BIOGAS POTENTIAL FROM FRESHWATER ALGAE

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Abstract. 54 biogas plants are working today in Latvia. Maize is the dominating crop for biogas production in Latvia, however, fertile or best arable land areas are used for maize cultivation traditionally. There is a need to investigate the alternative biomass for energy production. The aim of the investigation is evaluation the biogas and methane production from freshwater algae *Chlorella vulgaris*, cultivated using complex fertilizer Varicon (Study 1) or grown in wastewater (Study 2). The digestion process of algae was investigated in sixteen 0.75 l digesters, operated in batch mode at temperature 38 ± 1.0 ºC. The average specific methane yield per dry organic matter (DOM) from Chlorella vulgaris was 0.297 $l \cdot g_{DOM}^{-1}$ or 0.451 $l \cdot g_{DOM}^{-1}$ fertilised with Varicon or cultivated in wastewater respectively. The specific methane yield from maize Celido silage was 0.331 $l \cdot g_{DOM}^{-1}$ and from Tango silage it was 0.312 $l \cdot g_{DOM}^{-1}$. The specific methane yield from rye grass silage or mixed perennial grasses silage was 0.316 $l \cdot g_{DOM}^{-1}$ or 0.322 $l \cdot g_{DOM}^{-1}$ respectively. Algae Chlorella vulgaris biomass can replace maize silage or other silages for biogas production and can be successfully cultivated under the climatic conditions in Latvia.

Key words: anaerobic digestion, Chlorella vulgaris, maize silage, ryegrass, perennial grass, biogas, methane.

1. Introduction

The directive of the European Union lays down that 20 % of the energy must be produced from alternative energy resources (in Latvia 40 %) in year 2020. Most of the biomass will come from forest products, but it should be taken into account, that from 1 ha agricultural land more energy can be obtained compared to forest wood biomass increment per 1 ha in a year. One of the most promising energy resources is biogas, which can be obtained from cogeneration plants in the anaerobic fermentation process [1]. Latvia is already running 54 biogas cogeneration plants, and maize silage is the most common biomass used as feedstock, as it gives large quantity of biomass and good yields of biogas. Most of the biogas plants built in Latvia are relatively big and need a lot of raw materials for round year running. Many of biogas cogeneration plant owners do not have land for cultivation of raw materials and are forced to transport raw materials even from a great distance, so the price of biomass increases considerably. Competition on arable land areas increases and particularly frustrated are those farmers who based biogas production efficiency on cheap land rent. On the other hand, although Latvia has lots of unused or underused lands (around 360,000 ha in 2010), farmers, who do not own a biogas plant, put the pressure on the Ministry of Agriculture and the Ministry of Economy aimed to limit the use of arable land for biogas production. Therefore, production of raw materials from unused lands would be most supported and encouraged.

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 and whether this input can compete with other biomass, especially with maize silage. Freshwater algae (*Chlorella Vulgaris*) is one of the plants, that also gives great yields of biomass yield and hence could be used for biogas production. Biogas yield from algae *Chlorella vulgaris* in Latvia has not been studied so far. *Chlorella vulgaris* is a green algae growing in freshwater lakes. It can be used as a feed supplement for human and animal consumption. It strengthens the immune system, increases energy, slows down the aging processes, regulates sugar and cholesterol levels in the blood, improves liver and intestinal activity, helps remove heavy metals and toxins from the body. Algae contains 60 % protein, it is rich in minerals (iron, zinc, magnesium) and vitamins (E, B1, B2, B3), as well as it is the source of Omega 3 fatty acids. It contains high amounts of chlorophyll and is an excellent antioxidant.

Cultivation of algae *Chlorella Vulgaris*. The following factors should be taken into account: water, carbon dioxide, minerals and light required for cultivation. The optimal water temperature is 20-30 ºC, as algae grows slower at temperatures below 16 ºC and stops growing at temperatures above 35 ºC. The following methods are used for algae cultivation:

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

A cheaper and widely used method is cultivation in open ponds. The advantages of this method are simplicity and cheapness, but 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 the climate.

