

Microalgae Strains *Monoraphidium Griffithi* and *Chlorella sp.* for the Carbon Dioxide Capture from Biogas

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Abstract. One of the most important tools for mitigating climate change is biogas production. Increasing their use requires improving the energy value of biogas by reducing its carbon dioxide content while increasing the methane concentration in biogas. Biological purification of biogas using microalgae strains – merging biogas upgrading method where microalgae use carbon dioxide for their growth during the process of photosynthesis. A four-column photobioreactor was constructed with *Monoraphidium Griffithi*, *Chlorella sp.* microalgae strains, distilled water and MWH medium for purification of biogas. Experimental studies determined sizes, shapes and of microalgae cells, the pH changes of mediums used after biogas treatment and the composition of biogas before and after upgrading using photobioabsorber. Microalgae *Monoraphidium Griffithi* was estimated to have the greatest contribution to CO₂ reduction by decreasing from 31.0% to 10.0%. The smallest reduction in CO₂ was recorded when biogas was flowing through MWH medium. Experiments have shown that the absorption of biogas components results in the release of oxygen. As the biogas was flowing through all suspensions, the oxygen concentration increased from 3.6 to 5.2%.

Keywords: microalgae, biogas, photosynthesis, photobioabsorber, carbon dioxide, oxygen.

Introduction

Today, climate change is one of the most important global challenges that requires complex management, political and technological measures. Climate change is driven by greenhouse gas (GHG) emissions. Anaerobic digestion of organic waste is an important tool for mitigating climate change. The main components of biogas are methane (55–75%), carbon dioxide (20–35%), nitrogen (10%) and hydrogen sulphide (3000–5000 ppm) and other impurities. In order to improve the energy value of biogas and to use it more efficiently, it is necessary to reduce its carbon dioxide, remove hydrogen sulphide and increase the total methane concentration in biogas.

Currently, the production of biogas by anaerobic treatment of sewage sludge is of particular interest. Sludge is divided into two main groups: primary (physically and / or chemically treated) and secondary (biological treated). Sometimes a tertiary type of sludge is also produced, which is obtained after additional removal of organic matter (Fytily & Zabaniotou, 2008).

Biogas is produced from primary sludge. The yield of biogas depends not only on the technological process, but also on the composition of the organic raw material, especially nitrogen and phosphorus, fat and dry matter.

In Lithuania, as in the world, one of the most common sludge treatment methods is anaerobic sludge stabilization, which allows not only to reduce sludge content by up to 40% with respect to dry matter, but also to extract biogas, reduce pathogenic organisms, unpleasant odors (Vesilind, 2003; Pérez-Elvira et al., 2006). Methane accounts for more than 55% of the biogas produced and is therefore considered a valuable fuel that can be used in wastewater treatment plants and beyond, for example for electricity and heat production or as biofuel for transport (Budrys, 2006).

One of the main components of biogas from sewage sludge is carbon dioxide. Many different methods are used for purifying biogas from sewage sludge from carbon dioxide: physical absorption (with water), chemical absorption (with alkaline hydroxides, alkaline carbonates, ethanolamines) and others (with membranes, molecular filters) (Busca & Pistarino, 2003), however, other researchers are increasingly reporting data on adsorption methods (dry decarbonisation, carbonization rocks) (Johnsen et al., 2006; Hildenbrand et al., 2006; Stasiulaitienė, 2010; Sherman & Barak, 2000; Lackner et al., 1997).

The most widely used purification method is the physical/chemical absorption method as it requires less investment and is not complicated. The physical / chemical absorption method commonly involves high pressure and water

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absorber (Schieder et al., 2003; Rasi et al., 2008), where the cleaned biogas is fed into a purification column filled with Raschig rings, from below and using high pressure from above water.

Chemical absorption is determined by the reaction of CO₂ with absorbence – ethanolamine, alkali, potash. The most common purification technology is chemical absorption using alkanolamines (monoethanolamine (MEA) and N-methyl diethanolamine (MDEA) (Hoff et al., 2004)). Monoethanolamine solutions are widely used due to their properties: high efficiency, low cost and low uptake of hydrocarbons (Al-Marzouqi et al., 2008; Navaza et al., 2009; Fujii et al., 2009; Zaretskii et al., 2008).

