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VERIFICATION OF THE GREEN MICROALGAE BIOMASS USE FOR BIOGAS PRODUCTION

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The article reviews the energy potential of microalgae as an alternative raw material for anaerobic digestion. Currently, energy security is one of the main topics among researchers. The amount of generated fossil fuels is limited, it is a question of time when fossil fuels will not continue to be accessible at low cost. There is a need to find an alternative carrier of energy which will replace the fossil fuels in the World. Green microalgae can be proposed as a possible bio raw-material, which can be used as an input material in order to produce energy. Lots of alternative technologies of algae cultivation are currently being developed all over the world. There is a necessity to search for a sensible way to produce algal biomass for bioenergy purposes, while maintaining all requirements involved in environmental and economic issues. The research results presented in the science article show that microalgae biomass is the proper alternative material for biogas production with the method of anaerobic fermentation. We believe that these research results can contribute to the future development of all forms of renewable energy in the Slovak Republic.

Keywords: biomass, microalgae, anaerobic digestion, biogas

The search for new alternative input materials for biogas plants is currently highly topical because of growing capacity to produce maize silage which is limited and at the same time also impinges on economic issues (high cost input material). Slovak Republic committed to increase the use of Renewable Energy Resources (RERs) in gross final energy consumption from 6.7% (2005) to 14% by 2020. The expected total energy consumption from Renewable Energy Resources for 2020 is approximately 80 PJ. Moreover, the Renewable Energy Directive (2009/28/EC) puts more emphasis on renewable energy than on biofuels by noting that 10% of energy used in transport should be renewable by 2020 (EU, 2009). Considering that, we should raise our awareness of alternative energy resources (for example algal biomass).

Cultivation of microalgae biomass – phases of growth of algae

We can underline the following phases in the growth of microalgae (Edmundson and Huesemann, 2015):

Lag Phase – the process of adaptation of algal suspension (inoculum) in a growth medium (culture). Generally, the duration of the initial phase is basically proportional to the duration time of process of inoculation.

Exponential Phase – the most important phase in the whole algae growth, the phase where we are able to control algal growth, by changing the parameters and environmental conditions (spectrum of light, pH, temperature, nutrients content, circulation time) created in a bioreactor with algal suspension. The density of algal cells increases.

Stationary Phase – the phase where the speed of algal growth is stabilised. In this phase the limiting factors (solid particles blocking the light, high concentration of phosphorus, nitrogen) are balanced.

Senescent Phase – this phase is called "culture crash". In the period of the last phase (culture collapse) the level of nutrients and water quality is not sufficient to sustain the growth of new cells. The number of algal cells usually quickly diminishes.

Basic factors required for the cultivation of microalgae

One of the most essential elements for the general positive result in algae cultivation process is proper selection of microalgae species, which is the most appropriate for the specific application (differences in cell wall structure, oil content, and growth parameters).

Light and heat

The photosynthetic activity of microalgae is usually limited due to availability of light intensity, nutrients and technological design of culture system. The highest data for the outdoor cultivation of microalgae in the world performs 30–40 g of dry weight m^2/day (Goldman, 1979). Light exposure should be kept in an optimal range (light and dark cycle), light duration can have a huge influence on final concentration of biomass, content of proteins and fatty acids (Ren, 2014). As defined by Schlagermann et al. (2012), effectiveness of light conversion into biomass is determined by photo-conversion efficiency (PCE) which is a decisive

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parameter. It is characterised by energy obtained by the process of conversion in comparison with available sunlight delivered to the conversion process.

Mixing

Mixing is a crucial parameter during the process of algae cultivation (Richmond, 2004). The proper intensity of mixing is required to transfer biomass in water. Mixing can reduce the concentration of nutrients as well as the gradient of temperature (Vasumathi et al., 2012). Moreover, mixing is very important for the cell growth, it prevents sedimentation of algal cells, the attachment of cells to the walls of bioreactor (cultivation system) and formation of dead zones (Carvalho et al., 2006).

CO_2 (e.g. flue gas)

As defined, algae were universally accepted as the proper solution for monitoring the greenhouse gas emissions. The research has demonstrated the efficient uptake of CO_2 (the amount of 159 mg/l per/day with 93% of CO_2 consumption efficiency) (Tsai et al., 2017). The capacity of microalgae to fix CO_2 enables to allocate carbon in cells of algae (Klinthong et al., 2015), pH controls CO_2 supply, which means carbon capture (Ying et al., 2014). The biogas plant produces various types of off-gases that are rich in CO_2 and thus can be used for the production of different types of microalgae. The CO_2 content in the exhaust gases is usually between 3–15%. Exhaust gases from agricultural biogas plants have relatively higher levels of CO_2 (approximately 12%). These gases are suitable as the carbon source for the cultivation of microalgae (Van Iersel and Flammini, 2010).

pH

The pH of microalgae suspension is a very important factor which affects the algal growth. The unsuitable pH level can have a negative impact and can be the inhibiting factor during the process of biomass generation. Generally the acidic media (pH 5–7.5) are beneficial for freshwater eukaryotic algae (Razzak et al., 2013).

