from renewable sources like anaerobic digestion or power-to-gas. Strong empirical evidence of the technical feasibility of 100% renewable electricity systems, however, still needs to be demonstrated, and efforts to date seem to have substantially underestimated the challenge of excising fossil fuels from our energy supplies at the required capacity deployment rates (Heard et al., 2017). To enable high shares of variable and asynchronous renewable energy sources to enter the energy mix, it is clear that the entire electricity supply-and-demand system needs to be reinvented to ensure the same reliability as our current fossil fuel dominated systems. Some recent studies also concluded that the current global supply of several critical metals like neodymium and indium is insufficient to ensure the global energy transition to be in line with the goals of the Paris Agreement. Additionally, the dependency on foreign supply of these rare earth metals will

shift the geopolitical power from oil-dominated to critical metal-dominated countries (van Exter et al., 2018). To ensure sufficient supply of metals, we will need to reduce critical metal use through substitution, increase a circular design and recycling effort of these metals, and go for local mining.

The potential for the expansion of solar PV can be easily illustrated by looking at the average flow of solar energy that strikes the surface of the Earth (161 W m^{-2}). Assuming that all primary energy (13 511 million tonnes of oil equivalent in 2017 (BP, 2018) would be generated with PV technology at a solar panel efficiency of 20%, an area of 1.1 million km^2 of installed photovoltaic panels, or about 12% of the Sahara Desert, can suffice to empower the entire planet. Low-value land areas for installing the panels are available worldwide (*e.g.*, arid zones). Hence, these values indicate the huge need for increased production capacity of photovoltaic panels. The issue seems not to be feasibility (and on the long term even not viability), but whether governments, industry and consumers are willing to take the actions required to get to this renewable mix fast enough.

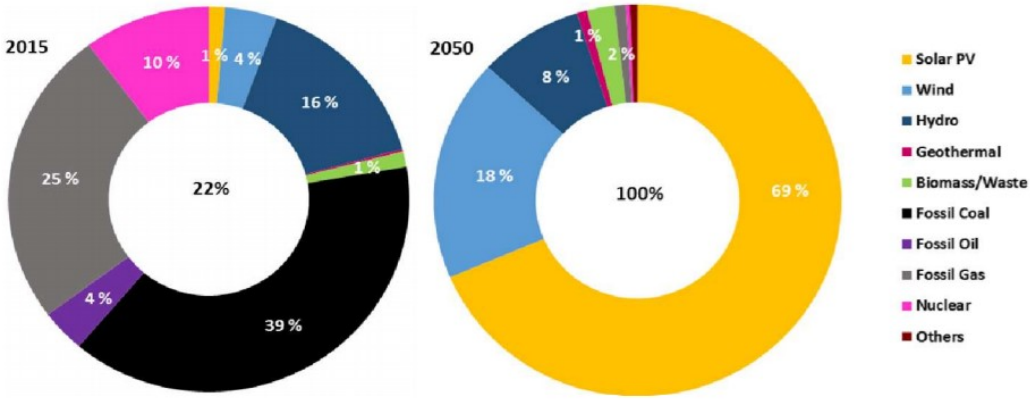

Figure 7.2 - 100% renewable electricity generation is technically feasible. Copied from Ram et al. (2017).

2. Anaerobic digestion: methane as a central building block chemical

A bio-based platform that has been discussed throughout this thesis is the anaerobic digestion (AD) of biomass to biogas. Even though AD has successfully been implemented at full scale, the products of AD have not yet been valorised to their full potential as renewable C and N-sources. AD has historically been positioned as an end-of-pipe technology to reduce the organic load of organic waste(water), rather than as a strategy for bio-production. However, AD is in fact a very efficient and flexible biomass conversion technology that has the potential to become the cornerstone of second-generation biorefineries, thereby outcompeting bioethanol and biodiesel production if it comes to input of energy and chemicals, and recovery efficiency of energy and carbon from the lignocellulosic biomass into the product (Cesaro & Belgiorno, 2015; De Meester et al., 2012). Moreover, the gaseous nature of the product makes product extraction to happen spontaneously and without cost.

We envisage renewable methane as a key compound in the carbon-based economy of the future, playing a crucial role in power balancing and bio-based production of fuels, chemicals and food/feed. Methane holds an intriguing place in bridging biological and chemical processes. By using renewable methane all current end-consumers of natural gas can take an important step towards CO_2 neutrality. Using ("green") renewable methane as feedstock in industrial production processes is probably the most straightforward approach to lower the CO_2 footprint of industry as a part of their fossil carbon consumption can be replaced by biomethane without major changes in their current petrochemical synthesis pipelines. In fact, methane is the drop-in chemical par excellence, having the ability to be used as a commodity that can be turned theoretically into every organic bulk commodity *via* reforming into CO. By using a well-defined blend of renewable and fossil methane, chemical plants can flexibly and gradually shift towards biomass as renewable resource in order to meet consumer's demands for sustainability. Especially the ability to transport gas to be used at virtually any place adds flexibility to the system, as not only the share of renewables in the transport and the heating sectors can be increased, but potentially also the chemical and food/feed sectors can be supplied with renewable methane produced through anaerobic digestion or biomethanation. The conceptual idea to take green methane from the existing network and use it as a readily available carbon and energy source for production opens new utilization options of biogas, *i.e.*, not just for heat and power production or as fuel for vehicle use (compressed natural gas, CNG), but also as a feedstock to produce high-value bio-based

chemicals (Moghaddam et al., 2016) or as reducing agent in steel making (Otto et al., 2017). Different biomethane utilization routes for production of platform chemicals are being proposed, starting from "green" C_1 based products with subsequent (bio)catalytic synthesis (Buelens et al., 2016; Charisiou et al., 2016). Hence, the conceptual idea of producing platform chemicals, which are conventionally derived from natural gas, from biomethane instead opens an inroad to an entire plethora of products that can be generated in a carbon-neutral way (Verbeeck et al., 2018).

2.1. Strengthening business cases for biogas

As the value of methane is low and conversion to power adds only marginal value, government incentives to ensure a sound economic argument are often required (Edwards et al. 2015). This study opens up new fields of applications where biogas has not played a major role so far. A centralized use of grid-injected biomethane is shown in Chapter 2 as the preferred route for chemical synthesis, while a decentralized approach for partial self-supply of animal feed from biogas and recovered ammonia is suggested in Chapter 4.

Centralized *vs.* decentralized biogas valorization? Chemicals *vs.* proteins?

In contrast to present biorefineries that require costly road transportation to large-scale processing facilities, anaerobic digesters mostly valorize biomass directly at their point of production. For low-value, and in many cases 'wet' waste streams, such as animal manures, slurries, food waste, AD is in fact the only strategy to valorize these organic waste streams on-site, as the high water content makes road transportation too costly. A key question in AD management is at which level the valorization of biogas should be implemented, *i.e.* centralized or decentralized. The key will be to recognize which value chain fits a specific installation, making optimally use of the resources that are available at the digester or chemical processing plant, as well as product demands of these facilities. For the chemical-case this means that we make CO or syngas where sufficient quantities of CO_2 feedstock are available and where the produced intermediates can be processed further into platform chemicals. The fact that *via* the existing natural gas grid renewable methane can be transported to any industrial end-user of this molecule makes it an interesting approach to valorize the biomethane produced and injected by hundreds of small-scale biogas plants in one central processing plant.

For farm digesters that typically treat manure and other on-farm feedstocks, it is clearly more beneficial to keep the nutrients in a loop close to the digester by upgrading *via* MP as this will enable partial self-supply of fodder, while locally dealing with nutrient surpluses. The upcycling of recovered nitrogen as microbial protein would offer important savings in terms of nutrient management, and could offset the higher production costs of MP compared to conventional protein sources.

Rather than being competitive technologies, grid injection of biomethane and MP production can in fact both be implemented at the biogas plant, giving rise to a full valorization of biogas carbon, *i.e.*, methane being sold to the grid as chemical feedstock and the CO_2 being up-cycled by means of hydrogen-oxidizing bacteria to microbial protein-rich biomass. This would create novel value chains for both biogas and digestate, strengthening the business case for anaerobic digestion as a driver for local C and N valorization from low-value waste.

2.2. Rethinking support schemes for biogas

Current subsidy schemes for biogas clearly focus on renewable and on-site power. Our calculations in Chapter 2, however, showed that as an energy producing technology, AD is not an economically strong case in itself and incentives are needed to keep the biogas sector alive. Fraunhofer estimated that the LCOE of current biogas power plants ranges between 100 and 150 € MWh^{-1} and highlighted that due to the maturity of the technology no further cost reductions have be expected (Fraunhofer, 2018). Now that energy systems based on renewables are competing head-to-head with fossil energy, and power costs are expected to decrease even further, green premiums for biogas will always be necessary to compensate for the higher production cost of biogas electricity compared to power from solar and wind. At present, a growing number of EU member states gradually shift incentives from combustion to upgrading and injection of biomethane into the natural gas grid. This shift is mainly driven by a growing demand for biomethane as green transportation fuel (CNG or LNG). Based on the outcome of the research presented here, it seems more meaningful to support the transition of the biogas industry to biomethane and MP production as much as they do now with renewable electricity, and shift the balance away from combustion to more value-added production.

A criterion that could help decision makers to evaluate different options for support is the emission reduction per € spent. In Chapter 2 it was calculated that for each MWh of electrical energy produced at an 'average' biogas plant, a subsidy of at least 40 € per MWh is needed to operate the AD plant at break-even, while injection of biomethane in the grid and its coupling to super-dry methane reforming can generate viable business cases for both facilities (the localized biogas as well as the centralized chemical production plant). The question will be of course whether companies are willing to pay for the more expensive 'green' methane as the lost profit will be taken into account in every business case with biomethane as carbon feedstock. At present, avoided carbon taxes will only partially offset the price difference between renewable methane and its fossil counterpart, thus, some additional incentives or regulation will be needed. One can think to support chemical plants that use this renewable feedstock at a higher cost, compensating for the price difference between natural gas and the sum of biomethane and revenues from avoided CO_2 cost. As the monetized damages from emitting CO_2 are expected to increase, incentivizing biomethane can become a cost-efficient way to reduce greenhouse gas emission. Current subsidies for biogas electricity in Flanders are roughly 90 € per MWh electrical energy produced, and, thus, an incentive of 472 € per ton methane is granted to CHP projects on biogas. Given the fact that methane combustion emits 0.65 ton CO_2 per MWh_e, the current green energy certificate scheme comes at a CO_2 abatement cost of 138 euro per ton CO_2 reduction.

3. Toward a CO_2-based bio-economy

3.1. CO_2 and H_2: the building blocks for renewable chemical production

Microbial production strains that are exploited for their biotechnological potential to fix CO_2 into valuable bio-based products are moving from concept to reality, with currently various emerging concepts being implemented at demonstration and even industrial scale. Hydrogen gas is envisaged as a key molecule in this CCU based bio-economy of the future, since (i) an extensive variety of microorganisms with a wide plethora of end-product metabolites can use this electron source for autotrophic growth, and (ii) its carbon-free production *via* water electrolysis enables to achieve a CO_2-neutral carbon/energy cycle.

In contrast to the fuel sector, which is dominated by emission reduction targets imposed by governments, the chemical sector is still mainly driven by market mechanisms. Companies and consumers are often not really committed to pay a premium on their daily-use goods, which means that the production of carbon-neutral chemicals has to be cheaper than, or provide an extra benefit over non-renewable petrochemically sourced compounds to convince industries to take the risk of an alternative supply chain over the well-established ones. The economic viability of new value chains is a critical requirement for widespread implementation of CCU. To make sure that CO_2-based production is not only a "green dream" alternative to petrochemistry, but possesses a realistic economic potential, costs minimization in each step of the value chain will be crucial, with a clear focus on low cost renewable power production. The most important factors that will determine the economic potential of a certain CO_2-based production route are: (i) the price of renewable electrons; (ii) the energetic efficiency and investment cost of electrolysis; (iii) concentration and purity of available CO_2 source; (iv) avoidance of CO_2 emission taxes and (v) the product value.

The price of renewable electrons

As the production cost of CO_2-based chemicals is directly determined by the cost of electrons, bringing down the renewable electricity cost will be crucial to compete with 'fossil' electrons. For hydrogen production via water electrolysis (or direct electron supply *via* a cathode), one mole of electrons (*i.e.,* a charge of 96 485 C) transferred at a cell voltage of 1.7 V equals an energy input of 4.56×10^{-2} kWh. If we assume a solar PV power cost of 50 € MWh^{-1} this equates to a price of 0.228 € per kmole of electrons (at E_{cell} = 1.7 V). When considering 8 moles of electrons are needed to produce 1 mole of acetic acid from CO_2, acetic

acid can be produced at a cost of 0.26 € per kg (not including CO_2 cost and any investment or operational costs). Currently electrons for acetic acid production via the Monsanto process (carbonylation of methanol) are derived from two carbon substrates: methanol (6 e^-) and CO (2 e^-) (Sunley & Watson, 2000). Considering a market value of 360 and 300 € per ton for methanol and CO respectively, the acetic acid production cost is estimated at 0.28 € per kg *via* petrochemical supply of carbon and electrons (not including investment or operational costs). This indicates that for this specific synthesis pathway, renewable electrons are already competitive with fossil electron sources. However, fossil feedstocks deliver next to the electrons also the carbon atoms for chemical synthesis, whilst for CO_2-based approaches costs are incurred with CO_2 capture and the production of H_2 *via* electrolysis.

Hydrogen production *via* electrolysis

The levelized cost of electrolytic hydrogen at current mainstream grid prices varies between 3.2 and 5.2 € per kg H_2, at least double to cost of H_2 production *via* steam reforming of fossil methane (Dincer & Acar, 2016). It is expected that through scale effects and completive procurement the cost of electrolyzers will end up in a similar cost curve to that of wind turbines and photovoltaic panels, bringing down the H_2 production cost below 2 € per kg if cheap electricity is used. This should be achieved by both CAPEX/OPEX reduction and improvements in energy efficiency in alkaline and polymer electrolyte membrane electrolysis technology. Long term estimations suggest substantial advancements in the coming years with the required electrical energy input evolving from 57 to 47 kWh per kg H_2 by 2030 (LHV efficiency climbing from 59 % to 71 %) and the average capital investment dropping from about 2090 € kW^{-1} down to 250 - 760 € kW^{-1} for fully mature PEM technology (Bertuccioli et al., 2014).

CO_2 separation cost

Costs for capturing CO_2 from flue gases through chemical absorption or pressure swing adsorption have been estimated in between 24 and 52 € per ton CO_2, depending on the CO_2 concentration in the gas and the required purity/composition for conversion (Yang et al., 2011). For example the biocatalysts used in anaerobic gas fermentation are sensitive to oxygen and trace contaminants like cyanide, acetylene and BTEX. However, biocatalysts are typically more tolerant to these gas contaminants than metal catalysts (Molitor et al., 2016). Large-scale stationary point sources like the combustion of coal (12-15 mol.% CO_2) or natural gas (3-4 mol.% CO_2), oil refining (8-9 mol.% CO_2), production of cement (14-33 mol.% CO_2)

and iron and steel (20-44 mol.% CO_2) are prime candidates for carbon recycling (Songolzadeh et al., 2014), with the latter being of special interest due to the high share of CO/CO_2, absence of O_2 and the relative low concentrations of contaminants in steel mill gases.