Biological purification of biogas using microalgae strains is based on the principle of photosynthesis, in which microorganisms use carbon dioxide in the photobioreactor for their vital processes and reproduction. Correspondingly, the concentration of methane in biogas increases, and so does the energy value of biogas. At the same time, biogas from sulfur hydrogen and ammonia is purified. Improving the quality of biogas by using microalgae strains is based not only on biological processes but also on physical and absorption, where a significant reduction in carbon dioxide concentration is achieved by using an absorbent solution – water with chemical elements (MWH (Modified Woods Hole Medium)) with microalgae suspension (microalgae suspension)).

Biodegradation of microalgae with biogas is not always considered to provide a high biogas upgradation efficiency, so system design improvements, medium optimization and appropriate microalgae are constantly sought. High absorbency strains include *Monoraphidium Griffithii* microalgae found in fresh water. Microalgae use carbon dioxide to maintain their vital functions and benefit from the biogas treatment process. This removes harmful components from biogas and reduces the cost of biomass production (Hendroko et al., 2013).

Although considerable efforts have been made to develop efficient and cost-effective photobioreactors, the high cost of installing and operating artificial light sources in conventional photobioreactors with artificial lighting systems remains a major problem (Chen et al., 2011).

The light intensity decreases rapidly due to the shading of the light due to the increased concentration of both the cell and the product or the formation of biofilm on the surface of the reactor vessels (Chen et al., 2008). In addition, while short light paths are theoretically favorable for achieving high light efficiency, conventional light sources cannot come in close contact with microalgae culture as they typically emit large amounts of heat. Therefore, the light conversion efficiency of conventional photobioreactors is limited. Due to the problems and limitations associated with conventional light sources, various photobioreactor designs with different illumination strategies have been developed to increase microalgae growth rate and oil / lipid content (Ma & Hanna, 1999).

As discussed above, the productivity of microalgae biomass production is often limited by the light energy supplied and the cell concentration.

A cost-effective, low-cost, durable, reliable, and highly efficient light source is required to produce a cost-effective unit. If the light source has a narrow spectral output that coincides with the absorption spectrum of photosynthesis, the emission of light at the wrong frequencies would be eliminated, thus improving overall energy conversion. Among the light sources currently available, LEDs are the only ones that meet the above criteria. Light-emitting diodes are light and small enough to fit in virtually any photobioreactor, with the other benefits of longer life, lower heat dissipation, higher conversion efficiency and tolerance on and off. In addition, LEDs have a narrow light emission spectrum of 20 to 30 nm, which can be matched to the needs of photosynthesis. Wang et al. (2007) found that the highest specific growth rates and biomass production were obtained using a red light LED in a photobioreactor for growth of *Spirulina platensis* (Wang et al., 2007).

Significant research into the efficiency and applicability of bio-biogas purification methods has been carried out by scientists from different countries: Q. Zhao, R. Ramaraj, N. Dussadee, Y. Lee, G. Mann, M. Schlegel, R. Schumann, R. Sakalauskas, V. Makareviciene and others. Research findings and detailed results allow for an accurate assessment of not only the potential of efficient biological treatment methods, but also their realistic application, taking into account existing and expanding infrastructure in different countries.

The aim of the study was to evaluate the efficiency of carbon dioxide purification from biogas using *Monoraphidium Griffithii* and *Chlorella sp.* microalgae strains.

1. Methodology

Two cultures of microalgae strains of different genera were selected for the study: *Chlorella sp.* and *Monoraphidium Griffithii*.

Chlorella sp. belongs to the Chlorella family, a genus of *Chlorella* comprising about 56 species.

Monoraphidium Griffithii – spindle-shaped microalgae found in freshwater bodies. It is important to note that they need a sunlight or artificial light source to absorb carbon dioxide much more efficiently than plants do. These algae are used in the production of both biodiesel and bioethanol.

Knowing these microalgae evaluation criteria – size, shape and amount of microalgae cells – the volume of the biomass in suspension can be calculated, the changes in the life cycle (decay, death) of microalgae and the efficiency of carbon dioxide purification.

