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Microalgae for biomethane production

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Abstract. Competition for arable land between food and energy producers has begun in Latvia. Biogas producers are seeking to use the hitherto unused land. There is a need to investigate the suitability of various biomasses for energy production. Maize is the dominating crop for biogas production in Latvia, but it is expensive to grow. The cultivation of more varied biomass with good economics and low environmental impact is thus desirable. Microalgae can be grown in pipes, basins and also in open ponds. This paper shows the results from the anaerobic digestion of microalgae *Chlorella vulgaris*, cultivated with fertilizer Varicon in open pond and harvested on 27 October and centrifuged (Study 1). The anaerobic digestion process was investigated for biogas production in sixteen 0.75 l digesters, operated in batch mode at temperature 38 ± 1.0 °C. The average methane yield per unit of dry organic matter added (DOM) from digestion of *Chlorella vulgaris* was 0.331 l g_{DOM}^{-1}. The second investigation (Study 2) used fresh biomass of *Chlorella vulgaris* harvested on 10–15 June with low dry matter content, as it was obtained from 4 m deep open pond without centrifugation. Anaerobic digestion process was provided in 4 digesters with volume of 5 l each. Average methane yield from the digestion of *Chlorella vulgaris* was 0.290 l g_{DOM}^{-1}, which is comparable to methane yield obtainable from maize silage or other energy crop silages. Microalgae *Chlorella vulgaris* can be successfully cultivated for biogas production from May to October or at least 170–180 days in a year under the agro-ecological conditions in Latvia.

Key words: anaerobic digestion, *Chlorella vulgaris*, biogas, methane yield.

INTRODUCTION

According to Directive 2009/28/EC, Annex I, Part A, the goal for Latvia is to increase the share of energy produced from renewable energy sources (RES) in gross final energy consumption from 32.6% in 2005 to 40% (1918 toe) in 2020 (Ministry of Economics, 2010). Most of the biomass will come from forest products, but it should be taken into account that 1 ha of agricultural land can be used to obtain more energy than compared to forest wood biomass increment per 1 ha in a year (Dubrovskis & Adamovics, 2012). One of the most promising energy resources is biogas, which can be obtained from cogeneration plants in anaerobic fermentation process (Dubrovskis & Plume, 2015). Latvia is already running 56 biogas cogeneration plants, and maize silage is the most common biomass used as feedstock, as it gives a large quantity of biomass and a good yield of biogas (0.5–0.6 l g_{DOM}^{-1}). Most of the biogas plants built in Latvia are relatively large (49 of them greater than 0.5 MWel) and need a lot of raw materials for year-round running. Many of the biogas cogeneration plant owners do not have land for the cultivation of raw materials and are forced to transport raw materials even from a great distance, therefore, the prices of biomass increase considerably (Dubrovskis & Plume, 2015).

Competition on arable land areas increases, which affects seriously those farmers who based biogas production efficiency on the cheap land rent. On the other hand, although Latvia has a lot of unused or underused land (around 360,000 ha in 2010), (Dubrovskis et al., 2011) farmers who do not own a biogas plant, put pressure on the Ministry of Agriculture and the Ministry of Economy aimed to limit the use of arable land for biogas production. Therefore, the production of raw materials from unused land would be most supported and encouraged (Dubrovskis & Plume, 2015).

Freshwater algae (*Chlorella vulgaris*) is one of the feedstock that also gives a great yield of biomass and hence could be used for biogas production. *Chlorella vulgaris* is a green algae growing in freshwater lakes. It can be used as a feed supplement for human and animal consumption also (Dubrovskis & Plume, 2015). For the cultivation of algae *Chlorella vulgaris*, the following factors should be taken into account: water, carbon dioxide, minerals and light. Optimal water temperature is 20–30 °C, as the algae grows slower at temperatures below 16°C and stops growing at temperatures above 35 °C (Chen, P.H., 1987). The following methods are used for algae cultivation:

- cultivation in open ponds;
- cultivation in closed basins;
- cultivation in photobioreactors.

The cheaper and more widely used method is cultivation in open ponds. The advantages of this method are simplicity and cheapness, but its shortcomings are worse light utilisation, water evaporation losses and CO_2 discharge into the atmosphere, as well as the need for large land areas and partial dependence on climate (Dubrovskis & Plume, 2015).