Algae biomass yield. 150-300 tons of algae were obtained from 5 ha sewage treatment pools during the Bio-crude Oil Demonstration Project activities in 2009 [2]. The really obtainable algae biomass harvest was 106 t/ha per year, as estimated in Ltd. Delta Riga experimental plant in 2014.

Volume of biogas obtainable from algae. Theoretically, a large biogas yield can be obtained from algae, if all organic matter can be conversed. The methane yield from a unit of dry organic matter of *Chlorella Vulgaris* may be in the range 0.63-0.79 l g_{DOM}^{-1} [3], as calculated theoretically according to Buswell equation [4; 5]. However, it is not possible to convert all the organic matter into biogas in practice. Biogas production depends on many factors and it should be taken into account that the algae cells have durable casing. Methane production from algae is investigated by many researchers [3; 4; 6-9], some results are shown in Table 1.

Table 1

Methane production from algae Chlorella

Algae	Methane (biogas) yield, l g_{DOM}^{-1}	Methane content, %	Reference
Scenedesmus And Chlorella-	0.17-0.32	62-64	Golueke et.al., 1957[5]
Chlorella Vulgaris	0.31-0.35	68-75	Sanchez and Travieso, 1993[10]
Chlorella-From Scenesm	0.09-0.136	69	Yen and Brune, 2007[2]
Chlorella Vulgaris	0.26-0.29	60-65	Liandong Zhu, 2013
Zofingiens of Chlorella	0.06-0.1	52-60	Liandong Zhu, 2013
Pyrenoidos of Chlorella	0.29	61-66	Liandong Zhu, 2013
Scenedesmus (centrifuged)	(0.510)	(66.03)	Skorupskaite, Makareviciene, 2014[11]
Scenedesmus (unfreezed)	(0.646)	(68.03)	Skorupskaite, Makareviciene, 2014[11]
Chlorella SP. (centrifuged)	(0.508)	66.75	Skorupskaite, Makareviciene, 2014[11]
Chlorella SP. (unfreezed)	(0.652)	67.98	Skorupskaite, Makareviciene, 2014[1]

2. Investigation of the potential of biogas production from algae

2.1. Materials, methods and description of the investigation

Algae from Delta Riga experimental units were used in the study. Equal quantities of algae biomass were filled in every of 14 bioreactors (20 g in R2-R7 and 25 g in R8-R15). Inoculum in the amount of 0.500 kg was filled in 2 reactors in every investigation. Each raw material was weighed carefully before filling in the bioreactor. Fermentation was continued in batch mode until biogas production ceases. Fermentation parameters, e.g., volume, composition, pH, inside and outside temperatures were registered every day in the experimental journal. Every digestate sample was weighed and its composition analyzed before the start and at the end of the fermentation process. The average volume of biogas released in bioreactors with inoculum (control) was subtracted from the biogas volume obtained from every bioreactor filled with inoculum and algae biomass.

2.2. Equipment

The volume of biogas production was studied using laboratory equipment consisting of 16 bioreactors.

Fermentation temperature was maintained 38 ± 1 ºC inside containers during the batch mode process. The mixture consists of 500 g inoculum (fermented cow manure) and added 20 g or 25 g biomass sample, placed into 0.75 l bioreactors for anaerobic fermentation. Dry matter, ash and organic dry matter content was determined for every sample mixture before filling 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 ashes weight analyses, ± 0.02 pH for pH (accessory PP-50), ± 0.05 l for gas volume, and ± 0.1 ºC 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 by help of a specialized unit

Shimazy at temperature 105 ºC, and ashing was performed in the oven Nabertherm at temperature 550 ºC using the standard heating program. Standard error was calculated by help of standardized data processing tools for each group of digesters.

2.3. 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, 2 below.