Prior to conducting research on the shape, size and quantity of the microalgae cells, they were grown in an algae growth reactor at the Nature Research Center. The growth time of microalgae is 20–30 days.

The algae were grown at room temperature at 22 ± 2 °C and illuminated with fluorescent lamps at ~ 250 $\mu\text{mol} / \text{m}^2$ white light for an average of 10 h. per day. Light intensity was measured using a Data Logger (Model LI-1400) LI190SA Quantum sensor.

Microalgae cell shape, size and quantity were studied using a Nikon ECLIPSE Ci-L optical microscope. Microscope resolution 1 μm , magnification up to 600x.

A microplate suspension of 256 cells is placed in a 0.0032 ml glass slide. Cover the specimen with a lid, beginning with the tip of the drop and carefully lowering the slide to distribute the suspension evenly. Place the specimen on the stage and press it against the holders. Using the microscope levers, the image is focused. By changing the objective sample, the sample is magnified 200, 400 and 600 times and analyzed. The image is output to the screen. The number of microalgae cells in the selected cells is counted. By determining the amount of microalgae cells in the selected cells, the average number of cells per cell is derived. The value obtained is multiplied by the number of cells in the cell. In this way, the number of microalgae cells per unit cell of known volume is obtained.

The same micro-algae cultures are still being studied for carbon dioxide purification from biogas. Prior to the assays, microalgae were inoculated with biogenic elements in MWH medium (Modified Woods Hole MBL Medium) for 14 days. Oxygen was supplied to the microalgae suspension during inoculation.

The required volume of the microalgae suspension is 4 liters as this volume contains a photobioabsorber column. The microalgae biotope is diluted with the nutrient medium in such proportion that the chlorophyll content is the same in all microalgae suspensions. The initial concentration of chlorophyll is 516 ± 10 mg / l.

Before filling the columns with microalgae suspensions, measure the physico-chemical parameters of the suspensions: pH, conductivity, Redox potential, TDS, total carbon, total organic carbon, inorganic carbon, total nitrogen concentration, chlorophyll concentration. In order to compare the effect of the nutrient medium and water on the purification of biogas from CO₂, the two columns of the photobioabsorber are filled with these suspensions. Since the photobioabsorbent laboratory consists of four columns, the columns are filled with four suspensions for biogas purification: suspension with *Monoraphidium Griffithii* microalgae culture, suspension with *Chlorella sp.*, microalgae culture, MWH nutrient medium, distilled water.

A laboratory bench and the course of the study were used for the studies.

The photobioabsorber constructed in the Department of Environmental Protection and Water Engineering of VGTU was used for the research. A schematic diagram of a photobioabsorber bench is shown in Figure 1.

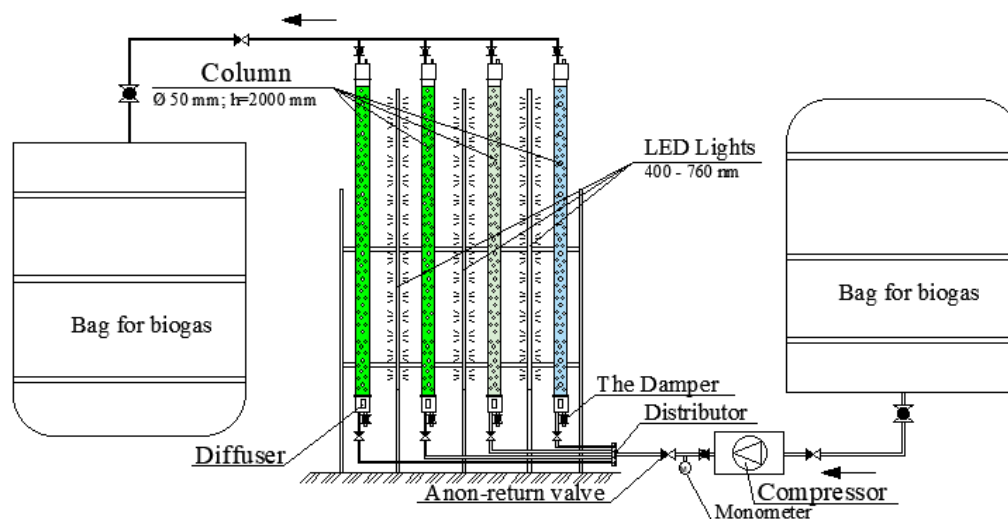


Figure 1. Photobioabsorber laboratory bench

Photobioabsorber – a microalgae strains filled biogas upgrading device to capture carbon dioxide from biogas, based on the principle of photosynthesis and absorption in liquid media.