Algae biomass yield: 150–300 tons (first year 150 t, but after adding CO_2 –300 t per year) of algae were obtained from 5 ha of sewage treatment pools during Bio-Crude Oil Demonstration Project activities in 2009 (Oilgae, 2016). *Chlorella vulgaris* obtainable biomass harvest was 106 t ha^{-1} per year, as estimated in Ltd. Delta Riga experimental plant by owners in 2014 (Dubrovskis & Plume, 2015).

Theoretically, a large biogas yield can be obtained from algae, if all of the organic matter can be conversed. Methane yield from a unit of dry organic matter of the *Chlorella vulgaris* may be in the range 0.63–0.79 l g_{DOM}^{-1}(Becker, 2004) as calculated theoretically according to Buswell equation (Symons & Buswell, 1933; Chen, 1987). However, in practice it is not possible to convert all of the organic matter into biogas. Biogas production depends on many factors and it should be taken into account that the algae cells have strong cell walls. The growing media and availability of nutrients may impose some impact on biogas yield. For example, former investigations have shown increased methane yield from algae grown in wastewater compared to algae fertilised with complex mineral fertiliser Varicon (Dubrovskis & Plume, 2015). Biogas and methane production from algae is investigated by many researchers (Symons & Buswell, 1933; Golueke et.al., 1957; Samson & LeDuy, 1986; Chen, 1987; Hernandez & Cordoba, 1993; Sanchez & Travieso, 1993; Mussgnug et al., 2010). Some of the research results on biogas and methane yield obtained from algae under different growing and treatment technologies are shown in Table 1.

Table 1. The methane production from algae *Chlorella sp*

Algae	Methane (biogas) yield, l g_{DOM}^{-1}	Methane content, %	Reference
Scenedesmus sp& Chlorella sp	0.17–0.32	62–64	Golueke et.al., 1957
Chlorella vulgaris	0.31–0.35	68–75	Sanchez & Travieso, 1993
Chlorella sp & Scenedesmus sp	0.09–0.136	69	Yen&Brune, 2007
Chlorella vulgaris	0.26–0.29	60–65	Liandong Zhu,2013
Chlorella zofingiensis	0.06–0.1	52–60	Liandong Zhu,2013
Chlorella pyrenoidosa	0.29	61–66	Liandong Zhu,2013
Chlorella sp+wws	(0.624)	66.61	Skorupskaite & Makareviciene, 2015
Chlorellasp+cm	(0.580)	59.63	Skorupskaite & Makareviciene, 2015
Chlorella sp (centrifuged)	(0.508)	66.75	Skorupskaite & Makareviciene, 2014
Chlorella sp (unfreezed)	(0.652)	67.98	Skorupskaite & Makareviciene, 2014
Chlorella vulgaris with Varicon as fertilizer	0.297	45.95	Dubrovskis & Plume 2015
Chlorella vulgaris with waste water as fertilizer	0.451	55.45	Dubrovskis & Plume 2015

Notes: biogas yield shown in brackets; wws – waste water sludge used as fertilizer; cm – cow manure used as fertilizer.

The objective of this study was to find out how much methane and biogas can be obtained from algae Chlorela vulgaris cultivated in open ponds under conditions different from normal growing conditions (cultivated on 20–27 October, harvested on 27 October, while the average daily water temperature was 12 °C during 20–27 October) and in a deep pond (4 m), when sun radiation is smaller and insufficient, and to estimate when freshwater algae can be cultivated for biogas production in climatic conditions of Latvia.

MATERIALS AND METHODS

Materials, equipment and methods in Study 1

Algae from the Delta Riga experimental unit harvested on 27 October was used in Study 1. Equal quantities of algae biomass were filled in each of the 14 self-made 0.75 l volume bioreactors (30 g in R2-R15) with 500 g of inoculum, which was taken from 110 l bioreactor working with cow manure continuously. Inoculum in the amount of 500 g was filled in two of the same self-made reactors only for control sample. Each raw material sample was weighted (by electronic moisture balance Shimazy and scales Kern FKB 16KO2) carefully before it was filled in the bioreactor. Fermentation was continued in batch mode until biogas production ceased. Fermentation parameters, *e.g.*, volume, composition, pH, inside and outside temperatures, were registered every day in the experimental journal. Each sample was weighted and its composition analysed before the start and at the end of the fermentation process. Average volume of biogas released in bioreactors with inoculum (control sample) was subtracted from biogas volume obtained from each bioreactor filled with inoculum and algae biomass.

