AlgaeBioGas

Algal treatment of biogas digestate and feedstock production

D4.2

Alternative solutions review

PUBLIC

Contract	ECO/12/333018
Instrument	CIP Eco-innovation - Pilot and market replication projects
Call Identifier	CIP-EIP-Eco-Innovation-2012
Project website	http://algaebiogas.eu/
Project coordinator	Robert Reinhardt
Task Leader	Ana Cerar
Contact e-mail	mailto:algaebiogas@algen.si
Start date of project	1. September 2013
Duration	36 months
Reporting Date	31. December 2015

Co-funded by the Eco-innovation Initiative of the European Union

European Commission | Executive Agency for Small and Medium-sized Enterprises

cip competitiveness and innovation framework programme 2007-2013

Table of figures

List of tables

1 Summary

AlgaeBioGas demo centre aims to establish successful algae-bacterial treatment of digestate on a demonstration scale. This report describes Task 4.2 which deals with possible alternative solutions for digestate pre-treatment to improve the treatment performance of AlgaeBioGas solution and to produce algal biomass for specific purpose.

Editorial note

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

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

Previous deliverables (partially) quoted in this document:

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

2 Project Abstract

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

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

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

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

3 Task Description and Objectives

The AlgaeBioGas demonstration centre was set up in order to provide relevant demonstration-scale data for algal-bacterial treatment of digestate from a biogas plant. Experience gained in this way is being used to improve our digestate treatment process and provide vital information for new AlgaeBioGas installations. Biogas digestate is a *left over* from anaerobic digestion; most of the organics that could be biologically degraded have already been converted to energy in the anaerobic digestion step. Treating biogas digestate is thus a particularly challenging problem, especially since it contains growth-inhibiting substances such as lignins and high concentrations of ammonia. Digestate is typically of a dark colour, which presents a practical limit on any photosynthetic activity. The algal-bacterial process deployed in AlgaeBioGas is different from the anaerobic digestion used in the biogas facility itself, and we are able to remove further organic materials in this way; moreover, the algae in particular primarily use up the inorganic materials present in the biogas digestate and thus represent a significant improvement over conventional treatment processes. Nevertheless, the process remains difficult, requiring a significant amount of both time and space to produce effective results, and any

pre-treatment steps that can be taken in order to enhance the performance of the algal-bacterial treatment process could represent a major advantage both for the performance of the AlgaeBioGas facility, the quality of the treated water and the quality of the resulting biomass for specific purposes, such as use as a food supplement, as animal feed or in bioplastics.

In this report we first examine why digestate pre-treatment may be required, then we describe the digestate preparation steps implemented at the demonstration centre. We describe some tests that we made in physical and/or chemical digestate pretreatment with a short review of the literature in this topic. Then we describe some of the potential uses of produced biomass, for which we would require digestate pre treatment: bioplastics, fish feed and food.

Some of the procedures that were tested at lab scale are being implemented at full scale at the demonstration centre.

From DoW (Task 4.2 Review of alternative solutions for treatment of digestate)

Experience gathered through different operating modes of the demonstration centre will be used to design alternative operating modes and configurations of the system that can serve special purpose. Such options will mostly not be tried on the demo system, but rather theoretically defined as future options and tested on the lab scale. The present ideas for such options include various types of pre-treatment, for example to remove heavy metals from the cycle, to perform a first treatment phase so that edible products can be grown in the second phase. Special species may be tried as the input to biorefinery.

Use of algal biomass for other feed & food purposes may be legally problematic: under present legislation (before end-of-waste directive) it was virtually impossible to use any products from waste for other productive uses. Some testing of highly processed digestate (heat and pressure sterilized, UV treated and filtered) as nutrient source for Spirulina production has already been made and we will elaborate on this. This may be the only feasible approach for production of organic algae (eco certificate) which are demanded as food supplement – the biogas substrates would have to be of organic origin. When such algae are used for fish or poultry food as a source of omega-3 fatty acids and protein this may well fit our scope. For many other high-value uses the risks involved in using nutrients of biological origin simply does not pay itself – when products selling at over 100€/kg are grown, highly purified nutrients are affordable that are well controlled thus enabling total quality control of the process. We believe that the border between low grade and high value products will slowly shift and experiments in this direction are potentially fruitful. Knowledge and experience in this area is at least needed as a marketing tool, even if all installations will only be growing biogas substrate – they will all be interested in the potential of higher value products. The objective of this task is more to list and define the options than to try them on large scale.

4 Digestate is a challenge

Biogas digestate is difficult to treat simply as a wastewater for several reasons:

- Biogas digestate is the substance that is left over from the biological production of methane and CO_2, which ideally consumes all organic matter that was possi-

ble to digest. This means that biogas digestate would ideally – if biogas production were optimal – only contain non-biodegradable organics.
- Biogas digestate is of a very dark colour, which prevents light from penetrating deep into the water and limits photosynthesis to only a shallow layer under the surface.
- Biogas often contains toxic levels of ammonia or other substances that block further biological activity.

These imply that biogas digestate treatment requires quite unusual microbial communities to remove the residual organics and the high concentrations of inorganic nutrients. The algal-bacterial community is a promising candidate, provided that:

- the retention times are sufficiently long so that the substance is suitably dilute,
- the illuminated surface is large enough so that the amount of light and hence the oxygen production are sufficient to allow the bacteria to do their work,
- the digestate load is low enough to avoid toxic conditions, and
- a microbial community with high levels of resistivity for such growth conditions is kept.

If the resulting algal-bacterial biomass from digestate treatment is recycled as a biogas substrate, we face additional challenges of potential accumulation of unwanted materials, such as heavy metals, completely indigestible organics and salts.

Biogas treatment is considered a hard problem also in the literature. Although there are many flavours of AD biogas (land-fill, anaerobic WW treatment (e.g. UASB), aerobic sludge treatment, food & biological waste processing, agricultural waste processing, energy crops) which face different problems in details, there are some common challenges, like digestate colour and digestate composition which are recently studied extensively (mostly in lab scale studies). We are mentioning just a few of them.

