

# Experimental system for the prevention of O<sub>2</sub>- and air contamination during biogas upgrading with phototrophic microalgae

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## Abstract

Several alternative biogas purification techniques are currently being examined for increasing the efficiency of the biogas production. Phototrophic microalgal strains have been tested for converting large quantities of CO<sub>2</sub> from biogas into algal biomass. However, nearly all studies on biogas upgrading with microalgae resulted in contamination of upgraded gas with O<sub>2</sub> caused by photosynthesis or air due to algal culturing in open ponds. To avoid impurities in upgraded biogas, we tested a discontinuous system at bench scale. We grew the well-studied green algae *Chlorella vulgaris* and the recently described green algae *Chloroparva pannonica* in a tubular photobioreactor with 3NBB medium. Subsequently, we used a detached gas scrubber to transfer CO<sub>2</sub> from biogas into dark-adapted microalgal suspensions. During the gas upgrading, the O<sub>2</sub>- and CO<sub>2</sub> concentrations in biogas were monitored and corresponding reaction kinetics of mass transfer from biogas into algal medium were determined. The upgrading experiments resulted in a virtually complete removal of CO<sub>2</sub> from all biogas batches. Simultaneously, no O<sub>2</sub> or air was added to the upgraded biogases. Furthermore, we found varying CO<sub>2</sub> kinetics which indicated an algal-specific effect upon the CO<sub>2</sub> removal from biogas. These findings proved the suitability of our experimental system for detailed studies on biogas upgrading with phototrophic microalgae, including their culture media. Moreover, we demonstrated the feasibility of bench-scale biogas upgrading with microalgae without simultaneous contamination of the upgraded gas.

**Keywords:** Bench-scale, Biogas upgrading, Gas contamination, Microalgae

## Zusammenfassung

### Versuchsaufbau zur Vermeidung von Sauerstoff- und Lufteinträgen während der Biogasaufbereitung mit phototrophen Mikroalgen

Gegenwärtig werden verschiedene Mikroalgen als alternatives Biogasaufbereitungsverfahren untersucht. Bei der Umwandlung von Biogas-CO<sub>2</sub> in Algenbiomasse treten jedoch in nahezu allen Studien Verunreinigungen der aufbereiteten Gase auf. Diese erfolgen in Form von Sauerstoff durch Photosynthese sowie Luft infolge der Algenkultivierung in Becken. Um Gaskontaminationen zu vermeiden, kultivierten wir *Chlorella vulgaris* und *Chloroparva pannonica* in einem Photobioreaktor. In einem separaten Gaswäscher überführten wir anschließend Biogas in die dunkel-adaptierten Algensuspensionen. Während der Gasaufbereitung wurden die O<sub>2</sub>- und CO<sub>2</sub>-Konzentrationen überwacht und entsprechende Kinetiken des Massentransfers von Biogas zu Algenmedium bestimmt. Die Experimente zeigten eine nahezu vollständige Entfernung des CO<sub>2</sub> aus allen eingesetzten Biogaschargen. Gleichzeitig wurde den aufbereiteten Gasen weder O<sub>2</sub> noch Luft hinzugefügt. Darüber hinaus fanden wir abweichende CO<sub>2</sub>-Kinetiken, die auf eine algen-spezifische Beeinflussung der CO<sub>2</sub>-Entfernung schließen lassen. Die Ergebnisse belegen die Eignung unseres Versuchsaufbaus für detaillierte Untersuchungen zur Biogasaufbereitung mit phototrophen Mikroalgen, einschließlich deren Kulturmedien. Zudem wurde die Machbarkeit der Biogasaufbereitung mittels phototropher Mikroalgen ohne die gleichzeitige Verunreinigung aufbereiteter Gase im Labormaßstab gezeigt.