Fermentation temperature was maintained at 38 ± 1 °C inside the containers during batch mode process. Dry matter, ash and organic dry matter content was determined for every sample mixture before being filled into the bioreactor. Measuring accuracies were the following: ± 0.2 g for inoculum and substrate weight (scales Kern FKB 16KO2), ± 0.001 g for biomass samples for dry matter, organic matter and ash weight analyses, ± 0.02 pH for pH (accessory PP-50), ± 0.05 l for gas volume, and ± 0.1 °C for temperature inside the bioreactor. Biogas composition, *e.g.*, methane, carbon dioxide, oxygen and hydrogen sulphide volume was measured with the gas analyser GA 2000. Dry matter was determined with the help of electronic moisture balance Shimazy at temperature 105°C. Dry organic matter was calculated from the weight of biomass ashes obtained in the oven Nabertherm at temperature 550°C using the standard heating program. Standard error for measurement data was calculated with the help of statistical data processing tools for each group of digesters.

Materials, equipment and methods in Study 2

Algae *Chlorella Vulgaris* cultivated in 4 m deep open pond (from Ltd. Delta Riga experimental plant) and fertilized with Varicon, harvested on 10–15 June and having low dry matter content was used in Study 2. The algae biomass was obtained from an open pond without centrifugation.

The methodology for biogas and methane potential estimation was the same as in Study 1. The only difference was the number (4) and volume (5 l) of bioreactors used in Study 2. All 4 bioreactors were filled with 1 kg of inoculum and 2 kg of tap water. 1 kg of algae biomass was added to bioreactors B2, B3 and B4. Inoculum (finished digestate from fermented cow manure) and water only was fermented in reactor B1 for control sample.

RESULTS AND DISCUSSION

In **Study 1**, biogas and methane data from all 16 bioreactors were used to calculate the average biogas and methane volume for each group of similar bioreactors filled in with the same sample replications. The results are summarized in Tables 2, 3, 4 and in Fig. 1, below.

The algae *Chlorella vulgaris* biomass samples investigated in the Latvia University of Agriculture Bioenergy laboratory contained the following complex substances: proteins 53.60%, lipids 18.51% and carbohydrates16.81%.

The results of raw algae biomass and inoculum analysis before fermentation are shown in Table 2.

Table 2. The results of the analyses of raw materials

Bioreactor	Raw material	Substrate pH	TS, %	TS, g	Ash, %	DOM, %	DOM, g	Weight, g
R1, R16	IN500	7.14	2.26	11.30	28.87	71.13	8.04	500.0
R2-R15	IN500 +A30	7.00	2.82	14.95	25.42	74.58	11.15	530.0
R2-R15	A30	5.95	12.18	3.65	14.78	85.22	3.11	30.0

Abbreviations: TS – total solids, Ash – ashes, DOM – dry organic matter, R1–R16 – bioreactors numbers; IN – inoculum, A– algae fertilised with complex fertiliser Varicon.

The algae biomass has a higher content of ashes compared to agricultural energy crops (maize silage 19–21%) (Dubrovskis et al., 2011), which can be explained by high minerals (complex fertiliser Varicon) doses used, but may be poorly utilized by algae in the growing process. This suggests that there are opportunities for the improvement of cultivation technologies and usage of optimised doses of fertilizer. Results of the analyses of finished fermented digestate are shown in Table 3.

