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BIOGAS UPGRADING USING MICROALGAE

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Summary and thesis outline

Biogas is a biofuel composed by a gas mixture, mainly methane and carbon dioxide, produced from anaerobic biodigestion of organic matter. Carbon dioxide must be removed from biogas to increase the calorific value and fulfill with the regulations for biogas injection in vehicle or gas grid. The main current technologies for biogas upgrading are liquid absorption, pressure swing adsorption, membranes and cryogenic separation. These technologies have been implemented to full scale and are effective in achieving CO_2 separation from CH_4. However, these methods have high investment and operational costs. As a new alternative for biogas upgrading, the use of photosynthetic microorganisms, such as microalgae, has been proposed. Such process would convert CO_2 contained in the biogas into microalgal biomass, generating two products: upgraded biogas and biomass.

As microalgae perform oxygenic photosynthesis, microalgae-based biogas upgrading process needs to be carefully designed and controlled in order to separate O_2 desorption from CO_2 absorption. An open-photobioreactor connected to mass transfer column was proposed.

To evaluate the technical feasibility of biogas upgrading using microalgae, the study of the following key topics was performed:

- Control of O_2 in the upgraded biogas (Chapter 3)
- Effect of pH change on the microalgae activity (Chapter 4)
- Effect of light/dark photoperiod on the biogas upgrading process (Chapter 5)
- Simultaneously CO_2 and H_2S removal from biogas using a microalgae culture (Chapter 6)

- Evaluation of key operational parameter of the process and estimation of biogas treatment capacity at large-scale using a mathematical model (Chapter 7).

The operation of an open photobioreactor connected to external bubble column for CO_2 absorption enabled the production of an upgraded biogas with low CO_2 and O_2 levels. Although during night microalgae did not perform photosynthesis, desorption of CO_2 from photobioreactor to atmosphere enabled high levels of CO_2 removal during periods without illumination. Additionally, H_2S and CO_2 could be simultaneously removed from biogas using a microalgae culture because H_2S could be oxidized to sulfate due to the high dissolved oxygen concentration in the photobioreactor. Therefore, this system represents a feasible alternative for biogas upgrading. However, biogas upgrading by microalgae has lower biogas treatment capacity per m^2 in comparison to traditional technologies. Therefore, the proposed system could be a feasible process in places where there is enough available land. The maximum biogas capacity of the photosynthetic biogas upgrading depends on the objective of the system. If the objective is only to upgrade biogas fulfilling the biomethane standards, a theoretical maximum biogas treatment capacity of 3.6 m^3 d^{-1} per m^3 reactor could be achieved. If the objective is to upgrade biogas and avoid the release of CO_2 into atmosphere, the maximum biogas treatment capacity should be reduced down to 0.12 m^3 d^{-1} per m^3 reactor.

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CHAPTER I

General Introduction

1. General Introduction

1.1. General Introduction

Probably fossil fuel will continue to be available at low cost for a long period of time, considering new extraction technologies and discoveries of new reserves. However, increasing effect of climate change on natural and human systems causes us to search clean and renewable energetic alternatives. Although there are several potential sources of renewable energy, biofuels have focused important interest and are expected to play a crucial role in global energy infrastructure in the future.

Bioenergy is an attractive energetic alternative due to low or no emission of greenhouse gases, because during their conversion and combustion, the same amount of CO_2 is emitted as it was absorbed during feedstock growth (Schubert and Blasch, 2010). It is possible to diversify fuel supply sources through bioenergy, it promotes development in rural zones and can be used in engines with little or no modifications (Mata *et al*, 2010).

Among various types of biofuels, biogas has been receiving increased interest. The main advantage of biogas production is the generation of energy from organic wastes, so it is possible to solve two problems: energetic crisis and waste treatment. Biogas is produced from anaerobic digestion of organic matter and is composed by a gas mixture, principally methane and carbon dioxide, with smaller amounts of hydrogen sulphide, ammonia, nitrogen and it is generally saturated with water vapor.

Biogas can be used with minimum or moderated levels of purification for heat and electricity production. However, many applications, such as vehicle and grid injection, require the removal of CO_2 in order to produce a gas of equivalent characteristics as that of natural gas. Removal of CO_2 increases the caloric value and decreases the relative

density of the gas, increasing the Wobbe index (Ryckebosch *et al*, 2011). There are regulations that establish the technical specifications of biogas for injection in gas grid or for use of biogas as vehicle fuel. These regulations indicate the limit of concentration of each component in biogas. For example, European standards establish a maximal CO_2 concentration in biogas between 2 and 6%. In the case of Chile, there is the standard NCh3213.Of2010, which indicates a maximal 1.5 – 4.5% concentration of inert gases (CO_2+N_2).

There are several conventional methods available for CO_2 removal from biogas, such as liquid absorption, pressure swing adsorption (PSA), membrane processes and cryogenic separation (Ryckebosch *et al*, 2011). Apart from these methods, researchers have proposed new approaches for biogas upgrading in the recent years. Among them is the use of photosynthetic microorganisms such as microalgae. The definition of microalgae includes all unicellular and simple multicellular microorganisms, considering both prokaryotic microalgae (cyanobacteria) and eukaryotic microalgae, e.g. green algae (Chlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophyta) (Richmond, 2004; Sialve *et al*, 2009; Brennan and Owende, 2010). Microalgae are able to capture solar energy, have high growth rates and can be adapted to different environmental conditions (Mata *et al*, 2010). The use of microalgae represents an attractive alternative for biogas upgrading because CO_2 is not only removed, but also transformed in biomass that can be used as feedstock for biofuel production.

Few articles about biogas upgrading by microalgae have been published. Published reports have informed efficiencies of CO_2 capture between 50 and 95%, demonstrating the potential of these photosynthetic microorganisms for biogas upgrading (Conde *et al*, 1993; Mandeno *et al*, 2005; Converti *et al*, 2009; Mann *et al*, 2009; Dousková *et al*, 2010; Kao *et al*, 2012). However, most these articles only show preliminary results that

only consider CO_2 removal efficiency and/or biomass productivity. Additional research needs to be performed to improve design and operational strategies of a photosynthetic biogas upgrading process, in order to obtain a technology that can be applied at full-scale.

To evaluate the technical feasibility of biogas upgrading, the study of the following key topics is needed:

Effect of photoperiod on the operation of a photosynthetic biogas upgrading system.

Full scale system of biogas upgrading by microalgae should be operated using natural photoperiods, because illumination by artificial light is obviously not feasible. In absence of light, microalgae have no energy to perform photosynthesis. Therefore, they can only carry out respiration, where CO_2 is released into culture medium (Granum and Myklestad, 2002). Thus, a photosynthetic biogas upgrading system cannot operate continuously. Hence, one could speculate that biogas could only be injected during the day and should be stored during the night.

Effect of pH on microalga culture

The pH is an important parameter in the operation of a photosynthetic biogas upgrading system, because pH influences on the carbon inorganic equilibrium and the microalgae activity. When CO_2 is dissolved in the aqueous phase, it can dissociate in HCO_3^- and CO_3^{-2} according to equation 1.1. The concentration of each carbon inorganic species depends on pH (Stumm and Morgan, 1995; Manahan, 2007; Kumar *et al*, 2011).

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{-2} + 2H^+ \quad (1.1)$$

The carbon inorganic dissociation causes the release of H^+, and as a result, pH decreases. The pH reduction affects the microalgae activity because most microalgae

growth in a pH range of 7 – 9, with optimal pH between 8.2 and 8.7 (Barsanti and Gualtieri, 2006). Therefore, CO_2 should be injected into the microalgae culture according to the carbon fixation velocity of the microalga cells to maintain a constant pH in the system. A pH control is fundamental for the operation of photosynthetic biogas upgrading.

Control of O_2 content in the upgraded biogas

Microalgae perform oxygenic photosynthesis. This means that 1 mole of O_2 is released per mol of CO_2 captured. Then, when operating closed-photobioreactors with direct biogas injection in the culture, oxygen will be released into the biogas, as the CO_2 is absorbed. Indeed when Converti *et al* (2009) studied biogas upgrading with *Arthrospira platensis*, they achieved a negligible content of CO_2, but a O_2 concentration of 10-24% in the purified biogas. Similar results were reported by Mann *et al* (2009), who despite achieving good levels of CO_2 removal (97%), observed oxygen levels in the range of 18-23% when working with *Chlorella vulgaris* in a spiral photobioreactor. Oxygen content in the biogas must be minimized, since mixtures with CH_4 are explosive when CH_4 content is between 5% and 60% (Hopp, 1994; Mandeno *et al*, 2005). Moreover, most standards for biomethane use require an oxygen content in upgraded biogas lower than 1% (Rutledge, 2005; Marcogaz, 2006). Then a microalgae-based biogas upgrading process needs be carefully designed and controlled in order to separate O_2 desorption from CO_2 capture. An alternative for controlling the oxygen concentration in the upgraded biogas is to separate the process in two stages, as a strategy to (partially) separate removal of CO_2 from desorption of O_2.

Capacity of microalgae to remove H_2S from biogas.

H_2S is a typical pollutant of biogas, with concentrations ranging from 1.0% v/v to 0.1% v/v (10000 – 1000 ppm_v). It has to be removed in order to avoid corrosion in compressors, gas storage tanks and engines and for health and safety reasons due to its high toxicity (Rasi *et al*, 2011; Ramos *et al*, 2013). H_2S is a gas highly soluble in water and can be spontaneously oxidize in contact with O_2. The products of the reaction can be elemental sulfur, thiosulfate or sulfate, depending on pH and sulfur/oxygen proportion (van der Zee *et al*, 2007). Algae have the ability to take up SO_4^{-2} and reduce it to amino acids (Barsanti and Gualtieri, 2006). Kao *et al* (2012) reported growth inhibition when exposing a mutant strain of *Chlorella sp* to a gas mixture containing 150 mg/L of H_2S. However, if the microalgae cultures are expected to high concentrations of oxygen, promoting conditions for a fast H_2S oxidation, inhibition is expected to play a minimal role. Then, additional research is necessary to study the fate of H_2S in the photosynthetic biogas upgrading system and evaluate the feasibility of simultaneous CO_2 and H_2S removal from biogas.

1.2. Hypotheses

Considering that:

- The biogas must be upgraded to fulfill the standards that regulate the biomethane injection in vehicle and natural gas networks.
- The microalgae have the ability to capture carbon dioxide by photosynthesis.
- Direct biogas injection in the microalgae culture is not a feasible process for biogas upgrading due to the production of treated biogas with high O_2 content.

The following hypothesis is proposed:

Physical separation of CO_2 removal from O_2 desorption in a two-stage process allows to control the CO_2 and O_2 content in upgraded biogas by microalgae culture.

1.3. General objective

- To evaluate the technical feasibility of biogas upgrading by microalgae culture.

1.4. Specific objectives

- To analyze the use of an open-photobioreactor connected to mass transfer column for obtaining upgraded biogas with CO_2 and O_2 contents fulfilling European regulations.
- To evaluate the effect of a day/night photoperiod on the operation of the biogas upgrading process by microalgae.
- To evaluate the capacity of H_2S removal from biogas of a microalgae culture.

CHAPTER II

A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading

Muñoz, R; Meier, L; Díaz, I; Jeison, D. (2015) A critical review on the state-of-the-art of physical/chemical and biological technologies for an integral biogas upgrading. *Reviews in Environmental Science and Bio/Technology*, 1-33.

A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading

Abstract

The lack of tax incentives for biomethane use requires the optimization of both biogas production and upgrading in order to allow the full exploitation of this renewable energy source. The large number of biomethane contaminants present in biogas (CO_2, H_2S, H_2O, N_2, O_2, methyl siloxanes, halocarbons) has resulted in complex sequences of upgrading processes based on conventional physical/chemical technologies capable of providing CH_4 purities of 88-98 % and H_2S, halocarbons and methyl siloxane removals > 99 %. Unfortunately, the high consumption of energy and chemicals limits nowadays the environmental and economic sustainability of conventional biogas upgrading technologies. In this context, biotechnologies can offer a low cost and environmentally friendly alternative to physical/chemical biogas upgrading. Thus, biotechnologies such as H_2-based chemoautrophic CO_2 bioconversion to CH_4, microalgae-based CO_2 fixation, enzymatic CO_2 dissolution, fermentative CO_2 reduction and digestion with in-situ CO_2 desorption have consistently shown CO_2 removals of 80-100 % and CH_4 purities of 88-100 %, while allowing the conversion of CO_2 into valuable bio-products and even a simultaneous H_2S removal. However, despite these promising results, most biotechnologies still require further optimization and scale-up in order to compete with their physical/chemical counterparts. This review critically presents and discusses the state of the art of biogas upgrading technologies with special emphasis on biotechnologies for CO_2 removal.

2. A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading

2.1. Introduction

Biogas represents a renewable energy source based on its high CH_4 content. This CH_4-rich gas is a byproduct from the anaerobic treatment of wastewaters, the organic fraction of municipal solid wastes (OMSW), livestock residues or organic agroindustrial wastes (Rasi, 2009). The composition of biogas is intrinsically determined by the carbon oxidation-reduction state of the organic matter present in the waste and the type of anaerobic digestion process, which in turn depend on the origin of the residue digested (Jönsson *et al*, 2003). For instance, the biogas recovered from conventional landfills is a complex mixture composed of CH_4 (35-65%), CO_2 (15-50%), N_2 (5-40%), H_2O (0-5%), O_2 (0-5%), H_2 (0-3%), CO (0-3%), H_2S (0-100 ppm_v), NH_3 (0-5 ppm_v), halogenated hydrocarbons (20-200 ppm_v Cl^-/F^-), volatile organic contaminants (0-4500 mg m^{-3}) and siloxanes (0-50 mg Si m^{-3}) (Jaffrin *et al*, 2003; Persson *et al*, 2006; Ajhar *et al*, 2010; Bailón and Hinge, 2012). A slightly simpler biogas is typically obtained from the anaerobic degradation of sewage sludge, livestock manure or agroindustrial bio-wastes: CH_4 (53-70%), CO_2 (30-47%), N_2 (0-3%), H_2O (5-10%), O_2 (0-1%), H_2S (0-10000 ppm_v), NH_3 (0-100 ppm_v), hydrocarbons (0-200 mg m^{-3}) and siloxanes (0-41 mg m^{-3}) (Persson *et al*, 2006; Soreanu *et al*, 2011; Bailón and Hinge, 2012). Carbon dioxide and nitrogen constitute the major contaminants of biogas (N_2 in the particular case of landfills), decreasing its specific calorific value and therefore its Wobbe index (Ryckebosch *et al*, 2011).

Biogas is currently used as a fuel for on-site heat, steam and electricity generation in industry, as a substrate in fuel cells, as a substitute of natural gas for domestic and

industrial use prior injection into natural gas grids and as a vehicle fuel (Rasi, 2009; Andriani *et al*, 2014; Thrän *et al*, 2014). In this context, biogas production in Europe accounted for 13.4 million tons of oil equivalent (≈10 % increase compared to 2012), which represented 52.3 TWh of electricity produced and net heat sales to heating district networks of 432 megatons of oil equivalent (EurObserv'ER, 2014). In addition, the actual European network of 14000 anaerobic digesters is expected to increase in order to supply up to 18-20 million m^3 by 2030 (3 % of the European gas consumption) according to the latest European Biogas Association's estimations (European Biogas Association, 2013).

The final use of biogas determines its composition and the type of upgrading process required. Thus, on-site biogas use in boilers for heat generation only requires H_2S removal below 1000 ppm_v and water removal prior to combustion (Bailón and Hinge, 2012). The use of biogas in internal combustion engines for combined heat and power generation (CHP) requires the removal of water, and H_2S, NH_3, siloxanes and halocarbons levels below 200-1000 ppm_v, 32-50 mg m^{-3}, 5-28 mg m^{-3} and 65-100 mg m^{-3}, respectively, depending on the manufacturer. Turbines and micro-turbines for CHP generation require very low contents of siloxane (0.03-0.1 ppm_v) and water (pressurized dew point -6.7 ºC below biogas temperature), but are able to stand high concentrations of H_2S (10000-70000 ppm_v) and halocarbon (200-1500 ppm_v Cl^-/F^-) (Soreanu *et al*, 2011; Bailón and Hinge, 2012). However, the most stringent quality requirements are encountered in biomethane for injection into natural gas grids and as a vehicle fuel, which often demands CH_4 concentrations > 80- 96 %, CO_2 < 2-3%, O_2 < 0.2-0.5 %, H_2S < 5 mg m^{-3}, NH_3 < 3-20 mg m^{-3} and siloxanes < 5-10 mg m^{-3} (Table 2-1).

Table 2-1.Technical specifications for injection of biogas in natural gas grid and use as a vehicle fuel (Marcogaz, 2006; Persson *et al*, 2006; Huguen and Le Saux, 2010; INN, 2010; Bailón and Hinge, 2012; BOE, 2013; Svensson, 2014)

Country	Sweden	Switzerland	Germany	France	Austria	Netherlands	Spain	Belgium	Czech Rep	California U.S.	Chile
CH_4 content (%)	97±1 (Type A)[1] 97±2 (Type B)	> 96[2] > 50[3]			> 96	> 80	> 95	> 85	> 95		> 88
Wobbe index (MJ Nm^{-3})	44.7–46.4 (Type A)[1] 43.9–47.3 (Type B)	47.9 - 56.5 (unlimited injection)	46.1 - 56.5[4] 37.8 - 46.8[5]	48.2 - 56.5[4] 42.5 - 46.8[5]	47.7 - 56.5	43.46 - 44.41	13.40-16.06 kWh m^{-3} (48.25-57.81 MJ m^{-3})			47.6–51.6	47.28 – 52.72
Water dew point (°C)	< t[6]–5 < -9 (at 200 bar)	-8 at MOP	Ground temp.	< -5 at MOP	< -8 (40 bar)	< -10 (8 bar)	2°C at 7 bar		< -10°C		
Water content max. (mg Nm^{-3})	< 32					< 32					
CO_2 (%)	< 3	< 4 [2] < 6 [3]	< 6	< 2.5[7]	< 2	< 6 (< 10–10.3 for regional grid)	2.5	< 2.5	< 5	3	
O_2 (%)	< 1	< 0.5	< 3	< 0.01[7]	< 0.5	< 0.5	0.01 (0.3[8])		< 0.5	< 0.2	< 1
CO_2+O_2+N_2 (%)	< 4 (Type A)[1] < 5 (Type B)										1.5 – 4.5 (CO_2+N_2)
H_2S (mg Nm^{-3})	< 15.2	< 5	< 5	< 5 (H_2S+COS)	< 5	< 5	15 (H_2S+COS)	< 5 (H_2S+COS)	< 7	88	-
Total sulfur (mg Nm^{-3})	< 23	< 30	< 30	< 30	< 10	< 45	50	< 30	< 30	265	< 35
Mercaptans (mg m^{-3})		< 5	< 6	< 6	< 6	< 10	17	< 6	< 5	106	
NH_3 (mg/Nm^3)	< 20	< 20	< 20	< 3	Technically free	< 3	< 3	< 3		< 0.001 % mol	-
Siloxanes					< 10 total silicon mg m^{-3}	< 5 ppm_v	< 10mg m^{-3}		< 6 mgSi m^{-3}	Commercial free or < 0.1 mgSi m^{-3}	
Halogenated compounds		< 1 mgCl m^{-3}	< 1 mgCl m^{-3}	< 1 mg m^{-3} (9) < 10mg m^{-3} (10)		< 50 mg m^{-3} (9) < 25 mg m^{-3} (10)	< 1 mg m^{-3} (9) < 10mg m^{-3} (10)	< 1 mg m^{-3} (9) < 10mg m^{-3} (10)	< 1.5 mg m^{-3} (Cl + F)	< 0.1 ppm_v	

(1) Type A: biogas as vehicle fuel – Engines without lambda control, type B: biogas as vehicle fuel – Engines with lambda control. (2) Unlimited gas injection in Switzerland; (3) Limited gas injection in Switzerland; (4) High calorific gas; (5) Low calorific gas; (6) Ambient temperature; (7). France allows some flexibility on parameters, O_2 and CO_2 content may be increased to 3 % and 11.3 %, respectively, under some conditions; (8) possible if the following conditions concur in the injection point: CO_2 < 2%, water dew point < -8°C, biogas injection flow rate into the main transport network never exceeds 5000 m^3h^{-1} (Possibility to inject higher flow rates are studied on a case by case basis); (9) Chlorine compounds; (10) Fluorine compounds.

With the biogas upgrading market and technologies rapidly evolving, a more frequent evaluation of the state-of-the art technologies available is necessary (Bauer *et al*, 2013b). In this context, most physical/chemical biogas upgrading technologies are still highly energy or chemical intensive, which has triggered the rapid development of biogas upgrading biotechnologies based on their superior economic/environmental sustainability. This paper critically reviews and discusses the state-of-the-art technologies for the removal of CO_2 with a special focus on the potential and limitations of biotechnologies based on the significant technological breakthroughs occurred in this field in the past 10 years.

CO_2 removal from biogas at industrial scale is nowadays performed by physical/chemical technologies based on their high degree of maturity and commercial availability, while the potential of biotechnologies has been assessed only at lab or pilot scale. However, while most physical/chemical units discharge the separated CO_2 to the atmosphere (prior off-gas post treatment to avoid the release of CH_4), biotechnologies allow for the bioconversion of CO_2 into valuable commercial products, at significantly lower energy costs.

2.2. Physical/chemical CO_2 removal technologies

Scrubbing with water, organic solvents or chemical solutions, membrane separation, pressure swing adsorption and cryogenic CO_2 separation dominate the biogas upgrading market nowadays. Figure 2-1 shows the participation in the market of these technologies.

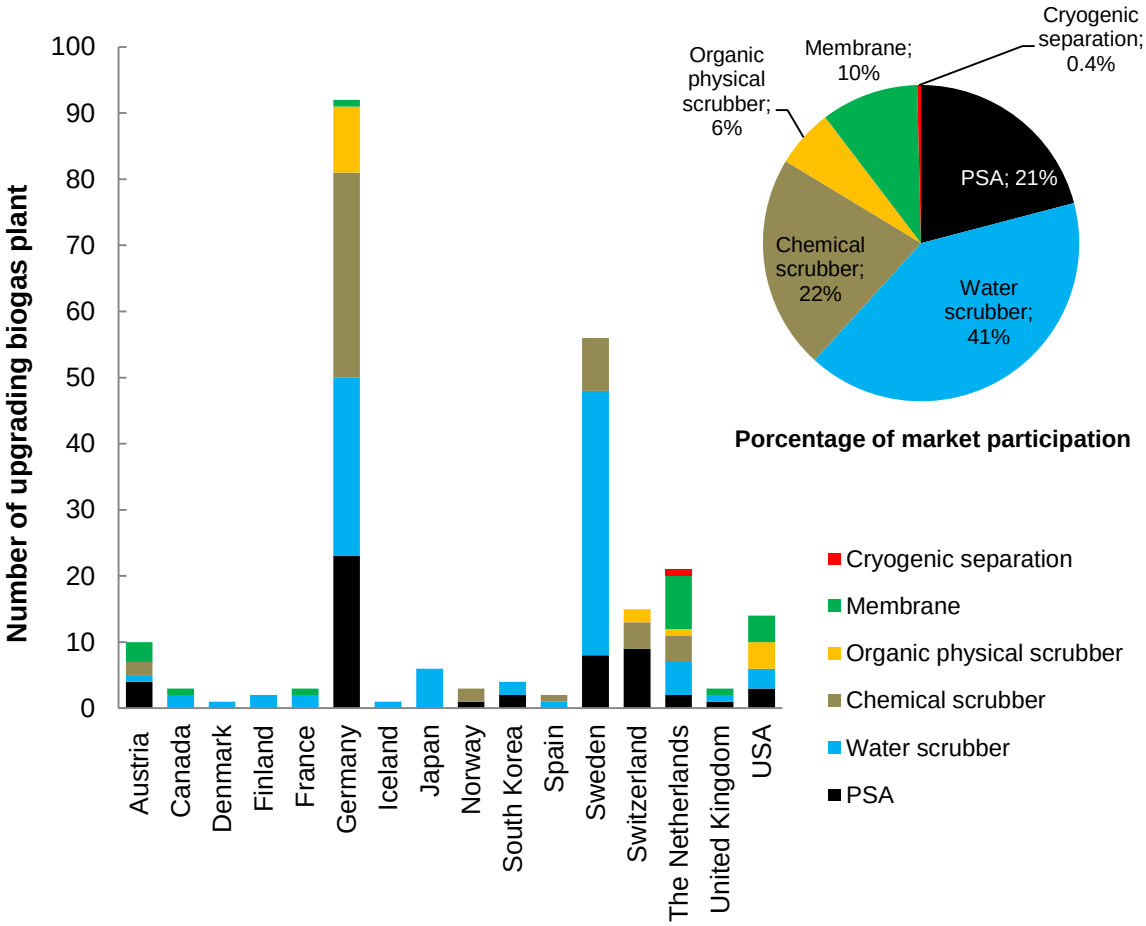

Figure 2-1. Biogas upgrading plants in operation classified according to the upgrading technologies and country (IEA-Bioenergy, 2013).

According to Figure 2-1, the principal producers of upgraded biogas are Germany and Sweden. The principal use of upgraded biogas in Germany is its injection into the gas grid. Contrarily, Sweden mostly uses the upgraded biogas as vehicle fuel. The most used technology for biogas upgrading is CO_2 absorption by water and chemical scrubber. This result can be attributed to that CO_2 absorption is one of the cheapest and simplest technologies. Then, pressure swing adsorption (PSA) and membrane separation are also used. Cryogenic separation is the newest of the current technologies with few operating plants in the United States, Sweden and The Netherlands. These technologies are discussed below:

2.2.1. Water Scrubbing

CO_2 removal via scrubbing with water as selective absorbent is a classical unit operation in chemical engineering based on the higher aqueous solubility of CO_2 compared to that of CH_4 (26 times higher at 25 ºC) (Sinnott, 2005). Water scrubbing is nowadays a mature technology with accounts for approximately 41 % of the global biogas upgrading market, being considered the upgrading method less sensitive to biogas impurities (Thrän *et al*, 2014). The availability of a low-cost water supply of sufficient quality often determines the water scrubber configuration implemented. For instance, CO_2 removal from biogas produced in wastewater treatment plants (WWTPs) has been performed in single-pass scrubbers using pressurized treated water (6-10 bar), which after absorption is sent back to the main water treatment line (Tynell *et al*, 2007). However, most modern units in landfills or OMSW treatment facilities are constructed based on a sequential pressurized CO_2 absorption in water (tap water quality) coupled to a two-stage stripping, which allows for water regeneration (Beggel *et al*, 2010; Bauer *et al*, 2013). CO_2 absorption is often carried out at 6-10 bar, although pressures in the range of 10-20 bar are also used (Ryckebosch *et al*, 2011). The first flash unit is operated at 2-4 bars, resulting in the emission of a CO_2 rich biogas (80-90% CO_2 and 10-20 % CH_4) that is returned to the absorption unit (Bauer *et al*, 2013b) (Figure 2-2 A). Water decompression to atmospheric pressure in the second stripping unit, often assisted by air injection, results in the final regeneration of the absorbent that is returned to the absorption unit (Kapdi *et al*, 2005; Patterson *et al*, 2011; Ryckebosch *et al*, 2011). The amount of water required ($m^3\ h^{-1}$) depends on the water pressure and temperature, and can be estimated as $Q_{biogas}/(H \times P)$, where Q_{biogas} (kmol h^{-1}) represents the raw molar biogas flow rate, H (M atm^{-1}) the Henry's Law constant and P (atm) the total pressure of operation. Surprisingly, it does not depend on the pH of water or on the CO_2

concentration in the raw biogas. Typical water flow rates of 0.1-0.2 $m^3_{water}\,Nm^{-3}_{biogas}$ are reported in single-pass scrubbers depending on the operational pressure (Persson, 2003), which are comparable to the 0.18-0.23 $m^3_{water}\,Nm^{-3}_{biogas}$ in units designed with water recycling (Bauer *et al*, 2013b). Higher operational pressures entail lower water flow rates, but higher pumping and compression costs and a reduced lifetime of the upgrading plant. Despite water recycling significantly reduces water consumption, 20-200 L h^{-1} are continuously purged to avoid the accumulation of detrimental byproducts.

Countercurrent operation is preferred regardless of the scrubbing configuration. Both absorption and desorption units are typically constructed with random packings such as Pall or Raschig rings to support an efficient gas-liquid mass transfer (Ryckebosch *et al*, 2011; Bauer *et al*, 2013). CH_4 and CO_2 concentrations in the upgraded biogas are normally > 96% and < 2%, respectively. CH_4 losses of 1-2 % and technical plant availabilities of 95-96 % are typically reported in technical literature for commercial full-scale facilities (10-10000 $Nm^3\,h^{-1}$) (Beil, 2009; Rasi, 2009; Patterson *et al*, 2011; Bauer *et al*, 2013b) (Table 2-2). Despite manufacturers guarantee 2 % methane losses with exhaust gas recirculation, losses of 8-10 % have been measured under regular operation, as a result of the non-optimized operation of the flash tank (Persson, 2003). Elemental sulfur accumulation, corrosion and odour nuisance also rank among the most important operational problems in water scrubbers derived from the simultaneous absorption of H_2S in water. Thus, despite this technology can cope with H_2S concentrations of 300-2500 ppm_v (depending on the manufacturer), H_2S removal is highly recommended prior to water scrubbing (Persson *et al*, 2006; Thrän *et al*, 2014). On the other hand, microbial growth (especially when using treated water in WWTPs) and foam formation in the packed bed constitute additional operational problems of this

technology, which result in a limited gas-liquid mass transport and require the use of antifoaming agents (although their cost is marginal) (Bauer *et al*, 2013b).

Investment costs in water scrubbers linearly decrease from 5500 to 2500 € $(Nm^3\ h^{-1})^{-1}$ when the design treatment capacity increases from 100 to 500 $Nm^3\ h^{-1}$, and remained relatively constant at 1800-2000 € $(Nm^3/h)^{-1}$ for plant capacities over 1000 $Nm^3\ h^{-1}$. On the other hand, the operating costs range from 0.11-0.15 € Nm^{-3} (200-300 $m^3\ h^{-1}$), which can be attributed to both energy consumption (decreasing from 0.3 kWh Nm^{-3} at 500 $Nm^3\ h^{-1}$ to 0.2 kWh Nm^{-3} at 2000 $Nm^3\ h^{-1}$) and annual maintenance costs (2-3 % of the investment costs), since the costs of consumables are often negligible (Urban *et al*, 2009; Patterson *et al*, 2011; Bauer *et al*, 2013b). In this context, the major energy demanding processes are gas compression (0.10-0.15 kWh Nm^{-3} in 6-8 bar modern facilities), water compression (0.05-0.1 kWh Nm^{-3}) and water cooling (0.01-0.05 kWh m^{-3}). The need for an off-gas treatment unit such as incinerators, activated carbon filters or biofilters to abate the H_2S and CH_4 stripped from the desorption tank entail additional costs not considered in the above discussion.

Table 2-2. Commercial upgrading technologies

	Technology	CH_4 (%)	CO_2 (%)	H_2S (%)	Methane loss	Costs	Power consumption	Examples	References
High pressure water scrubbing	**DMT Carborex®PWS** P= 8-10 bar CO_2 and H_2S removal Solvent regeneration: Flash tank in two steps: 1) 2-4 bar; 2) 1 bar. Air stripping unit and Biotrickling Filter.	>97%	< 2%	< 2 ppm_v	< 2%	0.105 € m^{-3} (250 $Nm^3 h^{-1}$) 0.052 € m^{-3} (2000 $Nm^3 h^{-1}$)	0.4-0.5 kWh m^{-3} produced gas	1) Zalaegerszeg, HU, Okoprotec (50-85 $Nm^3 h^{-1}$; WWTP) 2) Zwolle, NL, Nature Gas Overijssel (520 $Nm^3 h^{-1}$; green waste and other garbage) 3) Wijster, NL (1500 $Nm^3 h^{-1}$; Landfill)	DMT (2015)
	Malmberg COMPACT® CO_2 and H_2S removal Capacity: 100-3000 $Nm^3 h^{-1}$ Methane emissions are avoided by thermal oxidation in the process air.	>97%	1-2%		<1%	2 ct kWh^{-1} (250 $Nm^3 h^{-1}$) 1 ct kWh^{-1} (2000 $Nm^3 h^{-1}$)		1) Stockholm Vatten, Henriksdal (1400 $Nm^3 h^{-1}$; WWTP) 2) Jönköping Municipality, Sweden (150 $Nm^3 h^{-1}$; sludge digestion)	Malmberg (2014)
Chemical scrubbing	**OASEgreen™ Process (Bilfinger EMS GmbH)** Chemisorption with PuraTreat™ solvent CO_2 and H_2S removal Atmospheric pressure T° solvent regeneration: 106-110°C Capacity: 600- 10000 $Nm^3 h^{-1}$	>99%	< 1%	< 4 ppm_v	<0.05%	< 0.01 € kWh^{-1} of raw biogas		1) BUP´s Verbio (2 separate plants Schwedt and Zörbig; 6000 $Nm^3 h^{-1}$) 2) BUP Weltec (Arneburg; 1450 $Nm^3 h^{-1}$)	Bilfinger EMS GmbH (2014)
	LP Cooab-technique (Cirmac) Absorption by amines CO_2 removal Atmospheric pressure Exhaust-gas treatment is not necessary	99.5%			<0.1%		0.05 - 0.12 kWe Nm^{-3} raw gas	Gasslosa biogas plant in Boras, Sweden	Energy Transition–Creative Energy (2014)

Chemical scrubbing	**CApure™ process (Purac Puregas)** Absorption by amines CO_2 removal Atmospheric pressure 100 - 3000 raw biogas $Nm^3\ h^{-1}$	99%	0.20%	< 0.5 ppm_v	<0.1%		0.23 - 0.26 kWh Nm^{-3} raw gas (with heat recovery system)		Purac Puregas (2014)
Organic physical scrubbing	**Schwelm Biogas treatment plant** Capacity: 200-1600 $Nm^3\ h^{-1}$ Absorption by polyethylene glycol.	98%			<1%		0.21 kWh Nm^{-3} of raw gas		Schwelm Anlagentechnik GmbH (2014)
Pressure Swing adsorption	**Xebec PSA** P= 8-11 bar 9 vessel system with a patented rotary valve Previous H_2S removal Regeneration under vacuum pressure (typically 0.5 bar) Capacity: 100-10000 $Nm^3\ h^{-1}$ Removal CO_2 and water vapour	98%	1-2%					1)Scenic View Dairy, Fennville, Michigan (animal waste; 225$Nm^3\ h^{-1}$) 2)Rumpke Landfill Cincinnati,Ohio (7000 $Nm^3\ h^{-1}$)	Xebec (2014)
Membrane separation	**DMT Carborex® MS** Previous H_2S and water vapour removal P= 10 bar The off-gas contains over 99.5% CO_2. Removal CO_2 Gas/gas membrane	97-99%	1-3%		<0.5%	50 $Nm^3\ h^{-1}$ (0.432 ct Nm^{-3}); 200 $Nm^3\ h^{-1}$ (0.211 ct Nm^{-3})	< 0.22 kWh Nm^{-3}		DMT (2014)
	Biopower plant P = 16 bar Hollow fiber membrane Removal CO_2 Gas/gas membrane	96%			<1%			Biopower plant in Pratteln, Switzerland (210 $Nm^3\ h^{-1}$;high solids digestion, biowaste, yard waste)	Eisenmann (2014)

2.2.2. Organic Solvent Scrubbing

This technology, fundamentally similar to water scrubbing, uses polyethylene glycol-based absorbents (commercialized under trade names such as Selexol® or Genosorb®), which exhibit a higher affinity for CO_2 and H_2S than water. For instance, Selexol®, a mixture of polyethylene glycol dimethyl ethers, has a 5 times higher affinity for CO_2 than water (Tock *et al*, 2010). These solvents allow for a decrease in both the absorbent recycling rates and plant sizing, with the subsequent decrease in investment and operating costs (Petersson and Wellinger, 2009; Ryckebosch *et al*, 2011). Unlike water scrubbing, the use of organic solvents requires a gas condition step to remove water and several heating stages to promote an efficient desorption of CO_2 at 40 ºC (Figure 2-2 B). Both biogas and organic solvent are cooled down to 20 ºC prior absorption (Bauer *et al*, 2013b). The anticorrosion nature of the organic solvents does not require the use of stainless steel in the scrubber. Despite the advantages of this mature technology, its share in the biogas upgrading market is only 6% (Thrän *et al*, 2014).

A biomethane with CH_4 contents of 96-98.5 % can be consistently achieved in optimized full scale organic solvents scrubbers with a 96-98 % technical availability (Bauer *et al*, 2013b; Thrän *et al*, 2014). Similarly to water scrubbing, this technology results in CH_4 losses lower than 2 % (Persson *et al*, 2007). When biogas contains high concentrations of H_2S, solvent regeneration is conducted with steam or inert gas in order to avoid a sulfur-mediated solvent deterioration (Ryckebosch *et al*, 2011). However, a complete H_2S removal using activated carbon filters is often recommended prior to organic scrubbing.

The capital costs for implementation of organic scrubbers decrease from ≈ 4500 € $(Nm^3 h^{1-})^{-1}$ for 250 $Nm^3 h^{-1}$ plants to 2000 € $(Nm^3 h^{-1})^{-1}$ for design capacities of 1000 $Nm^3 h^{-1}$.

Constant capital costs of 1500 € $(Nm^3/h)^{-1}$ correspond to large upgrading plants with treatment capacities over 1500 $Nm^3\ h^{-1}$ (Bauer *et al*, 2013b). Process operating costs mainly derive from the electricity used for biogas compression and liquid pumping (0.2-0.25 kWh Nm^{-3}) and maintenance costs (2-3 % of the investment cost), since the heat required for absorbent regeneration is often obtained from the residual heat of the exhaust gases of the off-gas incineration units (Bauer *et al*, 2013b). Higher energy requirements in the range of 0.4-0.51 kWh Nm^{-3} can be found in technical literature (Berndt, 2006; Günther, 2007; Persson, 2007). On the other hand, the low vapour pressure of polyethylene glycol dimethyl ethers requires a minimum organic solvent make-up.

2.2.3. Chemical Scrubbing

Chemical scrubbing involves similar biogas-liquid mass transfer fundamentals to water/Selexol® scrubbing but a simpler process configuration and an enhanced performance derived from the use of CO_2-reactive absorbents such as alcanol amines (monoethanolamine, diethanolamine, etc.) or alkali aqueous solutions (NaOH, KOH, CaOH, K_2CO_3, etc.) (Andriani *et al*, 2014). According to a recent review of commercial technologies, a mixture of methyldiethanolamine and piperazine (aMDEA) constitutes the most popular amine absorbent nowadays, which is used at aMDEA/CO_2 mol ratios of 4-7 (Bauer *et al*, 2013b). This technology consists of a packed bed absorption unit coupled to a desorption unit equipped with a reboiler, which simplifies process configuration compared to their physical absorption counterparts (Figure 2-2 C). Both structured and random packings are employed since the risk of biomass growth is limited by the high pH of the amine solutions (Bauer *et al*, 2013b). Unlike water/Selexol® scrubbing, the formation of intermediate chemical species (CO_3^{2-}, HCO_3^-) mediated by the exothermic reaction of the absorbed CO_2 with the chemical

reagents present in the scrubbing solution results in an enhanced CO_2 absorption capacity and process operation at maximum CO_2 concentration gradients (Ryckebosch *et al*, 2011). This intensification in CO_2 mass transfer from biogas finally results in more compact units and lower absorbent recycling rates (Patterson *et al*, 2011). In addition, process operation at low pressure (1-2 bar in the absorption column and 1.5-3 bar in the stripping column) entails significantly lower energy requirements for biogas compression and absorbent pumping (Patterson *et al*, 2011). However, the high energy requirements for solvent regeneration (carried out at 120-150 ºC) have likely limited the share of this mature technology to 22 % of the global upgrading market (Thrän *et al*, 2014).

Like water scrubbing, chemical scrubbing is operated in a countercurrent flow configuration (Bauer *et al*, 2013b). CH_4 recoveries of 99.5-99.9 % can be achieved at a plant availability of 91-96 % due to the low solubility of CH_4 in alcanol amines (Beil, 2009; Ryckebosch *et al*, 2011; Bauer *et al*, 2013b). On the other hand, H_2S removal (often carried out in activated carbon filters) prior to amine scrubbing is highly recommended to prevent amine poisoning, although some commercial units can cope with biogas containing up to 300 ppm_v of H_2S. Foaming and amine degradation/losses rank among the most important operational problems along with corrosion issues (Bauer *et al*, 2013b).

The investment costs in chemical scrubbing linearly decrease from 3200 € $(Nm^3/h)^{-1}$ for design flow rates of 600 $Nm^3\ h^{-1}$ to 1500 € $(Nm^3/h)^{-1}$ for 1800 $Nm^3\ h^{-1}$ upgrading plants (Bauer *et al*, 2013b). While the costs associated to amine, antifoam and water make-up (3 mg Nm^{-3} for each compound) are marginal and the electricity requirements for gas compression and liquid pumping are moderate (0.12-0.15 kWh Nm^{-3}) (Günther, 2007;

Beil, 2009; Bauer *et al*, 2013b), the main operating costs derive from the energy required for amine regeneration (0.55 kWh Nm^{-3}).