Temperature

When the light intensity is reduced, temperature is the crucial parameter which has a huge impact on growth of algae. Temperature can affect the photosynthetic rates of different algae. As examined by Xiao et al. (2009) the temperature is an essential factor during algal growth, it determines intracellular processes, which can influence the final concentration of algal suspension. Temperature conditions can affect directly the growth rate of green microalgae (Singh, 2015).

Nutrients

The growth medium is aimed to supply the important inorganic factors, being in a further process the main components which build microalgae cells, these being: nitrogen, phosphorus, potassium and iron. The chemical estimation of the minimum content of the nutrients which have to be provided for the algae cultivation is specified in accordance with the molecular formula especially formulated for the biomass of algae: $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti, 2007).

Elimination of oxygen

Green microalgae produce oxygen in proportion to their growth. Oxygen should be removed, it is strongly connected to the activity of CO_2-fixing enzyme RuBisCO, which is responsible for the generation of biomass (competition between oxygen and enzyme) (Lodish et al., 2000; Haas et al., 2013).

Material and methods

The genus of microalgae *Chlorella sorokiniana* was selected for this study. The cultures of microalgae were cultivated in the laboratories of the Environmental Institute, Koš (Slovakia) within the biotechnological process conducted in an enhanced Bold's Basal medium (Andersen, 2005). For the cultivation of *Chlorella sorokiniana*, there was used a 10-liter bioreactor, which was later replaced by a 100 L bioreactor (10 L of algae suspension was added to 90 L of culture medium) while maintaining the optimum temperature between 25–28 °C. The bioreactor was not covered. The cool-white lamp (Sun-Glo T8 Fluorescent Aquarium Bulb, 30 Watt, 29.7 µmol/ s/m^2, 4200 K, 36 Inch) was used for the cultivation (photoperiod: 16 : 8, light:dark ratio). The proper pH level was kept between 7.0 and 7.3, using pH controller (digital pH CO_2 controller PH-201; electrode L: 100 mm, D: 10 mm; measuring range: 0.00 to

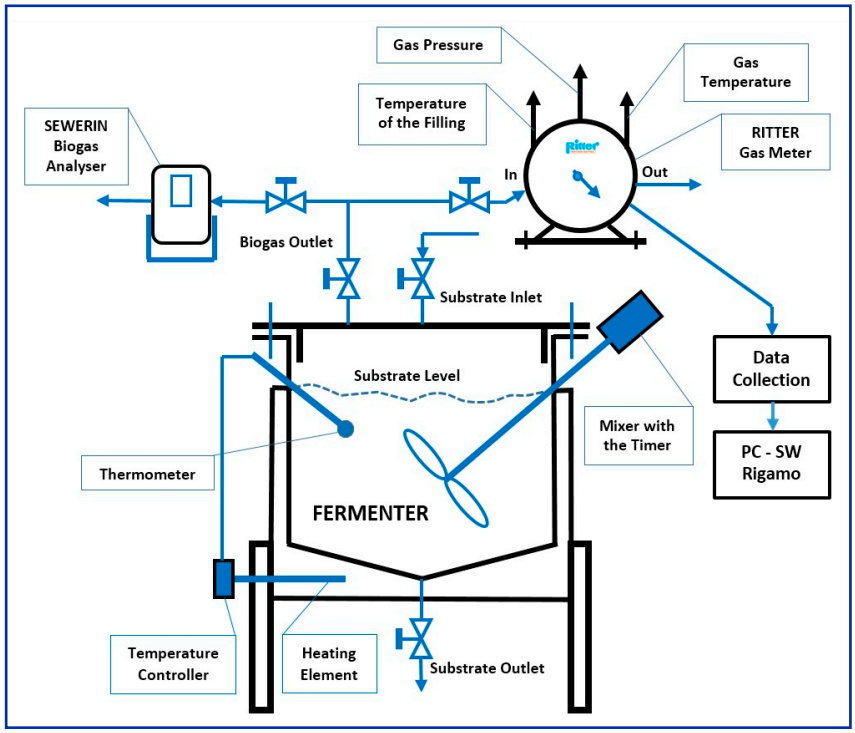

Figure 1 The technological scheme of the experimental fermenter 100 L
Source: author

Figure 2 The substrate: the inoculum + microalgae after filling of the fermenter
Source: author

14.00 pH; power supply: 230 V/50 Hz; dimensions: 100 × 20 mm). The algal suspension was circulated in the bioreactor (24 hours) with the use of CO_2 diffuser TURBO (diameter approx. 4.5 cm of height with base approx. 7.5 cm, height of the cover approx. 2.5 cm; power supply: 230 V).