Table 3. The results of the analyses of finished digestate

Bioreactor	Raw material	Substrate pH	TS, %	TS, g	Ash, %	DOM, %	DOM, g	Weight, g
R1	IN	7.16	2.18	10.86	29.05	70.85	7.69	498.1
R16	IN	7.15	2.20	10.96	29.10	70.90	7.77	498.2
R2	IN+A	7.18	2.12	10.90	26.72	73.28	7.99	515.0
R3	IN+A	7.17	2.18	11.24	26.57	73.43	8.25	515.2
R4	IN+A	7.16	2.15	11.11	25.90	74.10	8.23	515.6
R5	IN+A	7.18	2.16	11.14	26.36	73.64	8.20	515.0
R6	IN+A	7.18	2.17	11.21	25.98	74.02	8.30	515.6
R7	IN+A	7.16	2.17	11.20	26.13	73.87	8.27	516.0
R8	IN+A	7.19	2.18	11.24	26.49	73.51	8.26	515.6
R9	IN+A	7.20	2.20	11.36	26.10	73.90	8.39	516.4
R10	IN+A	7.20	2.20	11.36	26.05	73.95	8.40	516.2
R11	IN+A	7.21	2.22	11.47	25.88	74.12	8.50	516.8
R12	IN+A	7.17	2.18	11.26	26.09	73.91	8.32	516.2
R13	IN+A	7.21	2.15	11.08	26.44	73.56	8.15	515.0
R14	IN+A	7.18	2.18	11.22	26.45	73.55	8.25	515.4
R15	IN+A	7.18	2.17	11.20	25.89	74.11	8.30	516.0

It was calculated from Table 3 data that only a small part of inoculum's (R1, R16) dry organic matter (3.8% or 0.31 g) was biodegraded during the re-fermentation process, perhaps, due to plentiful presence of cells of microorganisms and complex humus substances persistent to biodegradation. Therefore, inoculum has little or no impact on the results of biogas production from added biomass. Algae dry organic matter was biodegraded by 82.63% during the anaerobic fermentation process. Biogas and methane yield from algae is shown in Table 4. The average volume of biogas (0.20 l) or methane (0.014 l) released in control bioreactors R1, R16 has already been subtracted from biogas volume from every bioreactor filled with inoculum and algae biomass in Table 4.

The relatively lower average methane content in biogas in bioreactors with 30 g algae biomass is explained by the fact that around 0.25 l of air remains in top of every bioreactor at beginning of anaerobic process. This air warms up and enters gas bags and was measured together with biogas during fermentation process. This effect is particularly evident in bioreactors with less added organic matter.

Table 4. Biogas and methane yield

Reactor	Raw material	Biogas, l	Biogas, l g_{DOM}^{-1}	Methane aver. %	Methane, l	Methane, l g_{DOM}^{-1}	Methane max, %
R1	IN	0.2	0.01	7.3	0.01	0.01	7.3
R2	IN+A	3.2	1.03	41.4	1.33	0.43	58.2
R3	IN+A	2.0	0.64	49.8	1.00	0.32	63.2
R4	IN+A	2.1	0.68	47.7	1.00	0.32	64.1
R5	IN+A	2.3	0.74	47.4	1.09	0.35	64.8
R6	IN+A	1.9	0.61	49.3	0.94	0.30	62.6
R7	IN+A	1.9	0.61	49.9	0.95	0.31	63.2
R8	IN+A	2.0	0.64	50.1	1.00	0.32	66.5
R9	IN+A	2.3	0.74	42.5	0.98	0.31	65.5
R10	IN+A	2.2	0.71	48.4	1.06	0.34	60.7
R11	IN+A	1.8	0.58	50.5	0.91	0.29	59.7
R12	IN+A	1.9	0.61	48.3	0.92	0.30	64.9
R13	IN+A	2.7	0.87	48.7	1.32	0.42	65.3
R14	IN+A	2.0	0.64	48.4	0.97	0.31	66.9
R15	IN+A	1.9	0.61	48.8	0.93	0.30	65.3
R16	IN	0.2	0.01	7.2	0.01	0.01	7.3
Average (R2-15)		2.16± 0.38	0.694± 0.12	47.69± 2.70	1.027± 0.14	0.331± 0.04	63.64± 2.57

Biogas and methane production from algae that was fertilized by complex fertilizer Varicon and harvested on 27 October is shown in Fig. 1.