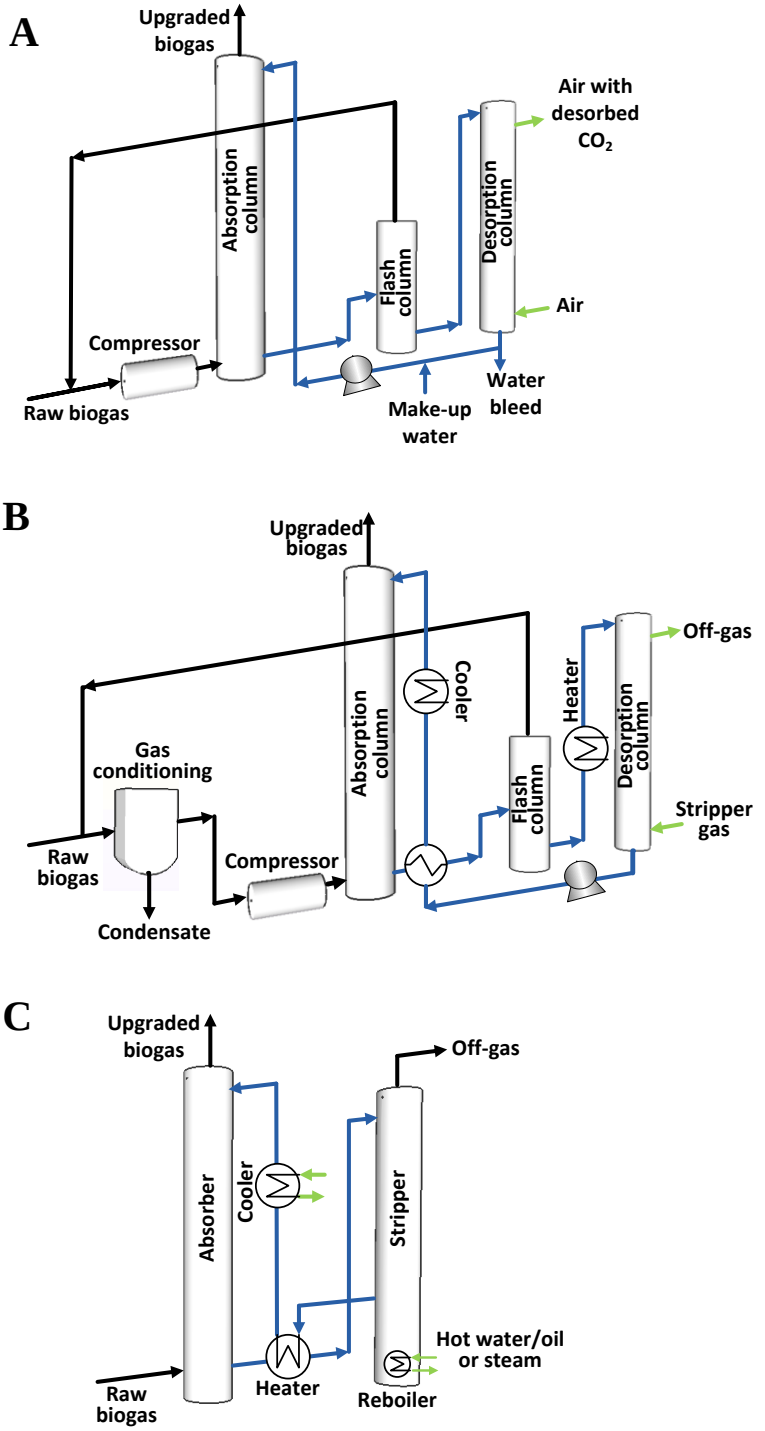

Figure 2-2. Biogas upgrading by liquid absorption. A) Water scrubbing; B) Organic solvent scrubbing; C) Chemical scrubbing. Adapted from Bauer *et al* (2013b).

2.2.4. Pressure swing adsorption

PSA is based on the selective adsorption of CO_2 over CH_4 onto porous adsorbents with a high specific surface area such as activated carbon, silica-gel, activated alumina, zeolite and polymeric sorbents (Patterson *et al*, 2011; Ryckebosch *et al*, 2011). Molecular size exclusion and adsorption affinity constitute the separation mechanisms of this technology. Molecular sieve adsorbents with average pore size of 3.7 Å are used to retain CO_2 molecules (molecular size of 3.4 Å) inside the pores, while excluding CH_4 molecules (molecular size of 3.8 Å). Hence CH_4 flows unretained through the interstitial spaces of the packed bed under continuous PSA operation, resulting in a CH_4 rich biogas (Patterson *et al*, 2011). Adsorbents such as activated carbon or zeolites base this selective CO_2/CH_4 separation on their higher CO_2 solid-gas partition coefficient compared to that of CH_4. Other adsorbents facilitate a faster diffusion of CO_2 molecules inside the adsorbent pores, kinetically excluding CH_4 retention inside the adsorbent (Bauer *et al*, 2013b). Apart from a high selective adsorption of CO_2, molecular sieves used in PSA must be non-hazardous, readily available, stable under long-term operation and must exhibit a linear adsorption isotherm (Bauer *et al*, 2013b). These adsorbents are often packed in vertical columns operated under a pressurization, feed, blowdown and purge regime, which requires the arrangement of 4 interconnected columns in parallel operating at any of the 4 stages described above (Figure 2-3). Column pressurization and biogas feeding are often carried out at 4-10 bars to increase CO_2 retention inside the pores. When the column gets saturated with CO_2, the blowdown phase commences by filling the adjacent previously regenerated adsorption column with the exiting gas from the saturated column (in order to reduce the overall energy consumption of the process), which represents the pressurization stage of this new operating adsorption column. The saturated column is finally vented to ambient pressure and purged with upgraded biogas

to complete the regeneration of the adsorbent bed. The exhaust gases from column purging are often recirculated to the biogas feed (Bauer *et al*, 2013b). This cycle of adsorption and regeneration (so called Skarstrom cycle) last for 2-10 min (Grande, 2011). PSA, originally developed in the 1960s for the separation of industrial gases, constitutes nowadays a mature technology with a market share of 21 % (Patterson *et al*, 2011; Thrän *et al*, 2014).

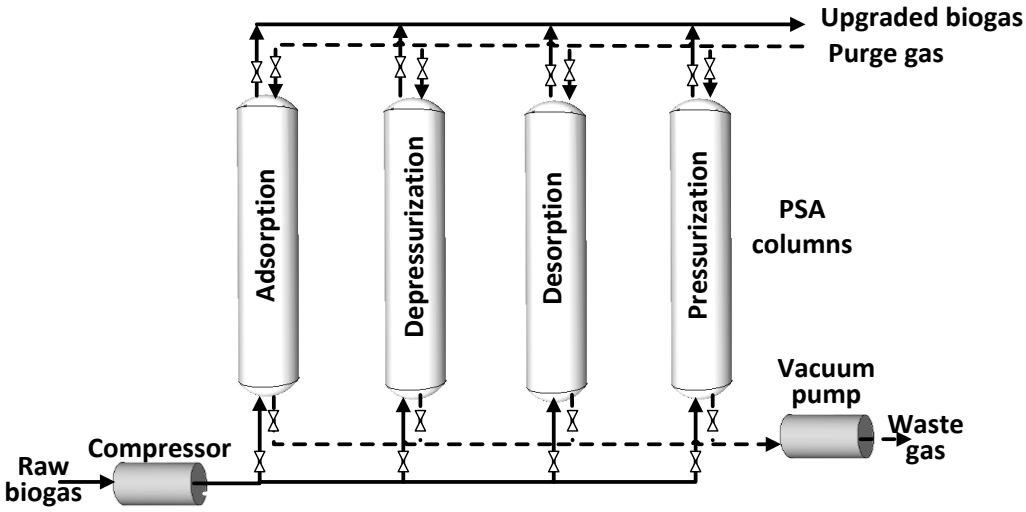

Figure 2-3. Biogas upgrading by Pressure Swing Adsorption (PSA). Adapted from Bauer *et al* (2013b).

Biomethane with a CH_4 purity of 96-98 %, recoveries of ≈98% and technical plant availabilities of 94-96 % are commonly reported in technical literature (Beil, 2009; Bauer *et al*, 2013b). H_2S and siloxanes irreversible adsorb onto the molecular sieves and are often removed using activated carbon filters during the biogas conditioning stage. The moisture content of the biogas is also removed by condensation prior to PSA (Bauer *et al*, 2013b).

Capital costs in PSA linearly decrease from 2700 € $(Nm^3/h)^{-1}$ at design flow rates of 600 $Nm^3\ h^{-1}$ to 1500 € $(Nm^3/h)^{-1}$ for plants with a capacity of 2000 $Nm^3\ h^{-1}$ (Bauer *et al*,

2013b). Electricity requirements for gas compression and biogas demoisturisation in the range of 0.24 to 0.6 kWh Nm^{-3} are typically reported in literature (Günther, 2007; Persson, 2007; Beil, 2009), although a recent cost survey limits electricity needs to 0.25-0.3 kWh Nm^{-3} (including catalytic oxidizers from the abatement of CH_4 off-gas emissions) (Bauer *et al*, 2013b). PSA does not entail additional costs derived from water make-up addition or heat for adsorbent regeneration.

2.2.5. Membrane separation

Membrane-based upgrading technologies are based on the principle of selective permeation of biogas components through a semi-permeable membrane (Bauer *et al*, 2013b). Conventional membranes for biogas upgrading retain CH_4 and N_2, and facilitate the preferential permeation of O_2, H_2O, CO_2 and H_2S with CO_2/CH_4 selectivity factors of up to 1000/1 (Ryckebosch *et al*, 2011). Polymeric materials such cellulose acetate are preferred for the manufacture of biogas separating membranes over non-polymeric materials because of their lower cost, easy manufacture, stability at high pressures and easy scalability (Basu *et al*, 2010). Recent breakthroughs in membrane manufacture driven by nanotechnology have increased membrane selectivity factors (and therefore methane recoveries) and renewed the interest in this classical natural gas upgrading technology (Bauer *et al*, 2013b). Membrane separation is in fact a mature technology (with a market share of 10 %) commercialized either in high pressure gas-gas modules or low pressure gas-liquid modules (Patterson *et al*, 2011; Thrän *et al*, 2014). Biogas is pressurized at 20-40 bars in gas-gas systems (although some commercial units also operate in the 6-20 bar range) resulting in a CH_4 rich retentate and a CO_2 rich permeate containing methane and trace levels of H_2S at atmospheric pressure (or negative pressures to increase the purity of the biomethane over 97 %) (Bauer *et al*, 2013b). Gas-gas units are manufactured under different configurations: single-pass membrane

unit or multiple stage membrane units with internal recirculations of permeates and retentates (Figure 2-4). On the other hand, gas-liquid systems are operated at atmospheric pressure (with the associated reduction in construction costs) with biogas and a CO_2-liquid absorbent separated by a micro porous hydrophobic membrane. Both fluids flow under counter current mode (Ryckebosch *et al*, 2011). Alcanol amines or alkali aqueous solutions are used as CO_2 liquid absorbents.

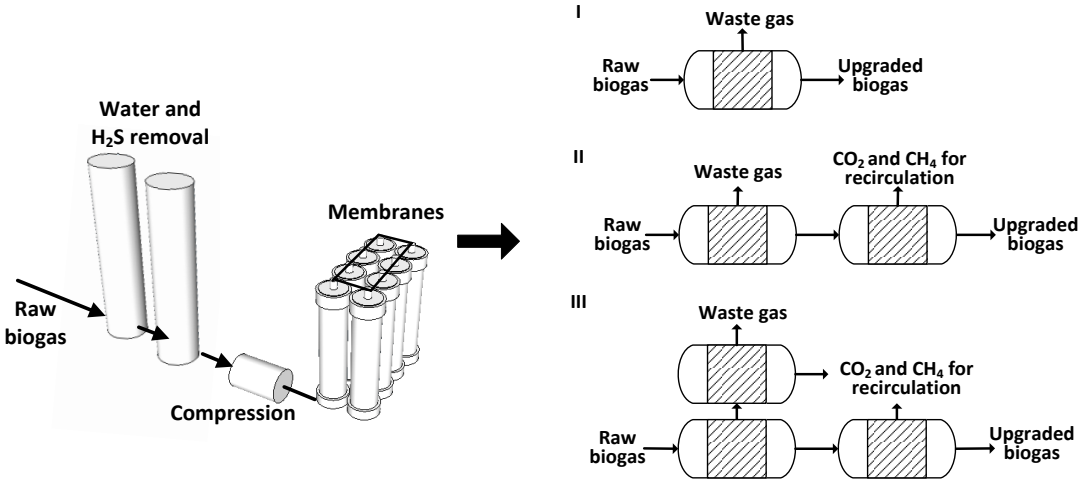

Figure 2-4. Biogas upgrading by membrane separation. Different configurations of gas-gas units: I) single-pass membrane unit, II) multiple stage membrane units with internal recirculation of permeate and III) internal recirculation of retentates. Adapted from Bauer *et al* (2013b).

CH_4 recovery in membrane-based upgrading systems depends on the membrane configuration used. Thus, CH_4 recoveries of 98-99 % can be achieved in gas-liquid units or two-stage gas-gas units with recirculation of the permeate from the second membrane module. Recoveries of 99-99.5 % require more complex designs with recirculation of both the permeate from the second stage and the retentate from the filtration of the permeate of the first module (Benjaminsson, 2006). The technical availability of this mature technology ranges from 95-98% (Beil, 2009; Bauer *et al*, 2013b). CH_4

concentrations of 96-98 % are guaranteed by most membrane manufacturers in gas-liquid or multiple-stage gas-gas units, while single-pass gas-gas units provide a biomethane with CH_4 concentrations of 92-94 % and off-gas permeates with CH_4 concentrations of 10-25 % that need to be further treated (Ryckebosch *et al*, 2011; Andriani *et al*, 2014). Higher pressures or higher membrane areas would be required to further increase the CH_4 concentration in the final biomethane. Biogas pre-treatment involving the removal of particles, H_2S, H_2O, VOCs, NH_3 and siloxanes by condensation and activated carbon filtration is highly recommended prior to membrane separation to avoid a rapid deterioration and clogging of the membrane (Patterson *et al*, 2011; Bauer *et al*, 2013b).

The investment costs of gas-gas membrane units rapidly increase from 2500 € $(Nm^3/h)^{-1}$ for design flow rates of 400 $Nm^3\ h^{-1}$ to 6000 € $(Nm^3/h)^{-1}$ when scaling down the process to 100 $Nm^3\ h^{-1}$ (Bauer *et al*, 2013b), remaining approximately constant at 2000 € $(Nm^3/h)^{-1}$ for plants with capacities over 1000 $Nm^3\ h^{-1}$. The operating costs of this technology are mainly determined by membrane replacement (5-10 years lifetime), biogas compression cost (0.2-0.38 kWh Nm^{-3}) and the cost associated to biogas pre-treatment (activated carbon replacement plus energy for condensation) (Benjaminsson, 2006; Beil, 2009; Bauer *et al*, 2013b). Costs in the range of 0.13-0.22 € Nm^{-3} are typically reported in literature (Hullu *et al*, 2008). Membrane-based upgrading exhibits slightly higher maintenance cost (3-4 % of the initial investment costs) compared to their physical chemical counterparts (2-3 %).

2.2.6. Cryogenic separation

The different liquefaction/solidification temperatures of the biogas components allow for a selective separation of H_2O, H_2S, CO_2 and CH_4 if the temperature of biogas is

stepwise decreased, which even allows for the generation of a liquefied biomethane (free of O_2 and N_2) at temperatures between -162 and -182 ºC (Bauer *et al*, 2013b). Cryogenic biogas upgrading can be conducted at constant pressure (10 bar) using a sequential temperature decrease to -25 ºC (where water, H_2S, siloxanes and halogens are removed in liquid phase), to -55 ºC (where most CO_2 is liquefied to facilitate its withdrawal from the upgrading unit and further commercialization) and finally to -85 ºC as polishing step (where the remaining CO_2 solidifies) (Ryckebosch *et al*, 2011). Process operation at high pressure avoids the sudden solidification of CO_2 below -78 ºC, which prevents operational problems derived from clogging of pipelines and heat exchanges (Bauer *et al*, 2013b). The most common operational procedure involves a preliminary biogas drying followed by a multistage compression (with intermediate cooling) up to 80 bar (Patterson *et al*, 2011; Ryckebosch *et al*, 2011). The pressurized biogas is stepwise cooled to -45 ºC and -55 ºC to promote the liquefaction of most CO_2, and finally expanded to 8-10 bar in a flash tank (-110 ºC) to facilitate biomethane purification via CO_2 solidification. Despite its synergies with the process of biomethane liquefaction, this technology is still not reliably commercialized at full scale and represents only 0.4 % of the upgrading market at a global level (Bauer *et al*, 2013; Bauer *et al*, 2013b; Thrän *et al*, 2014).

Cryogenic upgrading can provide a biomethane with a purity over 97 %, with methane losses lower than 2 % (Beil, 2009; Andriani *et al*, 2014). The emerging nature of this technology, with few operating plants in the United States, Sweden and The Netherlands, does not allow yet an accurate determination of its technical availability (Petersson and Wellinger, 2009; Bauer *et al*, 2013b). Water, H_2S, siloxanes and halogens must be removed prior to CO_2 removal to avoid operational problems such as pipe or heat exchanger clogging (Bauer *et al*, 2013b). On the other hand, no reliable

data for investment and operating costs of cryogenic upgrading plants is available, with the only estimation reported by Hullu *et al* (2008) to 0.4 € Nm^{-3}. There is also a large uncertainty on the estimations of the energy needs for this process, with values ranging from 0.42 to 1 kWh/Nm^{-3} (Benjaminsson, 2006; Bauer *et al*, 2013b).

2.3. Biological CO_2 removal technologies

CO_2 mass transfer from the biogas to a microbial or enzymatic broth followed by a CO_2 biological reduction constitutes the basis of most biotechnologies currently under research. Of them, H_2-assisted CO_2 bioconversion, microalgae-based CO_2 fixation, enzymatic CO_2 dissolution, fermentative CO_2 reduction and in-situ CO_2 desorption are discussed below:

2.3.1. Chemoautotrophic biogas upgrading

The chemoautotrophic microbial conversion of CO_2 to CH_4 is based on the action of hydrogenotrophic methanogens capable of using CO_2 as their carbon source and electron acceptor, and H_2 as electron donor in the energy-yielding reaction described by equation 2.1 (Strevett *et al*, 1995):

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \ (\Delta G_0 = -131 \text{ KJ}) \quad (2.1)$$

The bioconversion of CO_2 to CH_4 using an external H_2 injection has been used both in the upgrading of biogas to biomethane and in the reduction of CO_2 emissions from the electronic industry using the on-site hydrogen produced from the electrochemical treatment of its fluorhydric acid-containing wastewaters (Ju *et al*, 2008; Kim *et al*, 2013). Even syngas from coal or biomass gasification processes containing CO, H_2 and CO_2 can be upgraded to CH_4 based on the ability of some methanogens to convert CO

to CH_4 and CO_2 ($4CO + 2H_2O \rightarrow CH_4 + 3CO_2$). Microorganisms from the Archaeal domain such as *Methanobacterium* sp., *Methanococcus* sp., *Methanothermobacter* sp., *Methanosarcina* sp., *Methanosaeta* sp., *Methanospirillum* sp. and *Methanoculleus* sp. have been consistently found in stand-alone bioreactors or anaerobic digesters upgrading CO_2 to CH_4 via H_2 injection (Strevett *et al*, 1995; Luo *et al*, 2012b; Kim *et al*, 2013; Luo and Angelidaki, 2013; Wang *et al*, 2013). These autotrophic methanogens often exhibit an optimum pH interval of 6.5-8 under both mesophilic and thermophilic conditions, and can even remove part of the H_2S present in the biogas by assimilation into biomass. However, while thermophilic methanogens (55-88 ºC) exhibit higher bioconversion rates than their mesophilic counterparts (30-40 ºC), the latter can achieve a more complete conversion of CO_2 (Strevett *et al*, 1995). In addition, thermophilic methanogens often present lower growth yields (commonly defined as grams of biomass per mole of CH_4 formed), which ideally should be lower than 1 to promote the conversion of CO_2 to CH_4 rather than the formation of biomass. In this context, chemical compounds such as cyanide or alkylhalides have been shown to uncouple archaeal anabolism and catabolism, thus maximizing biomethane production (Strevett *et al*, 1995).

Most CO_2 bioconversion studies using H_2 as electron donor have been carried out at lab scale (0.05-100L) under mesophilic or thermophilic conditions in stirred tank, bubble column, packed bed or membrane bioreactors with synthetic mixtures of CO_2 and H_2 supplied at stoichiometric ratios (1:4) (Table 2-3) (Kim *et al*, 2013). The extremely poor aqueous solubility of H_2 (dimensionless gas-water Henry's law constant of 52) always limited the gas-water H_2 mass transfer rates and therefore the bioconversion of CO_2 to CH_4, which is known to occur in the aqueous phase containing the methanogenic community. In this regard, process operation under H_2 mass transfer limitation is known

to decrease the efficiency of CH_4 production at the expenses of an enhanced biomass formation (Strevett *et al*, 1995). This resulted in the need to operate the process at extremely high gas residence times (1-208 h) in order to achieve CH_4 concentrations in the upgraded biogas over 90 %, but entailed low volumetric CH_4 productivities ranging from 0.65 to 5.3 L CH_4/L_r d (Table 2-3). The few bioreactors reporting volumetric CH_4 production capacities sufficiently high to support a cost-efficient CO_2 bioconversion (54-470 L CH_4/L_r d) were operated during short periods of time at low gas residence times (0.02-0.13 h) but yielded CH_4 concentrations (30-50%) not suitable for injection in natural gas grids or direct use as autogas. In this context, the implementation of this bioconversion in high-mass-transfer gas phase bioreactors such as two-phase partitioning or Taylor Flow bioreactors could support an increase in the volumetric CH_4 productivities of up to 1 order of magnitude, as reported during the treatment of volatile organic contaminants (Kreutzer *et al*, 2005).

Table 2-3. Experimental studies on the chemoautotrophic CO_2 conversion to CH_4

Bioreactor configuration	**CO_2:H_2** (mol mol^{-1})	**Gas Residence Time** (h)	**Maximum CH_4 production**	**CH_4** (%)	**Reference**
Mesophilic sewage sludge STR digester (2 L) stirred at 200 rpm supplied with in-situ coke gas addition (92 %H_2/8% CO) via bubbleless membranes	0.11-0.24	13-22	1.45 L CH_4 gVS^{-1} d^{-1} 0.65 L CH_4 L_r^{-1} d^{-1}	90-99	Wang *et al* (2013)
Mesophilic biotrickling filter (27 L) with random packing and internal gas recycling supplied with synthetic CO_2:H_2 mixtures. Batchwise operation	0.25	2-10	1.17 NL CH_4 L_r^{-1} d^{-1}	94-98	Burkhardt and Busch (2013)
Mesophilic STR (100L) stirred at 70 rpm with sparging of residual H_2 and CO_2 gases	0.125-0.5 (0.2)*	42-208	4.1 L CH_4 L_r^{-1} d^{-1}	92	Kim *et al* (2013)
Thermophilic manure-whey STR digester (0.6 L) stirred at 150-300 rpm with in-situ H_2 supply via ceramic and column diffusers.	0.25	14	0.88 L CH_4 L_r^{-1} d^{-1}	75	Luo and Angelidaki (2013)
Thermophilic STR (0.6L) stirred 500-800 rpm with sparging of synthetic mixture of H_2:CH_4:CO_2 (60:25:15)	0.25	1-8	5.3 L CH_4 L_r^{-1} d^{-1}	90-95	Luo and Angelidaki (2012a)
Mesophilic STR (0.5 L) supplied with synthetic CO_2:H_2 mixtures	0.25	1	0.24 L CH_4 gVS^{-1} d^{-1} 2.4 L CH_4 L_r^{-1} d^{-1}	-	Ako *et al* (2008)

Mesophilic packed bed filter (7.8L) supplied with synthetic CO_2:H_2 mixtures	0.125-0.5 (0.2)*	3.8-6.5	1.34 L CH_4 L_r^{-1} d^{-1}	100	Lee *et al* (2012)
Mesophilic Hollow Fiber biofilm membrane bioreactor (0.195 L) supplied with synthetic CO_2:H_2 mixtures	0.25	1.2	4.6 L CH_4 L_r^{-1} d^{-1}	80-90	Ju *et al* (2008)
Thermophilic STR (2L) with sparging via membrane diffusion of synthetic biogas mixtures and H_2	0.27	0.13	-	96	Strevett *et al* (1995)
Thermophilic column packed bed reactor (0.2L) sparged with synthetic CO_2:H_2 mixtures	0.25	-	54 L CH_4 L_r^{-1} d^{-1}	-	Bugante *et al* (1989)
Thermophilic packed bed column (0.105 L) supplied downwards with a synthetic CO_2:H_2 mixture	0.25	0.033	105 L CH_4 L_r^{-1} d^{-1}	40-50	Jee *et al* (1988)
Thermophilic STR (1.5L) stirred at 320-1015 rpm supplied via sparging with a synthetic CO_2:H_2 mixture (batch and continuous)	0.25	0.012	76 L CH_4 L_r^{-1} d^{-1} (continuous) 470 L CH_4 L_r^{-1} d^{-1} (batch)	50%	Peillex *et al* (1988)
Thermophilic packed bed column (0.05 L) supplied downwards with a synthetic CO_2:H_2 mixture	0.25	0.02	144 L CH_4 L_r^{-1} d^{-1}	30	Jee *et al* (1987)

*- Optimum value

On the other hand, the studies evaluating the performance of the direct H_2 injection in the anaerobic digester are scarce (Luo *et al*, 2012b; Luo and Angelidaki, 2013). This process configuration can avoid the use of an additional external bioreactor for biogas upgrading (estimated to require 1/10 of the digester volume), and made the anaerobic digestion of cattle manure and acidic whey more robust towards sudden increases in organic loading rates, unexpectedly preventing the accumulation of Volatile Fatty Acids (VFA) likely due to its associated pH increase (Luo and Angelidaki, 2013). Indeed, the addition of H_2 into the above described digester did not decrease the activity of the acetate kinase, a key enzyme in the bioconversion of VFA to acetate, and increased the activity of the coenzyme F420 (involved in hydrogenotrophic and acetoclastic methanogenesis). Likewise, the injection of H_2 into the digester also resulted in a significantly higher microbial activity, as shown by the twice higher specific ATP content of the H_2 supplemented biomass compared to the mixed liquor of a similar digester deprived of H_2 (Luo and Angelidaki, 2013). The main limitation of this process configuration arises from the fact that anaerobic digesters are not designed to maximize the gas-liquid mass transfer (excessive mixing might damage the structure and functionality of anaerobic flocs), which might limit the performance of this in-situ approach of CO_2 bioconversion at large scale. Even small scale (0.6 L) stirred tank digesters provided with fine bubble diffusers only achieved a biomethane composition of 75%/6.6%/18.4% $CH_4/CO_2/H_2$. In addition, the consumption of CO_2 in the digester can mediate inhibitory pH increases if the alkalinity of the organic fed is not properly controlled, as reported by Luo *et al* (2012b) during the anaerobic digestion of cattle manure.

The use of H_2 to upgrade biogas entails a significant loss in energy efficiency and requires the enforcement of severe safety operating procedures in anaerobic digestion

plants as a result of the high flammability of hydrogen. However, the use of CH_4 as a fuel gas benefits from both the exiting gas distribution infrastructure and well established combustion technology, which represents the main reason to promote the production of CH_4 over H_2 (Wang *et al*, 2013). Water electrolysis from renewable energy sources (e.g. wind and solar power) represents nowadays the only environmentally friendly (large-scale) method to obtain H_2 for bioconversion of CO_2 to CH_4. In this context, it must be highlighted that the low density of H_2 often requires high storage volumes, while the technology for H_2 transportation and direct utilization is still under development. Therefore, H_2 transformation to biomethane, which can be injected into natural gas grids or employed as autogas, constitutes a very attractive alternative to chemically store an energy that would be otherwise lost. Finally, for chemoautotrophic biogas upgrading to be a sustainable and low cost technology, H_2 must be produced from water electrolysis using excess of electricity (typically during the night) or as a byproduct in a nearby facility (Kim *et al*, 2013).

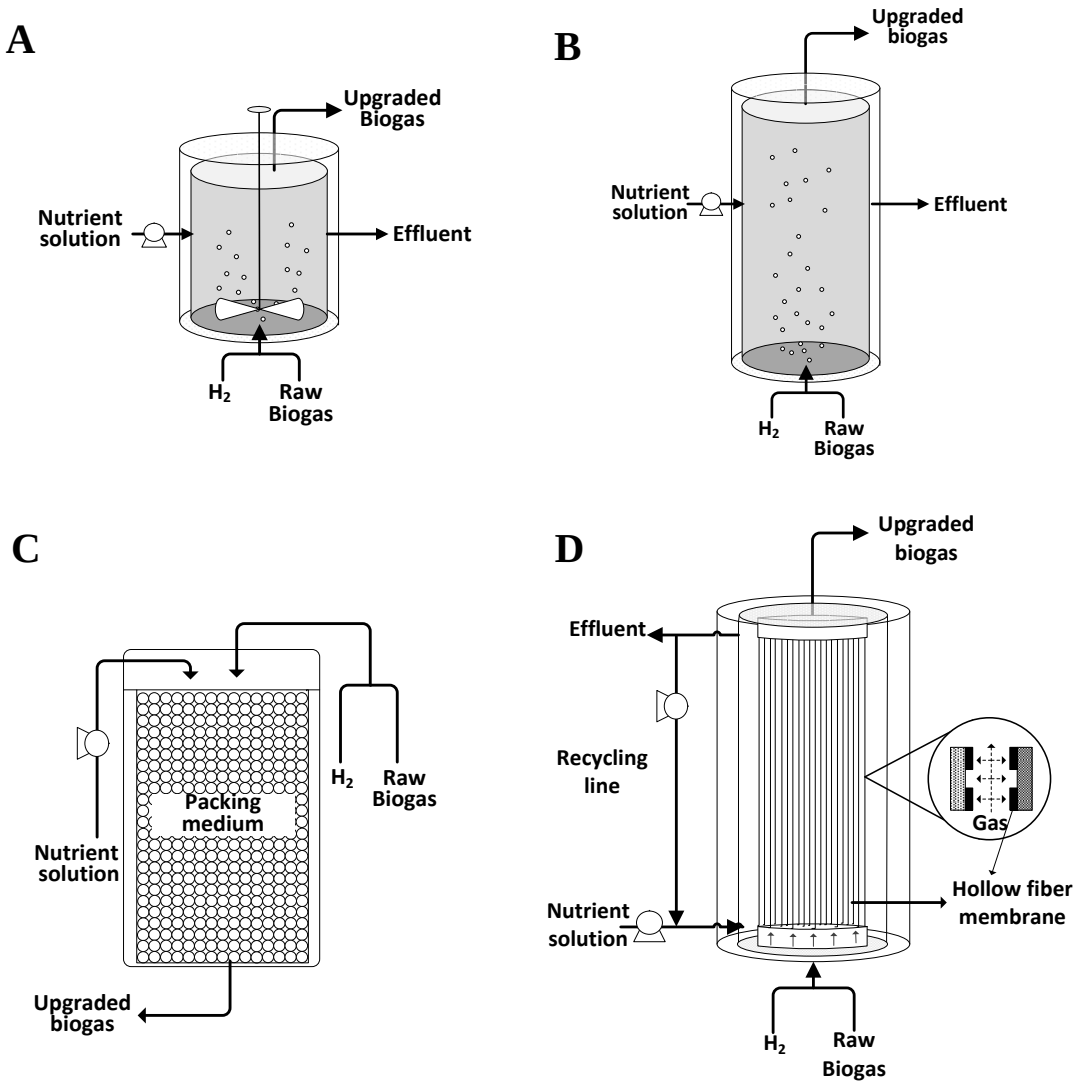

Figure 2-5. Reactor configurations for chemoautotrophic biogas upgrading. A) stirred tank; B) bubble column; C) packed bed, adapted from Jee *et al* (1988); D) membrane bioreactor, adapted from *Ju et al* (2008).

2.3.2. Photosynthetic biogas upgrading

Photosynthetic biogas upgrading relies on the ability of eukaryotic microalgae and prokaryotic cyanobacteria (commonly referred to as microalgae) to bioconvert the CO_2 present in the biogas into microalgae biomass using the electrons released during water photolysis (López *et al*, 2013). This redox CO_2 reduction process, namely oxygenic photosynthesis, can be represented by the overall equation 2.2:

$$CO_2 + H_2O + photons + nutrients \rightarrow O_2 + CH_{1.63}N_{0.14}O_{0.43}P_{0.006}S_{0.005} + waste\ heat \quad (2.2)$$

Such process requires the initial transport of the CO_2 from the biogas to a microalgae-containing aqueous phase. Likewise, approximately 1.8 g CO_2 are required per gram of microalgae produced. The low affinity for CO_2 of the enzyme RubisCO in microalgae ($K_M \approx$ 1-8 mg CO_2 L^{-1}) does not entail however any technical limitation during photosynthetic biogas upgrading as a result of both the relatively high levels of CO_2 allowed in most European biomethane legislations (3-6 %) and the presence of inorganic carbon-concentrating mechanisms in most microalgae (Raven *et al*, 2008). Despite any microalgae could eventually support photosynthetic biogas upgrading, *Chlorella, Arthrospira* and *Spirulina* species have been preferentially used in the lab and pilot scale studies conducted up-to-date, based on their tolerance to high CO_2 and pH levels (Table 2-4). In this context, while CO_2 gas concentrations of 5 % were traditionally considered inhibitory for microalgae growth, the intense research efforts conducted over the past 10 years in the field of CO_2-biomitigation from flue gases have resulted in the isolation of species tolerant to CO_2 concentrations of up to 60 % (Miyairi, 1995; Wang *et al*, 2008). The presence of H_2S in the biogas can inhibit microalgae growth, with H_2S concentrations over 100 ppm_v exhibiting inhibitory effects on *Chlorella* sp. growth (Kao *et al*, 2012). However, the synergistic occurrence of H_2S oxidizing bacteria and the chemical oxidation of H_2S in biogas upgrading photobioreactors (operating under non-sterile conditions at high dissolved oxygen concentrations) rapidly oxidizes this toxic sulfur compound into sulfate, which eventually prevents any H_2S-mediated microalgae inhibition in real applications (Bahr *et al*, 2014). On the other hand, methane does not exert any significant inhibitory effect

on microalgae growth in the concentration range of 20-80%, likely due to its low aqueous solubility and reactivity (Kao *et al*, 2012).

Provided a sufficient CO_2 mass transport from the biogas to the microalgal cultivation broth, the rate of CO_2 fixation, which itself determines the maximum biogas loading rate to be applied to the upgrading unit, is governed by environmental factors such as light availability, temperature, pH and dissolved O_2 concentration in the cultivation medium. Thus, the photosynthetic CO_2 fixation rate linearly increases when increasing light intensity up to a critical species-dependent saturation radiation (200-400 $\mu E\ m^{-2}\ s^{-1}$), remaining constant afterwards up to a critical photoinhibition value and deteriorating subsequently as a result of the damage in the microalgal photosystem II at high light intensities (Tredici, 2009). At this point it should be highlighted that light availability does not depend exclusively on the impinging light irradiation at the microalgae cultivation surface, but also on the biomass density and photobioreactor configuration (Muñoz and Guieysse, 2006). Most microalgae exhibit an optimum growth temperature in the range of 15 to 25ºC, although some species such as *Chlorella* can grow optimally at 30-35ºC, which are temperatures typically encountered in outdoor environments. On the other hand, while most microalgae present an optimum activity at pH 7-8, process operation at pH of 9-10 (optimal for cyanobacterial species such as *Spirulina platensis*) is desirable to maximize CO_2 mass transport from the biogas due to the acidic nature of this gas (Bahr *et al*, 2014; De Godos *et al*, 2014). Finally, high dissolved oxygen concentrations in the cultivation broth can mediate a competitive inhibition in the enzyme RubisCO (which also exhibits oxygenase activity) and oxidative damage in the photosynthetic apparatus of microalgae due to the formation of oxygen radicals.

The physical and biological mechanisms underlying CO_2 removal from biogas in photobioreactors are similar to those governing CO_2 capture from exhaust flue gases (Yan and Zheng, 2013; De Godos *et al*, 2014). Both processes have been implemented in open and closed photobioreactors (Table 2-4), which are designed to maximize light distribution, pH control, CO_2 supply and O_2 evacuation (Morweiser *et al*, 2010). Raceways, which constitute the most common configuration of open photobioreactors, are characterized by a simple construction and operation, and lower capital (2-20 € m^{-2}) and energy requirements (2-10 W m^{-3}) than their closed counterparts (Tredici, 2009; Craggs *et al*, 2012). However, raceways entail a poor light utilization efficiency (≈ 2 %), a high water footprint by evaporation (≈ 6 L m^{-2} d^{-1}) and large land requirements (López *et al*, 2013; De Godos *et al*, 2014). The higher photosynthetic efficiency of enclosed photobioreactors (4-6%), supported by their higher illuminated surface-volume ratio and turbulence, results in microalgae productivities of 0.4-1 g L^{-1} d^{-1}, but at the expenses of significantly higher energy consumptions (50-100 W m^{-3}) and investment costs (500-3000 € m^{-2}) (Acién *et al*, 2012). The number of studies evaluating the potential of microalgae-based biogas upgrading in photobioreactors is scarce, most of them being conducted indoors under artificial illumination and ambient temperatures (20-30 ºC) (Table 2-4). Bubble column and horizontal tubular photobioreactors, and raceways constructed with additional biogas scrubbing units rank among the preferred photobioreactor configurations evaluated. Most experimental units were capable of removing CO_2 with efficiencies higher than 80 %, providing a biomethane with CH_4 concentrations of ≈ 90% (Table 2-4). The gas residence times in the absorption units ranged from 0.03-0.3 h in outdoors photobioreactors to 0.7-96 h in indoor set-ups, which suggests that photosynthetic activity rather than CO_2 mass transfer limits the biogas upgrading capacity of photobioreactors. In this context, high biogas residence

times in the absorption unit or a direct scrubbing in the photobioreactor entails high O_2 concentrations in the upgraded biomethane (5-25 %). This constitutes one of the main limitations to be overcome in this novel biotechnology, due to its associated explosion hazards and to the fact that most biomethane regulations require O_2 levels below 0.5 % (Mandeno *et al*, 2005). In this context, the use of a 2-stage process based on biogas scrubbing in an external column interconnected to the photobioreactor via a variable microalgae broth recycling has been shown to support a satisfactory biogas upgrading with O_2 concentrations below 1 % (Bahr *et al*, 2014) (Figure 2-6). Nitrogen gas stripping from the cultivation broth, which results in N_2 concentration of 6-9% in the upgraded biomethane, has been also identified as a technical limitation to be overcome. Thus, the removal of N_2 from biomethane would be required in order to comply with biomethane regulations of some European countries such as Sweden, Spain or Austria that require CH_4 contents over 95 % (Persson *et al*, 2006; Huguen and Le Saux, 2010; Serejo *et al*, 2015). Finally, the CH_4 losses derived from the mass transfer of CH_4 from biogas to the recycling microalgal cultivation broth and its subsequent oxidation by the methanotrophs present in this aqueous medium were recently estimated to be <1% as a result of the low aqueous solubility of methane (Serejo *et al*, 2015).

