AlgaeBioGas

Algal treatment of biogas digestate and feedstock production

D3.7

Input, output and biomass analysis report

PUBLIC

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Instrument	CIP Eco-innovation - Pilot and market replication projects
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1 Summary

Editorial note

Deliverables in AlgaeBioGas project necessary build on and refer to previous deliverables. Our aim is to make them self-contained readable documents which necessary involves some replication of contents of previous deliverables, either as verbatim or summarized quotes. We are aware that such text is annoying to someone reading deliverables in series, so we have decided to set such text in lighter colour.

Thus, if you are reading just this text, please find contextual and reference information in lightly set sections; if you are acquainted with the project context (like a reviewer), please ignore the text set in light typeface.

Previous deliverables (partially) quoted in this document:

- DoW Description of work (Annex I of the Grant Agreement)
- D4.1 Case study operation assessment

2 Project Abstract

AlgaeBioGas project is focused to market introduction of algal-bacterial treatment of biogas digestate. Using algae we can recycle CO2 emissions and nutrients contained in the biogas digestate. Excess heat can also be productively used. Treated digestate is of such quality that it can be reused or released to the environment. Resulting biomass can be used as biogas substrate, possibly after extraction of specific components in biorefinery.

Classical biological (bacterial) waste water treatment successfully reduces the quantities of organic substances at the cost of significant CO2 emissions and significant energy consumption for aeration. Mineral nutrients, flushed with the liquid phase of digestate, are lost in the bacterial sludge which is frequently deposited, incinerated or discharged to the environment.

Algae hold a great potential because of their high growth rate, easy production, better utilization of sunlight compared to conventional plants, shorter lifecycles and independence from fertile agricultural land. Biogas plants are rich sources of mineral nutrients, CO2 and heat. By algal recycling we can close material cycles, provide feedstock for bio-refining various high value products and decrease competition between biogas and food use of agricultural crops.

The project aims to set-up the first application as a demonstration centre and prepare all prefabricated technology, organization and marketing tools to market replication projects. The technology demonstration centre is not only be able to demonstrate the technology in full size at a demanding customers site, but also provides on-site support for customer's testing, analysis, evaluation, training and other activities required as part of a complex project.

3 Task Description and Objectives

From DoW (Task 3.4 Input, output and biomass analysis)

Input (digestate) and output (water) will be sampled automatically and monitored daily. Produced algal biomass will be removed from the system. COD analyses of the input and output phase will be made, microscopic examination and biomethane potential of the biomass will be regularly measured. In some operating modes additional analysis plan will be made and executed. Such analyses may include element analysis, Chlorophyll content and similar.

During ordinary operating modes, a regular wastewater treatment monitoring will be done for obtaining a comparable set of performance parameters.

4 Overview of the process

From D4.1: The demonstration centre consists of the following subsystems:

- a greenhouse,
- main pond with mixing,
- inoculation pond with mixing,
- digestate separation and supply subsystem,
- CO2 (exhaust) cooling, dehumidification and supply subsystem,
- pond heating & cooling subsystem,
- sedimentation, harvesting and output water subsystem,
- control system with sensors.

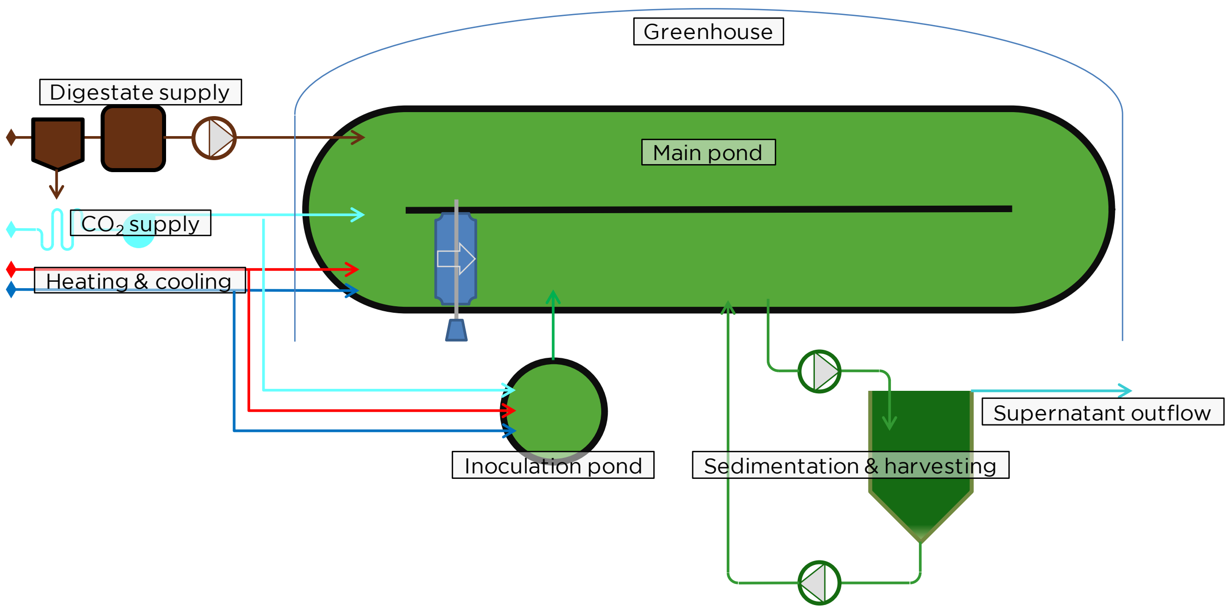

Figure 1 Demonstration centre scheme

Algae bacterial treatment of digestat takes place in the main pond, where algal-bacterial community uses nutrients present in digestat which results in growth of algal-bacterial biomass. Mixed algae and bacterial culture is maintained in inoculation pond and added to the main pond when necessary. Both ponds are continuously mixed. Biomass from the main pond is recycled through the sedimenter and harvested. The supernatant outflow from sedimenter is discharged to the sewage system. To assure optimal conditions for system operation, water level, CO_2 addition, water and air temperature in greenhouse are controlled.

Addition of digestate is controlled by several parameters (ORP, conductivity, pH, dissolved oxygen etc.) which is described in detail in D4.1.

5 Input: digestate

Analysis of digestate, input for ABG demo centre ponds are done regularly. Chemical Oxygen Demand (COD), ammonium concentration and pH are measured at least once per week. In general, ammonium concentration does not vary significantly, average ammonium concentration in digestate is 1400 mg/L. Chemical Oxygen Demand (COD), which is parameter used in classical waste water treatment, is usually between 6000 and 9000 mg O_2/L, except for a period of months between middle of September 2015 and middle of December 2015, when COD was lower, around 3000-4000 mg O_2/L. Changes in COD in that time occurred due to a longer retention time in anaerobic filter, which is a source of digestate for ABG demo centre. This means that during that mode, our system was in different mode than for the rest of operation, which probably had effect on operation in periods 6 to 10 (Table 3).

Digestate pH value is relatively stable around 7,6. Average values for input-digestate parameters are showed in Table 1.

Table 1 Average values for input-digestate

Time period	COD (mg O_2/L)	pH	NH_4-N
1.4.2014-13.9.2015	7.310	7,68	1.394
14.9.2015-14.12.2015	3.668	7,73	1.545
15.12.2015-17.3.2016	8.769	7,53	1.459

Values for digestate and outflow of water from ABG system, output, analysis are showed for time period between March 2015 and March 2016; with some exceptions for external analysis.

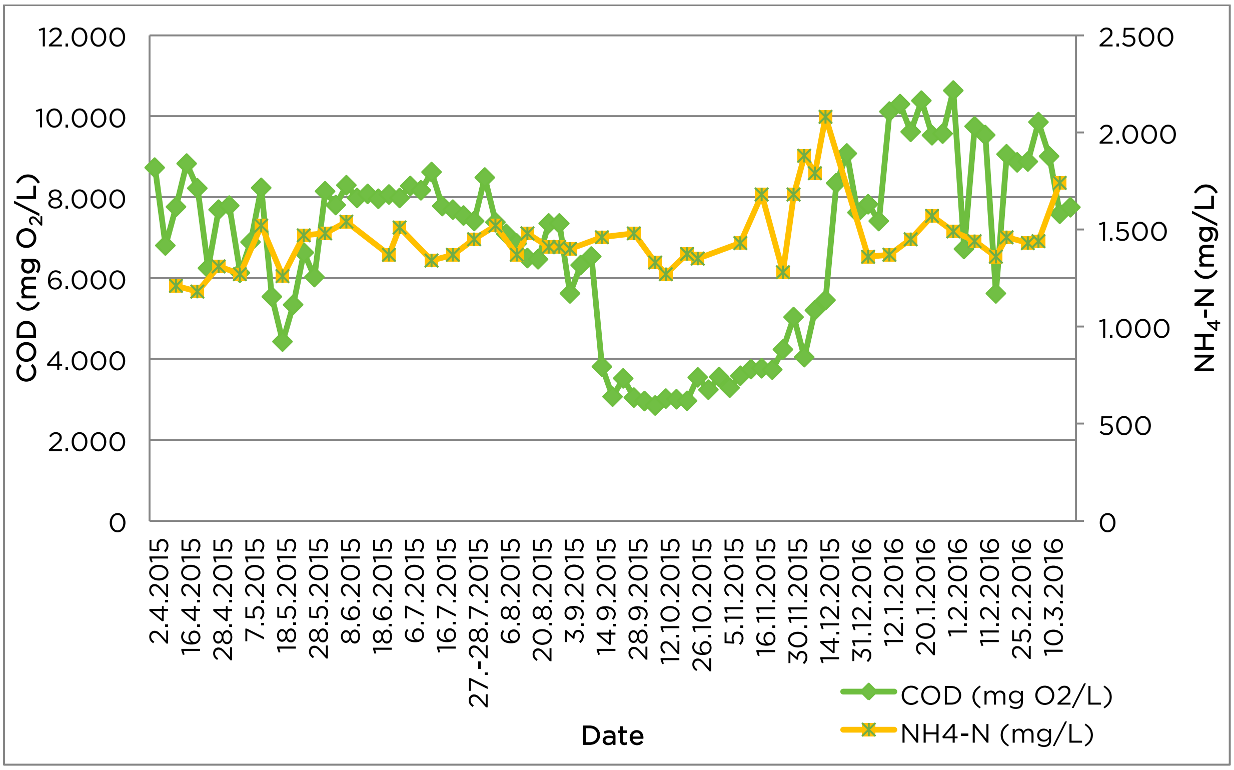

Figure 2 Digestate COD and NH_4-N concentration in 2015-2016

Table 2 Input-digestate analysis

Parameter	12.2.2015	25.1.2016	9.2.2016
COD (mg O_2/L)		8611	9033
Total N (mg/L)	1595	1900	2100
NH_4-N (mg/L)	1185	1691	1415
NO_3-N (mg/L)	<1	0,56	0,86
NO_2-N (mg/L)		<0,3	<0,3
PO_4-P (mg/L)		419	434
Total P (mg/L)	72	1658	1547
K (mg/L)	734	135	140
Ca^2+ (mg/L)	175		
Mg^2+ (mg/L)	73		
Na (mg/L)	7996		

By sending samples of digestate for analysis in external national laboratories (Table 2) we checked if in house analysis are in accordance with external analysis. Results showed that internal analyses are sufficient.

6 Output: water

After digestate is treated in the main pond, water from the main pond goes to sedimenter, where biomass is settled and harvested. Residual water is partially cycled back to the pond or discharged to the sewage system. Results for output water are showed for water coming from sedimenter after biomass settling. Due to insufficient operation of sedimenter, this water still contains some algal and bacterial cells. After we have done some laboratory tests we are positive that showed values for nitrogen, phosphorus and COD would be lover and in consistence with local regulations for water discharge to water body, if sedimentation would be improved.