**Schlüsselworte:** Labormaßstab, Biogasaufbereitung, Gasverunreinigung, Mikroalgen

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## 1 Introduction

Biogas typically consists of 40 to 75 % v/v methane (CH<sub>4</sub>), 15 to 60 % v/v carbon dioxide (CO<sub>2</sub>), water vapour, hydrogen sulphide and other trace gases, such as carbon monoxide, nitrogen and atmospheric oxygen (O<sub>2</sub>) (Andriani et al., 2014). Particularly, the CO<sub>2</sub> proportion influences the energetic properties of biogas since higher CO<sub>2</sub> levels result in a decreasing calorific value of the gas mixture. Further, the efficiency of four-stroke biogas engines decreases with rising CO<sub>2</sub> concentrations in biogas (Bari, 1996; Edelmann, 2001; Deublein and Steinhauser, 2011). Besides energetic aspects, high CO<sub>2</sub> concentrations increase the biogas volume and the corresponding storage space. Biogas storage at atmospheric pressure in large, plastic bags is associated with size- or transportation problems (Andrea et al., 2011; Khoiyangbam et al., 2011). Therefore, the generation of biogas generally benefits from a CO<sub>2</sub> separation procedure. Chemico-physical processes, e.g. pressure swing adsorption and amine scrubbing, are state of the art for industrial biogas upgrading (Pettersson and Wellinger, 2009). Besides these energy-intensive processes, biological approaches have high potential for CO<sub>2</sub> separation from biogas.

Particularly, photosynthetic microorganisms are well investigated in terms of removing CO<sub>2</sub> from gaseous waste streams (Benemann et al., 2003; Wang et al., 2008; Kumar et al., 2010). Moreover, several studies on biogas purification with photosynthetic microalgae have been conducted (e.g. Schmack et al., 2009; Kaštánek et al., 2010). However, apart from a sophisticated multi-species process (Bahr et al., 2014), the large-scale approaches resulted in contamination of the upgraded biogas. These contaminations were either oxygen (O<sub>2</sub>) due to algal photosynthesis or air caused by algal culturing in open ponds. As a consequence, studies on biogas upgrading with phototrophic microalgae focus on the optimal biomass, whereas effects of the algal medium on biogas upgrading are disregarded. In order to prevent contaminations of upgraded biogas and to examine potential influences of the algal medium on the process, we follow a small-scale approach. In a first step, we tested a bench-scale system in which biogas is upgraded by microalgal suspension without simultaneous oxygen- or air contamination. For this purpose, the green algae *Chlorella vulgaris* and *Chloroparva pannonica* served as model organisms and were cultured under monitored conditions in a tubular photobioreactor. Subsequently, biogas was upgraded with dark-adapted algal suspension in a detached gas scrubber and reaction kinetics of the O<sub>2</sub>- and CO<sub>2</sub> mass transfer from biogas into algal medium were determined.

## 2 Materials and Methods

### 2.1 Microalgal strain and culture medium

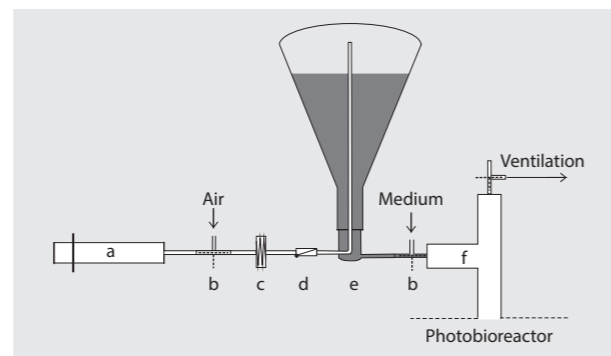
Two microalgal strains were grown as model organisms for biogas upgrading. The axenic *Chlorella vulgaris* 211-11B and the non-axenic *Chloroparva pannonica* 2358 were obtained from the SAG culture collection, University of Göttingen.

Both strains grew in 3NBB medium according to the recipe of Starr and Zeikus (1993). We selected unicellular and agitation resistant green algal strains (Trebouxiophyceae, Chlorophyta) due to the expected mechanical stress during biogas upgrading. Both strains were phototrophic in order to be independent from organic nutrients and the associated risk of contamination.

### 2.2 Culturing of microalgae

A closed tubular photobioreactor (PBR) was used to culture *Chlorella vulgaris* and *Chloroparva pannonica* constantly at 20 °C with a light-dark cycle of 16/8 h and a photon flux density (PFD) of 60 μE. The PBR had a total volume of 4.9 l and consisted of transparent borosilicate glass tubes with an inner diameter of 25 mm and a total length of 9 m. Its glass components were arranged helically and connected with flange mountings. The PBR was equipped with sampling- and effluent junctions, a vertical backflow as well as a headspace with junctions for medium- and inoculum inlet. Culture conditions were monitored online with temperature and pH sensors PHD 2 (PCE-Germany).