Algae Chlorella Vulgaris biomass samples investigated in the LUA Bioenergy laboratory contain the following complex substances: proteins 54.8 %, lipids 20.11 % and carbohydrates15.62 %.

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

Table 2

Results of the analyses of raw materials

Bioreactor / raw material	Substrate pH	TS, %	TS, g	Ash, %	DOM, %	DOM, g	Weight, g
R1, R16 IN	7.24	4.02	20.1	20.01	79.99	16.08	500
Av 20		20.20	4.04	21.96	78.04	3.15	20
R2-R7 IN+Av20	6.0	4.64	24.14	20.34	79.66	19.23	520
Av 25		20.20	5.05	21.96	78.04	3.94	25
R8-R15 IN+Av25	6.0	4.79	25.15	20.40	79.60	20.02	525

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

Algae biomass has a higher ashes content compared to energy crop biomass, that can be explained by high mineral (complex fertiliser Varicon) doses used but may be poorly utilized by algae in the growing process. This suggests that there are opportunities for the cultivation technologies improvement and usage of less doses of fertilizer. The results of the analyses of finished fermented digestate are shown in Table 3.

Table 3

Results of the analyses of digestate

Bioreactor/Raw material	Substrate pH	TS, %	TS, g	Ash, %	DOM, %	DOM, g	Weight, g
R1 IN	7.28	3.97	19.70	20.02	79.98	15.76	496.3
R16 IN	7.29	3.96	19.65	20.06	79.94	15.71	496.2
R2 IN+Av20	7.23	4.35	22.45	23.72	76.28	17.13	516.2
R3 IN+Av20	7.22	4.37	22.59	23.66	76.34	17.25	517.0
R4 IN+Av20	7.22	4.39	22.67	23.90	76.10	17.26	516.5
R5 IN+Av20	7.21	4.36	22.50	23.56	76.44	17.20	516.0
R6 IN+Av20	7.24	4.34	22.37	23.48	76.52	17.12	515.5
R7 IN+Av20	7.15	4.36	22.50	23.87	76.13	17.13	516.1
R8 IN+Av25	7.20	4.38	22.68	23.9	76.10	17.26	517.8
R9 IN+Av25	7.16	4.39	22.80	24.05	75.95	17.31	519.3
R10 IN+Av25	7.17	4.50	23.39	24.02	75.98	17.77	519.8
R11 IN+Av25	7.16	4.56	23.77	23.95	76.05	18.07	521.2
R12 IN+Av25	7.15	4.51	23.48	24.00	76.00	17.84	520.6
R13 IN+Av25	7.14	4.55	23.70	23.94	76.04	18.03	521.0
R14 IN+Av25	7.12	4.48	23.30	23.01	76.99	17.94	520.1
R15 IN+Av25	7.15	4.47	23.24	23.40	76.60	17.80	520.0

It was calculated from Table 3 data that only a small part of inoculum dry organic matter (0.76 % or 0.35 g) was biodegraded during the re-fermentation process, perhaps, due to plentful 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 62.85 % during the anaerobic fermentation process. Biogas and methane yield from algae *Chlorella Vulgaris* is shown in Table 4. Average volume of biogas (0.20 l) or methane (0.009 l) released in the control bioreactors R1, R16 is already subtracted from the biogas volume from every bioreactor filled with inoculum and algae biomass in Table 4.