6.1 Results of regular analysis

Table 3 shows different parameters for input (digestate) and output (water) for selected periods at the start of demonstration centre operation, in summer and in winter time. Summer time is defined from March to September, while winter time is defined from October to February, according to light availability and temperatures in Ljubljana (N 46° 3′ 5.1347″, E 14° 30′ 21.4758″).

Weather legend:

S	sunny
C	clouds/rain/fog/snow
SC	partly sunny (more sun)
CS	partly cloudy (more clouds)

Colum 10 shows average volume of digestate added per day in chosen time period.

Table 3 Input and output analysis for different time of year

INPUT (DIGESTATE)										OUTPUT (WATER)						
Time period	season	weather	COD (mg O_2/L)	N tot (mg/L)	NH_4-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	PO_4 (mg/L)	average V added/day (L)	COD (mg O_2/L)	NH_4-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	PO_4 (mg/L)	COD reduction (%)	dominant microbial species
1	start	SC	7486		1245				27	606	4,8	186,5	0,38	25,2	92	*Scenedesmus* and *Monoraphidium*
2	summer	S	6152		1380				206	213	41,3	216,0	1,11	16,8	97	*Monoraphidium*
3		S	8056		1510				321	239	46,0	163,0	0,43	21,9	97	*Monoraphidium*
4		S	8142		1510				165	579	68,8	276,5	0,32	30,8	93	*Monoraphidium*
5		SC	7350		1400				439	302	97,0	358,0	2,54	38,9	96	*Monoraphidium*
6	winter	C	3393		1374				474	625	236,0	304,5	>35,7	113,3	82	*Monoraphidium* and *Ankistrodesmus*
7		C	3423		1360				400	622	231,0	296,0		96,0	82	*Ankistrodesmus*
8		CS	3668		1430				264	276	22,9	94,0	>14,7	28,7	92	*Ankistrodesmus*
9		C	8735						51	257	71,4	98,0	>35,7	32,3	97	*Ankistrodesmus*
10		SC	4558		1658				93	246	140,0	130,3	13,00	34,5	95	*Ankistrodesmus*
11		S	8388		1340				0	396	49,0	162,0		45,0	95	*Ankistrodesmus*
12		SC	10208		1370				38		81,0	1,3	15,80			*Ankistrodesmus*
13		S	9550	1900	1570	0,56	<0,3	419	76	436	1,8	123,0	0,94	15,2	95	*Ankistrodesmus*
14		C	9643	2100	1440	0,86	<0,3	434	37	245	16,8	162,0	3,11	23,1	97	*Ankistrodesmus*
15		SC	8960		1460				51	274	25,2	167,0	0,38		97	*Ankistrodesmus*
16		C	8880		1430				63	386	34,1	196,0	0,14	38,7	96	*Ankistrodesmus*
17		C	9433		1440				36	374	35,4	182,0	0,05	41,3	96	*Ankistrodesmus*
18	summer	S	7670		1740				82	430	38,6	219,0	0,09	39,7	94	*Ankistrodesmus*

Chemical oxygen demand reduction is at average 94%, regardless of season.

Table 4 shows average % of dry matter, volume of harvested biomass and biomass production in kg on 100 m^2 per day for different time periods. Time periods were chosen according to available data. Since demonstration centre started fully operating in March 2015, we have yet to confirm tested settings in the last year of the project. AlgaeBioGas system is very complex, therefore several different settings had to be tried out in order to determine which parameters are most important for optimal operation, meaning treatment of maximum possible volume of digestate per day in selected season.

Table 4 Average harvest and biomass production per time period

Time period	Season	Weather	DM %	Harvest (L)/day	Biomass production (kg/100m^2/day)
1	start	SC	0,89	33	0,290
2	summer	S	1,18	37	0,440
3		S	0,92	43	0,400
4		S	0,51	0	0,000
5		SC	0,84	128	1,073
6	winter	C	1,84	0	0,000
7		C	0,74	51	0,378
8		CS	0,62	64	0,397
9		C	0,74	48	0,355
10		SC	0,46	64	0,292
11		S	0,27	3	0,008
12		SC	1,11	11	0,122
13		S	1,47	24	0,352
14		C	0,16	62	0,096
15		SC	1,24	36	0,439
16		C	1,45	26	0,384
17		C	1,28	17	0,219
18	summer	S	1,62	21	0,340

Based on preliminary results we calculated that we were so far able to process approx. 300 L of digestate in summer time and 150 L of digestate in winter time in 100 m^2 algal pond. Biogas plant produces approx. 80 m^3 of digestate per day, therefore for full treatment capacity we would need 3 ha of ponds in summer time and 6 ha of ponds in winter time, to be able to process all digestate produced on site.

6.2 Parameters for industrial water: legislation

Decree of the Government of the Republic of Slovenia »Decree on the emission of substances and heat in the discharge of waste water into waters and public sewage system« (OJ RS No. 12/14, 64/14, 98/15) lays down aims for the reduction of environmental pollution due to emissions of substances and heat generated during the discharge of sewage, industrial wastewater and rainwater, and their mixtures in water. The Decree establishes emission limit values of substances and heat, evaluation of emissions of substances and heat, measures preventing from emissions and heat in the discharge of waste water, measures to reduce emissions of substances and heat in the discharge of wastewater, and obligations of investors and operators of installations relating to obtaining an environmental permit for the operation of the plant, in accordance with European Community legislation.

Environmental quality standards and emission limit values for certain groups or families of pollutants are laid down as minimum requirements in EU Community legislation. The European Parliament and the Council determines the substances to be considered for action as a priority and on specific measures to be taken against pollution of water by those substances, taking into account all significant sources and identifying the cost-effective and proportionate level and combination of controls. Member States adopt measures to eliminate pollution of surface water by the priority substances and progressively to reduce pollution by other substances which would otherwise prevent Member States from achieving the objectives for the bodies of surface water.

Waste water from food industry pollution is quantified by COD, BOD, NH_4-H, fat, suspended solids, total P, total N, detergents, total dissolved solids, AOX, hydrocarbons, phenols. Digestate might contain some of the pollutants, if WWT sludge from food industry and other residues from food industry are treated in biogas plant.

According to our results (Table 3), we are well below limits for NH_4-N values for industrial waters, except for certain time periods where operation of demo centre was disturbed due to various reasons (technical errors, weather influence etc.).

Table 5 Limit values for industrial water in Slovenia

	Limit value for discharge of purified water	
Parameter and unit	to water body	to sewer
Insoluble solid mg/l	80	determined individually for each WWTP
COD mg/l	120	/
BOD5 mgO2/l	25	/
Total nitrogen mg/l	limit value is sum of limit value of ammonia nitrogen and nitrates	/
Ammonia nitrogen mg/l	10	200
Nitrite mg/l	1	10
Total phosphorus mg/l	2 (1 on water sensitive area)	/

Limit value for ammonia nitrogen for industrial waste water with discharge to sewage -municipal WWT is:

– if MWWT capacity is smaller than 2.000 PE limit value for NH_4-N is 100 mg/L,

– if MWWT capacity is 2.000 PE or bigge, limit value for NH_4-N is 200 mg/L.

6.3 Challenges

There are still some challenges left before ABG demo centre can operate under optimal conditions in selected time period. Some technical problems are still affecting regular measurements and analysis. One of those is lack of measurement for overflow in the sedimenter, which affects accurate estimation of digestate inflow to the ponds. Another problem is settling of the biomass in the sedimenter, which is insufficient, affecting residual water discharge to the environment.

One thing we will probably never be able to fully eliminate is human errors and operation disturbances such is power failure etc. But if the system is operating in the optimal mode for certain period and type of digestate, such occurrences should not be problematic on the long run.

7 Biomass

7.1 Microscopy

Microbial community in the ponds is monitored regularly, as mentioned in D4.1.

We followed changes in microbial community with regular (once per week) microscopy of samples from main and inoculation ponds. At the start of the system (September 2014) *Scendesmus* sp. (Figure 3) appeared to be dominant species, up until the end February 2015, when we first noticed new species (Figure 5), which was not added at the start of operation. Possible reasons for changes in microbial community are explained in chapter 4.1.1. We identified the new species as *Monoraphidium* sp. In March, ratio between *Scenedesmus* sp. and *Monoraphidum* sp. was approximately 1:1, while in May, *Monoraphidium* sp. became dominant (Figure 7). In May we also observed new species, which most likely is *Ankistrodesmus* sp. (a relative of *Monoraphidium*) Figure 9. *Monoraphidium* sp. and *Ankistrodesmus* sp. stayed dominant species up until November 2015 (Figure 10). The trend continued in January, February and March 2016: dominating culture is still *Ankistrodesmus* sp., every now and then some *Monoraphidium* sp. can be seen, together with *Chlorella* sp. , *Scenedesmus* sp. and diatoms. On certain periods we had problems with grazers (Figure 18), which multiplied rapidly at the beginning of March, but eventually the number of grazers lowered and is currently not affecting the algal growth significantly.

After harvesting, harvested biomass is periodically examined under the microscope as well. Harvested biomass in general reflects microbial composition in the ponds. However, cianobacteria are usually not present in the samples from the ponds, but can be seen in harvested biomass, as on Figure 6, Figure 8 and Figure 21. Flocs of bacteria are found in the harvested biomass, as well as some diatoms. Grazers are always seen in the harvest biomass, which is to be expected, but the types vary during season. Regularly seen are grazers such as Euplotes and Vorticella (Figure 8), alongside some other rotifer species and polychaeta (Figure 16).

