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THE ALGA *TRACHYDISCUS MINUTUS* AIDS PURIFICATION OF BIOGAS TO METHANE

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Summary: The alga *Trachydiscus minutus* was cultivated in a medium enriched with $NaHCO_3$ and periodically bubbled with bottled CO_2 for 10 min once per every two days. It was found that the alga was able to accumulate biomass under such conditions. An intrinsic ability of *T. minutus* is its easy precipitation, which implies that this alga could be used as a potential purifier of methane from biogas proposed in a model in which the alga was grown in a photobioreactor with mechanic stirring. Part of the algal suspension was periodically separated from the photobioreactor. The biomass was then precipitated and could be either returned back to the photobioreactor or separated as yield if the algal density was too high. The remaining cultural solution was placed under vacuum so that the dissolved oxygen could be removed. After that the solution was enriched with CO_2 from biogas. It was then returned to the photobioreactor because the content of $NaHCO_3$ was once again restored. Thus, methane can exit the absorption column purified due to its low solubility. The major outcome of the proposed procedure is the production of methane-rich and oxygen-free biogas which can be pressured and transported.

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INTRODUCTION

The yellow-green alga *Trachydiscus minutus* has been described as a useful new subject for photoautotrophic biotechnology (Iliev et al., 2010; Lukavský, 2012; Cepák et al., 2013). Several recently reported qualities characterize *T. minutus* as a microalga appropriate to be cultivated in a photobioreactor, especially in the temperate zone. Indeed, the alga shows satisfactory growth at low light intensity and optimal temperature 26-27°C, and such merits could be turned in real advantages. The lower optimal temperature means a maintenance of lower overall-heat-transfer and less energy waste in comparison to cyanobacterium *Spirulina* or the green algae *Chlorella*, *Dunaliella*, *Scenedesmus* whose optimal temperature is about 32-33°C. The alga *T. minutus*, which usually grows at low light intensity, is successfully cultivated in a vertical photobioreactor with folded

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area. One of the most useful qualities of *T. minutus* is its easy separation from the medium (Alexandrov et al., 2014). Besides, the alga has a higher percentage of fatty acids compared to many other studied algae. Nevertheless, the reason to involve *T. minutus* in the great quest of bio-fuels is neither the fair abundance of oil, nor the biodiesel euphoria (Petkov et al., 2012; Alonso and Maroto, 2012). We share the opinion that the photosynthetic function of vital alga cultures ought to be used in the production of bio-fuels. The algal biomass itself is a highly valued product and should be used in well known and much more effective ways. One could purify biogas to methane and decrease CO_2 emissions connecting the heterotrophic degradation of cellulose wastes and the photoautotrophic microalgae biomass production. Papers have been published concerning utilization of CO_2 from biogas and production of methane (Koh et al., 1988; Converti et al., 2009). Regretfully, the outcome is a gas mixture of methane and 10-24 % O_2 released by photosynthesis (Converti et al., 2009), but it is not safe working with such an explosive mixture. Besides, it cannot be compressed and transported. The aim of this paper was to find out how to utilize CO_2 rendering pure methane fit to be compressed.

MATERIALS AND METHODS

Cultivation of the alga

The yellow-green alga *Trachydiscus minutus* (strain Lukavský and Přibyl 2005/1) was grown as a mono-algal culture in 200 cm^3 flasks bubbling continuously with 3 $cm^3.s^{-1}$ air enriched with 0.5% CO_2, and also in glass flasks with a volume of 200 cm^3, mechanical stirring and periodic bubbling with pure bottled CO_2. The flask with mechanical stirring is the equivalent to the photobioreactor according to the proposed scheme in Figure 2. Cultivation was carried out at a temperature of 26°C and continuous illumination of 300 μmol m^{-2} s^{-1} provided by luminescent lamps (5 × 40 W). A previously described nutrition medium (Alexandrov et al., 2014) was used as a control, while the experimental variants included $NaHCO_3$ applied at concentrations of 3, 5 and 10 g dm^{-3}, respectively. Alga growth was estimated gravimetrically and calculated as dry weight of algal biomass per culture volume. The state of monoculture was checked microscopically once per day.