Table 4

Biogas and methane extraction

Raw material	Biogas, l	Biogas, l g_{DOM}^{-1}	Methane aver., %	Methane, l	Methane, l g_{DOM}^{-1}	Methane max, %
R1 IN	0.2	0.012	4.6	0.009	0.0005	-
R2 IN+Av20	1.8	0.571	46.43	0.743	0.235	60.3
R3 IN+Av20	1.6	0.508	40.93	0.573	0.182	50.6
R4 IN+Av20	1.6	0.508	44.79	0.627	0.199	57.7
R5 IN+Av20	1.9	0.603	39.47	0.671	0.213	58.5
R6 IN+Av20	2.3	0.730	45.90	0.964	0.306	65.3
R7 IN+Av20	2.0	0.635	54.22	0.976	0.310	69.6
R8 IN+Av25	3.0	0.761	51.96	1.455	0.369	62.1
R9 IN+Av25	2.8	0.711	54.88	1.427	0.362	70.5
R10 IN+Av25	2.9	0.736	54.74	1.478	0.375	71.0
R11 IN+Av25	2.3	0.583	55.81	1.172	0.297	69.7
R12 IN+Av25	2.6	0.660	58.95	1.415	0.359	71.2
R13 IN+Av25	2.4	0.609	48.57	1.020	0.259	60.5
R14 IN+Av25	2.8	0.711	49.96	1.299	0.330	66.6
R15 IN+Av25	2.8	0.711	54.62	1.420	0.360	67.3
R16 IN	0.2	0.012	3.5	0.007	0.0004	-
Average	-	0.646 ± 0.086	50.1 ± 0.90	-	0.297 ± 0.051	-

Relatively lower average methane content in gas in the bioreactors with 20 g algae biomass is explained by the fact that around 0.25 l of air remains in the top of every bioreactor at the beginning of the anaerobic process. This air warms up and enters the gas bags and it was measured together with biogas during the fermentation process. This effect is particularly evident in the bioreactors with less added organic matter.

In **Study 2** algae *Chlorella vulgaris* cultivated in wastewater (from Ltd. Delta Riga experimental plant) was used. The methodology and equipment for biogas and methane potential estimation was the same as in Study 1. Inoculum with added algae biomass was processed in 6 bioreactors and pure inoculum was fermented in R5 and R16 during the experiment. The results of raw biomass analysis before fermentation are shown in Table 5.

Table 5

Results of the analyses of raw materials

Bioreactor/raw material	Substrate pH	TS, %	TS, g	Ash, %	DOM, %	DOM, g	Weight, g
R5 IN	7.19	2.69	13.45	25.0	75.0	10.09	500.0
R16 IN	7.19	2.69	13.45	25.0	75.0	10.09	500.0
Aw23,6 R6 IN+Aw23.6	5.51 7.05	17.74 3.37	4.18 17.63	17.79 23.31	82.21 76.69	3.43 13.52	23.55 523.55
Aw25,2 R7 IN+Aw25.2	5.51 7.05	17.74 3.41	4.47 17.92	17.79 23.16	82.21 76.84	3.68 13.77	25.2 525.2
Aw25,4 R8 IN+Aw25.4	5.51 7.05	17.74 3.42	4.5 17.95	17.79 23.18	82.21 76.82	3.70 13.79	25.36 525.36
Aw24,8 R9 IN+Aw24.8	5.51 7.05	17.74 3.40	4.4 17.85	17.79 23.19	82.21 76.81	3.62 13.71	24.83 524.83
Aw25,1 R10 IN+Aw25.1	5.51 7.05	17.74 3.41	4.46 17.91	17.79 23.17	82.21 76.83	3.67 13.76	25.13 525.13
Aw24,9 R14 IN+Aw24.9	5.51 7.05	17.74 3.40	4.42 17.87	17.79 23.17	82.21 76.83	3.64 13.73	24.93 524.93

Abbreviations: IN – inoculum, Aw – algae cultivated in wastewater.

Biogas and methane production is shown in Table 6. Average volume of biogas (0.15 l) or methane (0.2 l) obtained in the control bioreactors R5, R16 is already subtracted from the biogas or methane volume released from every bioreactor filled with inoculum and algae mixture in Table 6.