3.2. Critical evaluation of CO_2-based bioproduction platforms

Capitalizing on the huge market opportunity for H_2-mediated conversion of CO_2 does not come without challenges, both of technological and legislative origin. There are plenty of approaches, products and CO_2 feedstocks that can contribute to solutions. However, there is no good overview of which commodity chemicals are the most attractive ones to produce from CO_2. The success of a value chain (feedstock – conversion step – product) will be determined by the market potential of the product as well as the number of conversion steps to product and the maximum yield per conversion step of a certain production pipeline. Considering economics, it is clear that competitiveness is not realistic since current product prices are highly distorted by direct and indirect subsidies for fossil carbon, also called externalities (IRENA, 2016). These “hidden costs” make it hard to evaluate the real market potential of a chemical, as the monetary value of environmental damages from the use of non-renewable carbon and energy sources is currently not (or only to a certain extent) passed on the polluter.

In what follows the economic potential of the emerging CO_2-based production platforms and products described in this work is evaluated based on (i) the production cost; (ii) the market value and market volume of the product, and (iii) the interchangeability of the product within existing production pipelines. Renewable drop-in chemicals that fit the chemical processes without too much adaption is of special interest, as existing infrastructure can be used.

Biomethanation

Considering the industrial production of H_2 using current best processes for water electrolysis, *i.e.* PEM or alkaline electrolysis, at an energy efficiency of 80%, the production of 1 kg H_2 (which has a specific energy content of about 40 kWh kg^{-1}) requires 48 kWh of electrical energy. At a forecasted average renewable energy price of 52 € per MWh, this corresponds roughly to an electricity cost of 2.5 € per kg H_2. If we consider a hydrogen-to-methane efficiency of 90 %, hydrogenotrophic biomethanation allows CH_4 production at an

electricity input of ~1.4 € kg^{-1} CH_4. Realizing that the current wholesale price of natural gas goes below 0.25 € kg^{-1}, methane from biomethanation, at present, cannot compete with inexpensive fossil methane. As the value of methane is low, H_2 needs to be produced very cheap to make a profitable business case for biomethanation. Our estimates highlight that biomethanation would hold economic potential at a projected electricity cost for hydrogen gas production of 0.45 € kg^{-1} H_2, corresponding to ~10 € MWh^{-1}. This clearly indicates that methanation will only be of economic interest as temporal low priced electricity is available to drive the CO_2 conversion into CH_4. The increasing penetration of renewable energy into the grid will pose severe unbalances between offer and demand, and thus, elegant and large-scale solutions to store these temporary energy surpluses are needed. Biomethanation offers an opportunity for energy storage in the form of a chemical energy carrier with an existing and widespread infrastructure. The power-to-gas (P2G) concept could partially decarbonize the natural gas grid while enabling the flexible integration of high shares of intermittent renewables into the future energy mix (Blanco et al., 2018; Schiebahn et al., 2015). Actually, it has been estimated that P2G becomes essential when the share of renewable energy reaches 80 %, and that in Europe more than 100 TWh of electrical energy would be wasted by 2050 if no large-scale storage mechanism if implemented (Bertsch et al., 2012). An important feature of P2G is that no full conversion of H_2 to CH_4 is needed as the natural gas grid can facilitate a H_2 blend concentration in between 5 and 25%, depending on the pipeline network and natural gas compositions (Haeseldonckx & D'haeseleer, 2007).

The theoretical storage capacity of our gas grid is immense (in Germany alone already over 200 TWh) (Hauer et al., 2013), but it needs to be recognized that the actual round trip efficiency of energy storage in the gas grid is at maximum only about 35% (±80% conversion efficiency from power to hydrogen, ± 90% from hydrogen to methane, and ±50% from methane to power). From an energy efficiency perspective it is thus more interesting to finally store the energy in the bounds of non-fuel chemicals, rather than combusting it at low overall efficiency. This can be done by directly making a non-fuel compound or by converting the methane to more complex base chemicals as proposed in Chapter 2. The calculations in this chapter showed that through CO or syngas production the inherently low value of methane produced *via* anaerobic digestion can be bypassed. When the calculations are repeated for CH_4 from methanation (at an assumed production cost of 1200 € ton^{-1} CH_4), the economic evaluation reveals that none of the reforming processes yield a positive profit at the current range of CO market prices, and that the economic viability of the super-dry

reforming process would be limited towards CH_4 produced at a cost of below 1000 € ton^{-1} CH_4 (Figure 7.3).

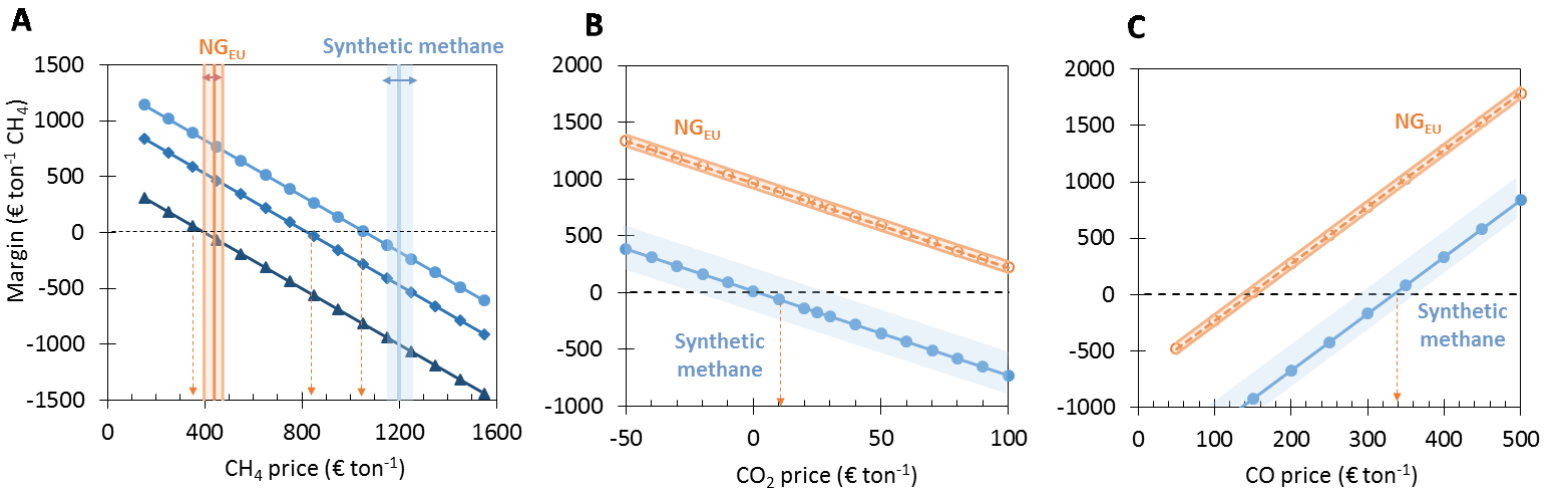

Figure 7.3. - Economic analysis for the production of chemicals from synthetic methane produced through (bio)methanation or natural gas. (A) Profit generated by producing H_2, CO/H_2 or CO as a function of CH_4 market price. (—▲—) H_2 production through sorption-enhanced steam reforming of methane; (—◆—) CO/H_2 production through dry reforming of methane; (—●—) CO production through super-dry reforming. The shaded vertical regions represent the variability in EU natural gas price (red) and biomethane price (blue). (B) Profit generated by producing CO as a function of CO_2 market price using a biomethane (—●—) or EU natural gas (--⊖--) feedstock. The shaded regions represent the variability in feedstock price of biomethane (blue) and natural gas in the EU (red).). (C) Profit generated by producing CO as a function of CO market price using a biomethane (—●—) or EU natural gas (--⊖--) feedstock. The shaded regions represent the variability in feedstock price of biomethane (blue) and natural gas in the EU (red).

In a future scenario where 100% of the energy would be provided by means of renewable sources, about 3700 Mton methane can be produced with the 100 TWh of intermittent energy, fated to be wasted if no storage is implemented. Combined with a global methane production potential from AD of 658 Mton, renewable methane production will theoretically be sufficient to achieve a full replacement of the current fossil methane consumption, *i.e.* around 2870 Mton natural gas year^{-1} (World Energy Council, 2016). This even leaves enough methane to produce ~ 1150 Mton microbial protein by means of methane oxidizing bacteria in an agriculture-free and virtually climate independent production pipeline (assuming 1 ton MP requires 1767 Nm³ methane) (Pikaar et al., 2018b).

Microbial electrosynthesis and gas fermentation

At best, Gildemyn and colleagues (2015) obtained a specific energy input of 19 kWh per kilogram of acetic acid produced and extracted in the microbial electrosynthesis system presented in this work. At an estimated global energy price of 52 € MWh^{-1} (Ram et al., 2017), this corresponds roughly to a power input of 1000 € ton^{-1} acetic acid, just to produce a dilute acetic acid stream of 1.35% acetic. Realizing that the current market price of acetic acid is ~400 € ton^{-1} (BCC, 2018), it is clear that the electrochemical losses in the reactor system should decrease drastically to bring down the operational cost of the process. The high energy losses observed in MES studies are partially linked to the indirect transfer of electrons from the electrode to the acetogens due to abiotic or biologically induced H_2 production (Gildemyn et al. 2016). The rapid uptake of H_2 by the cathodic biofilm can result in very low partial pressures near the electrode surface, enabling H_2 evolution at a less negative potential than in abiotic conditions. This would also partially explain the higher overpotentials observed at cathodes in MES studies that claimed a direct electron transfer mechanism (Jourdin et al., 2016b). And although the low solubility of H_2 could be circumvented by using an electrode, H_2 production in the fermentation broth is, at present, restricted to 'bio-friendly' conditions, *i.e.* an aqueous environment with low salt concentrations and a relatively neutral broth, *i.e.* non-ideal conditions for H_2 evolution. Therefore, one needs to critically evaluate whether MES *via* H_2 will ever be able to compete with the high H_2 evolution rates and high energy efficiencies of commercial electrolyzers (even at current densities > 1000 A m^{-2}). In this work, MES with *in situ* product extraction has been proposed as a secondary microbial electrochemical technology, supporting a H_2/CO_2 fermentation by: i) recovering the acetic acid in a clean and acid product solution; ii) balancing the pH of the fermentation broth *via in situ* OH^- production; and iii) providing additional H_2. In this perspective, MES and H_2/CO_2

based fermentation could strengthen each other, as higher production and extraction rates at a lower energy input can be considered.

In terms of industrial applications, CO and/or syngas fermentation is the most advanced process, with one commercial-scale ethanol-producing facility fully operational (in China, with Shougang Group, 46 kton) (Figure 7.4) and one under construction (in Belgium, with ArcelorMittal, 64 kton), both using steel mill off gases (blast furnace and/or basic oxygen gas). Three other LanzaTech projects are currently under development, using ferroalloy off-gases (in South Africa, with Swayana), refinery off gases (in India, with IndianOil), and gasified orchard wood and nutshells (in California, with Aemetis) (Simpson, 2018). With the commissioning of these first commercial CO-to-ethanol facilities it is clear that this research field has crossed the so-called 'valley of death' between demonstration and industrial scale. In these reactors the production is steered towards ethanol by operating the fermentation at low pH, high cell density (resulting in a nutrient limitation) and high CO partial pressure (resulting in a more negative redox potential) (Molitor et al., 2016). The rapid adoption of anaerobic gas fermentation by industry can be explained by (i) the presence of energy-rich waste gases containing CO (or CO + H_2) at these facilities, eliminating the need for costly H_2 production, and (ii) the product, bio-ethanol, having its status as drop-in fuel additive.

Figure 7.4 – First commercial scale gas fermentation plant commissioned in 2018 at the Jingtang Steel Mill in China. Picture copied from Simpson (2018).

It needs to be admitted that without ethanol's access to the transport market under renewable energy directives or without the CO-rich off gases that can be exploited as inexpensive feedstock, a positive financial return for the CO_2/H_2 case is not to be expected at current fossil carbon prices. Assuming an electricity cost of 2.5 € per kg H_2 and a 100% electron recovery in the target product, the power input equals 330 and 660 € per ton acetic acid and ethanol, respectively, solely for the production of a diluted product stream of typically 10 – 50 g L^{-1}. As product recovery and other operational costs are excluded from this basic cost calculation, it is clear how inexpensive fossil resources are and how difficult it is to create positive an economic return for CO_2 conversions, even when affordable renewable power is used.

However, to tap into the full potential of gas fermentation, CO_2-rich gases need be targeted following the H_2-route. As ethanol and acetic acid, the two major products of H_2/CO_2 fermentation, have a too low market value to offset the costs incurred with production and recovery, higher value products should be targeted for H_2-mediated fermentation. Two strategies are currently being exploited to overcome the low intrinsic value of these C_2 bulk chemicals: (i) expanding the product spectrum from autotrophic C_1 fermentation toward more value-added molecules, and (ii) (bio)chemical conversion of the natural metabolites of gas fermentation, ethanol and acetic acid, to higher value, more functional platform chemicals.

New fermentation products

The product spectrum can be expanded to virtually every compound for which a biological pathway can be constructed (using synthetic biology) (Molitor et al., 2017). But also the metabolic capabilities of native gas fermenting-acetogens reach beyond acetic acid and ethanol. However, these higher-end products are currently only detected as side-products during fermentation (Latif et al., 2014). Key is to give rise to a selective and efficient redirection of the electron and carbon flux towards the desired metabolite. 2,3-Butanediol is an example of a bulk commodity that has the potential to be produced by acetogens like *Clostridium ljungdahlii* or *C. autoethanogenum*, being of interest for commercialization due to the higher market value compared to acetic acid and ethanol. At present, production rates and titers remain however too low compared to acetic acid and ethanol (Köpke et al., 2011). The selection of the most appropriate microbial process for CO_2 conversion will always be a tradeoff between: (i) the market value of the product, (ii) the volumetric production rates

(keeping the bioreactor compact), (iii) the hydrogen (or electron) uptake efficiency (lowering the power input per kg product), (iv) the specificity towards the target compound (making the product separation easier) and (v) the energy input for gas-liquid mass transfer and product recovery.

Upgrading of ethanol and/or acetic acid

A biotechnological production platform of interest to go beyond acetate or ethanol is chain elongation. Chain elongation enables to convert short-chain carboxylates, such as acetate, into *n*-caproate (C6, hexanoate) with ethanol as a source of carbon, energy, and reducing equivalents (Spirito et al., 2014). Both pure and mixed cultures have shown potential to upgrade ethanol/acetic acid mixtures produced *via* syngas fermentation to longer hydrophobic carbon-chains which spontaneously separate from water at lower pH values around their pK_a (Angenent et al., 2016). It has been shown that syngas fermentation effluent can be directly used for biological chain elongation into caproic and caprylic acid using a pure culture *Clostridium kluyveri* (Gildemyn et al., 2017a). These medium-chain carboxylates are currently harvested from plant oils and animal fat and used as antimicrobials, fragrances, pharmaceuticals, and some other manufacture products (Agler et al., 2011). Recent research has also proposed to convert these biochemicals into liquid biofuels *via* Kolbe electrolysis (Khor et al., 2017; Urban et al., 2017). Kolbe electrolysis involves the removal of two carboxylate functional groups as CO_2, followed by the dimerization of the two chains to form a single chain. For example, caproic acid can be converted to *n*-decane (C10), a component of gasoline and kerosene.

