AlgaeBioGas

Algal treatment of biogas digestate and feedstock production

D3.8

Final technical report about the operation of the demonstration centre

PUBLIC

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1 Summary

Final technical report includes description of the process and operation based on the recorded data for pilot plant in period 1.9.2015 (M26) to 31.8.2016 (M36). Technical report about the operation of the AlgaeBioGas demonstration centre from start up 01.09.2014 (M13) to 31.08.2015 (M25) has been reported in deliverable D 3.5. Some important results are also presented for the whole operational period (M13 - M36). Results for last two months of operation are shown eg cleaning efficiency.

Results of microalgal treatment of liquid thermophilic supernatant digestate, from biogas plant, treating food waste (average COD 7400 mg O2/L, ammonia nitrogen 1400 mg/l), in algal pond (pilot system, outdoor covered on 89 m2 with 26 m3 raceway pond), showed sufficient nitrogen and phosphorous removal and COD reduction of 92% (COD of effluent reduced to 300 mgO2/L) by continuous operation. Cleaning efficiency is very dependable on available natural light. The process has been very stable in last two months of operation (M35-36) in summer period, when digestate supernatant (COD 5.600 mgO2/L. NH4-N 1.400 mg/L) has been cleaned to the parameters for waste water effluent to sewage system (containing max 200 mg/l ammonia) by hydraulic retention time 65 days (300 – 400 L input of digestate/day).

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)
D6.2	Detailed business plan
D3.1	Report on the demonstration centre operation – datasets M1 – M6
D3.2	Report on the demonstration centre operation – datasets M7 - M12
D3.3	Report on the demonstration centre operation – datasets M13 – M30
D3.4	Report on the demonstration centre operation – datasets M31 – M36
D3.5	Technical report on the demonstration centre operation M13 - M25
D3.6	Report on algal culture maintenance
D3.7	Input output and biomass anlaysis report

2 Project Abstract

AlgaeBioGas project is focused to market introduction of algal-bacterial treatment of biogas digestate. Using algae we can recycle CO_2 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 CO_2 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, CO_2 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 projects 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

AlgaeBioGas demonstration centre has been built in year 2014 and operated until August 2016. Demonstration centre is one of the pillars of the project, it is an installation of the technology at one of the project partners (company KOTO from Ljubljana, Slovenia) where we are able to demonstrate the technology in different working modes, measure the operating characteristics of the system in a realistic set-up, and above all show the installation to the potential customers to enable repeated installations.

From Dow:

The objective of work package WP3 is operation of the demonstration centre in various modes of operation under different loads and types of operation. Influence of weather will be carefully monitored. The main result of the operations work package will be collected performance results.

Operators will be responsible for monitoring the process according to the operating mode in force. Any unusual conditions that deviate from expected operation of the system will have to be analysed and a decision regarding the needed actions (if any) will have to be made. In addition to collected data, a visual check of the system will have to be made at least daily and again, any unexpected behaviour should be analysed, possibly using some additional measurements or analyses, and acted upon,

The demonstration centre will be able to run in different operating modes: water treatment mode, biomass generation mode are just two of the extremes. Adjustments on the throughput volume will be investigated; additions of specific algal culture, additions of flocculating agents, different sizes of digestate and condensate will be examined.

A basic inoculation photobioreactor will be included with the system to keep a viable clean algal culture ready for additional inoculation in case the algal bacterial culture is unbalanced. Since the demonstration centre will be used to test different operational modes and different cultures several separate photobioreactors will be operated in lab at Algen to keep cultures available. All cultures will be kept in sufficient quantities in duplicate, so that a quick recovery is always possible.

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.

Analyses include chemical and other analysis of inflow, outflow and culture media. Non exhaustive list of measured values: pH, NH_4-N, NO_3-N, NO_2-N, total N, Kjeldahl N, P, K, TOC, Cd, Cu, Ni, Pb, Cl, Mg, Na, Ca, S, Hg, dry mass, volatile solids, BOD_5, COD Chl a, Chl a+b, other heavy metals, hormone disruptors, antibiotics. Some basic methods are available in-house, but for accurate and certified analysis external labs will be used. Internal analyses will be calibrated and validated by results of certified analyses for routine use. Some of the above analyses also apply to the produced biomass; additional tests on the composition of the biomass (e.g. lipid content) are also relevant.

A report with the description of the datasets with minimal analysis will be prepared every 6 months during the demonstration centre operation.

4 Overview of demonstration centre operation

Demonstration centre construction was completed in August 2014. System was tested and started up in normal operation in September 2014. System operated continuously until end of August 2016. During whole operation period we have successfully demonstrated the technology inspite few process failures.

We have gained precious experience for process control and managing of technology and needed maintenance of whole system.

Demonstration centre was open for visitors and many visitor expressed their interest in circural technology MICROALGAE - BIOGAS and in technical details of ABG solution. Potential customers and researchers could see the technology operating in real industrial environment in sufficient scale.

Operation of Demo centre, located in Ljubljana (46°03′N 14°30′E, 299 m above sea, fog 91 days/year in period 1971-2000), Slovenia in southern Central Europe (Gauss – Krüger coordinates x= 102640, y= 470315) with continental climate fullfiled expectations. Technology is appropriate for mediteriane climate, locations with high number of sunny days.

The demonstration centre is proving the technical feasibility of the AlgaeBioGas process.

5 Operation

Reactor for algal treatment of digestate is **main pond (VB)** with following technological characteristics:

Raceway shaped pond, pond surface has 89 m2, water depth ~ 30 cm, active volume 26 m3. Active volume can be reduced during day due shadows (high pool edges, mixer) and limited light and evaporation.

Paddlewheel for mixing operates continuously at approximately 60% speed giving mixing water speed of approximately 0,3 m/s.

Gas diffuser for distribution of exhaust gas from gen-set (CO_2 source). A perforated pipe at the bottom of the pond covered by slanted plate of approx. size 2 x 3 m that prolongs the bubble path in the water (counter flow). In main pond is source of organic carbon provided with digestate and in normal operation is addition of CO_2 not necessary.

Figure 1 Main pond with paddlewheel mixer on the right and diffuser on the left (M36 and M31)

Inoculation pond (MB), round with diameter 3,2 m, water level between 30 and 40 cm, volume between 2,4 and 3,2 m^3; inoculation pond is mixed with propeller that operates at 35% speed; mixing is continuous.

Inoculation pond includes a central drainage **pit**; at its bottom a perforated pipe is installed for introduction of CO_2 (under the mixer). It is covered with thin mesh.

Figure 2 Vertical mixer in inoculation pond, inoculation culture

Both ponds are located in double foil **greenhouse** with all openings covered by insect nets. Automatic intra-foil pressure blower keeps foils separated as thermal insulation.

CO_2 supply subsystem consists of exhaust gas capturing from biogas engine via cooler, dehumidifier, filter, blower, 250 m piping, two more dehumidifiers and valves that deliver CO2 to the ponds.

Digestate separation and supply subsystem consists of pre-separating screw press filter, a 20 m^3 digestate buffer tank, delivery to time shared tricanting centrifuge with flocculant supply and 1m^3 equalization tank, anaerobic filter, a 50 m^3 equalizing tank, input valves, UV disinfector, and delivery valves to both ponds.

Harvesting and separation subsystem consists of valves for pond selection, a pneumatic recirculation pump, a 2,2 m3 clarifier with scrubber, return valves and pipes and overflow valves and pipes.

Pond **heating and cooling subsystem** consists of hot water supply from the existing infrastructure used for biogas digestor heating (at nearby biogas plant), heat exchanger, mixing valves, recirculation pump and pond selection valves. A powerfull (8 kW) water chiller is available for emergency cooling in case of need using the same structure. In addition to water cooling (which is not supposed to be used in normal operation) a strong fan (0.75 kW) and side-curtains are installed in greenhouse enabling cooling by evaporation.

5.1 Control system

Control system is the main interface to the demonstration system. In addition to enabling control of all aspects of operation the control system is essential for data collection and automation of the operation. Control system consists of more than 130 sensor inputs and control outputs. Main operational sensors include pH, DO, ORP and conductivity sensors (installed February 2015) in both ponds, PAR sensor (installed April 2015), many temperature and flow sensors.

Operator uses Scada system for daily control of devices and for monitoring the parameters, using PC in control/electro equipment container at the demonstration centre.

In Scada control system is several views. First picture show the scheme of complete algal treatment plant "1. BLOK SHEMA". On this picture is seen all equipment, installed at demonstration centre, plus storage tank for digestate located at biogas plant (CT). Operator can see the states of devices and their settings. We can control functioning of all process and we have overview of the main values of operational measurements. Manually can be set eg addition of water, set the digestate inlet and operation of the blower for CO2. All devices can be operate manually or automatically, depending on the needs of process.

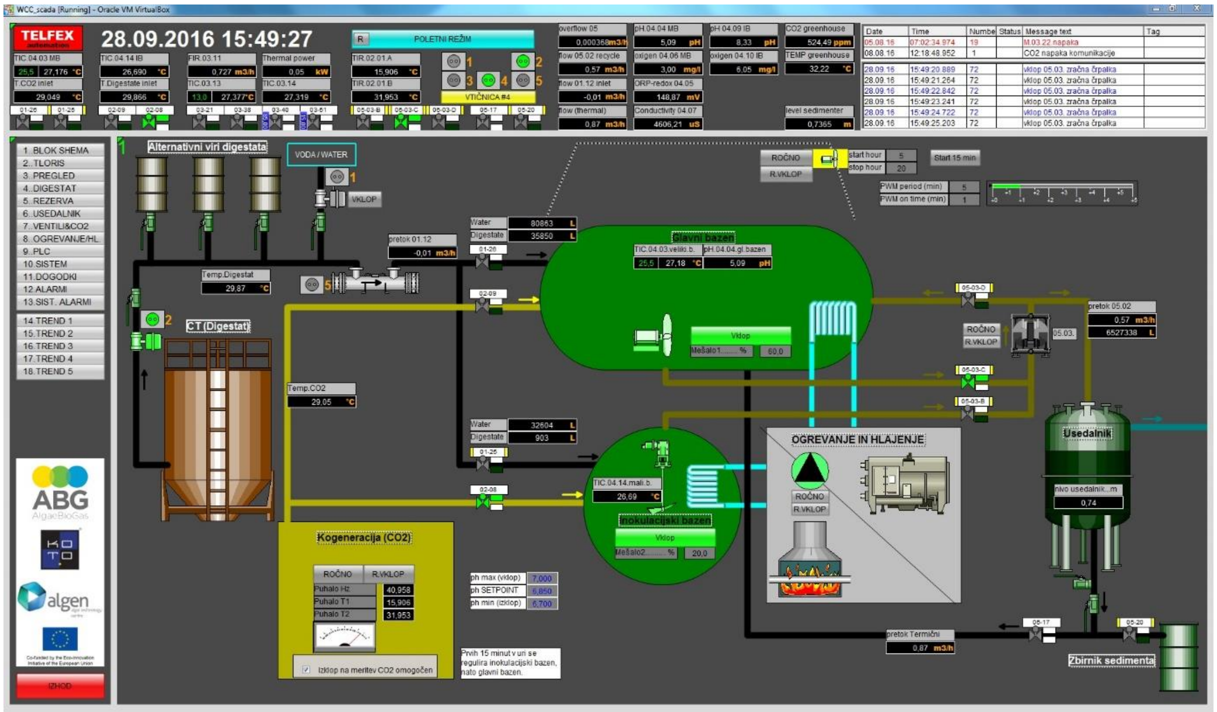


Figure 3 SCADA system

Other pictures (2. - 8.) show individual parts of system in details. There is subpicture for inflow of digestate in the pond, the picture of harvesting and separation system and the picture of heat and cooling system. On scheme Subpicture 6 ˝USEDALNIK˝ the parameters for sedimenter eg harvesting can be set. Level in sedimentation tank and parameters for the pump can be set, which enable overflow according to operational regime.

Figure 4 Subpictures with trends

We have set 5 different diagrams. There is history of all measurements and functioning of system. All data from the beginning of operation of the system are recorded. For checking of parameters different period of time can be set. Required value in required time can be chosen or min and max value or average value in period shown.

Figure 5 Sedimenter trends

SCADA control system is connected to internal KOTO VPN system. For connecting AlgaeBioGas demonstration centre to KOTO server SRV-IND following activities have been performed:

- creation of OPC communication between AlgaeBioGas centre and KOTO server SRV-IND
- adjustment of monitor from WinCC Scada system on format for SRV-IND server
- creation of web application

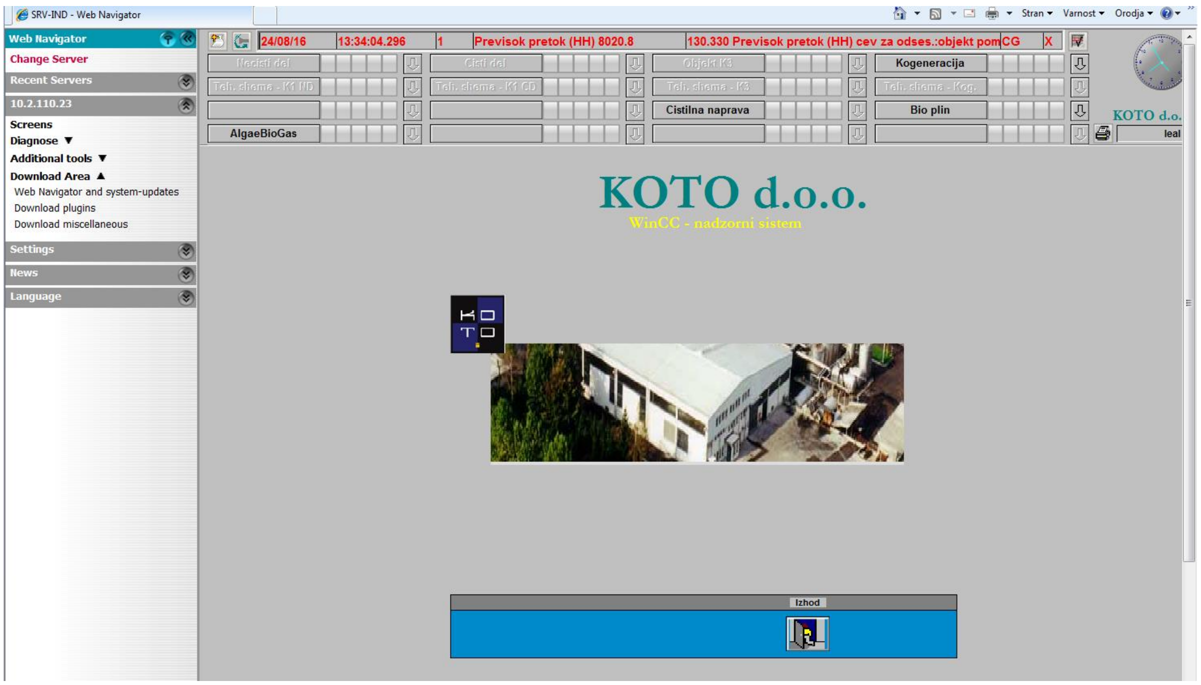


Figure 6 Entry page for WinCC Scada

Remote access to Scada is of paramount importance for demonstration system operation. KOTO project memebers have approval to access and can use access trough WinCC Scada from anywhere (home ect). All project members could used access trough Team viewer by emergency.

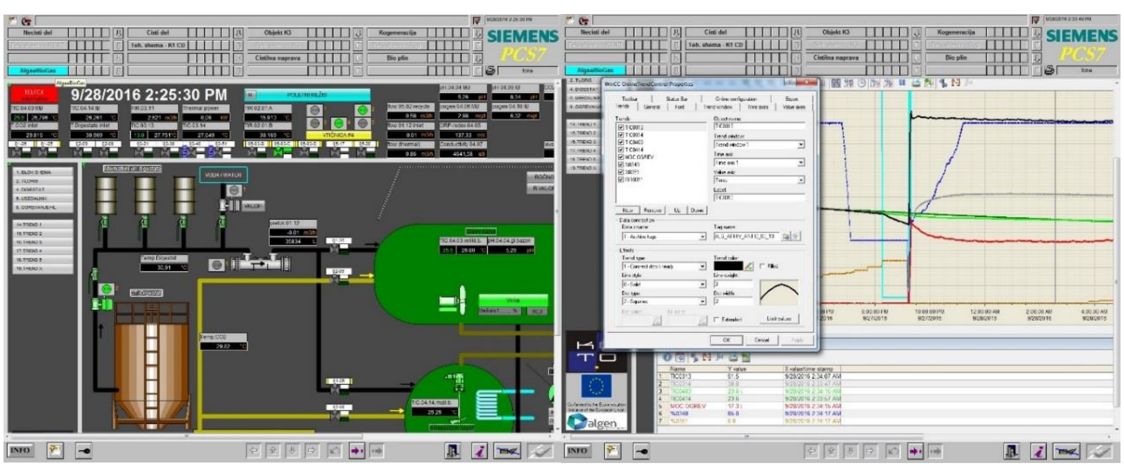


Figure 7 Pictures view trough WinCC Scada control system - scheme and diagram

Main control loops implemented in the control system are as follows:

pH control: for each pond pH is controlled by introduction of CO2 into the pond. Control loop is simple: when the (averaged) pH is below set threshold CO2 is demanded from the CO2 supply system and valve of the CO2 diffuser is open for specified amount of time.

Exhaust gas blower: RPM of the exhaust gas blower controlled when CO2 is demanded. There is a PID controller setting the RPM of the blower based on the compressed gas temperature. This is used to prevent meltdown of the (plastic) piping and protect the blower.

Pond temperature: recirculation pump is turned on when the pond temperature is far enough from the set value. Mixing valve is controlled by a PID controller to set the temperature of the inflowing water to the desired value. The allowed temperature range is defined so that warm water can not be above specified maximum or below specified minimum (this was done as a measure against concrete cracking).

Greenhouse fan: greenhouse fan is switched on when water temperature is above specified level.

Sedimenter level: sedimenter level is controlled by a PID controller from the level sensor. Controller throttles the valve after the pneumatic pump.

Pneumatic pump (sedimenter recycling pump) is controlled by a time sequence. There are two main cycles: daily cycle for stopping the sedimenter reflow overnight and shorter cycle for controlling the average flow rate. Pneumatic pump has clear lower and upper flow limits and actual rate is pre-set manually.

Digestate inflow is controlled by the most complex loop: there are several conditions that enable digestate inflow: ORP above pre-set threshold, dissolved oxygen above a pre-set threshold, conductivity under pre-set level and maximum digestate quantity has not been reached for a day. If all above conditions are met, the digestate flow is open for predefined time period and closed for predefined period that allows mixing in the pond.

5.2 Daily routine

AlgaeBioGas demonstration centre is monitored daily by the personnel that also operates the Biogas plant and employees working on AlgaeBioGas project. Each day process data were recorded in “diary”, visually checked the demo centre, performed harvest and did necessary cleaning (sensors ect).

A daily routine has been established, personnel has been instructed and trained. There is a 24/7 surveillance available for emergencies and a trained person is on-site at least daily. Operator checked the facility each day, also for weekends and holidays (man on duty).

Daily routine performed by biogas operator includes:

- water level checks; water is added in case of low levels
- mixers visual inspection
- colour checks of both ponds
- general visual inspection of the greenhouse
- adjustments to greenhouse ventilation (side curtains)
- heating / cooling system performance

- weather conditions
- samples of both pond cultures are taken on working days to the internal KOTO laboratory for analysis
- CO2 blower is checked (due to problems with condensate entering the blower)
- harvesting: sedimenter scrubber is stopped over the night, so harvesting is done from the sediment collected overnight; a prescribed volume of dense biomass is pumped to the harvest container, visual inspection of the density of the sediment is done, logging the duration of dense flow;
- sensor cleaning is done at every second day
- sensor calibration is done weekly or bi-weekly
- all activities are logged in a shared file.

In case of any unusual conditions operator informed Algaen or KOTO maintenance department. Employee on duty for weekend monitors operation, with checking of important parameters and acts if necessary (maintenance of temperature, ventilation, heating). Algaen daily monitors process with remote access to the control system and gives instructions for operation.

Analysis data from KOTO internal laboratory and external laboratories are enclosed in Appendix 9.

5.3 System operation

The following report covers period of AlgaeBioGas demo centre operation from M25 to M36. Overall we can say we successfully eliminated all major problems since M25 which hindered optimal operation of the demo centre. By fixing some small operational errors and optimising the conditions we achieved, what we see as optimal operation of demonstration centre in spring and summer 2016. Of course improvements are always possible and needed, but to be able to improve the operation we first need some basic information.

By analysing the data collected during 2 years of demo centre operation, we could see which technical aspects of the system needed improvement (most importantly sedimentor) as well as how environmental conditions (weather) affect operation in our geographic location.

Set parameters, controlling the input quantity of digestate, were tested during whole time of demo centre operation and it was established that the most important parameter is ORP, as well as max amount of digestate added, whereas conductivity did not seem to affect the system much. ORP Oxydation Reduction potential is typically measured to determine the oxidizing or reducing potential of a water sample.

<u>List of abbreviations used for set process parameters:</u>

- D_{add}= max volume of digestat added per day in liters
- Cond= max conductivity reached in µS
- ORP= lowest value of oxidation reduction potential in mV
- DO=lowest value of dissolved oxygen in mg O_2 per L

High load has been achieved in last month off operation in August 2016 under favourable weather conditions, average Temperature in reactor 26,7°C, when more than 300 L f digestate has been treated in HRAP and more than 80 L of microalgal biomass harvested per day.

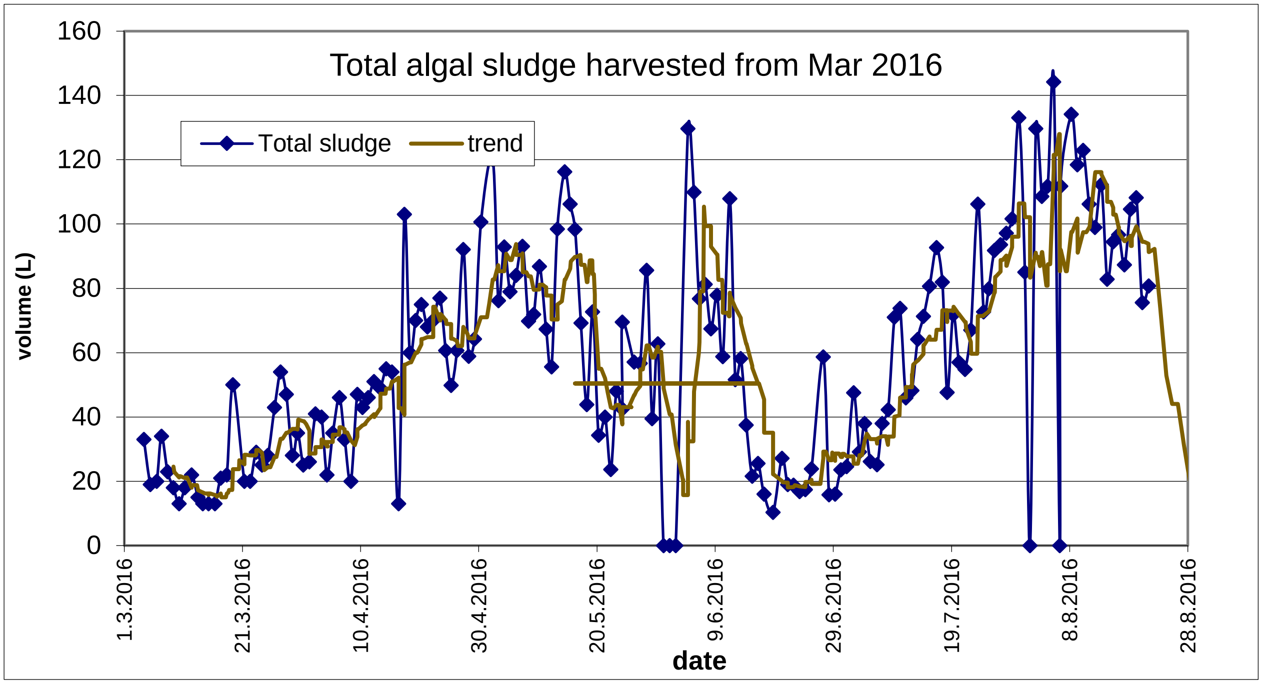


Figure 8 Total algal sludge volume (harvesting) from March 2016 to August 2016 – high load mode

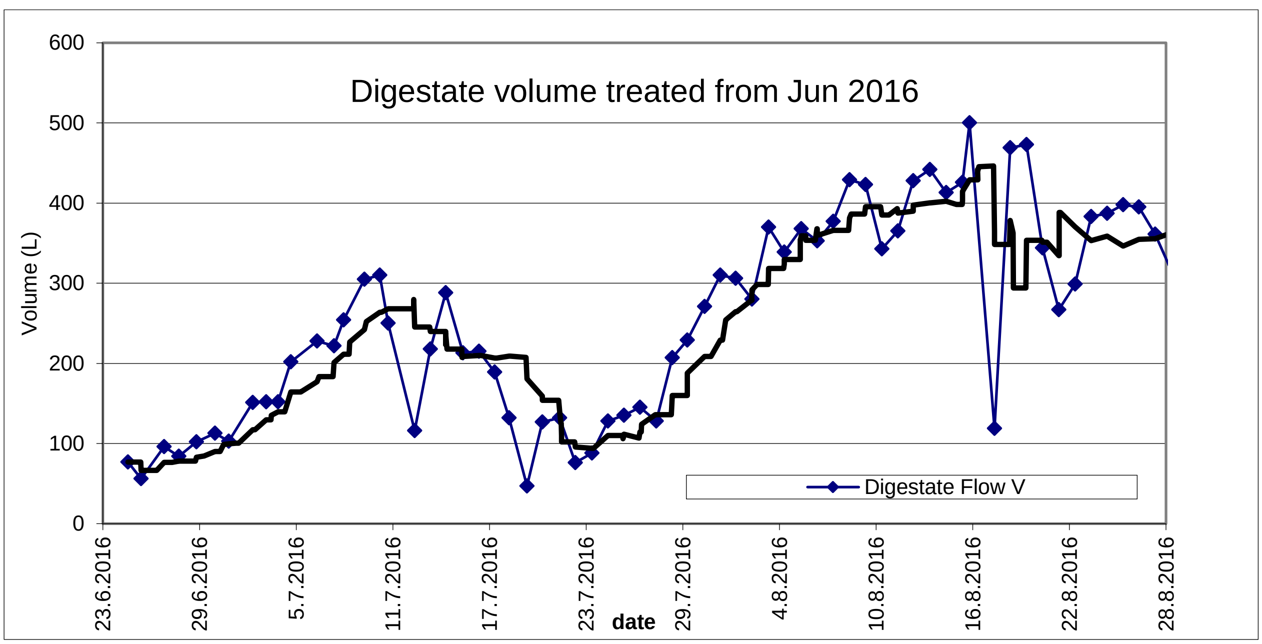


Figure 9 Digestate input in summer 2016

5.3.1 Autumn/Winter 2015/2016 (M25-M30)

In September (M25) and October (M26) 2015 average input of digestate to the ponds was 170 L and 350 L, respectively, with approx. 65 L of harvested sludge per day. At the beginning of November 2015 main pond was restarted (emptied and incoulated anew) due to bad condition of algal cultures and problems with dark colour of water, as a result of digestate (as seen in Figure 41). Set parameters for November 2015 were: D_{add}= 50 L/day, cond= 2000 µS, ORP=180 mV, DO=3,5 mg/L.