The main drawback of anaerobic digestion (AD) technologies is low efficiency in nutrients (phosphorus and nitrogen) removal from organic feedstock. Therefore, the liquid effluent from anaerobic digestion (liquid phase of digestate) needs to be further treated before discharging (Liu & Liu 2015). Widespread adoption of AD for bioenergy production may be limited by the massive quantities of AD effluent produced. Agronomic land application has been the primary AD effluent management technique. However, environmental restrictions due to pathogen accumulation, food safety, and nutrient runoff have necessitated the development of alternative technologies for digestate treatment (Sheets et al. 2015).

Dewatering techniques such as centrifugation are used at large scale facilities to separate raw AD effluent into liquid and solid fractions that are easier to handle and transport. Evaporation has been used to further reduce AD effluent volume and DAF (diffusion air flotation) is an effective method to remove solids. The restriction here is high energy demand of mechanical separation and evaporation that may limit their adaptation to large scale AD facilities which produce excess quantities of electricity and process heat. Small scale facilities could use less energy intensive methods such as passive filtration, but the variability of water distribution in AD effluent may negatively influence dewatering efficiency. The colloidal particles in digested manure may not be easily separated without polymer addition. Therefore, techno-economic and life cycle comparisons of dewatering techniques are needed (Sheets et al. 2015).

Turbidity in AD effluent may cause inadequate light penetration for sustained algae growth. Growth rate of *Chlorella* sp. in AD effluent declined linearly with increasing initial reactor turbidity. *Scenedesmus* sp. was inhibited by NH_3 at levels as low as 100 mg/L. To reduce the effects of turbidity and inhibitory NH_3, most researchers have diluted the liquid fraction of AD effluent to initial total nitrogen levels of less than 200 mg/L (Figure 1). This practice also diluted turbidity for improved light penetration. While the growth rate of algae can also be improved by optimizing the nitrogen to phosphorus (N/P) ratio, low nutrient content promotes lipid production. Studies have attempted to optimize the nutrient loading to maintain growth and high lipid yields (Sheets et al. 2015).

Algae biomass production using the liquid fraction of AD effluent as a nutrient source.

Algae strain	Working volume (L)	AD feedstock	TN in algae reactor		TP in algae reactor		Biomass production		Lipid content (% dry weight)	Reference
			Initial concentration (mg L^{-1})	Removal (%)	Initial concentration (mg L^{-1})	Removal (%)	Growth rate (d^{-1})	Biomass productivity (mg DW L^{-1} d^{-1})		
Chlorella sorokiana *Chlorella minutissima* *Scenedesmus bijuga*	0.06–0.2	Poultry litter	80–100[a]	50–80[a]	7–8[a]	70–75[a]	NR	71–75	3.5–9.6	Singh et al. (2011)
Chlorella sp.	0.1	Dairy manure	120–250[a,b]	76–83	15–30[a,b]	63–75	0.28–0.41	NR	9.0–13.7[c]	Wang et al. (2010)
Scenedesmus sp. AMDD	0.1	Vegetable, cow, swine, algae waste	23–28[a]	23–100	8.5–22[a]	13–99	0.85–1.66	NR	NR	Bjornsson et al. (2013)
Chlorella sp. UMN271	0.15	Swine manure	200–250[a]	22–34	10–55[a]	23–71	0.50–0.65[a]	0.04–0.08[a]	7.5–10.9[c]	Hu et al. (2012)
Chlorella sp. and microbial consortia	0.5	Wastewater sludge	225[a]	89[a]	120[a]	17[a]	NR	30[a]	NR	Yuan et al. (2012)
Scenedesmus sp.	1	Livestock waste	120	40–55[a]	NR	NR	0.04–0.09	46–57	NR	Park et al. (2010)
Nannochloropsis salina	1	Wastewater sludge	80–640	87–100	11.4–91.4	98–100	0.344–0.644	68–92	21–36	Cai et al. (2013)
Synechocystis sp.	1	Wastewater sludge	80–640	71.2–100	11.4–91.4	99–100	NR	41.3–150.9	11.8–13.5	Cai et al. (2013)
Chlorella sp.	2	Wastewater sludge	197	51	3.5	95	0.13	66[a]	NR	Wang et al. (2014)
Micractinium sp.				46		95	0.14	53[a]		
Chlorophycae and cyanobacteria	8	Wastewater sludge	45–80[a]	48–92[a]	5–11[a]	98[a]	NR	234	NR	Ruiz-Martinez et al. (2012)
Chlorella sp.	12	NR	481	78	51	73	NR	37–59	NR	Zhao et al. (2013)
Chlorella sp.	20[d]	Dairy manure	40–200[e]	72–98[e]	NR	55–58[e]	0.016[d]	22.8[d]	9.3–10.8[e]	Chen et al. (2012)
Mixed green algae culture	40	Dairy manure	16.3–30.5	96	1.8–2.6	98	NR	17.5	10–29	Woertz et al. (2009)
Spirulina platensis	100	Pig waste	28.2	80[a]	17.6	30[a]	NR	19.9[f]	4.2	Chaiklahan et al. (2010)

[a] Estimated from chart data.
[b] Total Kjeldahl Nitrogen (TKN).
[c] Total fatty acids (TFAs).
[d] Results from pilot-scale study.
[e] Results from laboratory-scale study.
[f] g m^{-2} d^{-1}.

Figure 1 Algae biomass production using the liquid fraction of AD effluent as a nutrient source (Sheets et al., 2015).

4.1 Process of digestate treatment at ABG Demo site

4.1.1 Digestate production

Digestate used in ABG demo centre comes from KOTO biogas plant. Capacity of biogas plant is 13.000 t/y biodegradable waste (food waste, biodegradable waste from households, flotation sludge); daily input of waste 35-40 t with biogas production 1,8 mio m^3/y; digestors 5.000 m^3 and anaerobic filter up to 200 m^3. From produced biogas electrical energy 4 GWh/y is produced and co produced heat is partly used (consumed heat app. 1,600 GWh/y). Renewable energy produced from biogas:

- Electricity 89% of produced electricity is used for internal needs
- Thermal power is efficiently used for steam production and heating in cold periods (more than 15% input energy).