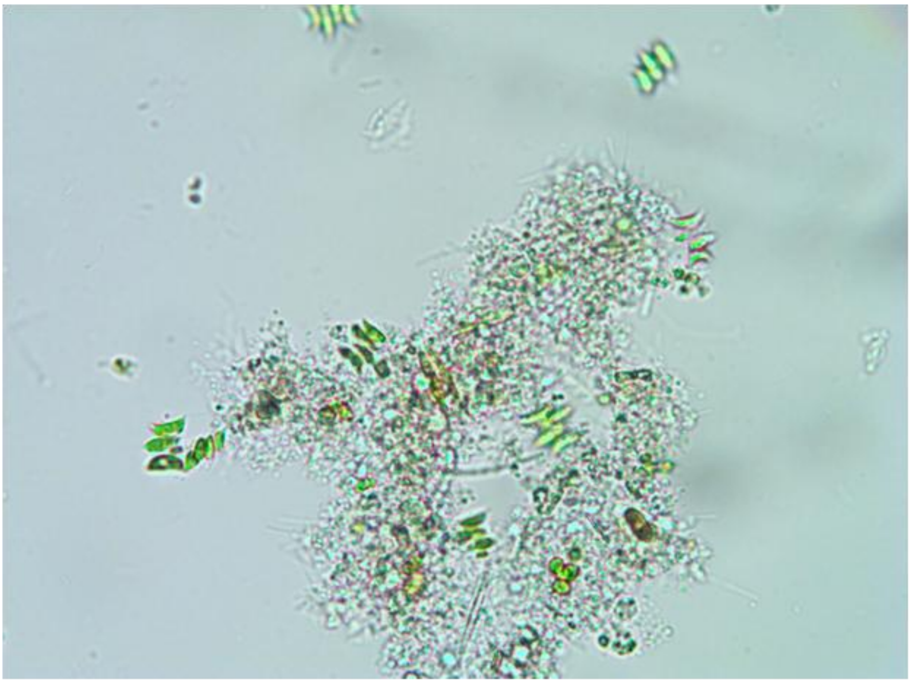

Figure 3 *Scenedesmus* sp. 100x, February 2015

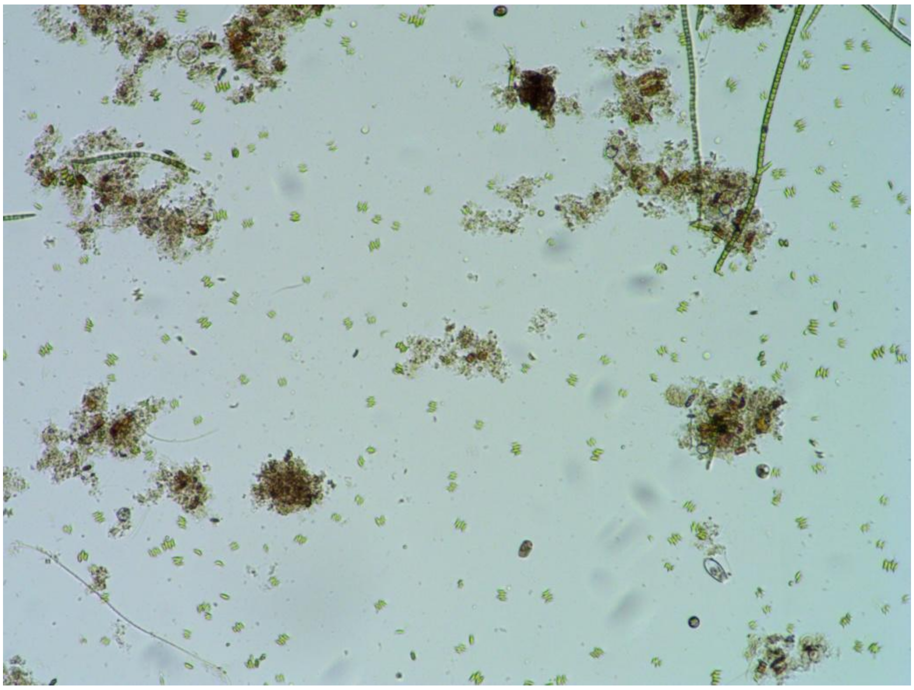

Figure 4 Biomass, 100x, February 2015

Figure 5 *Monoraphidium* sp. and *Scendesmus* sp. 400x, March 2015

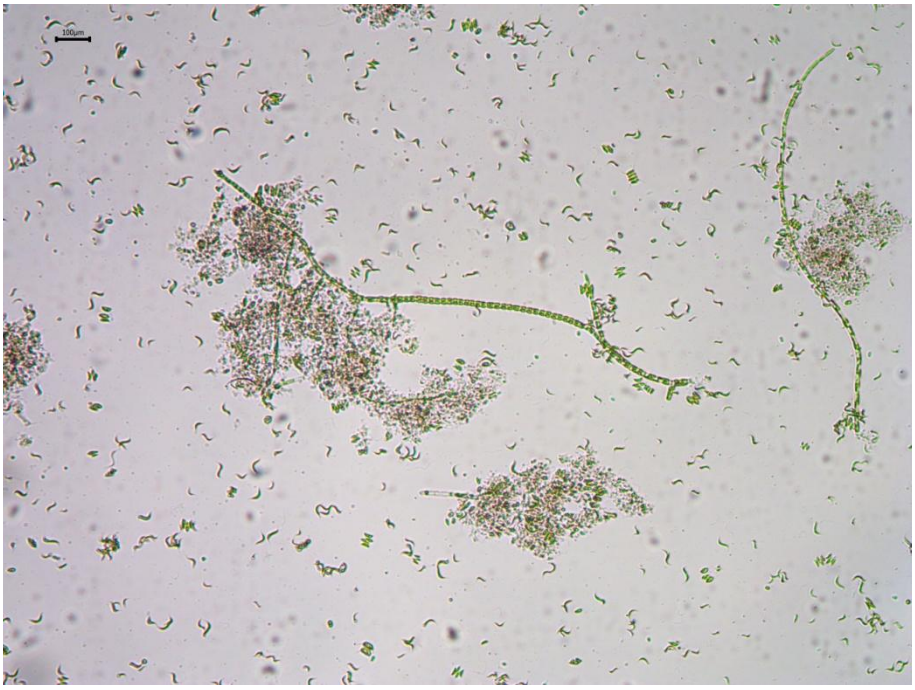

Figure 6 Biomass, 100x, March 2015

Figure 7 *Monoraphidium* sp. 400x, May 2015

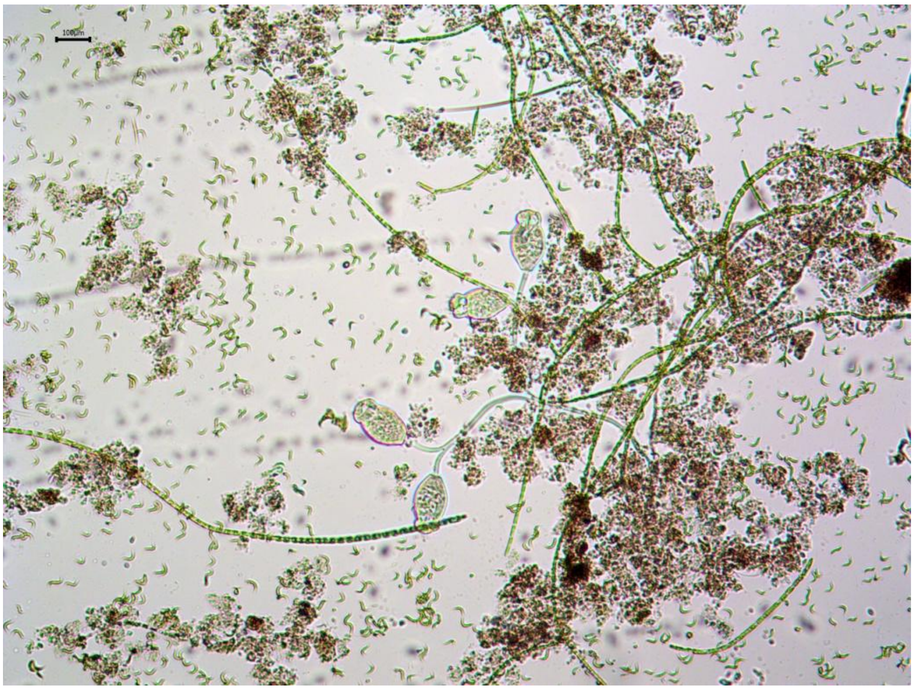

Figure 8 Biomass, 100x, May 2015

Figure 9 New species Ankistrodesmus 400x, May 2015

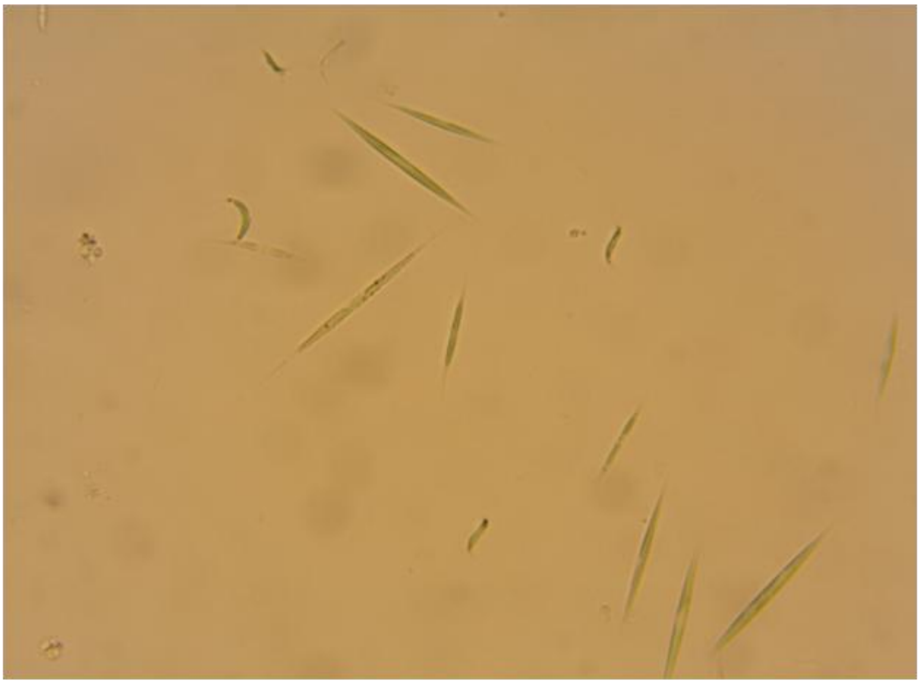