Another strategy to upgrade low-grade organics involves biphasic esterification, in which the aqueous carboxylic reacts with added alcohol in a water excluding phase to generate volatile esters. This pipeline was demonstrated for valorization of the acetic acid produced and extracted through microbial electrosynthesis, by the esterification to ethyl acetate in an ionic liquid (Andersen et al., 2016).

Recently, LanzaTech produced about 16,000 liters of on spec jet fuel from CO-derived ethanol *via* dehydration to ethylene, followed by an oligomerization and hydrogenation to long chain and energy dense hydrocarbons. The first batch of this jet fuel was blended with kerosene and used on a commercial Virgin Atlantic's flight from Orlando to London Gatwick, operated by a Boeing 747 aircraft (Burton, 2018).

4. High-rate bioreactor systems to the rescue?

Meeting future energy, chemicals and food/feed demand will be a key challenge. It has been estimated that we will need to increase total primary energy supply by 27 – 61%, global demand for petrochemicals by 40% and world food production by 60–70% in 2050. To reduce the negative environmental impact of current production processes necessitates the implementation of more sustainable technologies to secure the supply of energy, chemicals and food to 9.5 billion people.

Biomass feedstock is a viable alternative to finite fossil fuel resources to provide fuels and chemicals that have the potential to be more sustainable than their fossil fuel based counterparts. However, the biorefinery concept is challenged by a very low photon-to-product conversion efficiency of the photosynthetic pathway, with values of merely 1 – 2 % (Barber, 2007). At present, the highest production levels of C4 plants like *e.g.* energy maize are in the range of 10 – 20 tons dry matter per hectare per year. Considering an average carbon content in maize of 43.6 % (Loomis & Lafitte, 1987), yearly some 4.36 – 8.72 tons of carbon ha^{-1} can be captured and refined (based on a full conversion of the crop). Realizing that about 12.6 Gt C is needed to replace the current annual fossil carbon consumption, ~ 10 – 20 % of the land surface on Earth needs to be cultivated with the most efficient C4 crops. That is an area equal or double the current estimated arable land surface (Bruinsma, 2017). These numbers clearly indicate that cultivating biomass is rather an ineffective method for active carbon capture and that the shortened cycle with (bio)chemical refining of energy crops or organic waste residues still shows an enormous imbalance in the speed at which carbon is reduced by plants and consumed by man.

Even considering that biomass refining will be insufficient to meet current and future world energy demands, biorefineries will have their place in a CO_2-neutral world as agro-industrial side streams and organic waste will keep on requiring treatment. The key is, however, to recognize the real opportunity of each substrate-product combination and to find an effective niche for each specific bio-based product. Concerns that first generation biofuels had not delivered the environmental benefits they had promised initially (Gasparatos et al., 2013), the European Commission has the ambition to gradually phase out first generation biofuels –by reducing the cap on food-crop-based biofuels in the EU's

transport fuel mix– and make a turn to advanced (second, third or fourth generation) biochemicals and - fuels (European Parliament, 2018) (Aro, 2016).

Calling a halt to the bioenergy-driven agricultural expansion is critical to conserving natural ecosystems and global biodiversity, but also critical to secure food availability (Burneya et al., 2010). Efficiency improvements in the entire food-chain, intensification of cropland production and dietary changes toward less land-demanding food commodities are considered as major options to limit land area used for livestock production – which currently accounts for about 80% of total agricultural land use – and reduce livestock's environmental impact. An emerging technology discussed in this thesis is microbial protein production which tends to be more efficient in terms of nitrogen, water and land use, and can be grown at high rate on renewable carbon sources like methane, ethanol, organic waste streams and CO_2 (Pikaar et al., 2017). The direct CO_2 usage by the autotrophic HOB offers the potential to contribute to CO_2 avoidance relative to the conventional agricultural food supply line. The HOB route has, in principle, a CO_2 footprint that is negative, since anthropogenic CO_2 is fixed in microbial biomass, and renewable hydrogen is used as energy source. By harvesting solar photons *via* photovoltaic technology, an artificial, *i.e.* photosynthesis-independent, production pipeline can be set up, bypassing the current agriculture-based primary production. The direct conversion of solar energy and inexhaustible raw materials like water and CO_2 into a product will create new supply routes with a low environmental impact in terms of land and water footprint, greenhouse gas emission and nutrient management (Aro, 2016; Matassa et al., 2015a). Microbial bioconversions like methanation, MES/gas fermentation and HOB cultivation have the potential to become resource-efficient in-reactor based alternatives for anaerobic digestion, fermentation of lignocellulosic material and conventional land-based protein production, respectively (Figure 7.5). The high volumetric productivities by microbes grown in bioreactors enable production in well-engineered, intensive and confined fermentation reactor systems, offering the potential to short-cut the current unbalanced C and N cycles.

To adhere to the Paris Agreement of 2015, we will need to store about 4–5 Gt CO_2 annually. It needs to be recognized that the carbon capture and storage potential of CO_2-to-chemical routes is limited. A recent study concluded that chemical conversion of CO_2 will, at best, account 1% of the climate change mitigation challenge, due to limited amount of CO_2 that can be captured at reasonable cost and the limited demand for chemicals compared to

energy. Even a scaled-up enhanced oil recovery-CCS industry will likely only account for 4 – 8% of the mitigation challenge (Mac Dowell et al., 2017). From a perspective of mitigating anthropogenic climate change CO_2 utilization into chemicals seems, thus, highly insufficient. Nonetheless CCU provides an interesting approach to materialize CO_2 as carbon source for chemical synthesis without consumption of depleting fossil fuels. The mitigation potential of HOB-based protein production from CO_2 is not yet reported, but given the fact that current protein demand is approximately 202 million tonnes globally (Henchion et al., 2017), the production of microbial protein for food-feed applications can offer long-term perspectives to contribute to carbon capture and climate change abatement.

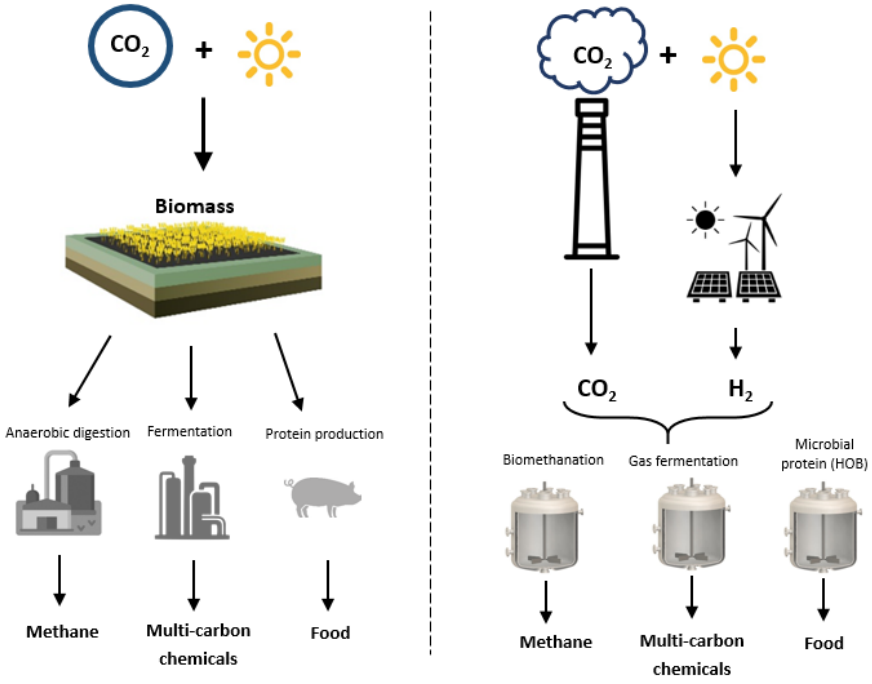

Figure 7.5 – Biomass *vs.* CO_2-based bioproduction routes.

5. Legislation and support for CCU

New innovative technologies for CO_2-based production are still more expensive compared to fossil energy sources and thus financial support schemes for renewable chemicals are needed to de-risk investment. Legislation and support will be important drivers to penetrate the competitive fossil fuel based market with CO_2-based chemicals by creating an investment climate for CCU. A long term vision on the role of CCU is needed as currently the added value of CCU for our economy is not recognized. At present day, the status of CCU is rather unclear, preventing a rapid adoption of CCU approaches by industry. Policymakers are unsure how to deal with the origin of the carbon in the waste gases since the CO_2 feedstock can be of fossil, biogenic or atmospheric origin. Although there is no scientific basis for distinguishing renewable carbon from CO_2, biomass and fossil sources, government support is limited to biofuels and biochemicals made from biomass. A key feature of renewable chemical production will be to provide a fair legislative and subsidiary system for each specific route from feedstock to product, that recognizes the actual CO_2 reduction potential of a certain pipeline, rather than a classification based on feedstocks or technologies. It is highly questionable why biofuels are heavily subsidized as today the renewable energy technologies are competitive with fossil energy sources, while biofuels and chemicals from CO_2 are not supported. A more efficient criterion to evaluate the environmental impact of a product is the use of the actual carbon footprint per product over its entire life cycle. In this way all CO_2 emitted is considered equal and taxes are being paid for the actual environmental damage. Internalization of economic, environmental and social externalities would facilitate a more powerful transition to an economy with alternative energy and carbon sources. The use of incentives focusing on efforts to decrease CO_2 emission. Replacing subsidies by a general carbon taxation is a more fair strategy to push to renewable technologies, avoiding selective support and the fluctuating willingness to maintain a certain support scheme. This would avoid the use of 'bio-labelling' and the use of complex schemes for premiums.

Taxation of carbon

Putting a price on each tone of CO_2 emitted with a carbon pricing mechanism is considered as a cost- effective economic instrument for emissions reductions, even with a modest carbon price. Along a carbon tax, a carbon pricing policy that aims to gradually cause a market response across an entire economy is emissions trading, like the EU-ETS ("Emissions Trading System") between European companies. ETS is a so-called "cap-and-trade" system.

Based on an emission ceiling ("cap") a certain number of emission allowances are assigned to every industrial installation (based on the product's benchmarked emissions and historical production), and these allowances can be traded on the market ("trade"). The current European CO_2 emission allowance price is fluctuating between 20 and 25 € per ton CO_2, but the longer view is that carbon taxes will increase up to 50 € per ton CO_2 to force larger concerns to reduce their emissions (Narassimhan et al., 2017). To illustrate the impact carbon pricing can have on certain carbon intensive sectors, the steel industry is taken as an example. Considering that the average CO_2 emission for primary steel making is around 1.9 tons of CO_2 per ton of steel produced (Kundak et al., 2009), the production cost will increase with roughly 100 € per ton steel. Realizing that the average EU carbon steel price is approximately 550 € per ton (SteelBenchmarker, 2019), carbon taxation will result in a significant price increase, putting themselves out of the market as long as there is no worldwide system for CO_2 pricing. In addition, this will result in so-called carbon leakage, where emitters in countries with strict emission requirements relocate their activities to less efficient installations in areas with less strict emission allowances, thereby undermining the environmental effectiveness of these programs. There are two options to avoid carbon leakage, by creating an international market for carbon or by taxing imported products on the same basis.

Integrating CCU in carbon pricing policies

For many sectors CCU is often the only way to reduce emissions in a cost effective, especially for energy and/or carbon intensive industries that generate CO_2 as part of their production processes (like steel, alloy and cement). The ability to reduce the carbon tax burden through CO_2 valorization will give industries a competitive advantage over competitors that do not valorize CO_2. Using CO_2 as a raw material to produce the core monomer building blocks from emission streams, carbon credits can be attained that can be traded as an additional source of income.

At present the ETS has not yet been modified to include the re-use of carbon from waste gas. At present day, industrial plants are monitored based on their fossil carbon input rather than their actual stack emissions, and thus, all emissions are allocated to the product the industrial plant is producing (being steel, cement, or any chemical or material). As the carbon is now transformed into a product rather than being emitted as CO_2, an amendment to the emission monitoring guidelines is highly recommended as this will result in a real incentive for carbon intensive industries. A valid CO_2 allocation system for CCU within the ETS

framework should be based on the actual GHG performance of the CO_2-based product over its entire life cycle, independently of the technology or the origin of the carbon or its emission. It is clear that current carbon taxation attempts fail to internalize the real environmental cost of GHG emissions, as only the damage caused by stack emissions is taken into account, and other carbon-intensive activities relative to fossil fuel use, such as mining, transportation and wastewater treatment, not. Furthermore, important sectors like transportation and agriculture are not included in these pricing strategies. Benchmarking will be crucial to evaluate the real savings in terms of environmental footprint when transitioning toward a sustainable technology. A portion of the revenue should be reinvested in new emission-reduction activities. In addition, it is not unthinkable that a part of the revenues will have to be allocated in ways that compensate the tax burdens falling onto (often lower income) populations.

6. More research required

Two novel value chains for biogas were discussed in this thesis. In the context of chemical production from grid injected biomethane, more research into the super-dry methane reforming process is needed, as at present day the process is still in an early research phase. Crucial aspects to be addressed in further research are the choice of reactor configuration as well as the further development of stable and performant oxygen storage materials and CO_2 sorbents. Efforts towards scaling up are necessary in order for the process to reach maturity in the coming years and attract attention by industry. As microbial-based feed formed through the fermentation of methane by methanotrophic microorganisms has already reached commercial scale, there is actually no big hurdle toward implementation at digester scale. In this recovery context, the real barriers are more from a legislative, economic and market uptake origin rather than scientifically.

Several syngas fermentation plants are being built at industrial scale, but currently only concern ethanol production from CO-rich waste gas. The production of short- and medium chain fatty acids from syngas or CO_2/H_2 is not yet commercialized, because subsidies are nowadays only warranted for renewable transportation fuels, but also because recovery technologies for carboxylates from fermentation broths have not reached industrial scale. After the proof of concept provided in this thesis, membrane electrolysis now must be demonstrated at a greater scale and critically analyzed. A demonstration scale reactor will bring more information on the power input, cell resistance, membrane lifetime and fouling behavior, flux and mass transfer phenomena, and enables to take a first step towards implementation.

Abstract

Human distortion of the Earth's long-term carbon cycle has by far exceeded the sustainability boundaries of our planet (**Chapter 1**). To sustain the ever-growing world population and reduce the current global environmental unbalances, more sustainable platforms to provide by 2050 about 9 billion individuals with energy, chemicals and food are needed. Renewably electricity production enables us to partially uncouple the carbon cycle from the energy cycle, and, thus, take an important step towards a decarbonized energy supply. However, the irreplaceable nature of carbon-containing compounds in an enormous variety of products makes it unrealistic to rapidly ban carbon from the entire supply of energy and chemicals and, thus, CO_2 production at the end of the product's life cycle cannot be avoided. Therefore, carbon capture and utilization (CCU) is a necessary measure to reduce CO_2 emissions at point sources and take the road to a CO_2-neutral world. CCU is a way to materialize CO_2 emissions and store low-carbon energy in the form of renewable carbon-based fuels and chemicals.

