Green energy from different feedstock processed under anaerobic conditions

V. Skorupskaitė¹, V. Makarevičienė¹,* G. Šiaudinis² and V. Zajančauskaitė³

¹Aleksandras Stulginskis University, Faculty of Forest Sciences and Ecology, Institute of Environment and Ecology, Studentų Str. 11, LT53361 Akademija, Kauno district, Lithuania; *Correspondence: virginija.skorupskaite@asu.lt
²Vėžaičiai Branch of Lithuanian Research Centre for Agriculture and Forestry, Gargždų str. 29, LT96216 Vėžaičiai, Klaipėda district, Lithuania
³Klaipėda University, Faculty of Marine Technology, Department of technological process. Herkaus Manto Str. 84, LT92294 Klaipėda, Lithuania

Abstract. The possible use of energy crops and aquaculture for bioenergy production has only recently become a research target, so there is little information on their properties and advantages. The aim of this study was to investigate the possible use of cup plant, as well as marine and freshwater algae (Scenedesmus sp and Chlorella sp.) for biogas production. Research of a batch anaerobic digestion process at a mesophilic temperature were performed using wet wastewater sludge, cattle manure, fresh microalgae biomass and dry marine algae, cup plant biomass and mixtures of these materials. The highest biogas yield (541.28 ml g⁻¹ VS) was obtained by using a new feedstock from the microalgae Scenedesmus sp. biomass. That yield was 1.4 times higher than the biogas yield from cattle manure and 15% lower than the biogas yield from wastewater sludge. It was found that adding microalgae biomass to a cattle manure substrate increases biogas production approx. 1.5 times. The highest methane concentration in biogas produced from microalgae ranges from 64.87% to 66.66% and exceeds the methane amount (64.26%) in biogas produced from wastewater sludge. The methane amount in biogas produced from cattle manure, cup plant and marine algae biomass is lower than 60%. In addition, it was found that it is possible to produce 5,092.3 m³ of biogas or 113 GJ of energy from 1 ha of harvested cup plant biomass.

Key words: biogas production, microalgae, marine algae, cup plant, cattle manure, wastewater sludge.

INTRODUCTION

Decomposition of organic matter is a natural process that has happened under anaerobic conditions since time immemorial. In the absence of oxygen and at certain temperature, certain groups of bacteria break down organic materials such as carbohydrates, proteins and grease into a gas composed mainly of methane (50–85%) and carbon dioxide (20–40%) (Herout et al, 2011; Ács et al., 2015).

The beginning of biogas production and its use for energy purposes started a few decades earlier (Buswell & Mueller, 1952) than the demand for renewable energy sources. Nowadays, the most prevalent sources of biogas production are conventional raw materials, such as wastewater treatment sludge, pig, poultry and cattle manure, and maize silage, while the overall production of biomass is growing. According to a report
by the European Biogas Association, over 13,800 power plants with a more than 7,400 MW$_{el}$ capacity were constructed in Europe in 2012.

In order to increase the quantitative and qualitative parameters of biogas, it is necessary to control some parameters, like temperature, pH, C:N ratio, substrate composition, etc.

In addition to the above-mentioned feedstocks, there are many potential candidates that could supplement or replace the raw materials of methane production. The possible use of terrestrial energy crops and aquaculture for biogas production has only recently become a research target, so there is little information on their properties and advantages.

Despite the fact that the use of crop biomass for the production of biogas has substantially increased in many European countries, maize (*Zea mays* L.) outweighs other species. The increase in maize cultivation has led to soil degradation, susceptibility to crop diseases and pests, and changes in the natural landscape (Robertson et al., 2008; Fletcher et al., 2010; Gansberg et al., 2015). One possible alternative might be the cultivation of perennial crops, which might have some superiority over the annuals due to lower amounts of energy needed for cultivation, which might substantially reduce the expenses (Jasinskas & Kryzeviciene, 2006). The most perspective are the species which can easily adapt to local agro-climatic conditions (McKendry, 2002).

Silphium species originate from North America (Huxley, 1992). In contrast, some European countries have conducted many important experiments with *Silphium* genus as a potential decorative, melliferous and fodder crop (Kowalski 2004; Kowalski 2007; Lehmkuhler, 2007).