For heating of algae pond we use hot water at 80°C from biogas engine.

Figure 2 Cogeneration unit, 526 kW at KOTO

Liquid effluent, digestate with 3-4% dry matter content production is 26.400 m^3/a (2014). After separation, 24.600 m^3 of digestate – centrate remains in liquid fraction and 1.800 m^3 in solid fraction (25% DMC). Centrate ~68 m^3/day is pumped to anaerobic filter (COD ~ 16.000 mg O_2/l, 0,5 - 1% DMC).

Figure 3 Digestate treatment- from top left to bottom left: fan separator, digestate container, tricanter centrifuge, anaerobic filter (AF), cooling tank (CT)

Figure 4 KOTO solid phase of digestate after separation

Figure 5 KOTO liquid phase of digestate after separation

Effluent from anaerobic filter could be stable source of nutrients for algae cultivation. Due to changes in waste flow to biogas plant, some variation in digestate quality might occur (colour, turbidity, nutrients, etc.).

Figure 6 Filter pack in anaerobic filter

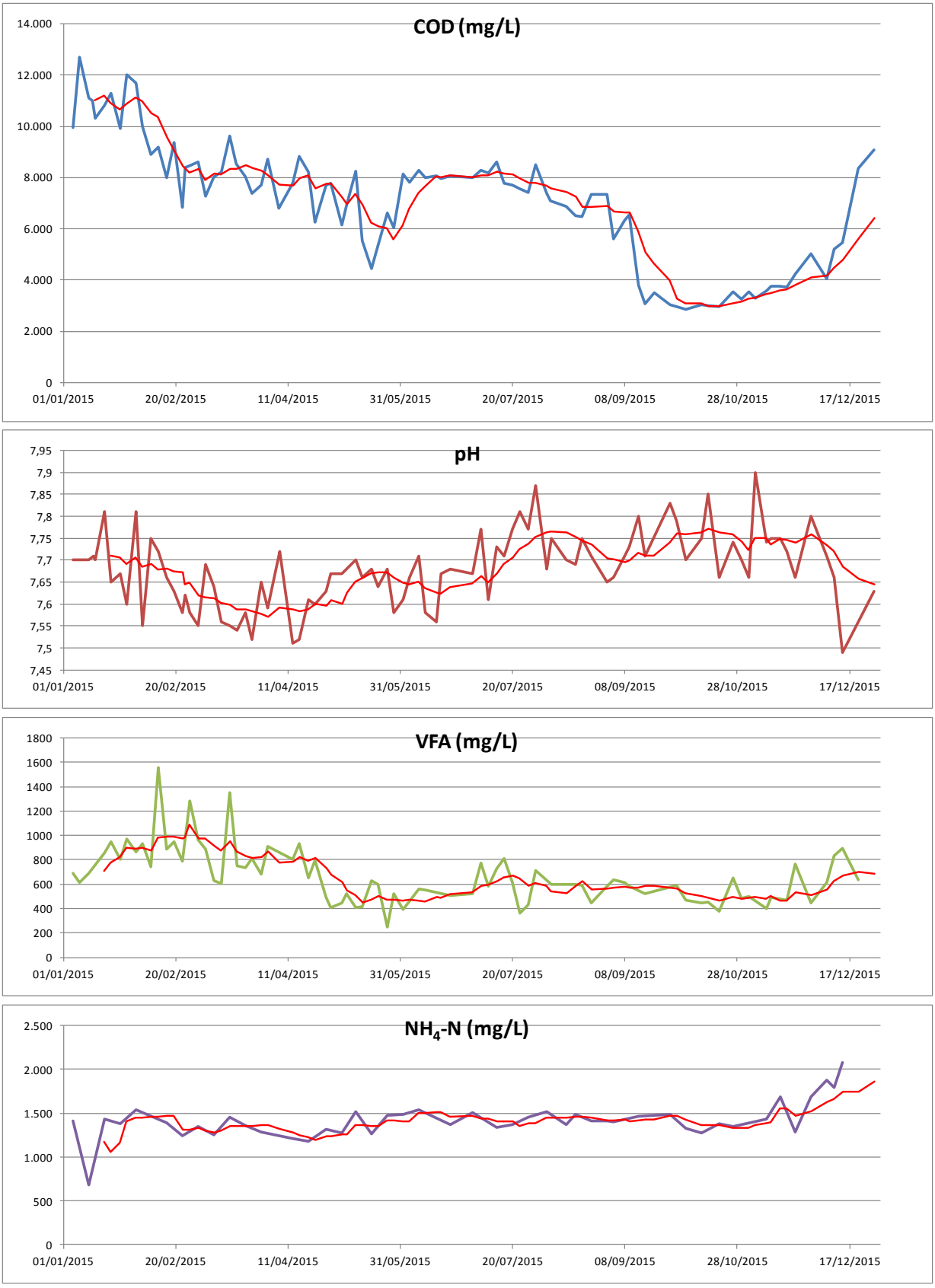

Figure 7 Digestate characteristics for 2015

4.1.2 AlgaeBioGas processing of digestate – overview

From D4.1 (Case study operation assessment)

Algae bacterial treatment of digestate takes place in the main pond, where algal-bacterial community uses nutrients present in digestate which results in growth of algal-bacterial biomass. Mixed algae and bacterial culture is maintained in inoculation pond and added to the main pond when necessary. Both ponds are continuously mixed. Biomass from the main pond is recycled through the sedimenter and harvested. The supernatant outflow from sedimenter is discharged to the sewage system. To assure optimal conditions for system operation, water level, CO_2 addition, water and air temperature in greenhouse are controlled. Digestate comes from the biogas plant using food and green waste as a substrate. Before entering the AlgaBioGas, digestate goes through anaerobic filter and UV sterilization.

The process itself is described in detail in previous deliverable D4.1.

4.1.2.1 Digestate processing

Amount of digestate added per day depends on the system mode of operation. During last year of testing, different amounts of digestate were added, varying from less than 100L per day and up to 500L per day.