Table 2-4. Experimental studies on biogas upgrading and CO_2 removal from flue gas in microalgal photobioreactors

Photobioreactor and absorption unit configuration	**Gas Residence Time* (h)**	**CO_2-RE (%)**	**Microalgae productivity ($g\ l^{-1}\ d^{-1}$)**	**O_2 (%)**	**N_2 (%)**	**CH_4 (%)**	**Reference**
Indoor 180 L raceway inoculated with a microalgae consortium and interconnected to a 2.5 L bubble column (1.65 m height) via algal-broth recirculation at a liquid to biogas ratio of 1:10. Synthetic Biogas (30%/69.5%/0.5% $CO_2/CH_4/H_2S$) supplied via porous diffuser.	1.4	82±2	0.079	1	6	88	Serejo *et al* (2015)
Indoor 180 L raceway inoculated with *Spirulina platensis* and interconnected to a 0.8 L bubble column (0.6 m height) via algal-broth recirculation at a liquid to biogas ratio of 1:1. Simulated biogas (30%/69.5%/0.5% $CO_2/N_2/H_2S$) supplied via porous diffuser.	0.7	86±5	-	0.2	-	-	Bahr *et al* (2014)
Indoor 1 L column photobioreactor stirred at 100 rpm supplied with real biogas (CH_4 70-72%, CO_2 17-19%) and inoculated with *Arthrospira platensis.*	96	100	0.041	10-24	-	-	Converti *et al* (2009)
Indoor 0.45 L enclosed tubular photobioreactor supplied with biogas (41%/57.5%/0.05% $CO_2/CH_4/H_2S$) inoculated with *Chlorella vulgaris*.	-	98	-	18-23	-	50-53	Mann *et al* (2009)
Indoor 15 L algal ponds inoculated with *Chlorella vulgaris* using a biolift absorption unit inside the pond	-	74-95	-	-	-	88-97	Conde *et al* (1993)

and supplied with real biogas (CH_4 55-71%, CO_2 44-48%, H_2S 1 %).							
Outdoor pilot raceway supplied with simulated biogas (40%/60% CO_2/N_2) using a countercurrent absorption sump (1 m deep) using a mixed microalgae population	-	>85	-	5.2-6	-	-	Mandeno *et al*. (2005)
Indoor 0.4-6 L bubble column photobioreactor inoculated with *Chlorella vulgaris* supplied with real biogas (CH_4-38-80%, CO_2-19-62%, H_2S-0.2 %).	0.16	-	2.6-3.8	3.5 <	-	-	Douskova *et al* (2010)
Outdoors 50 L bubble column photobioreactor (3 m height) inoculated with a mutant *Chlorella* strain supplied with biogas (20%/69%/0.005% $CO_2/CH_4/H_2S$) using intermittent biogas/air cycles (30 min/30 min)	0.06-0.3	74-85	0.3-0.32	-	-	86-91	Kao *et al* (2012)
Outdoor 100 m^2 raceway constructed with a 0.65 m^3 absorption sump (1 m deep) operated at a liquid recirculation rate of 0.22 m s^{-1} supplemented with flue gas (10.6 % CO_2) via membrane diffuser	0.2	96	0.088	>15	-	-	De godos *et al* (2014)
Outdoor 420 L raceway interconnected to a 1.4 L bubble column (3.1 m height) via water recycling from the HRAP. Abiotic experiment at pH 9-10	0.025	82-83	-	-	-	-	(Putt *et al*, 2011)Putt *et al* (2011)
Indoor 75 L open photobioreactor inoculated with *Nannochloropsis gaditana* and interconnected to a 0.7 L bubble column (2.2 m height) by continuous recirculation of microalgae culture at a liquid to biogas ratio of 1.8:1. Real biogas (72±2% CH_4; 28±2% CO_2) was supplied.	0.2	93	0.03	1.2	-	-	Meier *et al* (2015)

*Gas Residence Time estimated based on the volume of the absorption unit.

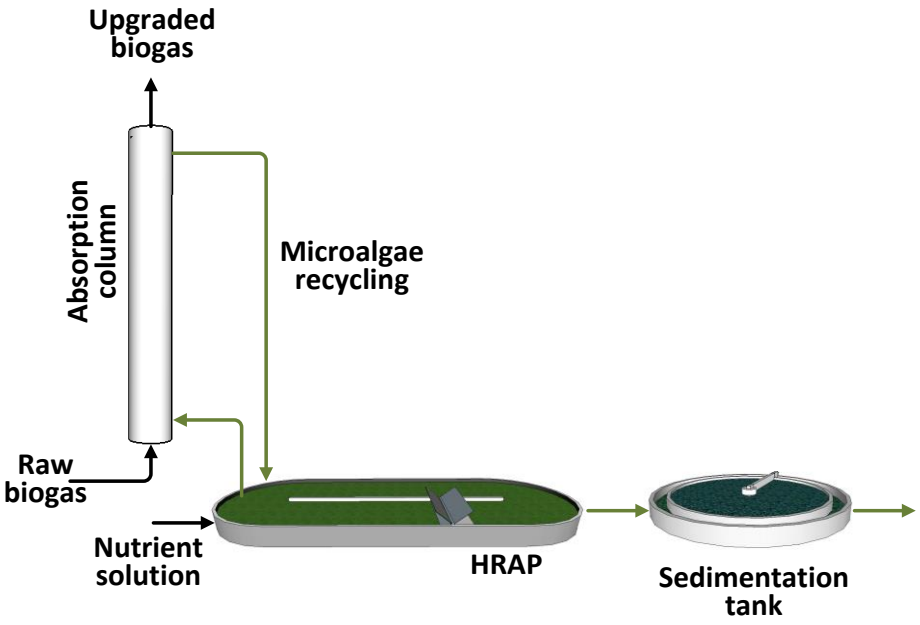

Figure 2-6. Biogas upgrading using microalgae cultures. Adapted from Bahr *et al* (2014).

Unlike most physical/chemical CO_2 absorption technologies, where CO_2 is separated from the biogas and discharged to the atmosphere, photosynthetic biogas upgrading allows the valorization of this CO_2 in the form of a valuable algal biomass. This microalgal biomass could be used as a feedstock for the production of biofuels (biogas, bioethanol or biodiesel) or high-added-value products (Alcántara *et al*, 2013). In this context, health-promoting molecules from *Chlorella* sp., β-carotenes from *Dunaliella salina*, pharmaceuticals, cosmetics and phycobiliproteins from *Spirulina platensis* or eicosapentaenoic acid from *Nannochloropsi*s sp. are already commercially available (Spolaore *et al*, 2006; Raja *et al*, 2008). An additional advantage of photosynthetic biogas upgrading is the possibility of simultaneously removing the H_2S present in the biogas based on its much higher solubility and rapid bacterial oxidation kinetics at the typically high dissolved oxygen concentrations present in photobioreactors (Bahr *et al*, 2014). Finally, the fact that residual nutrients from the anaerobic digester can support

microalgae growth brings an added environmental benefit to the process in term of biomitigation of the eutrophication potential of anaerobic digestion.

2.3.3. Other biological CO_2 removal methods

Fundamental studies on the use of the immobilized enzyme carbonic anhydrase resulted in a 99% pure biomethane (Mattiasson, 2005). This enzyme catalyses the reaction of CO_2 dissolution to bicarbonate in the blood and the reverse bioreaction of bicarbonate to CO_2 in the lungs (equation 2.3):

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^- \quad (2.3)$$

This technology was recently patented by CO_2 Solution Inc (CO2 solutions, 2014) and marketed for the removal of CO_2 from flue gases. However, the high production costs and low lifetime of the enzyme can limit the economic viability of this innovative biotechnology (Petersson and Wellinger, 2009).

The CO_2 reduction needed for biological biogas upgrading can be also accomplished by using the CO_2 present in the biogas as a carbon source during the anaerobic fermentation of sugars to succinic acid (Gunnarsson *et al*, 2014). Bacterial species such as *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, *Anaerobiospirillum succiniciproducens*, *Corynebacterium glutamicum* and some recombinant *Escherichia coli* can use glucose, xylose, arabinose, galactose, maltose, fructose, sucrose, lactose, mannitol, arabitol, sorbitol, or glycerol to produce succinic acid, which requires the fixation of 1 mol of CO_2 per mol of succinic acid produced. In a recent investigation, Gunnarson *et al* (2014) achieved an upgrading of biogas from 60% CH_4 to 95.4 % in a

pressurized (1.4 bar) lab-scale stirred tank reactor inoculated with *Actinobacillus succinogenes* using glucose as a carbon and energy source.

2.3.4. CO_2 removal by in-situ desorption

Biogas upgrading by *in-situ* desorption of CO_2 is based on the higher aqueous solubility of CO_2 compared with CH_4. This technology has been implemented on a novel anaerobic digester configuration (Figure 2-7) consisting of an external desorption unit, interconnected with the anaerobic digester. The anaerobic mixed liquor is continuously recycled to an aerated desorption unit, operated in countercurrent mode. The dissolved CH_4, H_2S and CO_2 are easily stripped out from the recycling sludge, which results in an overall decrease in the H_2S and CO_2 content in the biogas. However, the methane yield is lower as a result of CH_4 losses (Lindberg and Rasmuson, 2006; Nordberg *et al*, 2012). The higher content of CO_2 in the mixed anaerobic liquor (mainly present as bicarbonate) compared to that of CH_4 support the quasi-selective separation of CO_2 in the desorption unit. Lindberg and Rasmuson (2006) identified the air flow rate in the desorption unit as a key operational variable during the evaluation of the performance of this innovative biogas upgrading configuration, using a bubble column as external desorption unit. The higher the air flow rate, the lower the CO_2 and H_2S content in the upgraded biogas but the higher the CH_4 losses and the redox potential of the mixed liquor, which surprisingly did not cause any negative effect on the activity of the digester. Longer (but high enough to bring CH_4 concentration to the set point) sludge residence times in the desorption unit are recommended to maximize CO_2 removal from biogas while minimizing methane losses and the N_2 content in the biogas. Maximum CH_4 concentrations of 87 % with associated CH_4 losses of 8 % and biogas N_2 concentrations of 2 % (the main biogas pollutant being CO_2) were obtained by Nordberg *et al* (2012) in a pilot scale (15-19 m^3) digesters interconnected to 90-140 L

desorption units. Likewise, an external hollow fiber membrane (where degassing was driven by vacuum) was interconnected to a lab scale UASB reactor via mixed liquor recycling in a recent study by Luo and co-workers (2014), which resulted in a biomethane with CH_4 concentrations of ≈94 % and no disturbance on the COD removal or biogas yield.

Finally, it should be stressed that the fact that most biological CO_2 removal technologies are still in a lab or pilot scale limited the availability of both investment and operating cost data for the technologies discussed in section 2.2.

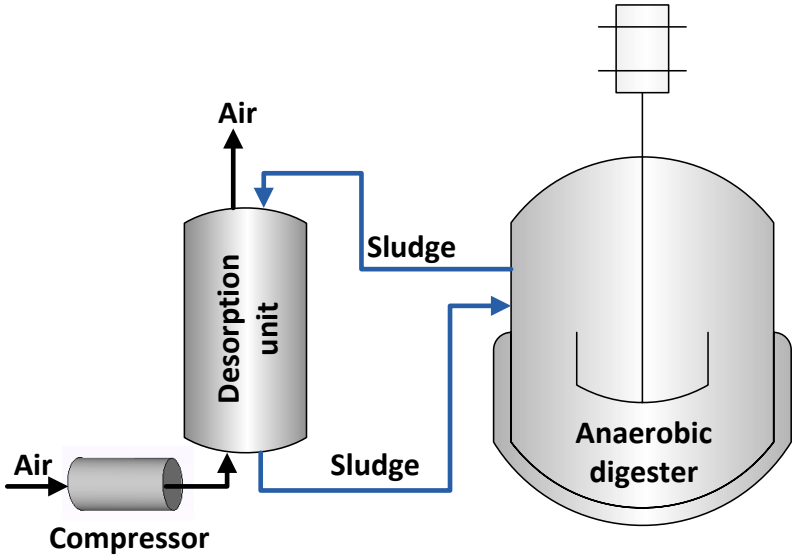

Figure 2-7. CO_2 removal by *in-situ* desorption in the anaerobic digester.

2.4. Conclusions

Physical/chemical technologies for biogas upgrading based on absorption, adsorption, chemical reaction, membrane separation or cryogenic separation are nowadays mature technologies capable of providing a biomethane suitable for injection into natural gas grids or use as autogas, with a limited room for technical and economic optimization (with the exception of membrane or cryogenic separation). However, their high energy and chemical requirements impose a severe limitation to the exploitation of the full

potential of biogas as a renewable energy source. In this context, biotechnologies such as algal-bacterial photobioreactors can provide a simultaneous CO_2 and H_2S removal in a single process, while bioconverting CO_2 into a valuable feedstock for the production of bioenergy or high added value products. The conversion of the electricity grid excess during the night into H_2, and its use as electron donor in chemolitotroph-based bioreactors can bioconvert the CO_2 from biogas into CH_4. Both technologies have been so far evaluated at lab and pilot scale, industrial scale testing and optimization being still necessary to show their full potential for biogas upgrading. Mass transfer limitations of CO_2 and H_2 have been identified as the main bottlenecks of algal-bacterial photobioreactor and chemolitotrophs-based bioreactors, respectively. Based on their high biogas pollutant removal efficiencies and robustness, research on innovative biogas-microbial community mass transfer strategies and process scale-up constitute the road map to the development of cost-efficient and sustainable biotechnological process for an integral upgrading of biogas.

CHAPTER III

Photosynthetic CO_2 uptake by microalgae: An attractive tool for biogas upgrading

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Photosynthetic CO_2 uptake by microalgae: An attractive tool for biogas upgrading

Abstract

The use of photosynthetic CO_2 reduction capacity of microalgae can be used for biogas upgrading. Such process would convert CO_2 contained in the biogas into microalgal biomass, generating two products: upgraded biogas and biomass. Growth rate of *Nannochloropsis gaditana* was determined in atmospheres containing different levels of CH_4 and CO_2. Results showed no effect of CH_4 rich atmosphere over microalgal development. CO_2 inhibition was observed only when microalgae culture was exposed to atmospheres containing 9% of CO_2. Direct contact of the biogas and the microalgal culture is not a feasible way to upgrade biogas, due to oxygen desorption to the gas phase. A two-stage process, involving a photo-bioreactor connected with a gas/liquid mass transfer unit showed to be an efficient way to remove CO_2 from the biogas, keeping low levels of oxygen in the upgraded biogas.

3. Photosynthetic CO_2 uptake by microalgae: An attractive tool for biogas upgrading

3.1. Introduction

Considering the need of modern societies to fulfill their increasing energetic needs, biogas has been receiving increasing interest as a renewable and sustainable source of energy. Biogas is the product of the anaerobic biodigestion of organic matter and it has a volume fraction of 55-75% of CH_4.

Biogas can be used with minimum or moderated levels of purification for heat and electricity production. However, many applications, such as vehicle use and grid injection, require the removal of CO_2 in order to produce a gas with equivalent characteristics as that of natural gas. Removal of CO_2 increases the caloric value and decreases the relative density of the gas, increasing the Wobbe index (Ryckebosch *et al*, 2011).

Regulations of European countries require different levels of biogas purification to enable biomethane injection in natural gas networks. For example, France, Austria, Sweden, Switzerland and Germany enable CO_2 levels up to 2.5, 3, 4, 6 and 6%, respectively (Marcogaz, 2006; Huguen and Le Saux, 2010). There are several methods available for the removal of CO_2 from biogas. Most common ones are liquid absorption, pressure swing adsorption (PSA), membrane filtration and cryogenic separation (Ryckebosch *et al*, 2011). These processes require considerable amount of energy, in the range of 200-700 Wh m^{-3}, and their operation may be complex (Patterson *et al*, 2011). Upgrading costs with traditional technologies are in the range 0.1-0.4 € m^{-3} (Patterson *et al*, 2011). Moreover, even though these methods are effective in achieving

CO_2 separation from CH_4, CO_2 is merely removed, not converted, being released to the environment.

An alternative way to remove CO_2 from biogas is the use of photosynthetic microorganisms, such as microalgae. Microalgae are able to capture solar energy, have high growth rates and can be adapted to different environmental conditions (Mata *et al*, 2010). When using microalgae for biogas upgrading, CO_2 is not only removed, but also transformed into biomass. Microalgal biomass can be then used as feedstock for biofuel production, such as biodiesel, bioethanol, or even more biogas (Singh and Gu, 2010). Biogas upgrading can be also integrated with wastewater treatment, since microalgae can be used to remove nitrogen and phosphorus from wastewater streams (Wang *et al*, 2008).

A process based on CO_2 capture by microalgae requires biogas components not exerting an inhibitory or toxic effect over these microorganisms. CH_4 is the main component of biogas, and the few reports available dealing with CO_2 capture from biogas mediated by microalgae did not report signs of toxicity or inhibition (Conde *et al*, 1993; Converti *et al*, 2009). To our knowledge, the only report directly dealing with CH_4 toxicity determination is the work of Kao *et al* (2012) who reported an 18% of growth rate reduction when exposing a mutant strain of *Chlorella sp* to a biogas containing 80% of CH_4.

Additionally, a process of microalgae-based biogas upgrading may involve exposing the microorganisms to a high CO_2 partial pressure. Even though CO_2 represents the source of carbon for microbial growth, high concentrations of this substrate can cause inhibition (Silva and Pirt, 1984; Farrelly *et al*, 2013). However, several reports indicate

that different strains of microalgae can grow at atmospheres containing 20% to 50% of CO_2 (Jeong *et al*, 2003; Ota *et al*, 2009; Ge *et al*, 2011).

Microalgae perform oxygenic photosynthesis. This means that 1 mole of O_2 is released per mol of CO_2 captured. Then, when operating closed-photobioreactors with direct biogas injection in the culture, oxygen will be released into the biogas, as the CO_2 is absorbed. Indeed when Converti *et al* (2009) studied biogas upgrading with *Arthrospira platensis*, they achieved a negligible content of CO_2, but a O_2 concentration of 10-24% in the purified biogas. Similar results were reported by Mann *et al* (2009), who despite achieving good levels of CO_2 removal (97%), observed oxygen levels in the range of 18-23% when working with *Chlorella vulgaris* in a spiral photobioreactor. Oxygen content in the biogas must be minimized, since mixtures with CH_4 may be explosive (Mandeno *et al*, 2005). Moreover, most standards for biomethane use require an oxygen content in upgraded biogas lower than 1% (Rutledge, 2005; Marcogaz, 2006). Then, a microalgae-based biogas upgrading process would need to be carefully designed and controlled in order to separate O_2 desorption from CO_2 capture. An alternative for controlling the oxygen concentration in the upgraded biogas is to separate the process in two stages, as a strategy to (partially) separate removal of CO_2 from desorption of O_2. This could be accomplished coupling an open-photobioreactor to a bubbling column for gas/liquid mass transfer.

This research was focused on the study of a process for photosynthetic biogas upgrading mediated by microalgae. *Nannochloropsis gaditana* was used as model microalgae. The effect of algae exposure to different atmospheres containing CH_4 and CO_2 was determined in order to identify potential inhibitory effects that could affect biogas upgrading. Finally, *N. gaditana* capacity to capture CO_2 was evaluated in lab scale continuous photobioreactors. This microalga was selected since it is characterized by

high growth rates, production of pigments, accumulation of lipids, among other positive characteristics (Rocha *et al*, 2003; Simionato *et al*, 2011).

3.2. Materials and methods

The effect of CH_4 and CO_2 on *N. gaditana* growth was evaluated exposing the microalgae culture to atmospheres containing different compositions of these gases, in batch photobioreactors. On a second phase, CO_2 absorption was evaluated in a continuous photobioreactor providing direct contact of the gas with the culture, in a single stage process. Finally, a two stages process was evaluated, providing gas/liquid contact in an external unit for mass transfer, which was connected with the photobioreactor by a continuous circulation flow.

3.2.1. Microalgae and culture medium

The microalgae *Nannochloropsis gaditana* CCMP-527 was provided by the Applied Microbiology Unit from Universidad de Antofagasta, Chile. Microalgae was cultivated using f/2 medium (Barsanti and Gualtieri, 2006) enriched with 12.0 mol m^{-3} $NaNO_3$ and 0.6 mol m^{-3} KH_2PO_4. Medium was prepared using seawater, collected from Chilean coasts of Araucanía region.

3.2.2. Effect of CH_4 and CO_2 on microalgae growth

A series of batch photobioreactors of 500 cm^3 were set-up for this purpose. All were air tight in order to enable their operation with atmospheres of controlled composition. Fluorescence lamps were used as source of light, providing an average light intensity of 60 μmol m^{-2} s^{-1}. Temperature was maintained at 20 ± 2°C. Potential CH_4 inhibition was tested using three gas mixtures: 0, 50 and 100% CH_4, balanced with N_2. Sodium bicarbonate was used as source of carbon, at an initial concentration of 1 g L^{-1}. CO_2

inhibition was tested using six gas mixtures: 0.3, 3, 6, 9, 15 and 30 % CO_2, balanced with N_2. In all cases, gas flow rate was adjusted to 100 mL min^{-1} using a peristaltic pump. Photobioreactors were operated without pH control. All experiments were done in duplicate. The specific growth velocity with the different atmospheres was determined through microalgal biomass concentration increase in time. Biomass concentration was determined by volatiles suspended solids (VSS).

3.2.3. Operation of a continuous single stage process for CO_2 capture.

To evaluate CO_2 capture when gas is injected directly into the microalgae culture, a continuous 2.2 L photobioreactor was operated. A gas with a composition of 70% N_2 and 30% CO_2 was injected into the photobioreactor, simulating biogas. Nitrogen was used at this step, instead of methane, for safety reasons. Gas injection was controlled on-line in order to provide a pH in the range 7.5-8.0. Gas recirculation (150 mL min^{-1}) was applied to provide mixing of the culture medium in the photobioreactor, and to enhance gas-liquid mass transfer. Applied dilution rate was 0.06 d^{-1} (feed flow of medium: 0.144 L d^{-1}). Samples from photobioreactor were periodically taken to measure biomass and dissolved inorganic carbon (DIC) concentrations. Composition of gas entering and leaving the system was also determined.

3.2.4. Operation of a continuous two stages process for CO_2 capture.

Photobioreactor connected to complete mixed unit for mass transfer.

A continuous 2.2 L photobioreactor was operated coupled to a 0.13 L vessel as gas/liquid mass transfer unit (Figure 3-1). Microalgae culture was continuously circulated between the photobioreactor and the mass transfer unit. The system was operated injecting a gas mixture simulating biogas (70% N_2; 30% CO_2) in the mass

transfer unit. Gas injection was controlled on-line in order to keep the photobioreactor pH in the range 7.5-8.0. Gas recirculation was applied in the mass transfer unit, so it was assumed that its hydraulic behaviour was that of a complete mix compartment. Applied dilution rate in the photobioreactor was 0.06 d^{-1} (feed flow of medium: 0.144 L d^{-1}).

An air flow of 210 mL min^{-1} was applied in the photobioreactor to provide mixing and a gas/liquid volumetric mass transfer coefficient (K_La) of 2.5 h^{-1} for O_2 and 2.3 h^{-1} for CO_2. Latter values may be considered representative of raceway reactors, since they present K_La values between 0.2 to 8 h^{-1} when 20 to 5 cm deep (Babcock *et al*, 2002). A K_La value typical of raceways was applied, since that cultivation system is the most applied for large scale microalgae cultivation.

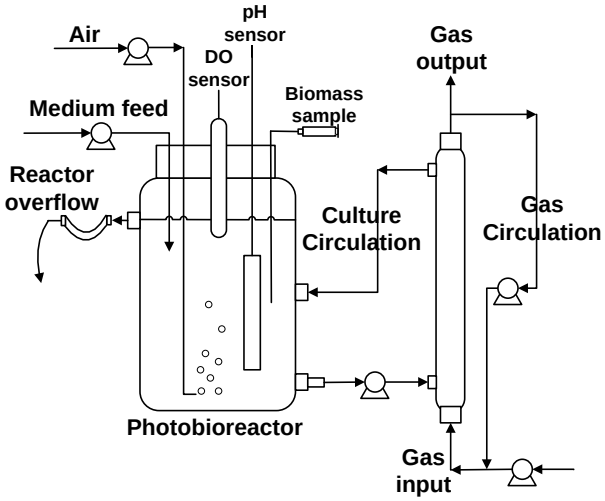

Figure 3-1. Experimental setup of the continuous photobioreactor connected with gas/liquid mass transfer unit.

Samples from the photobioreactor were periodically taken to determine biomass and dissolved inorganic carbon (DIC). Dissolved oxygen (DO) concentration was measured through an oxygen electrode placed in the photobioreactor. Gas samples were taken

from sampling ports located at the entrance and exit of the mass transfer unit for determination of gas composition.

Photobioreactor connected to bubble column operated in counterflow mode.

A continuous open-photobioreactor was operated at 25 ± 1°C. Continuous illumination was provided by means of cool white fluorescent light, at 100 ± 20 µmol m^{-2} s^{-1}. The photobioreactor consisted of a glass container of a 75 L. Its dimensions were 0.15 m depth, 0.50 m width and 1.0 m height. Applied dilution rate was 0.06 d^{-1} (feed flow of medium: 4.75 L d^{-1}). Aeration was applied into the photobioreactor for mixing. Resulting K_La for O_2 and CO_2 were 4 h^{-1} and 3.6 h^{-1}, respectively.

The photobioreactor was connected to a 0.7 L bubbling column operated in counterflow mode. Microalgae culture was continuously circulated between the photobioreactor and the column, by means of a peristaltic pump, in the similar way as when operating the system depicted in Figure 3-1, but applying a counterflow mode. Column dimensions were 2.2 m height and 0.02 m diameter. The system was operated injecting real biogas (72 ± 2% CH_4; 28 ± 2% CO_2) in the bottom of the column. Biogas was produced in a 4.5 L lab-scale UASB reactor. The anaerobic digester was operated at 30°C, and fed continuously at an organic loading rate (OLR as COD) of 5 g L^{-1} d^{-1}, using diluted wine as substrate.

Samples were periodically taken from the photobioreactor to determine biomass and DIC concentrations. Gas samples were taken from sampling ports located at the entrance and exit of the column for determination of gas composition.

3.2.5. Analytical methods

Volatiles suspended solids (VSS) and DIC concentrations were determined according to methods 2540 and 4500 of *Standard Methods* (APHA/AWWA/WEF, 1998), respectively. DO was determined by means of a portable DO meter (Thermo Orion 3-Star RDO). CO_2 and O_2 concentrations in the gas phase were determined by means of a PBI Dansensor Checkmate 9900 CO_2/O_2 Headspace. During the operation of the continuous photobioreactor connected to bubble column, gas composition was determined through a gas chromatograph with thermal conductivity detector (Perkin Elmer Clarus 500).

The gas/liquid mass transfer coefficient for O_2 (K_La_{O2}) was measured using the dynamic gassing-in method (Hulatt and Thomas, 2011), in seawater without biomass. CO_2 mass transfer coefficient (K_La_{CO2}) was calculated from values determined for O_2, through the relation $K_La_{CO_2} = 0{,}9K_La_{O_2}$ (Babcock *et al*, 2002).

3.3. Results and discussion.

3.3.1. *Effect of CH_4 and CO_2 on microalgae growth.*

Figure 3-2 presents growth curves measured with atmospheres containing different levels of CH_4 (0, 50 and 100%). Biomass growth was similar under all conditions. Growth stopped when biomass concentration reached a value close to 1 g L^{-1}, as a result of the consumption of the HCO_3^-/CO_2 present in the liquid phase. Indeed, when considering a biomass yield of 550 mg g^{-1} of CO_2 (Chisti, 2007), an initial bicarbonate concentration of 1 g L^{-1}, and initial biomass concentration of 0.4 g L^{-1}, a final biomass concentration in the range of 1 g L^{-1} can be predicted. A specific growth rate of 0.1 d^{-1} can be calculated from the data contained in Figure 3-2. It is then inferred then that an

atmosphere containing CH_4 did not produced any significant effect over the growth of *N. gaditana*.

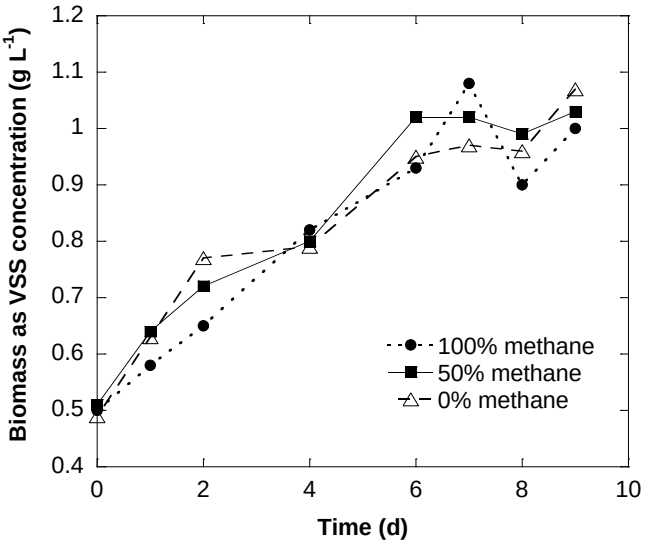

Figure 3-2. *N. gaditana* growth curves under different levels of CH_4 in the gas phase (Average values for the duplicates are presented).

Figure 3-3 presents the results of the batch cultures performed at different CO_2 levels. Results show that atmospheres containing CO_2 at 0.3, 3 and 6 %, generated a similar response. At these conditions, a specific growth rate of 0.16 d^{-1} was determined. However, when CO_2 level was 9 %, the specific growth velocity descended to 0.07 d^{-1}. When CO_2 level was 15 % or higher, clear signs of inhibition were observed.

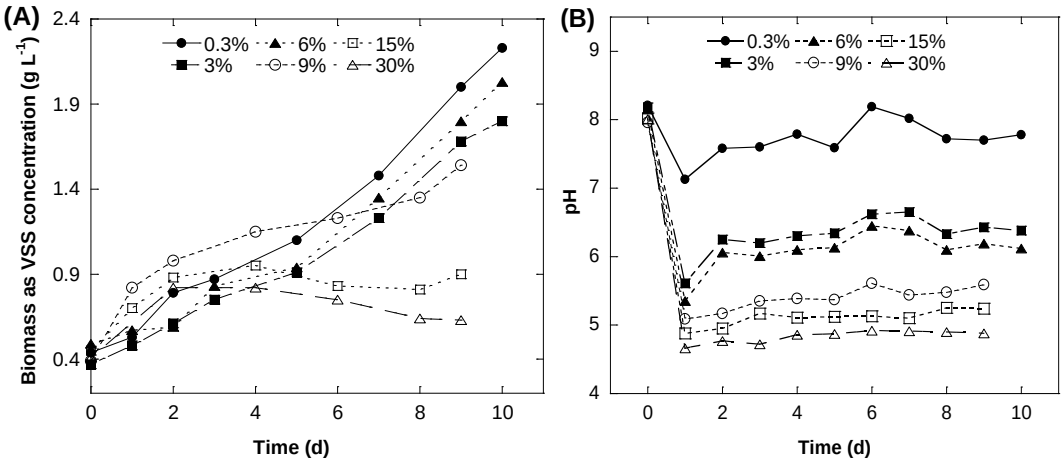

Figure 3-3. Effect of CO_2 on *N. gaditana.* A) Growth curves under different levels of CO_2 in the gas phase. B) pH microalgae culture under different levels of CO_2 in the gas phase (Average values for the duplicates are presented).

Growth inhibition may have been at high extent the result of pH, which was less than 5.0 for the highest tested CO_2 levels (see Figure 3-3B). As was commented before, pH was not controlled during this experiment, so it was determined at a high extent by the CO_2 absorption. pH values close to 8 are in general accepted as optimum for marine microalgae (Farrelly *et al*, 2013).

Different results have been reported dealing with the effect of high levels of CO_2 on the growth of the microalgae *Nannochloropsis*. In a study performed with *Nannochloropsis oculata*, the highest specific growth velocity was achieved with 2 % of CO_2. Whereas, when CO_2 level was 5 % or higher, biomass growth was completely inhibited (Chiu *et al*, 2009). However, in another study with the same microalgae, the biomass growth was only inhibited when exposed to a gas phase containing 10 % of CO_2, condition that caused a pH drop to a value around 5 (Hsueh *et al*, 2009). Other study observed inhibition of *Nannochloropsis salina* when the culture was exposed to 6.5 % of CO_2 or higher (Arudchelvam and Nirmalakhandan, 2012). Moreover, inhibition was not

observed in cultures of *Nannochloropsis* sp. exposed to 15 % of CO_2, obtaining a higher growth rate than a control culture with air bubbling (Jiang *et al*, 2011).

The results show that microalgae can be exposed to atmospheres containing important levels of CO_2, even without pH control. During the operation of a system for biogas upgrading, pH is expected to be control and composition of liquid and gas phases is expected to be far from equilibrium (as was not the case during previous experiments). Then it is expected that inhibition should not play a significant role during microalgae mediated biogas upgrading.

3.3.2. Operation of a continuous single stage process for CO_2 capture.

Figure 3-4 shows the operation of the 2.2 L photobioreactor operated with direct gas injection. Biomass concentration (VSS) was in the range 1.4-1.5 g L^{-1}. Considering the applied dilution rate, a biomass volumetric productivity of 0.1 g L^{-1} d^{-1} can be calculated. pH was controlled at 7.5 ± 0.3 through the input gas flow, which was kept in 1.0 L d^{-1}. This means the application of a volumetric gas load of 0.45 L d^{-1} per 1 L of microalgae. CO_2 concentration in the gas effluent was around 1 %. This means that 96 % of the CO_2 was removed from the gas influent. According to an inorganic carbon balance, 84 % of the CO_2 was fixed by the microalgae and 12 % was lost in the liquid effluent, dissolved as CO_2 or HCO_3^-/CO_3^{-2}, as is schematically represented in Figure 3-5. As a result, the biomass yield as measured as VSS was 529 mg g^{-1} of CO_2. This value was consistent with the theoretical yield reported by literature, which suggest a biomass yield about 500 to 667 mg g^{-1} of CO_2 (Sobczuk *et al*, 2000).

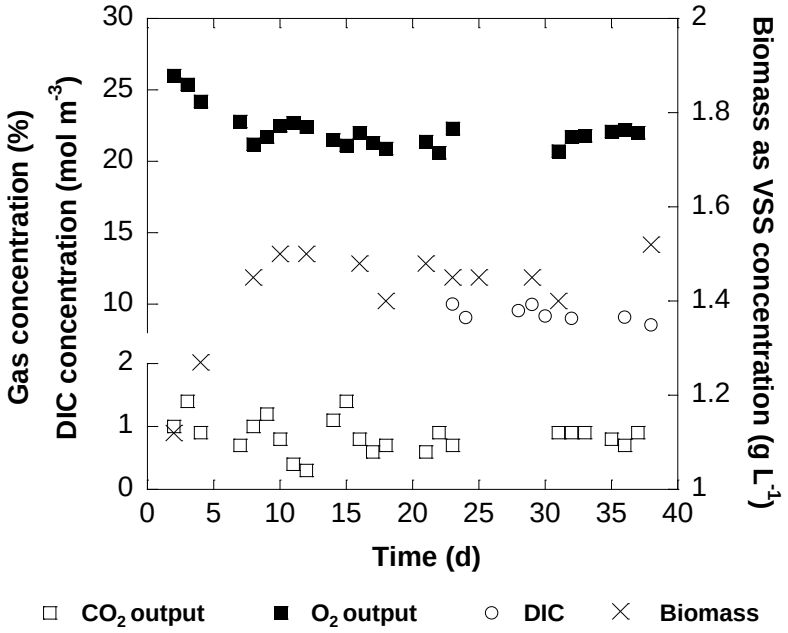

Figure 3-4. Operation of single stage continuous photo-bioreactor (Inlet gas composition: 70% N_2; 30% CO_2)

Oxygen concentration in the gas effluent was around 22 %, which was the result of the photosynthesis process. This result coincided with reports of Converti *et al* (2009) and Mann *et al* (2009), who obtained around 20 % of oxygen in a closed photobioreactor. A mass balance indicates that 0.96 mol of O_2 was produced for each mol of CO_2 consumed. Due to the low solubility of oxygen in seawater, only a negligible fraction was lost as dissolved oxygen in the liquid effluent of the photobioreactor, and most of the generated oxygen was desorbed to the gas phase.

Based on the high oxygen content observed in the gas effluent, it is concluded that a system providing direct contact of the biogas and the microalgae culture in a single stage, is not suitable for biogas upgrading, due to the formation of potentially explosive mixtures, and the obvious non compliance with regulations dealing with biomethane commercialization. It is inferred then that a successful process for biogas purification

requires the physical separation of the CO_2 absorption from the O_2 desorption, in a continuous two stages process.

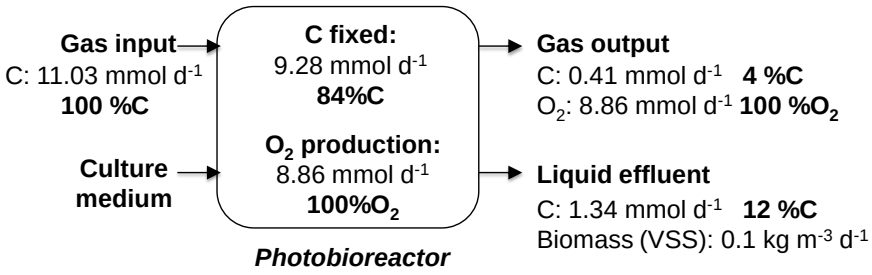

Figure 3-5. Carbon and oxygen mass balances during single stage continuous photobioreactor. (Photobioreactor volume: 2.2 L; Gas flow: 1.0 L d^{-1}, Gas composition: 70% N_2; 30% CO_2, liquid medium flow: 0.144 L d^{-1}; 28°C).

3.3.3. Operation of a continuous two stages process for CO_2 capture.

Figure 3-6 shows photobioreactor connected to a mass transfer unit (completely mixed). Reactor was started up for a period of 40 days. Then, two different levels of liquid phase circulation between the photobioreactor and the mass transfer unit were applied: 15.6 and 7.6 L d^{-1}. Biomass concentration was in the range of 1.0 to 2.0 g L^{-1}, during the operation of the system. Gas injection rate was 2.3 ± 0.4 L d^{-1}, which produced a photobioreactor pH of 7.7 ± 0.2. This means the application of a volumetric gas load of 1.0 L d^{-1} per 1 L of microalgae culture.

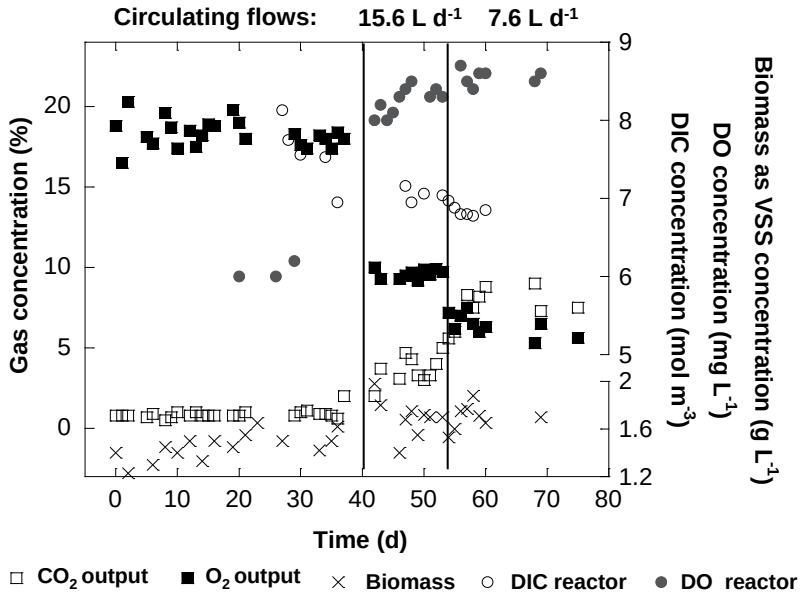

Figure 3-6. Operation 2.2 L continuous photobioreactor connected to mass transfer unit (Gas composition: 70% N_2; 30% CO_2).

Operation of the two-stage system showed to be able to promote lower levels of oxygen in the effluent gas, when compared to the single stage process. A gas effluent containing about 5 % of both O_2 and CO_2 was obtained, when applying a circulating flow of 7.6 L d^{-1} (Figure 3-6). Figure 3-7 presents a schematic representation of the mass transfer processes occurring in the two-stage system. CO_2 from the biogas can be either consumed by the microalgae, leave the system with the upgraded biogas, or be desorbed in the microalgae cultivation unit. The O_2 can either be desorbed in the cultivation unit, or be transferred to the mass transfer unit (dissolved in the liquid phase), where it can desorbed into the biogas.

Liquid flow between the photobioreactor and the column proved to be a relevant factor determining the relative magnitude of the phenomena described in Figure 3-7, determining then O_2 and CO_2 content of the gas effluent. As the circulating flow gets lower, the dissolved oxygen that is transferred to the mass transfer unit (dissolved in the

liquid) is lower, promoting a lower O_2 concentration in the upgraded biogas. However, a lower circulating flow reduces the mass transfer capacity, causing a rise in the concentration of CO_2 in upgraded gas.

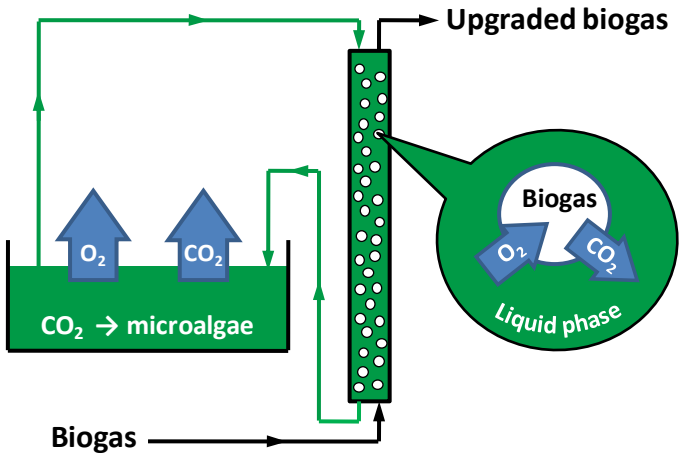

Figure 3-7. Schematic representation of mass transfer processes involved in two stage biogas upgrading process mediated by microalgae.

