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Purificación de biogás usando *Cianobacterias*

Purification of biogas using *Cyanobacteria*

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Resumen

Objetivo: Investigar técnicas biológicas para purificar biogás. **Metodología:** El biogás con bajo contenido de dióxido de carbono fue burbujeado en dos soluciones acuosas que contenían cianobacterias filamentosas de diferentes cepas de *Leptolyngbya sp*. Luego, los resultados obtenidos fueron comparados contra un blanco. **Resultados y Conclusiones:** El biogás que fue parcialmente purificado redujo su contenido de dióxido de carbono en una proporción de 20 % a < 10 % luego de estar en contacto con cianobacterias. A la vez, el oxígeno producido durante la fotosíntesis se mantuvo por debajo de los límites de explosión para la mezcla metano-oxígeno. En contraste, el blanco usado en el ensayo se saturó de dióxido de carbono, causando una caída en el pH conforme pasaba el tiempo. El contenido de metano en el biogás purificado, cuya pureza fue medida con un método volumétrico, superó el 90 %. Las dos cepas de cianobacteria usadas tenían una composición en base seca de proteína ≥ 25 % y en lípidos < 2 %.

Palabras claves: Biogás, reducción de dióxido de carbono, cianobacteria.

Abstract

Objective: Investigate biological techniques to purify biogas. **Methodology:** Biogas with low carbon dioxide concentration was bubbled in two aqueous mediums containing different strains of *Leptolyngbya sp*. filamentous cyanobacteria and the results were compared with a blank. **Results y Conclusions:** Biogas that was partially purified, reduced its carbon dioxide concentration further from 20 % to < 10 % after being in contact with cyanobacteria and, at the same time, the oxygen produced during photosynthesis was kept below the explosion limit for the methane-oxygen mixture. In contrast, the blank used to purify the biogas was saturated with carbon dioxide, causing a drop in pH as time elapsed. Methane content of the purified biogas was over 90 %. Its purity was measured with a volumetric method. The two strains of cyanobacteria used had a dry basis composition of protein ≥ 25 % and lipids < 2 %.

Keywords: Biogas, carbon dioxide reduction, cyanobacteria.

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Introducción

Biogas is a renewable fuel of which the main component is methane, with composition ranging from 56 % for potato haul to 71 % for fish waste [1]. Factors such as temperature, pH, ratio of carbon to nitrogen, load rate and retention period affect the amount of biogas and the quality of methane produced [2]. Components that have a negative impact on the combustion of biogas include carbon dioxide (CO2), ammonia (NH3) and hydrogen sulfide (H2S) [3]. To increase the energy density and to decrease corrosion problems, biogas is purified before use in combustion engines or injection into a natural-gas grid [4]. Various techniques have been used to purify biogas, ranging from scrubbing with water and glycol, chemical absorption, pressure swing adsorption, cryogenic separation and membrane separation [5, 6, 7]. As shown in table 1, the traditional techniques of purification can achieve purity over 93 % methane.

Table 1. Comparison of typical biogas purification techniques

Technique	CH4 Purity	Other
Pressure swing adsorption	> 96 %	Requires desulfurization and drying before entering the molecular sieve. Typical operational pressures 400 -700 kPa
Water scrubbing	~ 97 %	Removes CO2, H2S and NH3. Typical operational pressures 700 -1000 kPa.
Physical absorption with glycol ethers	93 % -98 %	Removes CO2, H2S, H2O and NH3. Typical operational pressure ~ 800. Solvent is regenerated at ~ 50 °C
Chemical absorption with amines	~ 99 %	Prior desulfurization step is recommended. Operates at low pressure. Amine is regenerated at ~ 120 °C – 160 °C
High pressure membrane separation	> 96 % two stages	Removes CO2, H2S, H2O and NH3. Prior desulfurization and drying are recommended. Operational pressures 800 - 3600 kPa.
Cryogenic	> 97 %	Dry biogas is compressed to 8000 kPa at -45 °C to remove CO2 then further cooling to -55 °C and expansion to 1000 kPa dropping temperature to -110 °C