Microbial technologies for the production of added-value products from CO_2 are emerging as potential approaches for CCU. Hydrogen gas produced *via* renewable-powered electrolysis is envisaged as a key energy vector to enable an efficient coupling of renewable electrons from the electricity grid with an autotrophic microbial metabolism. This work discusses biomethanation, gas fermentation, microbial electrosynthesis and microbial protein production by hydrogen-oxidizing bacteria as novel biotech platforms for the production of methane, multi-carbon organics and protein, respectively.

In addition, renewable methane produced through anaerobic digestion of waste biomass is suggested as a valuable C_1 stream which can be combined with CO_2 to provide CO, a versatile feedstock for synthesis of high-value platform chemicals (**Chapter 2)**. Technical modelling and detailed cost and revenue calculations for each step in the value chain (biogas production, upgrading, injection and valorization) reveals that *via* (super-)dry reforming of grid-injected biomethane to CO or syngas, the revenues from biogas could be increased drastically, to a point that, even without financial support and at present values and costs, an economically positive case can be created, thereby outcompeting the conventional

valorization of biogas and biomethane as fuel in a cogeneration plant. By coupling decentralized biomethane production to large-scale chemical synthesis *via* the existing natural gas infrastructure, chemical plants can reduce their CO_2 emissions in a cost-effective way, or, alternatively, increase the production capacity without considerably increasing CO_2 footprint, thereby still generating a product that gives profit, even when biomethane is used instead of cheaper natural gas. By using a well-defined blend of renewable and fossil methane, chemical plants can flexibly and gradually shift towards biomass as renewable resource in order to meet consumer's demands for sustainability. Anaerobic digesters have the potential to become the drivers of new biomass-based value chains, in which costly biomass transportation is avoided and industrial waste CO_2 is incorporated in chemicals and fuels. Based on the current global availability of biogas, it was calculated that CO production through super-dry reforming of biomethane could cover the current global methanol demand, assuming that a renewable source of H_2 would be available and all available biogas is upgraded to biomethane.

In **Chapter 3** an electricity-driven pipeline for the purification and upgrading of raw biogas to high-quality biomethane was proposed. The use of electrical energy to produce biomethane enables storage of intermittent renewable energy in the existing natural gas network. The proposed pipeline consists of two parts: (i) Membrane Electrolysis, an electrochemical treatment step where water electrolysis induces CO_2 and H_2S scrubbing while driving the dissociated anions across an ion exchange membrane, and (ii) Biomethanation, a process that makes use of hydrogenotrophic methanogens to convert residual CO_2 into additional CH_4 with H_2. Electrochemically-assisted scrubbing and stripping of CO_2 and H_2S resulted in high removal efficiencies (up to 100%), without addition of chemicals. The biogas leaving the electrolysis cell could be further upgraded *via* chemoautotrophic microbial conversion to a product gas with > 98% CH_4.

Nutrients are more and more regarded as potential new building blocks, but at present day, the products deriving from digestate achieve a market value that is too low to offset the costs incurred with the recovery. In **Chapter 4** an economically justified implementation of

resource recovery at digester scale was explored through up-cycling of recovered nitrogen as microbial protein. The direct assimilation of reactive nitrogen by means of methane-oxidizing and hydrogen-oxidizing bacteria was evaluated as a route to upgrade ammonia recovered from anaerobic digestion to high value microbial biomass that can replace conventional protein sources in livestock feed. The direct use of fast growing bacteria in the process of nutrient removal might create an interesting shortcut in the current inefficient nitrogen cycle. An economic analysis revealed that the methane-based microbial protein production route can compete with the soymeal-for-feed route, while the NH_3-H_2 route is only of micro-economic interest when a market price of fishmeal is considered. The process scheme here depicted would be capable to up-cycle up to 100% of the total nitrogen recovered from the treatment plant directly to valuable microbial biomass rich in edible protein. The possibility to implement such technologies on-site might enable partial self-supply of feed fodder.

Chapter 5 discussed the features of a microbial electrosynthesis (MES) system that uniquely couples production, extraction and concentration of acetic acid as a single organic acid in a solid-free extraction liquid. The electro-migration of dissociated acetic acid anions across an anion exchange membrane (AEM) was investigated on the hypothesis that the reactor with *in situ* extraction would have a higher production efficiency compared to conventional MES systems without *in situ* extraction. The comparative study demonstrated that membrane electrolysis can create a more stable reactor operation with and increased performance (32% higher production rate and efficiency), likely through the combined effect of a stable and high cathode pH (8.15 ± 0.15) and a low cathodic product concentration. Due to water displacement across the membrane, the product concentration in the AEM reactor (9 g L^{-1}) did not exceed the concentration in the classic reactor configuration with two compartments and a cation exchange membrane (10.5 g L^{-1}).

In situ extraction is valuable in MES to suppress production inhibition and recover the product, but it is also a challenging task considering the high energy input to drive the process from CO_2, the low production rates and the low extraction efficiency (now limited to 12.5% of the charge balancing). In **Chapter 6**, the use of an external fermenter to which the

electrochemical system could be coupled, was investigated as a strategy to increase the production rate and energy efficiency of the system. By injection of additional hydrogen gas (produced *via* water electrolysis) the charge balancing efficiency by acetate was increased up to 31 %, thus, making more efficiently use of the intrinsic ability to extract anions. The use of electrolytic H_2 lowered the power input for bioproduction (15 kWh kg^{-1} acetic acid) and increased the product concentration in the extraction liquid (up to 37 g L^{-1}). Furthermore, electrochemical water reduction in the cathodic chamber could completely replace chemical pH control with electrolysis products.

Samenvatting

De ontwrichting van de natuurlijke koolstofkringloop door menselijke activiteit heeft de duurzaamheidsgrenzen van onze planeet ver overschreden (**Hoofdstuk 1**). Om het hoofd te kunnen bieden aan de steeds groter wordende wereldbevolking, en tegelijkertijd de impact op ons milieu te verminderen, zijn er nieuwe technologieën nodig om tegen 2050 zo'n 9 miljard mensen op een duurzame wijze te voorzien van energie, chemicaliën en voedsel. Hernieuwbare elektriciteitsproductie stelt ons in staat om de koolstofcyclus gedeeltelijk los te koppelen van de energiecyclus, en dus een belangrijke stap te zetten naar een koolstofarme energievoorziening. Het onvervangbare karakter van koolstofhoudende verbindingen in een enorme verscheidenheid aan producten maakt het echter onrealistisch om koolstof zomaar uit het volledige aanbod van energie en chemicaliën te weren. De productie van CO_2 aan het einde van de levenscyclus van het product kan met andere woorden niet worden voorkomen. Daarom is koolstofafvang en -benutting een noodzakelijke maatregel om puntbron-gerelateerde CO_2-uitstoot te verminderen en de weg in te slaan naar een CO_2-neutrale wereld. Koolstofafvang en -benutting is een manier om CO_2-emissies te materialiseren en om koolstofarme energie op te slaan in de vorm van duurzame brandstoffen en chemicaliën

Microbiële technologieën voor de productie van hoogwaardige chemicaliën uit CO_2 treden steeds meer op de voorgrond als mogelijke benaderingen voor koolstofafvang en -benutting. Waterstofgas geproduceerd via elektrolyse van water wordt naar voor geschoven als een mogelijke energievector die een efficiënte koppeling mogelijk maakt van hernieuwbare elektronen uit het elektriciteitsnet en een autotroof microbieel metabolisme. Dit werk bespreekt biomethanisatie, gas fermentatie, microbiële elektrosynthese en microbiële eiwitproductie door waterstof-oxiderende bacteriën als nieuwe biotechnologische platformen voor de productie van respectievelijk methaan, meervoudige koolstofverbindingen en eiwitten.

Daarnaast werd hernieuwbare methaan, geproduceerd door anaerobe vergisting van afvalbiomassa, voorgesteld als een waardevolle C_1-stroom die kan worden gecombineerd met CO_2 om CO te produceren, een veelzijdige grondstof voor de synthese van hoogwaardige

platformchemicaliën (**Hoofdstuk 2).** Technische modellering en gedetailleerde berekeningen van de kosten en opbrengsten voor elke stap in de waardeketen (biogasproductie, opwaardering, injectie en valorisatie) tonen aan dat de benutting van biogas en biomethaan voor productie van warmte en elektriciteit aanzienlijke subsidies vereist, terwijl een chemische valorisatie van geïnjecteerde biomethaan via (super-)droge reforming naar koolstof monoxide of synthese gas een economisch positieve business case creëert, en dit zelfs zonder subsidies, aan de huidige waarden en kosten. Door de koppeling van een decentrale productie van biomethaan aan grootschalige chemische synthese via de bestaande aardgasinfrastructuur, hebben anaerobe vergisters het potentieel om nieuwe waardeketens in de chemische industrie aan te drijven, waarbij duur biomassatransport wordt vermeden en CO_2 wordt omgezet in chemicaliën en brandstoffen. De voorgestelde valorisatieroute stelt de chemische industrie in staat om hun CO_2-uitstoot te verlagen terwijl nog steeds een product wordt gegenereerd dat winst oplevert, zelfs wanneer biomethaan wordt gebruikt in plaats van goedkoper fossiel aardgas. Op basis van de huidige wereldwijde beschikbaarheid van biogas werd berekend dat de CO-productie door super-droge reforming van biomethaan de huidige wereldwijde vraag naar methanol zou kunnen dekken, ervan uitgaande dat een hernieuwbare bron van H_2 beschikbaar zou zijn en alle het beschikbare biogas wordt opgewaardeerd tot biomethaan.

In **Hoofdstuk 3** werd een door elektriciteit aangedreven strategie voor de opwaardering van biogas naar hoogwaardig biomethaan voorgesteld. Het gebruik van elektrische energie voor de productie van biomethaan maakt de opslag van intermitterende hernieuwbare energie in het bestaande aardgasnet mogelijk. De voorgestelde strategie bestaat uit twee opeenvolgende processtappen: (i) membraanelektrolyse, een elektrochemische behandelingsstap waarbij elektrolyse van water CO_2 en H_2S-wassing aandrijft terwijl gedissocieerde anionen door een anionen-uitwisselingsmembraan worden getransporteerd, en (ii) biomethanisatie, een proces dat gebruik maakt van hydrogenotrofe methanogenen om de resterende CO_2 om te zetten in extra CH_4 met behulp van elektrolytische H_2. Het elektrochemisch geassisteerde absorberen en strippen van CO_2 en H_2S resulteerde in hoge verwijderingsefficiënties (tot 100%), en dit zonder toevoeging van chemicaliën. Het biogas

dat de elektrolysecel verlaat, kan vervolgens verder worden opgewaardeerd via chemo-autotrofe microbiële omzetting in een productgas met > 98% CH_4.

Nutriënten worden meer en meer beschouwd als potentiële nieuwe bouwstenen, maar tot op heden hebben de producten die worden afgeleid uit digestaat, een marktwaarde die te laag is om de kosten van de herwinning te compenseren. In **Hoofdstuk 4** werd een economisch verantwoorde implementatie van grondstof hergebruik op de schaal van een mestvergister onderzocht door teruggewonnen stikstof op te waarderen tot microbieel eiwit. De directe assimilatie van reactieve stikstof door middel van methaan-oxiderende en waterstof-oxiderende bacteriën werd geëvalueerd als een route om ammoniak die werd teruggewonnen uit anaerobe digestie te upgraden naar hoogwaardige microbiële biomassa die conventionele eiwitbronnen in veevoer kan vervangen. Het gebruik van snelgroeiende bacteriën om herwonnen nutriënten te valoriseren, kan een interessante kortere weg creëren in de huidige inefficiënte stikstofcyclus. Een economische analyse bracht aan het licht dat de methaan-gebaseerde microbiële eiwitproductieroute reeds kan concurreren met de sojameel route, terwijl de NH_3-H_2-route enkel van micro-economisch belang is wanneer de marktprijs van vismeel kan worden bekomen. Het hier afgebeelde processchema zou in staat zijn om tot 100% van de totale hoeveelheid stikstof die uit de behandelingsinstallatie wordt teruggewonnen, direct op te waarderen tot waardevolle microbiële biomassa, rijk aan eetbaar eiwit.

In **Hoofdstuk 5** werden de kenmerken besproken van een microbieel elektrosynthesesysteem (MES) dat op een unieke wijze de koppeling van de productie, extractie en concentratie van azijnzuur in een propere extractievloeistof mogelijk maakt. De elektro-migratie van gedissocieerde azijnzuuranionen over een anion-uitwisselingsmembraan (AEM) werd onderzocht op de hypothese dat de reactor met in situ-extractie een hogere productie-efficiëntie zou hebben, vergeleken met conventionele MES-systemen zonder in situ-extractie. De vergelijkende studie toonde aan dat membraanelektrolyse een stabielere reactorwerking kan creëren, waarbij verhoogde prestaties worden waargenomen (32% hogere productiesnelheid en efficiëntie),

waarschijnlijk door het gecombineerde effect van een stabiele en hoge kathodische pH (8,15 ± 0,15) en een lage product concentratie in het kathode compartiment. Vanwege de waterverplaatsing over het membraan, overschreed de productconcentratie in de AEM-reactor (9 g L^{-1}) de concentratie in de klassieke reactorconfiguratie met twee compartimenten en een kationen-uitwisselingsmembraan (10,5 g L^{-1}) niet.

In situ-extractie is waardevol in MES om product-inhibitie te onderdrukken en het product terug te winnen, maar het is ook een uitdagende taak gezien de hoge energie-input om het proces aan te sturen, de lage productiesnelheden en de lage extractie-efficiënties (nu beperkt tot 12,5% van de ladingsbalans). In **Hoofdstuk 6** werd het gebruik van een externe fermentor waaraan het elektrochemische systeem werd gekoppeld, onderzocht als een strategie om de productiesnelheid en energie-efficiëntie van het systeem te verhogen. Door injectie van extra waterstofgas (geproduceerd via waterelektrolyse) werd de ladingsbalans-efficiëntie door acetaat verhoogd tot 31%, waardoor efficiënter gebruik werd gemaakt van het intrinsieke vermogen om anionen te extraheren. Het gebruik van elektrolytische H_2 verlaagde het benodigde energetische vermogen voor bioproductie (15 kWh kg^{-1} azijnzuur) en verhoogde de productconcentratie in de extractievloeistof (tot 37 g L^{-1}). Verder verving elektrochemische waterreductie in de kathode de chemische pH-regeling volledig door elektrolyseproducten.

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Appendix

Appendix 1 - Supplementary information for Chapter 2

Table S 1.1 - Main technical design data.

Biogas yield (Nm^{-3} biogas kg^{-1} fresh matter)	
Energy crops	210
Agricultural residues	100
Organic fraction municipal solid waste	100
Sewage sludge	80
Food waste	210
Manure	28
Biogas production rate (Nm^3 h^{-1})	1 000
Energy density methane (kWh Nm^{-3})	10.85
Energy density biogas (kWh Nm^{-3})	6.50
Mass density biogas (kg Nm^{-3})	1.22
Energy density biogas (MWh ton^{-1}_{biogas})	5.33
Raw biogas composition	
CH_4 content (vol.%)	60
O_2 content (vol.%)	0.1
N_2 content (vol.%)	0.4
H_2S content (ppmv)	50
CO_2 content (vol.%)	39.5

Table S 1.2. - Feed and product flow rates for super-dry reforming (SDR), dry reforming of methane (DRM) and sorption-enhanced steam reforming of methane (SESR).