Mass balances showed that at a circulating flow of 15.6 L d^{-1} around 68 % of generated oxygen by photosynthesis was desorbed from the photobioreactor (see Figure 3-8), so only 32 % of oxygen left the system with the biogas. The use of a two-stage process, although was efficient in providing a lower level of oxygen, promoted inorganic carbon release to the environment by desorption in the photobioreactor. Mass balances showed that around 46 % of the CO_2 absorbed in the column was lost in this way. Such rate of CO_2 desorption from the photobioreactor is the result of the concentration of dissolved CO_2 (0.1 mol m^{-3}), which is about 10 times higher than the concentration of dissolved CO_2 in equilibrium with air. Desorption of approximately half of the produced CO_2 explains why it was possible to apply a higher volumetric gas loading rate than that applied for the single stage process.

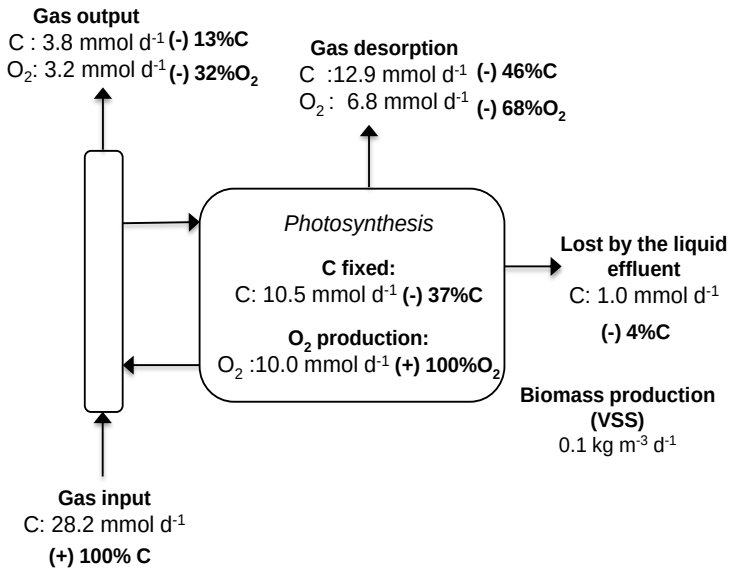

Figure 3-8. Carbon and oxygen mass balance in a continuous two stages process. The sign (+) indicates an inflow to the system and the sign (-) indicates an outflow of the system. (Gas composition: 70% N_2; 30% CO_2, Circulate flow: 15.6 L d^{-1}; Input gas flow: 2.3 L d^{-1}; 26°C).

Results presented in Figure 3-6 and Figure 3-8 were obtained working with a mass transfer unit presenting a completely mixed pattern, which is not the optimum situation from the point of view of gas-liquid mass transfer. It is then expected that the use of more efficient mass transfer equipment could improve biogas upgrading process. In order to test this hypothesis, a bigger scale photobioreactor was set up, connected to a 2.2 m bubbling column for mass transfer, as already described in materials and methods. The column was operated in counterflow mode, in order to provide a high driving force for CO_2 transfer, between biogas and microalgae. Four different liquid phase circulation flows between bubbling column and photobioreactor were studied: 14.4, 41.8, 72.0 and 115.2 L d^{-1}. Results are presented in Figure 3-9.

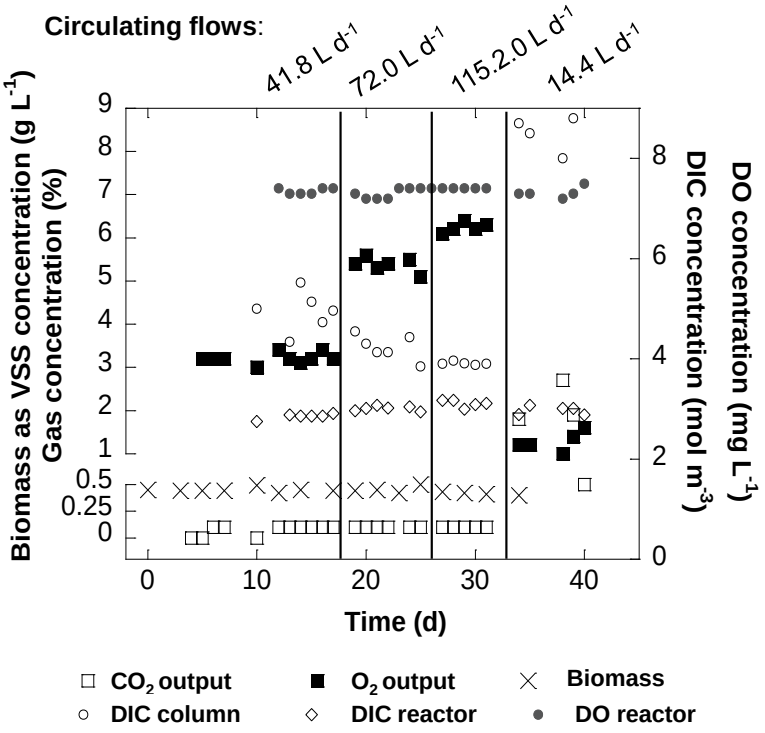

Figure 3-9. Operation continuous photobioreactor connected to bubbling column. (Biogas composition: 72 ± 2% CH_4; 28 ± 2% CO_2, DIC column refers to the condition in the liquid effluent of the column).

Biomass concentration (VSS) remained fairly constant during the operation, at 0.45 ± 0.03 g L^{-1} (Figure 3-9). This means that biomass volumetric productivity was 0.03 g L^{-1} d^{-1}. As expected, the utilization of more efficient mass transfer equipment produced an upgraded biogas with lower content of both O_2 and CO_2. Improvement of the CO_2 mass transfer, enabled the application of a lower liquid phase circulation rate reducing the amount of O_2 being transferred and desorbed in the column. At a circulating flow of 14.4 L d^{-1}, CO_2 and O_2 concentrations of 1.9 ± 0.6 % and 1.2 ± 0.1 % were achieved in upgraded biogas. At higher circulation rates, CO_2 absorption was almost complete, but O_2 concentration in upgraded biogas increased. Again, results indicate that liquid phase circulation is key operational factor determining the composition of the upgraded biogas.

Figure 3-10 shows the mass balance for the operation with a circulating flow of 14.4 L d^{-1}, the one producing the closest O_2 concentration to 1 %, requirement for most European regulations for biomethane. As shown in Figure 3-10, 81 % of the CO_2 contained in the biogas was captured by the microalgae, 13 % of carbon was lost in the liquid effluent from the photobioreactor and 6 % was not transferred and left the system in the upgraded biogas. Mass balances indicate that no desorption of CO_2 occurred in the photobioreactor. On the contrary, absorption of CO_2 from air occurred. Indeed, dissolved CO_2 in the photobioreactor (0.009 mol m^{-3}) was lower than that in equilibrium with the atmosphere. This was the result of the application of a low volumetric gas load (0.1 L d^{-1} per 1 L of microalgae), as a result of low biogas availability.

The CO_2 removal obtained during this research is in the range or higher than that reported by other authors. Conde *et al* (1993), Kao *et al* (2012) and Mandeno *et al* (Mandeno *et al*, 2005) achieved CO_2 removals of 74-95 %. 86 % and 87 %, respectively. The main difference is that in the system described in this research, such levels of CO_2 removal were achieved providing low levels of oxygen in the upgraded biogas. Indeed, Figure 3-10 shows that for a circulating flow of 14.4 L d^{-1}, 95 % of the photosynthetically generated O_2 was desorbed in the photobioreactor, and only 5 % left the system with the upgraded biogas. When analyzing the operation with circulating flows 41.8, 72.0 and 115.2 L d^{-1}, mass balances show that the oxygen desorbed in the photobioreactor was 89, 80 and 75% of the generated oxygen, respectively. An increase in oxygen desorption may be achieved by increasing gas/liquid volumetric mass transfer coefficient K_La. However, this may require increasing mixing levels in the photobioreactor, rising energy demands. It has to be reminded that applied K_La in the photobiorector was in the range of those typical for raceways. Therefore, the behavior

of the photobioreactor, in terms of gas/liquid mass transfer, can be considered equivalent of the one a raceway would present.

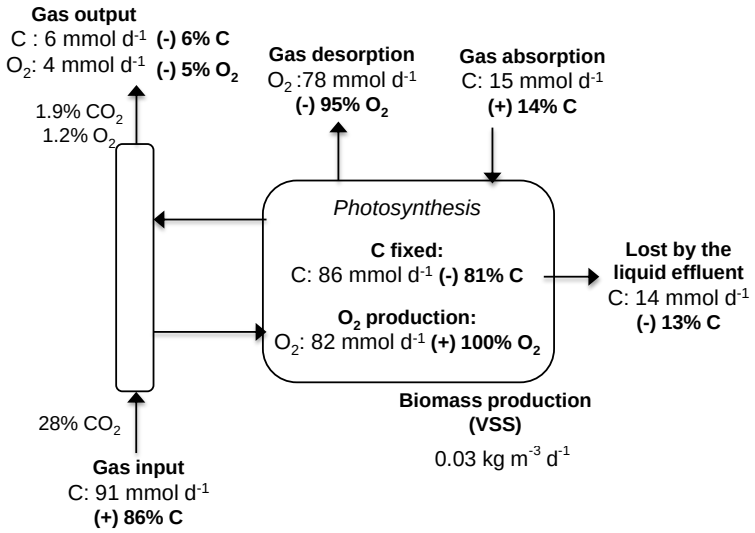

Figure 3-10. Carbon and oxygen mass balance in a continuous photobioreactor connected to bubbling column. The sign (+) indicates an inflow to the system and the sign (-) indicates an outflow of the system. (Circulate flow: 14.4 L d^{-1}; Biogas flow: 7.9 L d^{-1}; Biogas composition: 72 ± 2% CH_4; 28 ± 2% CO_2, 26°C).

Although the utilization of counterflow column improved the quality of upgraded biogas, microalgal culture was exposed to important pH changes when circulating through the column. Figure 3-11 shows the magnitude of pH difference between top and bottom of the column. Such pH changes may induce stress to the microalgae biomass. However, no evident changes in metabolic activity were observed during the 40 days of operation of the system. Supplementary research would be needed to clarify potential effect of this dynamic pH conditions.

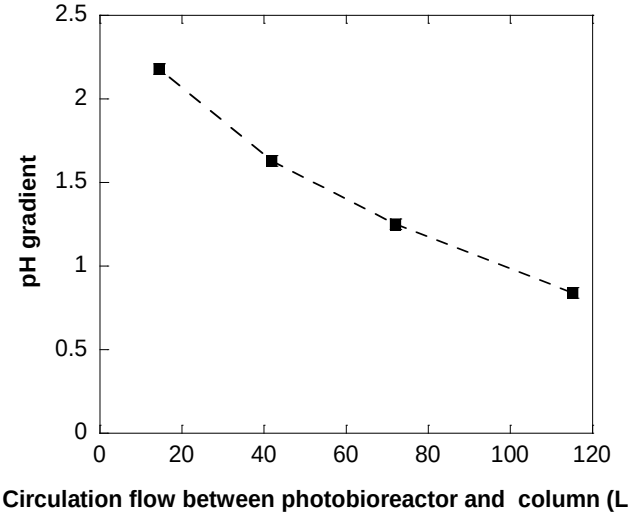

Figure 3-11. pH gradient magnitude along column when different circulate flows were applied.

A relevant potential advantage of biogas upgrading by microalgae, over the conventional methods, is the production of microalgae biomass, which can be used for the production of more biogas. For example, considering 1 m^3 of upgraded biogas (30 % CO_2), 0.15 kg of microalgae biomass may be produced as a result of CO_2 capture. Latter value was evaluated considering a biomass yield (VSS) of 529 mg g^{-1} of CO_2, 95 % of CO_2 removal and 50 % of CO_2 loss by desorption from the photobioreactor. The anaerobic conversion of such microalgae would theoretically generate 0.08 m^3 of biomethane, assuming a biomass COD of 1.5 g g^{-1} of SV, and a methane theoretical yield of 380 mL g^{-1} of COD. This means that the codigestion of the microalgae biomass, with the waste originating the biogas in first place, could increase biomethane production in 12 %.

3.4. Conclusions

- High content of CH_4 in biogas does not produce any clear inhibitory effect on *Nannochloropsis gaditana* activity.

- High CO_2 content of biogas should not produce microalgae biomass inhibition, as long as an upgraded biogas with low CO_2 level is achieved.
- Effective biogas upgrading with microalgae requires uncoupling CO_2 absorption from O_2 desorption.
- The operation of an open photobioreactor connected to external bubble column for CO_2 absorption, enables the production of an upgraded biogas with low CO_2 ad O_2 levels. Therefore, this system represents a feasible alternative for biogas upgrading.

CHAPTER IV

Effect of pH changes on microalgae-based biogas upgrading process

Effect of pH changes on microalgae-based biogas upgrading process

Abstract

An alternative way to remove CO_2 from biogas is the use of photosynthetic microorganisms, such as microalgae. As microalgae perform oxygenic photosynthesis, microalgae-based biogas upgrading process needs to be carefully designed and controlled in order to separate O_2 desorption from CO_2 absorption. An open-photobioreactor connected with mass transfer column was proposed. Although the utilization of counterflow column improved the quality of upgraded biogas, microalgal culture was exposed to important pH changes when circulating through the column. To clarify a potential effect of these dynamic pH conditions over the culture, the effect of pH change on the photosynthetic activity and PSII quantum yield was studied. Results showed that microalgae culture did not suffer a negative effect on the photosynthetic system of cells, because a high value of PSII efficiency was remained and the photosynthetic activity could be recovered.

4. Effect of pH changes on microalgae-based biogas upgrading process

4.1. Introduction

The operation of an open photobioreactor connected to counterflow bubble column for CO_2 absorption represents a feasible alternative for biogas upgrading because it enables the production of an upgraded biogas with low CO_2 and O_2 levels.

However, although the utilization of counterflow column improves the quality of upgraded biogas, microalgal culture is exposed to important pH changes when circulating through the column (Meier *et al*, 2015). Such difference is higher when reducing the circulating flow. Such pH changes are likely to produce a metabolic change on the microalgae culture.

The pH is an important parameter in the operation of a photosynthetic biogas upgrading system because pH influences on the carbon inorganic equilibrium and the microalgae activity. When CO_2 is dissolved in the aqueous phase, it can dissociate in HCO_3^- and CO_3^{-2}. The concentration of each carbon inorganic species depends on pH (Stumm and Morgan, 1995; Manahan, 2007; Kumar *et al*, 2011). The carbon inorganic dissociation causes the release of H^+, and as a result, pH decreases. The pH reduction affects the microalgae activity because most microalgae culture growth in a pH range of 7 – 9, with optimal pH between 8.2 and 8.7 (Barsanti and Gualtieri, 2006). Most of carbon is as bicarbonate in a pH range of 7 - 9. Although CO_2 is the substrate of the Rubisco enzyme, microalga cells can use bicarbonate as carbon source. Bicarbonate can be transformed into dissolved CO_2 by the enzyme carbonic anhydrase (CA) (Tchernov *et al*, 1997; Wang *et al*, 2008; Kumar *et al*, 2011). Although CO_2 dissolution causes pH decrease, the activity of CA causes pH increase outside the cell due to the transport of

hydroxide ions outside the cell in association with the capture of H^+ ions for the interior of the thylakoid membranes (Kumar and Das, 2012).

Given the importance of the pH on the microalgae cultivation, supplementary research is needed to clarify potential effect of this dynamic pH conditions on the culture.

The aim of this work is evaluate the effect of pH gradients expected in the column on the photosynthetic activity and PSII quantum yield. The photosynthetic activity refers to the oxygen released by the microalga from water photolysis under saturating PAR (photosynthetically active radiations) (Cuaresma *et al*, 2006). PSII quantum yield (Fv/Fm) reflects the performance of photochemical processes in PSII. PSII quantum yield ranges from 0.65 to 0.80 in healthy microalgae cultures (Richmond, 2004). Both analyses allow testing the condition of the photosynthetic system and the cell viability.

4.2. Methodology

4.2.1. Microalgae and culture medium

The microalga *Chlorella sorokiniana* was obtained from the culture collection of Central Research Services (CIDERTA) of the University of Huelva, Huelva, Spain. Microalgae were cultivated using modified M-8a medium (Mandalam and Palsson, 1998). All assays were carried out considering that optimal pH of *Chlorella sorokiniana* is 7.0.

4.2.2. Effect of pH change on the microalgae culture.

Batch photobioreactors of 200 mL were used, applying a light intensity of 90 µmol m^{-2} s^{-1}. Effect of pH changes on the microalgae culture was evaluated through two experiments:

- Change of pH by addition of HCl 3.7%. Three conditions were evaluated: control culture at pH 7.0; pH change from 7.0 to 5.0 when the culture is exposed to light; pH change from 7.0 to 5.0 when the culture is not exposed to light. Biomass concentration of 0.5 and 1.3 g L^{-1} were used. The effect was evaluated through photosynthetic activity and PSII quantum yield analysis.
- Change of pH by CO_2 injection. Three conditions were applied: control culture at pH 7 (DIC concentration of 12 mM); culture exposed to CO_2 injection and light; culture exposed to CO_2 injection without light. In the last two conditions, CO_2 was bubbled into the microalgae culture down to pH 5.8. Then, CO_2 injection was stopped and pH, PSII quantum yield and photosynthetic activity were determined. Biomass concentration of 1 g L^{-1} was used.

4.2.3. Determination of apparent affinity of microalgae

The use of inorganic carbon by *Chlorella sorokiniana* was studied by photosynthetic activity (PA) kinetics (oxygen release) at pH 5.0 and pH 7.0, applying different DIC concentrations into the electrode. The inorganic carbon was added in the form of $NaHCO_3$, partly converted into CO_2 as a function of the pH according to the chemical equilibrium $NaHCO_3/CO_2$ in water. The initial oxygen release rate was registered for each $NaHCO_3$ concentration added (Cuaresma *et al*, 2006). The apparent affinity constant (K_{DIC}) for inorganic carbon was calculated from graph of 1/PA versus 1/[DIC], according to the equation 4.1.

$$\frac{1}{PA} = \frac{K_{DIC}}{PA_{max}} * \frac{1}{[DIC]} + \frac{1}{PA_{max}} \qquad (4.1)$$

4.2.4. Analytical methods

Photosynthetic activity was determined by oxygen evolution using a Clark-type electrode. Oxygen release measurements were made under saturating white light (750 µmol m^{-2} s^{-1}) or darkness (endogenous respiration) at 25°C (Vaquero *et al*, 2012). Chlorophyll and carotenoid contents was determined by methanol extraction and visible spectrophotometry. Chlorophyll and carotenoid concentrations in the extracts were calculated by modified Arnon's equations (Lichtenthaler, 1987). PSII maximum quantum yield was measured using a pulse amplitude modulation (PAM) fluorometry with the saturating-pulse technique (Maxwell and Johnson, 2000). Dissolved inorganic carbon was analyzed by alkalinity determination according to method 4500 of standard methods (APHA/AWWA/WEF, 1998).

4.3. Results and discussion

Figure 4-1 shows the photosynthetic activity and PSII quantum yield of microalgae culture when pH decreased to 5.0 by addition of HCl, using 0.5 and 1.3 g L^{-1} of biomass concentration. The photosynthetic activity of the exposed light-culture decreased after 100 minutes since the pH change from 7.0 to 5.0. However, the PSII quantum yield remained around 0.6 and 0.7, values that correspond to healthy microalgae cultures (Richmond, 2004). When, pH was adjusted from pH 5 to pH 7 (Figure 4-1A), the photosynthetic activity of the culture was recovered, demonstrating that the cells did not suffer permanent damage. On the other hand, when pH was changed in darkness, no changes in the photosynthetic activity were observed.

Therefore, according the results in Figure 4-1, pH gradient in the column would not cause a negative effect on microalgae culture because the residence time of microalgae culture in the column is around 20 minutes and the column is operated in darkness.

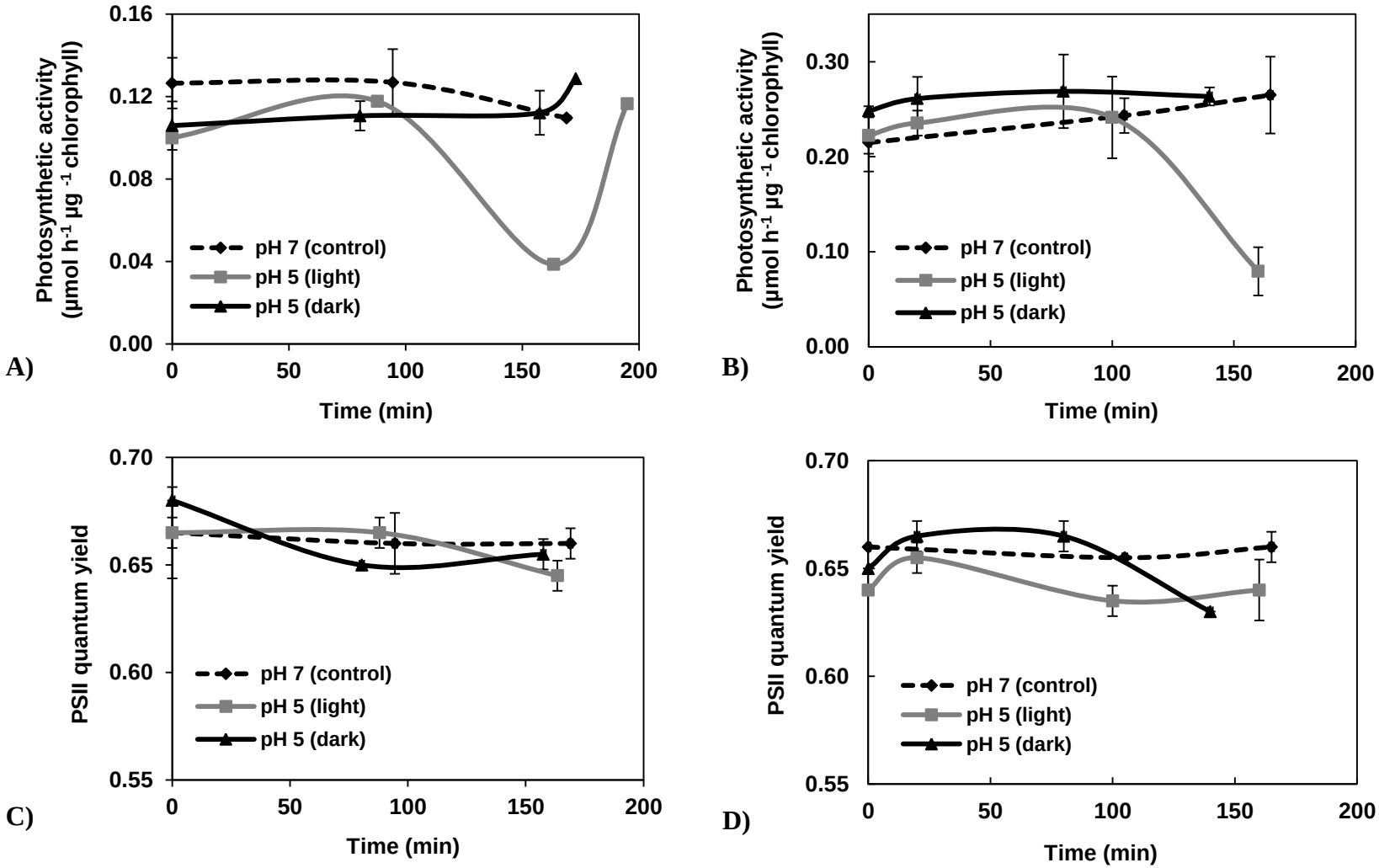

Figure 4-1. Effect of pH change on microalgae activity. A) Photosynthetic activity when a biomass concentration of 0.5 g L^{-1} was used. B) Photosynthetic activity when a biomass concentration of 1.3 g L^{-1} was used. C) PSII quantum yield when a biomass concentration of 0.5 g L^{-1} was used. D) PSII quantum yield when a biomass concentration of 1.3 g L^{-1} was used.

Figure 4-2 shows the photosynthetic activity and PSII quantum yield of microalgae culture when pH was decreased from 7.0 down to 5.8 by CO_2 injection, simulating the process occurring in the column (DIC concentration in the microalgae culture was tripled as consequence of CO_2 bubbling).

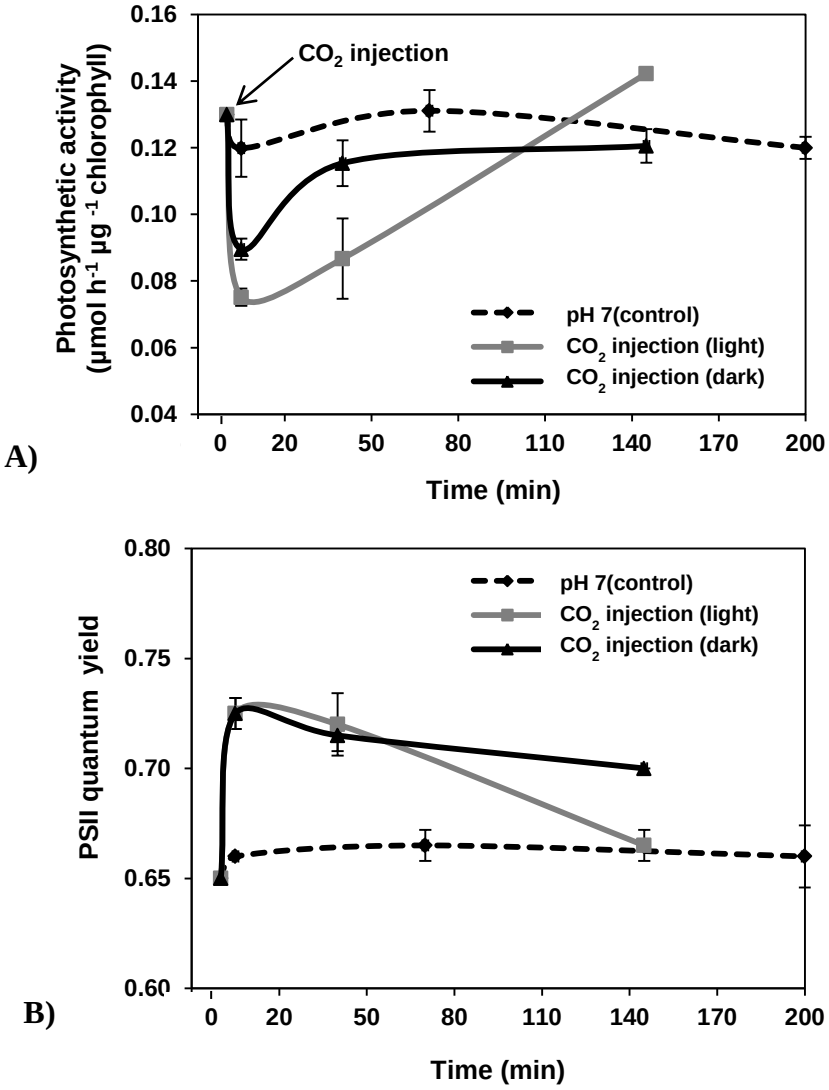

Figure 4-2. Effect of pH and DIC concentration changes on microalgae activity. A) Photosynthetic activity when CO_2 injection was applied. B) PSII quantum yield when CO_2 injection was applied.

In contrast with the situation of pH change by only acid addition (Figure 4-1A, Figure 4-1B), when pH was reduced by CO_2 injection, the photosyntethic activity decreased inmediately in the culture exposed to light and darkness (Figure 4-2A). This could mean

that CO_2 inhibits the microalgae activity by a different mechanism to pH decrease. The photosynthetic activity was recovered after CO_2 injection was stopped because pH increased and DIC concentration decreased in the culture medium due to the carbon fixation and desorption. Similar results were obtained with *Chlorella*, whose growth was inhibited when it was exposed to high CO_2 concentrations, but growth reappeared when the concentration was decreased (Hanagata, 1992).

As shown in Figure 4-2B, the CO_2 injection caused an increase in the PSII quantum yield. An increase in PSII yield means that a higher percentage of the absorbed light energy is used to photochemical process. This response could be attributed to an increase in the demand of reducing power (NADPH) to fix and reduce the higher carbon inorganic concentration in the culture medium (Papazi, 2008).

Therefore, according the results in Figure 4-2, when biogas is injected into the microalgae culture in the column, the photosynthetic activity of algal cells could decrease. However, the cells do not suffer damage in their photosynthetic system, maintaining a high value of PSII quantum yield. When microalgae come back to the photobioreactor, DIC concentration decreases due to photosynthesis and desorption, and the microalga cells recover their photosynthetic activity.

In order to study the preferred inorganic carbon source of *Chlorella sorokiniana*, the apparent affinity constant for dissolved inorganic carbon was determined. Figure 4-3 shows the photosynthetic activity as a function of the inorganic carbon concentration provided into the electrode cubet at pH 7 and pH 5. The apparent affinity constant for dissolved inorganic carbon (K_{DIC}) was 0.9 µM at pH 5 and 112.0 µM at pH 7. The $NaHCO_3$ added into algal samples at pH 5 is mostly in the form of CO_2. Therefore, the lower apparent K_{DIC} value at pH 5 than pH 7 suggests that this microalga has a higher

affinity for CO_2 than HCO_3^-. So, CO_2 would be the inorganic carbon source preferred by *Chlorella sorokiniana*. This result agrees with Williams *et al* (1996), who indicated that *Chlorella saccharophila* has an affinity for CO_2 which is 160 times greater than that for HCO_3^-. The highest affinity at pH 5 could suggest the expression of some concentrating mechanisms of CO_2 that could facilitate its fixation by Rubisco. According to Tsuzuki *et al* (1980), there are two possible ways by which CO_2 may be supplied to the *Chlorella* surface: CO_2 can be supplied from the culture medium by simple diffusion (direct supply of CO_2) or HCO_3^- formed from CO_2 can be converted again into CO_2 via the enzyme carbonic anhydrase (CA) and incorporated by the algal cells (indirect supply of CO_2).

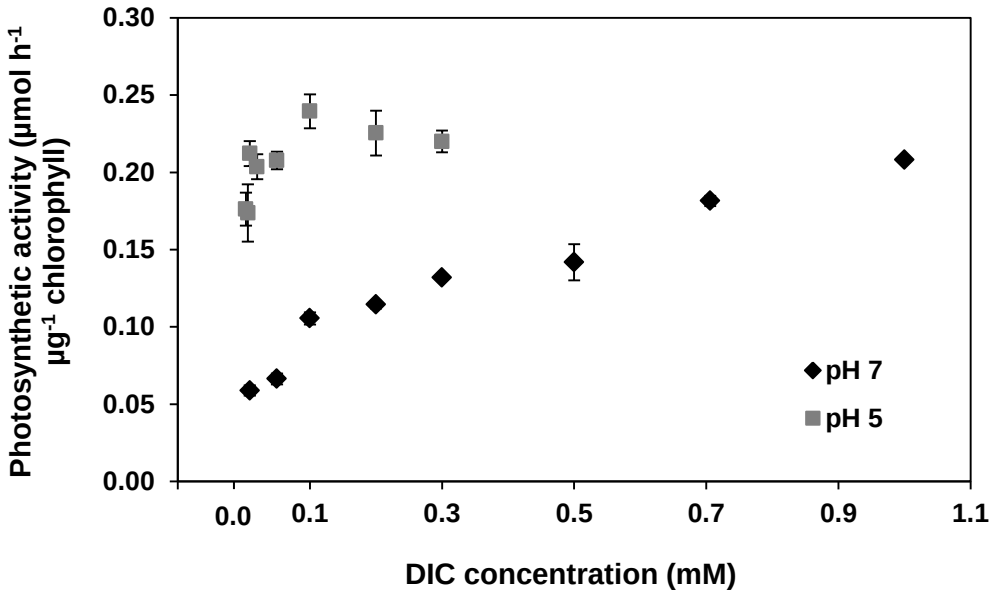

Figure 4-3. Photosynthetic activity curves of *Chlorella sorokiniana* at pH 7 and pH 5, as a function of DIC concentration.

Additional researches must be carried out to clarify the mechanisms affected in the algal cells due to the injection of a gas with high CO_2 concentration and/or a decrease of pH, considering that CO_2 is the preferred source of carbon for *Chlorella*. A possible

substrate inhibition may occur in an enzyme involved in the mechanism of carbon consumption. On the other hand, as result of the circulating flow between the photobioreactor and the column, a cell of microalga can be exposed to pH and DIC gradients several times during the operation of the system. So, it is interesting to study if these repeated changes have some additional effect on the microalgae activity.

4.4. Conclusions

- The pH gradients expected in the absorption column did not produce damage in the photosynthetic system of microalgae, because a high value of PSII efficiency was remained and the photosynthetic activity could be recovered.
- The CO_2 is the preferable source of carbon for *Chlorella sorokinana*. However, additional research must be carried out to study the mechanisms that are affected in the algal cells when a gas with high CO_2 concentration is applied.

CHAPTER V

Photosynthetic biogas upgrading using microalgae: effect of light/dark photoperiod.

Meier, L; Barros, P; Torres, A; Vilchez, C; Jeison, D. Photosynthetic biogas upgrading using microalgae: effect of light/night photoperiod. *Paper sent to Renewable Energy*

Photosynthetic biogas upgrading using microalgae: effect of light/dark photoperiod.

Abstract

The use of biogas for grid injection and/or as vehicle fuel requires its purification to obtain biomethane, a process normally referred to as biogas upgrading. This is usually accomplished by physical methods such as absorption or adsorption. The use of microalgae cultures has been proposed as a new alternative for CO_2 removal from biogas. Full-scale systems for biogas upgrading using microalgae should be able to deal with natural existing day/night photoperiods. This research evaluated the effect of a light/dark photoperiod on the operation of a photosynthetic biogas upgrading system at lab scale conditions. A system based on an open-photobioreactor connected to a mass transfer column was used for that purposes. Using a continuous biogas flow, an upgraded biogas with a CO_2 concentration between 4.5% and 2.0% and an O_2 concentration of 0.5% was obtained during the light/dark photoperiods, fulfilling the most of biomethane standards without stopping biogas injection during the dark period. Mass balances showed that CO_2 desorption was the main process behind its removal. CO_2 removal during the night was possible, under the tested conditions, as a result of inorganic carbon desorption from the photobioreactor and accumulation in the liquid phase.

5. Photosynthetic biogas upgrading using microalgae: effect of light/dark photoperiod.

5.1. Introduction

Biogas is the product of anaerobic bio-digestion of the organic matter and it consists mainly of CH_4 (55-75%) and CO_2 (25-45%). The use of biogas for grid injection and/or as vehicle fuel requires its purification to obtain biomethane (biogas upgrading). Indeed, most of the valid regulations dealing with biomethane use establish a maximal CO_2 concentration in upgraded biogas between 2 and 6% (Marcogaz, 2006; Huguen and Le Saux, 2010).

The use of microalgae cultures has been proposed as a new alternative for CO_2 removal from biogas. This is the result of their high growth rates, ability to grow in different environmental conditions and their capacity to take up nutrients from wastewaters (Wang *et al*, 2008; Mata *et al*, 2010; Singh and Gu, 2010). Direct biogas injection into the culture using a closed-photobioreactor is not a feasible process, because microalgae perform oxygenic photosynthesis, i.e., approximately 1 mol of O_2 is released per mol of CO_2 captured (Cuaresma *et al*, 2009). Then, depending on CO_2 content of biogas, O_2 concentrations around 20% in the resulting gas can be observed (Converti *et al*, 2009; Meier *et al*, 2015). Most standards for biomethane use require an O_2 content lower than 1% (Rutledge, 2005; Marcogaz, 2006). Therefore, the physical separation of the CO_2 absorption from the O_2 desorption, in a two-stage process, had been proposed. CO_2 and O_2 concentrations of 1.9 ± 0.6 % and 1.2 ± 0.1 % were achieved in the upgraded biogas produced at lab-scale using a counter-flow bubble column for mass transfer connected to an open-photobioreactor (Meier *et al*, 2015).

Full-scale systems for biogas upgrading using microalgae should be able to deal with natural existing day/night photoperiods. In the absence of light, microalgae have no energy to perform photosynthesis. Therefore, they can only carry out respiration, releasing CO_2 into the culture medium (Granum and Myklestad, 2002). Thus, it would be natural to expect that a photosynthetic biogas upgrading system would not be able to operate continuously. Hence, biogas could only be injected during the day, and should be stored during the night. To our knowledge, there are no reports dealing with the effect of photoperiods on biogas upgrading systems using microalgae. The aim of this study was to evaluate the performance of a two-stage system photosynthetic biogas upgrading process exposed to light/dark photoperiod, focusing on the CO_2 removal efficiency.

5.2. Materials and methods

5.2.1. Microalgae and culture medium

The microalga *Chlorella sorokiniana* was obtained from the culture collection of Central Research Services (CIDERTA) of the University of Huelva, Huelva, Spain. Microalgae were cultivated using modified M-8a medium (Mandalam and Palsson, 1998), prepared using tap water.

5.2.2. Experimental setup

An open-photobioreactor of 50 L was implemented (depth: 0.15 m, width: 0.50 m, height: 0.67 m). Illumination was provided by means of cool white fluorescent lights, which were programmed with an automatic on/off system in order to simulate a 12:12 light/dark period. During the light hours, four light intensities were used: 25, 50, 75 and 100 µmol m^{-2} s^{-1} (Figure 5-1). Photobioreactor was fed with medium only during light

hours, using a dilution rate of 0.1 d^{-1}. The feeding was stopped during darkness to avoid biomass wash out. Aeration was supplied continuously to the photobioreactor to facilitate desorption of generated oxygen by the photosynthesis and to enhance mixing of the microalgae culture. Aeration resulted in a gas/liquid volumetric mass transfer coefficients (K_La) of 0.56 h^{-1} and 0.50 h^{-1} for CO_2 and O_2 respectively.

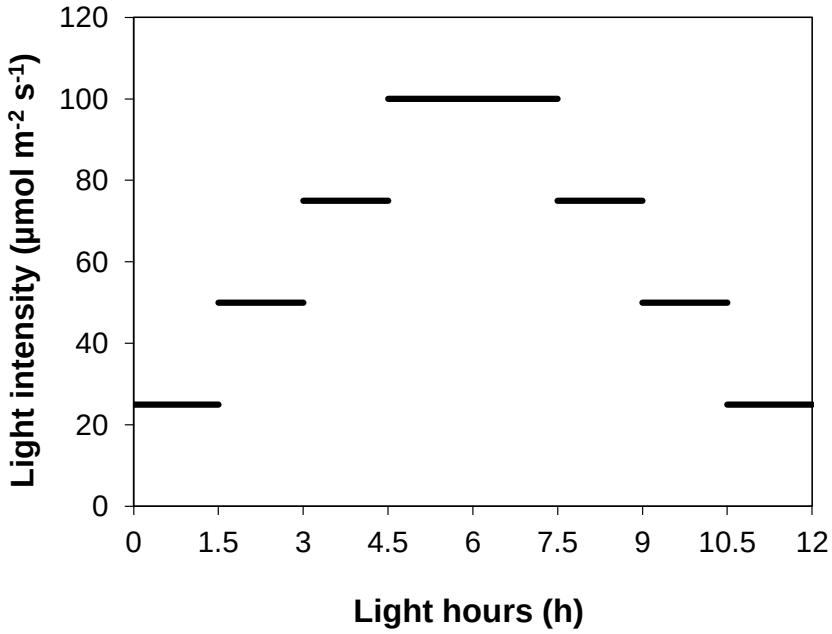

Figure 5-1. Light intensity applied to the photobioreactor during daylight hours.

The photobioreactor was connected with a bubble column operated in counter-flow mode (height: 3 m, diameter: 0.012 m). The system was operated injecting real biogas (65±1.5 % CH_4; 32.0±1.9 % CO_2) continuously at the bottom of the column, while microalgae culture was continuously circulated between the photobioreactor and the column by means of a peristaltic pump. A ratio of biogas flow over column-reactor circulation flow of 1.7 was applied. The residence time of microalgae culture into the bubble column was between 11 and 17 minutes. Figure 5-2 presents a schematic representation of the two-stage process: photobioreactor/absorption column.

Biogas was produced in a 50 L lab-scale anaerobic UASB digester, inoculated with sludge from a full scale UASB treating brewery wastewater. The reactor was fed continuously using diluted wine as substrate.

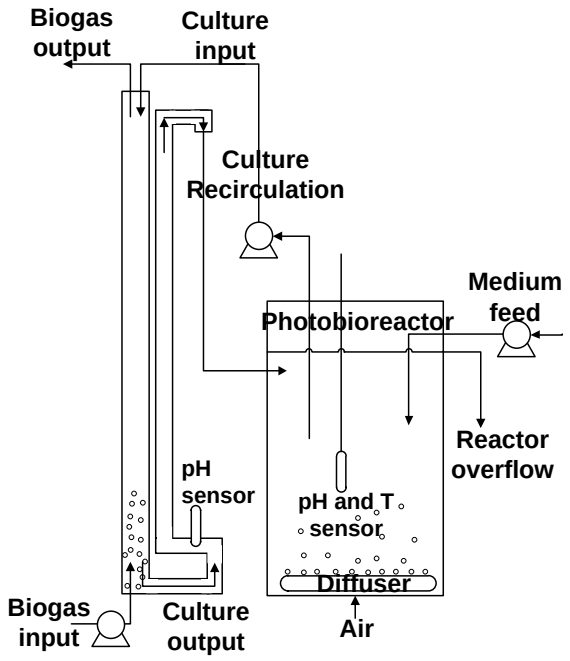

Figure 5-2. Two-stage process for biogas upgrading by microalgae.