Fuente: [7]

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Other non-traditional purification methods include the use of microorganisms such as microalgae and cyanobacteria [8, 9, 10]. The latter methods have an advantage over the former in decreased energy consumption and the production of biomass that could be used for food, biofuel generation, vitamins, fatty acids, biopolymers and dyes [11, 12]. An added benefit of using microorganisms such as cyanobacteria is that they can be used for bioremediation [13], allowing them to concurrently remove the CO2 in biogas and consume some pollutants such as nitrates, phosphates, carbonates and metal ions that are present in contaminated water. In our research the medium used is nearly neutral, while previous work carried by Mann et al. [8] used pH 5.5 while Converti et al. [9] used pH of 9.5, another difference is that the microorganisms discussed in this paper have an optimal growth temperature of ~ 50 °C, while the microorganisms used by Mann grow at 21 °C and Converti´s have an optimal growth temperature of 30 °C. Cyanobacteria can grow in a broad range of conditions, and some of them live in extreme environments in terms of temperature and chemical content [14]. Thermophilic microorganisms as cyanobacteria from thermal springs can be very successful when used in bioremediation as they have developed various adaptation strategies to cope with harsh environments [15]. Plant-based biofuels offer a potential for an expanding population with an increasing consumption of energy. This demand creates an opportunity for the use of biofuels such as bioethanol, biodiesel [16], biohydrogen and biogas produced by microalgae and cyanobacteria [17]. Despite the benefits of using biogas, there are perceived and real technological, economic, social and regulatory barriers that limit its use in combined heat and power (CHP) plants [18]. When cyanobacteria are used to purify biogas, the carbon dioxide is partly metabolized into oxygen; depending on the temperature and pressure of the mixture of methane and oxygen, a risk of explosion arises [19]. For this reason, prepurification might be necessary to keep oxygen levels below 12 %. Not all carbon dioxide in biogas is converted to oxygen by the cyanobacteria; part is used by the cyanobacteria to form tissue [20]. Cyanobacteria use active inorganic carbon uptake in at least four modes, including two bicarbonate transporters and two CO2 uptake systems associated with the operation of specialized NDH-1 complexes [21, 22]. NDH-1 complexes are proton-translocating NADH-quinone oxidoreductase enzymes that are capable to transfer electrons from an electron donor such as nicotinamide adenine dinucleotide (NADH) to a quinone molecule with the concomitant generation of adenosine triphosphate (ATP) which is used by the cells to transport energy. The constant intake of CO2 is reflected in the fact that carbon is the element most abundant in dry cyanobacteria; Gónzalez-López et al. [23] reported carbon mass values ranging from 35 % to 45 %. Other authors such as Sánchez-Mirón et al. [24] reported values closer to 50 %.

The objective of this work was to evaluate the ability of two thermophilic filamentous strains of Leptolyngbya sp cyanobacteria to remove CO2 from

biogas; these microorganisms grow in relatively low sodium chloride concentrations at approximately neutral pH and temperatures between 50 to 59 °C. The proved hypothesis was that these cyanobacteria can metabolize CO2 in biogas like Arthrospira platensis [9], but at neutral pH, and concurrently generate biomass than can have additional uses. The novelty of this research is that the biogas produced is suitable for compression because the oxygen content is below explosion limits.