From the 50 L biogas tank, the biogas obtained from the Vilnius sewage treatment plant is supplied by a compressor at a pressure of 200 mbar to four columns of 4 liters each. Each column is 2000 mm high and 50 mm in diameter. The distributor is equipped with valves that can regulate the flow of biogas into each column individually. Diffusers at the bottom of the column ensure uniform distribution of the biogas over the entire column cross-section, which disperses the biogas into small air bubbles, thereby improving the absorption and degradation of the elements contained in the biogas. The side illumination of the columns is equipped with LED light sources, which have regulators and the ability to select the required wavelength (400–760 nm) light. Biogas leaking columns are collected in a 2 m³ biogas collection tank.

Biogas for research was taken from AB Vilnius vandenys wastewater treatment plant. The biogas from the biogas storage tanks installed in the wastewater treatment plant is supplied by a compressor at a pressure of 100 mbar to a biogas tank with a capacity of 50 L. Composition of biogas taken from wastewater treatment plant: methane 51.5%, carbon dioxide 31.0%, oxygen 3.6%, hydrogen sulfide 2 ppm.

From the biogas tank, the compressor passes the biogas through the suspension columns. The biogas is supplied to the photobioabsorbent at a flow rate of 0.2 L / min. The photobioabsorber columns are illuminated by a white light LED source. The LED source is positioned 200 mm from the column to ensure that the light flux is evenly distributed throughout the column surface. The light intensity is adjusted so that the sharpness level of the columns reaches 500 lx.

The image of the laboratory bench is shown in Figure 2.

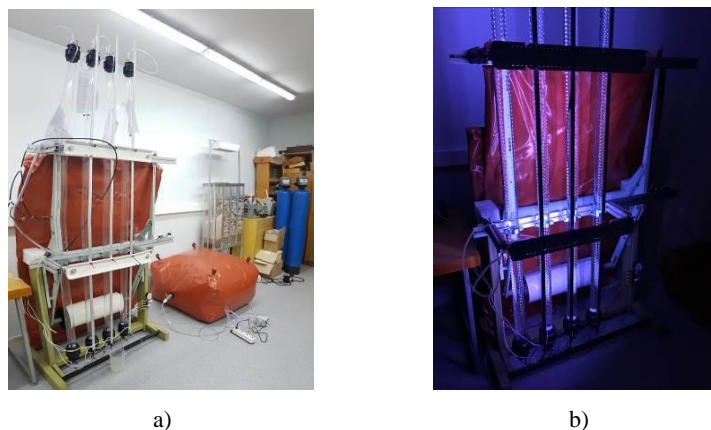


Figure 2. Laboratory bench (photobioabsorber): a) general view; b) lighting system view

Biogas is passed through photobioabsorber columns for 10 days. The biogas composition is measured every 3 days before and after each column. A sample of 1 L Tedlar is taken from the sampling points in the bench before and after each column to determine the biogas composition. The physical and chemical parameters mentioned in Table 2.2 of the suspension are measured every 5 days. The suspension is sampled from a laboratory bench in a 50 ml vial. The samples are transported in a mobile refrigerator for analysis to the laboratory of the Nature Research Center.

The dependence of the biogas cleaning efficiency on the suspension and the time of biogas passed through the photobioabsorber is investigated. This determines the dependence of the physico-chemical parameters of the suspensions on the time of biogas allowed through the photobioabsorber.