Figure 10 Microbial community 400x, November 2015

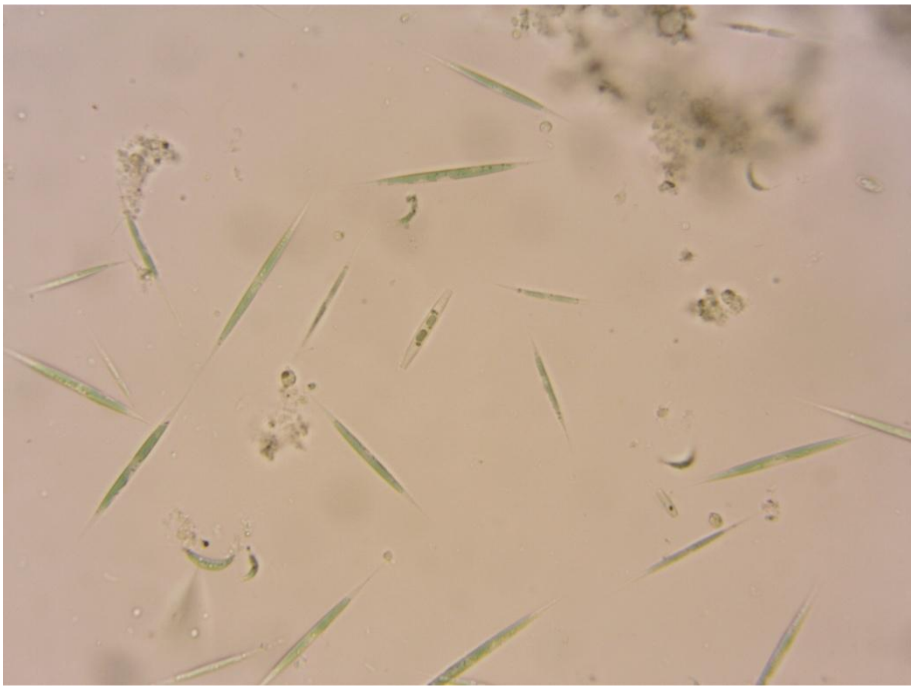

Figure 11 Biomass, 400x, November 2015

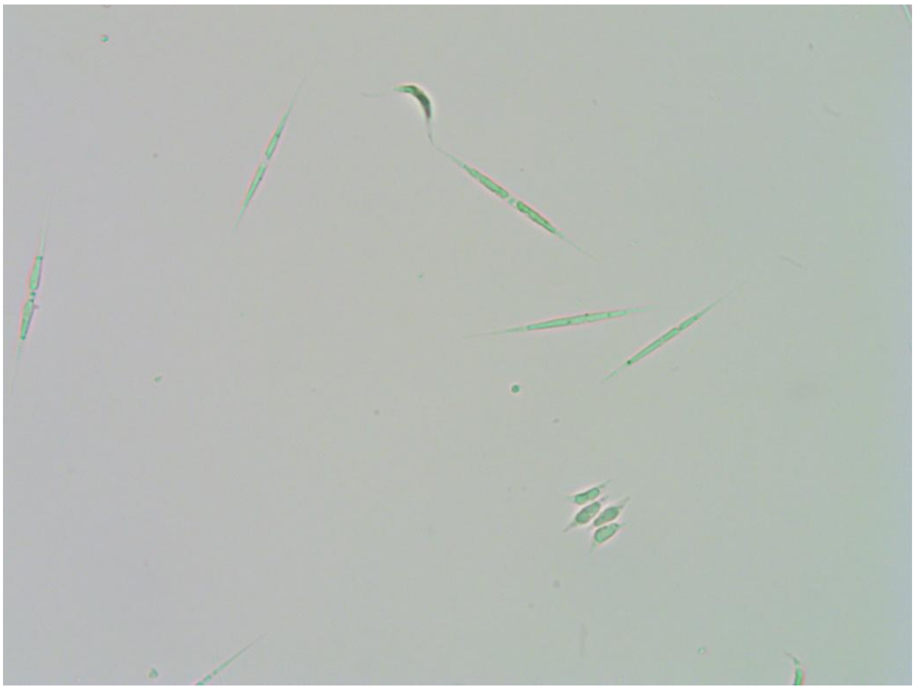

Figure 12 Microbial community, 400x, December 2015

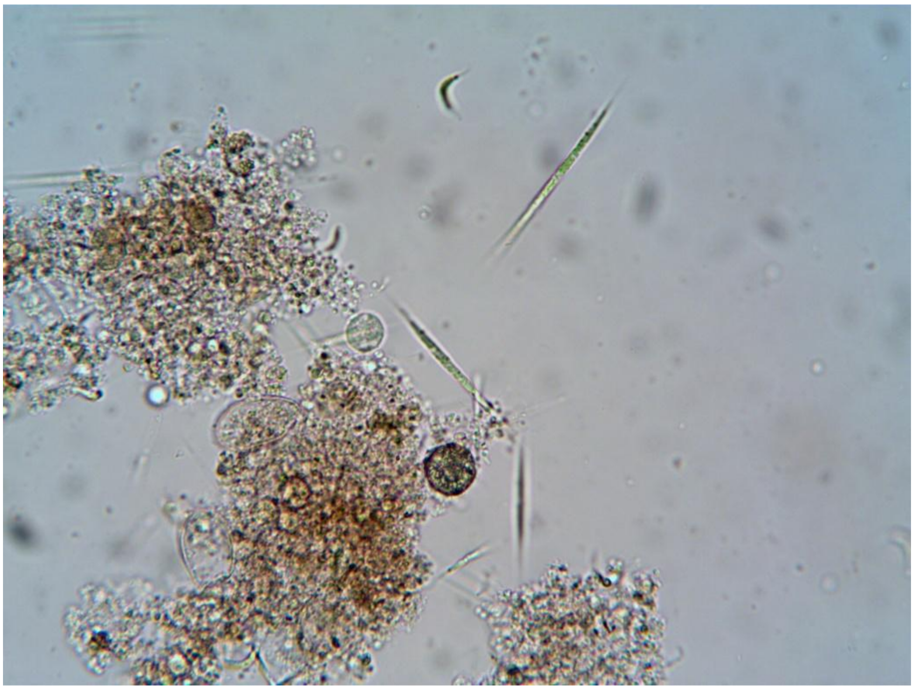

Figure 13 Biomass, December 2015

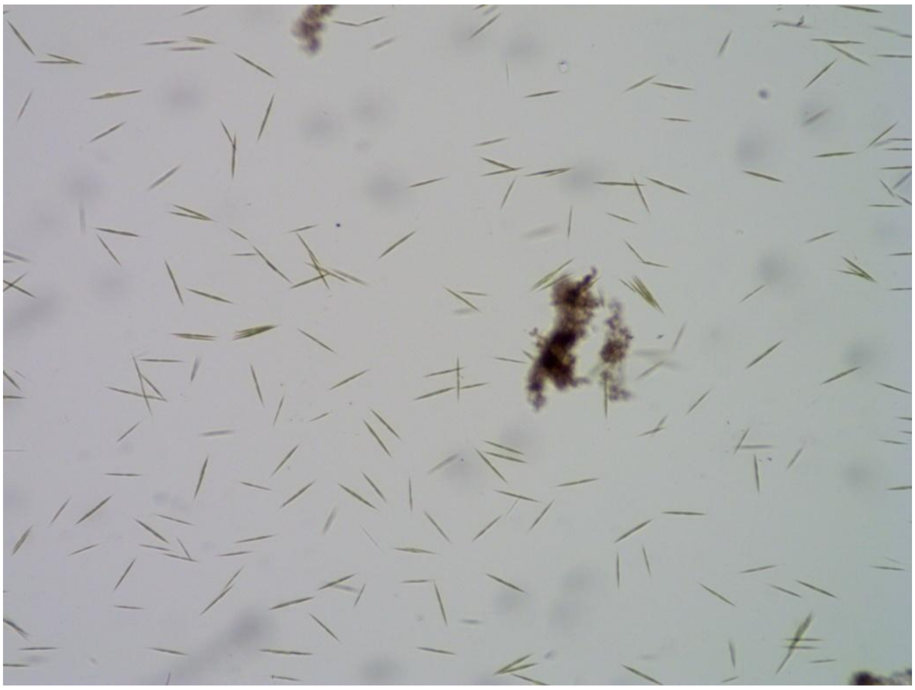

Figure 14 *Ankistrodesmus* sp., 100x, January 2016

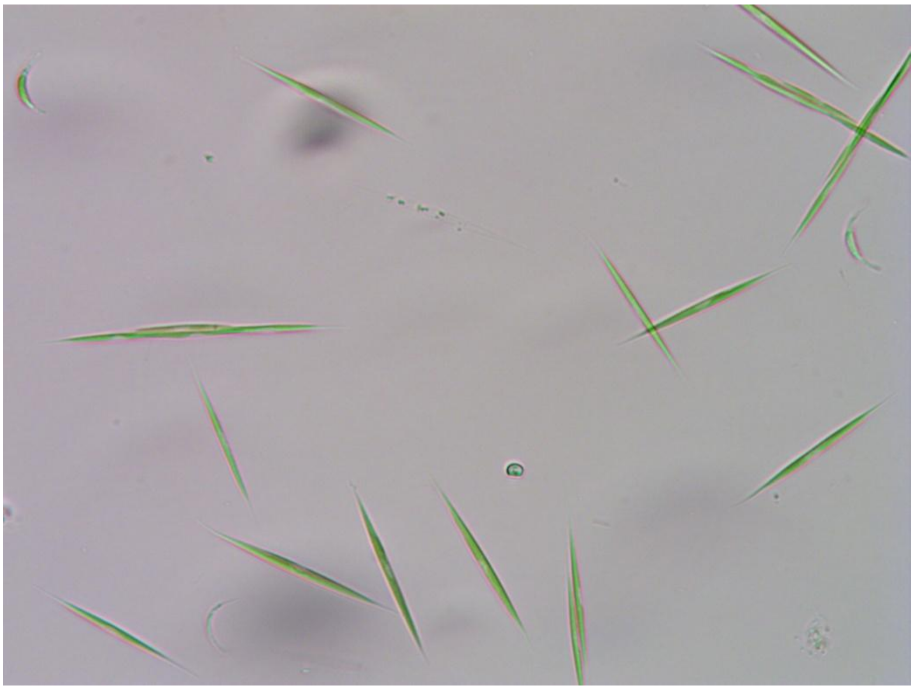

Figure 15 *Ankistrodesmus* sp. dominates in January, 2016 (400x)