Materials and methods

The cyanobacterial strains Leptolyngbya sp. 17M (sp.1) and Leptolyngbya sp. 7M (sp. 2) used in this study are uniserial, unbranched filamentous species that were isolated from the Miravalles thermal spring located in the Miravalles geothermal field 15 km north of La Fortuna de Bagaces, Guanacaste in the North-Western part of Costa Rica. Isolates were maintained in BG11 medium at 35°C in 200 rpm agitation [25]. Both cyanobacterial strains are part of the Thermophilic Cyanobacteria collection of the Research Center in Cellular and Molecular Biology (CIBCM, University of Costa Rica). The biogas was generated in a thermophilic biodigester located at the Fabio Baudrit Experimental Farm. To maintain oxygen at levels below the explosion limits, this biogas was prepurified from its original 69 % methane content to 80 % methane using a PVC column, 90 cm in length and 10 cm in diameter, containing 1 kg calcium oxide powder, 4 kg crushed charcoal (2 cm to 4 cm in diameter) and 0,5 kg of iron oxide powder (Fe2O3). Calcium oxide and charcoal help reduce humidity and carbon dioxide, while iron oxide is used to reduce the corrosive hydrogen sulfide from 100 mg/L to 1 mg/L and thus protect the compressor and storage tank. This prepurified biogas was later compressed and stored at 350 kPa (51 p.s.i.) before being bubbled into two cylinders (20 L) each containing previously grown cyanobacterial isolates (2.5 g dry mass each). The biogas was also bubbled through a blank solution containing only the aqueous medium used to grow the cyanobacteria; the medium was BG11 modified by Rippka et al [26]. The rate of bubbling biogas was 1 mL/s per container, continuously for 8 h under direct natural lighting and 25 °C. The CO2 in the biogas was assessed every 2 h using a variation of the volumetric method described by Abdel-Hadi [27], in which a sodium hydroxide (NaOH) solution (2 % m/m) reacted with CO2 and H2S present in the biogas; the methane in the sample is practically insoluble; its volume was measured 15 min after the sample injection, as shown in Fig. 1. The CO2 and pH were monitored every 2 h in all containers. Traces of H2S were measured every two hours with Sensorcon H2S Inspector.

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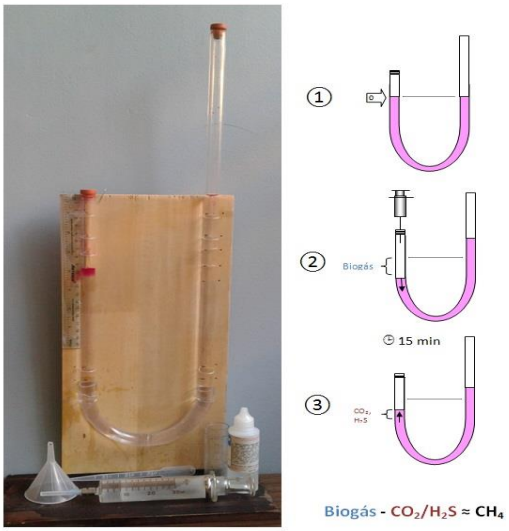
Figure 1. Volumetric method used to measure methane in biogas



This simple method was validated with a gas chromatograph (thermal conductivity detector, Carboxen 1000 column). The volumetric method tends to overestimate the methane content by 3 % because of the presence of nitrogen and hydrogen that are insoluble in the NaOH solution; their volumes are added to the methane volume.

After 8 h, the cyanobacteria were drained and dried at 60 °C. Triplicate samples were taken to determine the ash content using a muffle furnace at 575 °C for 18 h, as described by Van Wychem & Laurens [28]. Protein content was measured in triplicate (Kjeldah method AOAC-960.52). To convert the nitrogen percentage to protein, a factor of 5.95 was used as suggested by Gónzalez-López et al. [29] instead of 6.25 which is commonly used. The reason to use a smaller number is that not all the nitrogen containing compounds are proteins; in the case of cyanobacteria, the amount of pigments, DNA and chlorophyll increases nitrogen content compared to typical protein sources such as meat or dairy. Lipid extraction (Soxhlet) was performed in triplicate according to the conditions described by Bling & Dyer. [30].

Results and discussion

The conversion of CO2 to O2 is not equimolar, cyanobacteria reduced CO2 concentration from ~ 20 % to > 10 % in the prepurified biogas mixture and the final mixture contained only 6 % O2, which is below explosion limits. This result points to the partial use of CO2 by the cyanobacteria to form tissue as described by Riding [20]. Figure 2 supports that the cyanobacteria consume part of the CO2 present in the biogas. In contrast, the ability of the blank to

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continuously dissolve CO2 is decreased after 2 h. The results suggest that part of the carbonic acid formed when CO2 dissolved was metabolized by the cyanobacteria. The pH values in figure 3 are less for the blank, which indicates that the blank became saturated with CO2 of which most was in the form of carbonic acid. For all cyanobacteria samples, the pH stayed over 6, which indicates that the dissolved CO2 remained mainly in the form of bicarbonate. With a gas chromatograph, the analysis determined that the oxygen generated by the cyanobacteria remained below explosion limits and methane concentration was over 90 %. Initially, H2S concentration was 1 mg/L and none was detected after two hours.