Digestate is added from the digestate collection tank (output from anaerobic filter) by gravity flow. There is an electrically controlled valve with relatively long transition time, so we implemented digestate addition as a periodic process. Digestate addition is determined by the following settings:

- period length (in minutes)
- maximum amount per period (in litres)
- maximum amount per day as a safety measure (in litres)
- conductivity limit (in µS) – digestate is not added in the period if conductivity is above this limit,
- ORP limit (in mV) – digestate is not added in the period if ORP is below this limit,
- dissolved oxygen limit (in mg/L) – digestate is not added in the period if ORP is below this limit.

There are two modes of operation of the system:

- during inoculation procedure the total amount of added digestate is increased from 50, 100, 200 L/d to continuous mode in 4 weeks. The pulsed operation period is set to 120 and 60 min to spread the digestate pulses in a wider range of time.
- in continuous mode the pulsed operation period is set to minimal value (5 min) so the system can react as soon as possible to changing conditions.

Digestate composition changes over time, therefore we measure COD (chemical oxygen demand) to keep track of digestate nutrient value and system load.

During the first year of demo centre operation the COD of digestate varied between 7000 and 8000 mg O_2/L up until middle of September 2015, when quality of digestate changed due to the change at biogas plant operation. From September 2015 and until start of December 2015, the COD values varied between 3.000 and

4.000 mg O_2/L. By the end of December 2015, COD values are again around 8.000 mg O_2/L.

Digestate composition is analysed regularly as part of the biogas plant operation. Samples of digestate were also sent to outside laboratory for analysis in February 2015 and similar tests will be done early next year.

Table 1 Average values for different digestate parameters in ABG ponds

parameter	value
COD	7.800 mg O_2/L
Estimated BOD	5.000 mg O_2/L
VFA	650 mg/L
Ammonia	1350 mg/L
Temperature	38 °C
pH	7,8
Conductivity	1.350 mS/m
NO_2-N	< 1 mg/L
Ca	175 mg/L
Mg	73 mg/L
K	734 mg/L
Na	7.996 mg/L
P	72 mg/L

5 Alternative solutions

5.1 Pre-treatment of digestate

5.1.1 Chemical pre-treatment of digestate

It was expected that digestate color is an important factor of treatment performance. Digestate has a very strong colour, probably caused by humic substances, as mentioned in previous report D4.2. Strong brownish colour affects algae growth by limiting the light availability. Therefore, we carried out couple of tests, trying possible options for low cost and simple pre-treatment of digestate in order to reduce the effects of colour. We decided to test different concentrations of sodium hypochlorite (NaOCl), hydrogen peroxide (H_2O_2) and UV radiation.

5.1.1.1 Overview of the test

We used 30 ml of incoming digestate and pour it into plastic Petri dishes. We used one un-treated sample as control (no. 7) to compare results. For other pre-treatment's we used:

Table 2 Types of pre-treatment used in digestate pre-treatment test

Sample no.	Type of pre-treatment
1	1 ml H_2O_2 added
2	0,1 ml H_2O_2 added
4	1 ml H_2O_2 + 15 min UV
5	0,1 ml H_2O_2 + 15 min UV
7	Control
8	15 min UV
9	1 ml NaOCl (13%) added
10	0,1 ml NaOCl (13%) added

Samples were observed at the start of the test, 1 hour after start, 24 hours after start and 5 days after start. Changes were noted based on visual inspection of samples colour.

5.1.1.2 Results

The strongest effect was observed for sample #1, where change of colour was visible in minutes after adding H_2O_2. After 15 min, both samples with H_2O_2 changed colour. After 1 h sample 1 was even lighter in colour, for sample 2 there were no noticeable changes comparing to the colour from previous observation.

For sample 9, with NaOCl, barely observable change in colour was noticed after 5 min and a bit more after 1 h. There were no visible changes in sample 10 after 1 h. For samples treated with UV, there was change of colour right after addition of H_2O_2, which is consistent with previous results for samples 1 and 2. After 5 min under UV light, samples 4 and 5 were brighter, but probably only due to H_2O_2 addition, since there was no change in colour in sample 8. Contrary to the effects know from literature on advanced oxidation techniques (e.g. Ikehata 2006) UV light didn`t seem to have any effect on colour.

There was no significant change in colour observed after 24 hours, for any of the samples. Samples were observed after 5 days and there was no noticeable difference, therefore we concluded that the effect of this kind of pre-treatment can be seen quickly and are therefore useable just before digestate is brought to the pond.