On average 60 L of digestat per day was added (deviation from set value due to operation of the valve) and 10 L of sludge was harvested. By the end of November parameters were changed to : D_{add} =200L (gradually), ORP=200 mV, cond=2500 µS.

In December 2015 (M28) D_{add} was 100 L and digestat addition was stopped at the start of the month; consequently there was no harvesting. Due to technical problem with sedimenter overflow the pond was emptied at the end of the month and sedimenter was cleaned and repaired. Pond was reinoculated with fresh culture. At the end of December digestate inlet was 10 L/day up to start of January 2016 (M29).

It should be stated that every time ponds are restarted or major dilution appears for whichever reason, system needs to gradually adapt, meaning that level of digestate added is rinsing slowly over period of time. Adaptation usually takes longer in colder months.

In January 2016 D_{add} was raised from 10 L/day to 50 L/day and harvesting starts in second week with 10 L/day. Cond=1500 µS, ORP=120 mV, DO=3,5 mg/L.

Later we discovered that conductivity as high as 3000 µS and 4000 µS does not appear to affect the system. ORP was fixed at above 100 mV, according to the literature [1] to ensure nitrification. At the end of January 2016 D_{add} was approx. 80 L, with 30 L of harvesting.

In February 2016 addition of digestate was up to 80 L, with harvesting rising from 40 to 70 L/day. This is still considered to be acclimating period after last pond restart at the end of December. Set parameters remained the same as before. At the end of the month set levels were raised to D_{add}=100 L, cond=1600 µS, ORP 120 mV, DO=3,5 mg/L.

5.3.2 Spring 2016 (M31-M33)

In March 2016 (M31) level of digestate addition was lowered to 20 L; due to high levels of ammonia in the pond (Figure 10) D_{add} was 20-30 L with approx. 30 L of harvesting. In the middle of March set parameters were changed to D_{add}=40 L, cond= 1700 µS, ORP=120 mV, DO=3,5 mg/L. For short period D_{add} was 120 L, due to electricity blackout re-setting set values. Reset parameters to D_{add}=50L, cond=1600 µS, ORP=100 mV, DO=3,5 mg/L. Harvesting approx 40 L/day. At the end of the month cond= 1700 µS. Conductivity at this time was set according to levels reached in the ponds.

Improvements from SCADA: added comulatives-separate counters for water and digestate inlet in both ponds.

In April 2016 (M32) the system was operating nicely, probably due enough light (nice weather). Set parameters: D_{add}=50 L, harvesting approx. 43 L. Period of nice (sunny) weather and stable operation (12.4.-30.4., Figure 10), stable conductivity, pH and DO. At the end of the month D_{add}=70 L, approx. 80 L digestat added and 70 L of harvested sludge. Up until this point amount of harvested sludge was estimated manually, but from 23.4. onward harvested sludge was calculated using average flow and time of harvesting. Approx. 10 L of sludge was re-circulated back to the main pond in order to maintain community structure. Average D_{add} at the end of the month was 100 L, with approx. 85 L of harvested sludge.

In May 2016 (M33) set values were D_{add}= 130 L, cond=2100 µS. Interesting effect of oxygen levels rise was observed during night time in first half of the month. D_{add} was on average 130 L, with 105 L of harvesting. On 17.5.16 main pond overflowed due to operational mistake (water inlet during night). This resulted in some sensor deactivation: ORP was off for 3 days and conductivity sensor was broken (replaced in August). Process was stable, load D_{add} =180 L and 52 L of harvesting.

Another event affected operation in May 2016: paddlewheel broke on 30.5.16 which resulted in some system operation irregularities. Paddlewheel was changed on 3.6.16. We were glad to see that even though mixing was disturbed, DO levels did not fall below 2,7 mg/L. Usually DO levels at night are 3 mg/L and not lower. D_{add} was on average 160L (set D_{add} =250L).

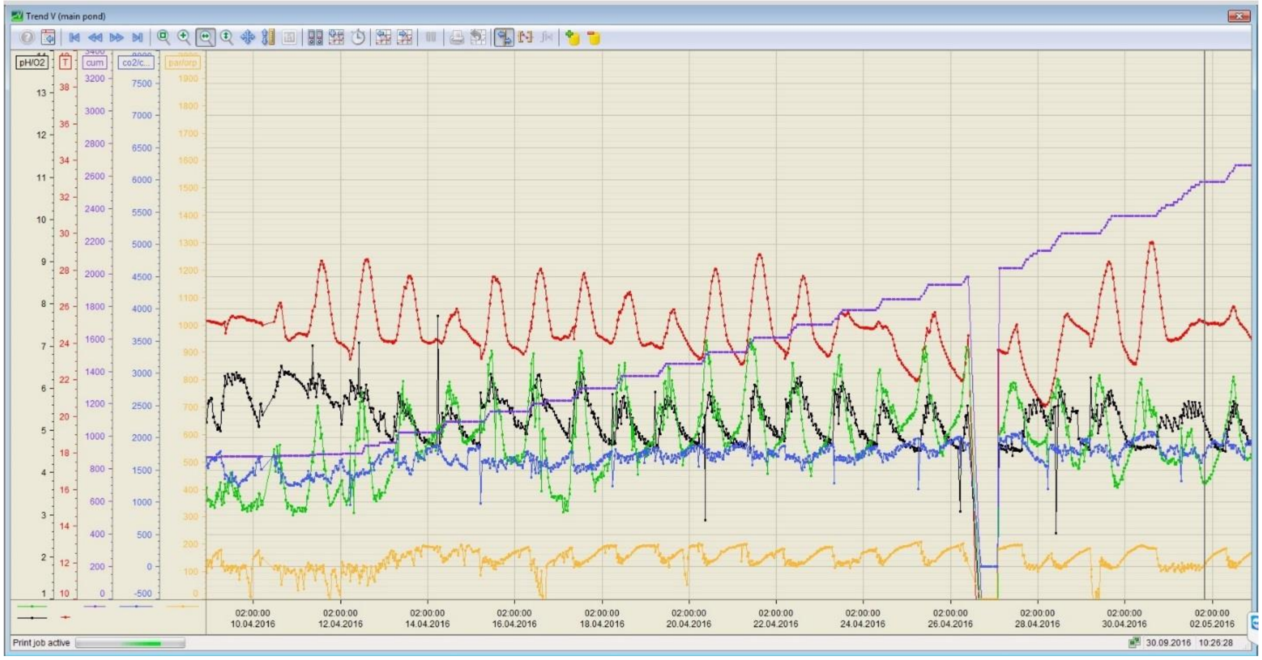


Figure 10: Optimal system operation in Spring 2016

5.3.3 Summer 2016 (M34-M36)

At the beginning of June 2016 (M34) drop in oxygen levels was observed, which was probably the effect of broken paddlewheel-no mixing resulted in forming sediment on the bottom with probably anaerobic conditions. Digestat was added until 11.6.16 then D_{add} was stopped. Approx.100 L of digestate was added to the system in that time, then digestate addition was stopped in order to allow the system to restore. From the end of June 2016 operation was normal. In the period right after paddlewheel broke harvest was high-100 L/day, then this lowered to 10 L/day until the end of the month. System recovered by the end of the month, which could be seen by DO and pH levels.

In July 2016 (M35), set value for D $_{add}$ was 150 L and system worked fine until the middle of the month. Then, due to bad weather, temperature of the air and consequently water suddenly dropped from 34°C to 25°C, resulting in less digestate addition for approx. 8 days (Figure 9). Digestate addition went from 200 L to 310 L before cold period and then to 110 L/day. After acclimation and raised temperatures digestate addition raised to 310 L/day again, harvesting was 85 L in both periods of higher digestate addition.

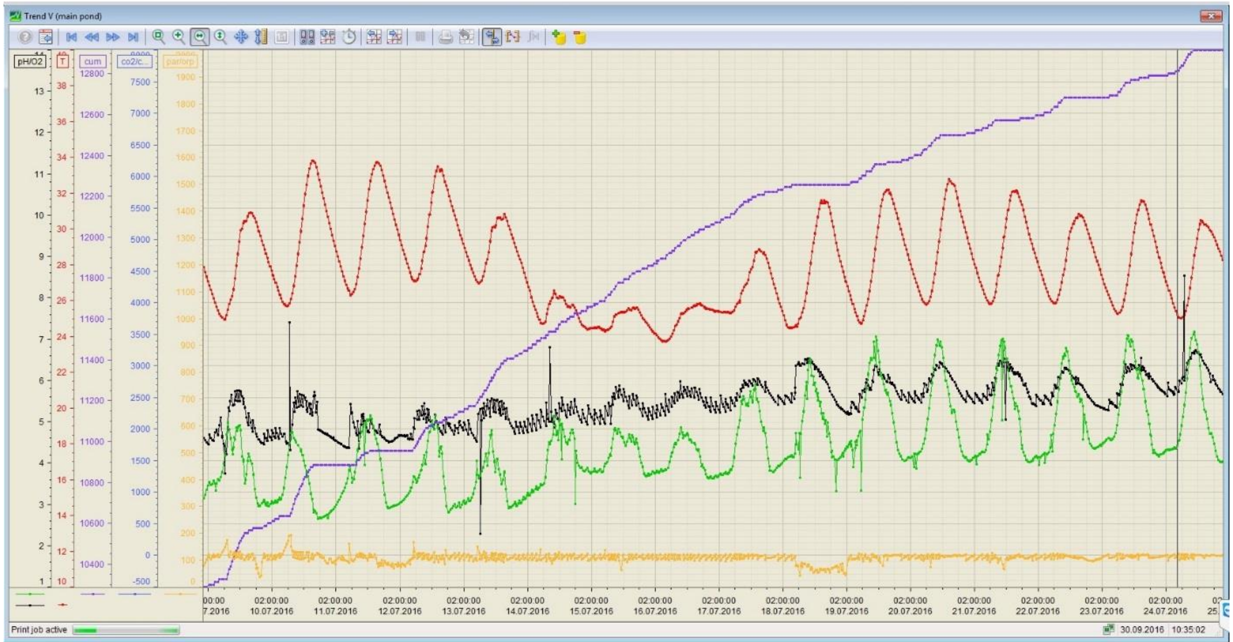


Figure 11: Temperature change VB 10.-24 July 2016

Generally, harvesting was raising from the end of June continuously towards the end of August (Figure 98); from 20 L to 90 L/day. From the middle of July system entered optimal mode of operation and is still working in this mode at the end of August and in September. Digestate input set value was 500 L, and up to 400 L was added per day.

At the start of August 2016 sedimenter was emptied and fixed in order to improve overflow control. Sensor for conductivity was replaced as well.

5.3.4 Operation settings in optimal conditions

Summer time (from June to September, depending on current weather) is considered optimal time for our system operation, taking into account Centre's geographic position. Whit enough sun light optimal operation of system was achieved. Set values controlling the addition of digestate were:

- Oxidation reduction Potential-ORP: 120 mV, according to literature to enable optimal nitrification.
- Conductivity: value not important according to results, ranged from 1500 µS to 4000 µS.
- Dissolved Oxygen (DO): 3,5 mg/L was set according to WWT standards. Even in periods out of optimal operation mode (lack of light etc.) DO levels never fall below 2,5 mg/L.
- Most important parameter for us was the maximum added volume of digestate, which was set to 500 L at the end. Addition of digestate was adapted according to operation in specific time.

At the end of May 2016 regular inoculation of the main pond started. 1500 L of inoculation pond culture was added to the main pond ever 7-14 days. With this, we also control evaporation in summer months, without adding water directly to the main pond. Water is added to the inoculation pond after inoculation.

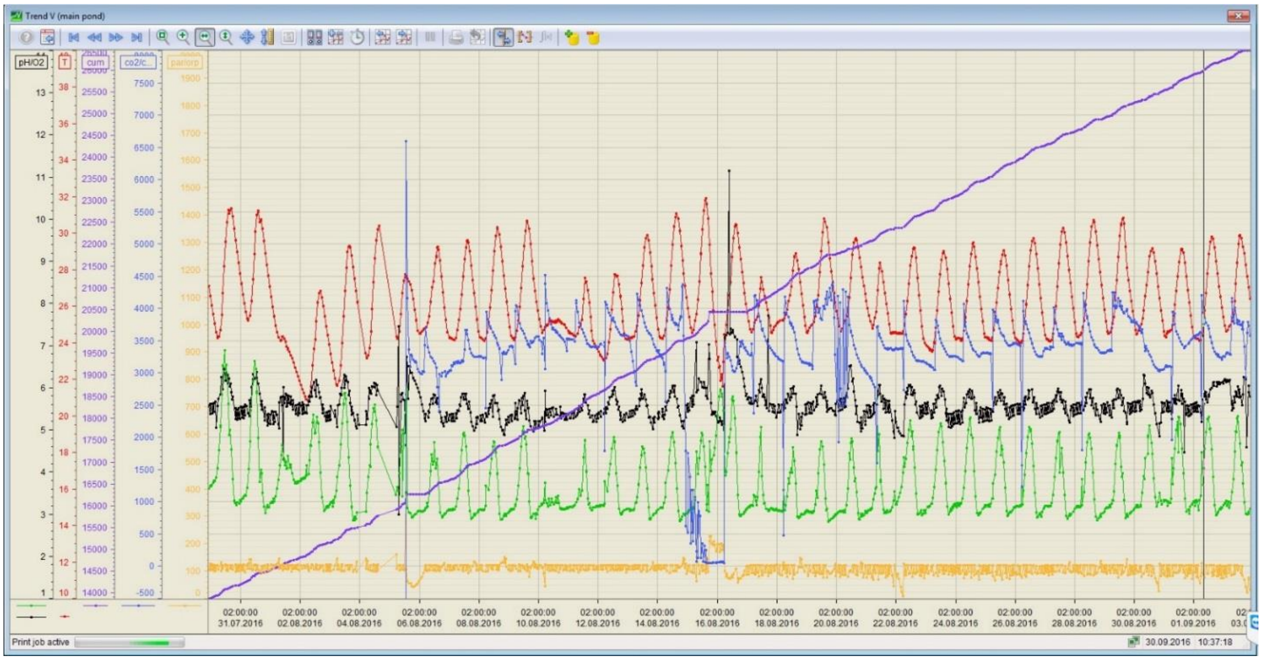


Figure 12 : Optimal system operation in August 2016

5.4 Measurements of important parameters

Online measured process parameters in main pond are temperature, digestate flow to the pond, pH value, photosynthetic active radiation PAR (above water surface), DO, ORP and Conductivity EC.

Recorded data at PC ALGAE are presented in deliverables Dataset (D3.3. and D3.4), exported from Scada with Siemens Connectivity pack. Other important process parameters were measured at internal KOTO laboratory and at external laboratories.

Table 1 Temperature in main pond VB monthly average, minimum and maximum (°C)

Month	**T (°C) average**	**T max (°C)**	**T min (°C)**
Sep 2014	25,4	25,8	25,2
Oct 2014	26,1	27,4	24,7
Nov 2014	25,8	26,7	22,6
Dec 2014	24,4	25,7	22,8
Jan 2015	20,6	21,6	19,5
Feb 2015	23,6	24,3	22,5
Mar 2015	21,5	24,8	18,7
Apr 2015	28,1	31,0	24,9
May 2015	28,4	29,5	27,8
Jun 2015	25,5	27,4	23,8
Jul 2015	28,5	31,8	25,5
Aug 2015	28,8	30,6	27,3
Sep 2015	25,3	25,5	24,8
Oct 2015	26,1	27,1	24,9
Nov 2015	24,8	26,2	23,6
Dec 2015	24,2	24,9	23,2
Jan 2016	23,2	24,5	21,9
Feb 2016	24,6	24,8	24,6
Mar 2016	23,8	25,2	22,0
Apr 2016	26,5	28,0	24,4
May 2016	24,7	28,2	20,8
Jun 2016	27,9	31,1	25,0
Jul 2016	24,7	25,7	23,7
Aug 2016	26,7	31,8	22,0

Digestate quality (pollutants COD and NH4-N) was normally COD 7000 - 8000 mgO2/l, ammonia nitrogen ~1400 mg/L with variatons during 2 years period, when HRAP operated in different modes:

- Jan – Feb 2015 high COD mode 11.100 mgO2/l
- Sep – Nov 2015 lowest COD mode 3300 mgO2/l due longer HRT in anaerobic filter AF
- Jun- Aug 2016 lower COD mode 5600 mgO2/l.

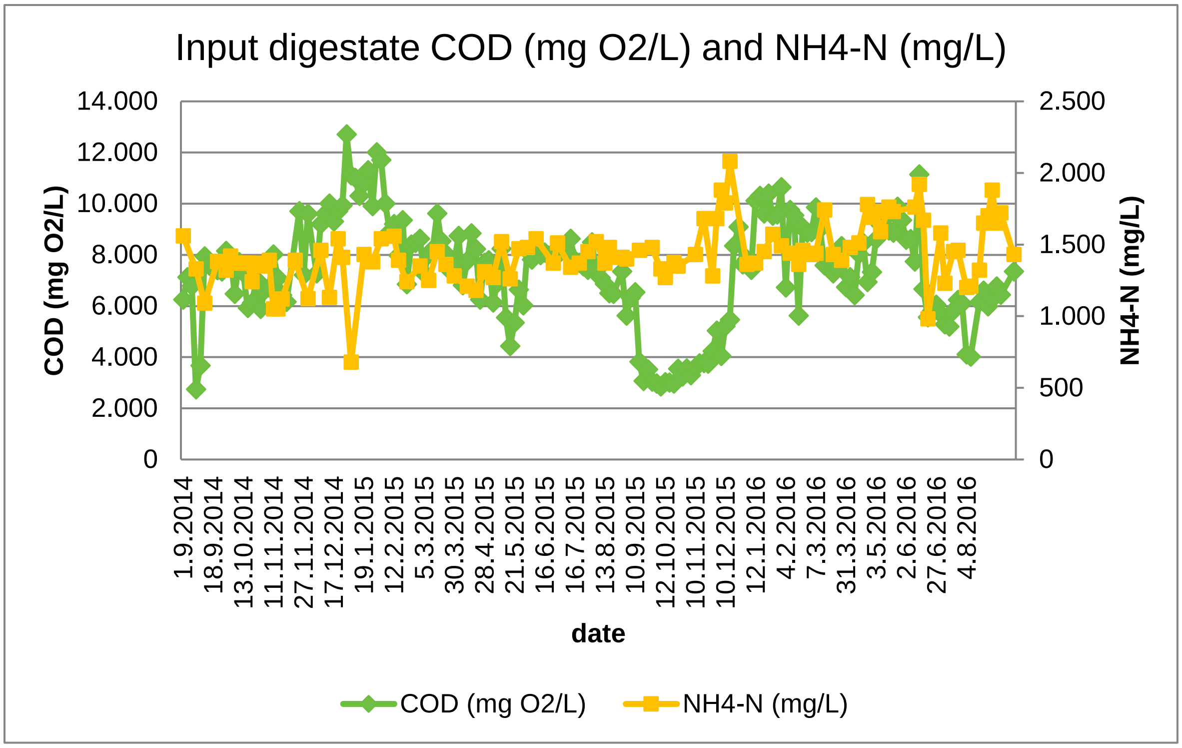


Figure 103 COD and NH4-N values for digestate

Unfiltered outflow have been analysed occasionally to see the difference between filtered and unfiltered COD value. By unfiltered sample is higher uncertainty regarding COD value by spectrophotometric method, because present particles in waste water. With sufficient sedimentation of microalgal biomass in sedimenter, outflow quality would correspond to quality of filtered sample. COD value in effluent was ~ 600 mgO2/L, and increased only by process failure end of year 2015.

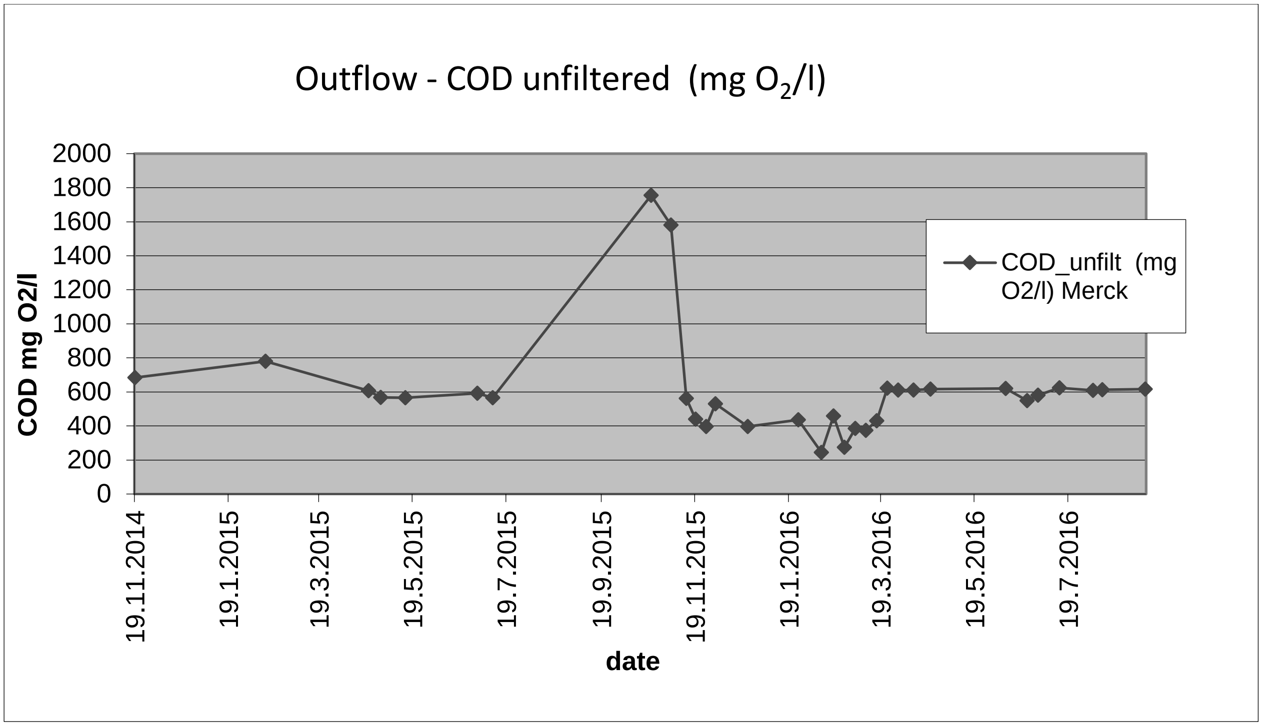


Figure 114 COD value in effluent ww the main pond internala anlsis results

Value pH of digestate has been favourable 7,5 - 7,9. After dewatering of digestate on centrifuge digestate supernatant was treated in anaerobic filter (AF mesophilic 35-38°C) and storage in intermediate tank CT.

