Experimental system for the prevention of O₂– 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 CO₂ from biogas into algal biomass. However, nearly all studies on biogas upgrading with microalgae resulted in contamination of upgraded gas with O₂ 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 CO₂ from biogas into dark-adapted microalgal suspensions. During the gas upgrading, the O₂- and CO₂ 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 CO₂ from all biogas batches. Simultaneously, no O₂ or air was added to the upgraded biogases. Furthermore, we found varying CO₂ kinetics which indicated an algal-specific effect upon the CO₂ 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


Schlüsselworte: Labormaßstab, Biogasaufbereitung, Gasverunreinigung, Mikroalgen
1 Introduction

Biofilms typically consist of 40 to 75 % v/v methanol (CH₃OH) to 60 % v/v carbon dioxide (CO₂), water vapour, hydrogen sulphide and other trace gases, such as carbon monoxide, nitrogen and atmospheric oxygen (O₂) (Jander et al., 2016). Particularly, the CO₂ proportion influences the energetic properties of biofilms since higher CO₂ levels result in a decreasing caloric value of the gas mixture. Further, the efficiency of four-stroke biogas engines decreases with rising CO₂ concentrations in biofilms (Bari, 1996; Edelmann, 2001; Deubel and Steinhauser, 2011). Besides energetic aspects, high CO₂ concentrations increase the biofilm volume and the corresponding storage space. Biofilm storage at atmospheric pressure in large, plastic bags is associated with size- or transport problems (Andrea et al., 2011; Khoiyanbarg et al., 2011). Therefore, the generation of biofilms generally benefits from a CO₂ separation procedure. Chemico-physical processes, e.g. pressure swing adsorption and amine scrubbing, are state of the art for industrial biofilm upgrading (Peterson and Wellingen, 2009). Besides these energy-intensive processes, biological approaches have high potential for CO₂ separation from biofilms.

2 Materials and Methods

2.1 Microalgal strain and culture medium

Two microalgal strains were grown as model organisms for biogas upgrading. The green alga Chlorella vulgaris and the red alga Chloroparva pannonica (Ozenda et al., 2010) were selected unicellular and aplanate resistant green algal strains (Trebsiophyceae, 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 cultivate 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 µmol. 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 NBMM medium via a peristatic 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⁻¹ provided by a micro membrane pump, model NPMF-830 D005 (Reichelt Chemietechnik, Germany). Air was filtered with sterile NaClO cleaned with ethanol (75 % v/v) and rinsed with autoclaved hot water and the inoculum was prepared at a density of approximately 248 · 10⁶ cells·ml⁻¹ provided by a micro membrane pump, model NPMF-830 D005 (Reichelt Chemietechnik, Germany). Air was filtered with sterile NaClO cleaned with ethanol (75 % v/v) and rinsed with autoclaved hot water and the inoculum was prepared at a density of approximately 248 · 10⁶ cells·ml⁻¹ provided by a micro membrane pump, model NPMF-830 D005 (Reichelt Chemietechnik, Germany). Within the scrubber, the algal suspension was separated from the biogas and a ventilation junction. A cooled (3 °C) liquid trap was installed to prevent condensation inside the pump and sensors. The gas flow was monitored by a flowmeter (AMW 3300V; Honeywell, USA). The experimental setup included a N₂-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 peristatic pump (Reefdoser 202, Aqua Medic, Germany). Within the scrubber, the algal suspension circulated anticlockwise for 40 min with a flow rate of 3.1 l·min⁻¹. 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 Chloroparva were performed with two batches of 34.33 mmol (ca. 840 ml) biogas with a mean content of 13.73 mmol CO₂ and 787 µmol O₂ before upgrading. In Chloroparva experiments, two batches of 34.33 mmol biogas with a mean content of 13.73 mmol CO₂ and 787 µmol O₂ 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⁻¹ to a maximum of approximately 40·10⁶ cells·ml⁻¹. Chloroparva pannonica showed a growth ratio of 0.49 d⁻¹ and a maximum cell density of approximately 248·10⁶ cells·ml⁻¹. Both growth curves were characterised by a short lag phase of approxi- mately two days, followed by a log phase of approximately five days. For both algal strains, the transition from exponents- tial growth to stationary growth phase was detected after circa seven culture days.

