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Process Integration of Algae Production and Anaerobic Digestion

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Combining algal cultivation and biogas (methane) generation is considered to be one of the prospective environmentally feasible options of creating perpetually renewable source of pure energy for industrial and human consumption. Methane and energy generated in anaerobic fermentation facilities can be utilized as replacement for fossil fuel energy, thus reducing greenhouse gas emissions. This is caused by the fact that carbon is a biologically degradable material and algae form an integral part of the carbon cycle. Carbon release into the atmosphere from biogas combustion is utilized by plants for their further growth.
Selection of optimal strain of algae depends on cost, efficiency, growth rate of algae, and difficulties during cultivation. The best cost-effective strain is *Chlorella*, green algae, because after extraction of lipids further utilization of algae is possible. The anaerobic digestion of *Chlorella vulgaris* and *Chlorella sorokiniana* was studied using batch digesters. Standard analysis of chemical oxygen demand (COD), solids, pH were performed. Biogas composition and production were also determined. The conversion of biomass to biogas ranged from 40 - 73 % in COD. In general it is important to select the strain of algae that contains the most of chlorophyll and lipids (reservoir substances of algae).

1. Introduction

Algae with cyanobacteria are the simplest organisms with autotrophy and undemanding requirements for growth (light, CO_2, N, P and K). These organisms may produce in a relatively short time large amounts of lipids, proteins and carbohydrates. The resulting products can be then used and processed for production of biofuels, respectively valuable secondary products. Many types of algae contain oil in the range of 20 - 50 % dry weight biomass. Because of theirs rapid growth potential, exponential growth allows them to double the biomass for a period shorter than 3.5 h. The biochemical composition of algal biomass can be modified changing growth conditions, thus oil yields can be significantly increased.
Algae can also produce valuable co-products such as proteins. Biomass can be used as feed, respectively fertilizer, or fermented to produce ethanol or methane. The importance of algae lies in their potential CO_2 fixation and utilization (1 kg of dry algal biomass utilizes about 1.83 kg of CO_2) and wastewater treatment from excess nutrients, and thus the products obtained from algae is of high value added. Other advantages of algae are the ability of their total yearly production and lower requirements on water supply in comparison with terrestrial plants, thereby reducing the burden on fresh water resources. Algae can be grown on set-aside land, which is not threatened food production, food and other products derived from crops (Costa, 2011; Brennan, 2010).

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The aim of this research work was the cultivation of selected algae and their digestion into biogas. The advantage of the proposed approach is the possibility of integration of CO_2 capture from combustion processes and the subsequent use of biomass to produce methane.

2. Experimental

The experiments were comprised of three parts: the cultivation of algae, the algae harvesting and digestion of algae to for biogas.

2.1 Algae strains

In the selection of suitable strains of algae, we have focused on those that have been used in several works dealing with the cultivation of production strains, their use in the production of biofuels, respectively other biotechnological processes Doušková (2010), Lakaniemi et al. (2012), Lakaniemi et al(2011), Jeong (2003), Illman (2000), Lee (1996), Janssen (2009), Takáčová (2012). We used two algae strains Chlorella vulgaris, sp.(CHV) and Chlorella sorokiniana (CHS), SHIHIRA et KRAUSS (SAG 211-8k, 211/8k CCAP, UTEX 1230, CCALA) for the tests.

2.2 Nutrient medium

Nutrient medium generally consist of a mixture of chemical salts and water. For the cultivation of algae was used two types of standardized culture media. BG-11 (blue - green medium) and BBM_{dn} (Bold's Basal Medium with doubled nitrate).

2.3 Microalgae production and harvesting

Algae were cultivated in plate photobioreactors, which consisted of prismatic container of gluted glass with the dimensions - length 110 cm, height 55 cm and 5 cm thickness. The panel was immersed in a slightly larger glass vessel filled with distilled water, which served as a water thermostatic bath. The temperature was maintained at 30 °C instantaneous water heaters. The whole culture of algae in the inner panel (working volume 22 L) was continuously aerated using an air compressor, and filtered air was mixed with extra CO_2 (3% v/v). The panel was continuously illuminated with fluorescent lamps on both sides of photobioreactors, the average luminance of 17,000 lux. In photobioreactors were cultivated a strain of algae *Chlorella sorokiniana*, in the growth medium BBM_{dn}, with baseline biomass inoculated at level 5×10^3 cells/mL. During cultivation, the algal suspension was recorded for the following parameters: cell density, growth rate, pH, temperature, dissolved oxygen, phosphate (PO_4^{3-}), nitrate (NO_{3-}), inorganic carbon, dry weight biomass and elemental composition of dry biomass of biogenic elements N, C, H, and S. Cultivation took 14 days, during which the fresh medium was added in the 4^{th} and 10^{th} day (by volume 4 L) due to sampling.