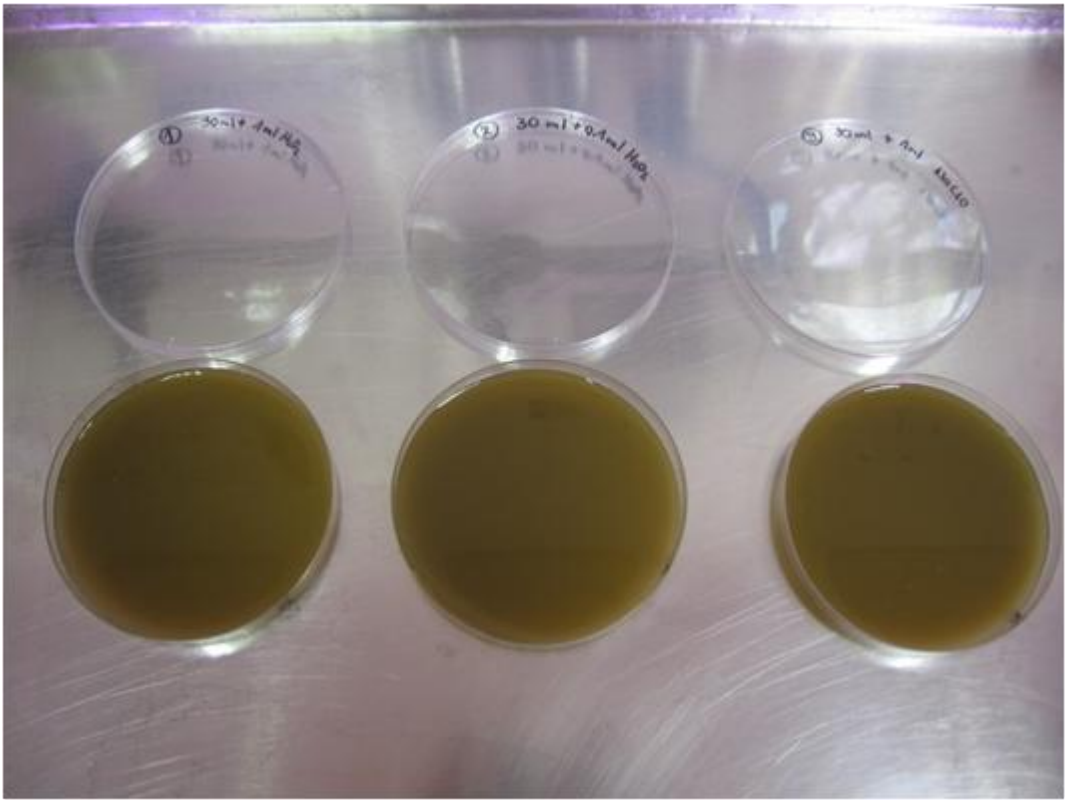

Figure 8 Digestate samples before start of the test

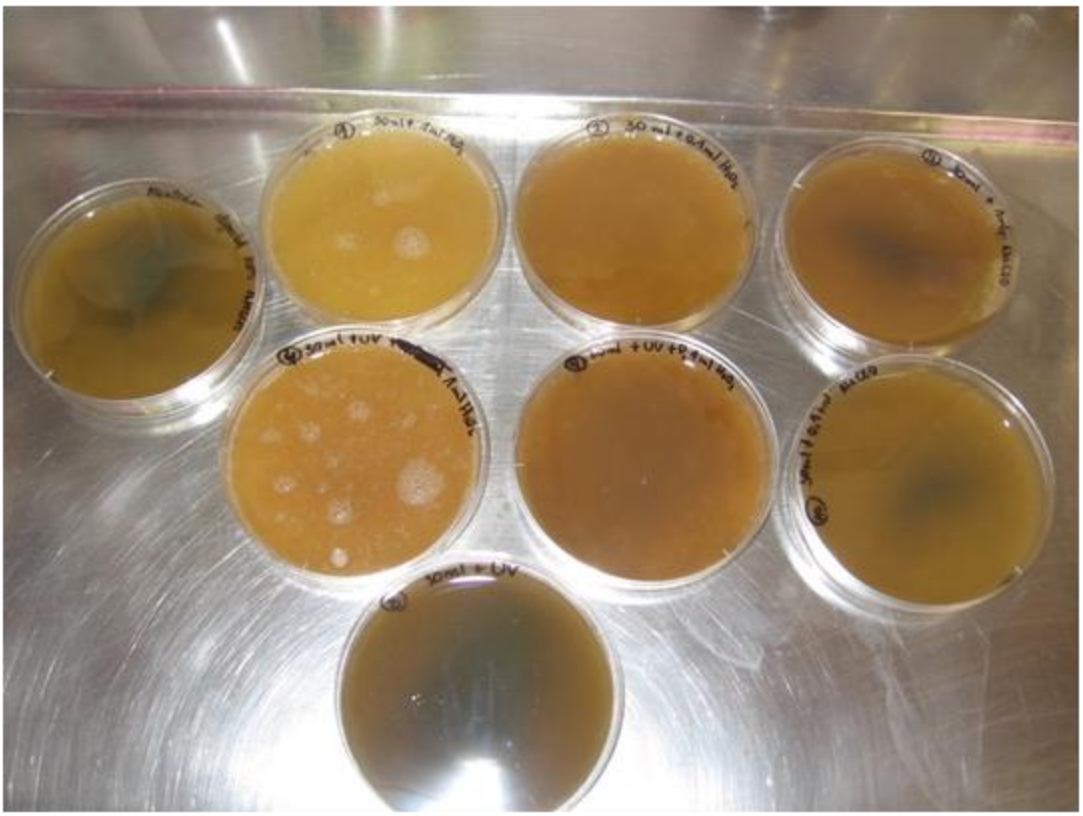

Figure 9 Samples after 1 hour

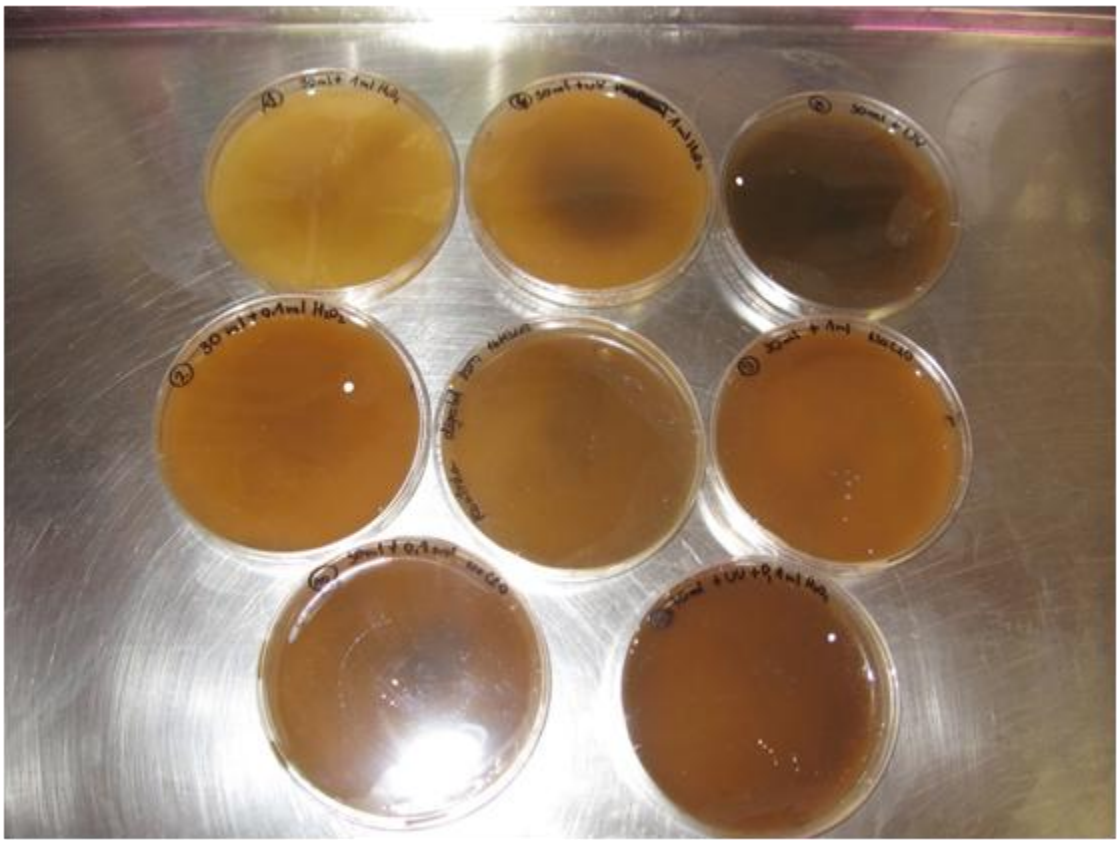

Figure 10 Samples after 24 hours

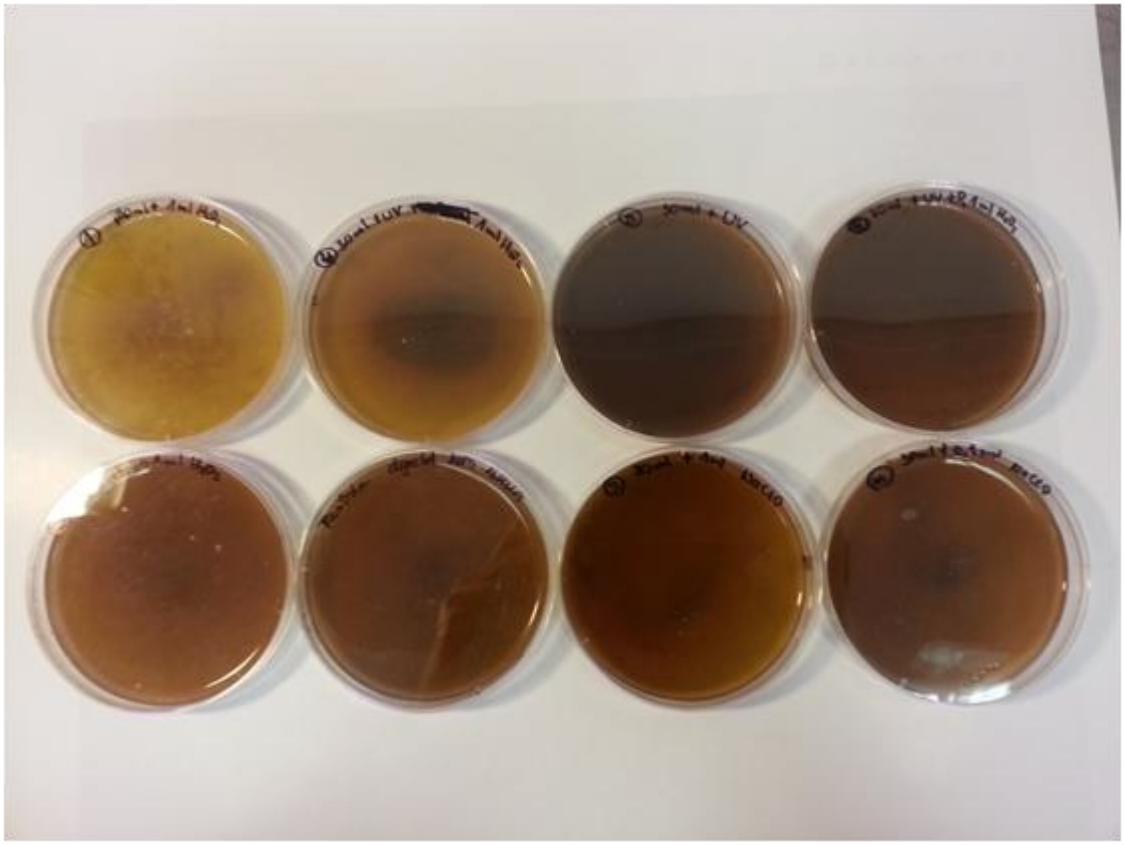

Figure 11 Samples after 5 days

5.1.1.3 Conclusions

Based on our pre-treatment test, the most effective way for pre-treatment of digestate (in order to affect its colour) is H_2O_2 in higher concentrations. We saw little change also with the highest concentration of NaOCl, but nowhere close to the effect of H_2O_2. Based on our observations, UV light does not affect colour of digestate. We will investigate economic feasibility of H_2O_2 treatment, repeat the tests with O_3 and repeat the UV irradiation tests before implementing this process at the demonstration centre. Initial tests comining elecrocoagulation (next section) and H_2O_2 treatment were very promising and will be further investigated.

5.1.2 Physical pre-treatment of digestate: Electrocoagulation

Liu and Liu (2015) suggested an integrated system of electrocoagulation and algal cultivation to treat a highly organic polluted wastewater—anaerobic digestion liquid effluent for reclaimed water and value-added algal biomass production. The integrated system synergistically takes advantages of both electrocoagulation and algal cultivation to enhance the efficiencies of wastewater treatment (Liu & Liu 2015).

Electrocoagulation is an electron driven coagulation method, which simultaneously coagulates and float solids in the solution. Electrocoagulation is performed by applying an electric current across metal plates that are submerged in water. Heavy metals, organics, and inorganics are primarily held in water by electrical charges. By applying another electrical charge to the contaminated water, the charges that hold the particles together are destabilized and separate from the clean water. The particles then coagulate to form a mass, which can be easily removed.