Culture experiments with *Chlorella* were performed under sterile conditions. For this purpose, the PBR was cleansed by circulating a sodium hypochlorite solution (1.5 % v/v) for one hour. Subsequently, the tubes were rinsed twice with sterilised deionised water for 30 min. The PBR was filled with sterile 3NBB medium via a peristaltic pump Ismatec IP-ISM 942 and microalgal suspension was inoculated through the headspace via a custom setup for inoculation (Figure 1). After inoculation, the algal suspension circulated constantly through the bioreactor. The suspension flow was driven by a constant airstream of 0.75 l·min<sup>-1</sup> provided by a micro membrane pump, model NPM NF-05D004 (Reichert Chemietechnik, Germany). Air was filtered with sterile syringe filter (Rotilabo®, Germany) and inserted through a cannula at the lowest part of the PBR. After passage of the tubing, the air was vented in the headspace.



**Figure 1** Custom setup for sterile inoculation of the photobioreactor; (a) gas syringe, (b) three-way valve, (c) sterile filter, (d) check valve, (e) conical flask with sterile algal suspension, (f) inoculum/medium junction at headspace of photobioreactor

Samples of algal suspension were taken daily from the bioreactor to determine biomass growth. The samples were filtered onto pre-weighted glass microfibre filters (WHATMAN, GF/F), dried at 60 °C for 24 hours and weighted again. In addition, algal growth was determined by triplicate cell countings using a Thoma counting chamber. The resulting cell densities are represented as mean and growth rates (μ) were calculated by stepwise optimization of the least deviation squares (Excel Solver). Losses of suspension by sampling and evaporation were compensated with 3NBB medium from an attached reservoir.

### 2.3 Biogas upgrading at bench scale

Carbon dioxide was separated from biogas in a darkened gas scrubber. The scrubber was built up of two circuits, which were detached from the photobioreactor (Figure 2 b). The first circuit consisted of a loop-shaped gas scrubber with online sensors for pH (PHD 2, PCE-Germany) and dissolved O<sub>2</sub> (GMH3630, Greisinger Germany). This liquid-circuit was interconnected to a gas-circuit, in which biogas was driven by a membrane pump, model NPM NF-830 D005 (Reichert Chemietechnik, Germany). Within the closed gas circuit, the biogas was transported through gastight hoses (Tygon®LP-1500) to a catalytic sensor for O<sub>2</sub>, model GMH3691+GGO369S (Greisinger, Germany), and an infra-red sensor for CO<sub>2</sub> (Pewatron Carbonoxy). A cooled (3 °C) liquid trap was installed to prevent condensation inside the pump and sensors. The gas flow was monitored by a flow meter (AMW 3300V; Honeywell, USA). The experimental setup included a N<sub>2</sub>-inlet for nitrogen purging, a bypass for the insertion of biogas and a ventilation junction.

Prior to the upgrading experiments, the gas scrubber was cleaned with ethanol (75 % v/v) and rinsed with autoclaved

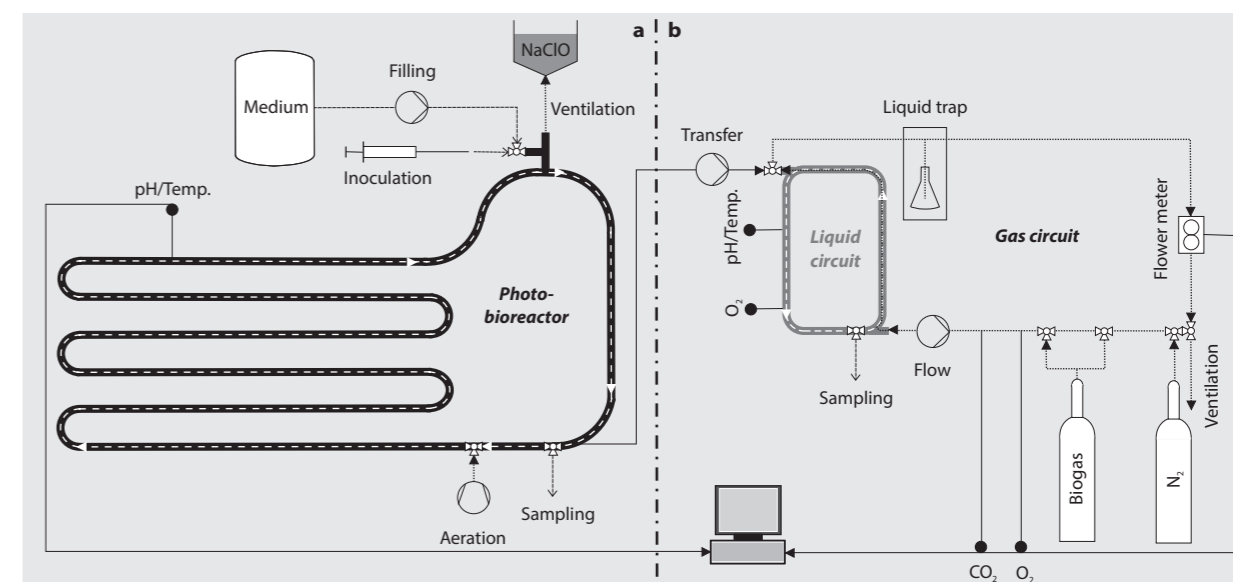
deionised water to remove contaminants. Subsequently, 520 ml of *Chlorella vulgaris*- and *Chloroparva pannonica* suspension, respectively, were pumped from the photobioreactor to the scrubber via a peristaltic pump (Reefdoser R02, Aqua Medic, Germany). Within the scrubber, the algal suspension circulated anticlockwise for 40 min with a flow rate of 3.1 l·min<sup>-1</sup>.