Gas samples were taken from sampling points located at the entrance and exit of the column for gas composition determination. Samples were periodically taken from the microalgae culture to measure dissolved inorganic carbon (DIC) and biomass concentration. As microalgae were transferred continuously from photobioreactor to the top of the column, it was assumed that the pH and DIC concentration were equal at those locations. The CO_2 concentration in the upgraded biogas, pH in the photobioreactor and pH in the column output were measured on line.

The system was started up during 15 days using continuous illumination. Then, system was operated during 30 days applying two different volumetric gas loads, using the already described light/dark photoperiod.

5.2.3. Carbon and oxygen mass balances

Carbon mass balances were performed to the column-photobioreactor system during light and dark periods. Inorganic carbon input to the system was evaluated considering an average CO_2 concentration of the raw biogas of 32%. The inorganic carbon output in the treated biogas was calculated from the experimental values measured during reactor operation. Inorganic carbon assimilation by microalgae was evaluated considering that 1 g of biomass (expressed as volatile suspended solids, VSS) fixes 1.83 g of CO_2 (Chisti, 2007). Inorganic carbon leaving the system with the liquid effluent of the photobioreactor was calculated from its average DIC concentration. Inorganic carbon production by respiration was evaluated considering that 15% of biomass grown in the light-time is lost during dark incubation (Geider and Osborne, 1989; Edmundson and Huesemann, 2015). Inorganic carbon leaving the system in the form of CO_2 by desorption from photobioreactor liquid phase was determined by the difference between inorganic carbon input and output.

The O_2 mass balance was performed for the light period. The O_2 production by microalgae was calculated considering that 1.33 g O_2 are produced per g of produced biomass (VSS). The O_2 output in the treated biogas and in the liquid effluent were evaluated from the experimental values measured during reactor operation. The O_2 desorption from photobioreactor was determined by the difference between O_2 input and output.

5.2.4. Microalgae dynamic response to light

The dynamic response to light of the microalgae was determined. This was done by measuring oxygen generation when microalgae was exposed to intermittent lighting conditions (light/dark cycles) in a batch assay, using a liquid-phase photosynthesis and

respiration meter (Oxygraph DW1/AD, Hansatech Instruments). A sample of 1.5 mL from a *C. sorokiniana* batch culture was incubated, and changes in oxygen concentration were determined, under saturating white light (500 µmol m^{-2} s^{-1} (Barsanti and Gualtieri, 2006)) and darkness, at 25°C (Vaquero *et al*, 2012).

5.2.5. Analytical methods

Volatiles suspended solids (VSS) and dissolved inorganic carbon (DIC) were determined according to methods 2540 and 4500 of *Standard Methods* (APHA/AWWA/WEF, 1998), respectively. Gas composition was determined through a gas chromatograph with thermal conductivity detector (Perkin Elmer Clarus 500). CO_2 concentration in the treated biogas was measured on line, using an infrared CO_2 sensor (Dynament, UK).

The gas/liquid global volumetric mass transfer coefficient for O_2 (K_La_{O2}) was measured using the dynamic gassing-in method (Hulatt and Thomas, 2011). CO_2 mass transfer coefficient (K_La_{CO2}) was calculated through the relation $K_La_{CO_2} = 0{,}9K_La_{O_2}$ (Babcock *et al*, 2002).

5.3. Results and discussion

After start-up was finished, the system was operated at a volumetric gas load of 1.44 L d^{-1} per L of microalgae culture. Under such condition, the CO_2 concentration in the upgraded biogas was in the range 8-17%. In order to reduce CO_2 content of upgraded biogas, the volumetric gas load was reduced to 1.0 L d^{-1} per L of microalgae culture. As a result, the CO_2 concentration in the upgraded biogas was reduced to the range 2-4.5%. Such content fulfills most of the European biomethane standards. An extract of the system operation is showed in Figure 5-3. The O_2 concentration in the upgraded biogas

was less than 1% throughout the operation of the system, demonstrating that the separation of CO_2 absorption and O_2 desorption in a two-stage process allowed the control of the O_2 concentration in the upgraded biogas.

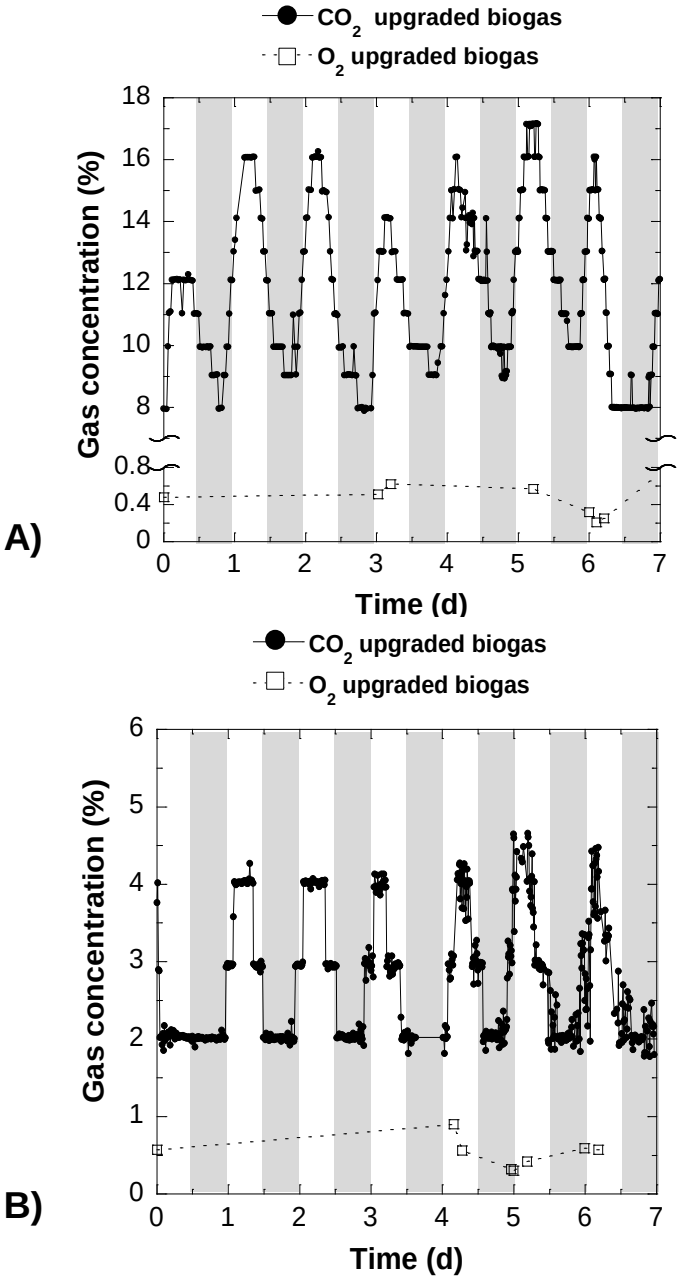

Figure 5-3. CO_2 and O_2 concentration in the upgraded biogas using 12:12 light/dark photoperiod. A) volumetric biogas load of 1.44 L d^{-1} per 1 L microalgae B) volumetric biogas load of 1.0 L d^{-1} per 1 L microalgae. White color: light period; grey color: darkness period.

As consequence of photosynthesis interruption during darkness period, pH decreased and DIC concentration slightly increased in the photobioreactor (Figure 5-4). Thus, a relevant decrease in CO_2 removal efficiency was expected during darkness period, as a result of the absence of photosynthesis. However, CO_2 concentration during both dark and light periods remained below 5%, at a gas load of 1.0 L d^{-1} per L of microalgae culture (Figure 5-3). Then, it is inferred that the absence of CO_2 uptake by microalgae during darkness did not cause an increase of DIC concentration that was enough to reduce the absorption capacity of the liquid phase.

According to the carbon mass balance, the CO_2 capture by microalgae only corresponded to 19 % of the CO_2 contained in biogas (Figure 5-5). Moreover, 57% of inorganic carbon entering the system in the biogas was lost by desorption from the photobioreactor to the atmosphere. Desorption was then the main mechanism of carbon removal from photobioreactor. Photobioreactor was not fed with liquid media during dark phase. Such condition and the absence of photosynthesis induced a small increase in the contribution of desorption to inorganic carbon removal (from 57 to 60%), and an increase on inorganic carbon concentration in the liquid phase, causing an accumulation accounting for 30% of the inorganic carbon entering the system (Figure 5-5). The dissolved CO_2 concentration in the photobioreactor was approximately 60 times greater than the saturation concentration of CO_2 in contact with air, which is the reason for the relevance of CO_2 desorption in the photobioreactor when analyzing inorganic carbon mass balance.

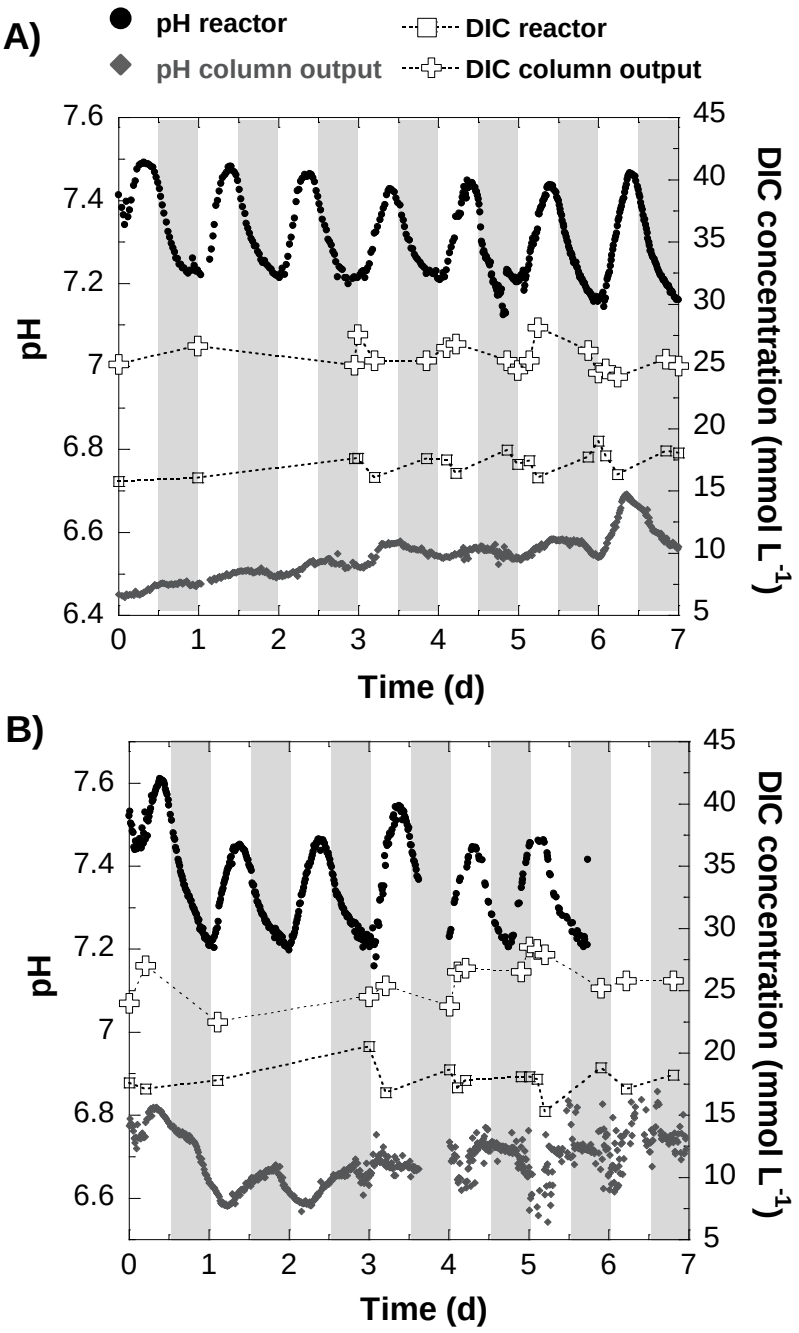

Figure 5-4. pH and DIC concentration in the reactor and in the output of the column using 12:12 light/dark photoperiod. A) Volumetric biogas load of 1.44 L d^{-1} per 1 L microalgae B) Volumetric biogas load of 1.0 L d^{-1} per 1 L microalgae (White color: light period; grey color: darkness period)

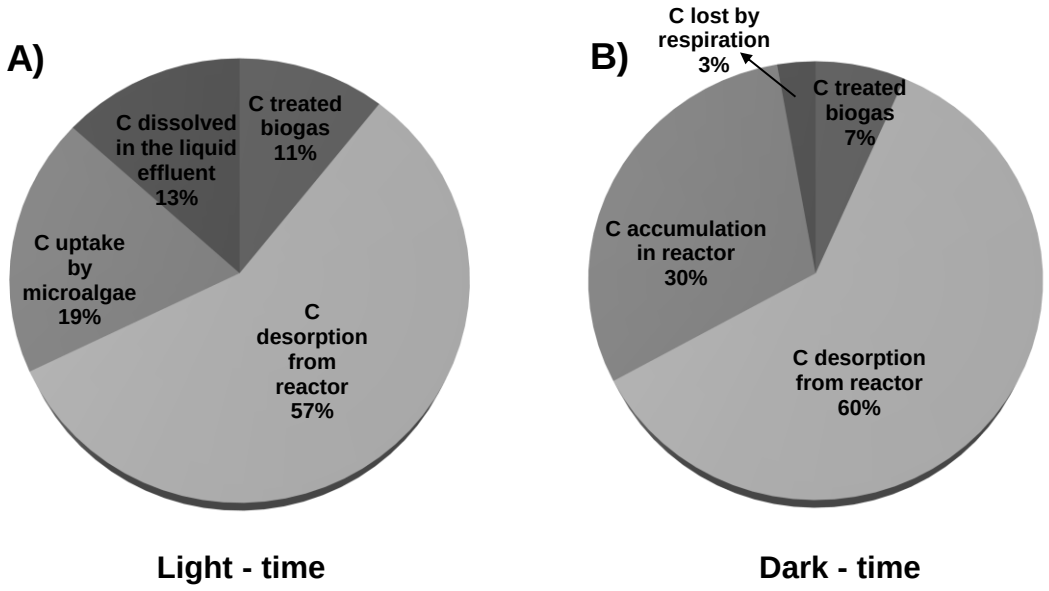

Figure 5-5. Inorganic carbon mass balance. It was considered that 100% of the inorganic carbon enters the system as CO_2 in the biogas. A) During 12 h light-time. B) During 12 h dark-time (volumetric biogas load: 1 L d^{-1} per 1 L of microalgae)

The resulting carbon mass balance can be representative of what may happen in a large-scale open-photobioreactor. The capacity of CO_2 absorption was obtained with a biomass productivity of 0.06 g L d^{-1} (biomass concentration of 0.6 g L^{-1}), which is representative of typical values obtained in open-photobioreactors. Raceways normally present biomass productivities between 0.05 and 0.1 g L d^{-1} (biomass concentration 0.3 - 0.5 g L^{-1}) (Pulz, 2001). Aeration in the photobioreactor resulted in a gas/liquid volumetric mass transfer coefficient (K_La) of 0.5 h^{-1}. Typical K_La values for raceways are between 0.2 to 8 h^{-1} when 20 to 5 cm deep (Babcock *et al*, 2002). So, an equal or superior desorption rate may be observed in a full scale systems. Indeed, Sydney *et al* (2014) reported that only 13-20% of the supplied CO_2 was absorbed in raceway ponds, when CO_2 was bubbled into the culture medium as carbon source.

Since the CO_2 capture by photosynthesis was not the main mechanism of CO_2 removal in the described system, the fluctuations in the CO_2 content in the upgraded biogas did not respond directly to the photosynthetic activity of the microalgae biomass. This resulted in good levels of CO_2 removal observed even during darkness period.

Observation of Figure 5-3 also reveals that CO_2 concentration in treated biogas began to increase only 7 hours after light was turned off. Subsequently, the maximum CO_2 concentration in treated biogas was obtained 4 hours after light was turned on, after which CO_2 began to decrease. Photosynthesis responds rapidly to light/dark cycles. Indeed, pH shifts were observed immediately after light was turned on or off. Therefore, the observed delay in the response of CO_2 concentration in the treated biogas would not be caused by a delay in the response of the photosynthetic system of cells. Figure 5-6 presents the rate of variation of oxygen concentration determined in a photosynthesis and respiration meter, when microalgae were exposed to intermittent light and dark periods. It is clear that the response of the microalgae is very fast, resulting in rapid changes in oxygen generation. Indeed, cells produced oxygen intensively and immediately after light was turned on, and when it was turned off, photosynthesis ceased rapidly and the oxygen concentration dropped. This behavior was similar to that reported by Yen *et al* (2004) in assays with microalgae *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942 cultivated under 12:12 light/dark periods. Then, the observed delay is not related with the metabolic response of algae.

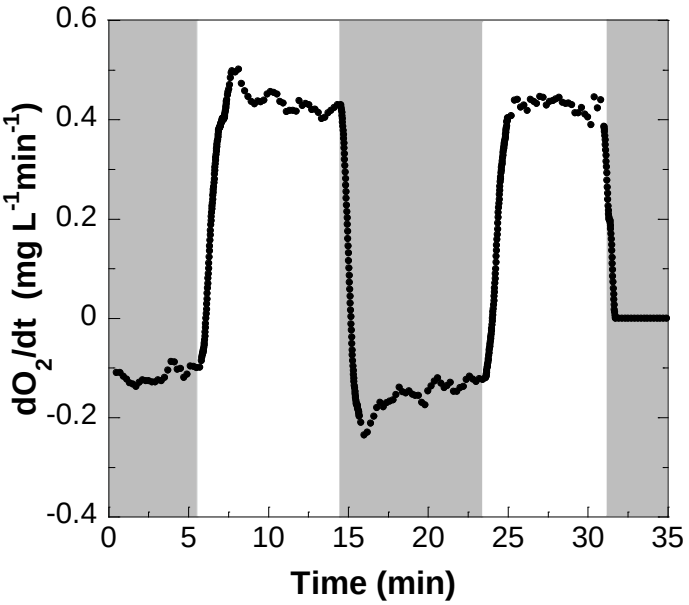

Figure 5-6. Dissolved oxygen consumption and generation rate in the microalgae culture to light and darkness exposition (White color: light period; grey color: darkness period). Biomass concentration: 0.1 g L^{-1}; 25°C.

The observed delay is most likely related with the physical phenomena of CO_2 desorption, which was the main factor determining inorganic carbon removal, as already stated. Phase equilibrium conditions depend on temperature. In other words, CO_2 solubility (C*) is a function of temperature, and it decreases as temperature increases. During the operation of the column-photobioreactor system, the temperature was not controlled, and fluctuated between 20 and 28 °C (Figure 5-7). As a result of temperature fluctuations, and considering a biogas CO_2 concentration of 32%, the equilibrium concentration (C*) for CO_2 in the liquid phase of column would move in the range 0.44-0.55 g L^{-1}, when temperature changes from at 28 to 20 °C. Then, the difference C*-$C_{CO2,L}$ was higher during darkness, as is represented in Figure 5-8. As shown in Figure 5-8, the difference C*- $C_{CO2,L}$ could fluctuate between 0.2 g L^{-1} during darkness period and 0.1 g L^{-1} in the light period, when evaluated at the bottom of the column. This would induce a much higher gas/liquid mass transfer capacity (from biogas to liquid

phase) in the column during darkness. It is inferred that this is the reason for having an upgraded biogas with lower CO_2 content during darkness (Figure 5-3).

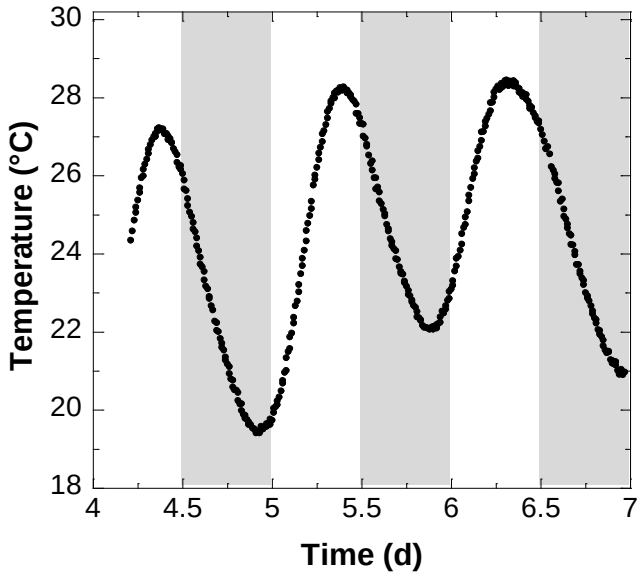

Figure 5-7. Temperature fluctuation in the photobioreactor using 12:12 light/dark photoperiod (White color: light period; grey color: darkness period).

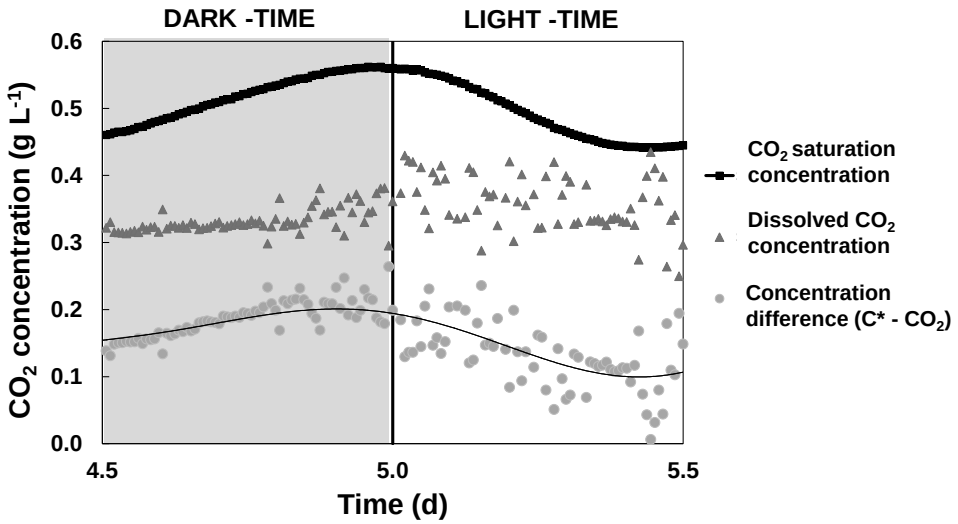

Figure 5-8. Dissolved CO_2 concentration fluctuation at the bottom of the bubble column using 12:12 light/dark photoperiod (White color: light period; grey color: darkness period).

In addition to the above, the observed delay could have been incremented by the effect of temperature on the growth kinetics of *Chlorella sorokiniana*, which is a high temperature strain (T_{opt}=38°C). Although photosynthesis responds rapidly to light/dark cycles (Figure 5-6), the biomass growth and CO_2 assimilation rate could have been limited by the lower temperatures (around 20°C) during the first daylight hours. When the temperature increased above 25°C, the growth rate could be higher and the photosynthetic CO_2 uptake enabled a decrease in the CO_2 concentration in the upgraded biogas (Vona *et al*, 2004; Cuaresma Franco *et al*, 2011).

It is clear then that the effect of temperature on the gas-liquid mass transfer is a key parameter that must be considered in the operation of a biogas upgrading system by microalgae. Temperature can fluctuated between 10 and 45°C in outdoor photobioreactors in temperate regions (Ras *et al*, 2013). These temperature changes will not only affect biomass growth kinetics, but also gas/liquid equilibriums and as consequence, the desorption/absorption rate of gases in the system.

On the other hand, oxygen was determined during light periods several times during the operation (Figure 5-3). That data was used to evaluate a mass balance for oxygen, which is presented in Figure 5-9. Only 9% of the generated O_2 by photosynthesis left the system with the upgraded biogas. The rest was desorbed in the photobioreactor. Due to the low solubility of O_2, only 1% of O_2 was lost in the liquid effluent, amount that was similar to that present dissolved in the input flow of fresh culture medium. Photobioreactor presented an average concentration of dissolved O_2 of 8.5 mg L^{-1}.

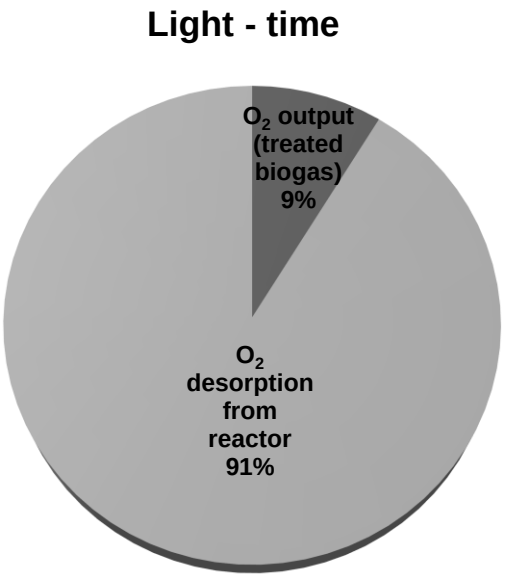

Figure 5-9. O_2 mass balance during 12 h light-time (volumetric biogas load: 1 L d^{-1} per 1 L of microalgae).

In summary, biogas could be injected continuously in an open-photobioreactor, without stopping biogas injection during night. Even a higher CO_2 removal capacity can be achieved overnight due to the gas solubility increase by a temperature decrease. However, it is important to consider that during night, CO_2 is not capture by microalgae biomass and only desorption and accumulation occurs. The described results may be considered representative of the operation of a large-scale system, as raceways ponds, because these systems are characterized by low biomass concentration, high carbon desorption rate from reactor and temperature fluctuations.

5.4. Conclusions

An efficiency of CO_2 removal from biogas of 89-93% was achieved throughout the light/dark cycles using a volumetric gas load of 1.0 L d^{-1} per 1 L of microalgae. Although during night microalgae did not perform photosynthesis, desorption of CO_2 from photobioreactor to atmosphere enabled high levels of CO_2 removal during periods

without illumination. The CO_2 removal efficiency was also affected by natural temperature changes between night and day, causing changes in the CO_2 equilibrium between liquid and gas phases. Lower temperatures increase solubility of CO_2, enhancing its removal in the absorption column. These phenomena enabled the continuous operation of the system, providing levels of CO_2 and oxygen in the upgraded biogas, which are compatible with most of European regulations for biomethane.

CHAPTER VI

Fate of H_2S in a photosynthetic biogas upgrading process

Fate of H_2S in a photosynthetic biogas upgrading process

Abstract

Some applications of biogas, such as vehicle and gas grid injection, require the removal of CO_2 and H_2S in order to produce a gas of equivalent characteristics as that of natural gas. An alternative way to remove CO_2 from biogas is the use of microalgae culture. In this work, the capacity of simultaneous CO_2 and H_2S removal from biogas using a microalgae culture was evaluated. Two continuous open-photobioreactors of 2 L were operated: reactor 1 (biogas with H_2S) and reactor 2 (biogas without H_2S). Each photobioreactor was connected to a bubbling column operated in counter-flow mode. Biogas was continuously injected at the bottom of the column, while microalgae culture was continuously circulated between the photobioreactor and the column. CO_2, O_2 and sulfur mass balances were performed. H_2S and CO_2 could be simultaneously removed from biogas using a microalgae culture. It was possible to remove 100% H_2S. The high dissolved O_2 concentration in the microalgae culture (9.5 mg L^{-1}) allowed a fast oxidation from H_2S to sulfate. A percentage of this produced sulfate could have been used by microalgae as source of sulfur. The oxidation of H_2S could have consumed 5% of O_2 entering the system and reduce the percentage of oxygen that left the system with the biogas. A volumetric gas load of 7.9 L d^{-1} per 1 L of microalgae could be applied with a CO_2 removal around 98%. Upgraded biogas with a composition of 0.7% CO_2 and 2.5% O_2 could be obtained.

6. Fate of H_2S in a photosynthetic biogas upgrading process

6.1. Introduction

Biogas is a biofuel produced from anaerobic digestion of organic matter and it is composed principally by CH_4 (35-70%) and CO_2 (15-50%) (Muñoz *et al*, 2015). Many applications of biogas, such as vehicle and gas grid injection, require the removal of CO_2 in order to produce a gas of equivalent characteristics as that of natural gas. There are regulations that establish the technical specifications of biogas for injection in gas grid or for use of biogas as vehicle fuel. For example, European standards establish a maximal CO_2 concentration in biogas between 2 and 6% (Huguen and Le Saux, 2010). As a new biological alternative for biogas upgrading, the use of microalgae culture has been proposed. Experimental assays have concluded that the use of an open photobioreactor connected to external bubble column for CO_2 absorption enables the production of an upgraded biogas with low CO_2 and O_2 levels. This configuration allows the physical separation of the CO_2 absorption from the O_2 desorption, restricting the amount of O_2 in the upgraded biogas (Meier *et al*, 2015).

However, apart from CO_2, biogas also has other impurities, such as H_2S. Later compound is produced by the anaerobic degradation of S-containing compounds (mainly proteins) and the reduction of anionic species (particularly SO_4^{-2}) contained in the wastes. The H_2S concentration in biogas is in a range between 0 – 10000 ppm_v (Muñoz *et al*, 2015). The H_2S has to be removed in order to avoid corrosion in compressors, gas storage tanks and engines and for health and safety reasons due to its high toxicity (Rasi *et al*, 2011; Ramos *et al*, 2013). Biomethane standards indicate a maximal H_2S concentration of 5 ppm_v in the biogas (Rutledge, 2005; Marcogaz, 2006).

Although desorption of O_2 is favored in the proposed system, a dissolved oxygen concentration above saturation can be reached (8.5 - 10 mg L^{-1}) during a photosynthetic biogas upgrading process. This high oxygen concentration could facilitate the oxidation of H_2S, allowing the simultaneous CO_2 and H_2S removal from biogas. The H_2S oxidation can be chemical or biological and can produce elemental sulfur, thiosulfate or sulfate, depending on pH and sulfur/oxygen proportion (van der Zee *et al*, 2007). Therefore, it is necessary to study the fate of H_2S in the photosynthetic biogas upgrading system and evaluate the feasibility of simultaneous CO_2 and H_2S removal from biogas.

6.2. Methodology

6.2.1. Experimental methodology

The microalga *Chlorella sorokiniana* was obtained from the culture collection of Central Research Services (CIDERTA) of the University of Huelva, Huelva, Spain. Microalgae were cultivated using modified M-8a medium (Mandalam and Palsson, 1998).

Two continuous open-photobioreactors of 2 L were implemented (0.136 m diameter). Each photobioreactor was connected with a bubbling column operated in counter-flow mode (dimensions: 1.71 m high and 0.012 m diameter) (Figure 6-1). Biogas was continuously injected at the bottom of the column, while microalgae culture was continuously circulated between the photobioreactor and the column, by means of a peristaltic pump (circulation flow: 15 L d^{-1}). Biogas with H_2S was injected into the system 1 (R_1) (composed by reactor 1 and its absorption column) and biogas without H_2S was injected into the system 2 (R_2) (composed by reactor 2 and its absorption

column). Biogas was washed in a zinc acetate solution before injection into the system R_2 to remove the H_2S content.

Illumination was provided by means of cool white compact fluorescent lamps (500 µmol m^{-2} s^{-1}). Aeration and mechanical agitation were supplied to the photobioreactor to facilitate desorption of generated oxygen in the photosynthesis and enhance mixing of the microalgae culture. Resulting K_La for O_2 and CO_2 were 8 h^{-1} and 7 h^{-1}, respectively. Applied dilution rate was 0.2 d^{-1} (feed flow of medium: 0.4 L d^{-1}).

Samples were taken from the culture medium to determine dissolved inorganic carbon, dissolved oxygen, sulfate, thiosulfate, sulfide and microalgae biomass concentration. Gas composition (O_2, CO_2, CH_4 y H_2S) at the inlet and outlet of the columns was measured.

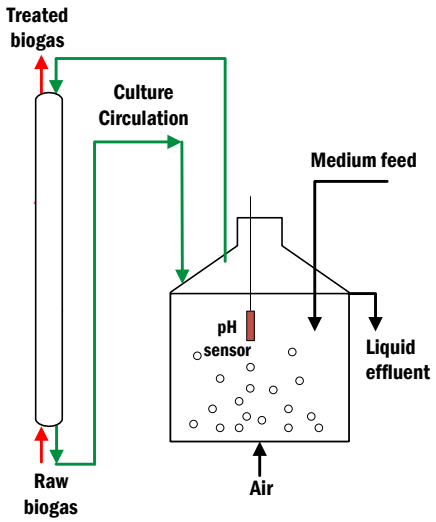

Figure 6-1. Two-stage process for biogas upgrading by microalgae.

Biogas was produced in a 4.5 L lab scale anaerobic UASB digester inoculated with sludge from a full scale UASB treating brewery wastewater. The reactor was fed continuously using diluted wine as substrate, adding Na_2SO_4 as sulfur source. Bicarbonate was added to feeding for regulating pH reactor.

6.2.2. Analytical methodology

Microalgal biomass concentration was determined by dry weight (APHA/AWWA/WEF, 1998) and optical density measurements at 680 nm (Gojkovic *et al*, 2013). The CH_4, CO_2 and O_2 content in the gas phase were determined by gas chromatograph with thermal conductivity detector (Perkin Elmer Clarus 580). To determine H_2S concentration in the gas phase, gas samples were stored in SKC FlexFoil PLUS bags and measured using RAE Systems gas detection tubes with a manual gas detection pump (50-100 mL sample size). Dissolved inorganic carbon was analysed by alkalinity determination according to method 4500 of standard methods (APHA/AWWA/WEF, 1998). Dissolved oxygen was determined by means of Hach HQd Portable Meter. Sulfate and thiosulfate concentrations were measured by ionic chromatography (Metrohm, Switzerland). Sulfide concentration was determined by method 8131-Methylene Blue using kits Hach. Samples were previously filtered and stabilized with an aliquot of a zinc acetate solution. The pH in the photobioreactor was monitored by an online pH electrode (HI 1230B Hanna Instruments), connected to a pH controller (BL 931700 Hanna Instruments). The gas/liquid mass transfer coefficient for O_2 (K_La_{O2}) was measured using the dynamic gassing-in method, using water without biomass. CO_2 mass transfer coefficient (K_La_{CO2}) was calculated from values determined for O_2, through the relation $K_La_{CO_2} = 0{,}9K_La_{O_2}$

6.3. Results and discussion

Operation of the photobioreactors included four stages. The systems were started up during the first stage (I), where dilution rate, circulation flow column-photobioreactor and the biogas flow were adjusted. Subsequently, the systems were operated using different volumetric gas loads. Volumetric gas loads of 2.5, 4.0 and 7.9 L d^{-1} per 1 L of

microalgae were applied during the stages II, III y IV. During the stage IV, the reactor volume was reduced to 1 L to increase the volumetric gas load.

6.3.1. Capacity of H_2S removal

Biogas with a concentration of H_2S between 2000-3000 ppm_v was injected into the system 1 (R_1). System 2 (R_2) was used as a control system, so biogas without H_2S was injected. System 1 was able to remove 100% of the incoming H_2S. Such removal was the result of H_2S oxidation to sulfate. As shown in Figure 6-2, 1.2 ± 0.2 mmol d^{-1} of sulfur left in the liquid effluent of system R_1 as sulfate. This amount of sulfur corresponded to the sum of the produced sulfate from H_2S oxidation (0.6 ± 0.1 mmol d^{-1}) and the sulfate content in the nutrients solution. The m8-a culture medium includes sulfate salts, mainly $MgSO_4 \times 7H_2O$. This nutrients solution was continuously injected in both photobioreactors (R_1 and R_2), which is equivalent to an input load of 0.7 mmol d^{-1} of sulfur (as sulfate) in the stages I, II and III.

The sulfate concentration in reactor R_1 was 1.5 to 3 times greater than reactor R_2, depending on the applied biogas flow. The sulfate concentration in R_2 only corresponded to the amount of sulfate added to the culture medium.

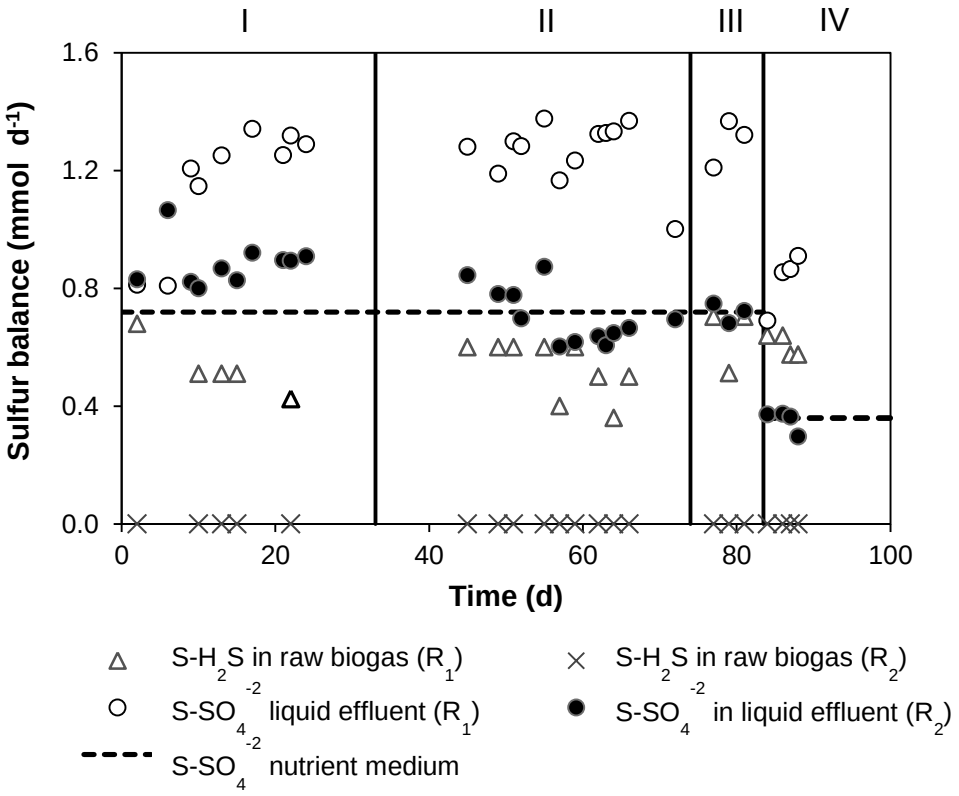

Figure 6-2. Sulfur inflow and outflow in the system with H_2S injection (R_1) and system without H_2S (R_2). H_2S raw biogas R_1: 2000-3000 ppm; H_2S raw biogas R_1: 0 ppm; Sulfate concentration in the liquid effluent: 3.3 ± 0.6 mmol L^{-1} and 1.7 ±0.3 mmol L^{-1} in R_1 and R_2.

H_2S has a high solubility in water (Henry constant 20°C: 5.15 x 10^2 atm). Total dissolved sulfur in water is a mixture of $H_2S_{(aq)}$, HS^- and S^{-2}. The proportions of species in the dissolved sulfide fraction in water are primarily a function of pH. Dissociation constants for sulfide species are pK_{a1}=6.9 and pK_{a2}= 12.75 (Faust and Aly, 1998; González-Sánchez and Revah, 2007). Thus, considering that the pH in the reactors was around 8.7, HS^- was the predominant specie. HS^- can react with oxygen, producing various oxydised forms of sulfur (Chen and Morris, 1972).

$$2HS^- + O_2 + 2H^+ \rightarrow 2H_2O + 2S^0 \qquad (6.1)$$

$$2HS^- + 2O_2 \rightarrow H_2O + S_2O_3^{-2} \qquad (6.2)$$

$$2HS^- + 4O_2 \rightarrow 2SO_4^{-2} + 2H^+ \qquad (6.3)$$

Therefore, the products of sulfur oxidation can be elemental sulfur, thiosulfate or sulfate, depending on pH and sulfur/oxygen proportion (van der Zee *et al*, 2007). Under oxygen limited conditions, that is at dissolved oxygen less than 0.1 mg/L or O_2/S_2^- ratio between 0.5 and 1.0, sulfur and thiosulfate are the major end-product of the sulfide oxidation (equations 6.1 and 6.2). Sulfate is formed when oxygen is in excess (O_2/S_2^- ratio > 1.0) (equation 6.3) (Janssen *et al*, 1995; Pokasoowan *et al*, 2009). The high dissolved O_2 concentration in the microalgae culture (9.5 mg L^{-1}) (Figure 6-3) allowed a fast oxidation from H_2S to sulfate. No presence of thiosulfate or sulfide was found in the microalgae culture.