Gas procedures

The supernatant from the mechanically stirred culture was separated from the biomass once per every two days. The cultural liquid was treated under vacuum for 10 min at 12 mm Hg (1.57 kPa) to expel photosynthetically produced O_2 and to simulate and test the viability of the proposed concept as shown in Figure 2. Finally, the liquid was saturated with bottled CO_2 for 10 min in order to restore the concentration of $NaHCO_3$.

RESULTS AND DISCUSSION

Growth of *Trachydiscus minutus* under high $NaHCO_3$ concentrations

The ability of *Trachydiscus* to grow at high concentrations of $NaHCO_3$ makes it particularly appropriate for the aim of biogas purification to methane. Two separate experiments with cultivation of the alga at high concentrations of $NaHCO_3$ were conducted. The first one aimed to

determine the concentrations under which the alga was able to grow satisfactorily. This experiment was carried out under continuous bubbling with air enriched with 0.5% CO_2 in order to ensure exponential growth. The results showed that $NaHCO_3$ concentrations of 10 g dm^{-3} and above were lethal, the algal cells died quickly within one or two days. The concentration of 5 g dm^{-3} suppressed the growth, but the algal cells didn't die and resumed their normal growth if the culture was returned to the standard medium. The concentration of 3 g dm^{-3} ensured intensive growth, high algal density and yield of more than 4 g dm^{-3} algal biomass in the final stages of cultivation, which was similar to the yield produced in the standard medium.

The second experiment included mechanical stirring and periodic pure CO_2 bubbling. A concentration of 3 g dm^{-3} $NaHCO_3$ was chosen based on the results shown above. The results confirmed that *T. minutus* was able to grow in the absence of continuous CO_2 supply. A short supply was provided once per every two days for 10 min with pure CO_2 bubbling through the cultural liquid separated from algal biomass. The accumulated biomass under optimum conditions (standard medium and continuous bubbling) increased 4.9-fold per eleven days, under continuous bubbling and in the presence of $NaHCO_3$ it increased 3.5-fold, while under mechanical stirring a 2.3-fold increase per eleven days was registered. One has to bear in mind that on a large scale the preferred initial density is higher than 2 g dm^{-3}, because we have observed in a previous study (Alexandrov et al., 2014) that there is a correlation between algal growth and density, and the value of 4 g dm^{-3} is optimal for *T. minutus*.

A comparison of the growth between the experiments with mechanical stirring, continuous bubbling and the control culture of *T. minutus* is shown in Fig. 1.

Separation of biomass and supernatant

As shown in Fig. 2, part of the algal suspension was poured out in two or several equal smaller cultivation units with a volume of about 5-10 % of the photobioreactor volume.