The flow was driven by 840 ml biogas, which was upgraded simultaneously by passing the algal suspension. The upgrading experiments were performed as duplicates within one day. Experiments with *Chlorella* were performed with two batches of 34.33 mmol (ca. 840 ml) biogas with a mean content of 13.73 mmol CO<sub>2</sub> and 787 μmol O<sub>2</sub> before upgrading. In *Chloroparva* experiments, two batches of 34.33 mmol biogas with a mean content of 13.73 mmol CO<sub>2</sub> and 743 μmol were tested. All upgrading experiments were performed at constant 20 °C and 1004 ± 2 mbar atmospheric pressure.

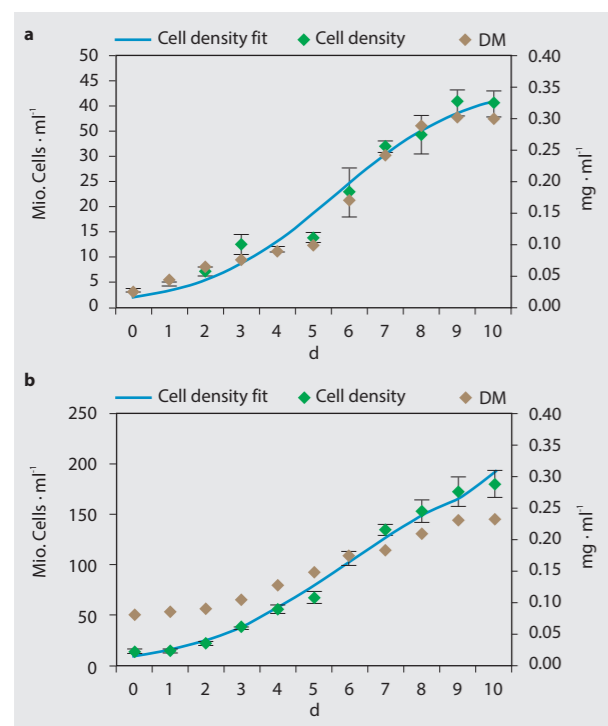
## 3 Results

### 3.1 Preparation of algal suspension

*Chlorella vulgaris* and *Chloroparva pannonica* were successfully cultured in our tubular photobioreactor at bench scale (Figure 3). *Chlorella vulgaris* grew with a ratio of 0.55·d<sup>-1</sup> to a maximum of approximately 40·10<sup>6</sup> cells·ml<sup>-1</sup>. *Chloroparva pannonica* showed a growth ratio of 0.49·d<sup>-1</sup> and a maximum cell density of approximately 248·10<sup>6</sup> cells·ml<sup>-1</sup>. Both growth curves were characterised by a short lag phase of approximately two days, followed by a log phase of approximately five days. For both algal strains, the transition from exponential growth to stationary growth phase was detected after circa seven culture days.



**Figure 2** Scheme of a bench-scale system for biogas upgrading. (a) Closed tubular photobioreactor for culturing 4.9 l phototrophic microalgae, at 20 °C with a light-dark cycle of 16/8 h and a PFD of 60 μE. (b) Darkened gas scrubber for upgrading 34.33 mmol biogas with 520 ml microalgal suspension, at constant 20 °C and 1004 ± 2 mbar atmospheric pressure.