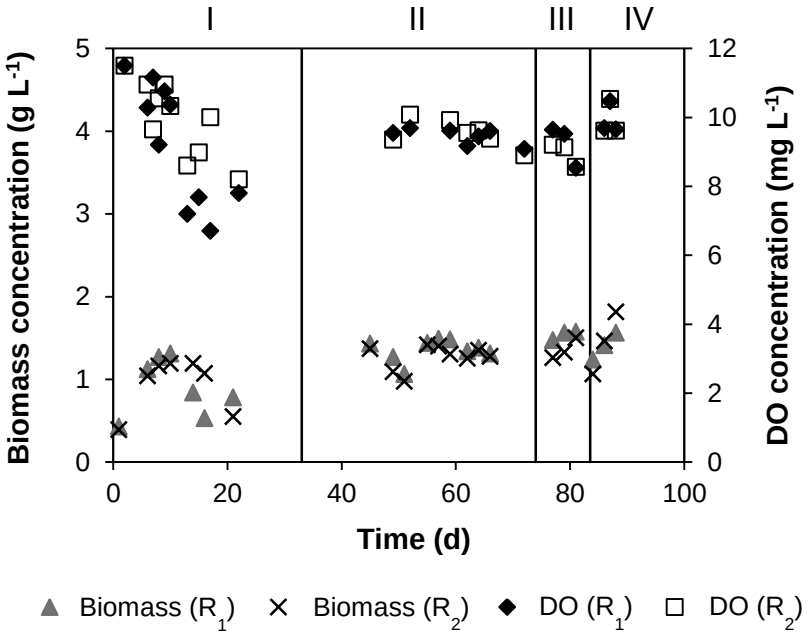

Figure 6-3. Biomass concentration and dissolved oxygen concentration during the operation of the reactors.

Algae have the ability to take up SO_4^{-2} and reduce it to amino acids (Barsanti and Gualtieri, 2006). The sulfur limitation reduces growth, decreases photosynthetic capacity, produces a rapid inhibition of ammonium uptake and elicits a substantial

increase in free non-sulphur aminoacids (Zhang *et al*, 2002; Carfagna *et al*, 2011). So, a percentage of this produced sulfate could have been used by microalgae as source of sulfur. Sulfur is present in freshwater algae at a ratio of about 1 S atom to 100 C atoms (0.15 – 1.96% by dry weight) (Barsanti and Gualtieri, 2006). In the case of *Chlorella sorokiniana,* Kumar *et al* (2014) indicated a sulfur content in biomass of 0.46% w/w.

According to sulfur mass balance in R_1 (Table 6-1), 46% of sulfur input corresponded to H_2S present in the biogas, and 54% is the result of the sulfate salts in the m-8a nutrient medium (mainly $MgSO_4$ x $7H_2O$). Due to the oxidation of H_2S, 92% of incoming sulphide was removed as sulfate in the liquid effluent. Considering a sulfur content in biomass of 0.46%, only 8% of the sulfur input could have been taken by microalgae.

Table 6-1. Sulfur mass balance (Considering stage II; biogas flow: 4.96 L d^{-1}; 28°C)

	System with H_2S (R_1)		**System without H_2S (R_2)**	
	mmol d^{-1}	%	mmol d^{-1}	%
Sulfur input:				
(+) Sulfur in gas inflow (raw biogas)	0.6	46	0.0	0.0
(+) Sulfur in medium culture inflow (m8a medium)	0.7	54	0.7	100
Sulfur output:				
(-) Sulfur in the liquid effluent (as $SO4^{-2}$)	1.2	92	0.6	86
(-) Sulfur fixed by microalgae	0.1	8	0.1	14
(-) Sulfur in gas outflow (upgraded biogas)	0.0	0	0.0	0.0

Kao *et al* (2012) reported growth inhibition when exposing a mutant strain of *Chlorella sp* to a gas mixture containing 150 ppm of H_2S. However, if there are conditions compatible with a rapid H_2S oxidation, microalgae will only be shortly exposed to H_2S, preventing inhibition. An average biomass concentration of 1.4 g L^{-1} was observed

throughout the operation of both reactors (Figure 6-3), and no signs of inhibition were detected in system R_1.

6.3.2. Control of oxygen in the upgraded biogas

Figure 6-4 shows the O_2 content of the raw and upgraded gas, for both tested systems. The O_2 concentration in the raw biogas was 0.4±0.2% throughout the operation of the two systems. Average O_2 concentration in the upgraded biogas was 2.5% and 2.7% for R_1 and R_2, respectively, during the stages II, III y IV. According to the biomethane standards, O_2 concentration must be reduced to less than 0.5% to fulfill most demanding standards, such as the regulations of Switzerland, Austria and The Netherlands (Marcogaz, 2006; Huguen and Le Saux, 2010).

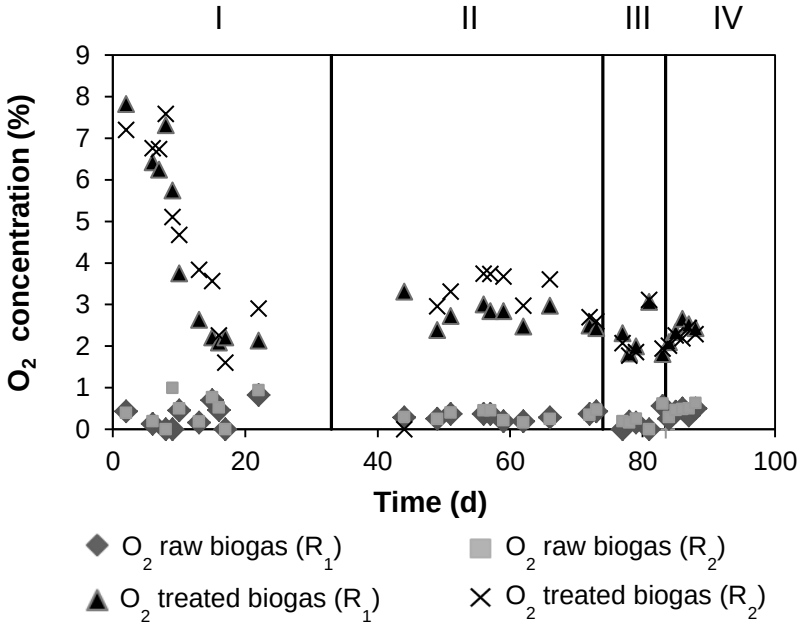

Figure 6-4. O_2 concentration in the biogas inflow and outflow of the systems (R_1: system with H_2S; R_2: system without H_2S).

Table 6-2 shows that around 82% of oxygen was desorbed from the photobioreactor. Only 3% and 4.3% of O_2 left the systems with the upgraded biogas during the stage II, R_1 and R_2 respectively. Considering the oxidation of H_2S to sulfate (equation 6.3), 2

mol of O_2 are needed per mol of oxidized H_2S (Chen and Morris, 1972). Thus, the oxidation of H_2S could have consumed 5% of O_2 entering the system.

Table 6-2. Oxygen mass balance (Considering II stage; biogas flow: 4.96 L d^{-1}; 28°C)

	System with H_2S (R_1)		**System without H_2S (R_2)**	
	mmol d^{-1}	%	mmol d^{-1}	%
Oxygen input:				
(+) Generated O_2 by microalgae	23.5	97.1	23.5	97.1
(+) O_2 in gas inflow (raw biogas)	0.6	2.5	0.6	2.5
(+) O_2 in medium culture inflow	0.1	0.4	0.1	0.4
Oxygen output:				
(-) O_2 desorbed from photobioreactor	19.9	82.2	19.8	81.8
(-) O_2 in gas outflow (upgraded biogas)	3.0	12.4	4.3	17.8
(-) O_2 used in oxidation of H_2S	1.2	5.0	0.0	0.0
(-) Dissolved O_2 in the liquid effluent	0.1	0.4	0.1	0.4

It was expected that H_2S oxidation would induce a higher reduction in the O_2 content in the upgraded biogas. However, the amount of O_2 in the circulating flow between the reactor and the column was seven times higher than the sulfide flow in the biogas at the column inlet (Figure 6-5).

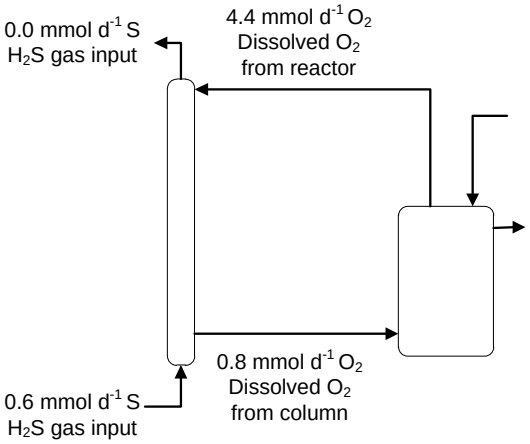

Figure 6-5. Diagram showing the O_2/sulfur ratio in the column (Stage II).

The removal of H_2S could be caused by chemical and/or biological oxidation (van der Zee *et al*, 2007). During biological oxidation, photoautotrophic or chemolithotrophic sulfide oxidizing bacteria use sulfide as an electron donor and convert it to sulfur or sulfate (Tang *et al*, 2009). Chemolithoautotrophic bacteria from the genera *Thiobacillae* and *Acidithiobacillae* have been reported to be the main organisms in the sulfide oxidation (González-Sánchez and Revah, 2007)

On the other hand, chemical oxidation has been reported to occur spontaneously in aqueous sulfide solutions with oxygen, leading to intermediate oxidation compounds such as elemental sulfur, polysulfides, sulfite and thiosulfate (Chen and Morris, 1972). However, chemolithotrophic rates are 10^4 times higher than the abiotic rate (using a trace metal clean solution) because sulfate formation from chemical sulfide oxidation requires large activation energy. Nevertheless, the addition of trace metals, in particular Fe^{+2} and Mn^{+2}, can increase the chemical sulfide oxidation rate such that the half-life is on the order of minutes. Also, the activation energy can be decreased by enzymatic reactions in microorganisms or by redox-mediating enzyme cofactors, released from cell lysis (Chen and Morris, 1972; van der Zee *et al*, 2007; Luther *et al*, 2011).

Further experiments should be performed to determine if the oxidation carried out in the system R_1 was mainly chemical or biological. Abiotic operation of the reactor is necessary to evaluate the possibility of H_2S oxidation to sulfate only by chemical oxidation. The culture medium has trace metals that may favor the chemical oxidation, acting as catalysts.

The reactors were not inoculated with a H_2S-oxidizing bacterial consortium, but the possibility of biological oxidation can not be rejected because the reactors were not operated under sterile conditions.

6.3.3. Capacity of CO_2 removal

Figure 6-6 shows the CO_2 content of the raw and upgraded gas, when biogas was injected with H_2S (R_1) and without H_2S (R_2). The CO_2 concentration in the raw biogas was 32±2% throughout the operation of the two systems. No significant differences were observed in the CO_2 concentration in the upgraded biogas, during the stages II, III y IV. A volumetric gas load of 7.9 L d^{-1} per L of microalgae culture could be applied with a CO_2 removal around 98%. Average values of CO_2 concentration of 0.7% and 1.1% in R_1 and R_2 were achieved, respectively. The CO_2 concentration in the upgraded biogas fulfills most of biomethane standards (Marcogaz, 2006; Huguen and Le Saux, 2010).

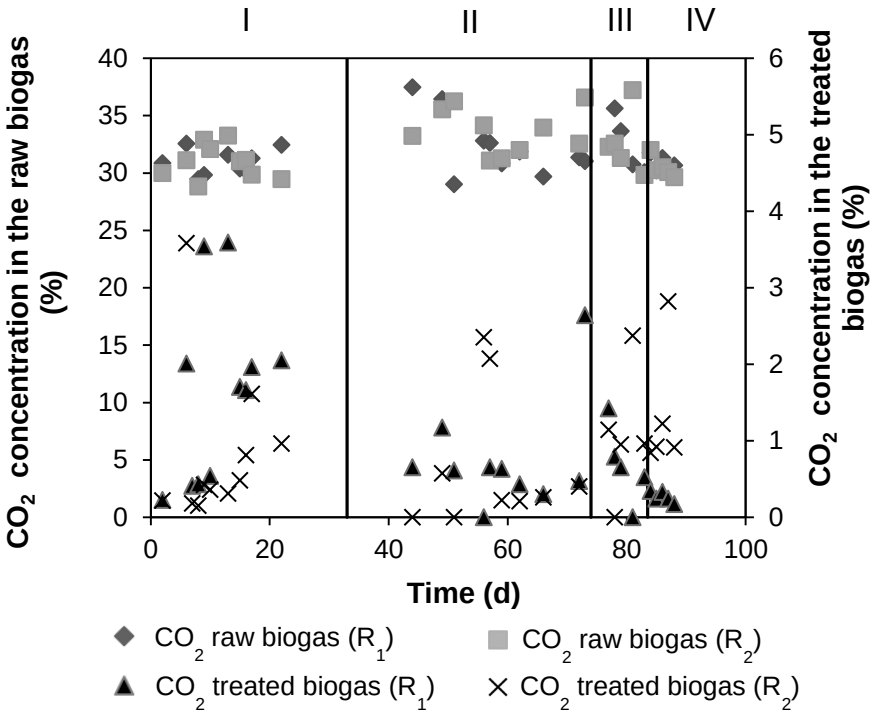

Figure 6-6. CO_2 concentration in the biogas inflow and outflow of the systems (R_1: system with H_2S; R_2: system without H_2S).

Table 6-3 shows the mass balance for inorganic carbon, applied to both tested systems. Evaluation was made considering the second stage (II) of the systems operation. The

CO_2 content in the raw biogas corresponded to 100% of the carbon that entered into the system. Only 37% of this carbon was fixed by the microalgae through photosynthesis, 12% was lost in the liquid effluent, dissolved as CO_2 or HCO_3^-/CO_3^{-2} and 50% was desorbed from photobioreactor. Such rate of CO_2 desorption from the photobioreactor was the result of the concentration of dissolved CO_2 (0.1 mmol L^{-1}), which is about 10 times higher than that at equilibrium the concentration of dissolved CO_2 in equilibrium with air. These results were similar to the carbon balance obtained when a similar two-stage process was operated using seawater and microalga *Nannochloropsis gaditana* (Meier *et al*, 2015)

Table 6-3. Inorganic carbon balance (Considering stage II; biogas flow:4.96 L d^{-1}; 28°C)

	System with H_2S (R_1)		**System without H_2S (R_2)**	
	mmol d^{-1}	%	mmol d^{-1}	%
Carbon input:				
(+) Carbon in gas inflow (raw biogas)	64.3	100	64.3	100
Carbon output:				
(-) Carbon desorbed from photobioreactor	32.3	50.2	32.0	49.8
(-) Carbon fixed by microalgae	23.5	36.5	23.5	36.5
(-) Dissolved carbon in the liquid effluent	7.6	11.8	7.6	11.8
(-) Carbon in gas outflow (upgraded biogas)	0.9	1.4	1.2	1.9

When the volumetric gas load increased to 4.0 and 7.9 L d^{-1} per L of microalgae, the desorption rate increased to 65 and 80% in the stages III y IV due to the increase of dissolved inorganic carbon in the photobioreactor (Figure 6-7). Dissolved inorganic carbon (DIC) concentration in the reactors R_1 and R_2 was around 18, 20 and 22 mmol L^{-1} in the stages II, III and IV. Bicarbonate was the predominant carbon species, as a

result of the operating pH (between 8.6 and 9.2). The injection of biogas-containing H_2S into the system R_1 did not cause any decrease in pH of the culture medium.

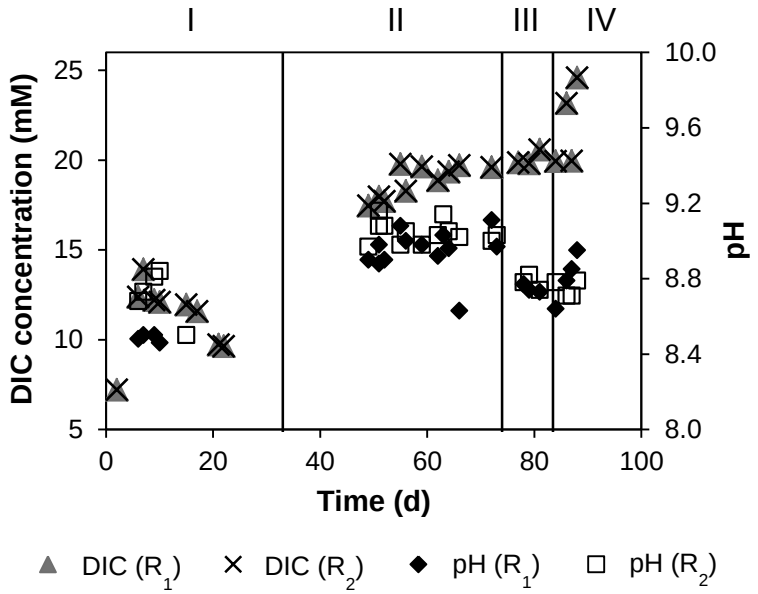

Figure 6-7. Dissolved inorganic carbon (DIC) concentration and pH in the photobioreactors R_1 and R_2 during the operation of the systems.

6.4. Conclusions

- H_2S and CO_2 could be simultaneously removed from biogas using a microalgae culture. In the tested system H_2S is oxidized to sulfate due to the high dissolved oxygen concentration in the photobioreactor.
- The presence of H_2S in the biogas did not affect CO_2 removal efficiency. 98% CO_2 removal was achieved in the systems with and without injection of H_2S.
- Under the tested conditions, the consumption of O_2 by the H_2S oxidation only cause a small decrease of the O_2 concentration in upgraded biogas. Since H_2S is almost completely transformed into sulfate, oxygen consumption would be the result of the relation between applied sulfide load and the amount of oxygen being transported from the bioreactor to the column.

CHAPTER VII

Does photosynthetic biogas upgrading have an opportunity?

Does photosynthetic biogas upgrading have an opportunity?

Abstract

Although experimental studies at lab-scale have indicated that the biogas upgrading using microalgae could be a feasible process, the design of a large-scale biogas upgrading plant using microalgae requires the identification of the key operational parameters and the determination of the maximum treatment capacity of the system. The aim of this work is to identify the principal operational parameters that affect the biogas upgrading system in order to evaluate the performance of the process. A mathematical model about microalgae culture for biogas upgrading considering a system composed of bubble column connected with an open-photobioreactor was developed. Simulations were carried out considering the values of parameters of raceway photobioreactor with microalga *Chlorella sorokiniana* and an industrial bubble column in order to evaluate the operation of a large scale system. A maximum volumetric gas load of 3.6 m^3 gas m^{-3} reactor d^{-1} could be injected into the system, because over this load the CO_2 exceeded 3%. Considering a volume/surface ratio of raceway of 0.25, a maximum capacity of 0.9 m^3 m^{-2} d^{-1} can be reached. Although a carbon removal efficiency from biogas of 90% could be obtained, around 84% of the removed carbon is lost by desorption from reactor. The maximum volumetric gas load could be reduced to 0.117 m^3 gas m^{-3} reactor d^{-1} for biogas treatment without carbon desorption. The main advantages of photosynthetic biogas upgrading over the traditional methods are lower investment and operational costs. However, one of the main disadvantages of the proposed system is its low biogas treatment capacity in comparison to traditional technologies.

7. Does photosynthetic biogas upgrading have an opportunity?

7.1. Introduction

Biogas is a biofuel produced from anaerobic digestion of organic matter and it is composed of a gas mixture, principally CH_4 (55-75%) and CO_2 (25-45%). The CH_4 is the component that gives the fuel characteristics to biogas, so better biogas quality is obtained when the CH_4 concentration is higher. Since CO_2 is the principal impurity of biogas, its removal is important for fulfilling the regulations, increasing the calorific value and avoiding operational problems. Upgraded biogas is a product of similar characteristics of natural gas and can be used as vehicle and household fuel. Regulations of European countries require a maximum CO_2 concentration in biogas between 2.5 and 6% to enable its injection in the natural gas networks (Marcogaz, 2006; Huguen and Le Saux, 2010).

The use of microalgae culture has been proposed as an innovative method to remove CO_2 from biogas. Microalgae are able to capture solar energy, have high growth rates and can be adapted to different environmental conditions (Wang *et al*, 2008). Experimental studies about biogas upgrading in microalgal photobioreactors indicate CO_2 removal efficiency around 74 - 98% (Conde *et al*, 1993; Mandeno *et al*, 2005; Converti *et al*, 2009; Mann *et al*, 2009; Kao *et al*, 2012).

Although a high CO_2 removal efficiency can be achieved, the microalgae perform oxygenic photosynthesis, whereby 1 mol of O_2 is released per captured mol of CO_2. Therefore, an upgraded biogas with around 20% of O_2 can be obtained, considering a biogas with a CO_2 concentration of 30% and direct injection into the microalgae culture in a closed photobioreactor (Converti *et al*, 2009; Mann *et al*, 2009; Meier *et al*, 2015).

The O_2 content in biogas must be minimized because CH_4/O_2 mixture is explosive and most standards require a maximal concentration of 1% in the biogas.

The use of an open-photobioreactor connected with a bubble column has been proposed to control the CO_2 and O_2 contents in the biogas. In this way, the CO_2 absorption is physically separated from the O_2 desorption, in a continuous two-stage process. The CO_2 is transferred from biogas to microalgae culture in the bubble column and CO_2 is fixed by microalgae in the photobioreactor. Desorption of the generated O_2 to the atmosphere is promoted in the open-photobioreactor, minimizing the release of O_2 by the upgraded biogas.

Experimental studies have been performed at laboratory scale. An upgraded biogas with 1.9% CO_2 and 1.2% O_2 was obtained using a 75 L open photobioreactor connected with a 0.7 L bubble column (2.2 m height) by continuous recirculation of microalgae culture (Meier *et al*, 2015). An upgraded biogas with 4% CO_2 and 0.2% O_2 was achieved using a 180 L raceway connected to a 0.8 L bubble column inoculated with a microalgal-bacterial consortium (Bahr *et al*, 2014). Although experimental studies have indicated that the biogas upgrading using microalgae could be a feasible process, the design of a large-scale biogas upgrading plant using microalgae requires the identification of the key parameters involved in the operation and their effect on the biogas quality, in order to evaluate the treatment capacity of the proposed system.

Taking advantage of existing mathematical model about microalgae growth rate and the theory of mass transfer in absorption column, it was possible to develop a mathematical model about microalgae culture for biogas upgrading considering a system composed of bubble column connected with an open-photobioreactor. By this mathematical model, the aims of this work are to evaluate the biogas upgrading capacity of the system at an

industrial scale and compare its performance with the traditional technologies. Thus, it is possible to analyze if the photosynthetic biogas upgrading have an opportunity in the market.

7.2. Methodology

7.2.1. Mathematical modeling

A mathematical model was performed to identify the main operational parameters and evaluate their effect on a biogas upgrading process. This mathematical model describes a system comprising a bubble column connected with an open-photobioreactor containing a culture of microalgae (Figure 7-1). In the proposed system the microalgae culture was circulated continuously between column and photobioreactor, so that the microalgae could use the absorbed CO_2 from biogas as a substrate and release O_2 into the environment. Table 7-1 shows the definition of the variables used in the mathematical model.

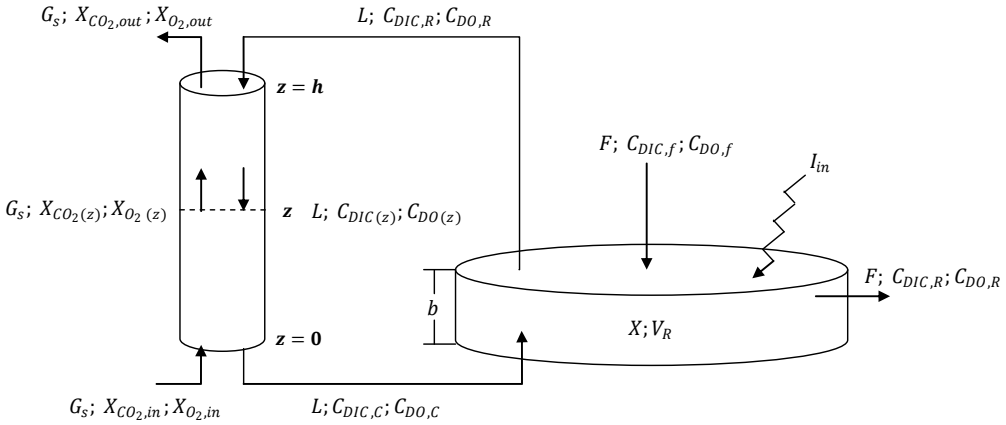

Figure 7-1. Bubble column connected to an open-photobioreactor.

Table 7-1. Definition of the main variables. (Here x_{CO_2} and x_{O_2} were the molar fractions in the gas).

Variable	Definition
G_s	Solvent CH_4 flow in the gas phase per cross-sectional area of the column (mol m^{-2} d^{-1})
G	Gas flow (L d^{-1})
L	Liquid recirculation flow between the column and photobioreactor (L d^{-1})
C_A	Liquid-phase concentration of A (mol L^{-1})
X_{CO_2}	Moles of CO_2 per mol of solvent CH_4 in the gas phase, that is, $X_{CO_2} = \frac{x_{CO_2}}{1-x_{CO_2}-x_{O_2}}$
X_{O_2}	Moles of O_2 per mol of solvent CH_4 in the gas phase, that is, $X_{O_2} = \frac{x_{O_2}}{1-x_{CO_2}-x_{O_2}}$
X	Biomass concentration (g L^{-1})
V_R	Volume of photobioreactor (L)
F	Feed flow of culture medium in the photobioreactor (L d^{-1})
I_{in}	Incident light intensity (µmol m^{-2} s^{-1})
b	Depth of the photobioreactor (m)
C, R, f	The subscripts C, R, f refer to the dissolved inorganic carbon (DIC) and dissolved oxygen (DO) concentration after passing through the absorption column, in the photobioreactor and in the feed input of culture medium, respectively.

7.2.1.1. Mathematical model of bubble column operation

It was considered a counter-flow bubble column with a gas feed flow at the bottom composed by CH_4 and CO_2, and a liquid feed flow at the top with dissolved inorganic carbon (DIC) and dissolved oxygen (DO) in its composition (Figure 7-1). It was assumed that there were only two possible transfers in the column, the transfer of CO_2 from the gas into the liquid (absorption) and the transfer of O_2 from the liquid into the gas (desorption), this implied that the concentration of CH_4 in the gas phase was constant along the column and that the gas could have O_2 in its composition at the top of the column. It was supposed that the column was always at steady state; however, the

variation of concentrations with the height of the column was considered. The following assumptions were considered:

(A1) each phase is in PF (piston flow), that means, that there is no axial mixing in the column, but complete radial mixing. Complete radial mixing implies that fluid properties, including velocity, are uniform across any plane perpendicular to the flow direction,

(A2) the temperature and the pressure are constant,

(A3) the operation is at steady state,

(A4) the two-film model is applicable,

(A5) the liquid flow rate is constant throughout the column; that is, the transfer of material from one phase to the other does not affect the rate of flow of the liquid phase (the dilute-system assumption),

(A6) the reversible reactions

$$CO_{2\,(aq)} + H_2O \leftrightarrow H^+ + HCO_3^-,$$

$$H_2O \leftrightarrow H^+ + OH^-,$$

take place in the liquid, and they are in chemical equilibrium, and

(A7) the liquid phase is not charged electrically (electroneutrality).

Considering a counter-flow bubble column, the following model can be obtained:

$$\frac{d}{dz}X_{CO_2} = \frac{(k_La)^c_{CO2}}{G_S}\left(\phi(C_{DIC}, Z) - H_{CO_2}P_0\frac{x_{CO_2}}{1+x_{CO_2}+x_{O_2}}\right), \quad (7.1)$$

$$\frac{d}{dz}\,C_{DIC} = \frac{(k_La)^c_{CO2}}{L}S\left(\phi(C_{DIC}, Z) - H_{CO_2}P_0\frac{x_{CO_2}}{1+x_{CO_2}+x_{O_2}}\right), \quad (7.2)$$

$$\frac{d}{dz}X_{O_2} = \frac{(k_L a)^C_{O_2}}{G_S}\left(C_{DO} - H_{O_2}P_0\frac{X_{O_2}}{1+X_{CO_2}+X_{O_2}}\right), \quad (7.3)$$

$$\frac{d}{dz}C_{DO} = \frac{(k_L a)^C_{O_2}}{L}S\left(C_{DO} - H_{O_2}P_0\frac{X_{O_2}}{1+X_{CO_2}+X_{O_2}}\right), \quad (7.4)$$

with boundary conditions

$C_{DIC}(h) = C_{DIC,R}$, $C_{DO}(h) = C_{DO,R}$, (top of the column)

$X_{CO_2}(0) = X_{CO_2,in}$, $X_{O_2}(0) = X_{O_2,in}$, (bottom of the column)

Here $\phi(C_{DIC}, Z)$ represents the concentration of dissolved CO_2 ($CO_{2(aq)}$), which depends on the DIC concentration and the concentration of cations Z that are not affected by chemical reactions. Z is the concentration of cations (mol L^{-1}) that are not affected by biochemical reactions and is constant along the column, $k_L a$ is the gas-liquid mass transfer coefficient, S is the cross-sectional area of the column, H is the Henry constant and P_0 is the absolute pressure in the column.

The $k_L a$ in the column was calculated from superficial gas velocity and physical properties of gas and liquid phase, according to equations 7.5 and 7.6 (Kantarci *et al*, 2005).

$$k_L a = 0.6\left(\frac{\upsilon}{D_{AB}}\right)^{0.5}\left(\frac{g\,D_T^2\,\rho_L}{\sigma}\right)^{0.62}\left(\frac{g\,D_T^3}{\upsilon_L^2}\right)^{0.31}\varepsilon_g^{1.1}\left(\frac{D_{AB}}{D_T^2}\right) \quad (7.5)$$

$$\varepsilon_g = 0.505 V_g^{\,0.47}\left(\frac{0.072}{\sigma}\right)^{2/3}\left(\frac{0.001}{\mu_L}\right)^{0.05} \quad (7.6)$$

Here υ_L is the kinematic viscosity of liquid phase, D_{AB} is the gas-liquid diffusion coefficient, D_T is the column diameter, ρ_L is the liquid density, σ is the surface tension, g is the gravitational acceleration, ε_g is the gas holdup, μ_L is the viscosity of liquid phase and V_g is the superficial gas velocity.

7.2.1.2. Mathematical model of open-photobioreactor operation

An open photobioreactor operating in continuous mode was considered. Constant stirring was supposed to keep the concentrations homogeneous in the medium. The mathematical model of photobioreactor was performed based on the mass balances of inorganic carbon, oxygen and biomass. The specific growth rate of microalgae was calculated considering a Monod kinetic with substrate (CO_2) and light limitation. The following mass balances for the photobioreactor were performed

$$\dot{X} = \mu_{(C_{DIC},X)}X - DX, \quad (7.7)$$

$$\dot{C}_{DIC} = -\frac{1}{Y_{CO_2}}\mu_{(C_{DIC},X)}X + D\left(C_{DIC,f} - C_{DIC,R}\right) + \frac{L}{V_R}\left(C_{DIC,C} - C_{DIC,R}\right) + (k_La)^R_{CO_2}\left(\phi(C_{DIC,R}, Z) - C^*_{CO_2}\right) \quad (7.8)$$

$$\dot{C}_{DO} = \frac{1}{Y_{O_2}}\mu_{(C_{DIC},X)}X + D\left(C_{DO,f} - C_{DO,R}\right) + \frac{L}{V_R}\left(C_{DO,C} - C_{DO,R}\right) + (k_La)^R_{O_2}\left(C_{DO,R} - C^*_{DO}\right) \quad (7.9)$$

$$\dot{Z} = D(Z_f - Z_R), \quad (7.10)$$

Here C_{DIC}, and C_{DO} denote the concentrations of the dissolved inorganic carbon (DIC) and dissolved oxygen (DO) (mol L^{-1}) respectively, $\mu_{(C_{DIC},X)}$ represents the specific growth rate (d^{-1}), $C_{DIC,f}$ and $C_{DO,f}$ are the input nutrient concentrations in the culture medium (mol L^{-1}) and D is the dilution rate. The terms $\frac{L}{V_R}(C_{DO,C} - C_{DO,R})$ and $\frac{L}{V_R}(C_{DIC,C} - C_{DIC,R})$ are referred as the recirculation terms and they represent the mass interchange between the column and the photobioreactor.

The specific growth velocity μ was defined according to equation 7.11 (Bernard, 2011).

$$\mu = \mu_{max} * \left(\frac{C_{CO_2,R}}{C_{CO_2,R} + k_{CO_2}}\right) * \frac{1}{aXb}\ln\left(\frac{I_{in} + k_I}{I_{in}\exp(-aXb) + k_I}\right) \quad (7.11)$$

Where μ_{max} is the maximum specific growth velocity, $k_{CO_2}$is the substrate saturation constant, b is the depth of the photobioreactor, a is the absorption coefficient of

microalgae biomass, I_{in} is the incident light intensity and k_I is the light saturation constant.

The relation between $(K_L a)^c_{CO_2}$ and $(K_L a)^c_{O_2}$ was calculated using the equation from Babcock *et al* (2002).

$$(k_L a)^c_{CO_2} = \sqrt{\frac{D_{CO_2}}{D_{O_2}}}(k_L a)^c_{O_2} \quad (7.12)$$

The photobioreactor model and the absorption column model are coupled in the boundary conditions and the recirculation terms.

7.2.2. Comparison of mathematical model with experimental results

The results obtained using the mathematical model were compared with experimental data. To verify the bubble column model, a counter-flow column of 3 m high and 0.012 m diameter was operated injecting real biogas (30±2% CO_2) and circulating water with different DIC concentrations. The $NaHCO_3$ was added to obtained different DIC concentration. Table 7-2 shows some of the applied conditions in the bubble column.

Table 7-2. Description of conditions used for verifying the bubble column model.

Condition	Biogas flow (L d^{-1})	Liquid flow (L d^{-1})	DIC concentration (mol L^{-1})	DO concentration (mol L^{-1})
1	24.5	23.8	0.017	0.0003
2	24.5	23.8	0.017	0.0000
3	24.5	23.8	0.015	0.0003
4	24.5	23.8	0.009	0.0003
5	24.5	37.8	0.017	0.0003
6	24.5	37.8	0.017	0.0000
7	40.3	23.8	0.016	0.0003
8	40.3	23.8	0.009	0.0003

The photobioreactor model was compared with experimental results obtained in a previous assay. This experiment was performed using a 2 L open-photobioreactor connected to a bubble column operated in counter-flow mode (dimensions: 1.71 m high and 0.012 m diameter). Real biogas (32% CO_2) was continuously injected into the bottom of the column, while microalgae culture was continuously circulated between the photobioreactor and the column (circulation flow: 15 L d^{-1}).

7.2.3. Simulations using mathematical model

Different conditions were simulated using mathematical model in order to evaluate the performance of the proposed system and identify the key operational parameters. Simulations were performed using Matlab 8.0 (Mathworks). Simulations were carried out considering the values of parameters of raceway photobioreactor, microalga *Chlorella sorokiniana* and an industrial bubble column in order to evaluate the operation of a large scale system (Table 7-3). Raceways are the most applied cultivation system for large scale microalgae production due to the lower costs of implementation and operation.

Table 7-3. Parameters used in the simulations.

Parameter	Data	Reference
Biogas composition	30% CO_2, 0% O_2	Data obtained experimentally from UASB anaerobic digester
μ_{max}	0.1 h^{-1}	
k_{CO_2}	2.04x10^{-6} mol CO_2 L^{-1}	Data obtained experimentally using *Chlorella sorokiniana* at pH 7.0.
I_{in}	2000 µmol m^{-2} s^{-1}	Average light intensity in Antofagasta, Chile (UTFSM, 2008).
a	500 m^2 kg^{-1}	Data obtained experimentally for *Chlorella sorokiniana* culture
k_I	200 µmol m^{-2} s^{-1}	Data for *Chlorella (*Hanagata, 1992)
$k_L a^R_{O_2}$	21.6 d^{-1}	Raceway photobioreactor (Mendoza *et al*, 2013)
$k_L a^R_{CO_2}$	19.4 d^{-1}	Calculated using equation 2.11
b	0.25 m	Typical depth of raceway (Scott, 2010; Mendoza *et al*, 2013)

7.2.4. Determination of capacity of the system

Since the maximum capacity of the system depends on its objective, the maximum flow of biogas that can be treated was calculated considering two scenarios: 1) Determination of the maximum biogas flow that can be treated fulfilling the biomethane standards when the objective is only to upgrade biogas. 2) Determination of maximum biogas flow minimizing the CO_2 desorption from photobioreactor into atmosphere when the objective is to upgrade biogas and avoid the release of CO_2 into atmosphere. According to most biomethane standards, a maximum concentration of CO_2 and O_2 of 3% and 1% in the upgraded biogas was considered in all calculations.

7.2.5. Comparison of the proposed system with traditional technologies.

The proposed system was compared with traditional technologies for biogas upgrading about costs and treatment capacity per m^2 based on the information available in the literature. The analyzed traditional technologies were water scrubbing, chemical scrubbing, pressure swing adsorption (PSA), membrane separation and cryogenic separation. Information about these technologies were obtained from Muñoz *et al* (2015).

To evaluate the cost of photosynthetic biogas upgrading, the microalgae cultivation in a raceway pond and a large-scale bubble column were considered. The costs of microalgae cultivation were obtained from Slade and Bauen (2013), Norsker *et al* (2011) and Rogers *et al* (2014). The investment cost of bubble column was calculated from construction of steel column of 10 m high.

7.3. Results and discussion

7.3.1. Comparison of mathematical model with experimental results.

To verify the results estimated by the bubble column model, a column operated only with water/HCO_3^{-2} recirculation was used. Water was used instead to microalgae culture for better control of the variables. No difference should be obtained because it is assumed that there is no photosynthetic CO_2 capture in the column due to the short residence time of microalgae inside the column and the absence of light.

Table 7-4 shows the calculated treated biogas composition using bubble column model and the experimental data. Although the model of the bubble column did not exactly replicate the experimental results, good agreement was observed in calculations with experimental data and a maximum absolute error for CO_2 and O_2 concentration of 2.2 % and 0.2 % was obtained, respectively.

Table 7-4. Comparison between gas composition obtained from experimental assays and bubble column model.

	CO_2 concentration (%)		**O_2 concentration (%)**	
Condition	Experimental data	Model data	Experimental data	Model data
1	5.4	5.4	0.50	0.43
2	5.4	5.3	0.00	0.00
3	7.0	6.7	0.35	0.50
4	9.1	6.9	0.46	0.45
5	1.2	1.3	0.63	0.51
6	1.2	1.3	0.00	0.00
7	13.5	13.6	0.23	0.25
8	13.0	13.0	0.18	0.25

In Figure 7-2, the fluctuation of pH and DIC concentration along of the column is showed. Since biogas was injected at the bottom of the column, pH decreased and DIC concentration increased, when water flowed from the top to the bottom of the column. Mathematical model represented this behavior and the values of pH and DIC concentration at the bottom of the column presented a maximum absolute error of 0.1 and 0.003 mol L^{-1}, respectively, under all tested conditions.

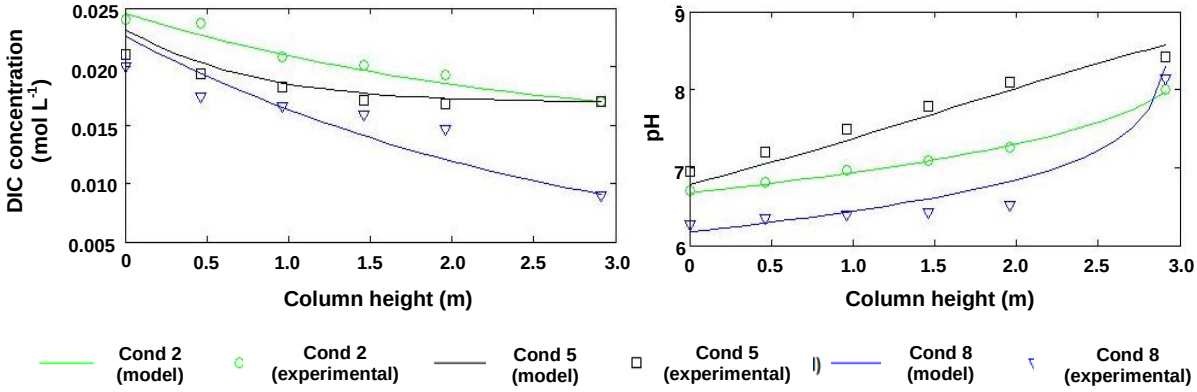

Figure 7-2. pH and DIC concentration along bubble column according to mathematical model and experimental results. Conditions (Cond) are described in Table 7-2.

To check the photobioreactor-column model, the treated biogas composition predicted by the mathematical model was compared with the results obtained previously in the operation of a column-photobioreactor system (Figure 7-3). The experimental system was operated for 90 days applying three volumetric gas loads. The mathematical model was not calibrated with experimental results, the growth kinetic parameters and mass transfer coefficients were obtained from literature and batch assays.