Application of analytical methods

The biogas composition was measured daily with the biogas analyzer INCA 4000, which provides methane (%), carbon dioxide (%), oxygen (%) concentrations and hydrogen sulfide (ppm). Appliance measuring ranges: 0–25% oxygen ($\pm 1\%$ tolerance), hydrogen 0–100 ppm hydrogen ($\pm 5\%$ tolerance), methane 0–100% ($\pm 1\%$ tolerance), carbon dioxide – 0–100 ($\pm 1\%$ error).

The pH is determined by a potentiometric method which is standardized. Measured according to LST ISO 10523: 1994. For pH determination, use the pH meter HI 98127, which measures the pH of the solution (measuring range 0 to 14, error $\pm 0,1$) and temperature (measuring range -5 to 60 °C, $\pm 0,5$ °C). The pH of the substrate is determined before and after the assay.

Illumination was measured using a meter Metrel Poly MI6401 with a measuring range (0 to 10.000) lx.

Measurements were made in three replicates. After obtaining the results of tests for a given parameter, its estimate is calculated, which is the arithmetic mean of the individual measurements. Knowing the arithmetic mean of the parameter, the experimental dispersion corresponding to the probability distribution of individual measurements was calculated. By calculation is founded the best estimate of the arithmetic mean dispersion, which is the experimental mean arithmetic dispersion equal to the normal distribution. After obtaining the arithmetic mean dispersion estimate, the experimental standard deviation of the arithmetic mean was calculated:

Microsoft Office Excel 2003 was used for statistical calculations. Arithmetic averages of measurements, experimental arithmetic variances, standard deviations of arithmetic mean were calculated using Microsoft Office Excel 2003 Statistical functions: AVERAGE, VARP, STDEV (standard deviation).

2. Results and discussion

By increasing the sample size to 200, 400 and 600 times the morphological composition of the microalgae was determined. The morphological composition studies show that the structure of the microalgae is not uniform. Studies have

shown that the microalgae examined are green in color due to their high chlorophyll content. Figure 3 shows the size and shape of the microalgae cultures *Chlorella sp.*, *Monoraphidium Griffithii* when the biogas was not allowed through the microalgae suspension.

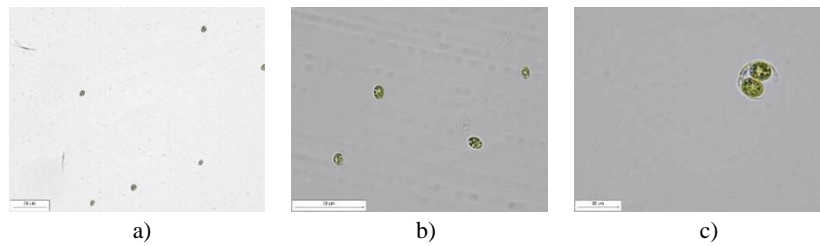


Figure 3. *Chlorella sp.* microalgae size and shape:
a) magnification 200x; b) magnification 400x; c) magnification 600x

Figure 3 shows that *Chlorella sp.* cells of the genus Algae were single, spherical or elliptical, with studies measuring 10 μm in diameter and 12 μm in length. During the studies, the number of microalgae cells present in the biogas purification system before the suspension was 542060 cells / ml.

Figure 4 shows that the microalgae of *Monoraphidium Griffithii* are elongated in shape. Consisting of two cells of equal size. The average cell length is 48 μm and the diameter is 3.7 μm . The cell volume is 275 μm^3 . The number of cells was 574320 cells / ml.

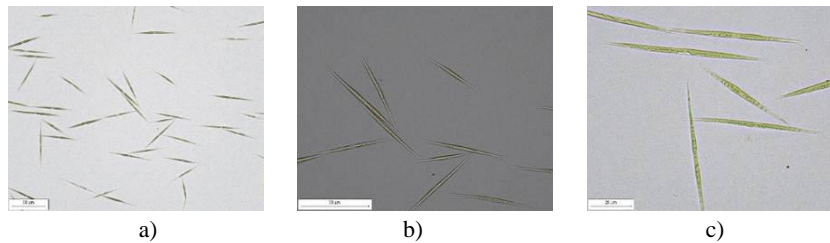


Figure 4. *Monoraphidium Griffithii* microalgae size and shape:
a) magnification 200x; b) magnification 400x; c) magnification 600x

During the research, the pH and purification efficiency of biogas were investigated by passing biogas through photobioabsorber columns with different suspensions. In this way, the influence of different suspensions on the biogas composition was evaluated.