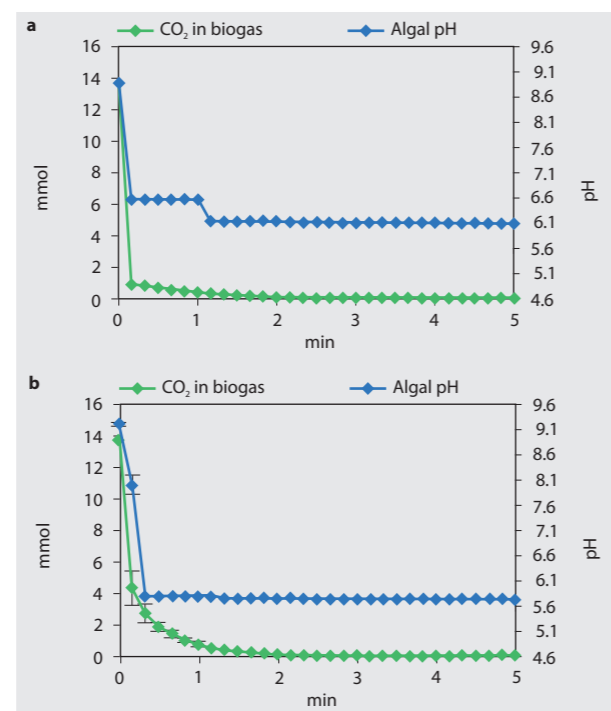
**Figure 3** Cell density in Mio. cells per ml ( $n = 3$ ) and algal biomass in mg per ml dry matter (DM) of (a) *Chlorella vulgaris* 211-11B and (b) *Chloroparva pannonica* 2358 in 3NBB-medium over a period of 10 days. Fit = fitted curve based on stepwise optimization of least deviation squares. Fed-batch cultures in a closed tubular photobioreactor at 20 °C, with a light-dark cycle of 16/8 h and a PFD of 60  $\mu\text{E}$ .

Axenic *Chlorella* was cultured with a start biomass of 0.03 mg dry mass · ml<sup>-1</sup>, whereas the non-axenic *Chloroparva* culture grew with a start biomass of 0.08 mg dry mass · ml<sup>-1</sup>. Regarding maximum biomass concentration, *Chloroparva* grew to 0.23 mg · ml<sup>-1</sup> and *Chlorella* to 0.30 mg · ml<sup>-1</sup>, both after nine culture days. The cell growth curves of *Chlorella* corresponded with their biomass growth curves. In contrast, the usage of non-axenic cultures led to deviations between biomass growth rates and cell growth rates during the preparation of *Chloroparva* suspension.

### 3.2 Biogas upgrading

All upgrading experiments with dark-adapted algal suspensions resulted in a virtually complete removal of the CO<sub>2</sub> from the biogas batch within a short time (Figure 4). In each trial, a biogas batch was upgraded for 40 min at a fixed gas to liquid ratio of 0.6 due to the construction of the gas scrubber. In tests with *Chlorella*, the CO<sub>2</sub> content of 34.33 mmol biogas dropped sharply by 94 % from 13.73 to 0.89 mmol within the first 10 seconds of the experiments. Subsequently, the CO<sub>2</sub> content decreased slightly to 0.02 mmol at 2:30 minutes and remained stable. Likewise, the pH of the *Chlorella* suspensions declined steeply from 8.91 to 6.57 during the first 10 seconds of the experiments. Subsequently, the pH dropped

further to 6.14 at 1:10 minute and remained constant at 6.10 from 2:10 minutes onwards. Biogas scrubbing in *Chloroparva* suspension was characterised by a nearly logarithmic decline of CO<sub>2</sub> by 99 % from 13.73 to 0.02 mmol within 2:50 minutes. The pH in *Chloroparva* cultures declined steeply from 9.22 to 5.79 within the first 20 seconds of the tests. Subsequently, the pH dropped to 5.75 at 1:20 minute and 5.74 at 2:20 minutes.