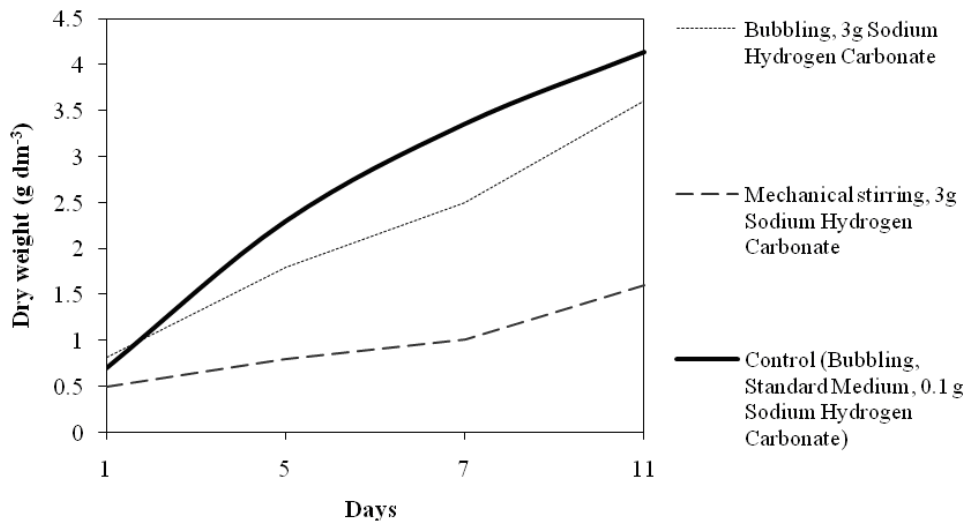

Figure 1. Growth of *Trachydiscus minutus*.

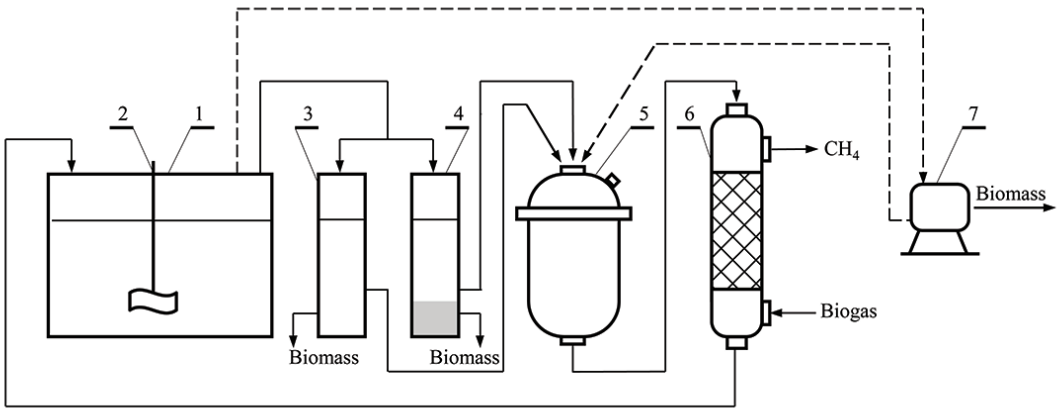

Figure 2. Purification of methane and algae biomass production. 1. Photobioreactor; 2. Mechanical stirrer; 3, 4. Units I and II; 5. Vacuum-receiver; 6. Absorption column; 7. Centrifuge.

While being in the smaller cultivation units, the biomass of *T. minutus* spontaneously precipitated and falled to the bottom. Our experiments showed that this process was partial and not complete, and further centrifugation at a low rate was still required as previously suggested (Alexandrov et al., 2014). The cultural liquid from the first unit was treated as described in the next entry. The separated algal biomass was added to the main cultivation unit when algal density was suboptimal, or it was periodically harvested as biomass yield. The same procedure was applied to the second cultivation unit or to each subsequent unit.

Removal of the solved O_2

As an outcome of photosynthesis, the concentration of O_2 reaches a mean value of 4-fold higher than its usual solubility at the same temperature and partial pressure (Richmond, 1986). Oxygen should not be present while working with methane because of the explosiveness of the mixture. In our experiments, the cultural liquid was placed under vacuum in order to expel the dissolved O_2. The pressure of the pump used was 12 mm Hg (1.57 kPa) and treatment duration was 10 min. This time is enough to evaporate O_2 and other dissolved gases, and after that water evaporation begins.

This little waste of labour and energy is well-paid with methane of high purity and safe work. The proposed concept was tested in the laboratory, in a vacuum flask, and it was proven that the dissolved air evaporated quickly from the cultural liquid, just within minutes. In a pilot plant for purification of methane it has to be carried out in a proper vessel designed to function under vacuum.