Figure 16 Biomass, 100x, February 2016

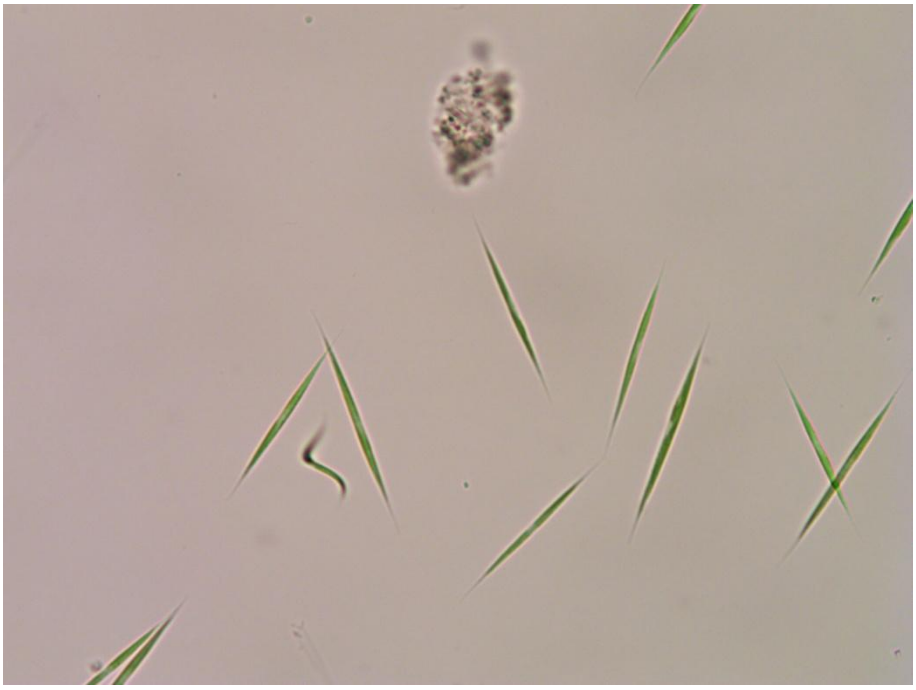

Figure 17 *Ankistrodesmus* sp. and *Monoraphidium* sp., 400x, February 2016

Figure 18 Grazers in the main pond, 400x, February 2016

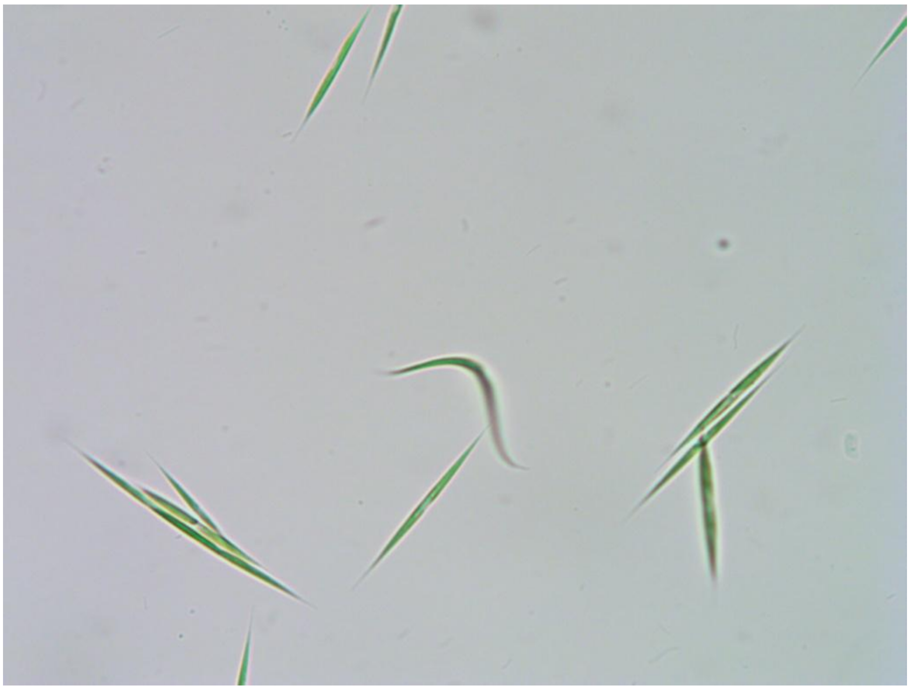

Figure 19 *Ankistrodesmus* sp. and *Monoraphidium* sp., 400x, March 2016

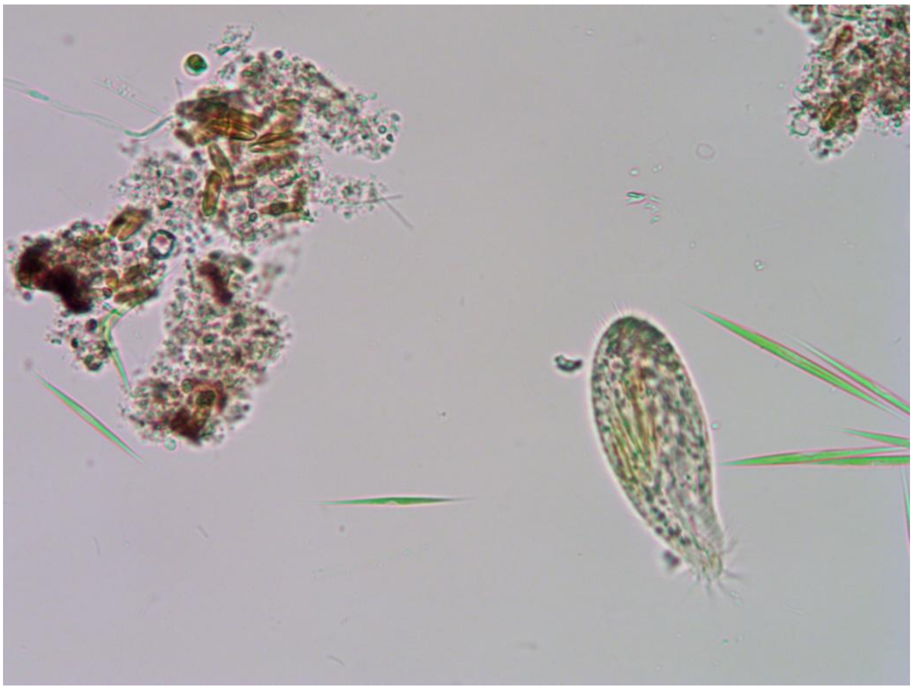

Figure 20 Grazers in the main pond, 400x, March 2016

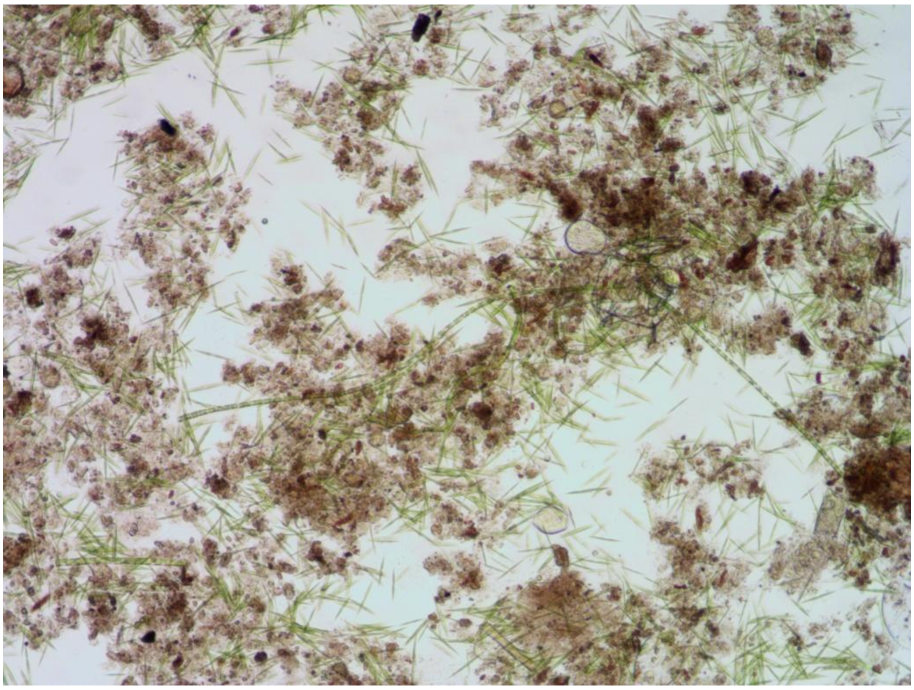

Figure 21 Biomass, 100x, March 2016

7.2 Analysis: composition

Biomass composition analysis was made in an external laboratory at the time of optimal operation of demo centre. We also measured amount of biomass regularly, by measuring total solids and chlorophyll (for algal biomass). Apart from that, several biogas tests were conducted, using harvested biomass. Biogas test are described in the next chapter.

Table 6 Biomass composition

Parameter	Unit	Fresh algal biomass		Dried algal biomass	
		12.2.2015	5.2.2016	12.2.2015	5.2.2016
DM	g/kg	63,8	8,4	1000	1000
water	g/kg	936	991,6	0	0
ash	g/kg	8,1	1,5	127	177
Volatile solids	g/kg	55,7	6,9	873	823
Total N	g/kg	4,8	0,65	75,02	77
Proteins	g/kg	30	4,03	469	479
Fibers	g/kg	9	0,76	133	90
Fats	g/kg	1,2	0,24	17,55	28
TOC	g/kg	31	3,65	485	434
C/N ratio	-	6,5	5,6	6,5	5,6
Ca	g/kg	0,97	0,2	15,2	23,7
Mg	g/kg	0,19	0,04	3,01	4,67
K	g/kg	0,53	0,1	8,39	11,7
Na	g/kg	0,25	0,08	4	9,76
P	g/kg	0,93	0,13	14,6	15,4
Cu	mg/kg	4,1	2,54	65	302
Zn	mg/kg	78	10,6	1229	1259
Cr	mg/kg	1,4	0,59	22,4	69,6
Ni	mg/kg	1,6	0,21	24,4	25,2
Pb	mg/kg	0,8	0,18	12	20,9
Cd	mg/kg	0,04	0,003	0,57	0,39
As	mg/kg	0,014	0,002	0,22	0,22

7.3 Biomethane potential tests

7.3.1 Biomethane potential of microalgae

Latest research results shows that algal biomass has good biomethane potential and that anaerobic treatment of algal biomass is feasible, if low costs production of algal biomass can be assured (Prajapati et al., 2014).

Practically methane potential is lower than theoretical and such deviations can occure because of macromolecular composition and properties of micro algal cell walls of different algal species (Passos et al., 2014).

Main limitation for anaerobic digestion (AD) biodegradation of algal biomass is resistant cell wall, which has to be broken before AD, ammonia inhibition and inhibition of VFA (volatile fatty acids) and LCFA (Long chain fatty acids). Co-fermentation is suggested for achievement of favourable C/N ratio in substrate for AD. Highest methane potential BMP 600 ml/gVS was reached for AD of mixed freshwater microalgae (non-defined species). Lowest determined methane potential BMP for freshwater microalgae ~70 ml/g VS has been determined for non-treated microalgae *Microcystis* sp.. Researchers have later determined BMP 153 ml/g VS for the same species of microalgae with optimal inoculum (Ward et al. 2014).

BMP tests for freshwater and saltwater species have shown methane production in the range between 50 – 310 ml CH_4/g VS. For microalgal biomass cultivated in waste water BMP of 170 ml CH4/g VS has been reached (Passos et al, 2014).

The effect of various pre-treatment strategies on methane yields following anaerobic digestion (AD) of five different microalgal strains was investigated by Bohutsky et al.. Pavlova_cf sp., *Tetraselmis* sp. and *Thalassiosira weissflogii* exhibited substantial methane yields of 0.4–0.5 L/g volatile solids (VS) without pre-treatment, providing up to 75–80% of theoretical values. In contrast, methane yields from *Chlorella* sp. and *Nannochloropsis* sp. were around 0.35 L/g VS, or 55–60% of the theoretical values, respectively. Alkali treatment was not effective and thermal pre-treatment only enhanced *Nannochloropsis* methane yields. Thermochemical pre-treatment had the strongest impact on biomass solubilisation with methane yields increasing by 30% and 40% for *Chlorella* and *Nannochloropsis*, respectively. The lipid content had a strong beneficial impact on the theoretical and observed methane yields as compared to protein and carbohydrate content. Other features such as cell-wall com-position are also likely to be important factors dictating algal biodegradability and methane yields addressed in part by thermochemical pretreatment (Bohutskyi at al. 2014)

Practically determined BMP of microalgal biomass is lower than theoretical and in range from 470 ml to 800 ml CH_4/g VS (Sialve et al., 2009). Microalgae with higher protein and carbohydrates content are theoretically poor substrate comparing to lipid reach algal biomass (Bohutskyi at al., 2014). Researchers have expressed results differently (presented results in different units, as biogas potential or methane potential, as degraded VS or lowered COD) (Ward et al., 2014).

With co-fermentation of algal biomass with carbon reach substrates optimal C/N equilibrium (20-30:1, min 16:1) could be maintained. Ammonia is essential for anaerobic bacteria and archaea in concentration 50-200 mg/l, but inhibitory critical concentration for NH_4-N is: for mesophilic 1 g/L and for thermophilic 1,9 g/L.

There are challenges for use of algal biomass in biogas production, which need to be addresses and solved, before use of algal biomass as AD substrate will be feasible:

1. Determination of potential species of microalgae (one or consortium), with high biomethane potential, efficient depollution of waste water and good potential for CO_2 uptake.

2. Detail research of AD of algal biomass for identification of key inhibitors for development of strategies to avoid inhibitory conditions with algae pre-treatment or/and co-fermentation with other substrates.

3. Transfer from laboratory to pilot system for further testing and development of technology. Testing of different bioreactors for AD (new designs) for better interaction between substrate and anaerobic microorganisms.

4. Technical – economic analysis for feasibility, fitoremediation of system for algae and biogas production and integration of algae cultivation for purpose of biogas upgrading (CO_2 uptake).

When mentioned obstacles will be overcome, algal biomass will become sustainable and efficient renewable energy source (Prajapati in sod., 2013)

7.3.2 Biogas tests with untreated biomass

Biogas potential tests of produced algal biomass in ABG Demonstration centre were done in KOTO laboratory.

Biogas potential test was set up under termophilic conditions in LAB digestors with volume 7 L (working volume 5 L). Biogas was measured with Wet tip gas meters (1 tip = 100ml). Methane content has not been determined.

Figure 22 Test digesters and gas meters, KOTO laboratory

7.3.2.1 Test no. 1

Inoculum was sampled at biogas plant KOTO DG and starved from 9.4. until 24.5.15. Algal biomass from ABG pilot system on 24.4.15 contained 4,51% of DMC and 81,79 VS in DM. Organic load was set to app 2 gVS/L inoculum.