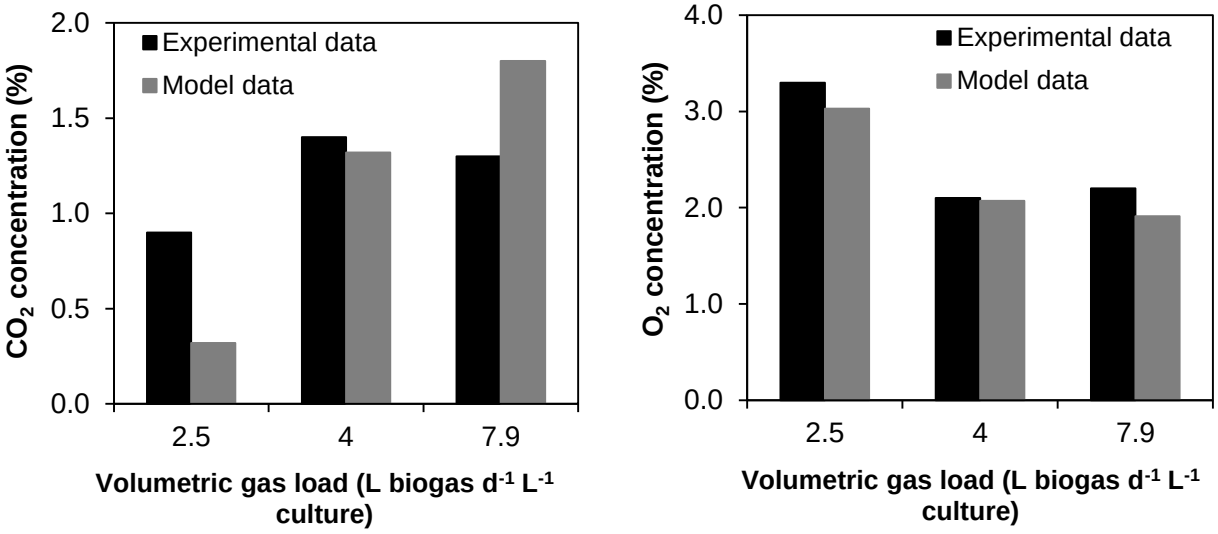

Figure 7-3. Comparison between gas composition obtained from experimental assays and bubble column-photobioreactor model.

As shown in Figure 7-3, the model simulations had a good agreement with the experiment data. The O_2 concentration adjusted better than the CO_2 concentration. However, the maximum absolute error in the CO_2 concentration only corresponded to 0.6%.

Taking into account that the proposed mathematical model was developed from validated models about mass transfer in absorption columns and microalgae growth model and since the proposed model could estimate the composition of treated gas with a lower error, it is considered that this model can be used to study the behavior of the system and evaluate its capacity at large scale.

7.3.2. Identification of key operational parameters

Important parameters in the operation of a biogas upgrading process by microalgae are dilution rate (D), mass transfer coefficient of photobioreactor (k_La_R), photobioreactor

volume (V_R), height of bubble column (H), volumetric gas load and the ratio between liquid recirculation flow and gas flow (L/G).

The dilution rate depends on the specific growth rate of the microalgae culture in the conditions of light intensity, temperature and nutrients concentration in the photobioreactor. Therefore, it is a parameter that is not easily manipulated to optimize the process. However, a lower dilution rate allows obtaining a higher concentration of biomass and consequently, allows capturing a greater percentage of carbon by biomass. In this work, a dilution rate of 0.2 d^{-1} was considered, according to experimental results using the microalga *Chlorella sorokiniana*.

On the other hand, mass transfer coefficient (k_La) in the photobioreactor depends on the design of the reactor. Raceway ponds are the most applied cultivation system for large scale microalgae production. Such photobioreactors generally have a mass transfer coefficient around 0.9 h^{-1} (Babcock *et al*, 2002; Mendoza *et al*, 2013). In this way, if the biogas upgrading process is carried out in a raceway pond, k_La is subjected to the mass transfer limitations of such reactors. However, simulations with different k_La showed that although a higher coefficient enables a better quality of treated biogas, it is produced an increase of the amount of CO_2 released into the atmosphere from photobioreactor.

Other parameters such as light intensity, temperature and raw biogas composition depend on the conditions in the place of operation and can not be optimized.

Therefore, only the effect of L/G, volumetric gas load, volume of photobioreactor and height of column were studied.

7.3.2.1. *Effect of photobioreactor volume (V_R) and height of column (H)*

The design of biogas upgrading plant requires optimizing the ratio between the photobioreactor volume (V_R) and the column volume (V_C). Figure 7-4 shows the maximum biogas flow (m^3 d^{-1}) that can be upgraded per m^3 of microalgae culture.

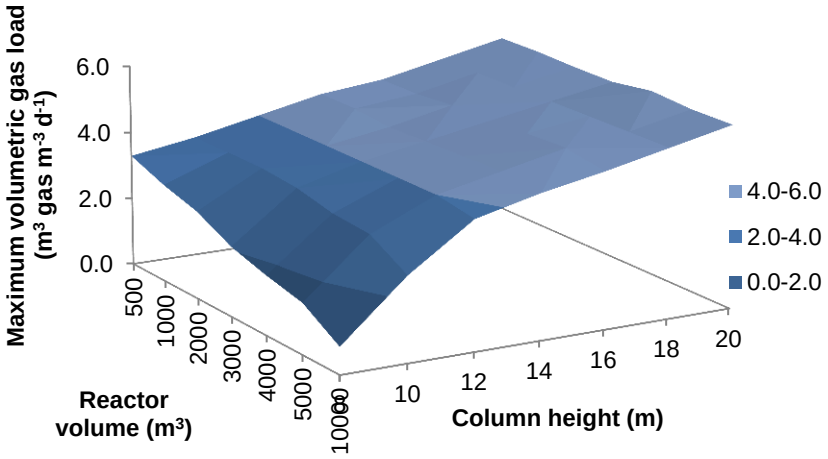

Figure 7-4. Effect of column height and photobioreactor volume on the biogas upgrading capacity of the proposed system.

The industrial bubble columns have heights between 10 and 30 m with a height/diameter (H/D) ratio between 5 and 10 (Kantarci *et al*, 2005; Shaikh and Al-Dahhan, 2013). As it is shown in Figure 7-4, when the column height is greater, the biogas treatment capacity increases. The treatment capacity of a 20 m bubble column can be 1.5 times higher than the capacity of a 10 m bubble column. However, the investment costs of a 20 m bubble column can be 6 times higher than the construction of a 10 m column. Building a higher column requires greater investment in structural support (concrete and ironwork). In addition, the cost of pumping of microalgae culture from reactor to bubble column can be duplicated. In all calculations a H/D ratio of 10 was chose to promote higher gas/liquid mass transfer.

On the other hand, Figure 7-4 shows that when the photobioreactor volume increases, the biogas upgrading capacity at low column heights is reduced. This capacity reduction occurs because the photobioreactor volume is oversized for the carbon removal capacity of the column. Therefore, a ratio between reactor volume and bubble column volume (V_R/V_C) between 100 and 300 is recommended.

Therefore, a bubble column of 10 m high and a (V_R/V_C) ratio lower than 300 were applied in all calculations.

7.3.2.2. Effect of ratio between liquid recirculation flow and gas flow (L/G) and volumetric gas load.

The volumetric gas load and the ratio between liquid recirculation flow and gas flow are the easiest parameters to manipulate for controlling the operation of the system. Figure 7-5 shows the effect of these parameters on the upgraded biogas quality.

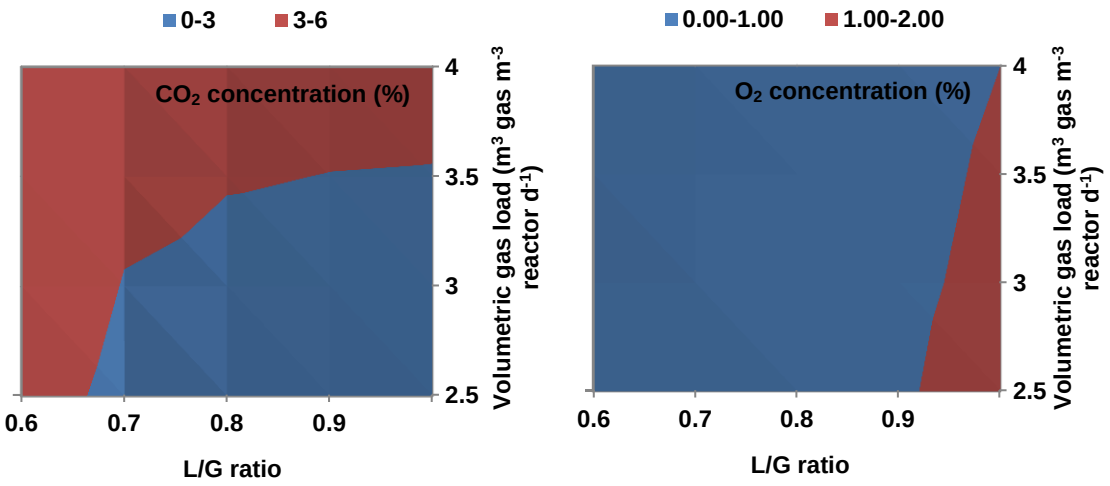

Figure 7-5. CO_2 and O_2 concentration in upgraded biogas using different volumetric gas loads and L/G ratios (H=10m, H/D=10, V_R=2000m^3).

Results indicate that liquid phase circulation is key operational factor determining the composition of the upgraded biogas. A higher liquid recirculation flow allows

increasing the mass transfer capacity causing a lower CO_2 concentration in the upgraded biogas. However, the increase of the recirculation flow produces an increase of the O_2 concentration in the upgraded biogas because the dissolved oxygen that is transferred to the bubble column (dissolved in the liquid) is higher. Thus, there is a compromise between a lower CO_2 concentration and a lower O_2 concentration. This result coincided with the experimental observations. In Meier *et al* (2015), CO_2 and O_2 concentrations of 1.9% and 1.2% were achieved in upgraded biogas when a circulating flow of 14.4 L d^{-1} was applied, while at higher circulation rates (115.2 L d^{-1}), CO_2 absorption was almost complete (0.1% CO_2), but O_2 concentration in upgraded biogas increased to around 6%.

When the gas flow is increased, the liquid recirculation flow has to be increased to maintain the gas/liquid mass transfer. As expected, a higher volumetric gas load produced an increase of CO_2 concentration in the upgraded biogas and a decrease of O_2 concentration. A concentration of CO_2 and O_2 lower than 3 and 1% can be achieved applying a volumetric gas load lower than 4 m^3 d^{-1} per m^3 reactor with a L/G ratio between 0.7 and 0.9.

Figure 7-6 shows the effect of L/G and volumetric gas load on the carbon mass balance. The CO_2 removed from biogas can be either consumed by the microalgae, leave the system as dissolved inorganic carbon in the liquid effluent, or be desorbed from the microalgae cultivation. As shown in Figure 7-6A, different L/G and/or volumetric gas load did not affect significantly the biomass productivity and consequently, the amount of CO_2 captured by biomass only changed 1.9 ± 0.2 mmol L^{-1} d^{-1} (Figure 7-6A). An increase of CO_2 removal efficiency caused by a higher L/G ratio did not produce an increase of biomass productivity because dissolved CO_2 in the photobioreactor is 2 to 3 orders of magnitude greater than substrate saturation constant (k_{CO_2}) in all tested conditions. So, microalgae are not limited by substrate, but they are only limited by

light. Therefore, when CO_2 removal efficiency increases due to a higher L/G, a greater amount of carbon is lost through desorption from the photobioreactor and through the liquid effluent as dissolved inorganic carbon (Figure 7-6B and Figure 7-6C).

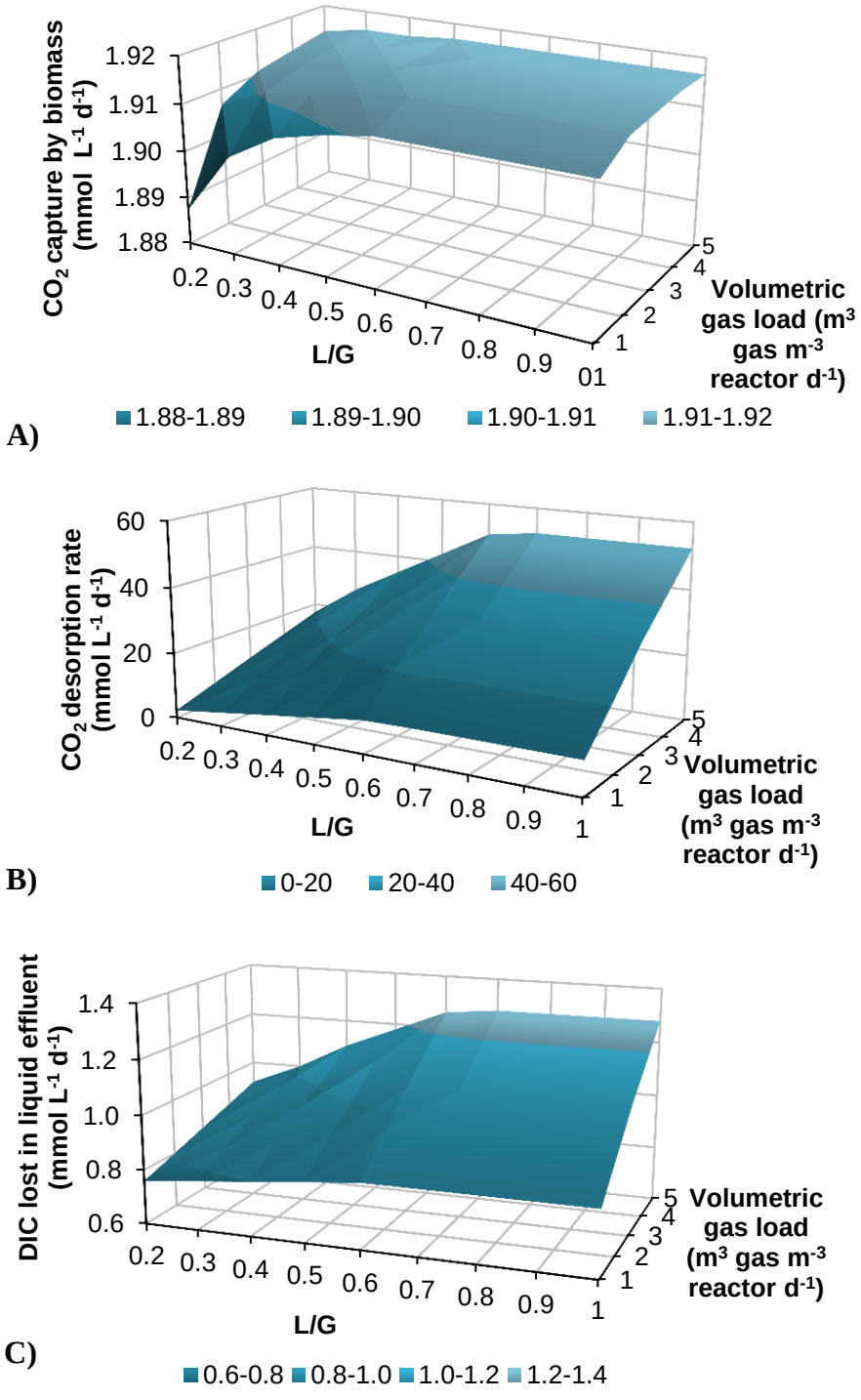

Figure 7-6. Effect of L/G ratio and volumetric gas load on biomass productivity (A), CO_2 desorption (B) and DIC loss in the liquid effluent (C).

7.3.3. Maximum capacity for biogas upgrading fulfilling the biomethane standards

The maximum capacity of the proposed system was evaluated considering a maximum CO_2 and O_2 concentration in the upgraded biogas of 3% and 1%, respectively. These limits were used according to the requirements of international standards about the use of biogas as biomethane.

Figure 7-7 shows the compliance area of biomethane standards (white color). A maximum volumetric gas load of 3.6 m^3 gas m^{-3} reactor d^{-1} could be injected into the system, because over this load the CO_2 exceeded 3%. Considering a volume/surface ratio of raceway of 0.25 (Table 7-3), a maximum capacity of 0.9 m^3 m^{-2} d^{-1} can be reached.

Applying this biogas flow, a superficial gas velocity of 10 cm s^{-1} is obtained in the column (D_c=1m). Although this gas velocity corresponds to a churn-turbulent flow regime (heterogeneous regime), this velocity is close to the lowest limit of the range of velocities applied in the industrial bubble column, which are usually operated between 10 and 40 cm s^{-1} (Krishna and Van Baten, 2001). This low superficial gas velocity allows to avoid the bubble coalescence and maintain a high gas mass transfer capacity (Kantarci *et al*, 2005).

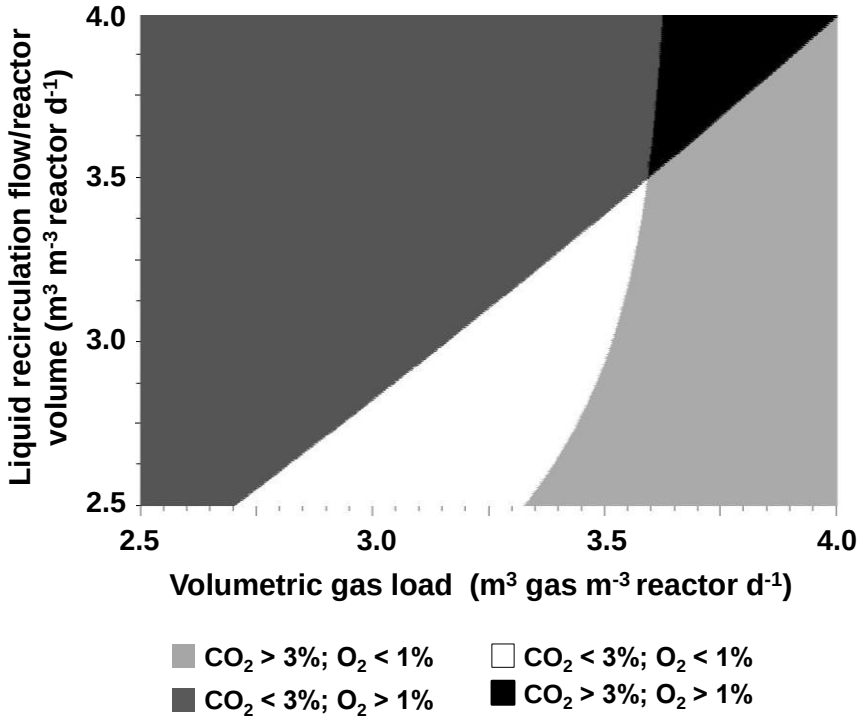

Figure 7-7. Determination of maximum capacity for upgrading biogas considering the biomethane standard requirements (H=10m, H/D=10, V_R=2000 m^3). Liquid recirculation and gas flow are represented per photobioreactor volume.

As it is shown in Figure 7-7, when the volumetric gas load flow is increased, the liquid recirculation flow has to be increased to maintain gas/liquid mass transfer. However, high liquid flow/gas flow (L/G) ratios cause a high O_2 concentration in the upgraded biogas.

Figure 7-8 shows a carbon mass balance in the system column-photobioreactor when a biogas load of 3.6 m^3 gas m^{-3} reactor d^{-1} is applied. Although a carbon removal efficiency from biogas of 90% could be obtained, a high fraction (around 84%) of the removed carbon is lost by desorption from reactor. Only 4% of carbon is captured by biomass. Raceway ponds are the most commonly used for large-scale microalgae cultivation for their lower investment and operating costs. However, they have the disadvantage of achieving low biomass productivity and, as consequence a low CO_2

capture rate can be obtained. So that the resulting carbon mass balance can be representative of what may happen in a large-scale open-photobioreactor. The capacity of CO_2 absorption was obtained with a biomass productivity of 12.5 g m^{-2} d^{-1}, which is representative of typical values obtained in open-photobioreactors. Raceways normally present biomass productivities between 10 and 15 g m^{-2} d^{-1} (Norsker *et al*, 2011; Rogers *et al*, 2014).

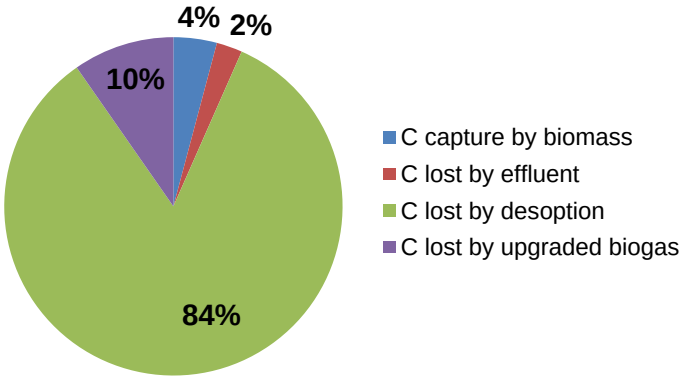

Figure 7-8. Carbon mass balance of system column-photobioreactor (3.6 m^3 gas m^{-3} reactor d^{-1}, H=10m, H/D=10, V_R=2000m^3, L/G=0.9).

If the system is only designed to purify biogas, CO_2 desorption into the atmosphere from the photobioreactor should not be a problem. However, if one of the objectives is to avoid the release of CO_2 into atmosphere (for example the sale of Certified Emission Reduction (CERs)), CO_2 desorption should be controlled.

7.3.4. Maximum capacity for biogas upgrading reducing CO_2 release into the atmosphere.

Although a high capacity of CO_2 removal from biogas can be reached, desorption is the main mechanism of carbon removal into the photobioreactor. Thus, only a low fraction

of the carbon removed from biogas is transformed into biomass and the remaining fraction is released to atmosphere.

To calculate the maximum capacity of the system without CO_2 release into atmosphere, the inorganic carbon assimilation by microalgae was evaluated considering that 1 g biomass (expressed as volatile suspended solids, VSS) fixes 1.83 g of CO_2 (Chisti, 2007).

Figure 7-9 shows the compliance area of biomethane standards avoiding the CO_2 desorption from photobioreactor into atmosphere (white colour). A maximum volumetric gas load of 0.117 m^3 gas m^{-3} reactor d^{-1} can be injected into the system without carbon desorption. Considering a volume/surface ratio of raceway of 0.25, only a maximum capacity of 0.03 m^3 m^{-2} d^{-1} can be reached. Composition of upgraded biogas using a biogas flow of 0.117 m^3 gas m^{-3} reactor d^{-1} was 0.01% of CO_2 and 0.81% of O_2.

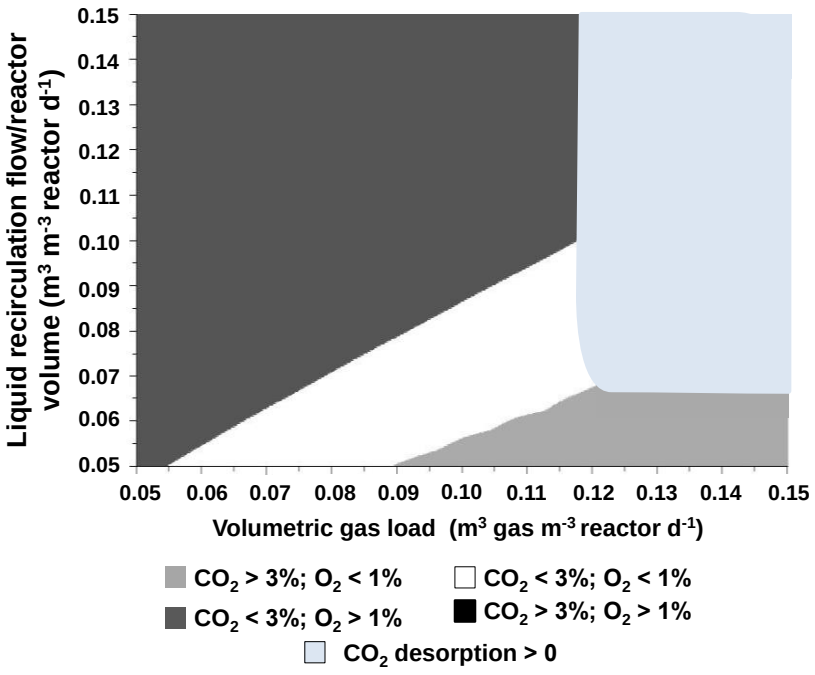

Figure 7-9. Determination of maximum capacity for upgrading biogas considering the biomethane standard requirements and avoiding the CO_2 desorption (H=10m, H/D=10, V_R=2000m^3). Liquid recirculation and gas flow are represented per reactor volume.

Figure 7-10 shows a carbon mass balance in the system column-photobioreactor when a biogas load of 0.117 m^3 gas m^{-3} reactor d^{-1} is applied. According to mass balance, if CO_2 release into atmosphere from reactor is avoided, 68% of carbon is captured by biomass and 32% is lost in the liquid effluent from reactor, mainly as bicarbonate. The carbon loss in the effluent is difficult to avoid because CO_2 is a highly soluble gas due to its ability to react with water to form $H_2CO_3/HCO_3^-/CO_3^{-2}$ depending on pH. At pH 8, the most inorganic carbon is as bicarbonate.

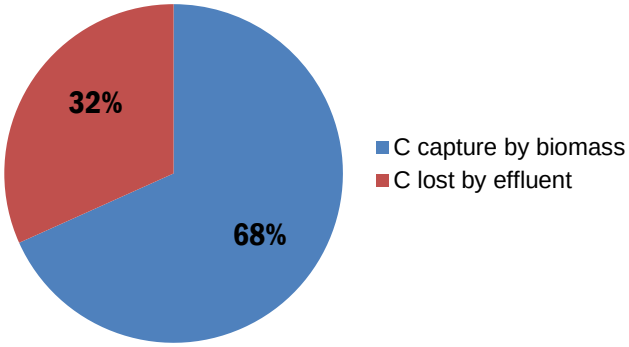

Figure 7-10. Carbon mass balance of system column-photobioreactor (0.117 m^3 gas m^{-3} reactor d^{-1}, H=10m, H/D=10, V_R=2000m^3, L/G=0.7)

Since the biogas flow was reduced to avoid the CO_2 desorption from photobioreactor, the column volume is oversized. Therefore, the column height can be decreased from 10 m to 7.5 m, maintaining the CO_2 removal efficiency around 100% and can be reduced to 6.5 m, decreasing the removal efficiency to 90% without failing to fulfill the biomethane standards (Figure 7-11). According to Figure 7-12, 6% carbon can be lost in the upgraded biogas if a 6.5 m bubble column is used. However, the CO_2 concentration in the upgraded biogas is lower than 3% and it is possible to fulfill the biomethane standards.

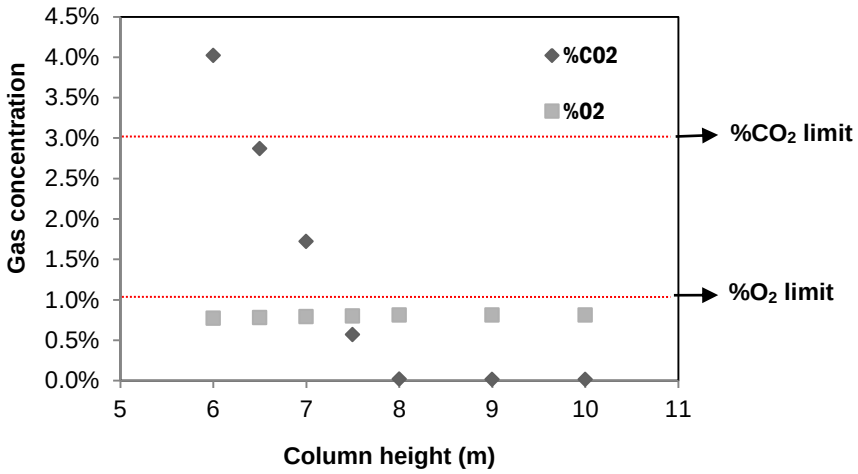

Figure 7-11. Gas concentration at different column height (0.117 m^3 gas m^{-3} reactor d^{-1}, H/D=10, V_R=2000m^3, L/G=0.7).

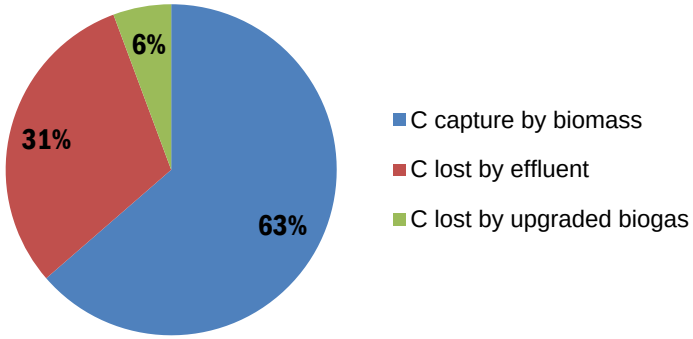

Figure 7-12. Carbon mass balance of system column-photobioreactor (0.117 m^3 gas m^{-3} reactor d^{-1}, H= 6.5 m, H/D=10, V_R=2000m^3, L/G=0.7).

7.3.5. *Comparison between the proposed system and traditional technologies for upgrading biogas.*

Table 7-5 and Table 7-6 show an approximation of the investment and operation costs of biogas upgrading using microalgae. The main investment cost of raceway ponds includes the photobioreactor construction (paddlewheels, liners and cover). Costs of other related facilities are also included, such as medium preparation unit, settler, harvest storage tank, instrumentation and control, piping, among others. The investment

cost of bubble column was calculated from construction of steel column of 10 m high. The main costs of column construction are the ironwork to support the structure and iron sheet. Concrete foundation, piping and paint were also considered.

The main operational costs are maintenance costs, electrical energy for pumping and paddlewheels operation and nutrients of culture medium.

Table 7-5. Investment costs of biogas upgrading using microalgae (€ per treated-biogas flow)

	Investment cost		**Basis of calculation**	**Reference**
Raceway ponds	11 – 14 € m^{-3} d^{-1}	264-336 € m^{-3} h^{-1}	9.6 – 12.6 € m^{-2} (0.9 m^3 m^{-2} d^{-1})	(Norsker *et al*, 2011; Slade and Bauen, 2013; Rogers *et al*, 2014)
Bubble column	8.19 € m^{-3} d^{-1}	197 € m^{-3} h^{-1}	59000 € (column 10 m high; 7200 m^3 d^{-1})	
Recirculation pump	0.91 € m^{-3} d^{-1}	22 € m^{-3} h^{-1}	6567 € (7200 m^3 d^{-1})	(KSB, 2016)
Total	20.1 – 23.1€ m^{-3} d^{-1}	**482-554 € m^{-3} h^{-1}**		

Table 7-6. Operational costs of biogas upgrading using microalgae (€ per m^3 of treated-biogas).

	Operational cost	**Basis of calculation**	**Reference**
Raceway ponds operation	0.003-0.022 €/m^3	0.2 – 1.6 € kg^{-1} (0.9 m^3 m^{-2} d^{-1}; 12.5 g m^{-2} d^{-1})	(Norsker *et al*, 2011; Slade and Bauen, 2013; Rogers *et al*, 2014)
Gas and liquid pumping	0.008 €/m^3		(KSB, 2016)
Total	**0.01 – 0.03 €/m^3**		

Table 7-7 shows the comparison between the proposed system and the traditional technologies for upgrading biogas based on costs and operational conditions. The main advantages of photosynthetic biogas upgrading over the traditional methods are the operation at atmospheric pressure, the injection of biogas without pre-treatment to remove H_2O and H_2S and lower investment and operational costs. However, one of the main disadvantages of the biogas upgrading process by microalgae is its low biogas treatment capacity per area in comparison to traditional technologies (Table 7-8). Traditional technologies used for CO_2 removal from biogas, such as high pressure water scrubber (HPWS) and pressure swing adsorption (PSA) are characterized by compact systems with high CO_2 removal capacities, which allow achieving the biogas treatment in a small space.

Table 7-7. Comparison between the proposed system and traditional technologies (Norsker *et al*, 2011; Slade and Bauen, 2013; Rogers *et al*, 2014; Muñoz *et al*, 2015).

	Photosynthetic upgrading*	**High pressure water scrubbing**	**Chemical scrubbing**	**PSA**	**Membrane**	**Cryogenic**
Pressure (bar)	atm	6 -10	atm	4 - 10	20-40 (gas/gas); atm (gas/liquid)	80
%CH_4	>90	>96	96-98.5	96-98	92-98	97
H_2O removal	no	no	yes	yes	yes	yes
H_2S pretreatment	no	no (but it is recommended)	yes	yes	yes	yes
Investment cost (€ Nm^{-3} h^{-1})	482 - 554 (≈300 Nm^3 h^{-1}) 60% raceway ponds costs; 40% bubble column costs	5500 - 2500 (100-500 Nm^3 h^{-1}); 1800-2000 (over 1000 Nm^3 h^{-1})	3200-1500 (600-1800 Nm^3 h^{-1})	2700-1500 (600-2000 Nm^3 h^{-1})	2500-6000 (400-100 Nm^3 h^{-1}); 2000 (1000 Nm^3 h^{-1})	
Cost price per Nm^3 biogas upgraded (€)	0.01 – 0.03 (≈300 Nm^3 h^{-1})	0.11 -0.15 (200-300 Nm^3 h^{-1})	0.1 - 0.28	0.25	0.12-0.22	0.4

* Considering a biogas treatment capacity 0.9 m^3 m^{-2} d^{-1}, biomass productivity of 12.5 g m^{-2} d^{-1} and a bubble column of 10 m high.

Table 7-8. Comparison of treatment capacity per m^2 among the proposed system and other technologies

Technology	Treatment capacity (m^3 m^{-2} d^{-1})	Area needed (m^2) to treat 1000 m^3 d^{-1}	Reference
Photosynthetic upgrading	0.9	1111	This work
Photosynthetic upgrading without CO_2 desorption	0.03	33333	This work
High pressure water scrubbing	71 - 750	1.3 - 13	(DMT, 2015)
Membrane	191-525	1.9 - 5.2	(DMT, 2014)
PSA	48-233	4.3 - 20.8	(Carbotech, 2016)

Therefore, the photosynthetic biogas upgrading can be an alternative in places where there is land available at low prices. Additionally, location of the proposed system should provide favorable conditions for the microalgae cultivation to maintain constant biomass productivity. The profitability of the system increases if the biogas upgrading process is inserted in a global project that involves the use of microalgae biomass in a productive process, such as, biofuel production.

7.4. Conclusions

The maximum biogas capacity of the photosynthetic biogas upgrading depends on the objective of the system. If the objective is only to upgrade biogas fulfilling the biomethane standards, a theoretical maximum biogas treatment capacity of 3.6 m^3 d^{-1} per m^3 reactor could be achieved using an open-photobioreactor connected to bubble column system. If the objective is to upgrade biogas and avoid the release of CO_2 into

atmosphere, the maximum biogas treatment capacity should be reduced down to 0.12 m^3 d^{-1} per m^3 reactor.

The photosynthetic biogas upgrading has lower investment and operation costs than the traditional technologies. However, biogas upgrading by microalgae has lower biogas treatment capacity per m^2 in comparison to traditional technologies. Therefore, the proposed system could be a feasible process in places where there is enough available land.

CHAPTER VIII

General discussion and conclusions

8. General discussion and conclusions

8.1. General discussion

The proposed system represents a feasible alternative for biogas upgrading. The use of a bubble column connected to an open-photobioreactor, such as a raceway pond, allows the control of O_2 and CO_2 in the upgraded biogas. Additionally, H_2S and CO_2 can be simultaneously removed using a microalgae culture because dissolved oxygen concentration in the culture medium allows the oxidation of H_2S to SO_4^{-2}.

There are two main advantages of biogas upgrading by microalgae over the traditional methods:

- The photosynthetic biogas upgrading has lower investment and operational costs. The lower costs of this alternative is because the system operates at atmospheric pressure, it does not need solvent regeneration and biogas does not need pre-treatment to remove H_2O and H_2S. Additionally, raceway pond is a simple technology characterized by low investment and operation costs (Section 7.3.5).
- Biogas upgrading by microalgae can be inserted to a global project that includes biofuel production and wastewater treatment (Figure 8-1). When using microalgae, CO_2 is not only removed, but it is also transformed to biomass. The generated biomass is not a waste, but it can be used as feedstock for biofuel production, such as biodiesel, biogas or bioethanol (Singh and Gu, 2010). Additionally, the microalgae culture can be used to treat wastewater due to its ability to remove nitrogen and phosphorus from water streams (Wang *et al*, 2008). In a process of such characteristics, the existing microalgae culture could

be used to promote biogas upgrading, reducing costs and providing a potential carbon source for growth of microalgae.

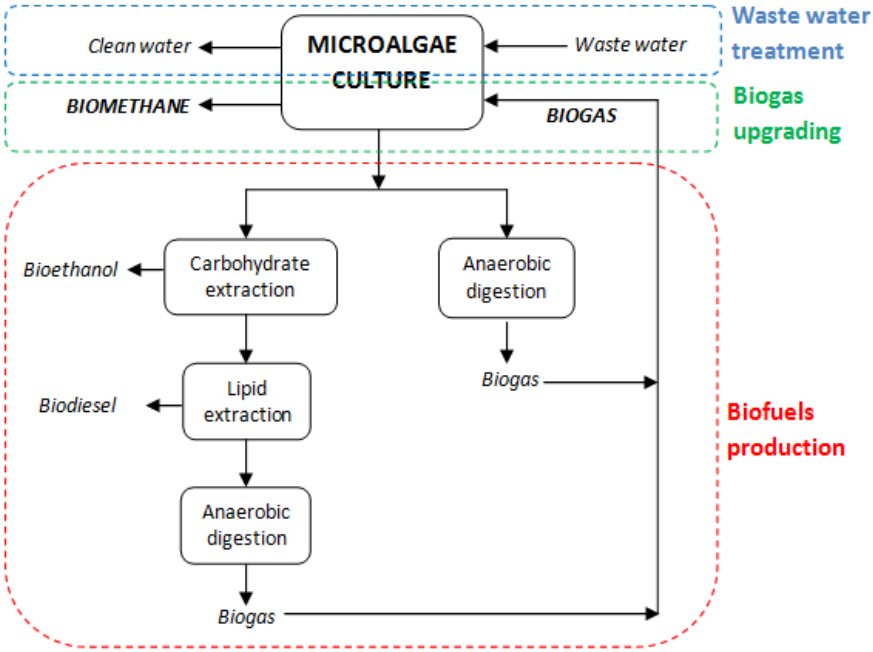

Figure 8-1. Global process for the use of microalgal biomass.

Although CO_2 uptake by microalgae is an attractive alternative for biogas upgrading, there are disadvantages and challenges to solve:

- One of the main disadvantages of the biogas upgrading process by microalgae is its low biogas treatment capacity per area in comparison to traditional technologies. Therefore, this technology is suitable in places where there is enough available land at low price (Section 7.3.5).
- Although the use an open-photobioreactor allows to control the oxygen concentration in the upgraded biogas, it promotes high rate of CO_2 desorption into atmosphere. Mass balances showed that the main mechanism of CO_2 removal in the photobioreactor was desorption into atmosphere, between 40 and 60% of the CO_2 absorbed in the column was lost in this way. Such rate of CO_2 desorption from the photobioreactor mainly is the result of the high

concentration of dissolved CO_2 in the liquid medium, which can be 10 – 60 times greater than the concentration of dissolved CO_2 in equilibrium with air (Sections 3.3.3, 5.3 and 6.3).

The CO_2 desorption can be a problem depending of the objective of the system. If the system is only designed to purify biogas, CO_2 desorption into the atmosphere from the photobioreactor should not be a problem. However, if one of the objectives is to avoid the release of CO_2 into atmosphere (for example the sale of Certified Emission Reduction (CERs)), CO_2 desorption should be controlled. The reduction of the volumetric gas load allows reduce the CO_2 desorption from photobioreactor. According to simulations using the mathematical model, the maximum flow of biogas that can be treated should be reduced by 97% to prevent desorption. Therefore, there is a compromise between a higher biogas treatment capacity and a lower CO_2 desorption rate. The decision depends on the objective of the system.

- Although raceway ponds are the most commonly used for large-scale microalgae cultivation for their lower investment and operating costs, they have the disadvantage of achieving low biomass productivity and, as consequence a low CO_2 capture rate can be obtained. Nevertheless, although the design of photobioreactor is optimized, the photosynthetic CO_2 capture has a limit. Only around 42% of the average solar radiation that reaches the Earth surface on a clear day is photosynthetically active radiation (400-750 nm, PAR) (Richmond, 2004; Barsanti and Gualtieri, 2006) and around 13.5-27% of the light energy captured by the cell is converted into chemical energy (between 8 and 16 moles of photons are required to convert 1 mol of CO_2 to 1 molecule of CH_2O) (Richmond, 2004; Weyer *et al*, 2010). Therefore, a theoretical photosynthetic

efficiency between 6 and 11% is calculated. Considering this theoretical photosynthetic efficiency and assuming an average PAR intensity of 2000 µmol m^{-2} s^{-1} for 8 h d^{-1}, a theoretical carbon capture rate between 158 - 317 g CO_2 m^{-2} d^{-1} can be obtained. This means that it could be possible to fix the CO_2 content of a biogas flow between 0.3 and 0.6 m^3 d^{-1} per m^2. Although this theoretical value is 10 times higher than the capacity obtained using a raceway pond, the treatment capacity is still much less than the capacities of traditional technologies (Section 7.3.5). This theoretical value considers ideal conditions of mass and light transfer. Table 8-1 shows a comparison of CO_2 rate of photobioreactors with theoretical efficiency photosynthetic.

Table 8-1. Comparison of CO_2 capture rate of photobioreactor with theoretical efficiency photosynthetic.