Table 6

Biogas and methane extraction

Raw material	Biogas, l	Biogas, l g_{DOM}^{-1}	Methane aver., %	Methane, l	Methane, l g_{DOM}^{-1}	Methane max, %
R5 IN	0.1	0.01	13.2	0.013	0.001	
R6 IN+A23.6	2.9	0.845	56.46	1.636	0.477	66.2
R7 IN+A25.2	2.9	0.788	57.01	1.653	0.449	70.6
R8 IN+A25.4	2.9	0.784	50.12	1.464	0.393	66.6
R9 IN+A24.8	3.0	0.829	57.05	1.711	0.473	71.9
R10 IN+A25.1	3.1	0.845	55.98	1.736	0.473	66.9
R14 IN+A24.9	2.9	0.797	55.43	1.608	0.442	67.8
R16 IN	0.2	0.02	13.0	0.026	0.003	
Aver. (R6-R14)		0.815 ± 0.085	55.34 ± 0.071		0.451 ± 0.042	68.33 ± 0.63

Average methane content in biogas was 55 %, and the maximum methane content reaches 68 %, that can be considered as good for direct usage of biogas in cogeneration engines or for further purification/upgrading.

Biogas and methane production from algae fertilized by the complex fertilizer Varicon or from algae cultivated in wastewater is shown in Fig. 1.

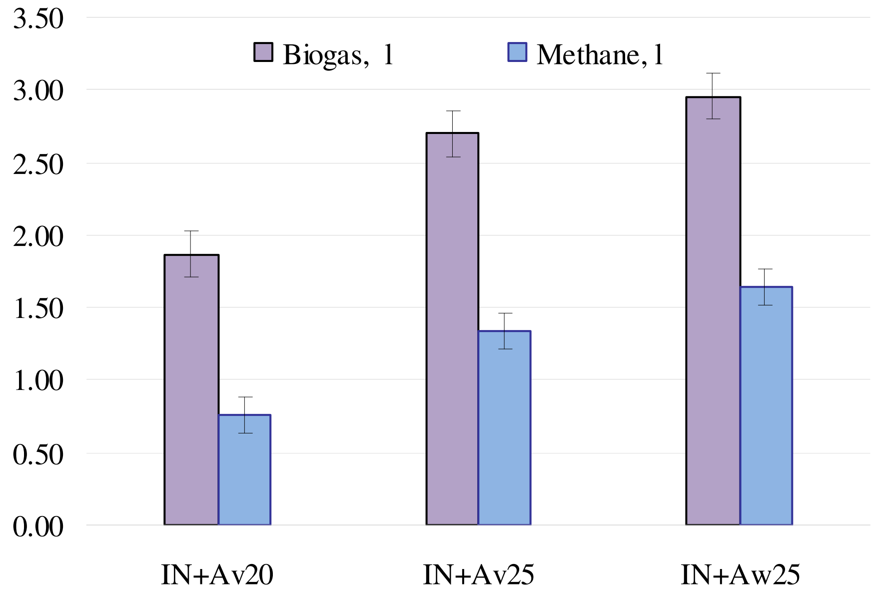

Fig. 1. **Biogas and methane production from algae;** IN – inoculum; Av – algae fertilized with Varicon; Aw – algae cultivated in wastewater

There is no significant difference (with probability 95 %) between biogas production in dependence on the fertilisation method, but significant difference exists for average biogas production between bioreactor groups having different initial organic load. Significant difference (with probability 95 %) exists also between the methane production values due to different fertilization or different initial organic load, see Fig. 1. The results can be even uniform, if the excess space in bioreactors (approx. 0.25 l) can be minimised.

The second study shows increased specific biogas and methane production from algae cultivated in wastewater compared to that obtained from algae fertilised by the complex fertiliser Varicon. This evidence can be explained by high mineral impurities from Varicon still existing in algae solution interfering with bacteria reproduction in the first study. Such the assumption is confirmed by higher total solid content in the first study, compared to that in the second study (see Tables 2, 5). Specific biogas and methane production, calculated per unit (1 g) of initial dry organic matter (DOM) are shown in Fig. 2.