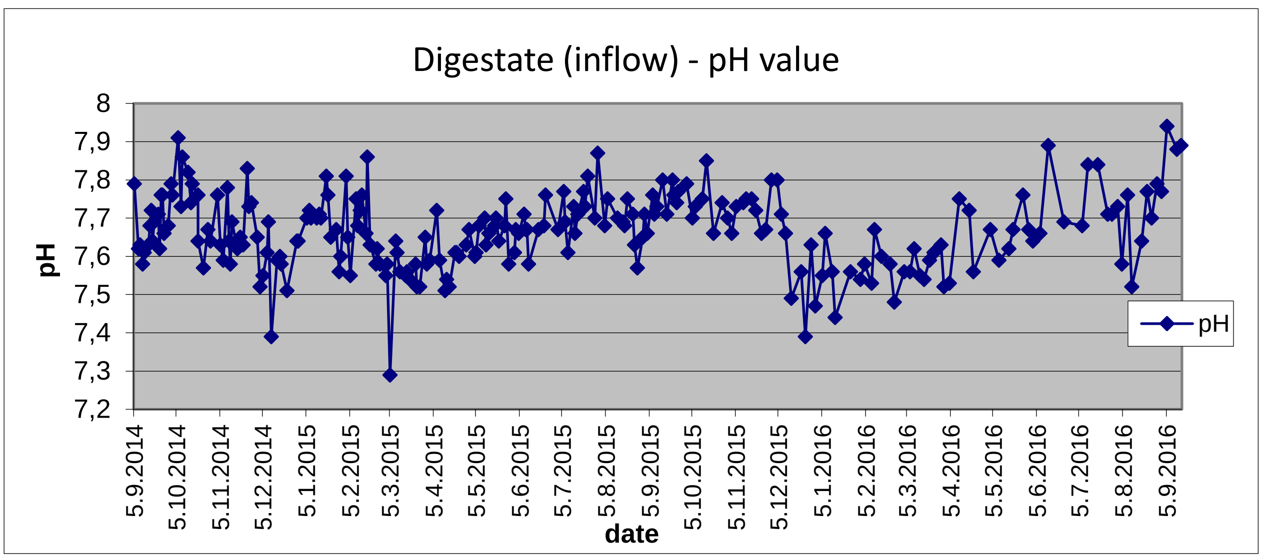


Figure 15 Digestate pH value measured at KOTO laboratory

Change in pH value shows change in process. Value pH in main pond HRAP was high pH 7- 8 in year 2014, and lowered to pH 5 - 6 in summer 2015, and after that only increased to 7,5 after restart in January 2016.

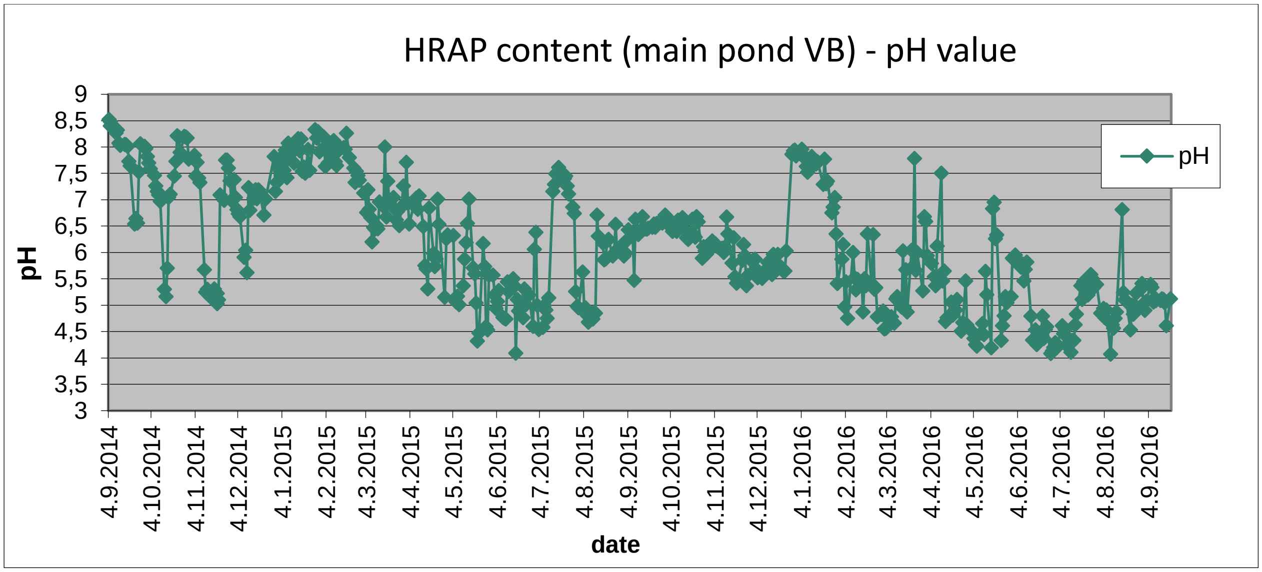


Figure 16 pH in the main pond VB

Process is very dependable oo available light for photosynthesis. In Winter time 2015/16 PAR value has been under 300 µmoles/m2/s. Stable process was achieved above PAR value 500 µmoles/m2/s.

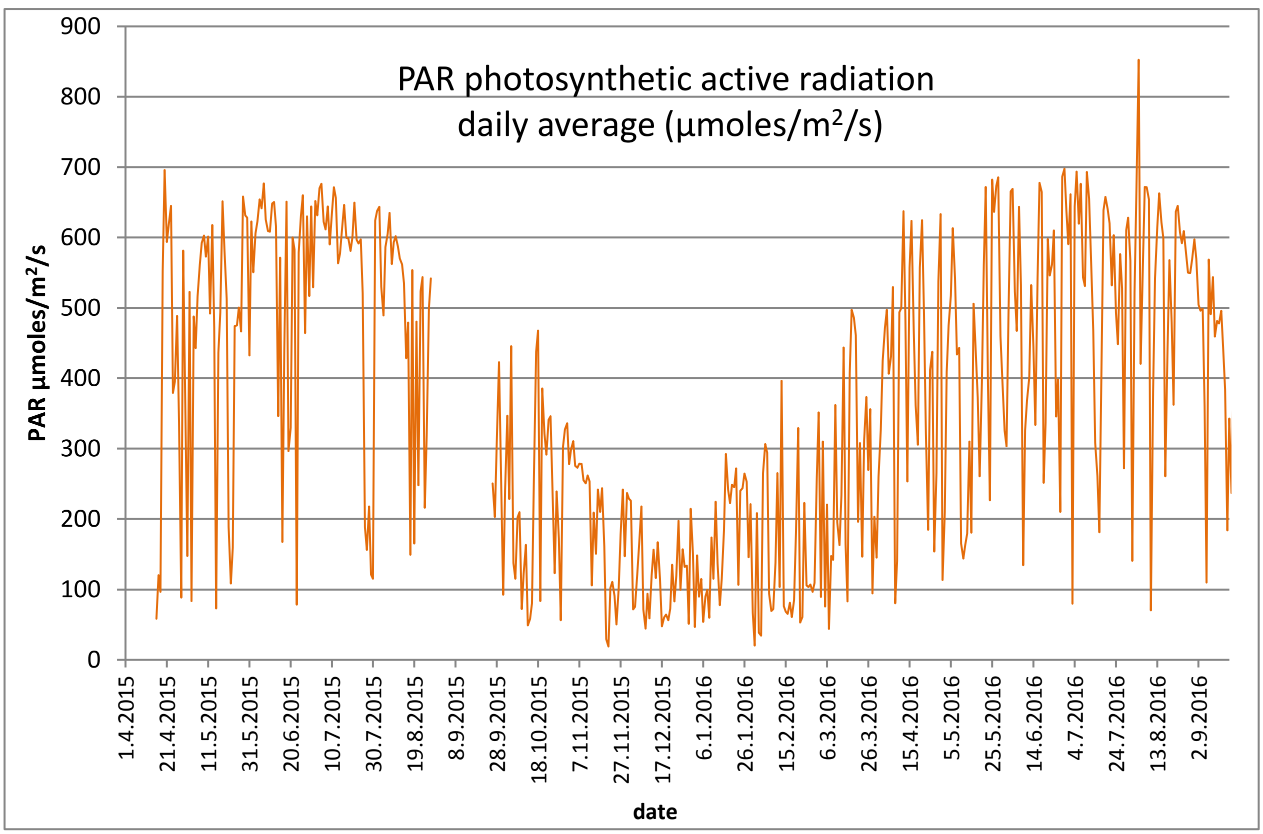


Figure 17 PAR above HRAP water surface-average daily average (µmoles/m2/s)

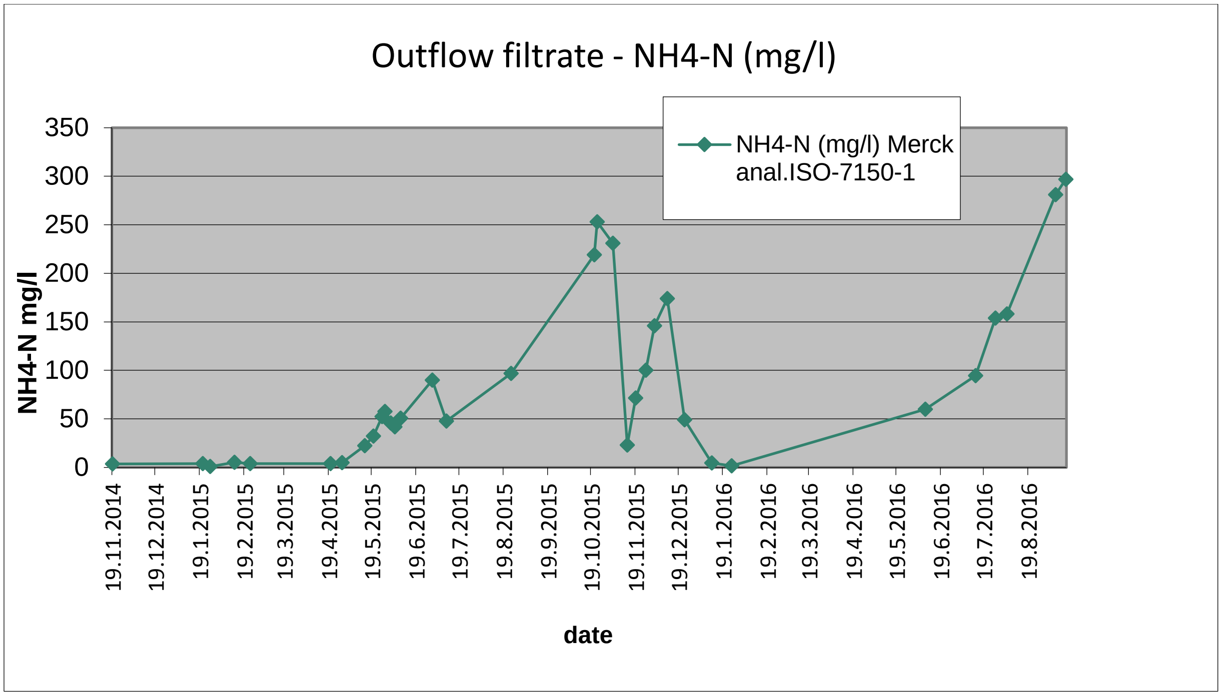


Figure 128 Outflow NH4-N values in effluent, internal analysis results

Ammonia nitrates concentration in waste water effluent has been below 200 mg/l, except in October 2015 when value has been slightly higher, up to 253 mg/l. Analysis of ammonia nitrogen concentration were performed in internal and external laboratory (M 34-36) and analysis results are comparable.

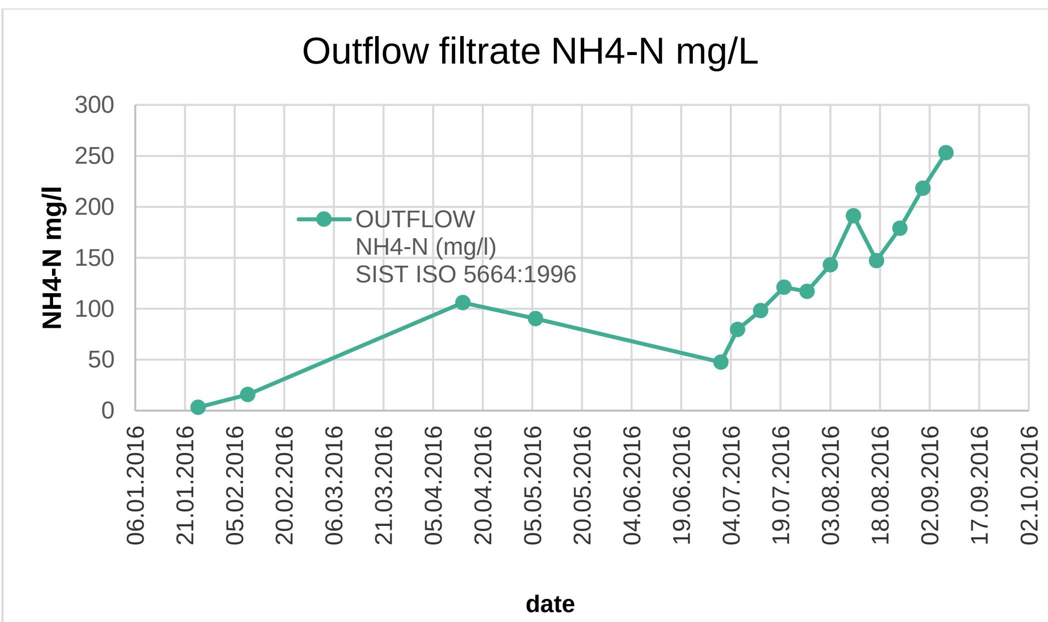


Figure 139 Outflow NH4-N values, external laboratory results

Nitrates and Nitrites concentration in effluent waste water were regulary analysed. Nitrites value in effluent was low, below 10 mg/l, except in second part of 2015.

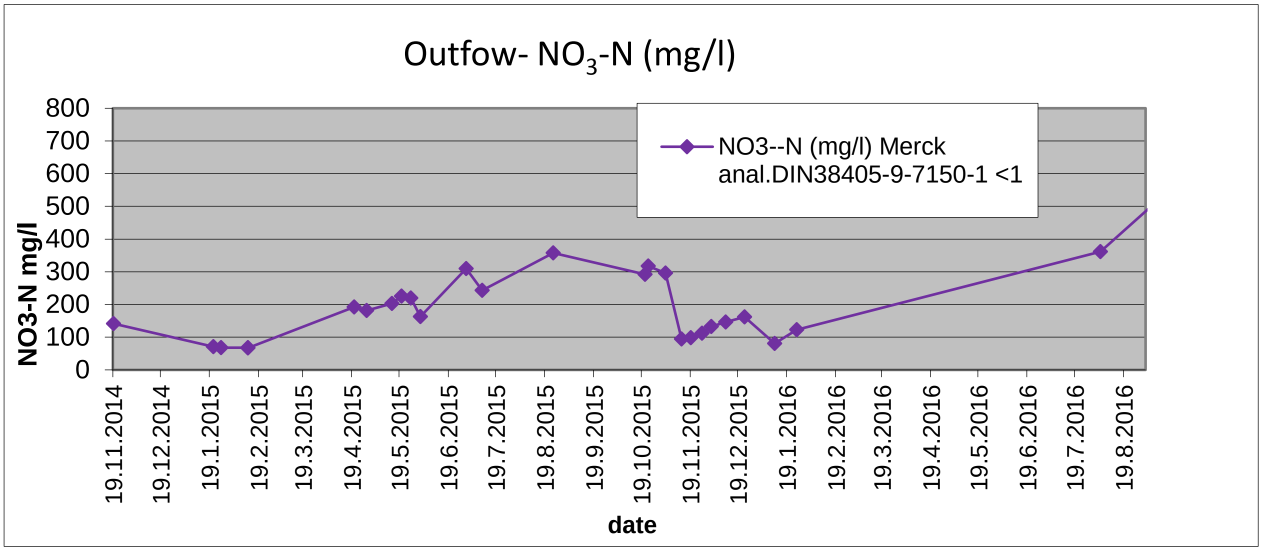


Figure 20 Outflow NO3-N values

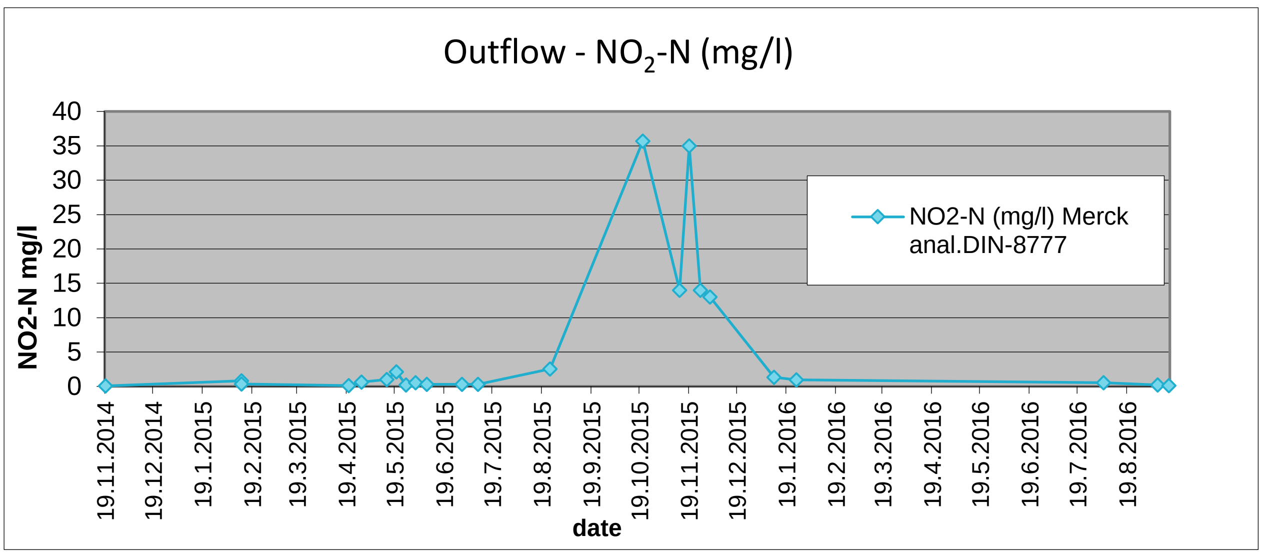


Figure 21 Outflow NO2-N values

Free Chlorine in effluent has been below 1000 mg/l, value only increased in October 2015.

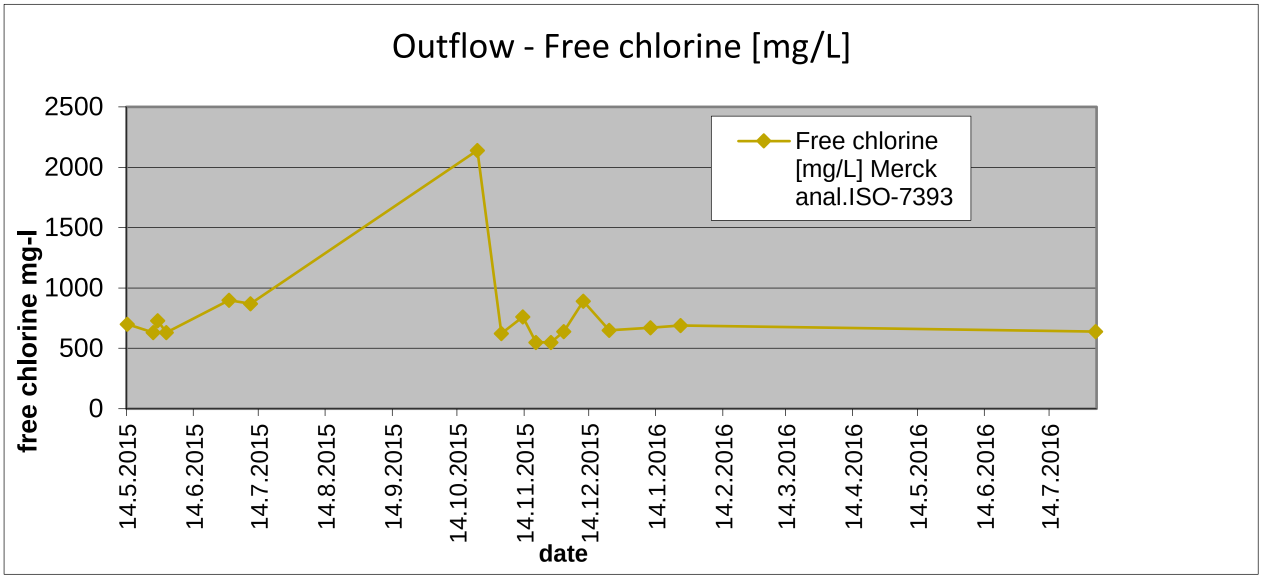


Figure 22 Outflow free chlorine values

Concentration of Phosphates in effluent shows if Phosphorous has been uptaken by microalgae. In October 2015 concentration of Phosphates in effluent has been higher due process disorder.

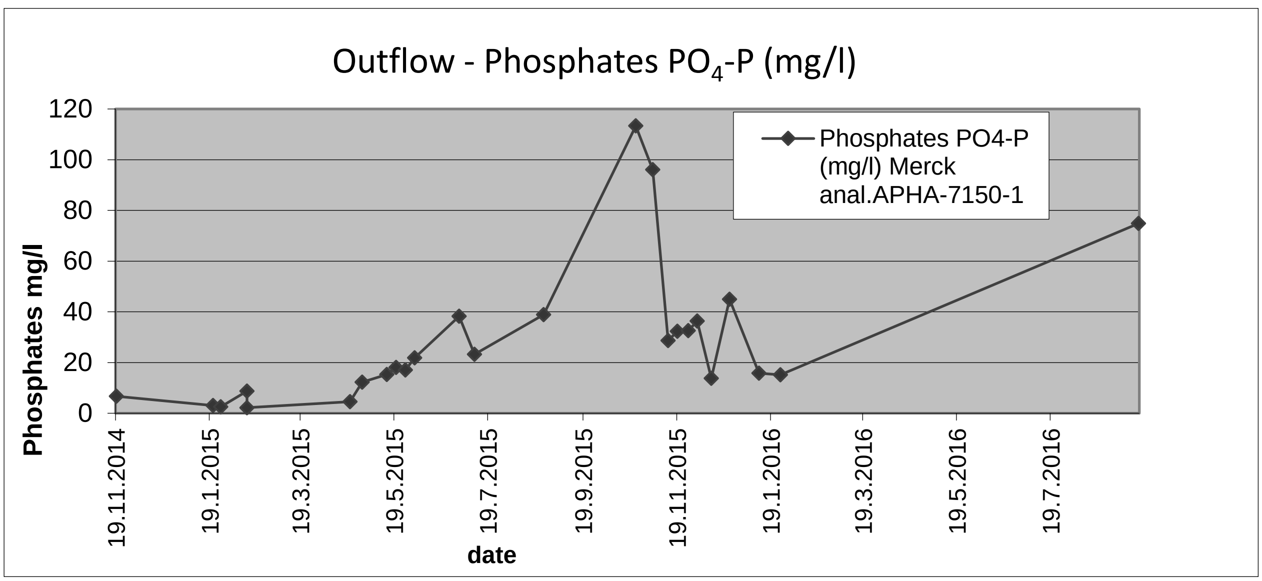


Figure 14 Outflow phosphates

Absobance in main pond has been measured since April 2015. Absorbance has been measured more often end of 2015 and last two months of operation. With monitoring of this parameter technologist could set the volume for harvest of biomass. More measurements were done Jun - Aug 2016.