Production of biogas from *Chlorella sorokiniana*

The microalgae biomass (3.5 L) from *Chlorella sorokiniana* (Figure 2), concentration of DM 1.05%, was processed during the comparative test of biogas yield in the workplace of the Department of Regional Bioenergy in Kolíňany. For our experiment we used the experimental fermenter (as presented on Figure 1) for batch tests.

The fermenter was filled with the inoculum taken from the biogas plant in volume of 97 L, where the microalgae (3.5 L) were added. The fermenter was constructed from the following parts: stainless steel tank (100 L of net volume), electric water heating, digital temperature control, electric low-speed mixer (12 cycles of mixing per day from 20–30 minutes). The value of the achieved biogas was recorded every hour. Each experiment directed to detection of the yield of biogas is carried out in the period of 30 days. After closing of the fermenter, it was set to auto mode control heating at 40 °C ±1 °C, as well as the automatic recording mode of the cumulative biogas production. The value of biogas production was recorded every hour. The processed outputs of individual endpoints are shown in the following tables and graphs.

Results and discussion

The processed outputs of the monitored parameters are given in the table and graphs. The values of monitored parameters and chemical composition of microalgae and inoculum are presented separately in Table 1. The cumulative production of the biogas is presented in Figure 3. The course of methane, carbon dioxide and hydrogen sulphide content in the biogas is showed in Figure 4.

The performed experiment has shown that algae *Chlorella sorokiniana* is a biomaterial, which can be used as an input material in order to produce biogas (as shown in Table 2). The significant result was in level of hydrogen sulphide which was low (267.32 ppm), the low content is important due to the fact that generated biogas will later require the

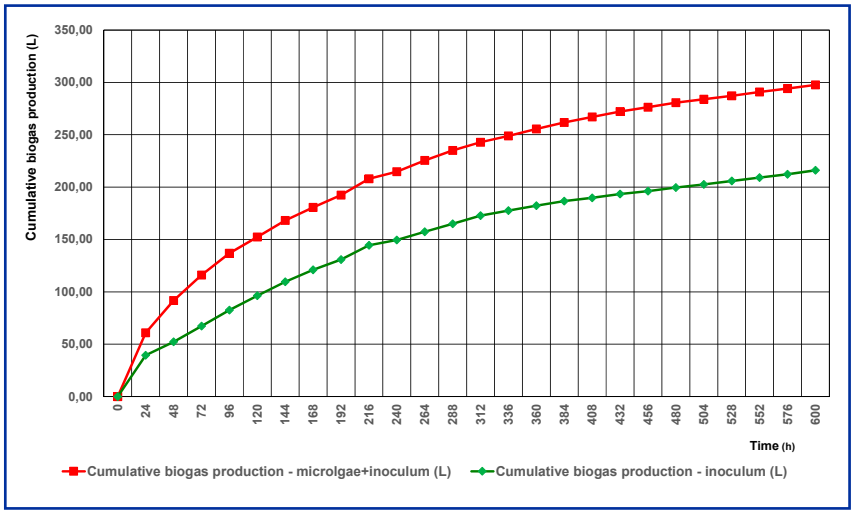

Figure 3 Comparison of the biogas cumulative productions from two substrates: microalgae *Chlorella sorokiniana* + inoculum and inoculum
Source: author

Table 1 The measurement of algae biomass *Chlorella sorokiniana* and inoculum

Input material (amount)	Temperature (°C)	pH	DM (%)	ODM (%DM)	COD (mg/L)	Ntot (mg/L)
Chlorella sorokiniana	20	8.2	1.05	73.91	13000	294
Inoculum	20	7.3	1.20	68.97	14000	300