	Biomass productivity (g m^{-2} d^{-1})	**CO_2 capture rate (g m^{-2} d^{-1})***	**Biogas treatment capacity (m^3 m^{-2} d^{-1})****	**Reference**
Raceway ponds	10 - 15	18 – 27	0.03 - 0.05	Biomass productivity according Norsker *et al* (2011) and Rogers *et al* (2014) and Eriksen (2008)
Closed photobioreactor	20 – 40	37 – 73	0.07 – 0.13	Biomass productivity according Eriksen (2008)
Theoretical efficiency photosynthetic	86 - 173	158 – 317	0.3 – 0.6	Assuming that between 8 and 16 moles of photons are required to convert 1 mol of CO_2 to 1 molecule of CH_2O (Richmond, 2004; Weyer *et al*, 2010).

* Considering that 1 g of biomass fixes 1.83 g of CO_2 (Chisti, 2007)
** Considering raw biogas with 30% of CO_2

8.2. Concluding remarks

The following conclusions can be proposed from this work:

- The operation of an open photobioreactor connected to external bubble column for CO_2 absorption, enables the production of an upgraded biogas with low CO_2 ad O_2 levels. Therefore, this system represents a feasible alternative for biogas upgrading.
- The recirculation flow of microalgae culture between bubble column and photobioreactor is a key operational parameter to control the biogas quality.
- The pH gradients expected in the absorption column did not produce damage in the photosynthetic system of microalgae, because a high value of PSII efficiency was remained and the photosynthetic activity could be recovered.
- Although during night microalgae did not perform photosynthesis, desorption of CO_2 from photobioreactor to atmosphere enabled high levels of CO_2 removal during periods without illumination. The CO_2 removal efficiency was also affected by natural temperature changes between night and day, causing changes in the CO_2 equilibrium between liquid and gas phases. Lower temperatures increase solubility of CO_2, enhancing its removal in the absorption column. These phenomena enabled the continuous operation of the system, providing levels of CO_2 and oxygen in the upgraded biogas, which are compatible with most of European regulations for biomethane.
- H_2S and CO_2 could be simultaneously removed from biogas using a microalgae culture. H_2S could be oxidized to sulfate due to the high dissolved oxygen concentration in the photobioreactor. The presence of H_2S in the biogas did not

affect CO_2 removal efficiency. 98% CO_2 removal was achieved in the reactors with and without injection of H_2S.

- The maximum biogas capacity of the photosynthetic biogas upgrading depends on the objective of the system. If the objective is only to upgrade biogas fulfilling the biomethane standards, a theoretical maximum biogas treatment capacity of 3.6 m^3 d^{-1} per m^3 reactor could be achieved using an open-photobioreactor connected to bubble column system. If the objective is to upgrade biogas and avoid the release of CO_2 into atmosphere, the maximum biogas treatment capacity should be reduced down to 0.12 m^3 d^{-1} per m^3 reactor.
- Biogas upgrading by microalgae has lower biogas treatment capacity per m^2 in comparison to traditional technologies. Therefore, the proposed system could be a feasible process in places where there is enough available land.

8.3. Future directions

The future challenges of this work are indicated below:

- The implementation of a two-stage process allowed to control the oxygen content in the upgraded biogas and to fulfill the biomethane standards. However, there is a compromise between a lower CO_2 concentration and a lower O_2 concentration. A lower recirculation flow photobioreactor-column allows reducing the oxygen content in the upgraded biogas, however, the mass transfer capacity is reduced and a higher CO_2 concentration in the upgraded biogas is obtained (Section 7.3.2.2). To reduce the oxygen content without affecting the CO_2 removal efficiency, other configurations should be evaluated. For example: cultivation of microalgae-bacteria consortium to promote the O_2 consumption in the photobioreactor; absorption of CO_2 in a liquid medium without microalgae

and subsequently to inject this DIC-enriched liquid medium into the photobioreactor, avoiding the recirculation of microalgae culture through the column; implementation of an additional step for O_2 removal from upgraded biogas; among others.

- The H_2S could be removed from biogas due to its complete oxidation to sulfate. Further experiments should be performed to determine if the oxidation carried out in the proposed system was mainly chemical or biological. Abiotic operation of the reactor is necessary to evaluate the possibility of H_2S oxidation to sulfate only by chemical oxidation.
- Although CH_4 is a slightly soluble gas, the high concentration of CH_4 in the gas phase of bubble column (50 – 95%) can promote its absorption in the microalgae culture and its subsequent desorption from photobioreactor into atmosphere. To calculate the CH_4 loss, a methane mass balance has to be performed in the proposed system. The main challenge is the determination of dissolved methane in the microalgae culture.
- Aeration was supplied to the photobioreactor to facilitate desorption of generated oxygen by the photosynthesis and to enhance mixing of the microalgae culture. However, the aeration also facilitates the N_2 absorption in the liquid medium. Further experiments have to be performed to evaluate the impact of this gas on the upgraded biogas quality.

CHAPTER IX

References

9. References

Acién, F. G., Fernández, J. M., Magán, J. J. and Molina, E. (2012). Production cost of a real microalgae production plant and strategies to reduce it. *Biotechnology Advances* 30, 1344–1353.

Ajhar, M., Travesset, M., Yüce, S. and Melin, T. (2010). Siloxane removal from landfill and digester gas - a technology overview. *Bioresource Technology* 101(9), 2913-2923.

Ako, O., Kitamura, Y., Intabon, K. and Satake, T. (2008). Steady state characteristics of acclimated hydrogenotrophic methanogens on inorganic substrate in continuous chemostat reactors. *Bioresource Technology* 99, 6305–6310.

Alcántara, C., García-Encina, R. and Muñoz, R. (2013). Evaluation of Mass and Energy Balances in the integrated microalgae growth-anaerobic digestion process. *Chemical Engineering Journal* 221, 238-246.

Andriani, D., Wresta, A., Atmaja, T. and Saepudin, A. (2014). A Review on Optimization Production and Upgrading Biogas Through CO_2 Removal Using Various Techniques. *Applied Biochemistry and Biotechnology* 172(4), 1909-1928.

APHA/AWWA/WEF (1998). Standard Methods for the Examination of Water and Wastewater. American Public Health Association / American Water Works Association / Water Environment Federation, Washington, DC.

Arudchelvam, Y. and Nirmalakhandan, N. (2012). Energetic optimization of algal lipid production in bubble columns: Part II: Evaluation of CO_2 enrichment. *Biomass and Bioenergy* 46, 765-772.

European Biogas Association (2013). Proposal for a European Biomethane Roadmap. Retrieved on January 5, 2015, from http://european-biogas.eu/wp-content/uploads/2013/11/GGG-Biomethane-roadmap-final.pdf.

Babcock, R. W., Malda, J. and Radway, J. C. (2002). Hydrodynamics and mass transfer in a tubular airlift photobioreactor. *Journal Applied Phycology* 14(3), 169-184.

Bahr, M., Díaz, I., Dominguez, A., González Sánchez, A. and Muñoz, R. (2014). Microalgal-biotechnology as a platform for an integral biogas upgrading and nutrient removal from anaerobic effluents. *Environmental Science Technology* 48(1), 573-581.

Bailón, L. and Hinge, J. (2012). Report: Biogas and bio-syngas upgrading. Retrieved on 8 December 2014, from http://www.teknologisk.dk/_root/media/52679_Report-Biogas%20and%20syngas%20upgrading.pdf.

Barsanti, L. and Gualtieri, P. (2006). Algae. Anatomy, Biochemistry, and Biotechnology. Taylor y Francis, New York.

Basu, S., Khan, A., Cano-Odena, A., Liu, C. and Vankelecom, I. (2010). Membrane-based technologies for biogas separations. *Chemical Society Reviews* 39(2), 750-768.

Bauer, F., Hulteberg, C., Persson, T. and Tamm, D. (2013b). Biogas upgrading – Review of commercial technologies. SGC Rapport 2013:270. Retrieved on 10 October 2014, from http://vav.griffel.net/filer/C_SGC2013-270.pdf.

Bauer, F., Persson, T., Hulteberg, C. and Tamm, D. (2013). Biogas upgrading – technology overview, comparison and perspectives for the future. *Biofuels, Bioproducts and Biorefining* 7(5), 499-511.

Beggel, F., Nowik, I. J., Modigell, M., Shalygin, M. G., Teplyakov, V. V. and Zenkevitch, V. B. (2010). A novel gas purification system for biologically produced gases. *Journal of Cleaner Production* 18, (Supplement 1), S43-S50.

Beil, M. (2009). Overview on biogas upgrading technologies. European Biomethane Fuel Conference, Goteborg, Sweden.

Benjaminsson, J. (2006). NYA Renings - Och Uppgraderingstekniker för biogas: Rapport SGC 163. Retrieved on 20 December 2014, from http://www.sgc.se/ckfinder/userfiles/files/SGC163.pdf.

Bernard, O. (2011). Hurdles and challenges for modelling and control of microalgae for CO_2 mitigation and biofuel production. *Journal of Process Control* 21(10), 1378-1389.

Berndt, A. (2006). Intelligent utilization of biogas - Upgrading and adding to the grid. CarboTech Engineering GmbH. Retrieved on 12 December 2014, from http://biogas-infoboard.de/pdf/presentation_CarboTech%20Engineering%20GmbH.pdf

BOE (2013). Resolución de 21 de diciembre de 2012, de la Dirección General de Política Energética y Minas, por la que se modifica el protocolo de detalle PD-01 «Medición, Calidad y Odorización de Gas» de las normas de gestión técnica del sistema gasista. BOE N° 6 (7 enero 2013). Ministerio de Industria, Energía y Turismo.: 889-892.

Brennan, L. and Owende, P. (2010). Biofuels from microalgae--A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews* 14(2), 557-577.

Bugante, E., Shimomura, Y., Tanaka, T., Taniguchi, M. and Oi, S. (1989). Methane Production from Hydrogen and Carbon Dioxide and Monoxide in a Column Bioreactor of Thermophilic Methanogens by Gas Recirculation. *Journal of Fermentation and Bioengineering* 67(6), 419-421.

Burkhardt, M. and Busch, G. (2013). Methanation of hydrogen and carbon dioxide. *Applied Energy* 111, 74–79.

Carbotech (2016). Biogas upgrading plants. Retrieved on 10 April 2016, from http://www.carbotech.info/content/dam/internet_carbotech/pdf/biogas_upgradingplants_77162.pdf.

Carfagna, S., Salbitani, G., Vona, V. and Esposito, S. (2011). Changes in cysteine and O-acetyl-l-serine levels in the microalga *Chlorella sorokiniana* in response to the S-nutritional status. *Journal of Plant Physiology* 168(18), 2188-2195.

Conde, J. L., Moro, L. E., Travieso, L., Sanchez, E. P., Leiva, A., Dupeirón, R. and Escobedo, R. (1993). Biogas purification process using intensive microalgae cultures. *Biotechnology Letters* 15(3), 317-320.

Converti, A., Oliveira, R. P. S., Torres, B. R., Lodi, A. and Zilli, M. (2009). Biogas production and valorization by means of a two-step biological process. *Bioresource Technology* 100(23), 5771-5776.

Craggs, R., Sutherland, D. and Campbell, H. (2012). Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. *Journal of Applied Phycology* 24, 329-337.

Cuaresma Franco, M., Buffing, M. F., Janssen, M., Vílchez Lobato, C. and Wijffels, R. H. (2011). Performance of *Chlorella sorokiniana* under simulated extreme winter conditions. *Journal of Applied Phycology* 24(4), 693-699.

Cuaresma, M., Garbayo, I., Vega, J. M. and Vílchez, C. (2006). Growth and photosynthetic utilization of inorganic carbon of the microalga *Chlamydomonas acidophila* isolated from Tinto river. *Enzyme and Microbial Technology* 40(1), 158-162.

Cuaresma, M., Janssen, M., Vílchez, C. and Wijffels, R. H. (2009). Productivity of *Chlorella sorokiniana* in a short light-path (SLP) panel photobioreactor under high irradiance. *Biotechnology and Bioengineering* 104(2), 352-359.

Chen, K. and Morris, J. (1972). Kinetics of Oxidation of Aqueous Sulfide by O_2. *Environmental Science & Technology* 6(6), 529-537.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances* 25(3), 294-306.

Chiu, S. Y., Kao, C. Y., Tsai, M. T., Ong, S. C., Chen, C. H. and Lin, C. S. (2009). Lipid accumulation and CO_2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration. *Bioresource Technology* 100(2), 833-838.

De Godos, I., Mendoza, J. L., Acién, F. G., Molina, E., Banks, J., Heaven, S. and Rogalla, F. (2014). Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases. *Bioresource Technology* 153, 307–314.

DMT (2014). The DMT Carborex® MS biogas upgrading system. Retrieved on 22 December 2014, from http://www.dirkse-milieutechniek.com/dmt/do/webPages/202356/Biogasupgrading_small_size.html.

DMT (2015). The DMT Carborex® PWS biogas upgrading system. Retrieved on 11 December 2015, from http://www.dirkse-milieutechniek.com/dmt/do/webPages/200941/DMT_TS-PWS_Biogas_upgrading.html.

Dousková, I., Kastánek, F., Maléterová, Y., Kastánek, P., Doucha, J. and Zachleder, V. (2010). Utilization of distillery stillage for energy generation and concurrent production of valuable microalgal biomass in the sequence: Biogas-cogeneration-microalgae-products. *Energy Conversion and Management* 51(3), 606-611.

Edmundson, S. J. and Huesemann, M. H. (2015). The dark side of algae cultivation: Characterizing night biomass loss in three photosynthetic algae, *Chlorella sorokiniana*, *Nannochloropsis salina* and *Picochlorum* sp. *Algal Research* 12, 470-476.

Eisenmann (2014). Biogas Plants. Retrieved on 20 December 2014, from http://www.eisenmann.com/en/products-and-services/environmental-technology/biogas-plants/biogas-upgrading.html.

Energy Transition-Creative Energy (2014). From biogas to green gas: Upgrading techniques and suppliers. Retrieved on 16 December 2014, from http://www.rvo.nl/sites/default/files/bijlagen/From%20Biogas%20to%20Green%20Gas%20-%20Upgrading%20techniques%20and%20suppliers.pdf.

Eriksen, N. T. (2008). The technology of microalgal culturing. *Biotechnology Letters* 30(9), 1525-1536.

EurObserv'ER (2014). Biogas Barometer. Retrieved on 20 December 2014, from http://www.energies-renouvelables.org/observ-er/stat_baro/observ/baro224_Biogas_en.pdf.

Farrelly, D. J., Everard, C. D., Fagan, C. C. and McDonnell, K. P. (2013). Carbon sequestration and the role of biological carbon mitigation: A review. *Renewable & Sustainable Energy Reviews* 21, 712-727.

Faust, S. and Aly, O. (1998). Chemistry of water treatment. CRC Press, New York.

Ge, Y., Liu, J. and Tian, G. (2011). Growth characteristics of *Botryococcus braunii* 765 under high CO_2 concentration in photobioreactor. *Bioresource Technology* 102(1), 130-134.

Geider, R. J. and Osborne, B. A. (1989). Respiration and Microalgal Growth: A Review of the Quantitative Relationship between Dark Respiration and Growth. *New Phytologist* 112(3), 327-341.

Bilfinger EMS GmbH (2014). Bio-gas upgrading process. Retrieved on 16 December 2014, from http://www.ems-clp.de/fileadmin/user_upload/pdf/BIS_EMS_Biogas_A4_EN_scrn.pdf.

Schwelm Anlagentechnik GmbH (2014). Biogas Conditioning. Retrieved on 16 December 2014, from http://www.schwelm-at.de/en/business-divisions/biogas/biogas-conditioning.html.

Gojkovic, Ž., Garbayo-Nores, I., Gómez-Jacinto, V., García-Barrera, T., Gómez-Ariza, J.-L., Márová, I. and Vílchez-Lobato, C. (2013). Continuous production of selenomethionine-enriched *Chlorella sorokiniana* biomass in a photobioreactor. *Process Biochemistry* 48(8), 1235–1241.

González-Sánchez, A. and Revah, S. (2007). The effect of chemical oxidation on the biological sulfide oxidation by an alkaliphilic sulfoxidizing bacterial consortium. *Enzyme and Microbial Technology* 40, 292-298.

Grande, C. A. (2011). Chapter 3: Biogas Upgrading by Pressure Swing Adsorption. In book: Biofuel's Engineering Process Technology. InTech,

Granum, E. and Myklestad, S. M. (2002). A photobioreactor with pH control: demonstration by growth of the marine diatom *Skeletonema costatum*. *Journal of Plankton Research* 24(6), 557-563.

Gunnarsson, I., Alvarado-Morales, M. and Angelidaki, I. (2014). Utilization of CO_2 fixating bacterium *Actinobacillus succinogenes* 130Z for simultaneous biogas upgrading and biosuccinic acid production. *Environmental Science & Technology* 48(20), 12464-12468.

Günther, L. (2007). DGE GmbH Presentation: Purification of biomethane using pressureless purification for the production of biomethane and carbon dioxide. INNOGAS. Retrieved on 12 December 2014, from http://www.dge-wittenberg.com/english/vortraege/DGE%20Fachtagung%20WB%202006%20teil1-EN.pdf.

Hanagata, N., Takeuchi, T., Fukuju, Y., Barnes, D., Karube, I. (1992). Tolerance of microalgae to high CO_2 and high temperature. *Phytochemistry* 31(10), 3345-3348.

Hopp, V. (1994). Fundamentos de Tecnología Química. Editorial Reverté S.A., Madrid.

Hsueh, H. T., Li, W. J., Chen, H. H. and Chu, H. (2009). Carbon bio-fixation by photosynthesis of *Thermosynechococcus sp*. CL-1 and *Nannochloropsis oculta*. *Journal of Photochemistry and Photobiology B: Biology* 95(1), 33-39.

Huguen, P. and Le Saux, G. (2010). Perspectives for a european standard on biomethane: a Biogasmax proposal. Retrieved on 10 October 2014, from http://www.biogasmax.eu/media/d3_8_new_lmcu_bgx_eu_standard_14dec10_vf__077238500_0948_26012011.pdf.

Hulatt, C. J. and Thomas, D. N. (2011). Productivity, carbon dioxide uptake and net energy return of microalgal bubble column photobioreactors. *Bioresource Technology* 102(10), 5775-5787.

Hullu, J., Maassen, J., Van Meel, P., Shazad, S. and Vaessen, J. (2008). Comparing different biogas upgrading techniques. Retrieved on 20 September 2014, from http://students.chem.tue.nl/ifp24/BiogasPublic.pdf.

IEA-Bioenergy (2013). Upgrading plant list Retrieved on June 09, 2013, from http://www.iea-biogas.net/_content/plant-list/plant-list.html.

INN (2010). NCh 3213. Of 2010. Biometano - Especificaciones, Santiago, Chile.

Jaffrin, A., Bentounes, N., Joan, A. M. and Makhlouf, S. (2003). Landfill Biogas for heating Greenhouses and providing Carbon Dioxide Supplement for Plant Growth. *Biosystems Engineering* 86(1), 113–123.

Janssen, A., Sleyster, R., van der Kaa, C., Jochemsen, A., Bontsema, J. and Lettinga, G. (1995). Biological Sulphide Oxidation in a Fed-Batch Reactor. *Biotechnology and Bioengineering* 47 327-333.

Jee, H., Nishio, N. and Nagai, S. (1988). Continuous CH_4 Production from H_2 and CO_2 by *Methanobacterium thermoautotrophicum* in a Fixed-Bed Reactor. *Journal of Fermentation Technology* 66(2), 235-238.

Jee, H., Yano, T., Nishio, N. and Nagai, S. (1987). Biomethanation of H_2 and CO_2 by *Methanobacterium thermoautotrophicum* in Membrane and Ceramic Bioreactors. *Journal of Fermentation Technology* 65(4), 413-418.

Jeong, M. L., Gillis, J. M. and Hwang, J.-Y. (2003). Carbon Dioxide Mitigation by Microalgal Photosynthesis. *Bulletin of the Korean Chemical Society* 24(12), 1763 - 1766.

Jiang, L., Luo, S., Fan, X., Yang, Z. and Guo, R. (2011). Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO_2. *Applied Energy* 88(10), 3336-3341.

Jönsson, O., Polman, E., Jensen, J., Eklund, R., Schyl, H. and Ivarsson, S. (2003). Sustainable gas enters the European Gas Distribution System. World Gas Conference, Tokio.

Ju, D., Shin, J., Lee, H., Kong, S., Kim, J. and Sang, B. (2008). Effects of pH conditions on the biological conversion of carbon dioxide to methane in a hollow-fiber membrane biofilm reactor (Hf–MBfR). *Desalination* 234(409-415).

Kantarci, N., Borak, F. and Ulgen, K. O. (2005). Bubble column reactors. *Process Biochemistry* 40(7), 2263-2283.

Kao, C.-Y., Chiu, S.-Y., Huang, T.-T., Dai, L., Hsu, L.-K. and Lin, C.-S. (2012). Ability of a mutant strain of the microalga *Chlorella* sp. to capture carbon dioxide for biogas upgrading. *Applied Energy* 93, 176-183.

Kapdi, S. S., Vijay, V. K., Rajesh, S. K. and Prasad, R. (2005). Biogas scrubbing, compression and storage: perspective and prospectus in Indian context. *Renewable Energy* 30(8), 1195-1202.

Kim, S., Choi, K. and Chung, J. (2013). Reduction in carbon dioxide and production of methane by biological reaction in the electronics industry. *International Journal of Hydrogen Energy* 38, 3488-3496.

Kim, S., Park, J.-e., Cho, Y.-B. and Hwang, S.-J. (2013). Growth rate, organic carbon and nutrient removal rates of *Chlorella sorokiniana* in autotrophic, heterotrophic and mixotrophic conditions. *Bioresource Technology* 144(0), 8-13.

Kreutzer, M. T., Kapteijn, F., Moulijn, J. A. and Heiszwolf, J. J. (2005). Multiphase Monolith Reactors: Chemical Reaction Engineering of Segmented Flow in Microchannels. *Chemical Engineering Science* 60, 5895–5916.

Krishna, R. and Van Baten, J. (2001). Scaling up bubble column reactors with the AID of CFD. *Chemical Engineering Research & Design* 79(283-309).

KSB (2016). Catálogo de productos. Retrieved on April 25 th, 2016, from www.ksb.com.

Kumar, K. and Das, D. (2012). Growth characteristics of *Chlorella sorokiniana* in airlift and bubble column photobioreactors. *Bioresource Technology* 116, 307 - 313.

Kumar, K., Dasgupta, C. N. and Das, D. (2014). Cell growth kinetics of *Chlorella sorokiniana* and nutritional values of its biomass. *Bioresource Technology* 167, 358-366.

Kumar, K., Dasgupta, C. N., Nayak, B., Lindblad, P. and Das, D. (2011). Development of suitable photobioreactors for CO_2 sequestration addressing global warming using green algae and cyanobacteria. *Bioresource Technology* 102(8), 4945-4953.

Lee, J., Kim, J., Chang, W. and Pak, D. (2012). Biological conversion of CO_2 to CH_4 using hydrogenotrophic methanogen in a fixed bed reactor. *Journal of Chemical Technology and Biotechnology* 87, 844-847.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology. R. D. Lester Packer, Academic Press. Volume 148: 350-382.

Lindberg, A. and Rasmuson, Å. C. (2006). Selective desorption of carbon dioxide from sewage sludge for in situ methane enrichment—part I: Pilot-plant experiments. *Biotechnology and Bioengineering* 95(5), 794-803.

López, J. C., Quijano, G., Souza, T. S. O., Estrada, J. M., Lebrero, R. and Muñoz, R. (2013). Biotechnologies for greenhouse gases (CH_4, N_2O, CO_2) abatement: state-of-the-art and challenges. *Applied Microbiology and Biotechnology* 97(6), 2277-2303.

Luo, G. and Angelidaki, I. (2012a). Integrated Biogas Upgrading and Hydrogen Utilization in an Anaerobic Reactor Containing Enriched Hydrogenotrophic Methanogenic Culture. *Biotechnology and Bioengineering* 109, 2729–2736.

Luo, G. and Angelidaki, I. (2013). Co-digestion of manure and whey for in situ biogas upgrading by the addition of H_2: process performance and microbial insights. *Applied Microbial Biotechnology* 97, 1373–1381.

Luo, G., Johansson, S., Boe, K., Xie, L., Zhou, Q. and Angelidaki, I. (2012b). Simultaneous hydrogen utilization and in situ biogas upgrading in an anaerobic reactor. *Biotechnology Bioengineering* 109(4), 1088-1094.

Luo, G., Wang, W. and Angelidaki, I. (2014). A new degassing membrane coupled upflow anaerobic sludge blanket (UASB) reactor to achieve in-situ biogas upgrading and recovery of dissolved CH_4 from the anaerobic effluent. *Applied Energy* 132, 536–542.

Luther, G. W., Findlay, A. J., MacDonald, D. J., Owings, S. M., Hanson, T. E., Beinart, R. A. and Girguis, P. R. (2011). Thermodynamics and Kinetics of Sulfide Oxidation by Oxygen: A Look at Inorganically Controlled Reactions and Biologically Mediated Processes in the Environment. *Frontiers in Microbiology* 2, 62.

Malmberg (2014). Upgrade biogas to biomethane with reliable technology. Retrieved on 11 December 2014, from http://www.malmberg.se/en/malmberg_biogas_en/malmberg_compact_en.

Manahan, S. (2007). Introducción a la química ambiental. Reverté Ediciones, México.

Mandalam, R. and Palsson, B. (1998). Elemental Balancing of Biomass and Medium Composition Enhances Growth Capacity in High-Density *Chlorella vulgaris* Cultures. *Biotechnology Bioengineering* 59, 605-611.

Mandeno, G., Craggs, R., Tanner, C., Sukias, J. and Webster-Brown, J. (2005). Potential biogas scrubbing using a high rate pond. *Water Science Technology* 51(12), 253-256.

Mann, G., Schlegel, M., Schumann, R. and Sakalauskas, A. (2009). Biogas conditioning with microalgae. *Agronomy Research* 7(1), 33-38.

Marcogaz (2006). Injection of Gases from Non-Conventional Sources into Gas Networks. Retrieved on 6 March 2014, from http://www.marcogaz.org/index.php/gas-utilisation.

Mata, T. M., Martins, A. A. and Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable & Sustainable Energy Reviews* 14(1), 217-232.

Mattiasson, B. (2005). Ekologisk lunga för biogasuppgradering. Retrieved on 28 December 2014, from http://www.sgc.se/ckfinder/userfiles/files/SBGF610401.pdf.

Maxwell, K. and Johnson, G. (2000). Chlorophyll fluorescence - a practical guide. *Journal of Experimental Botany* 51(345), 659 - 668.

Meier, L., Pérez, R., Azócar, L., Rivas, M. and Jeison, D. (2015). Photosynthetic CO_2 uptake by microalgae: an attractive tool for biogas upgrading. *Biomass and Bioenergy* 73, 102-109.

Mendoza, J. L., Granados, M. R., de Godos, I., Acién, F. G., Molina, E., Heaven, S. and Banks, C. J. (2013). Oxygen transfer and evolution in microalgal culture in open raceways. *Bioresource Technology* 137(0), 188-195.

Miyairi, S. (1995). CO_2 assimilation in a thermophilic cyanobacterium. *Energy Conversion and Management* 36, 763-766.

Morweiser, M., Kruse, O., Hankamer, B. and Posten, C. (2010). Developments and perspectives of photobioreactors for biofuel production. *Applied Microbiol Biotechnology* 87, 1291-1301.

Muñoz, R. and Guieysse, B. (2006). Algal-bacterial processes for the treatment of hazardous contaminants: A review. *Water Research* 40, 2799-2815.

Muñoz, R., Meier, L., Diaz, I. and Jeison, D. (2015). A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading. *Reviews in Environmental Science and Bio/Technology* 14(4), 727-759.

Nordberg, Å., Edström, M., Uusi-Penttilä, M. and Rasmuson, Å. C. (2012). Selective desorption of carbon dioxide from sewage sludge for in-situ methane enrichment: Enrichment experiments in pilot scale. *Biomass and Bioenergy* 37(0), 196-204.

Norsker, N.-H., Barbosa, M. J., Vermuë, M. H. and Wijffels, R. H. (2011). Microalgal production — A close look at the economics. *Biotechnology Advances* 29(1), 24-27.

Ota, M., Kato, Y., Watanabe, H., Watanabe, M., Sato, Y., Smith Jr, R. L. and Inomata, H. (2009). Fatty acid production from a highly CO_2 tolerant alga, *Chlorocuccum littorale*, in the presence of inorganic carbon and nitrate. *Bioresource Technology* 100(21), 5237-5242.

Papazi, A., Makridis, P., Divanach, P., Kotzabasis, K. (2008). Bioenergetic changes in the microalgal photosynthetic apparatus by extremely high CO_2 concentrations induce an intense biomass production *Physiologia Plantarum* 132, 338-349.

Patterson, T., Esteves, S., Dinsdale, R. and Guwy, A. (2011). An evaluation of the policy and techno-economic factors affecting the potential for biogas upgrading for transport fuel use in the UK. *Energy Policy* 39(3), 1806-1816.

Peillex, J., Fardeau, M., Boussand, R., Navarro, J. and Belaich, J. (1988). Growth of *Methanococcus thermolithotrophicus* in batch and continuous culture on H_2 and CO_2: influence of agitation. *Applied Microbiology and Biotechnology* 29, 560-564.

Persson, M. (2003). Evaluation of upgrading techniques for biogas. Rapport SGC 142. Retrieved on 5 August 2014, from https://cdm.unfccc.int/filestorage/E/6/T/E6TUR2NNQW9O83ET10CX8HTE4WXR2O/Evaluation%20of%20Upgrading%20Techniques%20for%20Biogas.pdf?t=YWt8bml5eTJsfDBCijpDYjFf2sE5_wGsjeuV.

Persson, M. (2007). Biogas upgrading and utilization as vehicle fuel European Biogas Workshop – The Future of Biogas in Europe III University of Southern Denmark.

Persson, M., Jönsson, O. and Wellinger, A. (2006). Biogas upgrading to vehicle fuel standards and grid injection. Retrieved on 1 April 2013, from http://www.iea-biogas.net/_download/publi-task37/upgrading_report_final.pdf.

Persson, M., Wellinger, A., Rehnlund, B. and Rahm, L. (2007). Report on Technological Applicability of Existing Biogas Upgrading Processes. Retrieved on 20 October 2014, from http://www.biogasmax.eu/media/report_on_technological_2007__041639600_1025_22052007.pdf.

Petersson, A. and Wellinger, A. (2009). Biogas upgrading technologies – developments and innovations. Retrieved on 5 June 2014, from http://www.iea-biogas.net/_download/publi-task37/upgrading_rz_low_final.pdf.

Pokasoowan, C., Kanitchaidecha, W., Krishna, B. and Annachhatre, A. (2009). Investigation on laboratory and pilot-scale airlift sulfide oxidation reactor under varying sulfide loading rate. *Journal of Environmental Science and Health. Part A: Toxic/Hazardous Substances and Environmental Engineering* 44(1), 87-98.

Pulz, O. P. (2001). Photobioreactors: Production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology* 57, 287-293.

Puregas, P. (2014). Biogas upgrading. Retrieved on 15 December 2014, from http://www.purac-puregas.com/technology/biogas-upgrading/.

Putt, R., Singh, M., Chinnasamy, S. and Das, K. C. (2011). An efficient system for carbonation of high-rate algae pond water to enhance CO_2 mass transfer. *Bioresource Technology* 102(3), 3240-3245.

Raja, R., Hemaiswarya, S., Kumar, N., Sridhar, S. and Rengasamy, R. (2008). A perspective on the biotechnological potential of microalgae. *Critical Reviews in Microbiology* 34, 77-88.

Ramos, I., Pérez, R. and Fdz-Polanco, M. (2013). Microaerobic desulphurisation unit: A new biological system for the removal of H_2S from biogas. *Bioresource Technology* 142, 633-640.

Ras, M., Steyer, J.-P. and Bernard, O. (2013). Temperature effect on microalgae: a crucial factor for outdoor production. *Reviews in Environmental Science and Bio/Technology* 12(2), 153-164.

Rasi, S. (2009). Biogas composition and upgrading to biomethane. Department of Biological and Environmental Science. Jyväskylä, Finland, University of Jyväskylä, Jyväskylä.

Rasi, S., Läntelä, J. and Rintala, J. (2011). Trace compounds affecting biogas energy utilisation – A review. *Energy Conversion and Management* 52(12), 3369-3375.

Raven, J. A., Cockell, C. S. and De la Rocha, C. L. (2008). The evolution of inorganic carbon concentrating mechanisms in photosynthesis. *Philosophical Transactions of the Royal Society B* 363, 2641-2650.

Richmond, A. (2004). Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Science, Oxford.

Rocha, J. M. S., Garcia, J. E. C. and Henriques, M. H. F. (2003). Growth aspects of the marine microalga *Nannochloropsis gaditana*. *Biomolecular Engineering* 20(4-6), 237-242.

Rogers, J. N., Rosenberg, J. N., Guzman, B. J., Oh, V. H., Mimbela, L. E., Ghassemi, A., Betenbaugh, M. J., Oyler, G. A. and Donohue, M. D. (2014). A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. *Algal Research* 4, 76-88.

Rutledge, B. (2005). California Biogas Industry Assessment White Paper. Retrieved on 7 March 2014 from http://www.calstart.org/Libraries/Publications/California_Biogas_Industry_Assessment_White_Paper.sflb.ashx.

Ryckebosch, E., Drouillon, M. and Vervaeren, H. (2011). Techniques for transformation of biogas to biomethane. *Biomass and Bioenergy* 35(5), 1633-1645.

Scott, J., Boriah V. (2010). Modeling algae growth in an open-channel raceway. *Journal of Computational Biology* 17(7), 895-906.

Schubert, R. and Blasch, J. (2010). Sustainability standards for bioenergy—A means to reduce climate change risks? *Energy Policy* 38(6), 2797-2805.

Serejo, M., Posadas, E., Boncz, M., Blanco, S., Garcia-Encina, P. and Muñoz, R. (2015). Tailoring biomass composition during the optimization of the integral upgrading of biogas in microalgal-bacterial processes. *Environmental Science and Technology* Submitted for publication.

Shaikh, A. and Al-Dahhan, M. (2013). Scale-up of Bubble Column Reactors: A Review of Current State-of-the-Art. *Industrial & Engineering Chemistry Research* 52(24), 8091-8108.

Sialve, B., Bernet, N. and Bernard, O. (2009). Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances* 27(4), 409-416.

Silva, H. J. and Pirt, S. J. (1984). Carbon Dioxide Inhibition of Photosynthetic Growth of *Chlorella*. *Journal of General Microbiology* 130(11), 2833-2838.

Simionato, D., Sforza, E., Corteggiani Carpinelli, E., Bertucco, A., Giacometti, G. M. and Morosinotto, T. (2011). Acclimation of *Nannochloropsis gaditana* to different illumination regimes: Effects on lipids accumulation. *Bioresource Technology* 102(10), 6026-6032.

Singh, J. and Gu, S. (2010). Commercialization potential of microalgae for biofuels production. *Renewable and Sustainable Energy Reviews* 14(9), 2596-2610.

Sinnott, R. K. (2005). Chemical Engineering Design. Elsevier Butterworth-Heinemann, Oxford

Slade, R. and Bauen, A. (2013). Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. *Biomass and Bioenergy* 53, 29-38.

Sobczuk, T. M., Camacho, F. G., Rubio, F. C., Fernández, F. G. A. and Grima, E. M. (2000). Carbon dioxide uptake efficiency by outdoor microalgal cultures in tubular airlift photobioreactors. *Biotechnology and Bioengineering* 67(4), 465-475.

CO_2 solutions (2014). Harnessing Nature for Efficient Carbon Capture. Retrieved on 2 January 2015, from http://www.co2solutions.com/.

Soreanu, G., Béland, M., Falletta, P., Edmonson, K., Svoboda, L., Al-Jamal, M. and Seto, P. (2011). Approaches concerning siloxane removal from biogas - A review. *Biosystems Engineering* 53, 8.1-8.18.

Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering* 101, 87-96.

Strevett, K. A., Vieth, R. F. and Grasso, D. (1995). Chemo-autotrophic biogas purification for methane enrichment: mechanism and kinetics. *The Chemical Engineering Journal and the Biochemical Engineering Journal* 58(1), 71-79.

Stumm, W. and Morgan, J. (1995). Aquatic Chemistry. John Wiley & Sons Inc, New York.

Svensson, M. (2014). Biomethane standards. Gas quality standardisation of biomethane, going from national to international level. European workshop Biomethane, Brussels.

Sydney, E. B., Novak, A. C., de Carvalho, J. C. and Soccol, C. R. (2014). Chapter 4 - Respirometric Balance and Carbon Fixation of Industrially Important Algae. In book: Biofuels from Algae. Elsevier, Amsterdam.

Tang, K., Baskaran, V. and Nemati, M. (2009). Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries. *Biochemical Engineering Journal* 44(1), 73-94.

Tchernov, D., Hassidim, M., Luz, B., Sukenik, A., Reinhold, L. and Kaplan, A. (1997). Sustained net CO_2 evolution during photosynthesis by marine microorganism. *Current biology : CB* 7(10), 723-728.

Thrän, D., Billig, E., Persson, T., Svensson, M., Daniel-Gromke, J., Ponitka, J., Seiffert, M., Baldwin, J., Kranzl, L., Schipfer, F., Matzenberger, J., Devriendt, N., Dumont, M., Dahl, J. and Bochmann, G. (2014). Biomethane – status and factors affecting market

development and trade. Retrieved on 20 December 2014, from http://www.bioenergytrade.org/downloads/t40-t37-biomethane-2014.pdf.

Tock, L., Gassner, M. and Maréchal, F. (2010). Thermochemical production of liquid fuels from biomass: Thermo-economic modeling, process design and process integration analysis. *Biomass and Bioenergy* 34(12), 1838-1854.

Tredici, M. R. (2009). Photobiology of microalgae mass cultures: Understanding the tools for the next green revolution. *Biofuels* 1, 143-162.

Tsuzuki, M., Shiraiwa, Y., Miyachi, S. (1980). Role of carbonic anhydrase in photosynthesis in *Chlorella* derived from kinetic analysis of $^{14}CO_2$ fixation. *Plant and Cell Physiology* 21(4), 677-688.

Tynell, Å., Börjesson, G. and Persson, M. (2007). Microbial Growth on Pall Rings: A Problem When Upgrading Biogas With the Water-Wash Absorption Technique. *Applied Biochemistry and Biotechnology* 141, 299 - 320.

Urban, W., Girod, K. and Lohmann, H. (2009). Executive Report: The German Market for Biomethane. Retrieved on 22 December 2014, from http://exportinitiative.dena.de/fileadmin/user_upload/Table_of_Contents_v3_Biomethan.pdf.

UTFSM (2008). Irradiancia Solar en Territorios de la Républica de Chile. Margen impresores, Santiago.

van der Zee, F. P., Villaverde, S., García, P. A. and Fdz.-Polanco, F. (2007). Sulfide removal by moderate oxygenation of anaerobic sludge environments. *Bioresource Technology* 98(3), 518-524.

Vaquero, I., Ruiz-Domínguez, M. C., Márquez, M. and Vílchez, C. (2012). Cu-mediated biomass productivity enhancement and lutein enrichment of the novel microalga *Coccomyxa onubensis*. *Process Biochemistry* 47(5), 694-700.

Vona, V., Di Martino Rigano, V., Lobosco, O., Carfagna, S., Esposito, S. and Rigano, C. (2004). Temperature responses of growth, photosynthesis, respiration and NADH: nitrate reductase in cryophilic and mesophilic algae. *New Phytologist* 163(2), 325-331.

Wang, B., Li, Y., Wu, N. and Lan, C. (2008). CO_2 bio-mitigation using microalgae. *Applied Microbiology & Biotechnology* 79(5), 707-718.

Wang, W., Xie, L., Luo, G., Zhou, Q. and Angelidaki, I. (2013). Performance and microbial community analysis of the anaerobic reactor with coke oven gas biomethanation and in situ biogas upgrading. *Bioresource Technology* 146, 234–239.

Weyer, K. M., Bush, D. R., Darzins, A. and Willson, B. D. (2010). Theoretical Maximum Algal Oil Production. *Bioenergy Research* 3(2), 204-213.

Williams, T., Colman, B. (1996). The effects of pH and dissolved inorganic carbon on external carbonic anhydrase activity in *Chlorella saccharophila*. *Plant, Cell and Environment* 19, 485-489.

Xebex (2014). BGX SOLUTIONS - Biogas Plants. Retrieved on 5 January 2015, from http://www.xebecinc.com/biogas-plants.php.

Yan, C. and Zheng, Z. (2013). Performance of photoperiod and light intensity on biogas upgrade and biogas effluent nutrient reduction by the microalgae *Chlorella* sp. *Bioresource Technology* 139, 292–299.

Yen, U.-C., Huang, T.-C. and Yen, T.-C. (2004). Observation of the circadian photosynthetic rhythm in cyanobacteria with a dissolved-oxygen meter. *Plant Science* 166(4), 949-952.

Zhang, L., Happe, T. and Melis, A. (2002). Biochemical and morphological characterization of sulfur-deprived and H_2-producing *Chlamydomonas reinhardtii* (green alga). *Planta* 214(4), 552-561.