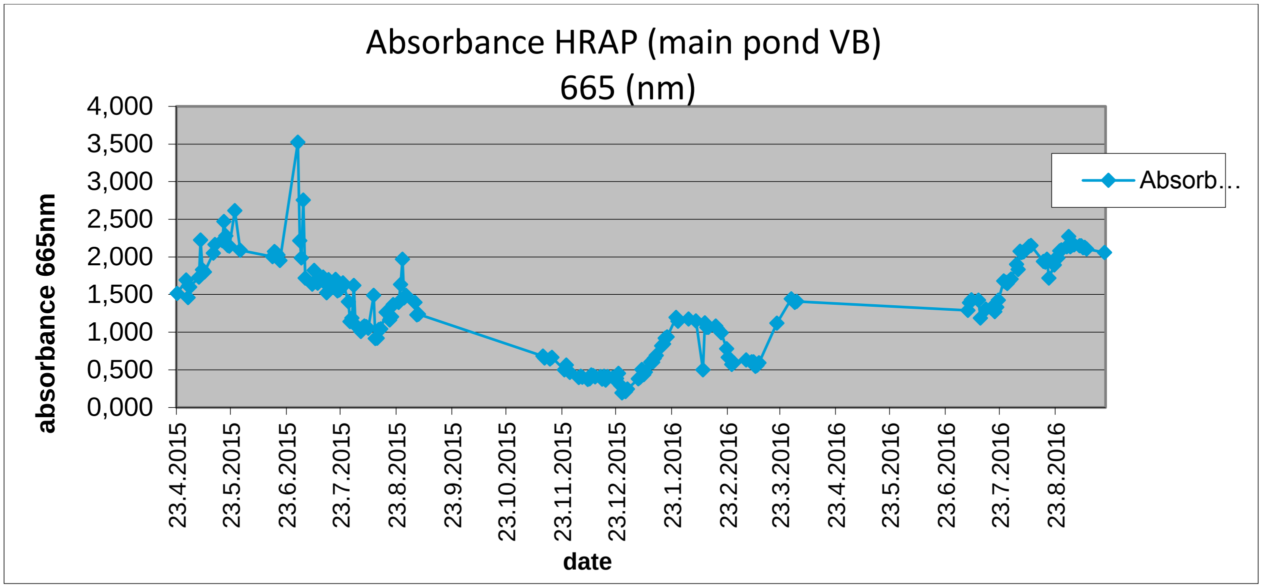


Figure 154 Absorbance in the main pond

For inoculation pond temperature (online) and pH value (internal laboratory) were measured.

In inoculation pond (MB) was pH value from start up until end of year 2015 around pH 8, in year 2016 has been pH value lower 6-7,5 but very stable. This enabled occasionally addition of CO2 (according to set value).

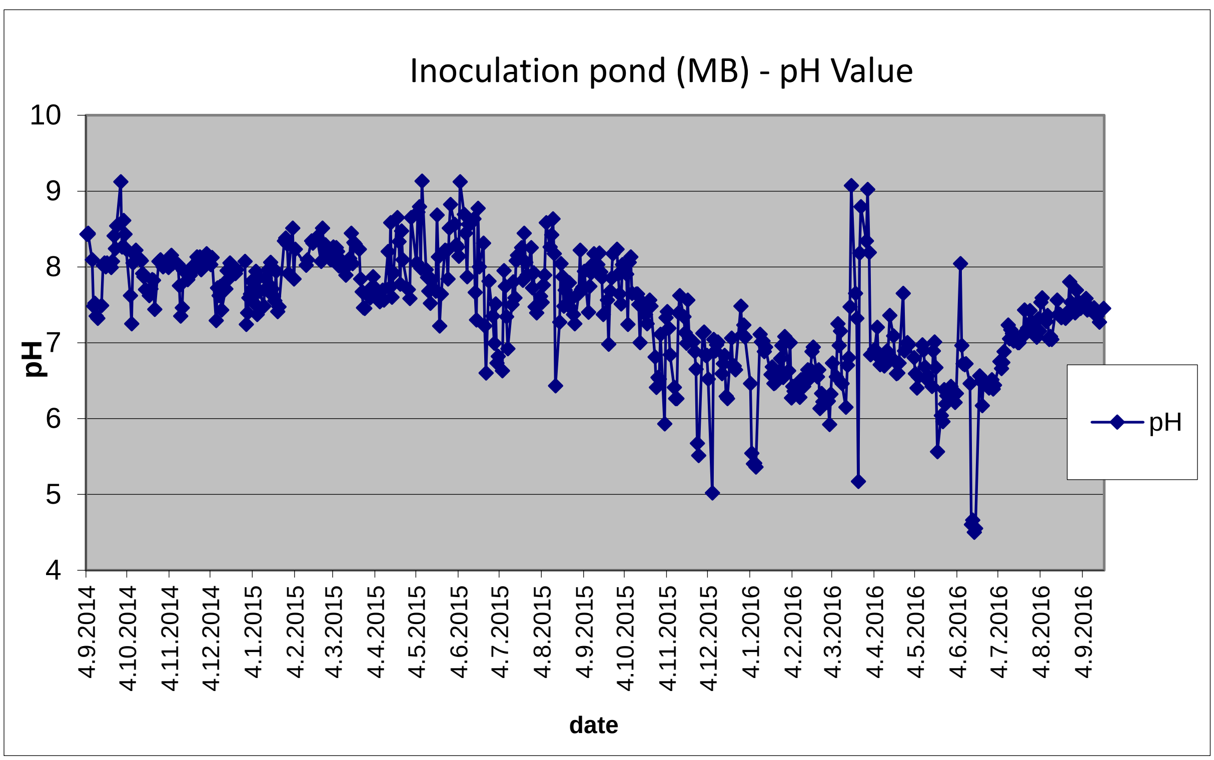


Figure 16 pH value in inoculation pond (MB)

Table 2 Temperature in inoculation pond per month, average, minimum and maximum (°C)

Month	T (°C) average	T max (°C)	T min (°C)
Sep 2014	25,3	25,6	24,9
Oct 2014	26,3	27,5	24,6
Nov 2014	25,5	26,8	23,7
Dec 2014	23,8	24,7	23,1
Jan 2015	20,8	21,2	20,6
Feb 2015	25,2	26,8	23,2
Mar 2015	25,3	28,3	22,2
Apr 2015	27,9	31,3	24,3
May 2015	28,3	29,7	26,8
Jun 2015	26,4	28,7	23,7
Jul 2015	30,1	32,7	27,8
Aug 2015	28,2	29,8	26,6
Sep 2015	25,7	25,9	25,1
Oct 2015	25,9	27,3	24,5
Nov 2015	24,8	26,9	22,8
Dec 2015	24,3	25,6	22,9
Jan 2016	23,6	24,4	21,5
Feb 2016	24,8	25,2	24,2
Mar 2016	24,1	25,3	22,3
Apr 2016	25,5	27,3	23,4
May 2016	25,2	28,8	21,7
Jun 2016	27,4	30,1	24,6
Jul 2016	24,8	26,3	23,5
Aug 2016	24,9	30,3	19,5
Sep 2016	23,5	26,9	23,6

5.5 Cleaning efficiency

Cleaning efficiency by algal treatment of digestate in year 2016, by high load ORL (200-300 m3 digestate/day), achieved in ABG main pond was 91.5 % for NH4-N removal and 92,1% for COD removal.

Table 3 NH4-N concentration in digestate (input) and outflow waste water, ammonia nitrogen removal

date	**DIGESTATE NH4-N (mg/l)** SIST ISO 5664:1996	**OUTFLOW** NH4-N (mg/l) SIST ISO 5664:1996	**Efficiency of algal treatment NH4-N removal %**
25.01.2016	1.691	3	99,81
09.02.2016	1.415	16	98,88
14.04.2016	1.546	106	93,14
06.05.2016	1.486	90	93,92
01.07.2016	1.540	48	96,92
06.07.2016	1.548	80	94,86
13.07.2016	1.522	98	93,56
20.07.2016	1.498	121	91,92
27.07.2016	1.287	117	90,91
03.08.2016	1.224	143	88,32
10.08.2016	1.097	191	82,59
17.08.2016	1.118	147	86,85
24.08.2016	1.597	179	88,79
31.08.2016	1.530	218	85,75
07.09.2016	1.653	253	84,69
average	**1.450**	**121**	**91,39**

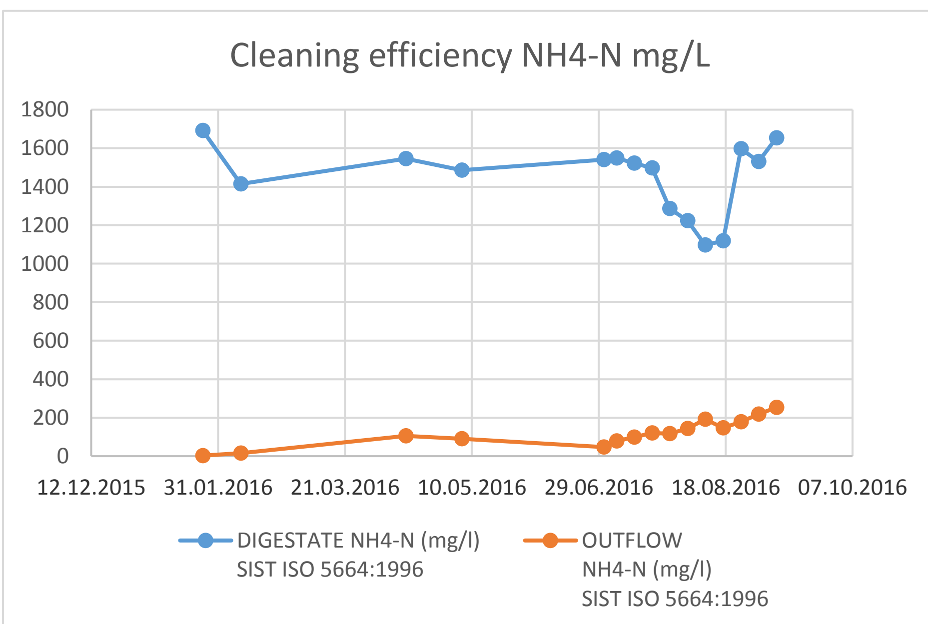


Figure 17 Treatment efficiency according to NH4-N concentration

Table 4 COD value of digestate (input) and outflow waste water, COD removal

date	**DIGESTATE COD (mg O_2/l)** SIST ISO 6060:1996	**OUTFLOW COD (mg O_2/l)** SIST ISO 6060:1996	**Efficiency of algal treatment COD removal %**
25.01.2016	8.611	453	94,74
09.02.2016	9.033	366	95,95
14.04.2016	6.331	357	94,36
06.05.2016	7.037	284	95,96
01.07.2016	5.987	322	94,62
06.07.2016	4.961	274	94,48
13.07.2016	4.842	346	92,85
20.07.2016	3.747	291	92,23
27.07.2016	4.661	317	93,20
03.08.2016	4.124	430	89,57
10.08.2016	2.317	446	80,75
17.08.2016	4.170	330	92,09
24.08.2016	5.127	493	90,38
31.08.2016	4.515	374	91,72
07.09.2016	4.439	460	89,64
average	**5.327**	**370**	**92,17**

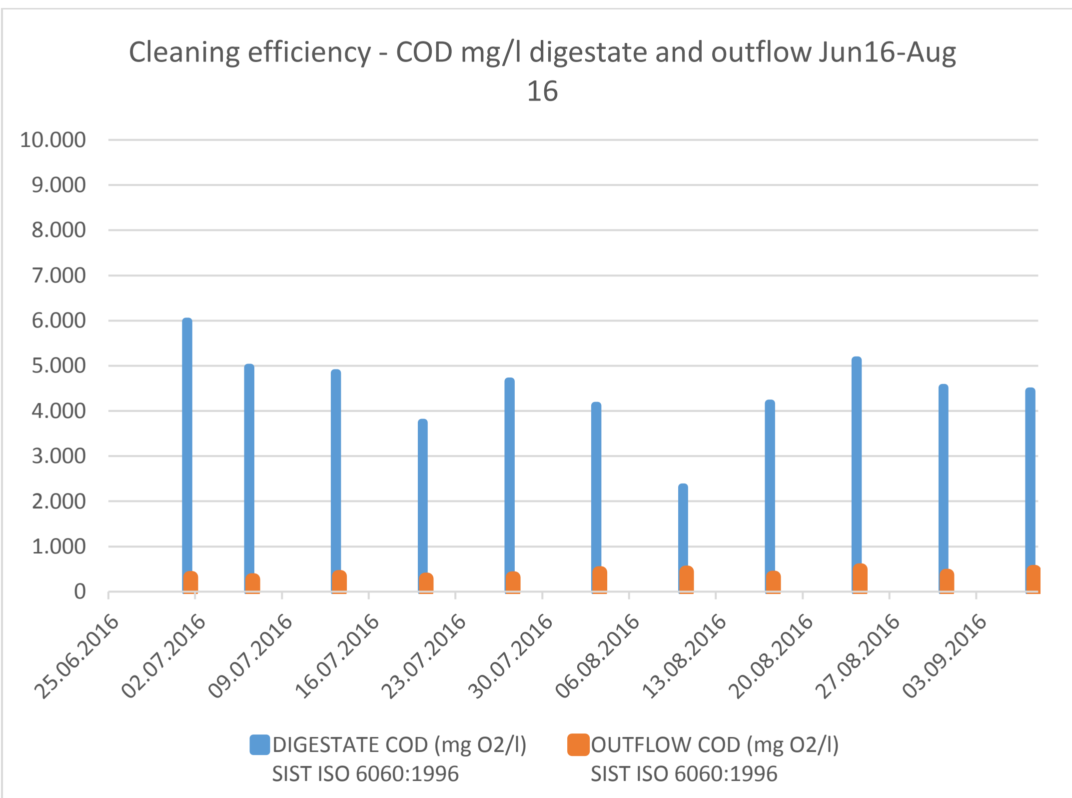


Figure 27 Treatment efficiency according to COD value

5.6 Biomethane potential of produced microalgal biomass

For better anaerobic biodegradability of microalgal biomass, biomass has been thermally pretreated and bioaugumented with goal to brake rigid microalgae cell walls. In mixed algal biomass grown in digestate used for BMP test were dominat species *Ankistrodesmus sp., Chlamydomonas sp.* and also noticed *Haematococcus sp.*.

Thermal pretreatment of mixed microalgae took place in autoclave (volume 5 m3) at KOTO pretreatment line for thermal treatment of food waste for industrial biogas production. Microalgae from pilot plant for algal treatment of digestate has been storaged in IBC container. Two full IBC containers of algal biomass and fresh water has been autoclaved under pressure 1,7 bar for 20 minutes above 100 °C. After batch thermal treatment biomass has been transported (shoot) to separator and sampled.

Biological bioaugumentation has been performed with anaerobic bacteria Strain *Clostridium thermocellum* (Viljoen et al. 1926, freeze dried) purchased from DSMZ, Germany. Bacteria gas been grown on liquid medium before bioaugumentation. During bioaugumentation anaerobic bacteria has been added into preheated mixure of microalgae, inoculum, buffer and anaerobic water.

Lab scale experiments were performed to deterime biomethane production by microalgal biomass anaerobic digestion in thermopilic process (55°C). Three BMP tests were performed in 2016. BMP test results show that is biomethane potential of algal biomass increased if algal biomass is thermally pretreated. Bioaugumentation with selected anaerobic bacteria also increased biodegradability of algal biomass.

Figure 32 Thermal treatment of algal biomass, samples for tests

BMP test 1.

Test with native and thermally treated algal biomass ABG was done at KOTO laboratory in June-July 2016.Inoculum was sampled at biogas plant KOTO DG3 and starved 16 days from 8.6.16 until 23.8.16. Algal biomass from ABG pilot system on 23.6.15 has been dewatered to 7,84% of DMC and contained 82,5 VS in DMC. Organic load was set to 2 g VS/l inoculum.

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

		tips						ml methane			
		treatment						glucose	alage	control	algae TP
		sample mass					g	10	155	0	155
		VS g/l inocukum						2,00	2,01	0,00	2,01
		VS g						10,00	10,03	0,00	10,03
		Vol, sample/Va %						0,20%	3,10%	0,00%	3,10%
date	time	1	2	3	4	5	6	**1**	**2**	**4**	**5**
23.6.16	15:21	0	0	0	0	0	0	0	0	0	0
24.6.16	7:02	2	3	0	0	3	2	200	300	0	300
25.6.16	7:25	17	5	0	2	10	7	1700	500	200	1000
26.6.16	7:00	19	7	0	2	17	13	1900	700	200	1700
27.6.16	8:28	19	8	0	4	18	17	1900	800	400	1800
28.6.16	8:15	20	10	0	4	19	18	2000	1000	400	1900
28.6.16	14:45	20	10	0	5	19	19	2000	1000	500	1900
30.6.16	12:55	22	12	1	6	21	20	2200	1200	600	2100
1.7.16	9:48	23	14	1	6	23	20	2300	1400	600	2300
2.7.16	8:35	25	15	1	6	26	21	2500	1500	600	2600
3.7.16	9:20	26	16	1	6	32	22	2600	1600	600	3200
4.7.16	8:35	27	16	1	6	39	23	2700	1600	600	3900
4.7.16	13:04	28	17	1	7	41	23	2800	1700	700	4100
6.7.16	12:55	31	19	1	7	54	27	3100	1900	700	5400
7.7.16	7:00	33	19	1	7	59	29	3300	1900	700	5900
7.7.16	15:50	34	19	1	7	61	30	3400	1900	700	6100
8.7.16	7:10	35	20	1	8	64	31	3500	2000	800	6400
8.7.16	13:20	35	20	1	8	65	32	3500	2000	800	6500
9.7.16	7:10	37	21	1	8	67	34	3700	2100	800	6700
10.7.16	6:55	38	21	1	8	68	37	3800	2100	800	6800
11.7.16	7:40	40	22	1	8	69	41	4000	2200	800	6900
12.7.16	11:15	41	23	1	8	70	45	4100	2300	800	7000
13.7.16	7:40	42	23	1	9	72	48	4200	2300	900	7200
13.7.16	14:00	42	23	1	9	73	49	4200	2300	900	7300
14.7.16	7:30	42	24	1	9	74	51	4200	2400	900	7400
14.7.16	14:00	43	24	1	9	74	52	4300	2400	900	7400
15.7.16	6:10	43	24	1	9	74	54	4300	2400	900	7400
15.7.16	13:00	43	24	1	9	74	55	4300	2400	900	7400
16.7.16	8:30	43	24	1	9	75	57	4300	2400	900	7500
17.7.16	10:11	43	25	1	9	75	60	4300	2500	900	7500
18.7.16	6:40	43	25	1	9	75	61	4300	2500	900	7500
18.7.16	14:00	43	25	1	9	75	63	4300	2500	900	7500
19.7.16	6:00	44	25	1	9	75	63	4400	2500	900	7500
19.7.16	14:00	44	25	1	9	75	63	4400	2500	900	7500
20.7.16	7:00	45	26	1	9	75	64	4500	2600	900	7500
20.7.16	14:00	45	26	1	9	75	65	4500	2600	900	7500
21.7.16	6:05	45	26	1	9	75	66	4500	2600	900	7500
21.7.16	14:00	45	26	1	9	75	66	4500	2600	900	7500
22.7.16	6:00	46	27	1	9	75	67	4600	2700	900	7500
22.7.16	13:00	46	27	1	9	75	67	4600	2700	900	7500
23.7.16	9:18	46	27	1	9	75	68	4600	2700	900	7500
24.7.16	10:05	46	27	1	9	75	68	4600	2700	900	7500
25.7.16	6:10	46	27	1	9	75	69	4600	2700	900	7500
25.7.16	14:00	46	27	1	9	75	69	4600	2700	900	7500
26.7.16	6:50	46	28	1	9	75	69	4600	2800	900	7500

Production in Digestor No. 3 and No. 6 wasn’t sufficient (gas leaking). Production of methane from algal biomass in digestor 2 and thermally treated microalgae are taken in account.

Table 6 Specific biomethane production (m3CH4/kg VS)

time day	Biomethane production m3/kg VS	
	Algae A 2-4	**Algae ATP 5-4**
0	0,000	0,000
1	0,030	0,030
2	0,030	0,080
3	0,050	0,150
4	0,040	0,140
5	0,060	0,150
5	0,050	0,140
7	0,060	0,150
8	0,080	0,170
9	0,090	0,199
10	0,100	0,259
11	0,100	0,329
11	0,100	0,339
12	0,120	0,469
13	0,120	0,519
14	0,120	0,539
15	0,120	0,559
16	0,120	0,569
17	0,130	0,589
18	0,130	0,598
19	0,140	0,608
20	0,150	0,618
21	0,140	0,628
22	0,140	0,638
23	0,150	0,648
24	0,150	0,648
25	0,150	0,648
26	0,150	0,648
27	0,150	0,658
28	0,160	0,658
29	0,160	0,658
30	0,160	0,658
31	0,160	0,658
32	0,160	0,658
33	0,170	0,658
34	0,170	0,658
35	0,170	0,658
36	0,170	0,658
37	0,180	0,658
38	0,180	0,658
39	0,180	0,658
40	0,180	0,658
41	0,180	0,658
42	0,180	0,658
43	0,190	0,658

Biomethane potential for native algal biomass was much lower 189 ml CH4/g VS (Digestor 2), than biomethane production by thermally treated microalgae 657 ml CH4/g VS. Glucose wasn't sufficiently degradated. Biogas production was recorded for 42 days. Thermally treated microalgal biomass has been sufficiently degradated in 14 days, normal RT in thermophilic process.

Table 7 Biomethane potential of native and thermally pretreated microalgae production

BMP	Algae native A	algae thermally pretreated ATP
ml CH4/g VS	189	657

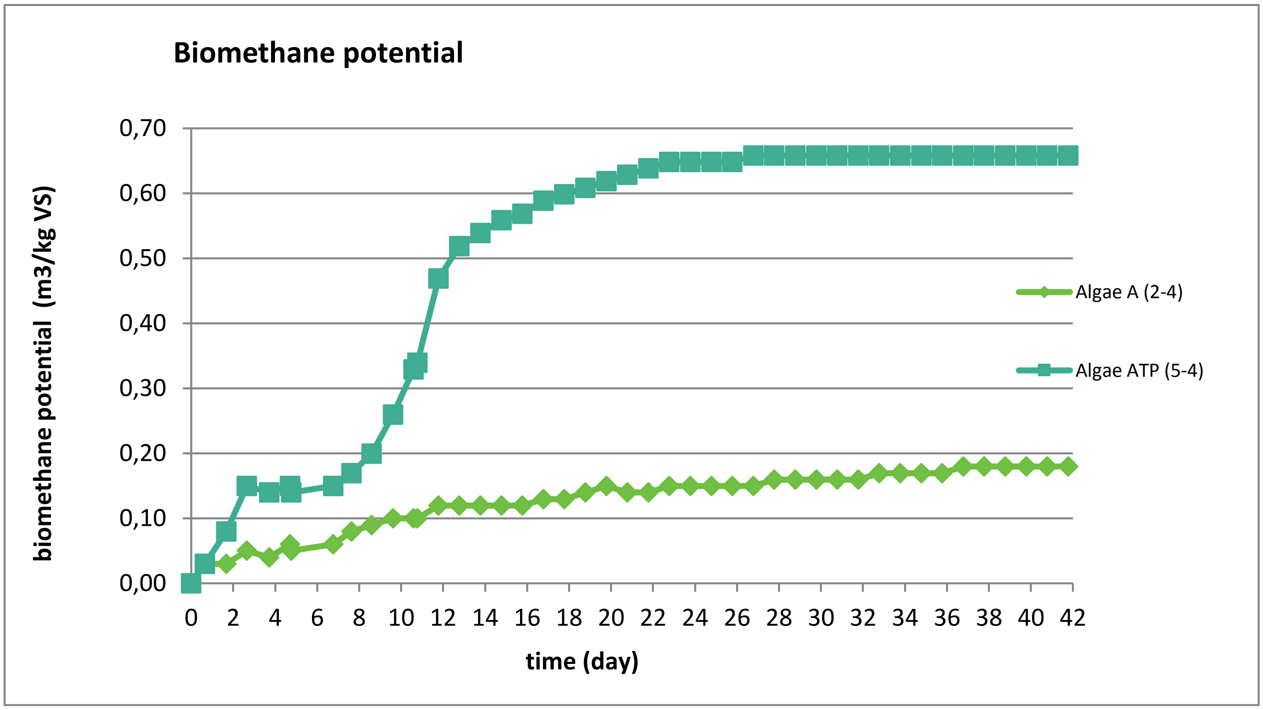


Figure 29 Biomethane potential of native and thermally treated algal biomass

BMP test 2

Test with ABG native (A), thermally treated (TPA and algal biomass bioaugumentation with bacteria C.t. ABA+C.t. was done in external laboratory in July - August 2016.