After cultivation of algae were settled down using flocculant $Al_2(SO_4)_3.18H_2O$ resp. by centrifugation (4,000 rev / min, 10 min). Sedimentation was performed by raising the pH to 11 with KOH. Liquid algae paste was dried in an oven at 105 °C. Before extraction of the dried algae were disintegrated in a laboratory mill Polymix A 10 fi. Kinematic. Extraction was carried out in 2055 Tecator Avanti apparatus at 130° C for one hour. Total lipids were determined gravimetrically.

For the extraction lipids from algae have been proven method of fine grinding dried algae and extraction with chloroform in Soxtec equipment. In the first stage the thimble with the sample of dry algae was immersed in boiling solvent, which allows faster extraction. The second stage continued by washing via condensing the solvent vapor.

The composition of fatty acid in the samples of extracted lipids was evaluated according to standard EN ISO 5509 and STN EN 14103. A sample of the extracted lipids was saponify with methanolic sodium hydroxide, mixture was then heated under reflux and the resulting soaps were converted to fatty acid methyl esters by reaction with methanolic solution of boron trifluoride. Prepared fatty acid methyl esters of C_8-C_{24} were separated by high-resolution capillary gas chromatography in a 100 m long columns with a polar stationary phase CP-Sil 88th

Determination of total carbohydrates was done by Anthrone methods.

2.4 Anaerobic digestion

The digester (glass column with 1 L volume) was continuously mixed with magnetic stirrer. The temperature was maintained at 40 - 41 ^{o}C in water bath. The reactor was fed with inoculum from biogas unit (with maize as a feedstock), Shelton medium containing nutrients and trace metals, demineralized water, algae paste or dry algae, silicone antifoam agent and $CaCO_3$. Biogas volume was measured by water displacement and then normalized normal condition. Biogas production is given in norm mL/ g volatile solids (273K, 1013 mbar).The biogas composition was determined by gas chromatography and/or by GA2000 analyzer. Total solid (TS), total volatile solid (TVS), pH, chemical oxygen demand were measured in regular interval.

3. Results and discussion

3.1 Algae production and composition

In the work was carried out cultivation of algae *Chlorella sorokiniana* (SHIHIRA et KRAUSS / SAG 211-8k, 211/8k CCAP, UTEX 1230) in a medium BBM_{dn} in flat panel photobioreactors with an average light intensity of 17 426 lux. Algae were collected by chemical flocculation and/or using a centrifuge and dried at 120 ^{o}C.After the harvesting and drying dry algae samples undergo to chemical analysis of the elemental composition of selected biogenous elements, the determination of total fat and carbohydrate, evaluation the composition of selected fatty acids present in lipid extracts of algae.

In terms of cultivation and biomass production value was reached 4.22 g/L. Lipid content was 18.63 wt%. of dry matter, total carbohydrate content was 7.66 wt% of dry. Lipids extracted with chloroform contain 20.66 wt%. saturated TAG (C16: 0 = 17.85 wt%.) and 77.78 wt%. of unsaturated TAG (C18: 2 n-6 = 36.08 wt%, C18: 3 n-3-11, 63 wt%). The elemental composition of dry matter after chemical flocculation using $Al_2 (SO_4)_3$ was as follows (in wt%.): N-6.00, C - 38.40 H - 7.48, S - 2.04. Higher sulfur originated from remains from flocculant. The comparative algae *Chlorella vulgaris, sp.* contains 7.92 % wt. total sugars, 10.41% wt. lipids. Lipids contained 17.15 wt%. saturated TAG (C16: 0 = 15.66% wt.) and 79.75 wt%. unsaturated TAG (C18: 2 n-6 = 24.18 wt%, C18: 3 n-3 to 19, 91 wt%).