	Feed (ton day^{-1})			Product (ton day^{-1})				Energy input	
Case	CH_4[a]	CO_2	H_2O	CO	H_2	CO_2	H_2O	(GJ ton^{-1} CH_4)	(GJ ton^{-1} CO_2)
SDR	176	1455	-	1234	-	-	396	21.0	2.55
DRM	176	485	-	617	44	-	-	16.3	5.94
SESR	176	-323	397	-	88	808	-	15.8	-

[a] The feed flow rate of CH_4 corresponds with 6000 Nm^3 h^{-1} (which in turn corresponds with 10000 Nm^3 h^{-1} biogas or about 10 large scale AD plants).

Table S1.3. - Biogas production cost compared to average and extreme reference systems.

Biogas production cost	Average	Min	Max
Feedstock cost (€ ton^{-1})[a]	**4.91**	2.00	50.00
Capital investment (€)[b]	**4 000 000**	3 500 000	4 500 000
CAPEX (€ year^{-1})[c]	**305 000**	266 875	343 125
OPEX (€ year^{-1})[d]	**300 000**	175 000	450 000
Biogas production (MWh year^{-1})[e]	**57 052**	57 052	57 052
Production cost (€ MWh^{-1})	**21.4**	9.8	41.1
Production cost (€ ton$^{-1}_{biogas}$)[f]	**114.4**	52.2	219.0

[a] Assumed that every substrate represents 20 % of the total biogas production. Assumed transport cost is 2.8 € ton^{-1}.

Only maize silage was assumed to have a feedstock cost (30 € ton^{-1}) (Balussou et al., 2012).

[b] Assumed investment: 3000 (Min), 4000 (Avg) and 5000 (Max.) € Nm^{-3} h^{-1} installed biogas capacity. Investment without investment subsidy or support.

[c] Calculated according to the annuity method with an interest of 5% and 20 years depreciation.

[d] Assuming 5 % (Min.), 7.5% (Avg.) and 10 % (Max.) of the total investment.

[e] Assumed methane content is 60 vol.% (Calculated under the assumption that no plant shutdown occurs).

[f] Assuming 4.91 MWh ton^{-1} $_{biogas.}$

Table S1.4. - Power and heat production cost contributions.

Power and heat production cost	Average	Min	Max
Installed power (kW_e)	**2500**	2500	2500
Efficiency (%)			
Electricity	**35**	35	35
Heat	**45**	45	45
Capital investment (€)[a]	**1 250 000**	1 000 000	1 500 000
CAPEX (€ $year^{-1}$)[b]	**231 250**	185 000	277 500
OPEX (€ $year^{-1}$)[c]	**100 000**	50 000	200 000
Production cost (€ MWh_e^{-1})	**17**	12	24
Production cost (€ ton^{-1})	**31.0**	22.0	44.6

[a] Assumed investment in the CHP plant is 500 € kW_e^{-1} (Lantz, 2012).
[b] Depreciation period of 5 years for the engine and 10 years for other installations. Engines represent approximately 35% of the investment.
[c] Assuming 20 € kW_e^{-1} (Min.), 40 € kW_e^{-1} (Avg.) and 100 € kW_e^{-1} (Max.).

Table S1.5. - Biomethane composition and technicalities of the gas upgrading unit and additional components for the different upgrading techniques.

Biomethane composition	*PWS*[a]	*PSA*[b]	*AS*[c]	*GP*[d]
Volume flow ($Nm^3\ h^{-1}$)	606.2	606.2	618.3	615.5
CH_4 content (vol.%)	97	97	97	97
O_2 content (vol.%)	0.47	0.07	0.16	0.08
N_2 content (vol.%)	1.57	0.33	0.65	0.65
H_2S content (ppmv)	0.68	0.26	0.44	0.33
CO_2 content (vol.%)	0.96	2.6	2.19	2.28
Technical parameters of biogas upgrading plant	*PWS*	*PSA*	*AS*	*GP*
Methane slip (vol.%)	2	2	0.04	0.5
Biomethane pressure (bar)	8	7	1	6
Technical parameters of grid injection	*PWS*	*PSA*	*AS*	*GP*
Length of biomethane pipeline (m)	100	100	100	100
Gas grid pressure (bar)	14	14	14	14

[a] *PWS = pressurized water scrubbing*
[b] *PSA = pressurized swing adsorption*
[c] *AS = amine scrubbing*
[d] *GP = gas permeation*

Table S1.6. - Estimated CAPEX contribution to reforming processes.

	Foster Wheeler, 2013 (Bressan & Davis, 2013)	Salkuyeh, 2017 (Salkuyeh et al., 2017)	Compagnoni, 2017 (Compagnoni et al., 2017)
Reforming process	SRM[a]	SRM[a]	SRE[b]
Plant capacity (ton CH_4 day^{-1})	553[c]	1814	55[c]
Capital investment (M€)[d]	85.7	217	16.2
Depreciation time (years)[e]	15	15	30
Interest (%)[f]	5	5	5
Percentage of time on stream (%)[g]	95	95	96
CAPEX (M€ $year^{-1}$)[h]	8.00	20.3	0.946
CAPEX (k€ day^{-1})	21.9	55.5	2.59
CAPEX (€ ton^{-1} CH_4)	41.8	32.2	49.0

[a] Steam reforming of methane (SRM)

[b] Steam reforming of ethanol (SRE)

[c] The reported plant capacity in terms of H_2 production was converted into CH_4 processing capacity by assuming a 3.1 mol H_2 mol CH_4^{-1} yield (based on our Aspen Simulations for SESR).

[d] The conversion factor between US$ and € was obtained from **https://www.statista.com/statistics/412794/euro-to-u-s-dollar- annual-average-exchange-rate/** taking into account the year of publication of the source data.

[e] The depreciation time was assumed 15 years in case it was not specified in the reference.

[f] The interest on capital investment was assumed to be 5%.

[g] Percentage of the time on stream is assumed 95% in case it was not specified in the reference.

[h] Calculated according to the annuity method taking into account the specific depreciation time and interest.

Table S1.7. - Overview of fuels, raw chemicals, bulk chemicals and other chemicals and their global production volume/capacity as well as the amount of carbon involved. The amount of CO that would be necessary to meet the production volume/capacity according to the reaction scheme of paragraph 2.3.3 is determined and linked to the amount of CO_2 that can be converted by SDR.

Fuels and raw chemicals	**Mt year^{-1}**	**Mt C year^{-1}**	**Mt CO yr^{-1}**	**Mt CO_2 year^{-1} [b]**	**%BMPP[c]**	**%SCE[d]**	**Year**
Coal production (World Energy Council, 2016)	7860	2360-6680					2015
Oil production (World Energy Council, 2016)	4400	3740	8800	10370	265	49	2015
Natural gas production (World Energy Council, 2016)	2870	2190					2015
	15130	**8290-12610**	**8800**	**10370**	**265**	**49**	
Bulk chemicals							
Ethylene (Boulamanti & Moya, 2017)[a]	154	132	308	363	9.3	1.73	2013
Propylene (Boulamanti & Moya, 2017)[a]	148	127	296	349	8.9	1.66	2013
Olefins (via MTO process) (Tian et al., 2015)	*11*	*9.4*	*22*	*26*	*0.7*	*0.13*	*2014*
Ethanol (Canilha et al., 2013)	68	35.4	82.5	98	2.5	0.46	2011
Methanol (Boulamanti & Moya, 2017)[a]	98	37	86	101	2.6	0.48	2013
Methanol (Boot-Handford et al., 2014)	55	20.6	48	56	1.4	0.27	2013
Formaldehyde (Bahmanpour et al., 2016)	30	12	28	33	0.8	0.16	2016
Acetic acid (Yang et al., 2013)	6.5	1.3	3	4	0.1	0.02	2013
	560	**365**	**852**	**1004**	**25.6**	**4.8**	
Other chemicals							
Phosgene (Jakobsson et al., 2015)	3	0.37	0.86	1.05	0.03	0.005	2014
Acetaldehyde (Eckert et al., 2006)	1	0.30	0.60	0.71	0.02	0.003	2006
Polycarbonate (Boot-Handford et al., 2014)	4	0.14	0.34	0.40	0.01	0.002	2014
Dimethylcarbonate (Araújo et al., 2014)	0.4	0.053	0.13	0.15	0.004	0.001	2014
	8.4	**0.86**	**1.9**	**2.31**	**0.06**	**0.011**	

[a] Global "capacity" is reported rather than the actual production volume

[b] Amount of CO_2 that can be converted into CO to meet the demand of chemicals/fuels when considering reaction stoichiometry of SDR: $1CH_4 + 3CO_2 = 4CO + 2H_2O$

[c] Percentage of the global biomethane production potential (assumed 658 Mt biomethane year^{-1}) necessary to provide CO for chemicals/fuels production by SDR, taking into account CH_4 necessary for providing process heat. The current production reaches around 3.5% of this production potential.

[d] Percentage of stationary CO_2 emission sources (estimation for 2015) that could be valorized by production of chemicals/fuels, taking into account that 1 mol CH_4 and 3 mol CO_2 are converted into 4 mol CO according to reaction stoichiometry.

[e] The reported range of Mt C/year originates from the highly variable carbon content of coal (ranging from 30 to 86 w% carbon).

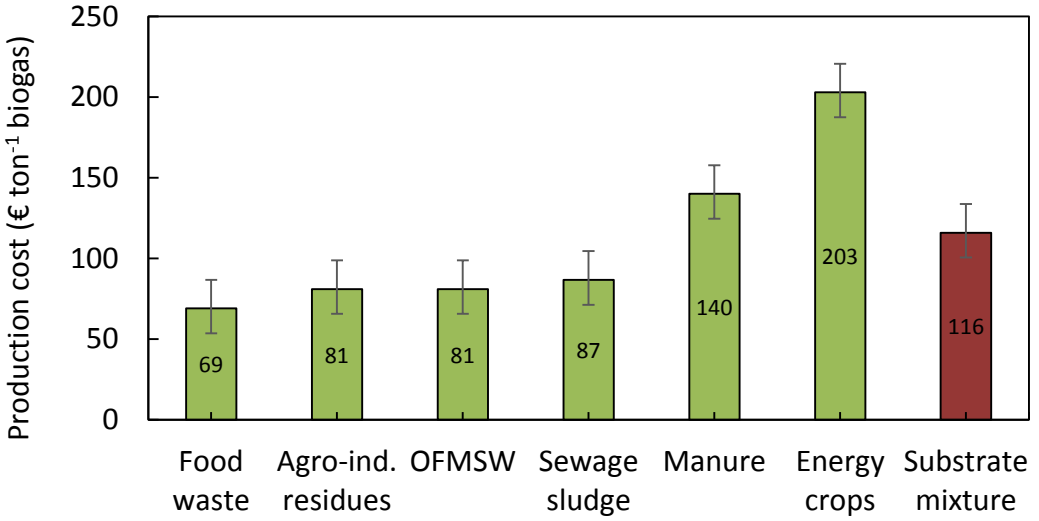


Figure S1.1. - Specific raw biogas production cost in € ton^{-1} biogas for 6 different feedstocks, and for co-digestion of the 6 substrates (every substrate represents 1/6th of the total biogas production).

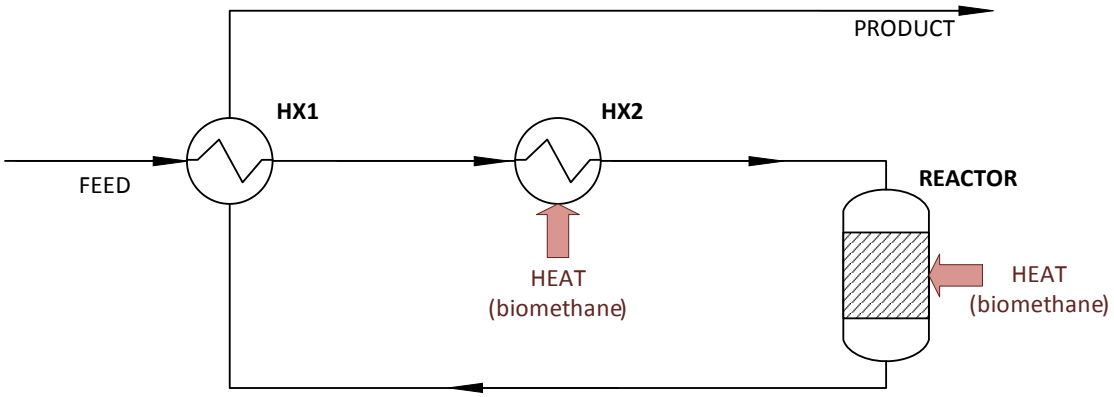


Figure S1.2. - Flowsheet used for Aspen Plus simulation of biomethane conversion to CO, syngas or H_2. (HX1: shell-tube heat exchanger, HX2: heat exchanger, REACTOR: RYield reactor.)

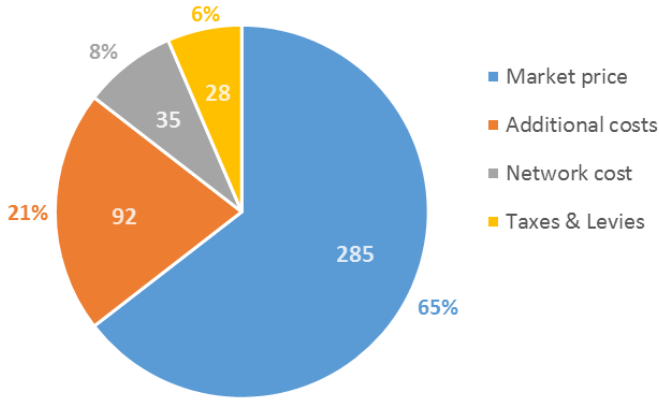


Figure S1.3. - Pie chart representing the contributions (percentual and in € ton^{-1} CH_4) that constitute the consumer price of natural gas for industrial end-users in 2015 (average for EU-28, 2015). Values are based on a report made by the European Commission (EC, 2016).

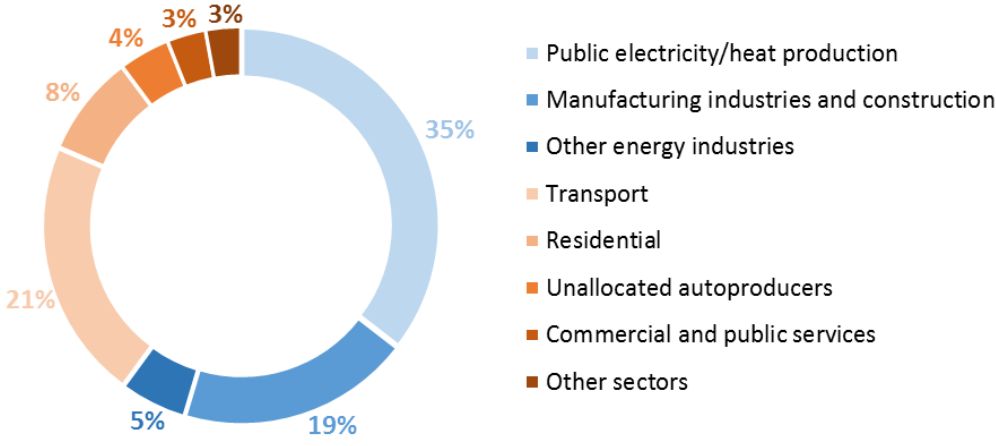


Figure S1.4. - Contributions to global CO_2 emissions: blue contributions constitute large stationary point sources, while orange and brown contributions constitute mobile sources as well as small stationary point sources (IPCC, 2005).