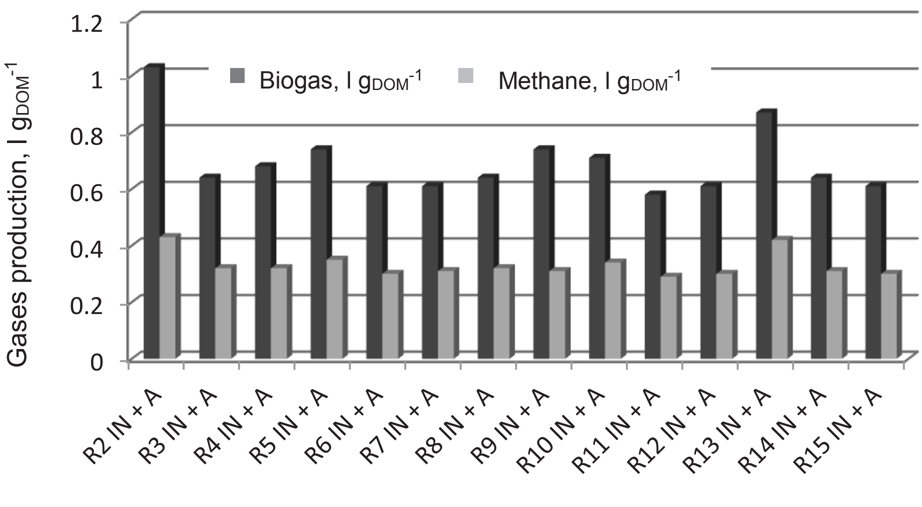

Figure 1. Biogas and methane production from algae; IN – inoculum; A– algae.

The results are comparable to those presented in Table 1 of the researchers who worked with *Chlorella vulgaris* results. The average methane yield is quite similar to the harvest derived from maize silage (0.332 l g_{DOM}^{-1}), rye grass silage (0.316 l g_{DOM}^{-1}) and perennial grass silage (0.322 l g_{DOM}^{-1}) in our previous studies (Dubrovskis & Plume, 2015).

Study 2 investigated the algae *Chlorella vulgaris* harvested on 10–15 June and obtained from an open pond without centrifugation (from Ltd. Delta Riga experimental plant) and fertilized with Varicon. The algae *Chlorella Vulgaris* biomass samples investigated in the LUA Bioenergy laboratory contained the following complex substances: proteins 48.7%, lipids 16.43% and carbohydrates 17.56%.The results are summarized in Tables 5, 6, 7 and in Fig. 2 below.

The results of raw biomass analysis before fermentation are shown in Table 5.

Table 5. The results of the analyses of raw materials

Bioreactor	Raw material	Substrate pH	TS, %	TS, g	Ash, %	DOM, %	DOM, g	Weight, g
B1	IN	7.41	3.14	31.40	22.93	77.07	24.21	1,000.0
	Water							2,000.0
B2	IN	7.41	3.14	31.40	22.93	77.07	24.21	1,000.0
	Algae	6.46	3.33	33.33	16.09	83.91	27.96	1,000.8
	Water							2,000.0
B3	IN	7.41	3.14	31.40	22.93	77.07	24.21	1,000.0
	Algae	6.46	3.33	33.31	16.09	83.91	27.95	1,000.2
	Water							2,000.0
B4	IN	7.41	3.14	31.40	22.93	77.07	24.21	1,000.0
	Algae	6.46	3.33	33.31	16.09	83.91	27.95	1,000.3
	Water							2,000.0

The results of analyses of finished fermented digestate are shown in Table 6.

Table 6. The results of the analyses of digestate

Bioreactor	Raw material	Substrate pH	TS, %	TS, g	Ashes, %	DOM, %	DOM, g	Weight, g
B1	IN+w	7.53	1.21	30.68	23.62	76.38	23.43	2536
B2	IN+w+A	7.08	1.16	35.40	25.21	74.29	26.47	3052
B3	IN+w+A	7.04	1.31	40.68	23.15	76.85	31.26	3105
B4	IN+w+A	7.11	1.19	36.02	29.79	70.21	25.29	3027

Abbreviations: w – water; A – algae; IN – inoculum

The biogas and methane yield from the algae *Chlorella vulgaris* is shown in Table 7. The average volume of biogas (2.9 l) or methane (0.621 l) released in control bioreactor B1 has already been subtracted from biogas volume from every bioreactor filled with inoculum and algae biomass in Table 7.