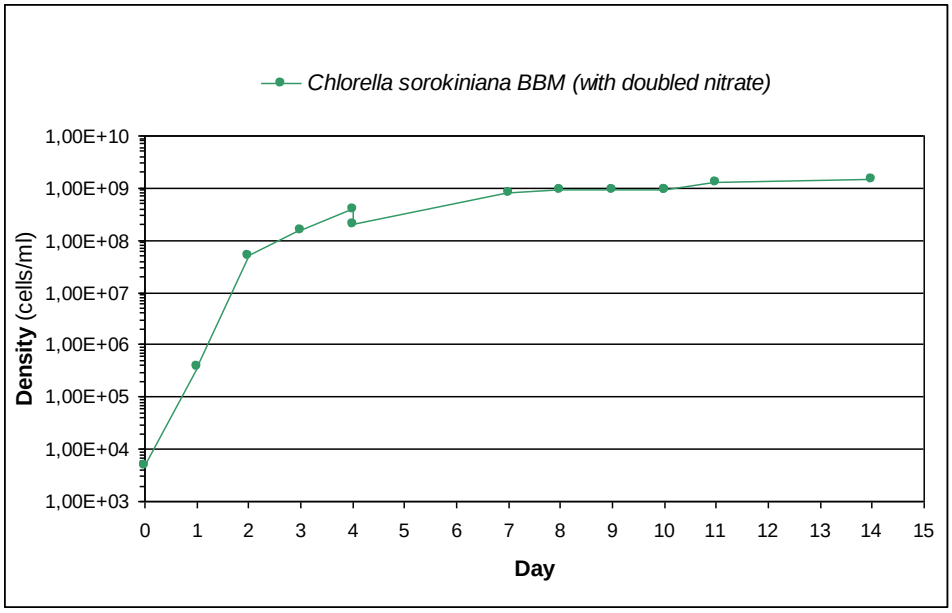

Figure 1: The growth curve (cell density)of algae strain Chlorella sorokiniana cultivated in a nutrient medium BBMdn using panel photobioreactor

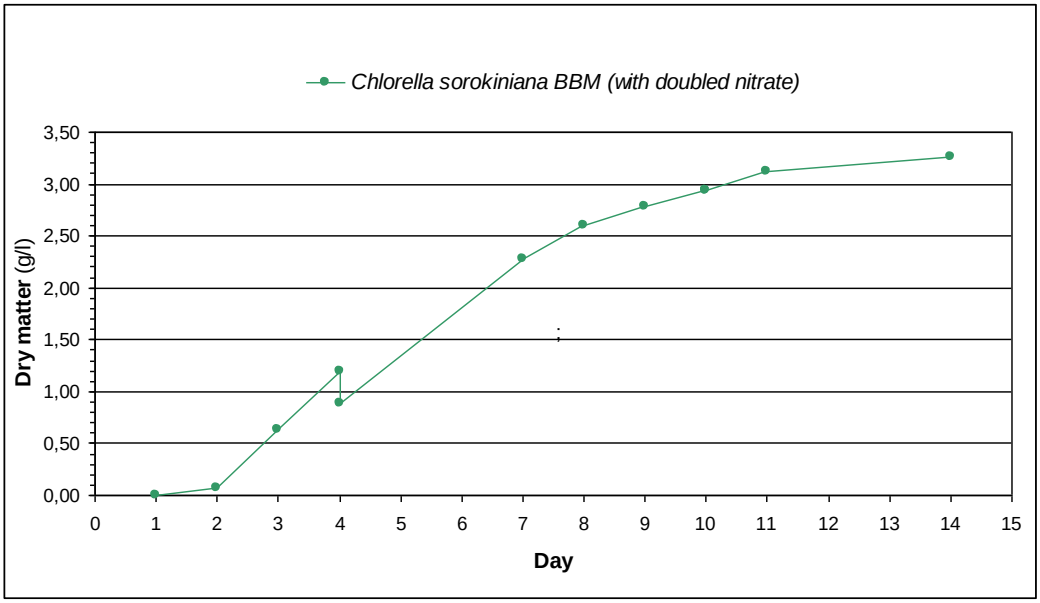

Figure 2: Dry matter of biomass algae Chlorella sorokiniana strain produced during cultivation in growth medium BBMdn using panel photobioreactors

An important difference was observed using two different growth media. Medium BG11 produced lower yields of biomass, higher share of total lipids. The content of total sugars was higher. The content of saturated and unsaturated fatty acids was also observed a dramatic difference in changing the cultivation conditions. Very high level saturated triacylglycerides, especially palmitic acid had been achieved with BG11 media.