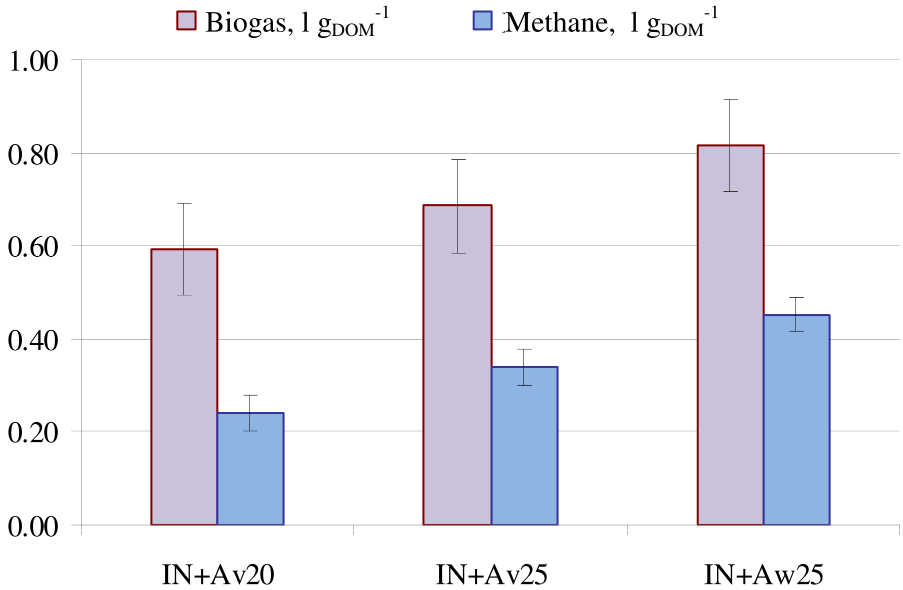

Fig. 2. **Specific biogas and methane production from algae:**
error bars represent 95 % confidence interval

There is no significant difference between specific biogas production, but the significant difference (with probability 95 %) exists between the specific methane production values due to different fertilization methods or different initial organic load, see Fig. 2.

2.4. Comparison of biogas production from algae *Chlorella Vulgaris* and energy crop silage

Many investigations on biogas production from different biomass and from different parts of plants were provided in the LUA laboratory of Bioenergetics. There is the increasingly widespread public view that agricultural arable land should only be used for production of food, and energy crops, particularly maize and grass silage, should not be grown on that land in the recent time. Unlike most energy crops, e.g., maize, algae can be cultivated on non-agricultural lands, poor soils or flood-lands, and it can be cultivated as an alternative biomass source for biogas producers. Summary results of laboratory investigations, using the same experimental methodology, for comparison of biogas and biomethane production from different biomass are given in Table 7.

Table 7

Specific biogas and methane production from different silage and algae biomass

Raw material	Biogas, l g_{DOM}^{-1}	Methane, l g_{DOM}^{-1}	Aver. methane, %
Maize Celido silage	0.563	0.332	59.10
Maize Tango silage	0.537	0.312	58.10
Rye grass silage	0.543	0.316	58.36
Perennial grasses silage	0.604	0.322	53.30
Algae fertilised with Varicon	0.646	0.297	45.95
Algae cultivated with wastewater	0.815	0.451	55.34

Comparison of the results is shown in Fig. 5. The results confirm the assumption that biogas and methane production from algae *Chlorella Vulgaris* can successfully compete with that obtainable from silage of different energy crops, including maize silage grown under the climatic conditions in Latvia.

A relatively short period of bioconversion is a great advantage of algae biomass. The anaerobic fermentation process period is around 50 days for maize silage, and 10-12 days only for algae *Chlorella vulgaris*. A possible direction for further research may be investigations of mixtures composed of algae, having short biodegradation time, with raw materials, e.g., chopped straw, sawdust, having long retention time and having a different carbon:nitrogen (C:N) ratio in anaerobic bioreactors.