Table 7 Organic load (VS g/5L), tips (no.) and biogas production (ml)

		tips						biogas (ml)				
		treatment						algae	algae	blind	control	control
		sample mass					g	270,37	270,14	0	10,0118	10,0267
		VS g/l inocukum						1,99	1,99	0,00	2,00	2,01
		VS g						9,97	9,97	0,00	10,01	10,03
		Vol, sample/Va %						0,18%	0,18%	0,00%	0,20%	0,20%
date	time	1	2	3	4	5	6	1	2	3	4	5
24.4.15	13:00	0	0	0	0	0	0	0	0	0	0	0
25.4.15	8:25	4	3	1	6	7	0	400	300	100	600	700
26.4.15	8:30	7	5	2	31	32	8	700	500	200	3100	3200
27.4.15	8:22	8	5	3	39	42	9	800	500	300	3900	4200
28.4.15	6:40	10	6	4	46	51	9	1000	600	400	4600	5100
29.4.15	6:40	13	8	6	54	60	9	1300	800	600	5400	6000
29.4.15	13:00	13	9	6	57	64	9	1300	900	600	5700	6400
30.4.15	6:40	13	10	6	62	69	9	1300	1000	600	6200	6900
30.4.15	15:00	13	10	6	64	72	9	1300	1000	600	6400	7200
1.5.15	8:05	14	11	7	70	76	9	1400	1100	700	7000	7600
2.5.15	8:00	14	11	8	76	82	9	1400	1100	800	7600	8200
2.5.15	11:50	14	11	8	77	82	9	1400	1100	800	7700	8200
3.5.15	8:30	15	11	9	82	86	9	1500	1100	900	8200	8600
4.5.15	11:15	16	12	11	85	90	9	1600	1200	1100	8500	9000
5.5.15	6:25	16	12	12	86	90	9	1600	1200	1200	8600	9000
6.5.15	6:30	18	13	12	87	90	10	1800	1300	1200	8700	9000
7.5.15	6:25	20	14	12	87	91	10	2000	1400	1200	8700	9100
8.5.15	7:20	24	15	12	89	91	10	2400	1500	1200	8900	9100
9.5.15	7:10	29	16	12	91	92	10	2900	1600	1200	9100	9200
10.5.15	8:58	36	18	12	93	93	10	3600	1800	1200	9300	9300
11.5.15	6:40	42	21	12	95	94	10	4200	2100	1200	9500	9400
11.5.15	11:50	44	22	12	95	94	10	4400	2200	1200	9500	9400
12.5.15	6:30	48	25	12	97	95	11	4800	2500	1200	9700	9500
13.5.15	6:30	54	32	12	99	95	12	5400	3200	1200	9900	9500
14.5.15	6:30	57	38	12	102	96	14	5700	3800	1200	10200	9600
15.5.15	6:30	59	45	12	104	97	16	5900	4500	1200	10400	9700
16.5.15	16:25	59	53	12	105	99	19	5900	5300	1200	10500	9900
17.5.15	16:25	59	56	12	106	100	22	5900	5600	1200	10600	10000
18.5.15	7:30	59	58	12	107	100	25	5900	5800	1200	10700	10000
19.5.15	8:10	59	59	13	108	102	29	5900	5900	1300	10800	10200
20.5.15	6:50	60	60	13	108	103	33	6000	6000	1300	10800	10300
20.5.15	15:00	60	61	13	108	103	35	6000	6100	1300	10800	10300
21.5.15	6:30	60	61	13	108	104	39	6000	6100	1300	10800	10400
22.5.15	9:10	60	62	13	108	105	44	6000	6200	1300	10800	10500
23.5.15	12:30	60	63	14	108	106	50	6000	6300	1400	10800	10600
25.5.15	6:05	60	64	14	109	106	57	6000	6400	1400	10900	10600
26.5.15	6:50	60	64	15	109	106	60	6000	6400	1500	10900	10600
27.5.15	6:00	60	64	15	109	106	61	6000	6400	1500	10900	10600
28.5.15	6:30	60	64	15	109	107	62	6000	6400	1500	10900	10700
29.5.15	6:25	60	64	16	109	107	63	6000	6400	1600	10900	10700

Digestor No. 6 did not reach sufficient production (gas leaking). Production of biogas from algal biomas in digestor 1 and 2 were comparable. Production of biogas from glucose in digestors 4-5 were comparable.

Table 8 Specific biogas production (m3/kg VS)

		biogas m3/kg VS		
time (min)	algae 1-3	algae 2-3	glucose 4-3	glucose 5-3
0	0,000	0,000	0,000	0,000
1165	0,030	0,020	0,050	0,060
2610	0,050	0,030	0,290	0,299
4042	0,050	0,020	0,360	0,389
5380	0,060	0,020	0,420	0,469
6820	0,070	0,020	0,479	0,539
7200	0,070	0,030	0,509	0,578
8260	0,070	0,040	0,559	0,628
8760	0,070	0,040	0,579	0,658
9785	0,070	0,040	0,629	0,688
11220	0,060	0,030	0,679	0,738
11450	0,060	0,030	0,689	0,738
12690	0,060	0,020	0,729	0,768
14295	0,050	0,010	0,739	0,788
15445	0,040	0,000	0,739	0,778
16890	0,060	0,010	0,749	0,778
18325	0,080	0,020	0,749	0,788
19820	0,120	0,030	0,769	0,788
21250	0,170	0,040	0,789	0,798
22798	0,241	0,060	0,809	0,808
24100	0,301	0,090	0,829	0,818
24410	0,321	0,100	0,829	0,818
25530	0,361	0,130	0,849	0,828
26970	0,421	0,201	0,869	0,828
28410	0,451	0,261	0,899	0,838
29850	0,471	0,331	0,919	0,848
31885	0,471	0,411	0,929	0,868
33325	0,471	0,441	0,939	0,878
34230	0,471	0,461	0,949	0,878
35710	0,461	0,461	0,949	0,888
37070	0,471	0,471	0,949	0,898
37560	0,471	0,481	0,949	0,898

38490	0,471	0,481	0,949	0,908
40090	0,471	0,491	0,949	0,918
41730	0,461	0,491	0,939	0,918
44225	0,461	0,501	0,949	0,918
45710	0,451	0,491	0,939	0,908
47100	0,451	0,491	0,939	0,908
48570	0,451	0,491	0,939	0,918
50005	0,441	0,481	0,929	0,908

Biogas potential for algal biomass was 473-503 l/kg VS (Digestor 1 and 2). For thermophilic conditions retention time is 13-15 days, KOTO full scale biogas plant operates with RT 20 days. Glucose was degradated (Digestor 4 and 5) in sufficient time with sufficient biogas production (915-948 L/kg VS). Microalgal biomass degradation began after 14 days AD. Biogas production was recorded for 35 days. Inhibition of degradation of organic matter of algae was noticed. Reason for that might be the unavailability of algal organic matter for anaerobic microorganisms (limited degradation of hydrolysis due to unbroken cell walls).

Table 9 Specific biogas production

Sample	m^3 biogas/t VS
Algae 1	473
Algae 2	503
Glucose 1	948
Glucose 2	915

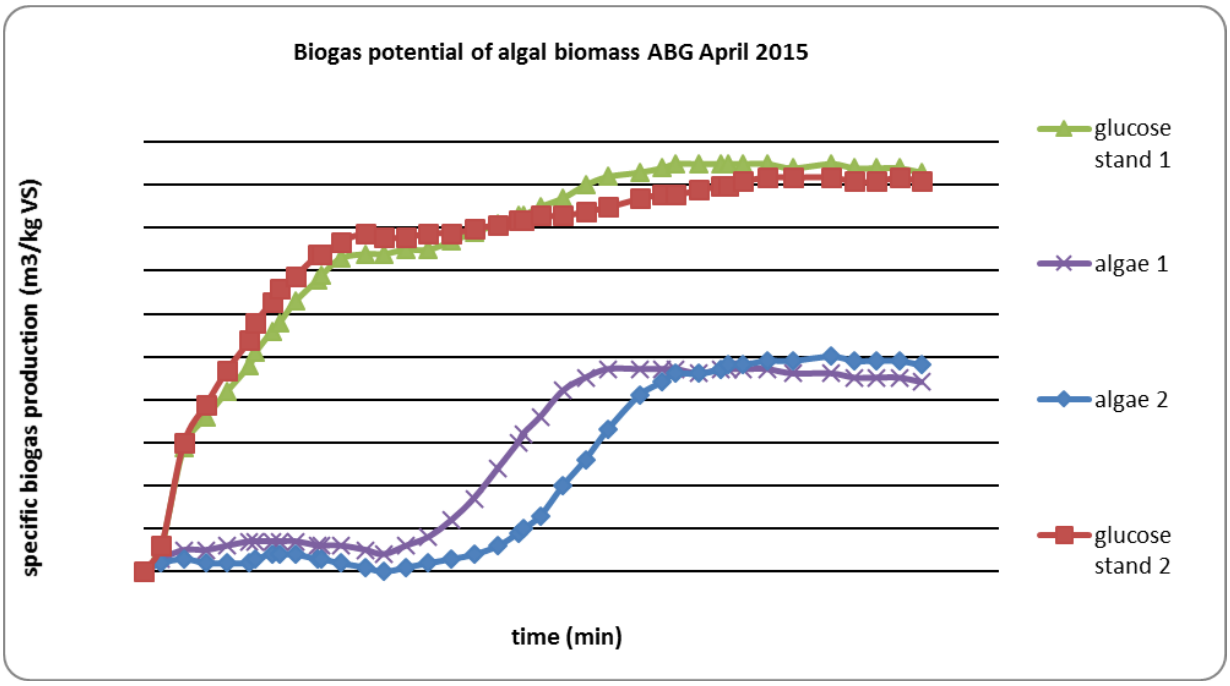

Figure 23 Specific biogas production of algal biomass and glucose (control)

7.3.2.2 Test no. 2

Inoculum was sampled at biogas plant KOTO DG and starved 17 days, from 13.10. until 30.10.15. Algal bio-mass from ABG pilot system on 30.10.15 contained 5,84% of DMC and 74,33 VS in DM. Organic load was set to app 2 gVS/L inoculum.

Table 10 Organic load (VS g/5L), tips (no.) and biogas production (ml)

		tips						biogas (ml)				
		treatment						algae	algae	glucose	glucose	blind
		sample mass					g	230	230	10	10	10
		VS g/l inocukum						2,00	0,00	2,00	2,00	2,00
		VS g						9,98	9,98	10,00	10,00	10,00
		Vol, sample/Va %						0,18%	4,60%	0,20%	0,20%	0,20%
Date	time	1	2	3	4	5	6	1	3	4	5	6
30.10.15	13:00	1	1	1	2	1	0	100	100	200	100	0
31.10.15	8:20	3	2	2	6	10	2	300	200	600	1000	200
1.11.15	8:00	4	2	4	26	23	3	400	400	2600	2300	300
2.11.15	5:30	5	2	5	31	24	5	500	500	3100	2400	500
3.11.15	12:00	6	2	8	38	24	7	600	800	3800	2400	700
4.11.15	6:45	7	2	9	42	24	8	700	900	4200	2400	800
5.11.15	7:35	8	2	9	43	24	10	800	900	4300	2400	1000
6.11.15	6:30	9	2	9	44	24	12	900	900	4400	2400	1200
9.11.15	6:50	9	2	9	47	27	14	900	900	4700	2700	1400
10.11.15	5:15	10	2	10	48	29	14	1000	1000	4800	2900	1400
11.11.15	6:20	10	2	10	50	33	14	1000	1000	5000	3300	1400
12.11.15	5:49	10	2	10	52	37	14	1000	1000	5200	3700	1400
13.11.15	6:15	10	2	11	55	41	14	1000	1100	5500	4100	1400
14.11.15	9:00	11	4	11	59	44	14	1100	1100	5900	4400	1400
15.11.15	8:15	12	7	12	63	46	14	1200	1200	6300	4600	1400
16.11.15	9:50	13	10	14	68	48	14	1300	1400	6800	4800	1400
17.11.15	9:40	15	12	15	72	48	14	1500	1500	7200	4800	1400
18.11.15	5:50	16	15	16	75	49	15	1600	1600	7500	4900	1500
21.11.15	9:00	24	17	25	84	49	16	2400	2500	8400	4900	1600
22.11.15	9:30	27	19	27	87	49	16	2700	2700	8700	4900	1600
23.11.15	7:45	29	20	30	89	49	16	2900	3000	8900	4900	1600
24.11.15	8:35	32	21	33	91	49	16	3200	3300	9100	4900	1600
25.11.15	8:50	35	21	36	93	49	16	3500	3600	9300	4900	1600
26.11.15	6:15	36	21	39	94	49	17	3600	3900	9400	4900	1700
27.11.15	5:50	41	21	41	94	49	17	4100	4100	9400	4900	1700
28.11.15	8:20	44	21	43	94	49	17	4400	4300	9400	4900	1700
29.11.15	8:10	44	21	45	95	49	18	4400	4500	9500	4900	1800
30.11.15	5:35	44	21	47	95	49	18	4400	4700	9500	4900	1800
1.12.15	11:40	44	21	50	95	49	19	4400	5000	9500	4900	1900
2.12.15	5:50	44	21	51	95	49	19	4400	5100	9500	4900	1900

Digestor No. 2 did not reach sufficient production (gas leaking; same as blind treatment). Production of biogas from algal biomas in digestor 1 and 3 were comparable. Production of biogas from glucose in digestors 4-5 were not comparable (noticed leaking in digestor 5).