3.2 Algae digestion

For digestion of algae, we selected three samples. Dried and disintegrated algae *CHV* was used for a comparison. For other tests we chose algae *CHS*, which in previous cultivations had higher lipid content. Since the disintegration and drying processes are energy intensive in one test, we used wet algae flocculated with $Al_2(SO_4)_3$ and in the other test was dried and disintegrated algae used.

Table 1 shows the biogas and methane yield in norm mL per g of total volatile solids. The highest biogas and methane yield was achieved in case of dry and milled algae *CHS*. In comparison with the algae *CHV* the yield of biogas is higher and is correlated with a higher content of lipids. In the case of wet algae *CHS* the lowest biogas yield was observed. One possible explanation is the inhibition by Al^{3+} ions.

Table 1: Average production of biogas and methane in normal mL/g TVS

Algae	Biogas yield, 1200 hours	Total biogas yield	Methane yield
Chlorella vulgaris, sp., dry & milled	162.2	221.1	189.0
Chlorella sorokiniana, wet	118.4	119.1	98.2
Chlorella sorokiniana, dry& milled	180.8	248.0	212.0

Figure 3 shows the cumulated biogas production during the whole experiment with three types of algae. Initial rate of anaerobic degradation was different in case of wet algae *CHS*. Biogas conversion yield fit exponential trend for all tested algae. Maximum total methane yield was in case of dry and milled *CHS*. Mechanical disruption of cell walls of algae has had a positive effect on the process which was reflected by a faster process of acidogenesis and higher production of biomethane. An important technological parameter is pH, which must be managed. At the beginning of digestion the pH was in

range 6.5 to 7.2, gradually increased to a value of 8.1 to 8.3 which remained all the time digestion. To suppress foaming, which occurred after 6 days, to add silicone defoamer has proven. The presence of protein caused formation a substantial amount of NH_3 and H_2S. In the case of algae *CHS* dry & milled NH_3 concentration was 5 times lower, which had a positive impact on the course of digestion and biogas yield.

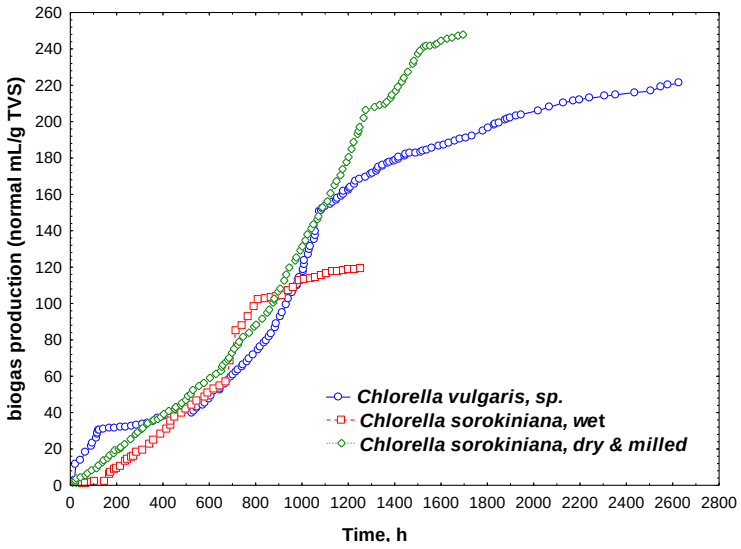

Figure 3: Comparison of cumulated biogas production

4. Conclusions

The integration technology cultivation of algae using waste CO_2 and algae digestion process has a great environmental and economic dimension. For the production of biomethane are more suitable algae which produce higher share of total lipids. A high concentration of nitrogen during anaerobic digestion of protein-containing algae have potential toxicity to methanogenic bacteria.
The fatty acids in the lipids are highly influenced by the composition of growth medium.

Acknowledgment

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