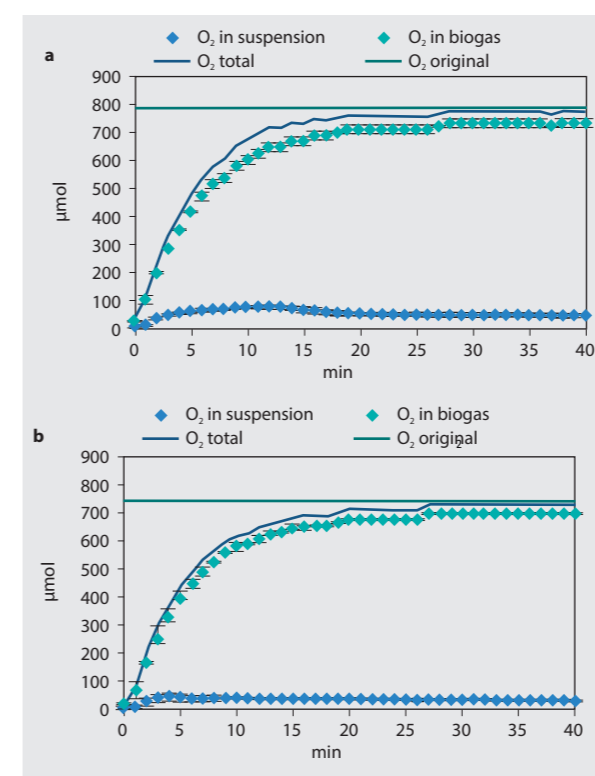


**Figure 4** Removal of CO<sub>2</sub> [mmol] from biogas batches and pH value of darkened microalgal suspension during the first 5 min of biogas upgrading experiments. (a) Batches of 34.33 mmol (840 ml) biogas with an original CO<sub>2</sub> content of 13.73 mmol scrubbed in *Chlorella vulgaris* suspension with a start pH of 8.91. (b) Batches of 34.33 mmol (ca. 840 ml) biogas with an original CO<sub>2</sub> content of 13.73 mmol scrubbed in *Chloroparva pannonica* suspension with a start pH of 9.22. Biogas sparged repeatedly through 520 ml algal suspension in a darkened, loop-shaped gas scrubber for 40 min. CO<sub>2</sub> contents measured in the gas compartment and pH values measured in the liquid compartment of the gas scrubber at constant 20 °C and 1004 ± 2 mbar atmospheric pressure.  $N = 2$ .

### 3.3 Biogas contamination

No air- or O<sub>2</sub> contaminations were detected subsequent to biogas upgrading experiments. During upgrading, the aggregated levels of O<sub>2</sub> in the liquid and gaseous compartment were consistently lower than the O<sub>2</sub> contents in biogas prior to upgrading (Figure 5). Biogas upgrading in *Chlorella* suspension resulted initially in an increase of dissolved O<sub>2</sub> in the algal suspension. The maximum of dissolved O<sub>2</sub> in algal suspension (72  $\mu\text{mol}$ ) was reached after 12 minutes of biogas upgrading. This increase was followed by a decrease to 42  $\mu\text{mol}$ , which was sustained through minute 40. The O<sub>2</sub>

content in biogas reached a maximum of 733  $\mu\text{mol}$  at minute 28 while passing the suspension repeatedly. Accordingly, the aggregated O<sub>2</sub> content in gas- and liquid compartment of the gas scrubber (O<sub>2</sub> total) increased to a steady maximum of 776  $\mu\text{mol}$  at minute 28. Likewise, biogas upgrading with *Chloroparva* suspension showed a steady increase of dissolved O<sub>2</sub> in algal suspension. A peak of 42  $\mu\text{mol}$  was measured after 4 minutes. Subsequently, dissolved O<sub>2</sub> declined to 30  $\mu\text{mol}$  during the further progress of the experiments. A maximum of 700  $\mu\text{mol}$  O<sub>2</sub> was detected in biogas after 27 minutes of passing the algal suspension repeatedly. As a result of the *Chloroparva* upgrading, the aggregated O<sub>2</sub> levels grew to a constant maximum of 732  $\mu\text{mol}$  at minute 27.



**Figure 5** Increase of O<sub>2</sub> content [ $\mu\text{mol}$ ] in 840 ml biogas and 520 ml darkened microalgal suspension during 40 minutes of biogas upgrading. (a) Batches of 34.33 mmol biogas with an original O<sub>2</sub> content of 787  $\mu\text{mol}$  scrubbed in *Chlorella vulgaris* suspension. (b) Batches of 34.33 mmol biogas with an original O<sub>2</sub> content of 743  $\mu\text{mol}$  scrubbed in *Chloroparva pannonica* suspension. O<sub>2</sub> content in biogas measured in the gas compartment and O<sub>2</sub> content in algal suspension measured in the liquid compartment of a darkened, loop-shaped gas scrubber at constant 20 °C and 1004 ± 2 mbar atmospheric pressure. Dotted line = original O<sub>2</sub> content of biogas prior to upgrading.  $N = 2$ .