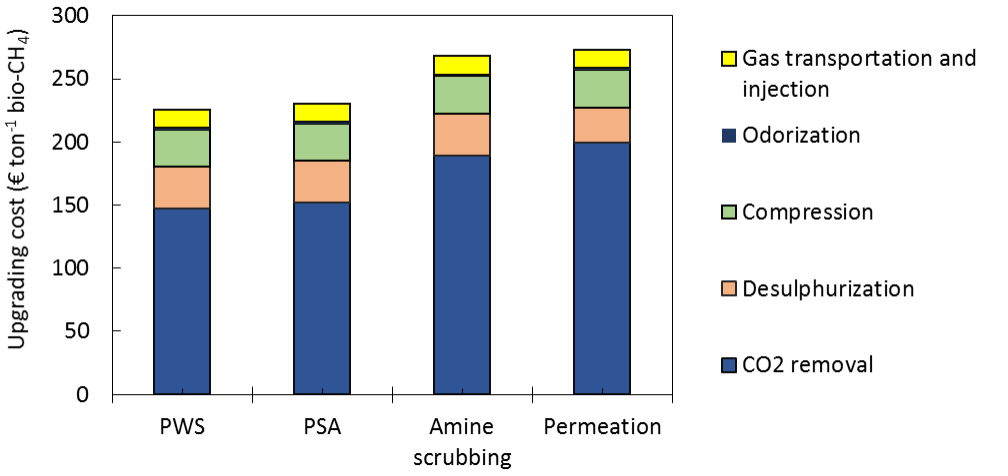


Figure S1.5. - The impact of the upgrading technology on the overall biogas upgrading cost (PWS = pressurized water scrubbing; PSA = pressurized swing adsorption).

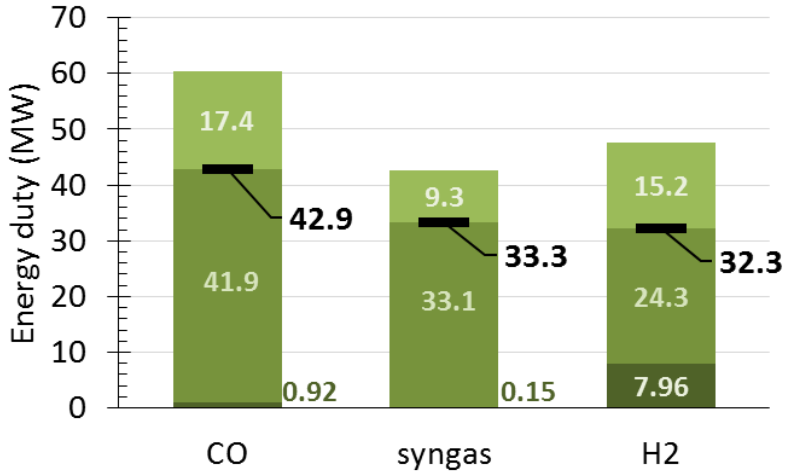


Figure S1.6. - Energy duty for heat exchanger HX1 (top light green), HX2 (lower dark green) and reforming reactor (middle green) in the CO production (SDR), syngas production (DRM) and H_2 production (SESR) case. The solid black line represents the net heat input, the sum of the energy duty for heat exchanger HX2 and the reactor.

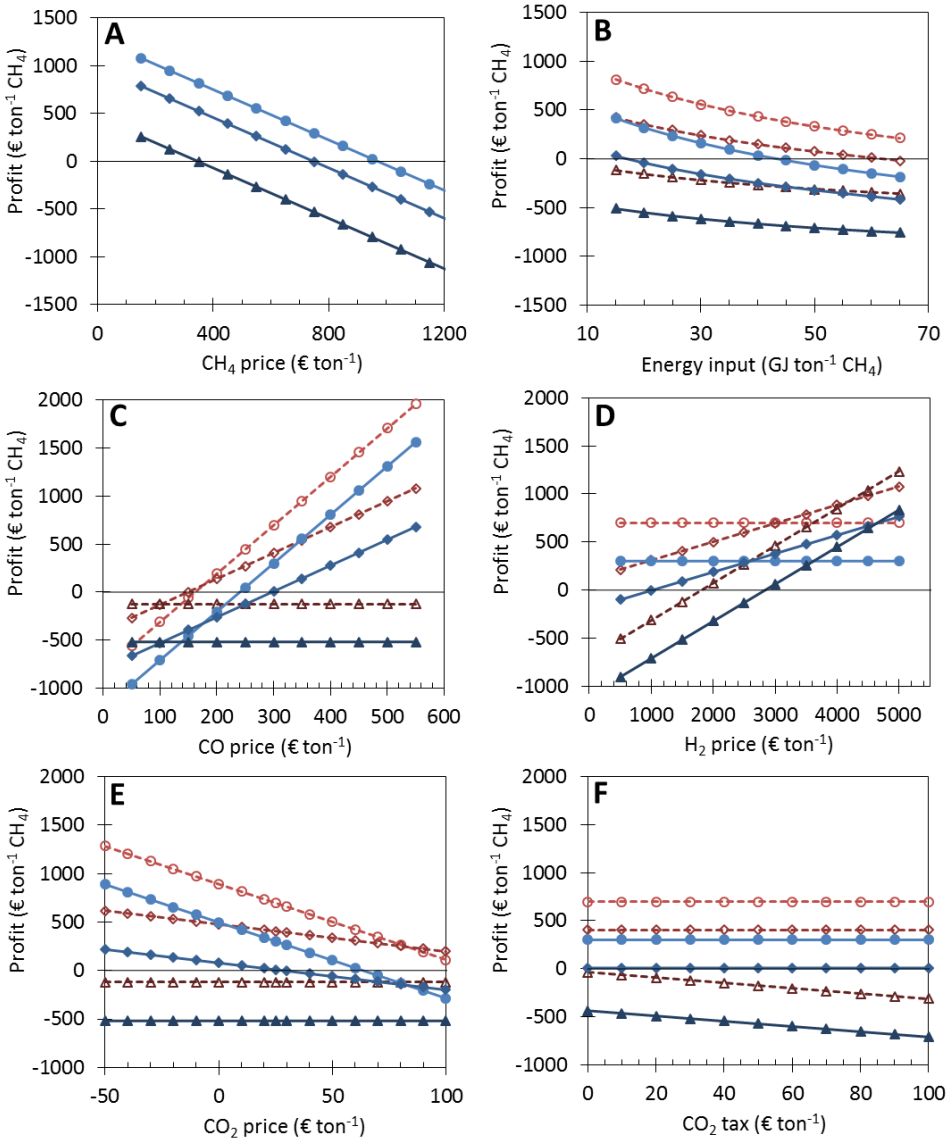


Figure S1.7. - Economic analysis for the production of chemicals from biomethane or natural gas. Effect of CH_4 price (A), required energy input (B), CO price (C), H_2 price (D), CO_2 price (E) and CO_2 tax (F) on calculated profit. Circles – super-dry reforming process (SDR); Diamonds – dry reforming of methane (DRM); Triangles – sorption-enhanced steam reforming of methane (SESR). Full symbols and full lines (blue) represent margin of the case studies with biomethane as source of CH_4, while hollow symbols and dashed lines (red) represent the margin of the case studies with natural gas (NG) as source of CH_4 in an EU context.

Appendix 2 - Supplementary information for Chapter 3

Table S 2.1 - General *Methanobacterium* growth medium for 1L (adapted from DSMZ 1523)

KH2PO4	0.50 g
MgSO4 x 7 H2O	0.40 g
NaCl	0.40 g
NH4Cl	0.40 g
CaCl2 x 2 H2O	0.05 g
Trace element solution SL-10*	10.00 mL
Vitamins solution**	1.00 mL
Na2S x 9 H2O	0.50 g

*** Trace element solution SL-10:**

HCl (25 %; 7.7 M)	10 mL
$FeCl_2$ x 4 H_2O	1.50 g
$ZnCl_2$	70 mg
$MnCl_2$ x 4 H_2O	100 mg
H_3BO_3	6 mg
$CoCl_2$ x 6 H_2O	190 mg
$CuCl_2$ x 2 H_2O	2 mg
$NiCl_2$ x 6 H_2O	24 mg
Na_2MoO_4 x 2 H_2O	36 mg
Distilled water	990 mL

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000 mL.

**** Seven vitamins solution:**

Vitamin B12	100 mg
p-Aminobenzoic acid	80 mg
D(+)-Biotin	20 mg
Nicotinic acid	200 mg
Calcium pantothenate	100 mg
Pyridoxine hydrochloride	300 mg
Thiamine-HCl x 2 H_2O	200 mg
Distilled water	1000 mL

Analytical techniques

Gas samples were analysed using a Compact GC (Global Analyser Solutions, Breda, The Netherlands). The first channel with He as carrier gas, consisted of a double channel with a Porabond Q precolumn and Molsieve 5A column for CH_4, O_2 and N_2 analysis and a Rt-QSBond precolumn and Rt-QSBond column for CO_2 measurement. The second channel, using N_2 as carrier gas, with a Porabond Q precolumn and Molsieve 5A column was used to measure H_2. Concentrations of gases were determined using a thermal conductivity detector with a lower detection limit of 100 ppmv, and were reported at STP (standard temperature, 273K, and pressure, 101325 Pa) conditions.

Performance parameters: calculations

1) $$CO_2\ flux = \ CO_2\ extraction\ rate\ = \frac{L\ CO_2\ extracted}{m^2_{membrane} day} = \frac{Q_{biogas,in}\ CO_{2,in} - Q_{biogas,out}\ CO_{2,out}}{m^2_{membrane} day}$$

2) $$CO_2\ removal\ efficiency\ = \frac{L\ CO_2\ extracted}{L\ CO_2\ injected} = \frac{Q_{biogas,in}\ CO_{2,in} - Q_{biogas,out}\ CO_{2,out}}{Q_{biogas,in}\ CO_{2,in}}$$

3) $$Current\ efficiency\ for\ HCO_3^-\ extraction\ = \frac{mole\ HCO_3^-\ extracted}{mole\ \ e^-\ supplied}$$

With $Q_{biogas,in}$ (L d^{-1}) and $Q_{biogas,out}$ (L d^{-1}) the influent and effluent gas flow rate in the cathode compartment, respectively, whereas $CO_{2,in}$ (%) and $CO_{2,out}$ (%) are the corresponding CO_2 concentrations in the influent and effluent gaseous cathodic streams, respectively.

Additional graphs

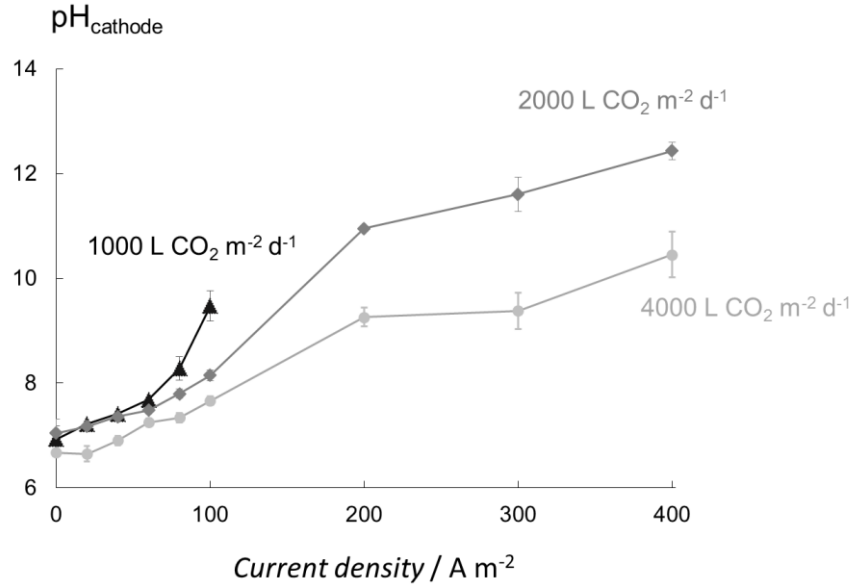
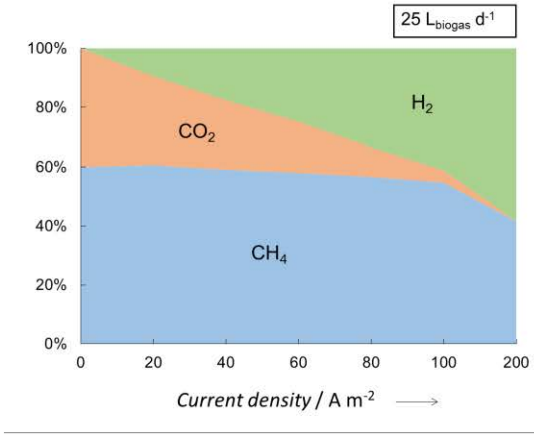
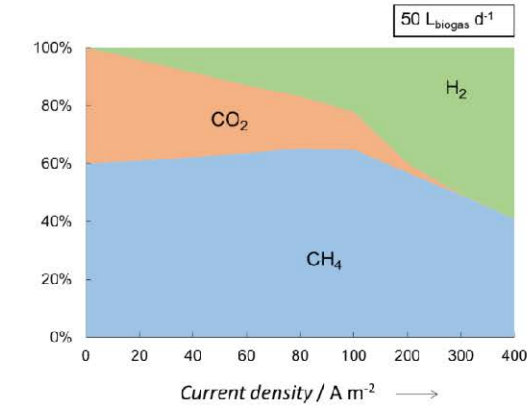


Figure S 2.1 – Cathode pH in function of CO_2 loading rate and current density. Average ± SD (n = 3, steady state conditions).

A



B



C

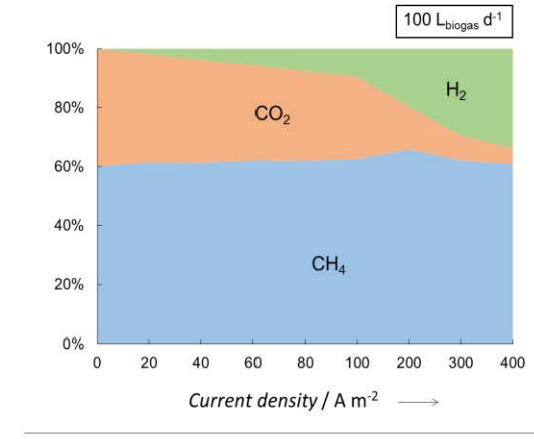


Figure S 2.2– Cathode off-gas composition after EBU treatment at 25 L_{biogas} d^{-1} (A), 50 L_{biogas} d^{-1} (B) and 100 L_{biogas} d^{-1} (C).