Figure 2. CO_2 concentration during biogas bubbling in two cyanobacteria strains and blank

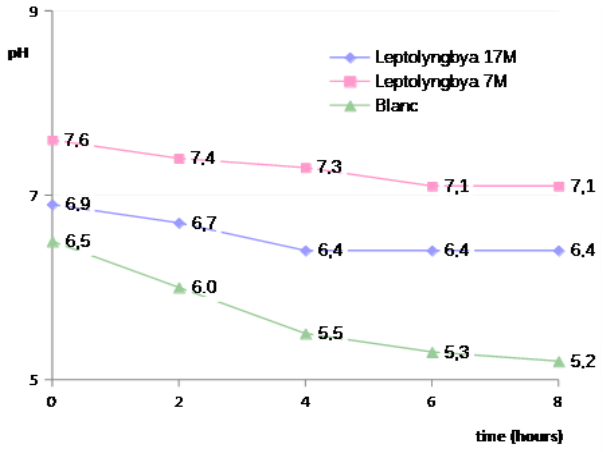

Figure 3. pH during bubbling of biogas in two cyanobacteria strains and a blank

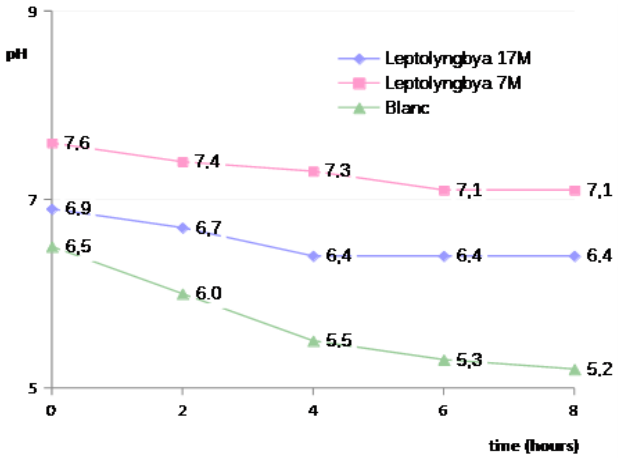

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Table 2 shows that both cyanobacteria have a large protein composition. Their flavor resembles Nori algae used in Japanese dishes. Further work is required to determine whether the product is suitable for animal or human consumption. For the unstressed conditions under which these cyanobacteria were grown, the amount of lipid is small, which makes it unsuitable for biodiesel production. It is expected that under stressed conditions, they would produce higher lipid content.

Table 2. Cyanobacteria composition, dry base

Sample	Ash Content /%	Protein /%	Lipid /%
Leptolyngbya sp. 17M	17	25	< 2
Leptolyngbya sp. 7M	16	29	< 2

Conclusions

The hypothesis was verified: the tested Leptolyngbya sp. cyanobacteria can metabolize most CO2 present in biogas. The mixture of methane and oxygen formed after the purification process remained below the explosion limits. The purified biogas is suitable for combustion and compression; its methane composition was > 90 %, close to traditional purification techniques. Because of the large protein content, these cyanobacteria have the potential for use as human or animal feed, but their small lipid content obtained under the conditions of growth makes them unsuitable for biodiesel. Further research is required to determine the optimal lighting, pH and temperature conditions for the conversion of CO2 to biomass but it is expected that different optimal conditions would be observed depending on the media and strain selected. There are opposing factors to be considered: the strains tested grow well at neutral pH but CO2 solubility increases at high pH; CO2 solubility also increases at low temperatures but the optimum grow temperature for the strains tested is between 50 °C to 59 °C. The effect of stress can also be analyzed to determine its influence in cyanobacteria lipid composition.

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