## 4 Discussion

The use of microalgae for lowering the CO<sub>2</sub> content in biogas has been studied extensively. Scrubbing of biogas in 15 l

*Arthrospira sp.* resulted in a CO<sub>2</sub> decrease from 44 to 48 % to 2.5 to 11.5 % in an unspecified biogas volume (Travieso et al., 1993). Similar results were achieved by Conde et al. (1993) with *Chlorella vulgaris*, under the same experimental conditions. Tietze et al. (2006) reported a CO<sub>2</sub> reduction as well as simultaneous O<sub>2</sub> formation in 34.9 l biogas after passing through 6 l of *Chlorella vulgaris* C-1 (IPPAS, Moscow) suspension. Their experiments were performed in two different illuminated gas scrubbers and resulted in a CO<sub>2</sub> reduction from 30.1 % to 6.1 % and 31.3 % to 7.6 %. Simultaneously, O<sub>2</sub> increased from 1.7 % to 26.6 % and 0.8 % to 25.4 %. Studies of Converti et al. (2009) resulted in a nearly complete removal of CO<sub>2</sub> from 140 to 200 l biogas when scrubbed in 1 l *Arthrospira platensis* suspension. The removal was accompanied by enrichment of O<sub>2</sub> in the upgraded gas in the range of 10 to 24 %. Similar results were achieved by scrubbing 3 l biogas in 450 ml *Chlorella vulgaris* 211 to 11B suspension by Mann et al. (2009). In these experiments the CO<sub>2</sub> proportion in biogas was lowered from 41 % to 1.2 % while O<sub>2</sub> increased from 1.0 % to 23.4 %. Schmack et al. (2009) reported a CO<sub>2</sub> removal of 92 % with a simultaneous increase of 2 % O<sub>2</sub> and 7 % N<sub>2</sub> to the cleaned gas.

Virtually all approaches in which photosynthetic microalgae were applied to biogas upgrading resulted in oxygenation of the upgraded gas. However, literature on the utilization of this type of product is scarce. Oxygenated biogas can be used, e.g., for the production of polyhydroxy butyric acid by methanotrophic organisms (Bäzold et al., 1998) or biochemical desulphurization of biogas (Krüger et al., 2007). Another promising process is based on the assimilation of CO<sub>2</sub> and O<sub>2</sub> by a microalgal-bacterial consortium (Bahr et al., 2014). However, this approach is H<sub>2</sub>S-dependent and still subject of optimization.

In the present study, CO<sub>2</sub> was successfully removed from biogas. The reaction kinetics of the CO<sub>2</sub> removal from biogas and the pH value of the algal suspensions during upgrading (Figure 4) indicate that CO<sub>2</sub> was nearly completely absorbed by the algal medium. The CO<sub>2</sub> mass transfer was primarily affected by the volumetric ratio of biogas and liquid upgrading suspension (G/L ratio) as well as the pH of the upgrading suspension. In our experiments, 99 % of CO<sub>2</sub> (13.72 mmol) were removed from biogas at a fixed G/L ratio of 0.6 at pH values of 8.91 (*Chlorella*) and 9.22 (*Chloroparva*). The comparison of our findings with the literature is limited due to missing information about molar concentrations of CO<sub>2</sub> as well as varying parameters of CO<sub>2</sub> mass transfer in other studies. For example, the pH values of the algal suspension ranged from 6.8 (Tietze et al., 2006) to 9.5 (Converti et al., 2009). In addition, G/L ratios were between 0.6 and 200. However, Schmack et al. 2009 reported a CO<sub>2</sub> removal from biogas of 92 %. The biogas was upgraded in a mixture of *Chlorella* and *Spirulina* at a pH of 8.8 and a G/L of 0.6. Compared to these findings, the CO<sub>2</sub> removal of 99 % in our experiments resulted in upgraded biogas with a higher calorific value and less of a requirement for storage space. The mass transfer of CO<sub>2</sub> from biogas to *Chloroparva* suspension was also almost complete (13.718 mmol). However, in contrast to the *Chlorella* experiments, we found higher pH shifts in algal

suspension and slightly delayed CO<sub>2</sub> decreases in biogas. The deviations indicate a biological effect on CO<sub>2</sub> removal from biogas since the upgrading experiments with both algal species were performed under identical physical and chemical conditions. Such an effect could be caused by carbon concentration mechanisms (CCM), which can be found in other Trebouxiophyceae (Meyer and Griffith, 2013). However, further analyses of algal biomasses and -media are required to determine the exact mechanisms of this algal-specific influence on the CO<sub>2</sub> mass transfer.