In order to determine how the pH of different suspensions changes through the biobleaching of the biogas produced by anaerobic digestion of sewage sludge, the pH concentration dependence of the duration of the biogas allowed through the photobioabsorbent was obtained when the photobioabsorber columns were filled with different compositions.

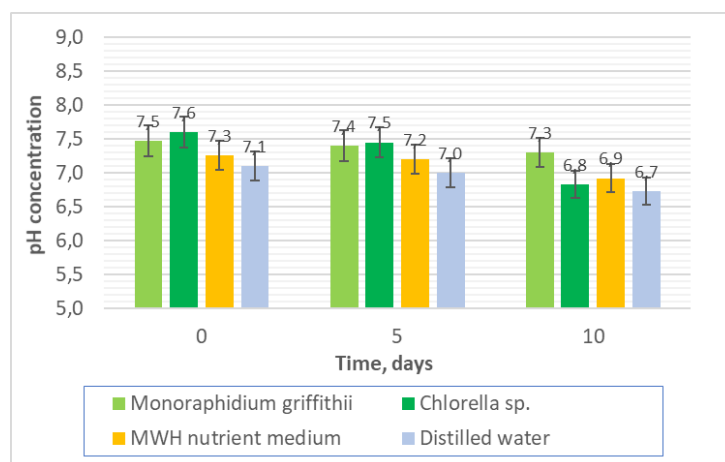


Figure 5. pH concentration dependence of biogas filtration time and different suspensions

The first two columns are filled with *Monoraphidium Griffithii* and *Chlorella sp.* microalgae cultures. It is noteworthy that the concentration of chlorophyll in microalgae was uniform and reached 516 ± 10 mg / l. At this concentration, the pH of the microalgae suspension at the start of the study was 7.5 and 7.6, respectively (Figure 5). Also, in order to evaluate the absorption of biogas contaminants in liquid media without microalgae cultures, the remaining two photobioabsorbent columns were filled with MWH nutrient medium and distilled water at pH 7.3 and 7.1, respectively.

Studies have shown that the pH of all suspensions is reduced by the passage of biogas through the photobioabsorber. The greatest decrease in pH after 10 days was observed in column-filled *Chlorella sp.* microalgae culture. After 10 days it reaches 6.8. The smallest reduction was found in column-filled culture of *Monoraphidium Griffithii* microalgae. Such acidification of the slurry is caused by chemical reactions of the compounds in the biogas, such as CO₂ or H₂S, with H₂O. This produces carbonic and sulfuric acids which also acidify the suspension itself. It is important to note that according to the literature, the pH of biogas is also acidic and averages between 6.6 and 6.8.

The initial methane concentration in the biogas was 51.5% (Figure 6). After passage of the biogas through the suspension with *Monoraphidium Griffithii* microalgae culture, the methane concentration increased to 63.2%. Between 5 and 10 days of biogas injection, the concentration of methane increased slightly, reaching 64.8%. Lower methane concentrations were fixed by passage of biogas through suspension with *Chlorella sp.* microalgae culture. After 10 days the methane concentration increased to 63.2%.

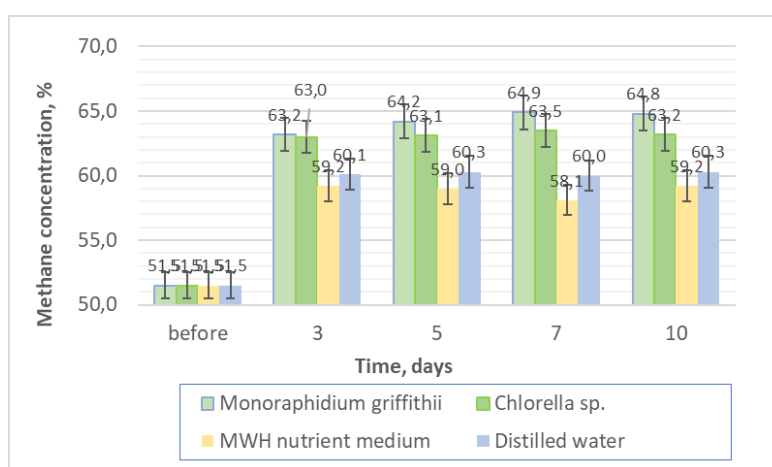


Figure 6. Methane concentration dependence on biogas flow time and different suspensions