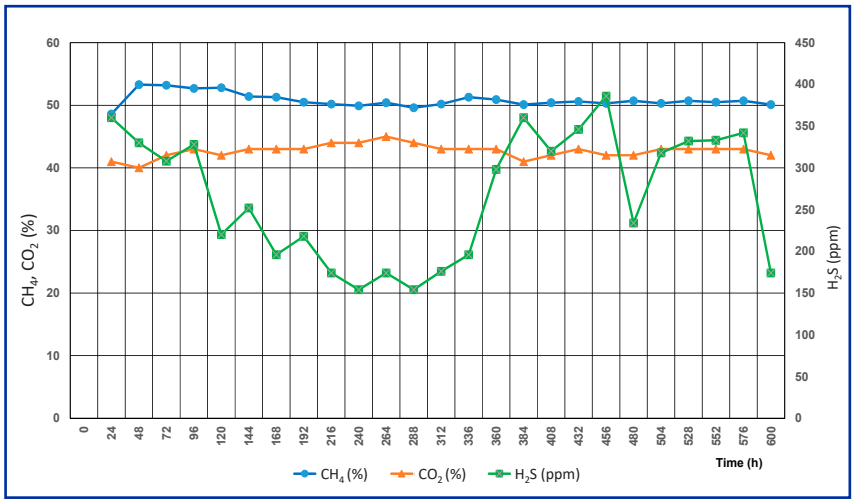

Figure 4 The course of methane, carbon dioxide and hydrogen sulphide in the produced biogas (author)

minimum desulphurisation. The average daily production of biogas was 11.90 L (in total 297.60 L). The conversion of biomass to the value of dry matter and organic dry matter (DM %, ODM %) of microalgae in the fermenter was as follows:

DM = 0.037 kg of DM of substrate

ODM = 0.027 kg of ODM of substrate

The average overall production of biogas (BP) on the unit of DM and ODM of the substrate was as follows:

BP production = 2.215 m^3/kg of DM

BP production = 2.997 m^3/kg of ODM

In comparison with results from the same experiment, with the use of liquid manure (inoculum 97 L, 1.20% DM) in the content of: pig liquid manure (80%) and cattle manure (20%). The total biogas production (in total 216.08 L) was 0.186 m^3/kg per unit of DM with the methane content of 50.83%. The results are presented in Table 2.

Comparing our results with biogas production achieved from maize silage (from previous experiments), the production of biogas was 0.689 m^3/kg per unit of DM and 0.954 m^3/kg per unit of ODM. Our experiment with the use of *Chlorella sorokiniana* gave the result of higher productivity, which means 2.215 m^3/kg per unit of DM and 2.997 m^3/kg per unit of ODM. However, we should also note the value of obtained hydrogen sulphide in produced biogas which was low (267.320 ppm), the low content is very important due to the fact that the generated biogas will not later require desulphurisation process. Based on our experimental results we should underline the high amount of achieved methane (CH_4) in biogas produced from microalgae *Chlorella sorokiniana*, which was 50.83%. Comparing our results with other research testing the potential of microalgal biomass for biogas production we can notice lower methane contents. Based on research results performed by Wang et al. (2013) they stated 19% of improved methane yield in case of *Chlorella* sp. (41% of DM), as well as taking into account the results provided by Olsson et al. (2014) where there was achieved 18% of improved methane yield in case of mixture *Chlorella* sp. and *Scenedesmus* sp. (37% of DM). The obtained results have shown that biomass of green microalgae *Chlorella sorokiniana* can be used as an input material to produce biogas with the method of wet fermentation. Green biomass can produce quite high values of methane (CH_4), and obtained biogas contains low values of hydrogen sulphide (H_2S), as indicated in Table 2. The obtained results give us a positive view into the future, it shows that there is a way to replace the traditional raw materials with algal biomass, which can be cultivated and harvested through the whole year, regardless of weather conditions and land area.

Conclusion

The results of the research collected in this science article allow formulating the following conclusion that microalgae biomass from *Chlorella sorokiniana* is the proper input material for biogas production, it generates the biogas with high methane content and low content of hydrogen sulphide. We believe the work will contribute to the comprehensive program for the use of all forms of renewable energy in the National Research Area.

Table 2 The average calculated values and the comparison of composition of the produced biogas from microalgae and liquid manure

Substrate (input material)	Total biogas production (L)	Average dose of substrate (kg)	Average biogas production per unit of DM (m^3/kg)	Average biogas production per unit of ODM (m^3/kg)	Average methane content (%)	Average carbon dioxide content (%)	Average hydrogen sulphide content (ppm)
***Chlorella sorokiniana* 3.5 L**	81.520	0.037	2.215	2.997	50.830	42.680	267.320
Liquid manure 97 L	216.080	1.164	0.186	0.269	50.830	41.720	253.240

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