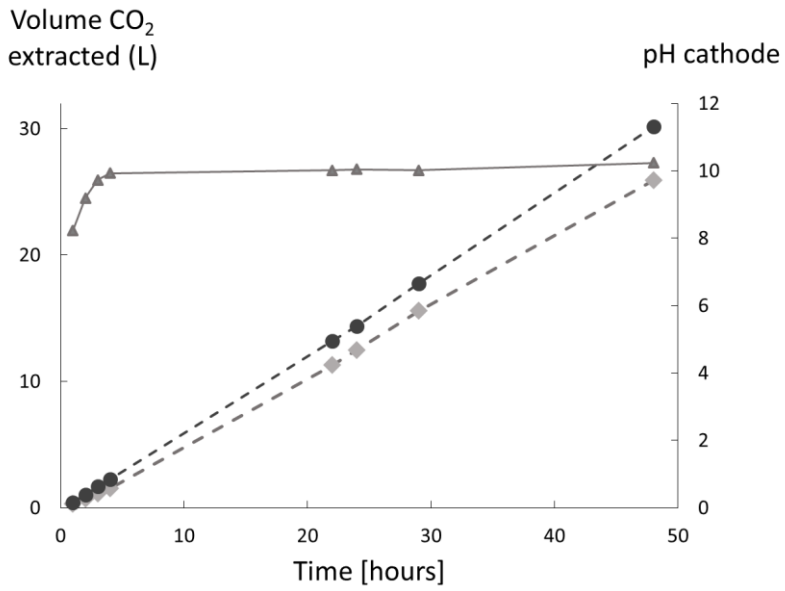


Figure S 2.3 – pH of the catholyte over a 48 hr experiment and volume of CO_2 extracted across the AEM based on anode and cathode measurements. Average ± SD (n = 3, steady state conditions).

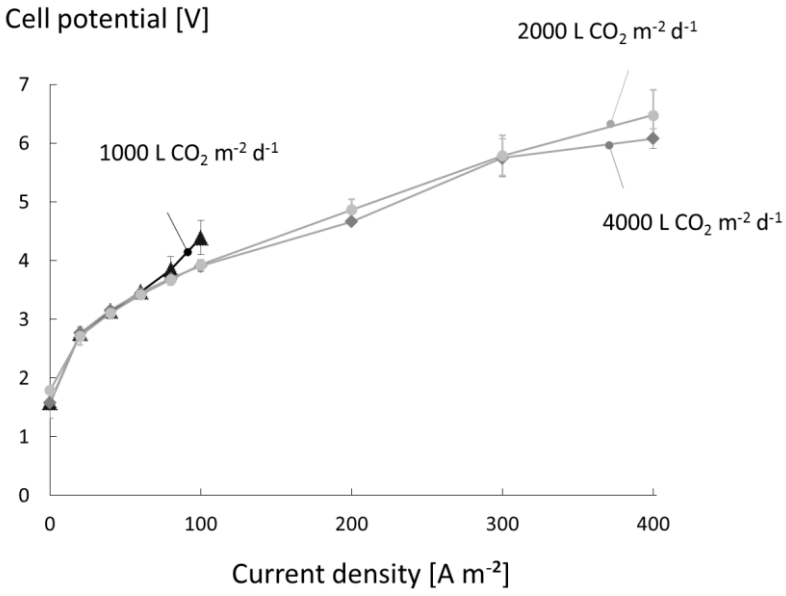


Figure S 2.4 – Cell potential in function of current density. Average ± SD (n = 3, steady state conditions).

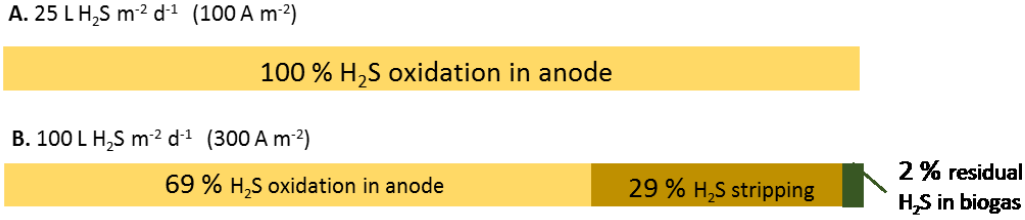


Figure S 2.5 – Sulfur mass balance over the electrochemical cell for a H_2S loading rate of (A) 25 and (B) 100 L H_2S m^{-2} d^{-1}.

Appendix 3 - Supplementary information for Chapter 4

Table S3.1. - Main technical design data and general assumptions.

Feedstock mix	**Biogas yield [a] (Nm^{-3} biogas kg^{-1} FM)**	**N content [b] (g TKN-N kg^{-1} FM)**	**w.% of feedstock (on FM basis)**
Maize silage	210	2.07	10 %
Crops residues	100	0.83	10 %
Food waste	190	4.06	10 %
Pig manure	28	6.30	70 %
Weighted average of the mix	*69.4*	*5.11*	

Biogas production rate (Nm^3 h^{-1} / ton day^{-1})	500 / 14.75	
Biomass input (ton FM day^{-1})	172	
Total solids input (ton TS day^{-1}) [c]	25.4	
Nitrogen mass balance		*Recovery efficiency*
TKN-N loading rate to AD (kg N day^{-1})	880	
NH_4^+-N release rate in AD (kg N day^{-1})	660	75 % [d]
NH_4^+-N recovery in liquid digestate (kg N day^{-1})	528	80 % [a]
NH_4^+-N recovery through stripping (kg N day^{-1})	475	90 % [e]
Energy density methane (kWh Nm^{-3}), $_{LHV}$	10.85	
CH_4 content raw biogas (vol.%)	60 %	
Energy density biogas (kWh Nm^{-3})	6.50	
Mass density biogas (kg Nm^{-3})	1.22	
Energy density biogas (MWh ton^{-1}_{biogas})	5.33	

**FM = fresh material*

[a] (Weiland, 2010)

[b] (Drosg et al., 2015; Pintucci et al., 2017)

[c] TS content of substrates: manure (8.7 %), corn & crop residues (33 %) and food waste (20.8%) (Pintucci et al., 2017)

[d] (De Vrieze et al., 2019)

[e] (Menkveld & Broeders, 2018)

Table S 3.2 - Estimation of the production cost of input materials for MP production at digester scale.

1. **Biogas production cost**	*Minimum*	***Average***	*Maximum*
Capital investment (€)[a]	1 500 00	**2 000 000**	2 500 00
CAPEX (€ ton^{-1} biogas)[b]	*18*	***25***	*31*
OPEX (€ year^{-1})[c]	75 000	**150 000**	250 000
OPEX (€ ton^{-1} biogas)	*14*	***28***	*46*
Feedstock cost (€ ton^{-1} FM)[d]	2	**5.28**	10
Feedstock cost (€ ton^{-1} biogas)	*23*	***62***	*117*
Production cost (€ ton^{-1} biogas)	*56*	***115***	*194*
Production cost (€ ton^{-1} methane)[e]	*156*	***326***	*550*

[a] Assumed investment: 3000 (Min), 4000 (Avg) and 5000 (Max) € Nm^{-3} h^{-1} installed biogas capacity. Investment without investment subsidy or support.
[b] Depreciation period of 20 years, 3 % interest rate.
[c] Assuming 5 % (Min), 7.5% (Avg) and 10 % (Max) of the total investment per year.
[d] Maize silage was assumed to have a feedstock cost of 32.8 € ton^{-1} FM and food waste a cost of 20 € ton^{-1} FM (including transportation). Agricultural residues and manure were assumed to come in for free.
[e] Assumed methane content is 60 vol.%.

2. **Ammonia recovery cost**	*Minimum*	***Average***	*Maximum*
CAPEX (€ ton^{-1} N recovered)	*230*	***345***	*460*
OPEX (€ ton^{-1} N recovered)[a]	*770*	***1 155***	*1 540*
Total N recovery cost (€ ton^{-1} N recovered)	*1 000*	***1 500***	*2 000*

[a] Based on an influent stream containing a NH_3-N concentration of > 4 g L^{-1}.

3. **Hydrogen production cost**	*Minimum*	***Average***	*Maximum*
Total production cost (€ ton^{-1} H_2)	*1 800 [b]*	***2 600 [a]***	*4 400 [c]*

[a] Future predicted levelized cost for hydrogen production using PEM electrolysis at an electricity price of 44 € per MWh. (Ayers et al., 2010)
[b] (Gökçek, 2010)
[c] Current cost for hydrogen production using PEM electrolysis at an electricity price of 44 € per MWh. (Ayers et al., 2010)

4. **Oxygen production cost[a]**	*Minimum*	***Average***	*Maximum*
Total production cost (€ ton^{-1} O_2)	*26*	***30***	*34*

[a] Only for MOB case, as for the HOB case oxygen is produced *via* electrolysis, and costs are allocated to hydrogen.

5. **Microbial protein production**	*Minimum*	***Average***	*Maximum*
Capital investment (€ m^{-3} $_{reactor}$)	*3 000*	***5 000***	*8 000*
OPEX (€ ton^{-1} MP)	*100*	***200***	*300*

*6. **Dewatering and drying***	*Minimum*	***Average***	*Maximum*
Centrifugation (€ ton MP^{-1})	-	***70***	-
Spray drying (€ ton MP^{-1})	-	***90***	-

[b] (Pikaar et al., 2018b)

Table S 3.3 - Comparison between methane-oxidizing bacteria and hydrogen-oxidizing bacteria used in MP production.

	Methane-oxidizing bacteria	*Hydrogen-oxidizing bacteria*
Cell yield (g CDW g^{-1} COD)	0.19 [a]	0.28 [b]
Protein content (% Protein on CDW)	60 % [c]	75 % [d]
Volumetric production rate (kg CDW m^{-3} h^{-1})	4 [e]	2.28 [f]
N content (% N on CDW)	12 %	12%

[a] (Higgins et al., 1981)
[b] (Ishizaki & Tanaka, 1990)
[c] (Yazdian et al., 2005)
[d] (Volova & Barashkov, 2010)
[e] (Ritala et al., 2017)
[f] (Tanaka et al., 1995)

Table S 3.4 - Input feed to produce 1 ton MP *via* HOB *vs.* MOB.

	Feed (ton)					**Product (ton)**
Case	CH_4	CO_2	NH_3	H_2	O_2	Microbial protein
MOB	2.20	-	0.20	-	2.50	1
HOB	-	3.31	0.16	0.79	2.05	1

Appendix 4 - Supplementary information for Chapter 5

Experimental setup for the reactor with *in situ* extraction

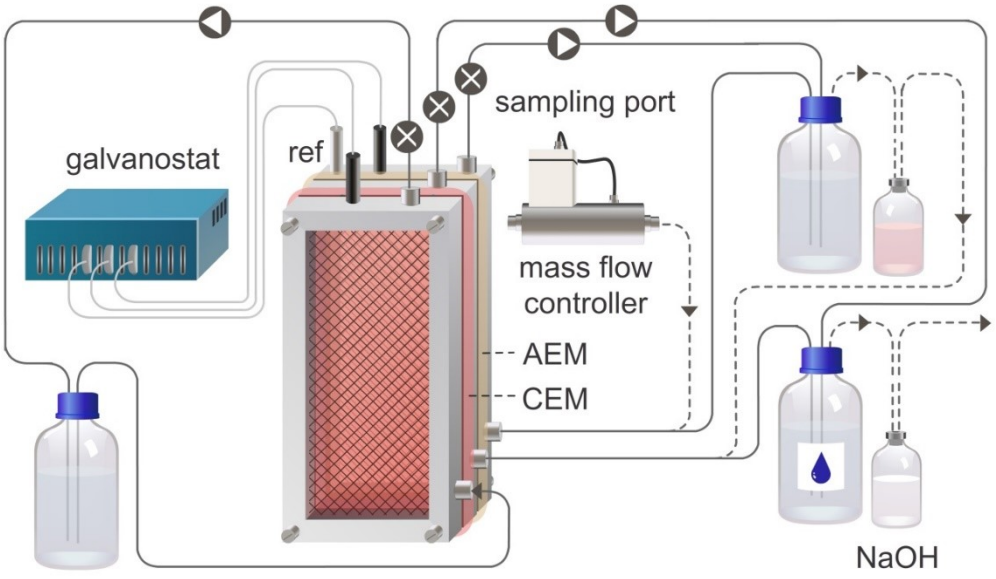


Figure S 4.1 - Reactor setup for simultaneous biological production and extraction of acetate from CO_2 and electrical current. Full black lines show liquid streams, dotted lines gas streams (from mass flow controller N_2/CO_2 – 90/10 %) and grey lines electrical connections. Anodic and cathodic compartments are represented on the foreground and background of the reactor, respectively. The middle compartment serves as extraction compartment for recovery of acetate as acetic acid. Copied from Gildemyn et al. (2015). AEM: anion exchange membrane; CEM: cation exchange membrane.

Carboxylic acid content per compartment

Table S 4.1 - Products in the reactor with anion exchange membrane (AEM, extraction) at the end of the 43 day production cycle. SCCA: short chain carboxylic acid.

	Catholyte (mg SCCA L^{-1} – mmol C)	Extraction (mg SCCA L^{-1} – mmol C)	Anolyte (mg SCCA L^{-1} – mmol C)
Formic acid	0 – 0	470 – 5.90	56 – 0.34
Acetic acid*	540 – 2.47	8881 – 85.05	2583 – 12.04
Propionic acid	0 – 0	100 – 0.78	20 – 0.07
Butyric acid	0 – 0	0 – 0	0 – 0
Ethanol	0 – 0	0 – 0	0 – 0
Final volume (mL)	270	565	275

* Acetic acid represented 95.8 % of all organic carbon contained in dissolved products at the end of the batch cycle.

Table S 4.2 - Products in the reactor with cation exchange membrane (CEM) at the end of the 43 day production cycle. SCCA: short chain carboxylic acid.

	Catholyte (mg SCCA L^{-1} – mmol C)	Anolyte (mg SCCA L^{-1} – mmol C)
Formic acid	0 – 0	0 – 0
Acetic acid*	10389 – 61.63	37.5 – 0.21
Propionic acid	72.16 – 0.35	0 – 0
Butyric acid	69.96 – 0.28	0 – 0
Ethanol	18.96 – 0.41	0 – 0
Final volume (mL)	270	275

* Acetic acid represented 97.6 % of all organic carbon contained in dissolved products at the end of the batch cycle.

Table S 4.3 - Products in the reactor with bipolar membrane (BPM) at the end of the 43 day production cycle. SCCA: short chain carboxylic acid.

	Catholyte (mg SCCA L^{-1} – mmol C)	Anolyte (mg SCCA L^{-1} – mmol C)
Formic acid	0 – 0	0 – 0
Acetic acid*	8092 – 63.01	767 – 4.16
Propionic acid	97 – 0.61	0 – 0
Butyric acid	70 – 0.37	14 – 0.05
Ethanol	3.59 – 0.08	0 – 0
Final volume (mL)	270	275

* Acetic acid represented 97.3 % of all organic carbon contained in dissolved products at the end of the batch cycle.

Acetic acid concentration per compartment

Due to water displacement across the membranes, the concentrations in the compartments do not reflect the efficiency of the reactors, as the mass of acetic acid that is present in the compartments is dependent on the volume of the solution. The volume in the compartments was monitored during sampling.