It should be noted that the flow of biogas through distilled water increased the methane concentration to 59.2%. We can assume that the increase in methane in the photobioabsorber is greatly influenced by the absorption processes taking place there. The solubility of carbon dioxide in water depends on its pressure and temperature. Microalgae increased the methane concentration to 64.8% after 10 days. Microalgae cultures contribute to improving the quality of biogas emitted by anaerobic treatment of sewage sludge. The methane concentration increased from 51.5% to 59.0% when biogas was injected via MWH medium. However, unlike suspensions with microalgae cultures, they remained stable.

Studies have shown that biogas, when passed through suspensions of the microalgae *Monoraphidium Griffithii* and *Chlorella sp.* in microalgae cultures, methane concentration increases. In the photobioabsorber, both absorption and biological purification methods have an effect on improving the quality of biogas.

Studies have also been carried out to evaluate the changes in carbon dioxide concentration in biogas as they pass through different suspensions (Figure 7). Studies have shown that the passage of biogas through all the suspensions studied decreases the concentration of carbon dioxide.

Studies have shown that passing biogas through distilled water reduced CO₂ concentration from 31.0 to 13.7%. With the release of biogas through distilled water over a longer period of 10 days, the CO₂ concentration increases to 15.0%. CO₂ absorption decreases due to the reduced solubility of CO₂ in the liquid medium. Biogas passage through micro-algae suspension slurries showed the greatest reduction in CO₂. Microalgae culture of *Monoraphidium Griffithii* had the greatest impact on CO₂ reduction. The passage of the biogas through the suspension with *Monoraphidium Griffithii* microalgae culture reduced the CO₂ concentration from 31.0 to 12.7%. Biogas flow through this suspension reduced the CO₂ concentration to 10.0% for 10 days. The smallest reduction in CO₂ was recorded when biogas was injected via MWH medium. Its effect on CO₂ reduction was worst.

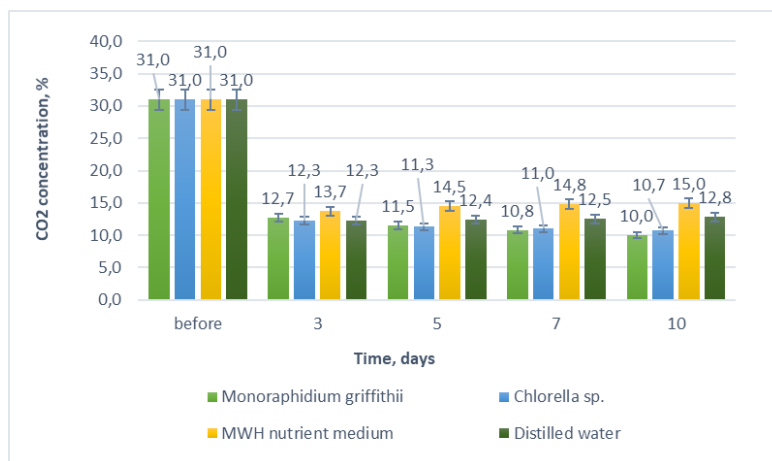


Figure 7. Dependence of carbon dioxide concentration on biogas flow time and different suspensions