A = microalgae

ATP = thermally pretreated microalgae,

ABA-C.t. = bioaugumentataion microalgae + *Clostridium thermocellum*

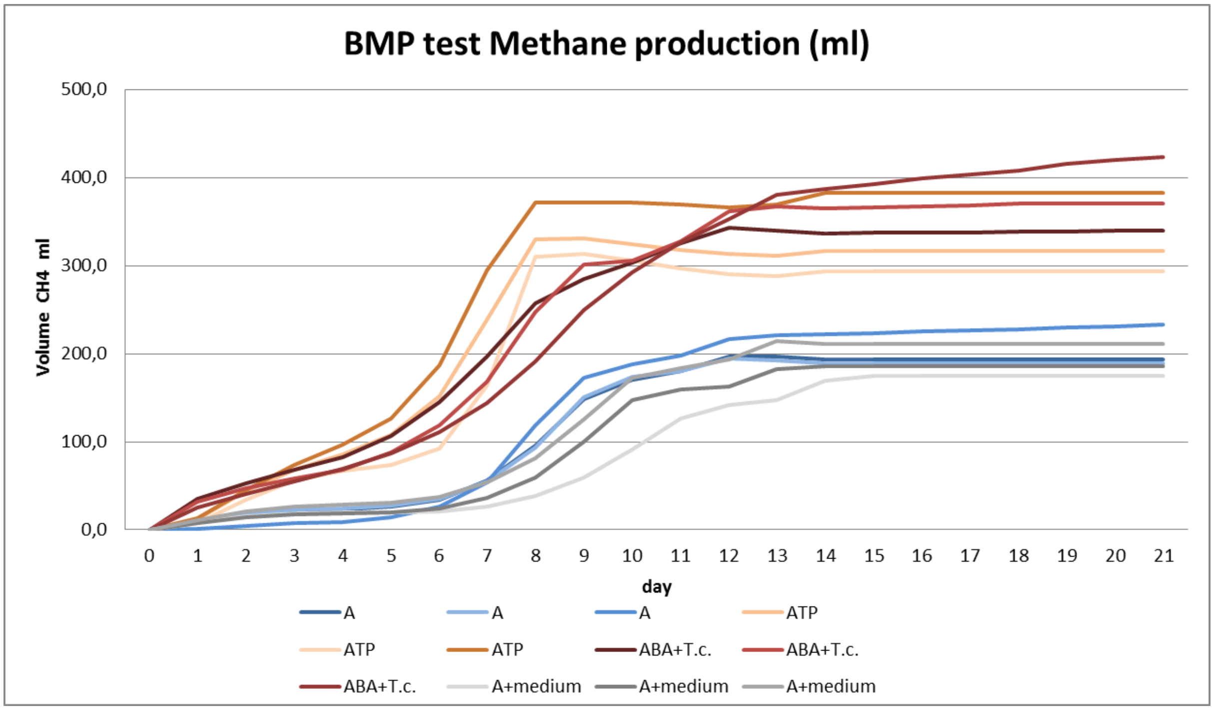


Figure 30 Biomethane potential of untreated algal biomass and thermally treated algal biomass

Table 8 Biomethane production of native A, thermally pretreated ATP and bioaugumented microalgal biomass ABA-C.t.

Mark of test mixture	g VS substrate / 2 g VS inoclum	V CH4 total (mL)	V CH4 per 1 g VS substrate
A	1,30	204,85	157,49
ATP	1,30	330,75	254,49
ABA+C.t.	1,30	377,95	290,58

Legend: A = microalgae, ATP = thermally pretreated microalgae, ABA-C.t. = bioaugumented microalgae (*Clostridium thermocellum*)

Biomethane potential of bioaugumented algal biomass with bacteria *Clostridium thermocellum* 290,6 mL CH_4/g VS was the highest. By non treated microalgae it was produced 157,5 mL CH_4/g VS. By thermally pretreated microalgae was production 254,5 mL CH_4/g VS, higher than by non treated microalgae. Organic load by BMP test was set to 1,3 g VS substrate/2g VS inoculum.

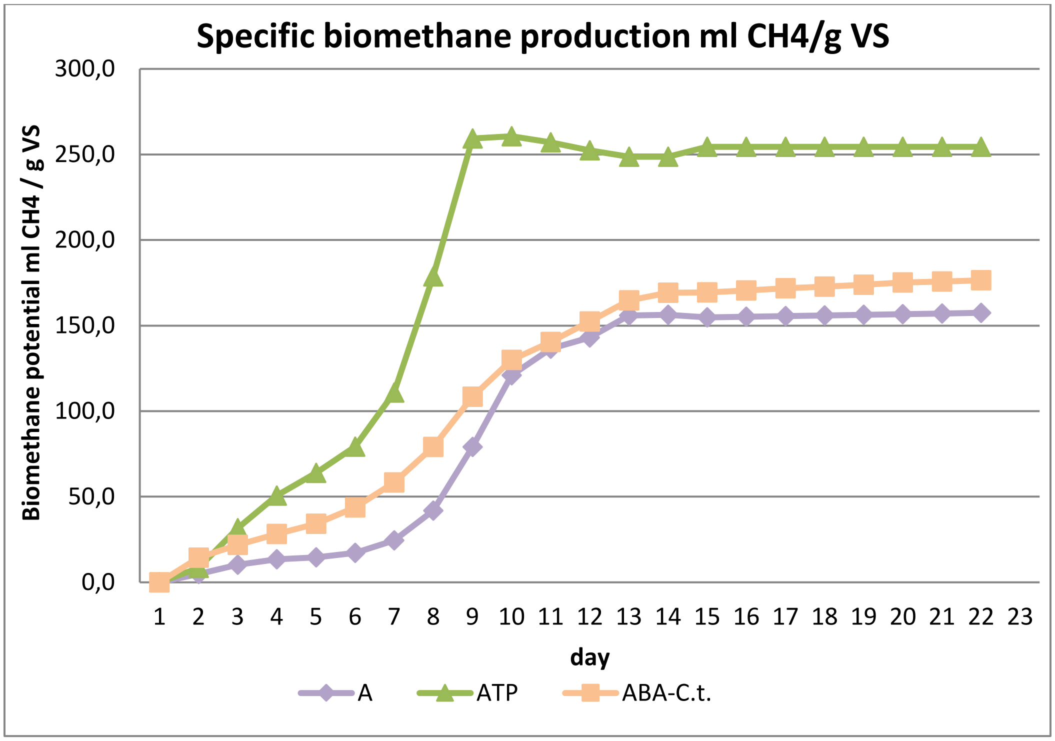


Figure 31 Biomethane potential of native, thermal treated and biological treated microalgal biomass

BMP test 3

Test with same native, thermally treated and algal biomass ABG bioaugumentation with bacteria C.t. (still ongoing, day 20 of measured biomethane production) in external laboratory, started in August 2016. Higest methane production is observed by thermally pretreated microalgae.

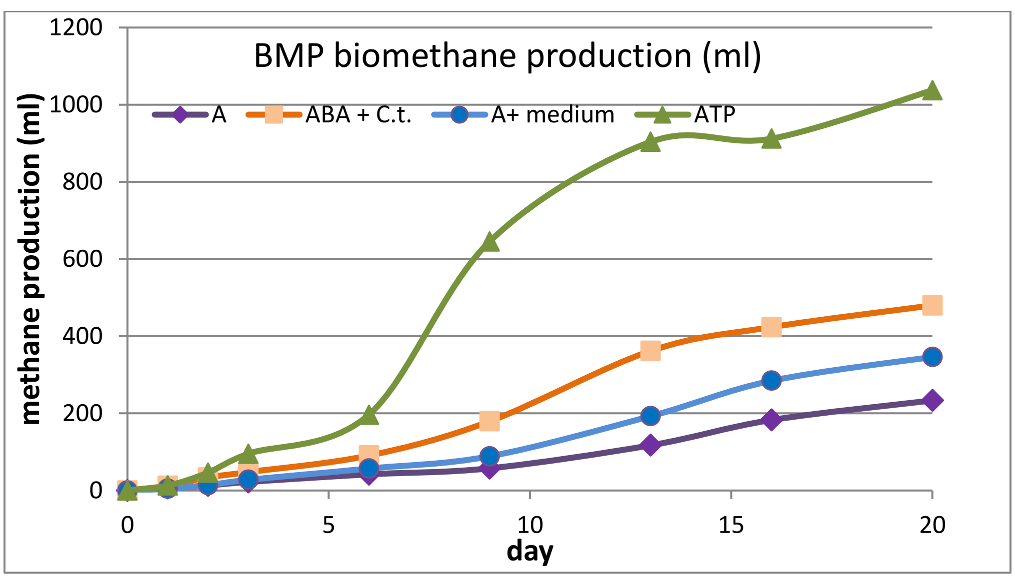


Figure 32 Biomethane production of native, thermal treated and biological treated microalgal biomass

5.7 Biomass yield

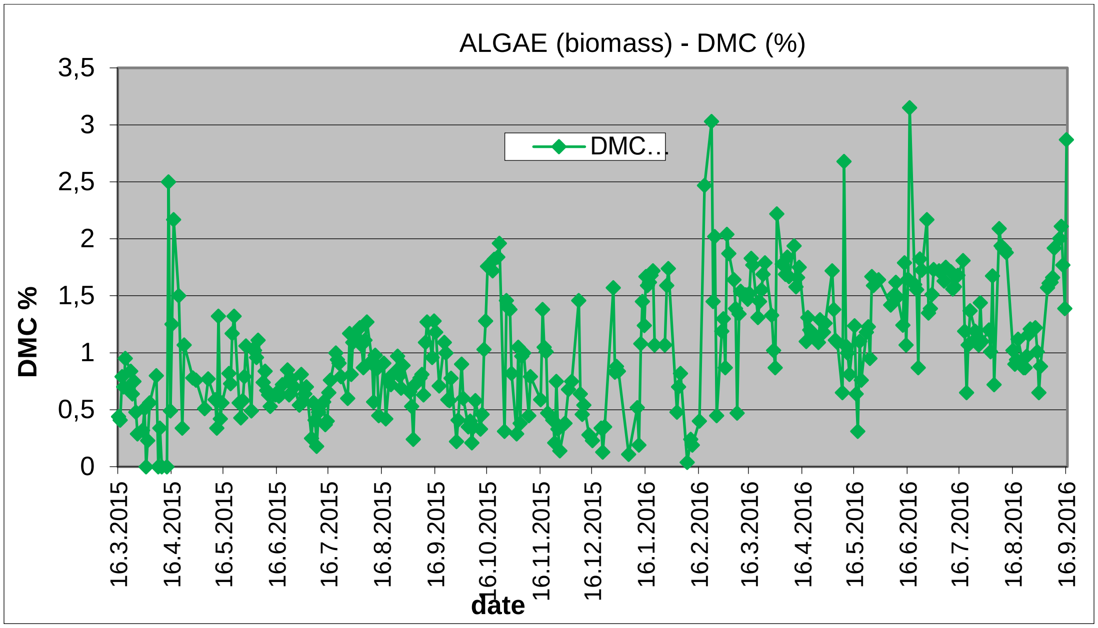


Figure 33 DMC of produced algal biomass

Algal biomass has been harvested each day manually, except in period when process wasn't stable. Algal biomass contained 0,5- 2% DMC.

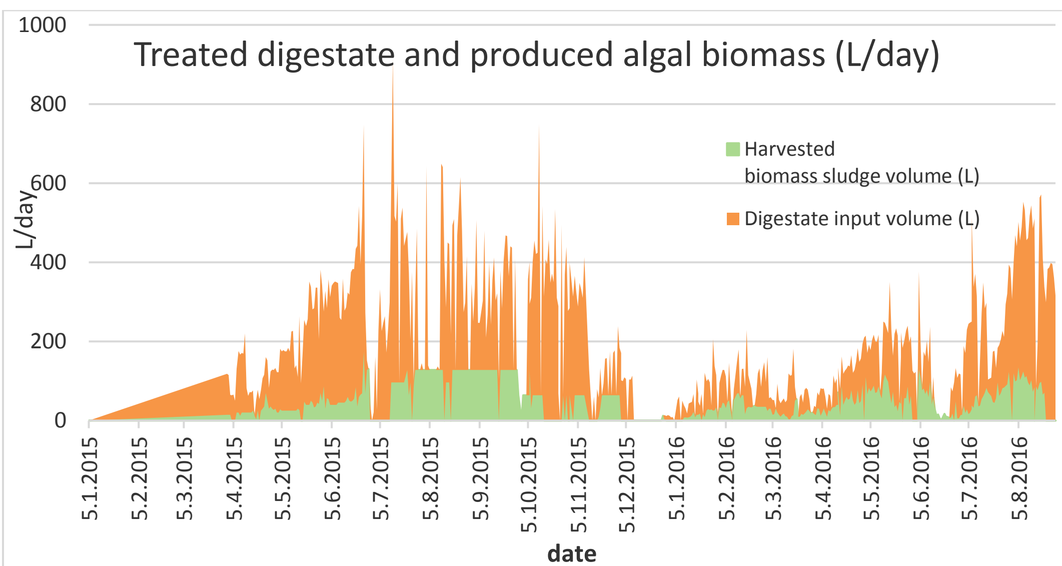


Figure 34 Treated digestate and produced algal biomass (L/day)

5.8 Pathogens

Microbiology analysis of ABG samples were performed in 2016. Samples for microbiology analysis were sampled in ABG demo centre in year 2016 on June 13., June 17., August 5. and August 23. Samples were sampled on 4 different locations in ABG system:

1. BIOGAS DIGESTATE – biogas digestate before entering the ABG system
2. UV TREATED BIOGAS DIGESTATE – biogas digestate sampled after UV light treatment
3. ABG MAIN POND – sample of main pond in ABG system
4. OVERFLOW FROM THE SEDIMENTER – output, supernatant from the sedimenter

Result are presented in Table .

Results show changes of microbiology parameters during sampling period. Samples analysed in June are negative on all tested parameters while samples analysed in August show presence of *Salmonella*, *Enterococci* and *E. coli*.

Biogas digestate sampled on August 5. is positive on *Salmonella* and *Enterococci*. *E. coli* was not detected in biogas digestate. In biogas digestate samples after UV treatment Enterococci were not detected, while *Salmonella* was still detected. Presence of *E. coli* and *Enterococci* was confirmed in samples from main ABG algae pond, while only *Enterococci* were detected in samples overflow from the sedimenter.

Microbiological analyses of samples sampled on August 23. show presence of *Salmonella* in biogas digestate and in biogas digestate treated with UV light while no *Enterococci* and no *E. coli* were detected. Samples of main ABG pond and overflow from the sedimenter were positive on *Enterococci* and negative on *Salmonella* and *E. coli*.

Salmonella are gram negative bacteria which can be found in/on many animals and in the environment. Many different serovars of Salmonella genus are pathogenic and can cause serious health issues in humans. Due to this reason Salmonella species have to be removed from the WW during WW treatment process. Bacteria from genus *Salmonella* are non-spore-forming bacteria and are quite sensitive on changes in environment. Due to this reason *Salmonella* cells can be destroyed with pasteurisation using T above 60 °C for 10 min or 70 °C for 1 min. In company KOTO all material entering in the anaerobic fermentation is sterilised at 130 °C and high pressure 3 bar for min 20 minutes. Sterilisation process removes all microorganisms from the substrate and presence of *Salmonella* in biogas digestate is highly unlikely.

Presence of *Salmonella* in biogas digestate samples in August 2016 is result of contamination of biogas digestate with WW. The centrifuge for separation of biogas digestate on solid and liquid phase is shared with biological WWTP, where all WW's from company are collected and treated. Bacteria from genus *Salmonella* can form biofilms which are hard to remove from pipes of the system and could present a problem to operator and additional costs in ABG demonstration centre.

In ABG demo centre UV lamp is installed on entry pipe to the algae pond for removal of microbiological contamination and potential pathogens. *Salmonella* was detected in samples of biogas digestate before and after UV treatment. This indicates that present UV lamp is not adequate for *Salmonella* removal, however at the same time UV lamp remove *Enterococci* in the same samples. *Salmonella* bacteria can be attached to particles or are present in small flocs in the biogas digestate, in which UV light cannot penetrate. For total

Salmonella removal from biogas digestate additional UV lamp or longer UV lamp could be mounted on the entry pipe. Identified *Salmonella* in the biogas digestate is *Salmonella enterica subsp. enterica* O:7, which is not the most pathogenic serovar such as O:9 or O:12, and do not present serious threat for human health of operators. *Salmonella* serovar O:7 is common serovar in poultry.

Nevertheless *Salmonella* was detected in biogas digestate, microbiology analyses showed no presence of *Salmonella* in samples of ABG main pond and overflow from the sedimenter. This indicates that the conditions in ABG main pond are not favourable for *Salmonella* and that *Salmonella* cannot survive in the algal pond. Negative impact for *Salmonella* removal can be caused by biological factors such as presence of microalgae and other microorganisms which have negative impact on *Salmonella* and physio-chemical factors such as sunlight, high concentration of dissolved oxygen and salinity. We can conclude that ABG system is safe for operators regarding *Salmonella*.

Microbiological parameters *Enterococci* and *Escherichia coli* are indicator parameters for faecal pollution of water. The process in WWTP should remove the vast majority of *Enterococci* and *E.coli* from the discharge from WWTP. In ABG demonstration centre *Enterococci* and *E. coli* were detected in samples sampled on August 5. and August 23., while samples sampled in July were negative on all microbiology parameters.

Enterococci were detected in biogas digestate samples sampled on August 5. and were removed from biogas digestate with UV lamp. The results show presence of *Enterococci* in main ABG pond with almost the same concentration as in biogas digestate as well as in samples of overflow from the sedimenter. Furthermore *E. coli* was detected only in samples of main ABG pond on August 5. This results indicates possible contamination with *Enterococci* and *E. coli* from outside sources. The source of contamination can be insects, visitors, operators of the ABG demo centre and common use of equipment (containers, buckets etc.) from biogas plant and WWTP, which is often used for manual operation such as returning of microalgae sediment from the sedimenter. High concentration of *Enterococci* was detected in samples of overflow from the sedimenter last sampling on August 23. This result stands out from the other results. *Enterococci* are gram positive bacteria and can survive in water environment and can accumulate in the sedimenter. This could be the reasons for high concentration of *Enterococci* in overflow from the sedimenter. *Enterococci* usually don’t multiply in water environment, especially in the sedimenter, where they don’t have optimal conditions (T, pH, salinity etc).

Samples of the overflow from the sedimenter present discharge from the ABG centre and microbiology parameters of the discharge should not exceed limiting parameters determined by local regulations. By Slovenian legislation only microbiological parameters of water discharges from municipial WWTP are regulated. ABG system can’t be ranked as municipial WWTP and the limiting values of microbiology parameters can’t be compared. Since regulations for industrial or similar biogas digestate treatment process don’t exist, we will compare microbiology parameters from ABG centre with existing parameters valid for municipial WWTPs.

The limiting values of microbiological parameters in discharges from WWTPs are determined by the Decree on the emission of substances in waste water discharges from municipal wastewater treatment plants (Official Gazette of RS, No 98/15) and are for *Enterococci* 400 cfu/100 mL for discharge to freshwaters and 200 cfu/ 100 mL for discharge to sea. Limiting values for *E. coli* are 1000 cfu/100 mL for discharge to freshwaters and 500 cfu/ 100 mL for discharge to sea. Table 9 show comparison of measured microbiology parameters in ABG centre with limit values determined by the Decree on the emission of substances in waste water discharge from municipal wastewater treatment plants (Official Gazette of RS, No 98/15). However ABG demonstration centre is not municipal wastewater treatment plant and shown microbiological parameters are not valid for ABG system.

Table 9 Limit values for waste water effluent to freshwater or sea

Microbiology parameter	**Unit**	**Limit**		**Sample - overflow from the sedimenter**			
		freshwater	**sea**	13.Jun.2016	17.Jun.2016	5.Aug.2016	23.Aug.2016
Enterococci	cfu/100 mL	400	200	0	0	21000	159000
Escherichia coli	cfu/100 mL	1000	500	0	0	0	0

Table 10 Analysis results of four samples, four replications

	Sample	Analysis	13. June 2016	17. June 2016	5. August 2016	23. August 2016	METHOD
1	**BIOGAS DIGESTATE in-flow to ABG**	*Salmonella*	Negative in 25 g sample	Negative in 25 g sample	Positive in 25 g sample; Salmonella enterica subsp. Enterica O:7 (S-312/16)	Positive in 25 g sample; Salmonella enterica subsp. Enterica O:7 (S-342/16)	ISO 6579:2002/ Amd 1:2007
		Enterococci	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Positive in 0.1 g (60 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Bacteriological test
		E.coli	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Bacteriological test
2	**UV TREATED BIOGAS DIGESTATE**	*Salmonella*	Negative in 25 g sample	Negative in 25 g sample	Positive in 25 g sample; Salmonella enterica subsp. Enterica O:7 (S-313/16)	Positive in 25g sample; Salmonella enterica subsp. Enterica O:7 (S-343/16)	ISO 6579:2002/ Amd 1:2007
		Enterococci	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Bacteriological test
		E.coli	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Bacteriological test
3	**ABG MAIN POND**	*Salmonella*	Negative in 25 g sample	Negative in 25 g sample	Negative in 25 g sample	Negative in 25 g sample	ISO 6579:2002/ Amd 1:2007
		Enterococci	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Positive in 0.1 g (55 CFU/g)	Positive in 0.1 g (<10 CFU/g)	Bacteriological test
		E.coli	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Positive in 0.1 g (<40 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Bacteriological test
4	**OVERFLOW FROM THE SEDIMENTER**	*Salmonella*	Negative in 25 g sample	Negative in 25 g sample	Negative in 25 g sample	Negative in 25 g sample	ISO 6579:2002/ Amd 1:2007
		Enterococci	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Positive in 0.1 g (210 CFU/g)	Positive in 0.1 g (1590 CFU/g)	Bacteriological test
		E.coli	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Negative in 0.1 g (<10 CFU/g)	Bacteriological test

5.9 Microbiology

Initial microalgal inoculum has been described in report D 3.5. Introduction of other species (besides inoculated) in semi open pond was noticed during the 20 month operation. Algae are brought to the media by wind, dust, visitors and small insects (in spite of insect nets noticed some in greehouse).

Microbial community in the AlgaeBioGas system has been regularly microscopically monitored (Am-Scope, model T690C-PL). Monitoring includes sampling of inoculation pond and raceway pond, microscopy and identification of microalgae species. Results of selected dates are shown in 19 in Appendix 10.

Reasons for changes in microalgal community are changing conditions of ABG process, which were necessary during the optimisation of the demo centre. Important factor is seasonal change of weather and temperature variation, illumination and humidity in different seasons of the year.

Detailed microbial community analysis is described in D3.7 *Input, output and biomass analysis*, up to March 2016. As seen in Table 19, *Ankistrodesmus* sp. dominated in the ponds until summer, accompanied by *Monoraphidium* sp. and sometimes some *Scenedesmus* sp. and *Chlorella* sp., as well as cianobacteria, but later was mostly present as a cianobacterial bloom on top of the ponds. From July 2016 change in community is seen, since *Scendesmus* sp. starts to appear in higher numbers and by the end of August 2016 *Scenedesmus* sp. (mostly *Scenedesmus acuminatus* and some *Scenedesmus quadricauda*) dominate together with *Ankistrodesmus* sp. and *Monoraphidium* sp., in approx. same proportions. At the end of July 2016 another new species is observed and by the end of August is present in moderate numbers. We were unable to identify this species. It appears as 3 to 4 smaller circular green cells together. In the optimal operation mode (Summer 2016) microscopy revealed that algal cells are nice and green (no depredating cells seen as in some periods of November 2015 and later) and culture in the ponds is dense, which was also confirmed by absorbance measurements of the samples taken daily.

As detailed described in D3.6 *Report on algal culture maintenance*, we can conclude that microbial community in the ponds changes according to season and consequently operation mode (different amounts of digestate-nutrients and light availability). There seems to be 2 prevail types of cultures: winter and summer one. In the winter one (October to March) *Ankistrodesmus* sp. dominates, while in the summer one (April-August) *Scenedesmus* sp., *Ankistrodesmus* sp. and *Monoraphidium* sp. dominate. Cianobacteria are also present in higher numbers in the summer time.

6 Observations, problems, performance

6.1 Problems

There are several problems we were facing during demonstration centre operation and which should be addressed in the future, if possible:

- Sedimentation in current sedimenter is not sufficient, due to sedimenter design,
- SCADA system (control system) resets periodically which affects set values,
- electricity blackout-resets set parameters like in March 2016 and April 2016, backup system should be in place,
- sensors malfunction due to pond level drop-affects the inlet of digestate (like in 30.3.16).

6.2 Improvements from M26

In period M25 – M36 the following maintenance work has been performed:

- New mixer for main pond with stronger axis

The axe of first mill mixer was under - dimensioned. Because of that the axe has been broken several times and consequently other parts of mixer were destroyed. We designed new stronger mixer which operates continuously since repair.

- Repair of sedimenter ring

Sedimenter ring was leaking on the bottom. Additional welding on the entire circumference was done. We tried to seal it with silicon but it wasn't good enough.