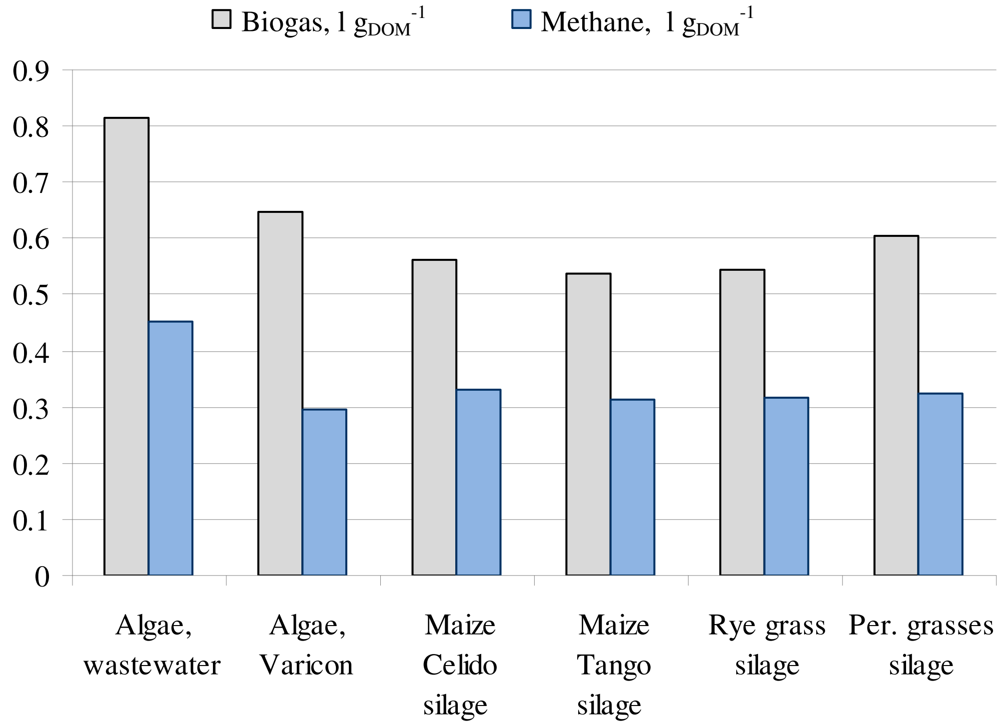

Fig. 3. **Comparison of biogas and methane production from various raw biomasses**

According to the estimation performed by the representatives of Ltd. Delta Riga, algae cultivation according to their technology can provide *Chlorella Vulgaris* biomass yield 106 t per 1 ha pond area. This biomass contains up to 15.45 t dry organic matter or 12,600 m^3 biogas (6,972 m^3 methane) production potential. Average maize silage yield is 50-60 t (17.0-20.0 t dry organic matter, at DOM percentage 35 %) from 1 ha arable land under the soil and climatic conditions of Latvia, and from this biomass up to 9,350-11,230 m^3 biogas or 5,520-6,620 m^3 methane can be obtained. Further investigations may be provided to establish optimal mineral composition, moisture, mixing mode and other parameters for improvement of algae anaerobic digestion.

3. Conclusions and proposals

1. The biogas and methane yield obtainable from algae biomass cultivated at Ltd. Delta Riga experimental plant is relatively higher compared to other agricultural biomass for biogas and methane production.
2. The biogas and methane yield from algae cultivated in the wastewater of sewage treatment facilities (Study 2) is by 21 % and 34 % higher compared to the biogas and methane yield from algae cultivated with the complex fertilizer Varicon (Study 1).
3. Algae *Chlorella Vulgaris* from 1 ha pond area give higher biogas yields compared to that obtainable from maize or grass 1 ha area. In addition, land used under ponds may be not appropriate for agricultural use.
4. The results of the investigation show that *Chlorella Vulgaris* is very good alternative biomass to replace or to complement other raw materials for biogas production.
5. The study of frozen and unfrozen confirmed that algae can be stored in frozen conditions without losing the ability to produce biogas and methane, however, the best method is fresh algae utilisation during its normal growing period (170 days period in a year at the climatic conditions of Latvia).

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