The electrocoagulation treated waste water had low turbidity with better light penetration (108 NTU) to enable algal growth. The algal cultivation had high removal efficiencies of phosphorus (99.4%) and nitrogen (88.2%). The dissolved iron in the electrocoagulation treated wastewater enhanced lipid accumulation of the algae. Their results shows that total phosphorus and nitrogen in the reclaimed water were 0.78 g/L and 35.5 mg/L and the harvested algal biomass had 35% of lipid, 53% of protein, and 6.4% of carbohydrates. This method could be interesting solution for agricultural wastewater treatment that turns waste water from an environmental liability into a valuable asset (Liu & Liu 2015).

We did only qualitative lab testing of several electrocoagulation methods on digestate with electrodes made of Aluminium, Iron and Titanium in all combinations. Aluminium gave excellent results in flocculation, Aluminium and Titanium performed even better in temporary flotation of the flocs. As we do not want to introduce Aluminium ions to the solution (this is frequently undesirable), we were trying similar methods with Iron but with much more mediocre results. We were unable to confirm results by Liu & Liu 2015, but we recently obtained very interesting results with combination of electrocoagulation and H_2O_2 oxidation. We decided to build a flow through chamber for electrocoagulation to be used at the demonstration site, but this is not complete yet.

Figure 12 Quick performance of Al-Ti electrofloculation: significant amount of particles flotate after just 30 s treatment

Figure 13 Al-Ti electrocoagulation after approx 20 s

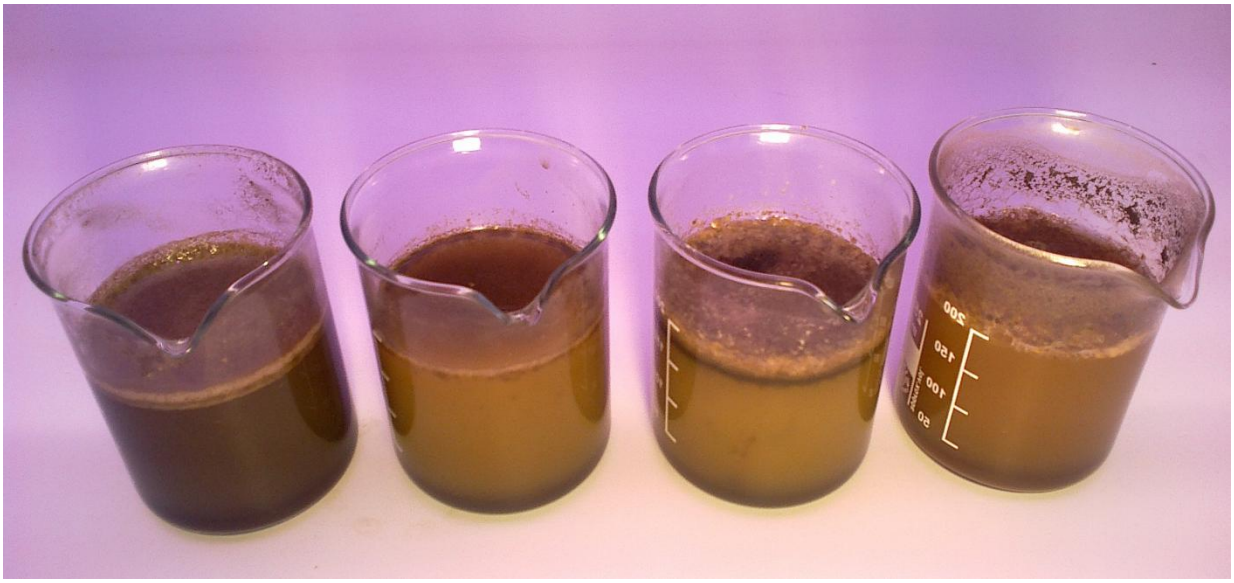

Figure 14 Electrocoagulation tests 1h after treatment (settled foam), L to R: Fe, Al-Al, Al-Ti, Fe+H_2O_2

We also performed a number of electrocoagulation tests on produced algal biomass with similar results. Implementation of these technologies to the demonstration centre will be done simultaneously with the DAF harvesting (a replacement or addition to sedimentation which is being planned).

5.1.3 Biological pre-treatment of digestate: use of algae for heavy metals removal

In order to use digestate as source of nutrients for algae intended for food and feed purposes, heavy metals content must be below legally set limits. As we discuss in chapter 5.3.2, use of algae grown on digestate for fish feed might be limited due to high concentrations of heavy metals in digestate. Solution for this problem is to pre-treat digestate with algae and using the resulting effluent as a nutrient source for algal biomass, which is later used for fish feed or food (Spirulina). The idea here is to add another pond to the ABG system: first pond is used to pre-treat digestate with algae in order to remove heavy metals, remove algal biomass with sedimentation and channel algae-free effluent to the main pond, used for growth of algal biomass of interest. Of course tests need to be done in order to find the right set of parameters: how much digestate can be treated in the first pond to achieve sufficient heavy metals removal and still leave enough nutrients for algae in the main pond.

As seen in literature, algae are good biosorbents. In previous years we also conducted some lab scale experiment, using compost water and testing for heavy metal removal with algae. Test gave positive results since *Chlorella* sp. successfully removed zinc from compost water.

Heavy metals are not biodegradable and tend to accumulate in living organisms. Many heavy metals ions are known to be toxic or carcinogenic. Toxic heavy metals of particular concern in treatment of industrial waste waters include zinc, copper, nickel, mercury, cadmium, lead and chromium (Fu & Wang 2011). Biosorption is a sorption process, where biomaterial or biopolymer is engaged as sorbent. Organic ligands or functional groups such as carboxyl, hydroxyl, sulphate, phosphate and amine groups have the dominant role in removal of various heavy metal contami-

nants. One of the biosorbets known since the 70s are algal-based biosorbents, especially marine algae. Advantages of use of algal biomass is their high uptake capacity, low cost, renewability and ready abundance of biomass in many parts of the world. Marine algae are divided in brown, red and green algae. The differences in biosorption between groups occur due to differences in the cell wall, where biosorption occurs. Green algae mainly have cellulose in the cell wall and a high content of proteins is bonded to the polysaccharides. These compounds contain functional groups such as amino, carboxyl, sulphate and hydroxyl, which play important roles in biosorption.