- Several improvements of flow sensors (sedimenter over-flow)
- Software improvements
- New pump and pipe for algae biomass
- New solenoid valve for inlet water
- Valve for sampling of algae biomass
- Fixing of CO2 supplier in the pond
- Renew of CO2 cooling exchanger
- Cleaning of CO2 blower.

CO_2 addition: CO_2 was collected in cooper pipes which continuously broke (several holes present), therefore pipes were changed for stainless steel which solved the problem. CO_2 addition to main pond was limited due to the presence of organic carbon present in digestate. Test were done trying to distinguished the optimal amount of digestate added in order to assure CO_2 addition, but final conclusion was that CO_2 addition is of minor importance in comparison with addition of digestate.

One of the problems observed was also SCADA system restart, which erased all the set parameters. This error was fixed.

In February 2016 (M30) fan in the greenhouse was connected to PAR sensor in order to lessen the effect of fog on light penetration. Light in winter is already scarce in this geographic region and due to substantial temperature difference in the greenhouse, compared to the outside temperature, a lot of fog was forming in the greenhouse, obstructing the light source for algae. Fan was turned on when light appeared.

6.3 Possible future improvements

Through changing different parameters and taking into account outside factors (weather, microbial community, digestate specifics) we established set of parameters for optimal operation mode at load of 500 L of digestate per day. Of course, improvements are always possible and once we have the system in optimal operation mode several factors could be improved:

- **Sedimentation** is the main drawback of the system and we believe DAF (diffusion air flotation) would improve the sedimentation. More successful harvesting would result in less algae in the water, hence more light availability and possibly bigger input of digestat, up to 800 L.
- **Optimisation of temperature control**; when air starts to cool down, control of water temperature must be switched on immediately, to prevent bigger drops in temperature.

- Measuring system for **overflow** from sedimenter needs to be improved for small flows and surges of ww.
- Separate **pipes** for water and digestate addition to ensure better control of the added volume.

6.4 Electricity consumption

Table 11 electricity consumption units with installed power

Unit	Installed power
Chiller	8 kW
Pump	0,3 kW
Mixer in pond VB	2,2 kW
Mixer in pond MB	1,5 kW
Fan greenhouse	0,5 kW
Air conditioner – container	1 kW
Sedimenter	0,25 kW

The biggest electricity consumption unit is chiller. Chiller has been installed for cooling of ponds in summer time. Chiller haven't operated a lot. Chiller operated only in summer 2015, when electricity consumption was the highest (Jul – Sep 2015 941-1112 kWh/ month). For that reason were highest electricity consumption in July 2016. The largest share of electricity consumption go to mixers in pounds.

For heating and cooling of container with Electro equipment and control room we installed air condition. Higher electricity consumption was observed in some winter months. Air condition electricity consumption would be minor by scale up of the system.

Table 12 Electricity consumption at Demo centre (Oct 2014- Aug 2016)

Month/year	**Meter state** kWh	**Consumption** kWh
2014		
Oct	320,34	559,75
Nov	962,51	642,17
Dec	1919,36	956,85
2015	1919,36	
Jan	2779	859,64
Feb	3531	752
Mar	4156	625
Apr	4741	585
May	5256	515
Jun	5859	603
Jul	6971	1112
Aug	7968	997
Sep	8909	941
Oct	9644	735
Nov	10219	575
Dec	11139	920
2016	11139	
Jan	11943	804
Feb	12625	682
Mar	13303	678
Apr	13815	512
May	14288	473
Jun	14871	583
Jul	15666	795
Aug	16468	802

7 Conclusion

We have demonstrated technology in continuous operation for 2 years in outdoor system. The location in Ljubljana is not fully appropriate for ABC technology because of climate conditions (often fog in the morning). First winter we were optimising the process and second winter it was extremely rainy. Undo process difficulties demo plant operated continuously and with recorded data and analysis results we managed to define optimal process conditions and to test the technology in appropriate scale in real environment.

Gained experience at operation of demonstration centre are key for new successful projects for algal treatment of digestate. With additional knowledge about digestate treatment process management is team highly competent for new projects.

The demonstration centre has proved the AlgaeBioGas process is technically feasible and performs well. Further research is needed to improve technical details and to prepare solution for the full scale plant for algal treatment of digestate, for which we can guarantee the continuous efficient performance.

8 Appendix Analytical methods used

Methods for internal analysis were reported in D 3.5. In internal laboratory KOTO additional analytical methods used were:

KOTO Absorbance

spectrophotometric method. Sample is homogenised and poured in 1 cm cuvette. Abosrbance is measured by 665 nm. Measurement is done in three re-applications. (Merck, nova 60)

KOTO Chloride

Measuring range: 2.5 – 250.0 mg/L Cl-

Chloride ions react with mercury (II) thiocyanate to form slightly dissociated mercury (II) chloride . The Thiocyanate released in the process in turn reacts with iron (III) ions to form red iron (lll) thiocyanate that is determined photometrically. The method is analogous to EPA 325.1 and APHA 4S00-Cl^-E. (Merck, nova 60) spectrophotometric method (ISO 8466-1 in DIN 38402 A51) with kuvette test.

In external laboratories they use following standard and other methods.

Algal biomass chemical analysis were performed in External laboratory with following methods:

The sample of algae biomass was oven dried at 60 °C to obtain the air-dried sample. This sample was homogenized and analysed. The dry sample was further dried at 105 °C and the weight losses during first (60 °C) and second drying (105° C) were taken into account to calculate the total water content and dry matter of the sample.

By ashing the sample at 550°C we obtained the ash content/remain after ignition. The loss on ignition/total organic matter of the sample was calculated by subtracting the ash content from the dry matter content.

The nitrogen content was determined by Kjeldahl method and the content of crude protein calculated by multiplying the N content by factor 6,25 (this factor is used for animal feed).

The fiber content was determined as the insoluble residue of an acid hydrolysis of the sample followed by an alkaline one by Fibercap method.

Solvent (petroleum ether) extraction was used to determine the crude fat content.

For the determination of carbon content the wet (sulfochromic) oxidation of the sample was used and the final spectrometric determination was used.

C/N ratio was calculated from separate results for both elements.

The residue after ashing was dissolved in hidrochloric acid and in this solution the content of Calcium (Ca), Magnesium (Mg), Potassium (K) and Sodium (Na) was determined by flame atomic absorption spectrometry (FAAS). Phosphorus (P) was analysed in the same solution using Molibdo-Vanadate spectrometric method.

Aqua regia extraction was applied and the content of metals: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) was determined in the extract using flame (FAAS) and electro-thermal atomic absorption spectrometry (ETAAS).

For analysis of digestate supernatant (inflow) and outflow waste water from algal pond/sedimenter in external laboratory following Standard analysis methods have been used:

- NH4-N (mg/L) SIST ISO 5664:1996,
- DMC (mg/L) SIST EN 14346:2007, method A"
- ash (mg/L) SIST EN 15169:2007
- COD (mg O2/L) SIST ISO 6060:1996"
- TN (mg/L) mOV070:2014, int. method "
- NO3N (mg/L) SIST EN ISO 10304-1:2009, ISO 10304-1:2007/Cor1:2010
- NO2N (mg/L) SIST EN ISO 10304-1:2009, ISO 10304-1:2007/Cor1:2010
- Chlorides Cl- (mg/L) SIST EN ISO 10304-1:2009, ISO 10304-1:2007/Cor1:2010
- PO4 3-(mg/L) SIST EN ISO 6878:2004 section 8 modified
- TOC (mg/l) SIST ISO 8245:2000.

Bacteriology: Pathogens were analysed in external laboratory with standard and ‘in house’ methods:

Salomonellae spp. SOP 208; version 3, mass spectrometry, White-Kauffman-Le Minor SOP 7, version 5

Determing no. of bacteria *E. coli* on agar 3M Petrifilm: E. coli and Coliform Count Plates, (dilution 10-1 and 10-2 in saline solution), incubation 37°C/24 - 48 h

Determing No of *Enterococae* on agar (Slanetz and Bartley Agar), (dilution 10-1 and 10-2 in saline solution), incubation 37°C/24 - 48 h

For BMP test in external laboratory AMPTS II Bioprocess control equipment has been used.

9 Appendix Analysis results

Digestate characteristics

Characteristics of digestate on inflow to HRAP slightly changed (M13-M36), in average COD of digestate has been 7330 mg/l, pH 7,7 and ammonia nitrogen 1.440 mg/l.

Table 13 Composition of digestate/inflow (COD, pH value, VFA, NH4-N), analysis results, internal laboratory

Date	COD (mg O_2/l)	estimated BOD (mg O_2/l)	pH	VFA mg/l	NH4-N mg/l
4.and 5.8.14	2.660	1.748	7,75	503	1.410
6.and 7.8.14	2.660	1.748	7,72	483	
7.and 8.8.14	2.240	1.472	7,70		
11.and 12.8.14	2.310	1.518	7,80	478	1.320
18.8.2014	5.765	3.788	7,68	575	1.470
21.8.2014	5.895	3.873	7,88	531	1.420
25.8.2014	5.950	509	7,87	509	1.510
27.8.2014	6.068	3.987	7,71	502	1.490
1.9.2014	6.250	4.106	7,74	501	1.560
3.9.2014	7.125	4.681	7,65	616	
4.9.2014	6.785	4.458	7,68	560	
9.9.2014	2.740	1.800	7,63	573	1.330
10.9.2014	3.665	2.408	7,58	525	
15.9.2014	7.940	5.217	7,68	630	1.091
17.9.2014	7.585	4.983	7,72	676	
18.9.2014	7.440	4.888	7,64	695	
22.9.2014	7.390	4.855	7,71	640	1.380
25.9.2014	7.340	4.822	7,76	603	1.380
29.9.2014	8.145	5.351	7,68	600	1.320
6.10.2014	7.960	5.230	7,91	614	1.420
7.10.2014	6.465	4.248	7,73		
9.10.2014	7.710	5.065	7,86	643	1.370
13.10.2014	7.290	4.790	7,82	477	1.370
16.10.2014	5.925	3.893	7,79	450	1.370
20.10.2014	6.120	4.021	7,76	548	1.240
24.10.2014	7.025	4.615	7,57	544	1.370
27.10.2014	5.885	3.866	7,67	460	1.350
29.10.2014	6.620	4.349	7,64	527	1.350
3.11.2014	6.725	4.418	7,76	391	1.390
11.11.2014	8.005	5.259	7,64	754	1.050
13.11.2014	7.085	4.655	7,69	497	1.050
17.11.2014	6.425	4.221	7,62	587	1.120
19.11.2014	6.155	4.044	7,65	587	
21.11.2014	6.156	4.044	7,63	446	
24.11.2014	6.156	4.044		586	1.390
25.11.2014	9.700	6.373	7,73		

Date	COD (mg O_2/l)	estimated BOD (mg O_2/l)	pH	VFA mg/l	NH4-N mg/l
27.11.2014	7.205	4.734	7,74	448	
1.12.2014	9.600	6.307	7,65	550	1.122
3.12.2014	9.600	6.307	7,52		
5.12.2014	7.270	4.776	7,55		
8.12.2014	9.200	6.044	7,61	587	1.460
11.12.2014	9.600	6.307	7,39		
15.12.2014	10.000	6.570	7,59	572	1.131
17.12.2014	9.300	6.110	7,60	431	
30.12.2014	9.700	6.373	7,64	438	1.540
5.1.2015	9.930	6.524	7,70	690	1.410
8.1.2015	12.700	8.344	7,70	610	
12.1.2015	11.100	7.293	7,70	688	680
14.1.2015	11.000	7.227	7,71		
15.1.2015	10.300	6.767	7,70		
19.1.2015	10.800	7.096	7,81	858	1.430
22.1.2015	11.300	7.424	7,65	948	
26.1.2015	9.900	6.504	7,67	806	1.379
29.1.2015	12.000	7.884	7,60	974	
2.2.2015	11.700	7.687	7,81	866	1.540
5.2.2015	10.000	6.570	7,55	932	
9.2.2015	8.900	5.847	7,75	746	
12.2.2015	9.200	6.044	7,72	1.557	
16.2.2015	7.975	5.240	7,66	884	1.390
19.2.2015	9.355	6.146	7,63	948	
23.2.2015	6.850	4.500	7,58	788	1.240
24.2.2015	8.400	5.519	7,62		
26.2.2015	8.455	5.555	7,58	1.280	
2.3.2015	8.625	5.667	7,55	966	1.350
5.3.2015	7.270	4.776	7,69	890	
9.3.2015	8.015	5.266	7,64	628	1.250
12.3.2015	8.205	5.391	7,56	608	
16.3.2015	9.610	6.314	7,55	1.350	1.450
19.3.2015	8.540	5.611	7,54	748	
23.3.2015	8.035	5.279	7,58	733	1.360
26.3.2015	7.385	4.852	7,52	807	
30.3.2015	7.700	5.059	7,65	680	1.280
2.4.2015	8.730	5.736	7,59	910	
7.4.2015	6.805	4.471	7,72		
13.4.2015	7.760	5.098	7,51	800	1.210
16.4.2015	8.830	5.801	7,52	930	
20.4.2015	8.220	5.401	7,61	653	1.180
23.4.2015	6.245	4.103	7,60	792	
28.4.2015	7.690	5.052	7,63	488	1.310
30.4.2015	7.790	5.118	7,67	404	
5.5.2015	6.130	4.027	7,67	448	1.270
7.5.2015	6.890	4.527	7,68	520	
11.5.2015	8.230	5.407	7,70	410	1.520
14.5.2015	5.540	3.640	7,66	412	
18.5.2015	4.430	2.911	7,68	629	1.260

Date	COD (mg O_2/l)	estimated BOD (mg O_2/l)	pH	VFA mg/l	NH4-N mg/l
21.5.2015	5.340	3.508	7,64	598	
25.5.2015	6.630	4.356	7,68	250	1.470
28.5.2015	6.025	3.958	7,58	520	
1.6.2015	8.145	5.351	7,61	394	1.480
4.6.2015	7.805	5.128	7,66	460	
8.6.2015	8.295	5.450	7,71	558	1.540
11.6.2015	7.980	5.243	7,58	552	
16.6.2015	8.080	5.309	7,56		
18.6.2015	7.960	5.230	7,67		
22.6.2015	8.065	5.299	7,68	510	1.370
2.7.2015	7.980	5.243	7,67	525	1.510
6.7.2015	8.275	5.437	7,77	770	
9.7.2015	8.170	5.368	7,61	582	
13.7.2015	8.620	5.663	7,73	728	1.340
16.7.2015	7.780	5.111	7,71	808	
20.7.2015	7.690	5.052	7,77	614	1.370
23.7.2015	7.545	4.957	7,81	358	
27.-28.7.2015	7.410	4.868	7,77	427	1.450
30.7.2015	8.485	5.575	7,87	712	
4.8.2015	7.380	4.849	7,68		1.520
6.8.2015	7.095	4.661	7,75	597	
13.8.2015	6.855	4.504	7,70		1.370
17.8.2015	6.495	4.267	7,69	598	1.480
20.8.2015	6.465	4.248	7,75	590	
24.8.2015	7.350	4.829	7,71	443	1.410
31.8.2015	7.350	4.829	7,65	580	1.410
3.9.2015	5.620	3.692	7,66	632	1.400
8.9.2015	6.320	4.152	7,71	615	
10.9.2015	6.532	4.292	7,73	580	
14.9.2015	3.810	2.503	7,80		1.460
17.9.2015	3.070	2.017	7,71	525	
21.9.2015	3.520	2.313			
28.9.2015	3.045	2.001	7,83		1.480
1.10.2015	2.960	1.945	7,79	587	
5.10.2015	2.850	1.872	7,70	470	1.330
12.10.2015	3.020	1.984	7,75	442	1.270
15.10.2015	3.005	1.974	7,85	450	
20.10.2015	2.965	1.948	7,66	379	1.374
26.10.2015	3.545	2.329	7,74	650	1.350
30.10.2015	3.240	2.129	7,70	484	
2.11.2015	3.555	2.336	7,66	498	
5.11.2015	3.290	2.162	7,90		
10.11.2015	3.585	2.355	7,74	398	1.430
12.11.2015	3.750	2.464	7,75	488	
16.11.2015	3.770	2.477	7,75		1.680
19.11.2015	3.735	2.454	7,72	468	
23.11.2015	4.235	2.782	7,66	764	1.280
30.11.2015	5.035	3.308	7,80	442	1.680
7.12.2015	4.045	2.658	7,71	614	1.880

Date	COD (mg O_2/l)	estimated BOD (mg O_2/l)	pH	VFA mg/l	NH4-N mg/l
10.12.2015	5.205	3.420	7,66	832	1.790
14.12.2015	5.455	3.584	7,49	896	2.080
21.12.2015	8.345	5.483	7,56	637	
28.12.2015	9.080	5.966	7,63		
31.12.2016	7.620	5.006	7,47	814	
5.1.2016	7.825	5.141	7,00	676	1.360
7.1.2016	7.410	4.868	7,46	646	
12.1.2016	10.115	6.646	7,56		1.370
14.1.2016	10.300	6.767	7,44	617	
18.1.2016	9.615	6.317	7,77		1.450
20.1.2016	10.385	6.823	7,44		
25.1.2016	9.530	6.261	7,60	637	1.570
28.1.2016	9.570	6.287	7,52		
1.2.2016	10.635	6.987	7,54	617	1.490
4.2.2016	6.720	4.415	7,58		
9.2.2016	9.750	6.406	7,53	460	1.440
11.2.2016	9.535	6.264	7,67	682	
16.2.2016	5.620	3.692	7,60	762	1.360
22.2.2016	9.060	5.952	7,58	472	1.460
25.2.2016	8.860	5.821	7,48		
29.2.2016	8.880	5.834	7,44		1.430
7.3.2016	9.855	6.475	7,56	648	1.440
10.3.2016	9.010	5.920	7,62	494	
14.3.2016	7.590	4.987	7,55	583	1.740
17.3.2016	7.750	5.092	7,45		
21.3.2016	7.265	4.773	7,59	336	1.430
24.3.2016	7.455	4.898	7,61	378	
29.3.2016	8.330	5.473	7,63	412	1.390
31.3.2016	6.730	4.422	7,52		
4.4.2016	7.120	4.678	7,53	419	1.480
7.4.2016	6.420	4.218	7,59		
11.4.2016	8.025	5.272	7,75		1.510
14.4.2016	8.465	5.562	7,68		
18.4.1900	6.935	4.556	7,60		1.780
25.4.2016	7.320	4.809	7,46		1.690
3.5.2016	8.595	5.647	7,67		1.730
9.5.2016	9.160	6.018	7,59		1.590
12.5.2016	9.420	6.189	7,66		
16.5.2016	8.945	5.877	7,62		1.760
23.5.2016	8.860	5.821	7,81		1.730
26.5.2016	9.875	6.488	7,76		
30.5.2016	9.350	6.143	7,67		
2.6.2016	8.590	5.644	7,64		
7.6.2016	8.610	5.657	7,66		
9.6.2016	7.735	5.082	7,73		1.760
13.6.2016	11.135	7.316	7,89		1.920
16.6.2016	6.655	4.372	7,84		1.670
20.6.2016	5.555	3.650	7,85		980
23.6.2016	5.812	3.818	7,69		

Date	COD (mg O_2/l)	estimated BOD (mg O_2/l)	pH	VFA mg/l	NH4-N mg/l
27.6.2016	6.045	3.972			
30.6.2016	5.690	3.738	7,68		1.580
4.7.2016	5.280	3.469	7,81	384	1.230
7.7.2016	5.190	3.410	7,68		
11.7.2016	5.825	3.827	7,84	532	1.450
18.7.2016	5.360	3.522	7,84	458	2.870
25.7.2016	6.235	4.096	7,71	502	1.460
1.8.2016	6.095	4.004	7,73	523	
4.8.2016	4.100	2.694	7,58		1.200
8.8.2016	4.015	2.638	8,03		1.210
11.8.2016		0	7,52	547	
18.8.2016	6.140	4.034	7,64		1.320
22.8.2016	6.590	4.330	7,77	304	1.650
29.8.2016	5.980	3.929	7,79	394	1.700

Waste water on input to algal treatment (digestate) has been analysed for several parameters, with additional frequent measurements in external laboratory in last two months of operation.

Table 14 : Composition of digestate, analysis results, external laboratory

date	**DMC (mg/l)** SIST EN 14346:2007, method A	**COD (mg O2/l)** SIST ISO 6060:1996	**TN (mg/l)** mOV070:2014, int. method	**NH4-N (mg/l)** SIST ISO 5664:1996	**NO3N (mg/l)** max SIST EN ISO 10304-1:2009, ISO 10304-1:2007/Cor1:2010	**NO2N (mg/l) max** SIST EN ISO 10304-1:2009, ISO 10304-1:2007/Cor1:2010	**Chlorides Cl- (mg/L)** SIST EN ISO 10304-1:2009, ISO 10304-1:2007/Cor1:2010	**PO4 3- (mg/L)** SIST EN ISO 6878:2004 section 8 modified	**TP (mg/L)** SIST EN ISO 6787:2004 section 8 modified	**TOC (mg/l)** SIST ISO 8245:2000
25.01.2016		8611	1900	1691	0,56	0,3	1658	419	135	
09.02.2016		9033	2100	1415	0,86	0,3	1547	434	140	
14.04.2016		6331	1800	1546	0,2	0,3	1377	23,6	110	
06.05.2016		7037	2000	1486	0,2	0,3	1356	248	103	
01.07.2016	14399	5987	1693	1540	0,2	0,3	1353	173,6	73,8	1559
06.07.2016	6784	4961	1703	1548	0,27	0,3	1170	121,5	48,8	1223
13.07.2016	8586	4842	1600	1522	0,2	0,3	777	107	53	1371
20.07.2016	8099	3747	1700	1498	0,2	0,3	933	139	54	1315
27.07.2016	6468	4661	1300	1287	0,2	0,3	932	148,8	60	1361
03.08.2016	6410	4124	1300	1224	0,2	0,3	548	166,2	66	1175
10.08.2016	5278	2317	1200	1097	0,2	0,3	934	114,1	47,5	845
17.08.2016	5788	4170	1243	1118	0,2	0,3	915	143,8	60	978
24.08.2016	7305	5127	1761	1597	0,2	0,3	712	143,8	67,5	1497
31.08.2016	7165	4515	1768	1530	0,2	0,41	1043	101,7	46	1177
07.09.2016	8568	4439	1880	1653	0,2	0,3	1215	181	40	1558

Outflow waste water after algal treatment – effluent characteristics

Waste water on outflow from sedimenter has been analysed for several parameters, with more frequent measurements in external laboratory in last two months of operation.

Table 15 Analysis results of outflow waste water, external laboratory

Date	**DMC (mg/l)** SIST EN 14346: 2007, method A	**ash (mg/l)** SIST EN 15169: 2007	**VS mg/l**	**COD (mg O_2/l)** SIST ISO 6060:1996	**TN (mg/l)** mOV070:2014, int. method	**NH4-N (mg/l)** SIST ISO 5664:1996	**NO_3N (mg/l)** max SIST EN ISO 10304-1:2009, ISO 10304-1:2007/Cor1:2010	**NO_2N (mg/l) max** SIST EN ISO 10304-1:2009, ISO 10304-1:2007/Cor1:2010	**Chlorides Cl- (mg/L)** SIST EN ISO 10304-1:2009, ISO 10304-1:2007/Cor1:2010	**PO4 3- (mg/L)** SIST EN ISO 6878: 2004 section 8 modified	**TOC (mg/l)** SIST ISO 8245: 2000
25.01.2016				453	130	3,18	95	1,3	137	22,5	
09.02.2016				366	190	15,9	136	1,3	179	27,9	
14.04.2016				357	380	106	301	0,3	383	46,8	
06.05.2016				284	360	90,3	294	0,3	355	46,3	
01.07.2016	2438	1370	1068	322	293	47,5	257	0,3	306	44,6	76
06.07.2016	3342	1554	1788	274	385	79,5	307	0,34	379	47,1	99
13.07.2016	3206	1837	1369	346	430	98	324	0,6	428	53,3	123
20.07.2016	3634	1888	1746	291	500	121	366	0,78	453	55,8	132
27.07.2016	3775	1865	1910	317	440	117	327	0,3	482	48,4	128
03.08.2016	4266	2280	1986	430	580	143	458	0,7	545	55,8	145
10.08.2016	4892	2352	2540	446	650	191	518	0,44	672	62	158
17.08.2016	3925	2076	1849	330	568	147	432	0,55	590	42,2	128
24.08.2016	4755	2373	2382	493	660	179	446	0,3	567	50,8	148
31.08.2016	4909	2541	2368	374	706	218	560	0,3	656	54,6	157
07.09.2016	5612	2857	2755	460	804	253	563	0,3	907	59,5	168

Microalgal biomass

Produced biomass has been analysed for several parameters, including nutrient value and heavy metals, intensively in last two months of operation.