Many authors state that various Silphium species could produce a high annual biomass yield (Kowalski, 2004; Kowalski, 2007; Šiaudinis et al., 2012). Although there had been some suggestions that its biomass could potentially be used for biogas production, *Silphium* genus only gained attention as a potential crop for biogas production in the recent years (Voigt et al., 2012; Jasinskas et al., 2014; Mast et al., 2014). All in all, in the light of the advances in technology and the use of biomass for bioenergy purposes, this topic warrants further research (Gansberger et al., 2015).

Another source material for biofuel production could be algae. Recently, an important issue for researchers has been the investigation of possible uses of algae biomass for bioenergy production due to its advantages, such as fast growth, high carbon dioxide fixation ability, treatment of wastewater, and the lack of competition for arable land (Mata et al, 2010; Brennan & Owende, 2010; Podkuiko et al, 2014).

The goal of our study was to investigate the applicability of new non-traditional materials for biogas production, as well as to compare the quantitative and qualitative indicators of the biogas produced from these feedstocks with properties of biogas produced from conventional materials.

The aim of our research was to: 1) investigate the quantitative and qualitative parameters of new non-traditional materials (cup plant, algae) and their suitability for biogas production; 2) compare the properties of the biogas produced from new feedstock to that produced from conventional ones (manure, wastewater).
MATERIALS AND METHODS

Cup plant (Silphium perfoliatum L.) has been cultivated at an experimental long-term energy crops site at the Vėžaičiai branch of the Lithuanian Research Centre for Agriculture and Forestry (Western Lithuania, 55°43′N, 21°27 E) since 2008 (Fig. 1). Before the beginning of the experiment, the following characteristics of upper soil layer (0–20 cm) were determined: pH$_{KCl}$ 4.25–4.45, mobile P$_{2}$O$_{5}$ 35–120 mg kg$^{-1}$, mobile K$_{2}$O 140–209 mg kg$^{-1}$, hydrolytic acidity 21.9–62.1 mequiv kg$^{-1}$ and mobile Al 10.7–50.9 mg kg$^{-1}$.

![Figure 1. Cup plant at flowering stage.](image)

The experiment was composed of two factorial designs with three levels of soil liming (not limed, limed at 3.0 t ha$^{-1}$ and 6.0 t ha$^{-1}$ rates) and three levels of nitrogen fertilization (0, 60 and 120 kg ha$^{-1}$ N). Lime application was done once just before the beginning of the experiment. Nitrogen fertilization (with ammonium nitrate) was performed each year. The rates of phosphorus (with a single superphosphate) and potassium (potassium chloride) fertilizers were equal for all the treatments – 60 kg ha$^{-1}$ P$_{2}$O$_{5}$ and 60 kg ha$^{-1}$ K$_{2}$O.

Cup plant seedlings at 2–3 leaf stage were planted at the experimental site in 2008. From 2009 onwards, cup plant biomass yield was cut annually using a rotary reaper (Claas, Germany) at full maturity stage, which falls to the end of September. In addition, in 2014, samplings for dry mass qualitative analysis were carried out during cup plant’s flowering stage (on 3 June). The biomass that was selected for analysis came from cup plants grown in the soil that had received no liming or N fertilization, as it was grown using the lowest energetic expenses, in natural soil and under natural climatic conditions.

Marine algae biomass was gathered at the coast of the Baltic Sea, at the Šventoji river mouth on 3 July 2014. It was visually estimated that red algae (Rhodophyta) was the prevailing species (Fig. 2).
The mixture of primary and redundant active wastewater sludge was purchased from JSC Kauno vandenys (Kaunas).

Cattle manure was acquired from a local farmer. Before it was used for biogas production, cattle manure was stored at 5 °C temperature in a refrigerator in order to avoid biological decomposition.