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

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 µmol. (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.

Both strains grew in NBMM medium according to the recipe of Starr and Zeikus (1993). We selected unicellular and aplanate resistant green algal strains (Trebsiophyceae, 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.
Axenic Chlorella was cultivated with a start biomass of 0.03 mg dry mass ml$^{-1}$, whereas the non-axenic Chlorella pannonica grew with a start biomass of 0.08 mg dry mass ml$^{-1}$. Regarding maximum biomass concentration, Chlorella grew to 0.23 mg ml$^{-1}$ and Chlorella to 0.30 mg ml$^{-1}$, 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 Chlorella pannonica suspension.

### 3.2 Biogas upgrading

All upgrading experiments with dark-adapted algal suspensions resulted in a virtually complete removal of CO$_2$, whereas the non-axenic Chlorella pannonica culture grew with a start biomass of 0.08 mg dry mass ml$^{-1}$. Regarding maximum biomass concentration, Chlorella grew to 0.23 mg ml$^{-1}$ and Chlorella to 0.30 mg ml$^{-1}$, 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 Chlorella pannonica suspension.

#### 3.3 Biogas contamination

No air- or O$_2$- contaminations were detected subsequent to biogas upgrading experiments. During upgrading, the aggregated levels of O$_2$ in the liquid and gaseous compartment consistently lower than the O$_2$ content in biogas prior to upgrading (Figure 5), biogas upgrading in Chlorella suspension resulted initially in an increase of dissolved O$_2$ in the algal suspension. The maximum of dissolved O$_2$ in algal suspension (72 µmol) was reached after 12 minutes of biogas upgrading. This increase was followed by a decrease to 42 µmol, which was sustained through minute 40.

**4 Discussion**

The use of microalgae for lowering the CO$_2$ content in biogas has been studied extensively. Scrubbing of biogas in 15 l Arthrospira sp. resulted in a CO$_2$ decrease from 44 to 48 % to 2.5 % to 11.5 % in an unspecified biogas volume. According to these findings, the aggregated O$_2$ content in gas- and liquid compartment of the gas scrubber (O$_2$ total) increased to a steady maximum of 776 µmol at minute 28. Likewise, biogas upgrading with Chlorella suspension showed a steady increase of dissolved O$_2$ in algal suspension. A peak of 42 µmol was measured after 4 minutes. Subsequently, dissolved O$_2$ declined to 30 µmol during the further progress of the experiments. A maximum of 706 µmol O$_2$ was detected in biogas after 30 minutes of passing the algal suspension repeatedly. As a result of the Chlorella suspension upgrading, the aggregated O$_2$ levels grew to a constant maximum of 732 µmol at minute 27.

**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) Chlorella pannonica 2358 in 3NBB-medium over a period of 10 days. Fit = fitted curve based on stepwise optimization. Error 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 µE.

**Figure 4**

Removal of CO$_2$ ([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$_2$ 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$_2$ content of 13.73 mmol scrubbed in Chlorella 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$_2$ 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.

**Figure 5**

Increase of O$_2$ content ([µmol]) 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$_2$ content of 787 µmol scrubbed in Chlorella vulgaris suspension. (b) Batches of 34.33 mmol biogas with an original O$_2$ content of 743 µmol scrubbed in Chlorella pannonica suspension. O$_2$ content in biogas measured in the gas compartment and O$_2$ 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$_2$ content of biogas prior to upgrading. N = 2.
Inhibition of oxygen consumption by upwelling in a darkened culture. After this, we detected reactions of increased oxygen and carbon 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 increase in oxygen and carbon dioxide was observed, indicating that further studies on the deposition of both gases were not determined. Also, the contact time for the phase transition of oxygen and carbon dioxide during gas scrubbing needs to be optimized so that the height of the gas scrubber and coalescence 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 on the algal and coagulation media in upgrading processes. 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 so that 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 CO₂ and O₂ mass transfer 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 buffer substances, which cannot be used as carbon sources by microalgae, and biogas upgrading with alkaline algal suspensions.

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