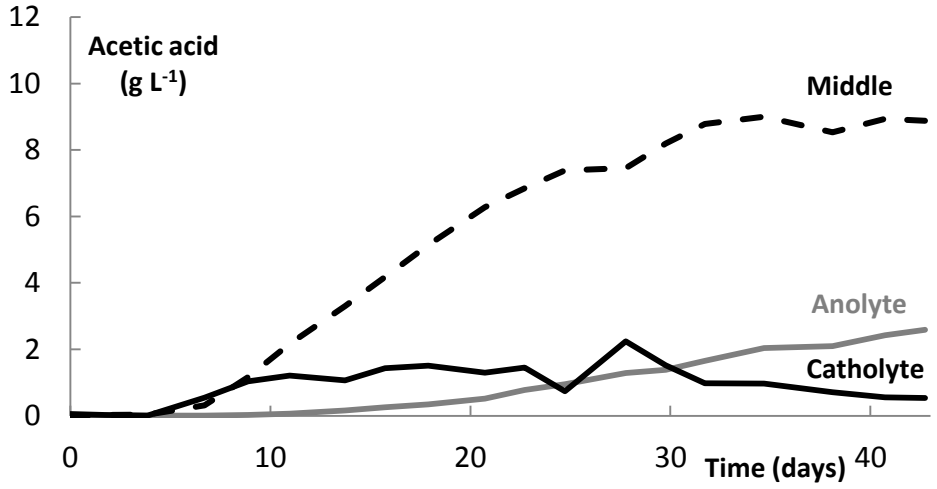


Figure S 4.2 - Acetic acid concentration in the catholyte (black line), middle compartment (black dotted line) and anolyte (gray line) of the reactor with anion exchange membrane (AEM, extraction).

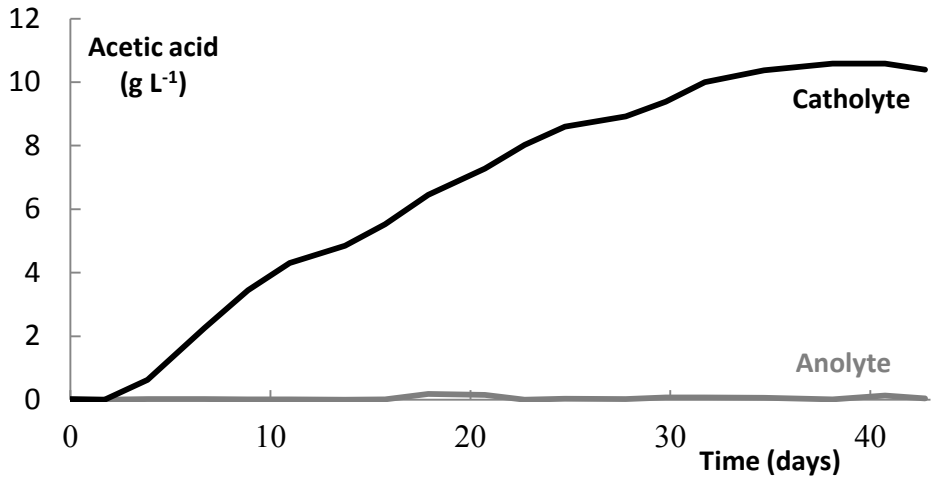


Figure S 4.3 - Acetic acid concentration in the catholyte (black line) and anolyte (gray line) of the reactor with cation exchange membrane (CEM).

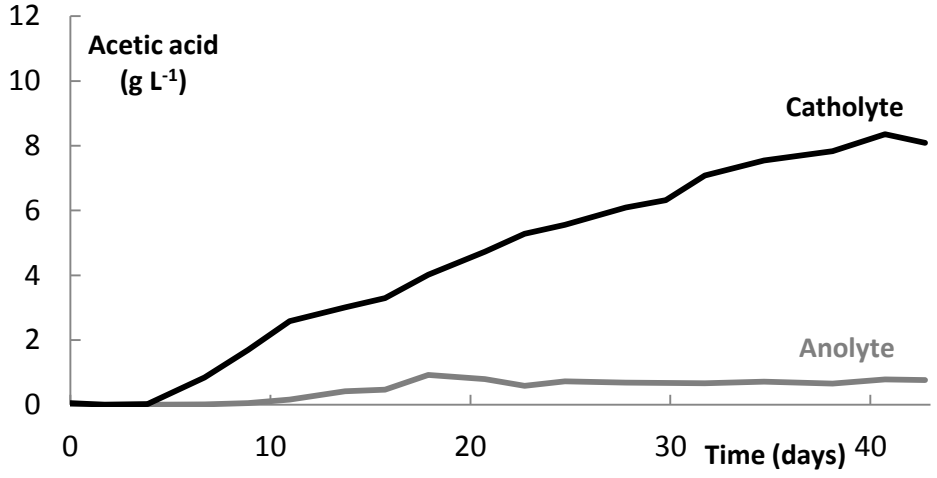


Figure S 4.4 - Acetic acid concentration in the catholyte (black line) and anolyte (gray line) of the reactor with bipolar membrane (BPM).

Electron balance for MES

Table S 4.4 - Electron balance for the three reactor types at the end of the experiment. Acetic acid, other organic products and H_2 were measured during the run. For the reactor with cation exchange membrane (CEM), values in italic are estimations. H_2 concentrations increased above the theoretical maximum after day 32. Acetic acid was not further produced, so probably this additional H_2 was produced *via* biomass fermentation. AEM: anion exchange membrane; BPM: bipolar membrane.

Electron sink (%)	AEM reactor	CEM reactor	BPM reactor
Acetic acid	41	25	28
Other organics	1.3	0.8	0.9
H_2	28	*57*	52
Other (*e.g.* biomass)	30	*17*	19
Sum	*~100*	*~100*	*~100*

Undissociated acetic acid concentrations

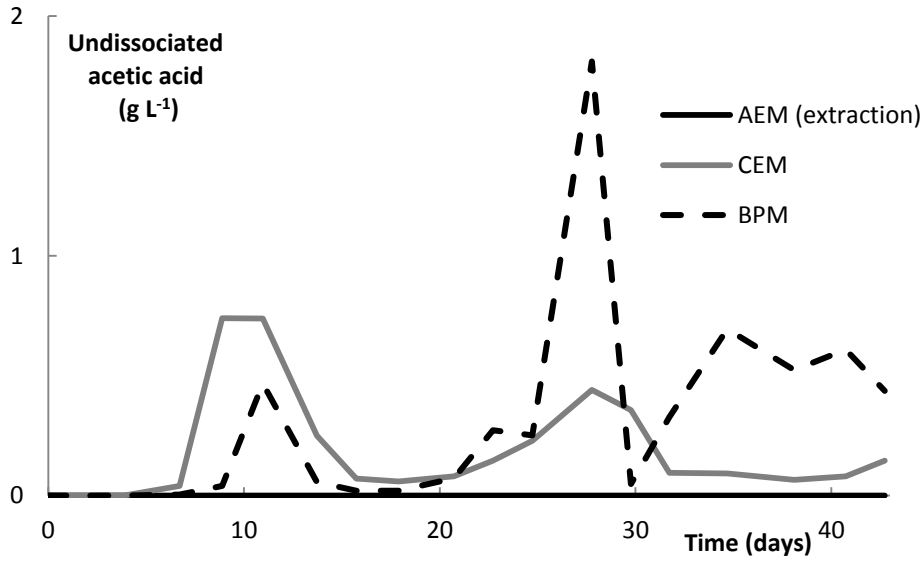


Figure S 4.5 - Concentration of undissociated acetic acid in the catholyte of each reactor (anion exchange membrane (AEM, full black line); cation exchange membrane (CEM, gray line); bipolar membrane (BPM, black dotted line)). The concentration of undissociated acetic acid in the catholyte of the reactor with extraction (black line) remained below 1 mg L^{-1} and is therefore not visible in the graph.

Reactor charge balances

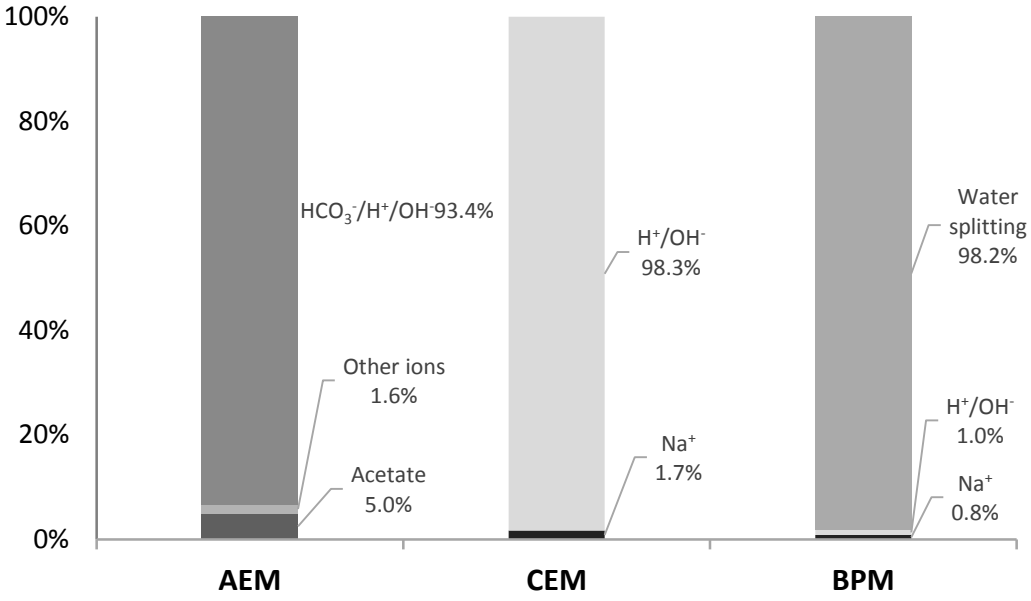


Figure S 4.6 - Charge balancing in the three reactor systems. For the setup with bipolar membrane (BPM) there is almost no ion transport across the membranes. Water splitting on the BPM was, thus, the dominant process. AEM: anion exchange membrane; CEM: cation exchange membrane.

Curriculum Vitae

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International peer reviewed publications

Diaz Nieto, C.H., Palacios, N.A., **Verbeeck, K.**, Prevoteau, A., Rabaey, K. & Flexer, V. (2019). Membrane electrolysis for the removal of Mg^{2+} and Ca^{2+} from lithium rich brines. Water Research, 154, 117-124. (IF 7.051)

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Philips, J., **Verbeeck, K.**, Rabaey, K., & Arends, J. (2015). Electron transfer mechanisms in biofilms. In K. Scott & E. H. Yu (Eds.), Microbial electrochemical and fuel cells : fundamentals and applications (pp. 67–113). Cambridge, UK: Elsevier. Woodhead Publishing.

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De Vrieze, J.*, **Verbeeck, K.***, Pikaar, I., Boere, J., Wijk, A., Rabaey, K. & Verstraete, W. (2019) The hydrogen gas bio-based economy and the production of renewable building block chemicals, food and energy. Submitted to New Biotechnology. *Equal contribution

Publications intended for publication

Verbeeck, K., De Vrieze, J., Pikaar, I., Verstraete, W. & Rabaey, K. (2019) Upcycling recovered resources from anaerobic digestion through microbial protein production. In preparation.

Oral presentations

Presenting author is market in **bold**.

Verbeeck, K., Gildemyn, S., & Rabaey, K. (2017). Increased concentration and current efficiency for *in situ* extraction of acetic acid in microbial electrosynthesis from CO_2. 6th General meeting of the International Society for Microbial Electrochemistry and Technology. Lisbon, 12 – 14 September.

Verbeeck, K., De Vrieze, J., & Rabaey, K. (2017). Electrochemical CO_2 Extraction as Innovative Pre-treatment Method for Biological Biogas Upgrading. 15th World Conference on Anaerobic Digestion. Beijing, 17 – 20 October.

Gildemyn, S., **Verbeeck, K.**, Jansen, R., & Rabaey, K. (2016). *In situ* membrane electrolysis enables high-rate production and electrochemical pH control in microbial electrosynthesis of acetic acid from carbon dioxide. 3rd European meeting of the International Society for Microbial Electrochemistry and Technology. Rome, 26 – 28 September

Verbeeck, K., Gildemyn, S., Jansen, R., & Rabaey, K. (2016). Integrated production and extraction of acetic acid from CO_2 using microbial electrosynthesis. 20th National Symposium for Applied Biological Sciences. Antwerp, 5 February.

Rabaey, K., Verbeeck, K., Patil, S.A., Arends, J.B.A., Gildemyn, S. (2016) From electro-fermentation to microbial electrosynthesis: rapid production of carboxylic acids at high titer. 3rd AP-ISMET International Society for Microbial Electrochemistry and Technology Meeting. Busan, 31 August – 2 September.

Gildemyn, S., Verbeeck, K. , Patil, S.A., Slabbinck, R., Andersen, S.J., Prévoteau, A., Arends, J.B.A., Rabaey K. (2015) Integrated production, extraction and concentration of acetic acid from CO_2 through microbial electrosynthesis. 5th international meeting of the International Society for Microbial Electrochemistry and Technology Meeting. Tempe, Arizona, 1 – 4 October.

Rabaey, K., Gildemyn, S., Verbeeck, K., Arends, J.B.A., Guo, K., Patil, S.A., Prevoteau, A. (2015) How useful is electricity as driver for microbial processes? 5th international ISMET International Society for Microbial Electrochemistry and Technology Meeting. Tempe, Arizona, 1 – 4 October.

Gildemyn, S., Verbeeck, K., Slabbinck, R., Andersen, S.J., Prévoteau A., Rabaey, K. Integrated production, extraction and concentration of acetic acid from CO_2 through microbial electrosynthesis. (2015) 1st IWA Resource Recovery conference, Ghent. 30 August – 2 September.

Gildemyn, S., Verbeeck, K., Andersen, S., **Rabaey, K**. Microbial electrosynthesis - electricity driven bioproduction and extraction. (2014) 2nd EU-ISMET International Society for Microbial Electrochemistry and Technology Meeting. Alcala, Spain. 3 – 5 September.

Gildemyn, S., Verbeeck, K., Andersen, S., **Rabaey, K**. Microbial electrosynthesis - electricity driven bioproduction and extraction. (2014) 2nd AP-ISMET International Society for Microbial Electrochemistry and Technology Meeting. Singapore, 21 – 24 July.

Scientific awards

Best Poster Presentation, 1st prize, AOG RENEW conference, January 21th, 2014, Ghent University, Belgium. ***In situ* extraction of microbial electrosynthesis products**.

Professional activities during PhD research

- Tutor of 4 Master students.
- Guide for master students visiting the wastewater treatment facilities in Kluizen.
- Responsible for several lab rooms and lab equipment.
- Internal services for CMET:
    - Organization of teambuilding event for the lab.
    - Presentations on 'Day of Europe' in Ghent, Belgium (2017 & 2018).
    - Art project: 'Spark of Life' with Teresa Van Dongen.
- External services for CMET:
    - 2014 – Covestro: Study on slime formation in a cooling water basin
    - 2017 – Covestro: Treatment of saline waste water streams to minimize organics

Courses and workshops

- Transferable skills:

    Effective scientific communication by Jean-Luc Doumont (Principiae, (2015)
    Effective Graphical Displays by Jean-Luc Doumont (Principiae, 2016)
    Leadership foundation course (2017)
    Resource Recovery Workshop IWA (2015)

- Specialist courses:

    Francqui lectures and master class by Prof. David Sedlak (2015

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Kristof

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