Freshwater microalgae *Scenedesmus sp.* and *Chlorella sp.* were isolated from Lithuanian lakes. A laboratory stand equipped with magnetic stirrers was used for microalgae batch cultivation. Both microalgae species were cultivated under mixotrophic growth conditions at a room temperature (22 ± 2 °C) mixing, in plastic cylinders with a working volume of 3 L. The duration of microalgae cultivation was 30 days. *Scenedesmus sp.* and *Chlorella sp.* were grown under ~250 μmol photons m² s⁻¹ of illumination using white fluorescent lamps for 10 h a day. Light intensity was estimated using a data logger (model LI-1400) with a LI-190SA Quantum sensor. Mixotrophic growth was carried out by growing microalgae *Scenedesmus sp.* in a modified BG11 medium (Skorupskaite et al., 2015) with adding 5 gL⁻¹ of technical glycerol purchased from JSC Rapsolia (Lithuanian producer of biodiesel). *Chlorella sp.* was cultivated in a growth medium composed of modified BG11 + 2 gL⁻¹ technical glycerol.

After cultivation, microalgae biomass was concentrated by centrifugation for 15 minutes at 4,700 rpm using a Thermo Scientific Heraeus Multifuge X3R centrifuge. The amount of volatile solids in microalgae biomass and other materials was measured by drying the feedstock in an oven at 105 °C to a constant weight and then by burning dry matter in a muffle oven at 550 °C for 2 hours.

Elemental composition of microalgae biomass was determined using CHNS-O Elemental Analyser (Perkin Elmer 2400 Series). Dry *Scenedesmus sp.* and *Chlorella sp.* biomass was pulverized using a mortar, weighed on thin foil (app. 2 mg), placed in an elemental furnace, combusted in a pure oxygen environment at 975 °C, analysed, and the weight of each element was calculated.

Both cup plant and marine algae green biomass were dried and recalculated to air-dry mass (DM). Chemical analyses of both crops were done based on samples dried at 105 °C for a total C and N content estimation. A dry combustion (*Dumas method*) was
used to measure organic carbon content (C). Total nitrogen content (N) was measured using the Kheldahl method.

The biogas production research was carried out using a feedstock made from wet wastewater sludge and cow manure, dry cup plant and marine algae, wet freshwater microalgae, and mixtures of these materials. Since cup plant and marine algae biomass can be stored and transported, such types of biomass was used dry for biogas production research. Wet freshwater microalgae biomass was used in the research, because drying of the biomass consisting of such type of algae requires a lot of energy and could significantly increase energy consumption during biogas production process.

Substrates containing different materials and mixtures (every species of freshwater microalgae with wastewater sludge and microalgae with cow manure 50% + 50%; marine algae with cup plant with a proportion 50% + 50% and 30% + 70% were prepared so that each of substrate should contain 0.25 g of volatile solids.

100 ml syringes (Hohenheim fermentation test) were used as small biogas reactors. Syringes were filled with a homogenized mass mixture composed of 30 ml of inoculum and substrate. The digested wastewater treatment sludge was used as an inoculum. Inoculum without substrates was used as a negative control sample. In order to keep them at the same temperature during the whole experiment, all syringes were placed on a thermostatically controlled laboratory shaker (Fig. 3). Anaerobic conditions in bioreactors were created by pushing air out of the syringes. All syringes were closed using tube clips.

Figure 3. Biogas production in syringes.

Biogas was produced at 37 °C temperature for approx. 25 days. The amount of produced biogas was measured while keeping the syringe in a vertical position. Composition of biogas was measured through gas chromatography using a Clarus 580 GC chromatograph (Perkin Elmer) equipped with a thermal conductivity detector. All experiments were performed in triplicate.
RESULTS AND DISCUSSION

The average cup plant dry mass yield per season during 2009–2014 was 13.15 t ha\(^{-1}\) (Fig. 4). In the first harvestable year (2009), cup plant yield was small. But in the following years, biomass yield was high and relatively similar. These results are in accordance with the results of other authors (Filatov et al., 1986).

Both of the investigated factors, the liming and nitrogen fertilization had a significant effect on cup plant dry mass yield.

![Figure 4. Cup plant dry mass yield.](image)

The application of 6.0 t ha\(^{-1}\) CaCO\(_3\) lime significantly increased cup plant productivity – the average cup plant yield was 14.70 t ha\(^{-1}\) (or 25% higher in comparison to the control sample). Similarly, the application of 120 kg ha\(^{-1}\) N rate increased dry mass yield up to 14.56 t ha\(^{-1}\) on average (or 24.80% higher in comparison to samples that had not received nitrogen treatments).