Table 7. Biogas and methane yields

Reactor	Raw material	Biogas, l	Biogas, l g_{DOM}^{-1}	Methane aver. %	Methane l	Methane, l g_{DOM}^{-1}	Methane max, %
B1	IN+w	2.9	0.12	21.4	0.62	0.03	21.4
B2	IN+w+A	15.5	0.56	54.0	8.37	0.30	66.5
B3	IN+w+A	14.2	0.51	52.0	7.37	0.26	65.3
B4	IN+w+A	16.1	0.58	53.0	8.53	0.31	64.7
Average (B2-B4)		15.27 ± 0.97	0.546 ± 0.04	52.95 ± 1.00	8.088 ± 0.63	0.290± 0.03	65.48 ± 0.92

The biogas and methane yields are lower compared to those obtained in Study1. Biogas and methane yield from the algae cultivated in 1 m deep pond and harvested on 27 October compared to the biogas and methane yield from the algae cultivated in 4 m deep pond and harvested on 10–15 June is higher by 27.1% for biogas and by 14.14% for methane.

This could be explained by the algae's lower content of lipids and proteins. The algae was cultivated in 4 m deep pond during 10–15 June, when sun radiation level is high, but obviously, mixing was not good enough. Another reason may be the lack of centrifugation providing some destroying of algae used in Study1.

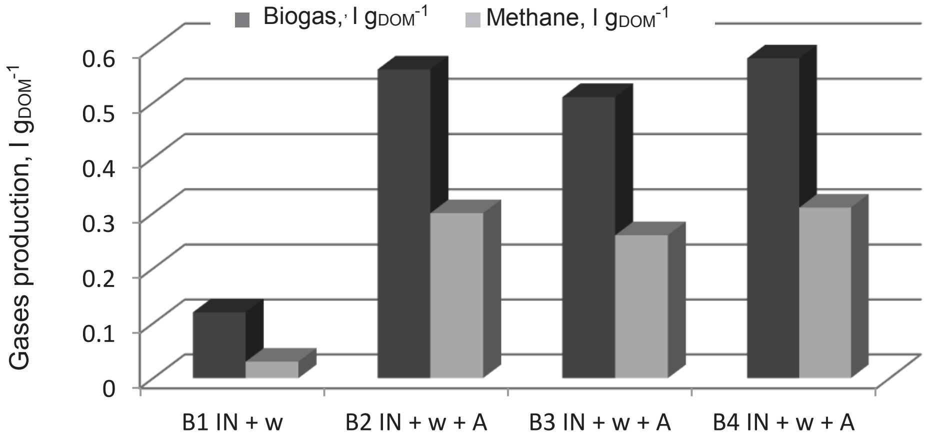

Figure 2. Biogas and methane production from algae; IN – inoculum; A – algae; w – water.

Further anaerobic fermentation investigations should deal with the combination of microalgae *Chlorella vulgaris* biomass having low C:N ratio of 7.53 (Skorupskaite et al., 2015) with agricultural wastes having high C:N ratio *e.g.*, straw (150), sawdust (208), etc. (Dubrovskis & Adamovics 2012). Such a combination can establish an important part of nitrogen, carbon, and other plant nutrients' life cycles, including capturing the leaching nitrogen from wastewater by algae biomass, biomethane production from combined substrates in optimised anaerobic fermentation process and returning of plant nutrients into the soil with digestate.

CONCLUSIONS

Biogas and methane yield obtained from algae biomass cultivated at Ltd. Delta Riga experimental plant under conditions different from normal growing conditions is comparable to that obtainable from other agricultural biomasses (maize, rye grass and perennial grasses silages) used for biogas and methane production in our previous research (Dubrovskis & Plume, 2015).

The study of methane production from algae harvested in summer or autumn period confirmed that algae can be utilised during its normal growing period from May till October or at least 170–180 days period in a year at the climatic conditions of Latvia.

The results of the investigation show that the algae *Chlorella vulgaris* is a prospective alternative biomass, suitable to replace or complement traditional feedstock, *e.g.*, maize silage or energy crops in biogas and methane production.

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