Saturation of the clear solution with CO_2 from biogas

In general, biogas is a mixture of 55-65% methane, 30-40% CO_2 and less than 1% other gases which has been well documented through decades (Golueke et al., 1957; Sharma et al., 1988; Briand and Morand, 1997; Fantozzi and Buratti, 2011). The solubility of CO_2 in the medium at the temperature of cultivation (26°C) was 1.3

g dm^{-3}, and this was about 60-fold higher in comparison to methane. Besides, the restoration of $NaHCO_3$ required CO_2 about 0.78 g dm^{-3}. As a sum of the solubility and the chemosorption (1.3 + 0.78), the real capacity of the solution to absorb CO_2 was above 2 g dm^{-3}. The solubility of CH_4 was merely 0.023 g dm^{-3}, which was 87-fold lower than the total effect of CO_2.

The decrease of solution pH indicated that $NaHCO_3$ was gradually restoring to the initial concentration. Both chemosorption and relatively higher solubility of CO_2 facilitate the separation of methane. The thermal effect of chemosorption is negligible for the biological process. It is too simple to change the temperature of the cultural liquid. In fact, the thermal income from the Sun is multifold higher than the thermal effect of chemosorption. The described processes are expected to work similarly not only in laboratory conditions, but also during large scale cultivation.

Due to the substantial difference between the solubility of methane (0.023 g dm^{-3}) and CO_2 (1.3 at 26°C) we used pure CO_2 from a bottle instead of biogas to restore the initial concentration of $NaHCO_3$ and to ensure good growth of the algal culture with mechanical stirring.

As a conclusion, the proposed procedure should lead to the production of methane with a negligible concentration of CO_2, free of O_2 and ready to be compressed and transported. The losses of methane were equal to its solubility in water (23 g m^{-3}).

Advantages and disadvantages of using *Trachydiscus minutus*

The alga *T. minutus* is not unique with its bias to sedimentation and HCO_3^- nutrition. For example, the cyanobacterium *Gloeocapsa* answers to these requirements (Gacheva, 2013) and can also be used for methane purification. In order to make the method universal with regards to algae, which are not easily yielding to separation, one can use a small centrifuge or a vacuum-filter (Fig. 2). For example, *Scenedesmus* was successfully cultivated at 5 g dm^{-3} $NaHCO_3$ and the growth was the same as at CO_2 bubbling (Georgiev et al., 1977). Taking into consideration the huge experience with *Scenedesmus,* it can be the proper alga for methane purification, where all other operations remain the same as it was described above. However, centrifugation should be performed for complete separation from the liquid.

It can be pointed out that *T. minutus* is easily contaminated with *Scenedesmus*. Our experiments showed that the alga was much more susceptible to contamination when cultivated with CO_2 bubbling and not with mechanical stirring. The culture, constantly being in contact with the rushing air, could easily be contaminated. It is hard to keep great volume of air sterile, and our experiments showed that even the air filters were not able to prevent cultures from contamination. On the contrary, mechanical stirring doesn't require constant bubbling, and in our particular case, the cultures remained uncontaminated during the time of the experiment. Nevertheless, even if some contamination occurs, it does not change the outcome. The purification of methane is going on even if there is contamination, and the mixed culture *Trachydiscus*/*Scenedesmus* is also a valuable one for purification. Moreover, our experiments confirmed that if the algal density of *Trachydiscus* was above 5 g dm^{-3}, the

occasional contamination with cells of *Scenedesmus* remained about 5 % keeping this constant *Trachydiscus*/*Scenedesmus* ratio for a longer period.

CONCLUSION

Microalgal cultures can be used for effective purification of methane from biogas. The algal strain has to be apt to HCO_3^- nutrition. The obtained biogas is oxygen free. The product is liable to compression.

Our work in this field also yielded some other results not related to biogas, but related to cultivation of *Trachydiscus minutus*. The fact that the alga is able to grow in $NaHCO_3$ without constant bubbling, only with mechanical stirring and a brief exposure to CO_2, is very important regarding its susceptibility to contamination with green algae. Growth in such conditions can decrease contamination chances greatly as the culture is not in contact with non-sterile air.

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