Unlike previous reports on biogas upgrading with microalgae, we removed CO<sub>2</sub> from biogas without simultaneous oxygenation. During upgrading, the aggregated amounts of dissolved O<sub>2</sub> in algal media and gaseous O<sub>2</sub> in biogases were constantly lower than the original O<sub>2</sub> content in biogases prior to upgrading (Figure. 5). The apparent increase of O<sub>2</sub> in biogas until minute 28 (*Chlorella*) and 27 (*Chloroparva*) was a result of the biogas distribution within the gas scrubber and did not reflect the actual amount of substance in the system. In contrast, the absorption of O<sub>2</sub> in algal media was detected in real-time. The transition of O<sub>2</sub> into media was feasible since the media were undersaturated with O<sub>2</sub> which was a result of dark adaption prior to biogas upgrading. We assume that a part of the dissolved O<sub>2</sub> was respired by the dark-adapted algae since the aggregated O<sub>2</sub> levels in the gas scrubber were 1.4 % (*Chlorella*) and 1.5 % (*Chloroparva*) below the original O<sub>2</sub> contents. The respiration, in turn, lead to the formation of CO<sub>2</sub> during the upgrading process. However, the minimal CO<sub>2</sub> input did not have a significant impact on the overall CO<sub>2</sub> removal.

In our photobioreactor, we grew *Chlorella vulgaris*, which is a well-studied organism with respect to carbon dioxide removal from gaseous waste streams (Yun et al., 1996; Keffer and Kleinheinz, 2002; Douskova et al., 2009). In addition, we tested the recently described *Chloroparva pannonica* (Somogyi et al., 2011) for its growth characteristics in a photobioreactor. The photobioreactor cultures yielded 0.30 g·l<sup>-1</sup> (*Chlorella*) and 0.23 g·l<sup>-1</sup> (*Chloroparva*) dry matter. At the start of culturing, cell densities and biomasses of *Chloroparva* were higher than in *Chlorella*. On the one hand, this resulted from the comparatively small *Chloroparva* cells, which hindered matching the cell densities of the *Chlorella* cultures during the preparation of the photobioreactor inoculum. On the other hand, the *Chloroparva* cultures were not axenic and their biomass could not be used for matching the start biomass of the *Chlorella* cultures. Hence, the differences in algal growth characteristics in the photobioreactor cannot be clearly attributed to a specific factor. Nevertheless, the bench-scale photobioreactor proved to be suitable for the preparation of algal suspension for biogas upgrading. We grew *Chlorella* without contamination since sterilisable components and a custom setup for sterile inoculation were applied. Moreover, we demonstrated that *Chloroparva* can be grown in a photobioreactor.

Different from Schmack et al. (2009) and Bahr et al. (2014), we applied a discontinuous procedure to avoid contamination of upgraded biogas. We prevented air-contamination by culturing microalgae in a detached photobioreactor and

inhibited oxygenation by upgrading in a darkened scrubber. In addition, we determined reaction kinetics of O<sub>2</sub>- and CO<sub>2</sub> mass transfer from biogas into algal suspension in an innovative gas scrubbing loop. The present study also revealed potential for improvement of the system. During our experiments, a small quantity biogas was upgraded and, so far, effects on the methane content in the biogas were not determined. Also, the contact time for the phase transition of O<sub>2</sub> and CO<sub>2</sub> during gas scrubbing needs to be optimized since it was limited by the height of the gas scrubber and large bubble sizes. Finally, discontinuous processes are generally time-intensive. Despite its few technical limitations, our experimental system proved to be appropriate for studying the effects of microalgae and their culture media on biogas upgrading. These studies comprise the enhancement of the upgrading performance since the energy surplus from upgraded biogas determines the energy expenditure for its upgrading. In addition, valuable algal strains will be studied since a profitable algal production can compensate potential costs of biogas upgrading.

## 5 Conclusion

The present study describes a bench-scale system for detailed studies on the effects of microalgal strains and -media on the biogas upgrading process. In particular, the effects of the algal medium upon the CO<sub>2</sub>- and O<sub>2</sub> mass transfers during biogas purification, which are largely disregarded in the literature, have been illustrated. Moreover, we demonstrated that the recently described green alga *Chloroparva pannonica* can be grown in a bench-scale photobioreactor. With our experimental upgrading system we created the basis for future studies on biogas upgrading with phototrophic microalgae. These studies include analyses of buffering substances, which cannot be used as carbon sources by microalgae, and biogas upgrading with alkaline algal suspensions.

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