Carbon and nitrogen (C:N) ratio in the feedstock is one of the most important factors that influence biogas quality and production output. According to Schnürer & Jarvis, (2009), the maximum volume of biogas is released when the C:N ratio varies between 10 and 30. The higher the C:N ratio (from 10 to 30), the better the formation of fatty acids as well as the methanogenesis stimulation. The results of our experiments (Fig. 5) showed that the highest C:N ratio was found in cup plant biomass; meanwhile the lowest C:N ratio was found in microalgae Chlorella sp. biomass. It seems that C:N ratio in Scenedesmus sp. biomass is optimal for biogas production. Taking into consideration of these results, it could be stated that Scenedesmus sp. microalgae and marine algae biomass can be used for biogas production without adding of any kind of co-substratum.

This study investigated biogas production from conventional feedstock and new potential raw materials like cup plant, marine algae and freshwater microalgae. Different types of biomass and mixtures of it were used for the study.
The first experiment on biogas production was carried out using both conventional raw material like wastewater sludge and cattle manure, and new feedstock material such as microalgae and marine algae. The results of the quantitative analysis showed that potential new raw materials could be promising for biogas production (Fig. 6). Out of those, the highest biogas yield (541 ml g\(^{-1}\)VS) from new raw materials was achieved using *Scenedesmus sp*. In comparison, that is about 28% more than the biogas yield from cow manure and 16% less than the biogas yield obtained from wastewater sludge. Similar results (524 ml g\(^{-1}\)VS) were derived from *Chlorella sp*. Cup plant biogas yield was 421 ml g\(^{-1}\)VS. Results of comparatively small yields of biogas produced from cup plant and marine algae can be explained by a smaller amount of moisture, which could inhibit biocenose activity (Schnürer & Jarvis, 2009) because the cup plant and marine algae biomass was dried before use.
Further analyses of biogas production were performed using mixtures of substrata (Fig. 7). Conventional raw materials were mixed with freshwater microalgae biomass, and marine algae were mixed with cup plant in different ratios. The results of the experiments reveal that aquaculture feedstock could be used for biogas production as sole substrate as well as co-substrate. A 50% of addition of freshwater microalgae to cattle manure improved the biogas yield 1.49 and 1.19 times in the case of *Chlorella sp.* and *Scenedesmus sp.*, respectively. The presented results revealed that there is no big difference between the biogas yield from wastewater sludge and its mixtures with microalgae *Scenedesmus sp.* and *Chlorella sp.*, therefore it can be stated that wet microalgae biomass could be used as additional feedstock to wastewater sludge substrata without adverse effects to the biogas yield.

The 50% addition of cup plant biomass to the marine algae substrate improves quantitative properties of biogas production approx. 1.4 times.

![Figure 7. Dependence of biogas yield on different mixtures of substrata.](image)

The examination of the composition of produced biogas (Fig. 8) revealed that methane content in biogas, produced from different substrata, varied between 57.53% and 66.66%. The highest methane content was found to be in the biogas produced from microalgae *Scenedesmus sp.* and *Chlorella sp.* Biogas produced from these microalgae species contain 64.87% to 66.66% of methane. The lowest methane yields (< 60%) were fixed in the biogas produced from cow manure, cup plant and marine algae. The addition of wet microalgae biomass into the wastewater sludge or cattle manure substrate increases methane content of produced biogas by approx. 2%. In this case, 1 m³ biogas containing 2% more methane has an additional 1 kJ of energy.
According to the results of our research, 1 ha of harvested cup plant biomass could produce 5,092.3 m$^3$ biogas, which contains 58.62% of methane. Such amount of biogas is equal to 113 GJ of energy.

**CONCLUSIONS**

To summarize the results of biogas production from wastewater treatment sludge, cattle manure and cup plant, marine and freshwater algae, it has to be noted that new types of biomass sources could be added to the conventional raw materials of methane production. Fresh concentrated microalgae biomass as a supplement to cattle manure substrate helps to improve not only quantitative (approx. 1.49 times), but also qualitative (increase of methane amount from 2 to 5%) indicators in biogas production. Biogas produced from a new type of feedstock contains a relatively high concentration of methane and it could be used for co-generation systems.

**REFERENCES**