Analysis results of produced fresh algal biomass and dried algal biomass showed relatively stable chemical composition. High values of zinc have been observed (up to 1333 mg/kg DMC). C/N ratio of produced algal biomass was 6:1. Heavy metals concentration in biomass has not increased in 1,5 years of operation.

Table 16 Dry algal biomass analysis results, external laboratory

parameter	unit	date						average
		12.2.2015	5.2.2016	13.7.2016	21.7.2016	5.8.2016	17.8.2016	
DMC	g/kg	1000	1000	1000	1000	1000	1000	**1000,0**
Water	g/kg	0	0	0	0	0	0	**0,0**
Ash	g/kg	127	177	189	238	226	262	**203,2**
Volatile solids	g/kg	873	823	811	762	774	738	**796,8**
TN	g/kg	75,02	77	69	66	67	70	**70,7**
Protein	g/kg	469	479	431	412	418	439	**441,3**
Raw fibers	g/kg	133	90	91	63	72	50	**83,2**
Fat	g/kg	17,55	28	17	13	19	17	**18,6**
TOC (C_{org})	g/kg	485	434	424	388	380	318	**404,8**
C/N ratio	-	6,5	5,6	6,1	5,9	5,7	4,5	**5,7**
Ca	g/kg	15,2	23,7	19,4	24,1	23,2	25,3	**21,8**
Mg	g/kg	3,01	4,67	4,33	6,57	5,62	6,95	**5,2**
K	g/kg	8,39	11,7	18,3	31	27,2	36,8	**22,2**
Na	g/kg	4	9,76	16,5	26,6	23,5	35,5	**19,3**
P	g/kg	14,6	15,4	13,7	15,5	15,3	13,9	**14,7**
Cu	mg/kg	65	302	173	169	142	90,7	**157,0**
Zn	mg/kg	1229	1259	771	1333	1115	1103	**1135,0**
Cr	mg/kg	22,4	69,6	18,7	19,6	20,9	8,4	**26,6**
Ni	mg/kg	24,4	25,2	17	22,1	17,9	18,6	**20,9**
Pb	mg/kg	12	20,9	12,4	11,6	12,1	9,6	**13,1**
Cd	mg/kg	0,57	0,39	0,36	0,56	0,39	0,3	**0,4**
As	mg/kg	0,22	0,22	0,17	0,19	0,19	0,24	**0,2**

Table 27 Fresh algal biomass analysis results

parameter	unit	date					
		12.2.2015	**5.2.2016**	**13.7.2016**	**21.7.2016**	**5.8.2016**	**17.8.2016**
DMC	g/kg	63,8	8,4	13,6	10,4	13,4	8,8
Water	g/kg	936	991,6	986,4	989,6	986,4	991,2
Ash	g/kg	8,1	1,5	2,6	2,5	3	2,3
Volatile solids	g/kg	55,7	6,9	11	7,9	10,4	5,9

Table 18 Harvested biomass and added digestate per day (L) and weather conditions

Date	Harvested biomass sludge volume (L)	Digestate input volume (L)	Weather
1.4.2015	15	103	sunny/rainy/ cloudy
2.4.2015	15	100	sunny/windy
3.4.2015	15	50	sunny
4.4.2015		64	rainy
5.4.2015		66	partly cloudy /windy/low T
6.4.2015		50	sunny/windy
7.4.2015	20	111	sunny/windy
8.4.2015	20	156	sunny
9.4.2015	12	155	sunny
10.4.2015	20	151	sunny
11.4.2015	20	149	sunny
12.4.2015	20	200	sunny
13.4.2015	20	62	sunny
14.4.2015	20	42	cloudy
15.4.2015	20	52	sunny
16.4.2015	20	51	sunny
17.4.2015	25	51	cloudy
18.4.2015	12	0	rainy/cloudy
19.4.2015	3	50	sunny
20.4.2015	25	53	sunny
21.4.2015		54	sunny
22.4.2015	25	52	sunny
23.4.2015	30	77	sunny
24.4.2015	36	80	cloudy
25.4.2015	70	90	cloudy
26.4.2015	50	102	sunny/cloudy
27.4.2015	30	53	cloudy
28.4.2015	36	88	rainy/cloudy
29.4.2015	30	99	sunny
30.4.2015	27	101	sunny/clouds
1.5.2015	30	103	cloudy
2.5.2015	20	102	cloudy/sunny
3.5.2015	20	102	cloudy
4.5.2015	28	153	sunny
5.5.2015	25	150	sunny

6.5.2015	25	153	sunny
7.5.2015	25	150	sunny
8.5.2015	25	150	sunny
9.5.2015	25	159	sunny
10.5.2015	25	149	sunny
11.5.2015	25	200	sunny
12.5.2015	25	202	sunny
13.5.2015	25	107	sunny
14.5.2015	25	115	rainy
15.5.2015	30	80	rainy
16.5.2015	27	237	cloudy
17.5.2015	0	94	cloudy
18.5.2015		168	sunny
19.5.2015	48	206	sunny
20.5.2015	40	199	sunny
21.5.2015	40	217	rainy
22.5.2015	40	311	rainy
23.5.2015	50	288	rainy
24.5.2015	45	236	cloudy
25.5.2015	33	251	sunny
26.5.2015	36	300	sunny
27.5.2015	45	291	sunny
28.5.2015	42	164	sunny
29.5.2015	70	311	sunny
30.5.2015	50	292	sunny
31.5.2015	87	188	cloudy
1.6.2015	30	298	sunny
2.6.2015	57	227	sunny
3.6.2015	54	303	sunny
4.6.2015	40	272	sunny
5.6.2015	40	301	sunny
6.6.2015	40	307	sunny
7.6.2015	40	311	sunny
8.6.2015	40	310	sunny
9.6.2015	45	302	sunny
10.6.2015	45	215	sunny
11.6.2015	45	209	sunny
12.6.2015	45	314	sunny
13.6.2015	45	233	cloudy
14.6.2015	50	237	cloudy
15.6.2015	60	264	cloudy
16.6.2015	50	268	cloudy

17.6.2015	48	327	rainy
18.6.2015	50	333	sunny
19.6.2015	55	328	sunny
20.6.2015	84	350	cloudy
21.6.2015	36	406	sunny
22.6.2015	60	482	sunny
23.6.2015	66	328	cloudy
24.6.2015	69	447	sunny
25.6.2015	175	573	sunny
26.6.2015	69	204	sunny
27.6.2015	128	24	cloudy, rainy
28.6.2015	130	0	cloudy, sunny
29.6.2015	0	41	sunny
30.6.2015	0	0	foggy
1.7.2015	0	21	sunny
2.7.2015	0	161	sunny
3.7.2015	0	27	sunny
3.7.2015	0	27	sunny
4.7.2015	0	229	sunny
5.7.2015	0	332	sunny
6.7.2015	0	226	sunny
7.7.2015	0	247	sunny
8.7.2015	0	264	sunny
9.7.2015	0	172	rainy
10.7.2015	0	230	sunny
11.7.2015	0	299	sunny
12.7.2015	96	499	sunny
13.7.2015	96	816	cloudy/rainy
14.7.2015	96	421	sunny
15.7.2015	96	407	sunny
16.7.2015	96	505	sunny
17.7.2015	96	0	sunny
18.7.2015	96	414	sunny
19.7.2015	96	442	sunny
20.7.2015	96	345	sunny
22.7.2015	128	349	sunny
23.7.2015	64	310	sunny
24.7.2015	96	108	sunny
25.7.2015	0	376	sunny
26.7.2015	87	0	rainy
27.7.2015	128	193	cloudy
28.7.2015	128	357	rainy
29.7.2015	128	0	cloudy
30.7.2015	128	0	rainy
31.7.2015	128	17	cloudy
1.8.2015	128	0	cloudy
2.8.2015	128	0	cloudy
3.8.2015	128	514	sunny
4.8.2015	128	11	foggy
5.8.2015	128	0	sunny
6.8.2015	128	0	sunny
7.8.2015	128	0	sunny
8.8.2015	128	0	sunny
9.8.2015	128	0	sunny
10.8.2015	128	9	sunny
11.8.2015	128	0	sunny
12.8.2015	128	521	sunny
13.8.2015	128	512	sunny
14.8.2015	0	403	sunny

15.8.2015	96	190	sunny
16.8.2015	96	354	sunny/rainy
17.8.2015	96	350	rainy
18.8.2015	0	218	cloudy/sunny
19.8.2015	128	366	cloudy / rainy
20.8.2015	128	381	cloudy
21.8.2015	128	0	sunny
22.8.2015	128	340	sunny
23.8.2015	128	412	sunny
24.8.2015	128	487	sunny/cloudy
25.8.2015	128	351	rainy
26.8.2015	128	146	fog
27.8.2015	128	171	fog
28.8.2015	128	287	fog, sunny
29.8.2015	128	0	sunny
30.8.2015	128	0	sunny
31.8.2015	128	218	sunny
1.9.2015	128	122	sunny
2.9.2015	128	207	cloudy/sunny
3.9.2015	128	379	cloudy/sunny
4.9.2015	128	119	cloudy
5.9.2015	128	119	cloudy
6.10.2015	128	167	sunny
7.9.2015	128	175	fog
8.9.2015	128	351	fog
9.9.2015	128	82	fog
10.9.2015	128	139	fog
11.9.2015	128	273	cloudy
12.9.2015	128	114	fog
13.9.2015	128	228	fog
14.9.2015	128	284	cloudy
15.9.2015	128	176	cloudy
16.9.2015	128	194	sunny
17.9.2015	0	104	fog
18.9.2015	128	252	sunny
19.9.2015	128	155	cloudy
20.9.2015	128	276	cloudy
21.9.2015	128	340	sunny
22.9.2015	128	338	sunny
23.9.2015	128	236	cloudy
24.9.2015	128	312	rain
25.9.2015	128	309	rain
26.9.2015	128		cloudy
27.9.2015	128	282	cloudy
28.9.2015	128		sunny
29.9.2015	64		sunny
1.10.2015			sunny
2.10.2015	64		cloudy
3.10.2015	64		cloudy, rain
4.10.2015	64		cloudy
5.10.2015	64	293	cloudy
6.10.2015	64	337	fog
7.10.2015	32	279	rainy
8.10.2015	64	341	rain
9.10.2015	64	391	cloudy
10.10.2015	64	357	cloudy
11.10.2015	64	360	rainy
12.10.2015	64	686	cloudy
13.10.2015	64		rain

14.10.2015	64	478	rain
15.10.2015	0	313	rain
16.10.2015	0	407	rain
17.10.2015	0	396	sunny
18.10.2015	0	457	sunny
19.10.2015	0	348	cloudy
20.10.2015	0	372	fog
21.10.2015	0	350	fog
22.10.2015	0	534	fog, sunny
23.10.2015	0	311	sunny
24.10.2015	0	289	fog
24.10.2015	0	69	
25.10.2015	0		fog
26.10.2015	0	494	fog
27.10.2015	64		fog
28.10.2015	0	368	fog
29.10.2015	64	375	rain
30.10.2015	0	277	fog
31.10.2015		284	fog
31.10.2015	64	335	fog
2.11.2015	0	313	fog
3.11.2015	64	302	fog
4.11.2015	64	260	sunny
5.11.2015	64	224	fog
6.11.2015	64	284	fog
7.11.2015	64	268	fog
8.11.2015	64	246	sunny
9.11.2015	64	349	fog
12.11.2015		128	sunny
13.11.2015		63	fog, sunny
14.11.2015		0	sunny
15.11.2015		61	sunny
16.11.2015		0	sunny
17.11.2015		79	fog
18.11.2015	10	48	sunny
19.11.2015	32	101	cloudy
20.11.2015	64	0	cloudy
21.11.2015	64	30	cloudy
22.11.2015	64	92	cloudy
23.11.2015	64	94	fog
24.11.2015	64		cloudy
25.11.2015	64	111	sunny
26.11.2015	64		snow
27.11.2015	64	107	sunny
28.11.2015	64	113	sunny
29.11.2015	64		
30.11.2015	64	175	sunny
1.12.2015	64	120	sunny
2.12.2015	0	171	sunny
2.12.2015	0	102	fog
3.12.2015	0	100	fog
4.12.2015	0	109	fog
5.12.2015	0	105	sunny
6.12.2015	0	0	cloudy
7.12.2015	0	104	sunny
8.12.2015	0	104	fog
9.12.2015	0	113	sunny
10.12.2015	0	0	sunny
11.12.2015	0	0	fog

12.12.2015	0	0	fog
14.12.2015	0	0	cloudy
15.12.2015	0	0	sunny
16.12.2015	0	0	fog
17.12.2015	0	0	fog
18.12.2015	0	0	cloudy
19.12.2015	0	0	sunny
20.12.2015	0	0	sunny
21.12.2015	0	0	sunny
22.12.2015	0	0	sunny
23.12.2015	0	0	sunny
24.12.2015	0	0	sunny
25.12.2015	0	0	sunny
26.12.2015	0	0	fog
27.12.2015	0	0	fog
28.12.2015	12	0	fog
29.12.2015	2	12	fog
30.12.2015	0,2	11	fog
31.12.2015	0	14	cloudy
1.1.2016	0	12	cloudy
2.1.2016	0	8	cloudy
3.1.2016	0	9	cloudy
4.1.2016	0		snow
5.1.2015	0		snow
6.1.2016	0	52	snow
7.1.2016	0	61	sunny
8.1.2016	0	0	sunny
9.1.2016	0	50	sunny
10.1.2016	7	32	cloudy
11.1.2016	8	23	sunny
12.1.2016	7	11	sunny
13.1.2016	8	35	fog
14.1.2016	10	35	foggy
15.1.2016	14	33	rainy
16.1.2016	22	45	sunny
17.1.2016	8		sunny
18.1.2016	15	89	sunny
19.1.2016	15	53	sunny
19.1.2016	15	33	sunny
20.1.2016	18	19	sunny
21.1.2016		67	sunny
22.1.2016	16		sunny
23.1.2016	9	112	light snow
24.1.2016	26	74	sunny
25.1.2016	29	76	sunny
26.1.2016	27	69	sunny
27.1.2016	28	10	sunny
28.1.2016	25	181	sunny
29.1.2016	26	112	sunny
30.1.2016	33	82	cloudy
31.1.2016	0		cloudy
1.2.2016	33	62	
2.2.2016	32	81	cloudy
3.2.2016	45	83	cloudy
4.2.2016	43	85	cloudy
5.2.2016	32	80	sunny
6.2.2016	47		cloudy
7.2.2016	51	95	cloudy
9.2.2016	0	0	cloudy, rainy
10.2.2016	52	27	rainy

12.2.2016	68	81	snow
13.2.2016	70	110	snow/sunny
14.2.2016	56		cloudy
15.2.2016	64	66	rain
16.2.2016	64	67	cloudy
17.2.2016	16	69	rainy
18.2.2016	31	198	cloudy
19.2.2016	35	80	cloudy
21.2.2016	33	47	sunny
22.2.2016	36		cloudy
23.2.2016	37		cloudy
24.2.2016	36	31	cloudy, rainy
25.2.2016	35	84	sunny
26.2.2016	35	97	rainy
27.2.2016	35	50	sunny
28.2.2016	35	92	sunny
29.2.2016	35	112	rainy
1.3.2016	35	62	rainy
2.3.2016	24	136	rainy
3.3.2016	30	27	rainy
4.3.2016	30	29	cloudy
5.3.2016	15	37	rainy
6.3.2016	16	36	rainy
7.3.2016	31		rainy
8.3.2016	20	73	rainy
9.3.2016	15	38	cloudy
10.3.2016	10	34	sunny
11.3.2016	15	35	cloudy
12.3.2016	18	38	cloudy
13.3.2016	11	35	cloudy
14.3.2016	10	42	cloudy
15.3.2016	3	116	sunny
16.3.2016	3	110	sunny
17.3.2016	11	104	sunny
18.3.2016	14	167	sunny
19.3.2016	50	35	sunny
20.3.2016	54		sunny
21.3.2016	10		sunny
22.3.2016	10	50	sunny
23.3.2016	17	50	sunny
24.3.2016	15	30	sunny
25.3.2016	18	60	sunny
26.3.2016	23	60	rainy
27.3.2016	28	50	cloudy
28.3.2016	24	30	cloudy
29.3.2016	18		cloudy
30.3.2016	20		sunny
31.3.2016	17	20	sunny
1.4.2016	14	20	sunny
2.4.2016	31	40	cloudy
3.4.2016	30	33	sunny
4.4.2016	12	70	sunny
5.4.2016	20	60	sunny
6.4.2016	31	50	sunny
7.4.2016	20	40	sunny
8.4.2016	10	20	cloudy
9.4.2016	27	40	rainy
10.4.2016	23	40	sunny
11.4.2016	31		sunny
12.4.2016	34	80	sunny

13.4.2016	34	60	sunny
14.4.2016	40	66	cloudy
15.4.2016	40		sunny
15.4.2016	71	63	sunny
16.4.2016	13	67	sunny
17.4.2016	87	75	sunny
18.4.2016	40	78	sunny
19.4.2016	50	76	cloudy
20.4.2016	60	71	fog
21.4.2016	53	86	sunny
22.4.2016	55	82	sunny
23.4.2016	77	88	cloudy
24.4.2016	50,7	70	cloudy
25.4.2016	39,8	88	cloudy
26.4.2016	45,7	103	cloudy
27.4.2016	77,1	106	cloudy
28.4.2016	43,8	104	snow
29.4.2016	49,2	108	sunny
30.4.2016	90,6	104	foggy
1.5.2016		210	rainy
2.5.2016	110,2	104	rain
3.5.2016	66,2	105	cloudy
4.5.2016	82,9	109	sunny
5.5.2016	74,0	104	rain
6.5.2016	79,0	125	sunny
7.5.2016	88,1	129	sunny
8.5.2016	69,8	130	sunny
9.5.2016	71,9	127	foggy
10.5.2016	86,8	130	cloudy
11.5.2016	67,3		
12.5.2016	55,6	65	rainy
13.5.2016	98,5	152	sunny
14.5.2016	116,3	131	rain
15.5.2016	106,2	129	rain
16.5.2016	98,3	123	sunny
17.5.2016	69,2	281	foggy
18.5.2016	43,9	187	sunny
19.5.2016	72,7	91	cloudy, rain
20.5.2016	34,3	185	cloudy
21.5.2016	40,0	193	sunny
22.5.2016	23,7	191	sunny
23.5.2016	48,1		sunny
24.5.2016	42,5	118	cloudy
24.5.2016	69,5	153	cloudy
25.5.2016		184	sunny
26.5.2016	57,1	143	sunny
27.5.2016	56,6	171	
28.5.2016	85,6	154	
29.5.2016	39,4	158	
30.5.2016	62,7	150	cloudy
31.5.2016	0,0	126	rain
1.6.2016	0,0	119	cloudy
2.6.2016	0,0	123	cloudy
3.6.2016			sunny
4.6.2016	129,7	247	sunny
5.6.2016	109,8	96	cloudy
6.6.2016	76,8		cloudy
7.6.2016	81,2	93	sunny
8.6.2016	67,4	95	sunny
9.6.2016	77,8	124	cloudy

10.6.2016	58,8	89	rain
11.6.2016	97,9	139	cloudy
12.6.2016	41,6		rainy
13.6.2016	58,1	52	sunny
14.6.2016	27,5		sunny
15.6.2016	11,5		rain
16.6.2016	15,5		sunny
17.6.2016	5,9		rain
18.6.2016	0,3		
20.6.2016	17,1		rain
21.6.2016	8,9		sunny
22.6.2016	8,7		sunny
23.6.2016	6,8		sunny
24.6.2016	7,4	77	sunny
25.6.2016	17,9	56	sunny
26.6.2016	0,0	96	sunny
27.6.2016	48,7	84	cloudy
28.6.2016	5,8	102	fog
29.6.2016	5,9	113	sunny
30.6.2016	13,5	103	sunny
1.7.2016	14,5		sunny
2.7.2016	37,5	151	sunny
3.7.2016	19,1	152	rainy
3.7.2016	19,1	152	rain
4.7.2016	28,0	202	sunny
5.7.2016	16,1	228	sunny
6.7.2016	20,1	228	cloudy
7.7.2016	28,0	222	sunny
7.7.2016	38,0	476	sunny
8.7.2016	32,3	305	sunny
9.7.2016	61,0	310	cloudy
10.7.2016	63,8	250	sunny
11.7.2016	36,0		sunny
12.7.2016	38,3	116	sunny
13.7.2016	54,1	218	sunny
14.7.2016	61,3	288	cloudy
15.7.2016	70,6	213	sunny
16.7.2016	82,7	215	cloudy
16.7.2016	82,7	189	cloudy
17.7.2016	72,0	32	sunny
19.7.2016	61,8	47	fog

20.7.2016	47,0	127	sunny
21.7.2016	44,8	132	sunny
22.7.2016	57,1	76	sunny
23.7.2016	96,2	88	sunny
24.7.2016	62,7	128	sunny
25.7.2016	69,8	135	cloudy
26.7.2016	81,8	145	sunny
27.7.2016	83,6	128	sunny
28.7.2016	87,2	207	cloudy
29.7.2016	91,7	229	sunny
30.7.2016	123,0	271	sunny
31.7.2016	75,0	310	sunny
1.8.2016	0,0	306	sunny
2.8.2016	119,7	280	fog
3.8.2016	98,6	370	sunny
4.8.2016	101,8	339	sunny
5.8.2016	134,2	368	sunny
6.8.2016	101,8	353	sunny
7.8.2016	112,0	377	sunny
8.8.2016	124,1	429	sunny
9.8.2016	108,5	423	sunny
10.8.2016	113,0	343	cloudy
11.8.2016	96,2	365	cloudy
12.8.2016	88,9	428	fog
13.8.2016	102,2	442	cloudy
14.8.2016	72,9	413	sunny
15.8.2016	84,5	426	sunny
15.8.2016		500	sunny
16.8.2016	86,6		fog
17.8.2016	77,3	119	cloudy
18.8.2016	94,6	469	cloudy, fog
19.8.2016	98,2	473	sunny
20.8.2016	65,5	344	sunny
21.8.2016	70,8	267	rain cloudy
22.8.2016	0,0	299	sunny
23.8.2016	0,0	383	sunny
24.8.2016	0,0	387	sunny
25.8.2016	0,0	398	sunny
26.8.2016	0,0	395	sunny
27.8.2016	0,0	361	fog
28.8.2016	0,0	316	fog

10 Appendix: Microscopy

If not described differently algae are described as :

Ankistrodesmus (long, green “sticks”, spikes at the end; bigger size approx.70 µm.

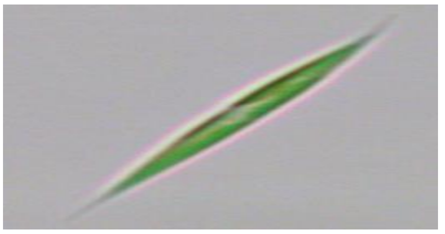


Figure 35 *Ankistrodesmus* sp., 400x

Monoraphidium: smaller than *Ankistrodesmus*, crescent shapes, sometimes spiral (cca 20 µm)

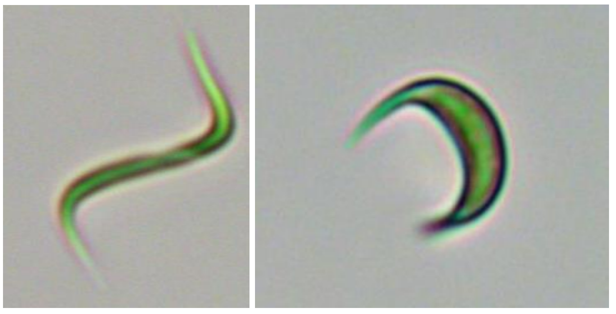


Figure 186 *Monoraphidium* sp., 400x

Chlorella: circular green cells, smaller than *Monoraphidum*

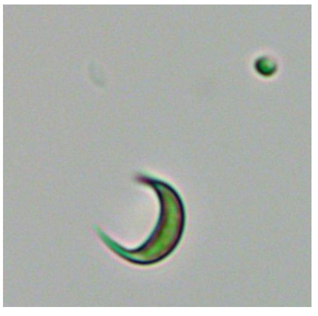


Figure 37 *Monoraphidium contortum* and *Chlorella* sp., 400x

Scenedesmus sp.:

Figure 198 *Scenedesmus quadricauda*, 400x

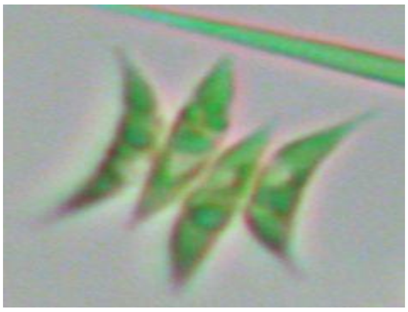


Figure 209 *Scenedesmus acuminatus*, 400x

Diatoms: approx. size of *Monoraphidium*, oval, with straight ends, cell wall distinguished; light color.