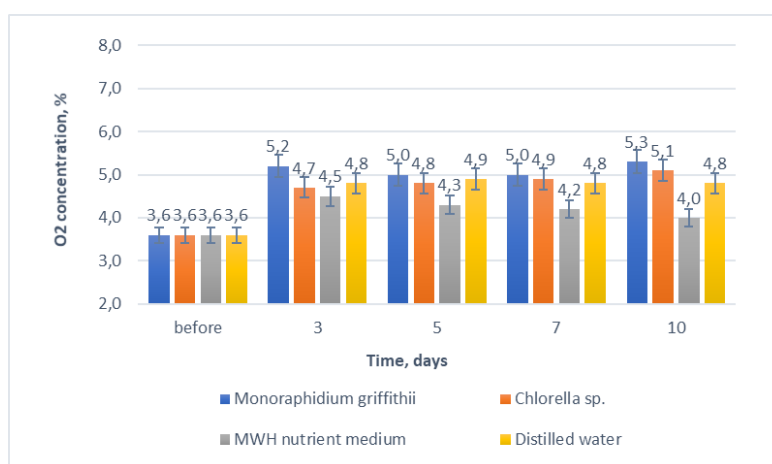


Figure 8. Dependence of oxygen concentration on biogas flow time and different suspensions

The reduction in CO₂ and the high efficiency of its purification in biogas can be explained by the process of photosynthesis and absorption. The solubility of CO₂ in water is higher compared to CH₄ – 1.45 g / l, which results in a higher proportion of CO₂ being absorbed. Also, microorganisms such as algae are the gases needed to support vital functions during photosynthesis. During photosynthesis, microalgae are capable of synthesizing organic compounds from inorganic materials. Organic substances are synthesized from carbon dioxide and water and oxygen is released as a by-product. In this process, high-energy carbohydrate glucose is synthesized from low-energy materials, carbon dioxide and water (Zhao & Su, 2014):



Increased oxygen concentration in biogas indicates that CO₂ absorption processes occur in suspensions (Figure 8).

During which the absorption of CO₂ in the nutrient medium increases the concentration of oxygen. Microalgae cultures also increase concentration.

Studies have shown that the absorption of biogas components results in the release of oxygen. As the biogas was passed through all the suspensions, the oxygen concentration increased from 3.6 to 5.2%. The highest concentration of oxygen was recorded by passage of biogas through suspension with micro-algae culture of *Monoraphidium griffithii*. The lowest concentration of oxygen – 4.5% – was recorded when biogas was injected via MWH medium. It is noteworthy that the oxygen concentration in the biogas increased during the 10-day passage of biogas through suspensions with microalgae cultures. This indicates that microalgae are active and capable of decomposing CO₂ by releasing oxygen. At this interval, the oxygen concentration did not change or decrease after 10 days passage through distilled water and MWH nutrient medium. This reflects the reduced absorption of biogas components in these suspensions.

Conclusions

1. Studies have shown that the pH of all suspensions is reduced by the flow of biogas through the photobio-absorber. The greatest drop in pH was found in column-filled *Chlorella sp.* microalgae culture (pH 7.6 to 6.8). The smallest reduction was found in column-filled culture of *Monoraphidium Griffithii* microalgae.
2. Methane concentration increased from 51.5% to 63.2% in the first 3 days after biogas treatment with *Monoraphidium Griffithii* microalgae. Such changes may have been caused by the absorption of CO₂ in biotechnology.
3. Biogas flow through suspension with *Chlorella sp.* microalgae after 10 days, the concentration of methane increased from 51.5% to 63.2%. The flow of biogas through distilled water increased the methane concentration to 59.2%. Therefore, the increase in methane in the photobioabsorber is greatly influenced by the absorption processes taking place there.
4. Studies have shown that the flow of biogas through distilled water reduced CO₂ concentration from 31.0% to 13.7%. After 10 days of biogas, the CO₂ concentration increases to 15.0%. CO₂ absorption decreases due to the reduced solubility of CO₂ in the liquid medium.
5. Microalgae culture of *Monoraphidium griffithii* had the greatest impact on CO₂ reduction. The flow of biogas through the suspension with this culture reduced the CO₂ concentration from 31.0 to 10.0%. The smallest reduction in CO₂ was recorded when biogas was injected via MWH medium.
6. Studies have shown that the absorption of biogas components results in the release of oxygen. As the biogas was passed through all the suspensions, the oxygen concentration increased from 3.6 to 5.2%. The highest concentration of oxygen was recorded by passage of biogas through suspension with micro-algae culture of *Monoraphidium Griffithii*. The lowest concentration of oxygen – 4.5% – was recorded when biogas was injected via MWH medium.

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