Table 11 Specific biogas production

	biogas m3/kg VS		
time (min)	algae 1-6	algae 3-6	glucose 4-6
0	0,010	0,010	0,020
1160	0,010	0,000	0,040
2580	0,010	0,010	0,230
3870	0,000	0,000	0,260
5760	-0,010	0,010	0,310
6825	-0,010	0,010	0,340
8315	-0,020	-0,010	0,330
9690	-0,030	-0,030	0,320
14030	-0,050	-0,050	0,330
15375	-0,040	-0,040	0,340
16880	-0,040	-0,040	0,360
18289	-0,040	-0,040	0,380
19755	-0,040	-0,030	0,410
21360	-0,030	-0,030	0,450
22755	-0,020	-0,020	0,490
24290	-0,010	0,000	0,540
25720	0,010	0,010	0,580
26930	0,010	0,010	0,600
31440	0,080	0,090	0,680
32910	0,110	0,110	0,710
34245	0,130	0,140	0,730
35735	0,160	0,170	0,750
37190	0,190	0,200	0,770
38465	0,190	0,220	0,770
39880	0,240	0,240	0,770
41470	0,270	0,260	0,770
42890	0,260	0,270	0,770
44175	0,260	0,290	0,770
45980	0,250	0,310	0,760
47070	0,250	0,321	0,760

Biogas potential for algal biomass was low, 271-311 L/kg VS (Digestor 1 and 3). For thermophilic conditions retention time is 13-15 days, KOTO full scale biogas plant operates with RT 20 days. Glucose (digestor 4) was degraded in insufficient time (degradation in 25 days, normal 700-800 m^3/t VS in 14 days) with lower biogas production (770 L/kg VS) which indicates low quality of inoculum. Biogas production has been recorded for 41 days. No degradation of organic matter was noticed until day 21, possibly because organic matter of algae was not available to anaerobic microorganisms (limited hydrolysis). New dominant algae species *Monoraphidium* sp. might be difficult to degradate.

Table 12 Biogas production, test no. 2

Sample	m^3 biogas/t VS
Algae 1	271
Algae 2	311
Glucose 1	770

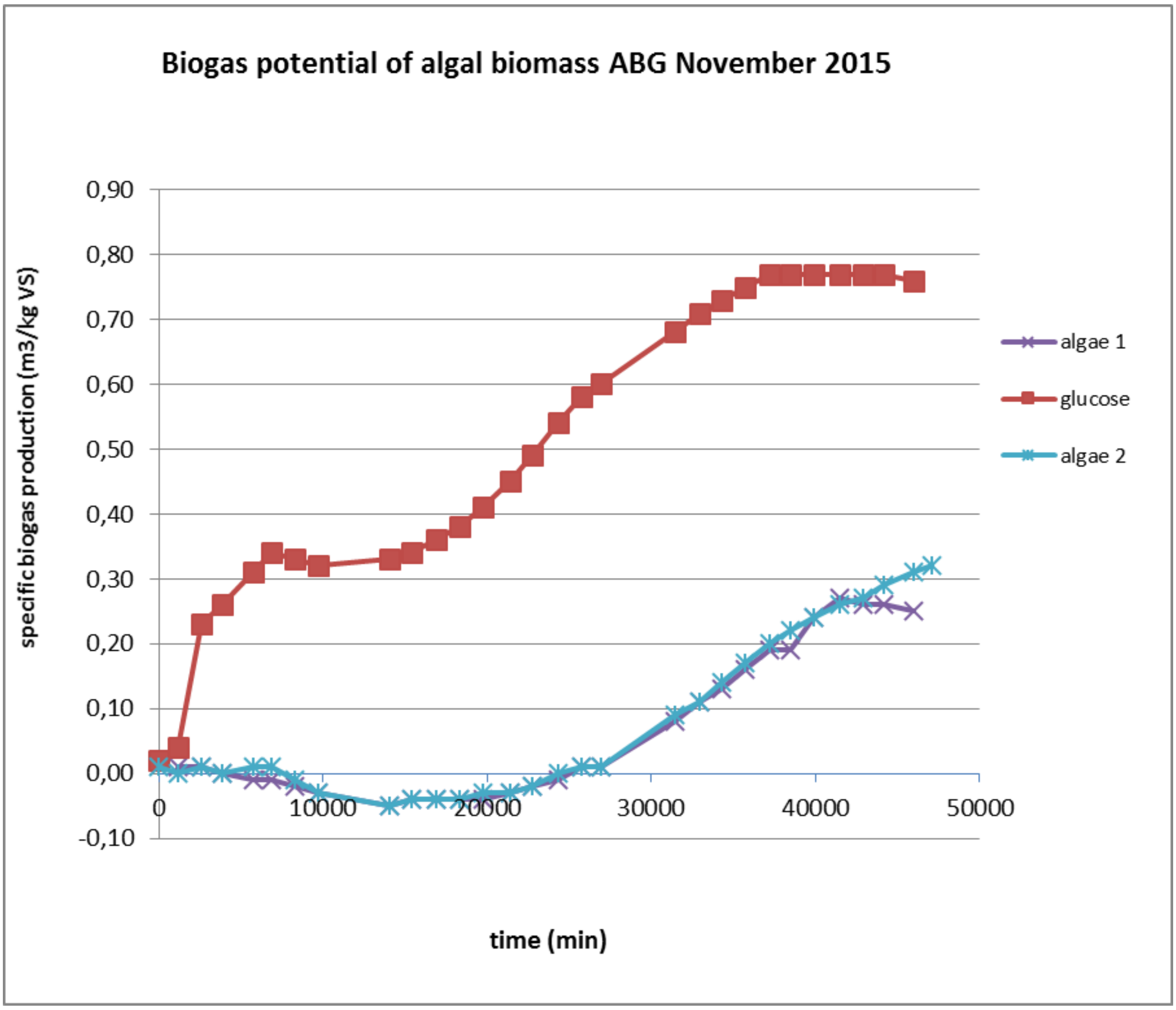

Figure 24 : Specific biogas production of algal biomass (Digestor 1 and 3) and control (glucose)

7.3.3 Biogas test with pre-treated biomass

Algal biomass grown on digestate can be used as source of biogas, but as we noticed during our tests, certain pre-treatement is needed in order to obtain better results and higher biogas yields. Currently algal biomass produced in demo centre ponds is subject to following pre-treatments:

- Thermal pre-treatment : algal biomass is heated for 3 hours at 90°C, with periodical mixing

Algal biomass was harvested on 18th of November 2015. Biomethane tests started on 21.1.2016.

Figure 25 shows methane production for tests with algae subjected to thermal pre-treatment (labelled “TA” on figure) and non-treated algae (labelled “A”). Line labelled “ST” shows positive control-glucose. As seen on picture, there is no significant change in methane production for treated or fresh algae. Pretreated sample has slightly bigger production, but not as much as expected.

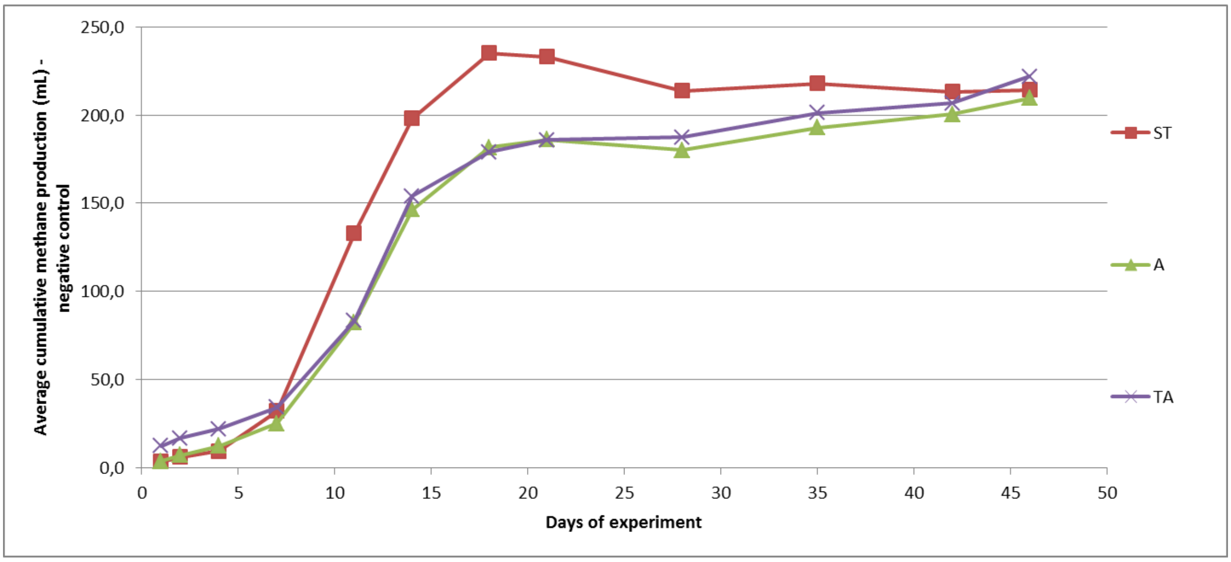

Figure 25 Methan production of pre-treated algal biomass

- Microbial pre-treatment: microorganism *Pseudobutyrivibrio xylanovorans* has been added to algal biomass during biogas tests

We hoped to speed up degradation of algal cells and shorten retention time. However, according to first results, pre-treatment speeds up degradation of algal cells, but does not shorten retention time on increase production.

Due to complications during test runs, we are unfortunately unable to present final results at the moment, but we hope to have positive results at the start of April 2016.

- Pretreatment ABG biomass for BMP test: autoclaved at 121 °C, 1,2 bar, 15 min.

Inoculum was sampled at biogas plant KOTO DG3 on 23.2.2016 and starved 21 days, from 23.2. until 15.3.16. Algal biomass from ABG pilot system on 2.3.16 contained 6,57% of DMC and 28,6% VS in DM (sample No.1).

Sample of algal biomass No. 3 has been thermally pre-treated (at 125°C, 11,25 mbar, 15 min).

Organic load was set to app 2-2,5 g VS/L inoculum. For experimental setup we have used traps with NaOH for CO_2 capture and measured methane production for digestors DG 1-DG 5. For No. 6 DG biogas production has been measured.