Figure 40 Diatom, 400x

Table 19 Microbial community in ABG ponds (Nov 2015-Avg 2016)

Date	Pond	Colour&density	Dominant species	Other species	Grazers
7.10.2015	VB		*Monoraphidium* sp., *Ankistrodesmus* sp.		
21.10.2015	VB		*Monoraphidium* sp., *Ankistrodesmus* sp., *Chlorella* sp.		
	MB		*Ankistrodesmus* sp.	*Monoraphidium* sp., diatoms	
28.10.2015	VB		*Monoraphidium* sp., *Ankistrodesmus* sp.		
	MB		*Ankistrodesmus* sp.	*Monoraphidium* sp.	
4.11.2015	VB		*Monoraphidium* sp., *Ankistrodesmus* sp.		
	MB		*Ankistrodesmus* sp.	diatoms, cianobacteria, *Monoraphidium* sp.	
	BM		*Ankistrodesmus* sp., cianobacteria, protozoa, fungi		
9.11.2015	VB	scarce, brown	*Monoraphidium* sp., *Ankistrodesmus* sp., *Chlorella* sp.		
	MB	dense, green	*Ankistrodesmus* sp.		
12.11.2015	VB	light green	*Ankistrodesmus* sp., *Monoraphidium* sp., cianobacteria, diatoms		
	MB	green	*Ankistrodesmus* sp.		
18.11.2015	VB		*Ankistrodesmus* sp., *Monoraphidium* sp.	diatoms, *Chlorella* sp., *Scenedesmus* sp.	cilliates
	MB	dense, green	*Ankistrodesmus* sp.		
26.11.2015	VB	brown-green	*Ankistrodesmus* sp., *Monoraphidium* sp.	diatoms	
	MB	green	*Ankistrodesmus* sp.	cianobacteria, *Scenedesmus* sp., *Chlorella* sp.	
	BM		*Ankistrodesmus* sp., *Monoraphidium* sp., cianobacteria, diatoms	protozoa	

Date	Pond	Colour&density	Dominant species	Other species	Grazers
2.12.2015	VB	light brown	*Ankistrodesmus* sp., *Monoraphidium* sp.		
	MB	green	*Ankistrodesmus* sp.		
	BM		*Ankistrodesmus* sp., *Monoraphidium* sp., cianobacteria, *Chlo-rella* sp.		
9.12.2015	VB	scarce, brown	*Ankistrodesmus* sp., *Monoraphidium* sp.	*Chlorella* sp.	ciliates
	MB	green, dense	*Ankistrodesmus* sp.		
16.12.2015	VB	scarce, brown	*Ankistrodesmus* sp., *Monoraphidium* sp.		
	MB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Scenedesmus* sp.	
	BM		*Ankistrodesmus* sp., *Monoraphidium* sp., cianobacteria, dia-toms		
21.12.2015	VB	scarce	*Ankistrodesmus* sp., *Monoraphidium* sp.		
	MB	dense	*Anksitrodesmus* sp.		
23.12.2015	VB	scarce, brown	*Ankistrodesmus* sp., *Monoraphidium* sp.	*Scendesmus* sp., other	
	MB	green	*Ankistrodesmus* sp., *Monoraphidium* sp., *Scenedesmus* sp., cia-nobacteria		
24.12.2015	VB	scarce	*Ankistrodesmus* sp., *Monoraphidium* sp.		
	MB	dense	*Ankistrodesmus* sp.		
26.12.2015	VB	scarce, green	*Ankistrodesmus* sp.	*Monoraphidium* sp.	
	MB	dense, green	*Ankistrodesmus* sp., *Monoraphidium* sp.		
29.12.2015	VB		*Ankistrodesmus* sp.	*Monoraphidium* sp.	
	MB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Chlorella* sp.	
31.12.2015	VB		*Ankistrodesmus* sp.	*Monoraphidium* sp., *Chlorella* sp.	
	MB		*Ankistrodesmus* sp.	*Monoraphidium* sp., other	

Date	Pond	Colour&density	Dominant species	Other species	Grazers
6.1.2016	VB	scarce, light green	*Ankistrodesmus* sp.	*Monoraphidium* sp.	
	MB	dark green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Chlorella* sp., diatoms, cianobacteria	
13.1.2016	VB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Chlorella* sp.	
	MB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Chlorella* sp.	
20.1.2016	VB	green	*Ankistrodesmus* sp.	*Monoraphidium* sp.	
	MB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp., diatoms	
27.1.2016	VB	dense, dark green	*Ankistrodesmus* sp.	*Monoraphidium* sp., diatoms	
	MB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Chlorella* sp.	
3.2.2016	VB	dense, dark green	*Ankistrodesmus* sp.	*Monoraphidium* sp., diatoms	
	MB	scarce, green	*Ankistrodesmus* sp.		
10.2.2016	VB	dark green	*Ankistrodesmus* sp.	*Monoraphidium* sp., other	
	MB	dense, green	*Ankistrodesmus* sp.	*Chlorella* sp.,diatoms	
17.2.2016	VB	dense, dark green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Scendesmus* sp.,other	cilliates
	MB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Chlorella* sp., cianobacteria	
	BM		Ankistrodesmus sp., Chlorella sp., Scendesmus sp., diatoms		rotifera, polychaeta, copepoda
24.2.2016	VB	dense	*Ankistrodesmus* sp.	*Monoraphidium* sp., diatoms	rotifera, cilliates
	MB	dense	*Ankistrodesmus* sp.	diatoms	cilliates
2.3.2016	VB	gren-yellow, dense	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Chlorella* sp.	rotifera,etc.
	MB	green, dense	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Scendesmus quadricauda, Chlorella sp.*	
	BM		*Ankistrodesmus* sp., diatoms, cinobacteria, *S. quadricauda*		rotifera

Date	Pond	Colour&density	Dominant species	Other species	Grazers
9.3.2016	VB	green,-brown, dense	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Scendesmus* sp., *Chlorella* sp., diatoms	rotifera,cladocera
	MB	dense, dark green	*Ankistrodesmus* sp.	*Chlorella* sp., diatoms	rotifera, cilliates
	BM		*Ankistrodesmus* sp.	*Chlorella* sp., diatoms	rotifera,euplotes, polychaeta
16.3.2016	VB	light green,dense	*Ankistrodesmus* sp.	*Monoraphidium* sp., *S. quadricauda, S. acuminatus, Chlorella sp.*	cilliates adn others
	MB	dark green	*Ankistrodesmus* sp.	*Chlorella* sp.	others, euplotes
	BM		*Ankistrodesmus* sp., *Chlorella* sp., diatoms		polychaeta, copepoda
23.3.2016	VB	dense, dark green	*Ankistrodesmus* sp.	*Monoraphidium* sp.	
	MB	scarce, light green	*Ankistrodesmus* sp.	diatoms	
	BM		*Ankistrodesmus* sp.	cianobacteria, diatoms	polychaeta, euplotes
30.3.2016	VB	dense, green	*Ankistrodesmus* sp.		
	MB	green	*Ankistrodesmus* sp.	*Monoraphidium* sp.	cilliates
	BM		*Ankistrodesmus* sp.	cianobacteria	
13.4.2016	VB	dense, dark green	*Ankistrodesmus* sp.	*Monoraphidium* sp., cianobacteria, *Scendesmus* sp., *Chlorella* sp.	grazers
	MB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp., cianobacteria	grazers
	BM		*Ankistrodesmus sp.*, cianobacteria		grazers
28.4.2016	VB	dense, dark green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *S. quadricauda, S. acuminatus, Chlorella* sp., cianobacteria, diatoms	grazers
	MB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp., cianobacteria	grazers

Date	Pond	Colour&density	Dominant species	Other species	Grazers
4.5.2016	VB	dense, dark green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Scendesmus* sp., *Chlorella* sp., cianobacteria	grazers
	MB	green	*Ankistrodesmus* sp.		
	BM		*Ankistrodesmus* sp., cianobacteria		polychaeta, euplotes
11.5.2016	VB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Scendesmus* sp., *Chlorella* sp.	vorticella, ciliates,other
	MB	dense, green	*Ankistrodesmus* sp.	*S.quadricauda*, cianobacteri	grazers
	BM		*Ankistrodesmus* sp., *Chlorella* sp., cianobacteria		polychaeta
18.5.2016	VB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp., cianobacteria, new unindentified algae	euplotes
	MB	dense, green	*Ankistrodesmus* sp., cianobacteria	*Monoraphidium* sp., *S. quadricauda*, cianobacteria	grazers undefined
	BM		*Ankistrodesmus* sp.		euplotes, others
25.5.2016	VB	dark greeen	*Ankistrodesmus* sp.	*Monoraphidum* sp., *Scednesmus* sp., *S. quadricauda*, cianobacteria, others	cilliates, vorticella, grazers, euplotes
	MB		*Ankistrodesmus* sp.	*Monoraphidium* sp., cianobacteria	grazers, euplotes, others
	BM		*Ankistrodesmus* sp., cianobacteria, other		
1.6.2016	VB	dense, green	*Ankistrodesmus* sp.	*Monoraphidium* sp.	
	MB	dense, dark green	*Ankistrodesmus* sp.	*Monoraphidum* sp., *Scednesmus* sp., cianobacteria	

Date	Pond	Colour&density	Dominant species	Other species	Grazers
8.6.2016	VB	green-brown	*Ankistrodesmus* sp.	*Monoraphidum* sp., *Scednesmus* sp.	vorti-cella, other
	MB	green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *S. quadricauda*	vorti-cella, other
	BM		*Ankistrodesmus* sp., cianobacteria		not iden-tified, new spe-cies
16.6.2016	VB		*Ankistrodesmus* sp.	*Monoraphidum* sp., *Scednesmus* sp.	yes
	MB	dense, green	*Ankistrodesmus* sp.	*Monoraphidum* sp., *Scednesmus* sp.	no
	BM		*Ankistrodesmus* sp., cianobacteria		
22.6.2016	VB	brown-green	*Ankistrodesmus* sp.	*Monoraphidum* sp., *Scednesmus* sp., cia-nobacteria	yes
	MB	dense, green	*Ankistrodesmus* sp.	*Monoraphidum* sp., *Scednesmus* sp., cia-nobacteria, other (*Chlorella* ?)	
	BM		*Ankistrodesmus* sp., cianobacteria		yes
1.7.2016	VB	dense, dark green	*Ankistrodesmus* sp., *Scendesmus* sp. (*S. acuminatus*)	*Monoraphidium sp.*, *Chlorella* sp.	yes
	MB	dense, green	*Ankistrodesmus* sp.		some
5.7.2016	VB	dense, dark green	*Ankistrodesmus* sp., *Scendesmus* sp.	*Monoraphidium sp.*, *Chlorella* sp.	poly-chaeta
	MB		*Ankistrodesmus* sp.	*Scenedesmus* sp., *Monoraphidium* sp., *Chlorella* sp.	yes
13.7.2016	VB	dar green	*Ankistrodesmus* sp., *Scendesmus* sp.	*Monoraphidium* sp., cianobacteria	cilliates (hoostic ha), other
	MB	dense, green	*Ankistrodesmus* sp.	*Scenedesmus* sp. (*S.quadricauda*), *Monoraphidium* sp.	no

Date	Pond	Colour&density	Dominant species	Other species	Grazers
20.7.2016	VB	dark green	*Scendesmus* sp. (*S. acuminatus*), *Ankistrodesmus sp.,Monoraphidium* sp.	*M.contortum, some new species, Chlorella sp. (?)*	yes
	MB	green	*Ankistrodesmus* sp.	*Monoraphidium* sp., *Scenedesmus* sp. (*S. quadricauda, S.acuminatus*), *Chlorella* sp., cianobacteria	yes
	BM		*Ankistrodesmus* sp., cianobacteria, *Scenedesmus* sp., *Chlorella* sp.		a lot, polychaeta
27.7.2016	VB	dense, dark green	*Ankistrodesmus* sp., *Scendesmus* sp. (*S. acuminatus*)	*Monoraphidium sp.*, *Chlorella* sp.	yes
	MB	green	*Ankistrodesmus* sp.	*Monoraphidum* sp., *Scednesmus* sp.	no
3.8.2016	VB	dense, greeen	*Scendesmus* sp. (*S. acuminatus*)	*Ankistrodesmus* sp., *Monoraphidium* sp., new species (4xcircular)	yes
	MB	green	*Ankistrodesmus* sp.	*Monoraphidum* sp., *Scednesmus* sp., cianobacteria	
17.8.2016	VB	dense, dark green	*Ankistrodesmus* sp., *Scendesmus* sp.	*Monoraphidium* sp., new species (ciruclar)	polychaeta
	MB	light green	*Ankistrodesmus* sp.	*Monoraphidium* sp., cianobacteria	yes
25.8.2016	VB	dense, dark green	*Scenedesmus* sp. (*S.acuminatus*)	Ankistrodesmus sp., Monoraphidium sp., new species (4xcircular)	polychaeta
	MB	green	*Ankistrodesmus* sp.	*Monoraphidum* sp., *Scednesmus* sp., cianobacteria	no
	BM	brown	cianobacteria, algae, grazers-eggs?		
31.8.2016	VB	dense, dark green	*Ankistrodesmus* sp., *Scenedesmus* sp., *Monoraphidium* sp.	cianobacteria, new species (4 circular green cells)	grazers, paramecium
	MB	light green	*Ankistrodesmus* sp.	*Monoraphidum* sp.	
	BM		cianobacteria, algae, grazers-eggs?		a lot

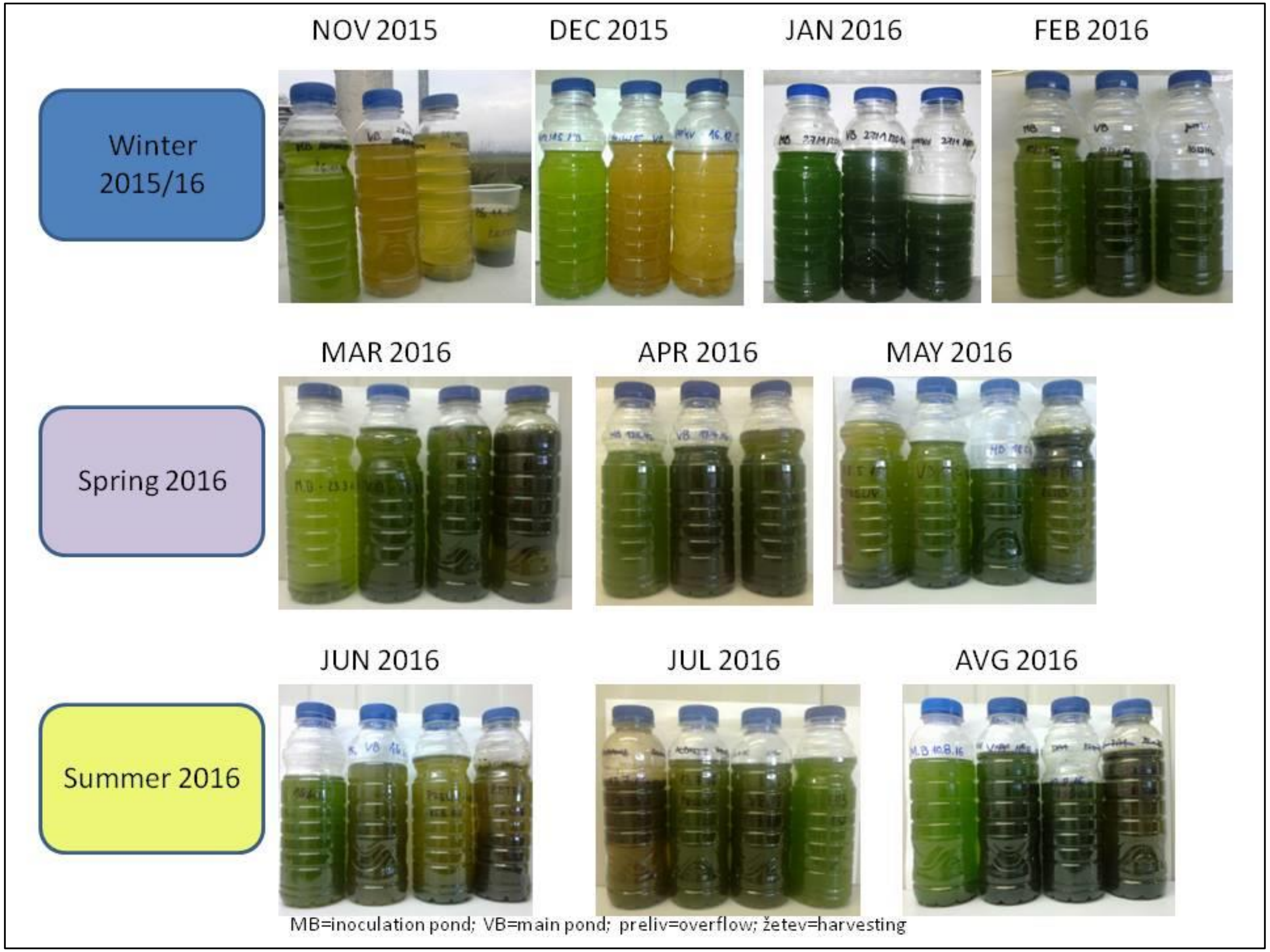
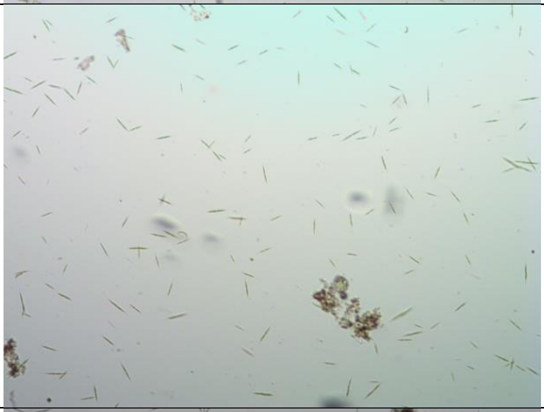
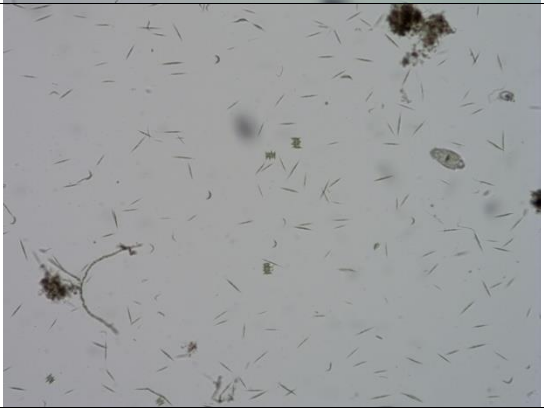
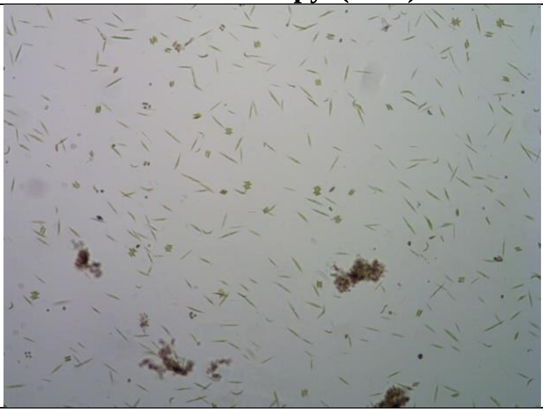


Figure 41 Change in colour of water in the ABG ponds from November 2015 to August 2016

Table 20 Change in microbial community structure and density from April 2016 to August 2016

Month	Microscopy (100x)
April 2016	
May 2016	
June 2016	
July 2016	

Month	Microscopy (100x)
August 2016	

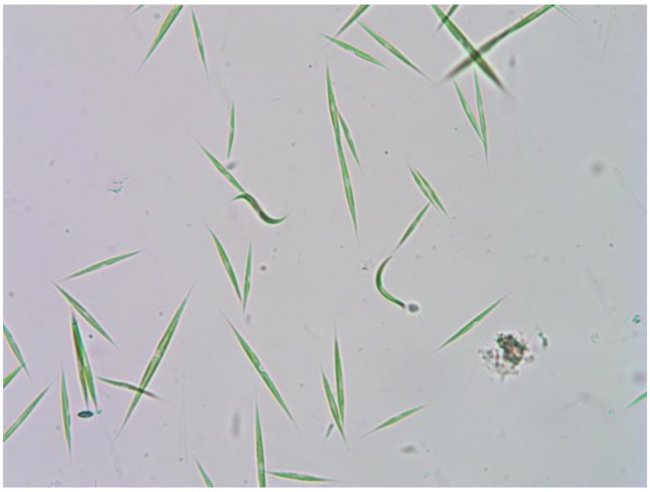


Figure 42 Microbial community in the main pond, April 2016

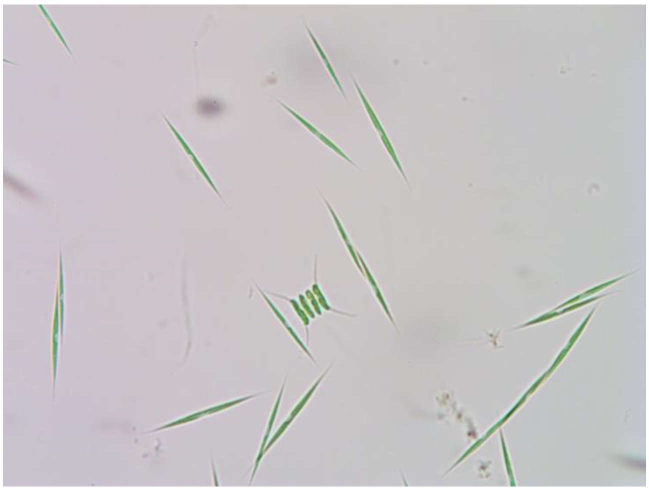


Figure 43 Microbial community in the main pond, May 2016

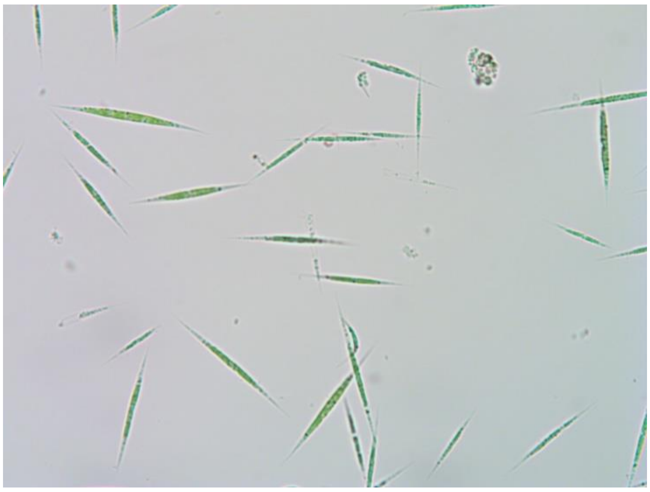


Figure 44 Microbial community in the main pond, June 2016

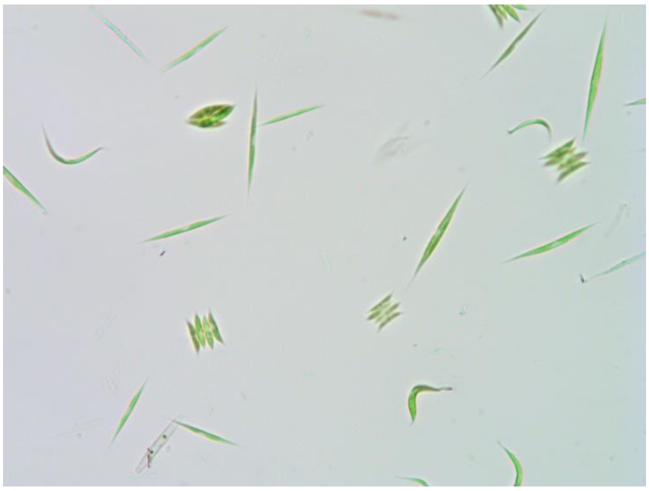


Figure 45 Microbial community in the main pond, July 2016

Figure 46 Microbial community in the main pond, August 2016

11 Literature

[1] Environmental, Y. S. I. (2008). ORP Management in wastewater as an indicator of process efficiency. YSI, Yellow Springs, OH http://www. ysi. com/media/pdfs/A567-ORP-Management-in-Wastewater-as-an-Indicator-of-Process-Efficiency. pdf (accessed on 15.08. 13).