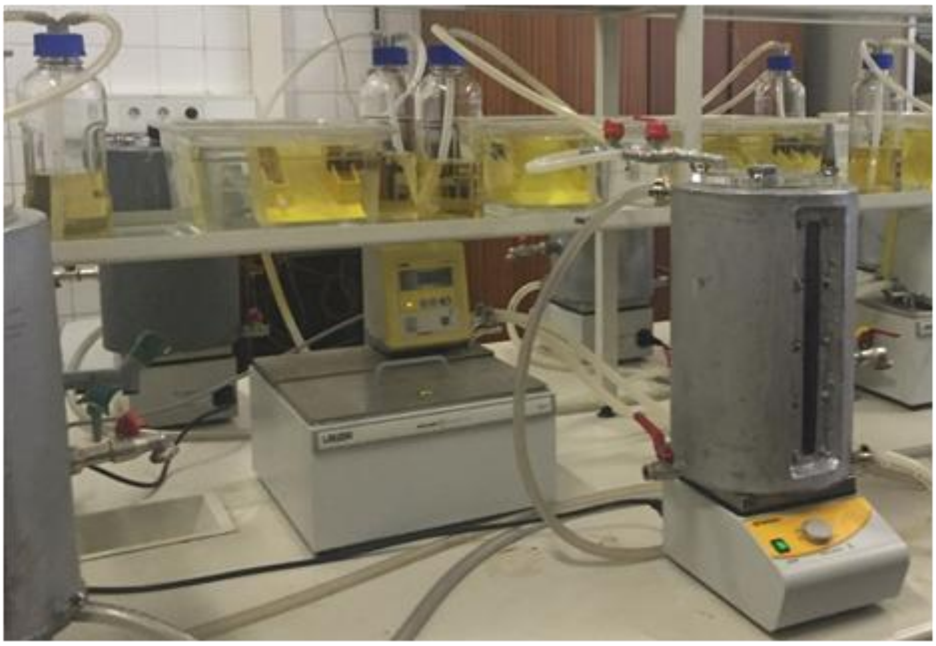

Figure 26 Laboratory test digestors with NaOH trap for CO_2 removal, at KOTO laboratories

Table 13: Organic load (VS g/5L), tips (no.) and methane (DG 1,2,3,5) and biogas production (DG 6) (ml)

		tips						ml methane				ml biogas
		treatment						algae	blind	algae HT	glucose	glucose
		sample mass					g	222,44	0	220,54	10,0094	10,0382
		VS g/l inocukum						2,41	0,00	2,48	2,00	2,01
		VS g						12,07	0,00	12,40	10,01	10,04
		Vol, sample/Va %						4,45%	0,00%	4,41%	0,20%	0,20%
date	time	1	2	3	4	5	6	1	2	3	5	6
15.3.16	14:00	0	0	0	0	0	0	0	0	0	0	0
16.3.16	7:45	3	1	3	2	2	5	300	100	300	200	500
17.3.16	7:35	4	1	4	3	15	23	400	100	400	1500	2300
18.3.16	7:40	5	2	6	4	17	25	500	200	600	1700	2500
21.3.16	7:10	6	5	8	4	19	32	600	500	800	1900	3200
22.3.16	11:40	6	5	10	5	21	35	600	500	1000	2100	3500
23.3.16	14:20	7	6	11	5	22	38	700	600	1100	2200	3800
24.3.16	14:20	7	7	12	5	24	41	700	700	1200	2400	4100
25.3.16	8:30	8	7	13	5	24	43	800	700	1300	2400	4300
26.3.16	11:00	8	7	14	6	26	46	800	700	1400	2600	4600
27.3.16	11:00	10	9	15	7	27	48	1000	900	1500	2700	4800
28.3.16	11:00	10	9	16	7	28	51	1000	900	1600	2800	5100
29.3.16	8:30	11	9	16	8	28	53	1100	900	1600	2800	5300
30.3.16	8:10	11	10	19	8	28	55	1100	1000	1900	2800	5500

Digestor No. 4 did not reach sufficient production (gas leaking; lower than gas production in blind sample).

Production of biogas from algal biomass in digestor 1 and thermally pre-treated algal biomass in digestor 3 were recorded for 14 days. Production of methane from glucose in digestor 5 was low. Production of biogas from glucose in digestor 6 has been lower than normal: 448 ml on 14th day (normally 700-800 ml on 14th day). For glucose the calculated average methane content is 57%, varied from 40-68%.

Table 14 Specific methane production (DG 1,3,5) and biogas production (DG 6)

		m^3/kg VS			
		methane			biogas
time (min)	algae 1-2	alge HT 3-2	alge 4-2	glucose 5-2	glucose 6-2
0	0,000	0,000	0,000	0,000	0,000
1065	0,017	0,016	0,008	0,010	0,040
2495	0,025	0,024	0,017	0,140	0,219
3940	0,025	0,032	0,017	0,150	0,229
8230	0,008	0,024	-0,008	0,140	0,269
9940	0,008	0,040	0,000	0,160	0,299
11540	0,008	0,040	-0,008	0,160	0,319
12980	0,000	0,040	-0,017	0,170	0,339
14070	0,008	0,048	-0,017	0,170	0,359
15660	0,008	0,056	-0,008	0,190	0,389
17100	0,008	0,048	-0,017	0,180	0,389
18540	0,008	0,056	-0,017	0,190	0,418
19830	0,017	0,056	-0,008	0,190	0,438
21250	0,008	0,073	-0,017	0,180	0,448

Biogas potential for algal biomass was low until 14^{th} day of the test (Digestor 1 and 3). For thermophilic conditions retention time (RT) is 13-15 days, KOTO full scale biogas plant operates with RT 20 days. Glucose (digestor 5) has been partly degradated (190 m^3 of methane and 440 m^3 of biogas per t VS; normally 700-800 m^3 biogas/t VS in 14 days) which indicates low quality of inoculum (possible long pre-incubation period). Low degradation of organic matter was noticed until day 14. Test is ongoing and gas production will be measured for another 10 days.

Table 15 BMP test results until day 14

Sample	m^3 biomethane/t VS
Algae 1	21
Algae heat treated 3	59
Glucose 1	190

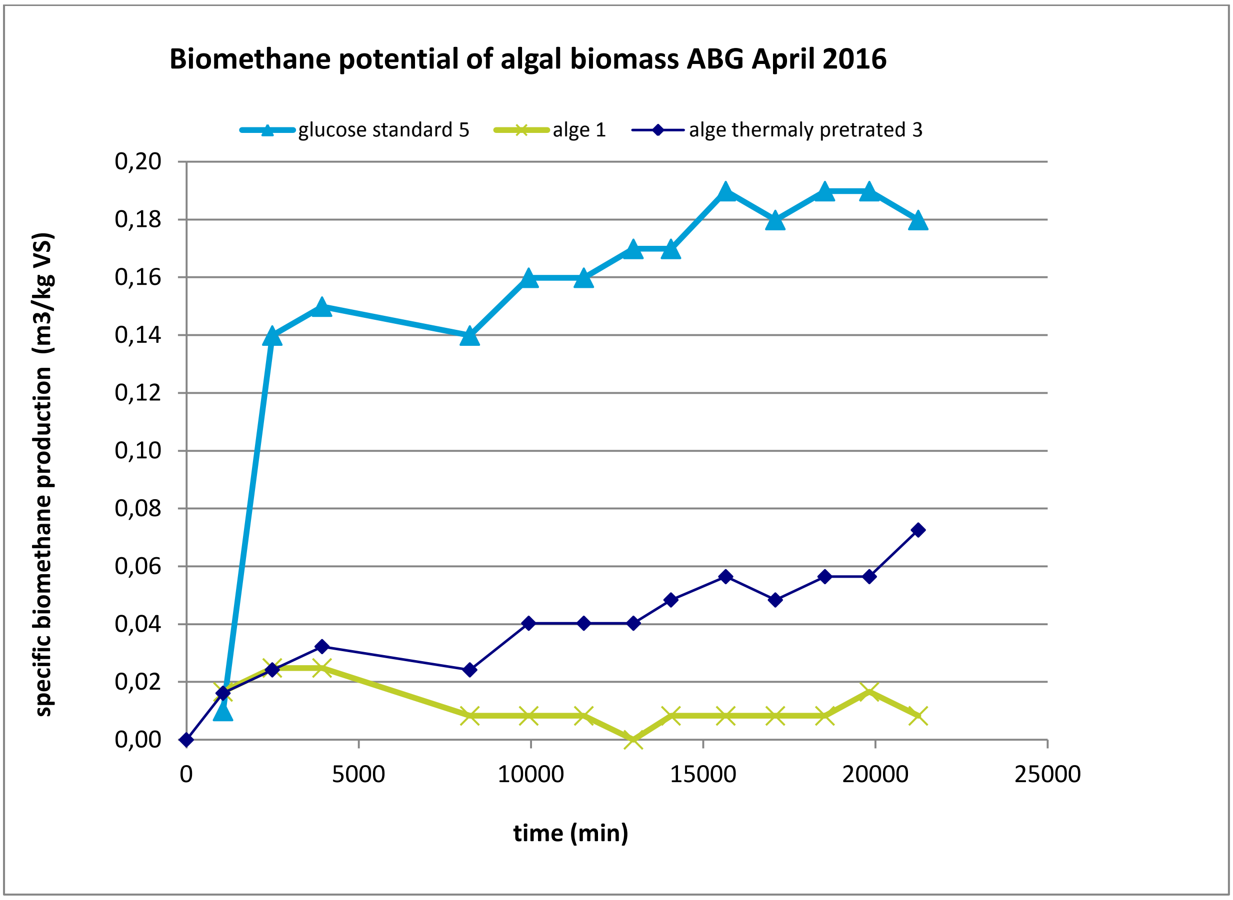

Figure 27 : Specific biomethane production of algal biomass (Digestor 1, 3) and control (glucose 5)

Anaerobic digestion BP (biogas potential) and BMP (biomethane potential) tests in thermophilic AD conditions show that fresh algal biomass is difficult to degradate. Anaerobic degradation has shown good potential, max determined biogas potential was 503 L/kg VS, but the time of degradation was too long. Better biodegradability was observed after 14 days or later.

Thermally pre-treated algal biomass in this experiment was not degradated any faster than fresh algal biomass. Further biomethane potential (BMP) tests of fresh and pre-treated algal biomass will be carried out until September 2016 with improved laboratory experimental setup.

We are speculating that dominant algae species *Ankistrodesmus* sp. in algal biomass might be difficult to degradate, so the composition of algal biomass might be the reason for low biomethane yields.

8 Conclusions

After more than a year of AlgaeBioGas demonstration centre operation we have obtained a lot of data, which need to be interpreted. In this report we showed first concrete analysis, but in order to confirm certain occurrences we will need another season of operation (last year of the project). In 2015 we tested several operating modes and coped with quite some technical difficulties. We have noticed that light availability has great effect on operation of the ponds. Microbial community, however, has only drastically changed 3 times since the start of operation and it has been stable for more than 6 months now. We will carefully monitor the community in the upcoming months, to confirm if this species dominates in all seasons.

Digestat-input analysis showed that COD and ammonium content are quite stable, which is good news for us, since algae were able to adapt to it. Water-output analysis showed that several factors are to be taken in to the account when talking about efficient digestate treatment. So far, we have successfully lowered nitrogen and phosphorus levels on the output from the ponds. Values for ammonium are most of the time well under limit values for discharge of industrial waters.

We used first results to calculate the area of algal ponds needed to successfully treat daily amount of digestate produced in biogas plant in the scope of demo centre. To be able to treat all the digestate produced in a day, we would need 3 ha of algal ponds in this climate. Produced biomass on 1 ha would be 11 tonnes per year.

Biomass produced in ABG demo centre is currently used for biogas production. Biogas and biomethan test were made and results showed that biogas production is limited by slow algal biomass degradation, possibly because of algal cell walls composition. To improve biogas yields we subjected algal biomass to thermal pretreatment and preliminary results show no or only slight improvement in biogas production. New tests with improved biomass pre-treatment will be made.

To conclude, we can say that ABG demonstration centre is working adequately, although efficiency and yields have not yet reached our expectations and we are working on improvements.

9 References

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