Heavy metal biosorption is metabolism-depended, so it typically occurs rapidly, especially for uptake of cationic metal ions. Most of cationic metal uptake takes places in first 20-60min, followed by relatively slow uptake process. For anionic contaminants biosoprtion is much lower, typically it would take more than half a day to few days to reach the biosorption equilibrium. Biosorbtion is affected by pH, at high pH higher cationic metal uptake occurs (pH 4-6), anionic heavy ions removal is better at lower pH.

Brown algae are the most extensively studied among the marine algae biomass and can effectively remove toxic metal ions such as lead and chromium. Maximum biosorbtion capacities for brown algae are quite high, ranging from 0.39 to 1.66 mmol/g. One of the best performing algae to remove heavy metals is *Saragassum* sp. Precious metals and radioactive metals may also be well accumulated by algae. Red and green algae can also remove heavy metals such as lead, copper, cadmium, zinc and chromium, but performance of both is far below that of brown algae.

Cell wall structure of marine algae (alginate and fucoidan) is responsible for heavy metal sequestration. Key functional groups present in brown and green algae play a dominant role in metal binding: carboxyl, hydroxyl, sulphate, phosphate and amine groups. The ion-exchange mechanism has been found to play a dominant role for the biosorbents that originate from seawater environment. The ion-exchange occurs between heavy metals and light metals (mainly Ca^{2+} and Mg^{2+}). The alginates of brown algae have a higher uptake for divalent cations (Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}). In red algae, sulphated polysaccharides (galactanes) are mainly responsible for the complex formation of metal ions (He & Chen 2014).

Reports mention biosorbiton of Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+} for green algae *Chaetomorpha linum*, *Caulerpa lentillifera, Ulva lactuca* and *Cladophora fascicularis* (Fu & Wang 2011). Romera et.al. tested biosorbtion of Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} for 6 different algae: green (*Codium vermilara*, *Spirogyra insignis*), red (*Asparagopsis armata, Chondrus crispus*) and brown (*Fucus spiralis, Ascophyllum nodosum*) and compared it with fungi and bacteria. They proved that algae were able to remove heavy metals equally or better. Brown and red algae proved to be the most efficient for removing heavy metals; 1mmol metal/g of biomass recovery by brown algae. Green algae show lower levels of metal recovery, one reason for this might be that there is less probability of having two adjacent carboxylic groups at the right distance to allow metal bond between them, as it happens with alginates. All tested algae showed similar affinities for metals tested, except for lead, where each biomass seemed to behave differently. Affinity for lead was much higher than for any other metal, especially with green and brown algae. Sorption capacity was the greatest with lead, followed by cadmium, copper, zinc and nickel.

Amount of metals anchored on the surface of algae depends on the number of active sites present and how easily they can be accessed, therefore suitable biosor-

bent and working conditions must be chosen for specific situation (Romera et al. 2007).

At the demonstration centre we are not well equipped to do two phase algal processing. We intend to do a biosorption test on ground dried biomass from the existing pond as this can be done relatively easy. Depending on these result we intend to set-up a protocol for measurements of selected heavy metal concentration and establish a small portable pond for this kind of testing.

5.2 Bioprocess alternatives: species selection and biorefinery concept

Another alternative option for digestate treatment process is to select specific parameters of the process in order to assure growth of specific species of algae, using a biorefinery approach.

5.2.1 Biorefinery

Biorefinery is a facility, which integrates biomass conversion processes and equipment to obtain energy, biofuels and high value chemicals from biomass. In case of algae, biomass can be used as source of energy (anaerobic digestion, hydrogen, bioethanol, biodiesel; although not all of them are economically feasible), source of proteins, vitamins (*Spirulina*, *Chlorella* as food additives), pigments (astaxhantin, β-carotene…), cosmetics, bioplastic etc. (Trivedi et al. 2015).

In the scope of biorefinery concept, taking into account which species can be grown on waste product such is digestate, is important. In our case, *Monoraphidium* sp. has prevailed and studies have shown that this species is suitable feedstock for biodiesel (Holbrook et al. 2014). Some species of *Monoraphidium* have lipid content of 40% of the cell weight. Same species was also used in production of astaxanthin for pawns pigmentation and results were comparable to common astahxantin producer, *Hematoccocus* sp. *Monoraphidium* also contains high level of vitamins, pantothenic acid and β-carotene (Fujii et al. 2010). However, potential use of *Monoraphidium* sp. biomass in this case would require some sort of pre-treatment of digestate, due to higher levels of heavy metals, as found in our aquaponic tests described later in this report.

5.2.2 Species selection

AlgaeBioGas demonstration centre is operated with naturally evolving algal community. It was started with a rich mix of cultures and species that grow well under pond conditions prevailed. We do occasional back inoculation from main pond to the inoculation pond and we bring mix of cultures maintained in the lab to the inoculation pond from time to time to maintain versatility.

Over time one or two species dominate. During the last half year of operation, two species seem to be dominant in the ponds: *Monoraphidium* sp. and *Ankistrodesmus* sp. are dominant at the moment. At the start of the centre operations, several *Scenedesmus* sp. have been working well in the ponds, some of them can still be found in current community.

At the start of the project we speculated about putting different species in to the ponds, as a way of establishing biorefinery concept. Deducting from our observa-

tion so far, if we want the system to work and process sufficient amounts of digestate, algal community must be adapted to digestate. We researched which algal species have been grown on digestate and their potential for use in biorefinery concept. Species and their possible use in biorefinery concept are shown in Table 3. Colored species are known to be able to grown on digestate.

Table 3 Different types of algae and their possible use in biorefinery

Algae	Use
Chlamydomonas	bioethanol
Porphyridium sp.	Pharmacology (anti-inflammatory skin treatment)
Arthrospira	Pycocyanin (pigment), cosmetics (skin care)
Chlorella	Food-addditive, nutraceuticals (EPA), vitamin C, cosmetics, bioethanol
Dunalliella salina	Beta Carotene
Hematoccocus pluvialis	astaxanthin
Euglena gracillis	Vitamin E
Rhizoclonium sp, *Oedonium* sp.	Bioremediation of heavy metals
Monoraphidium sp.	biodiesel

5.3 Alternative use of algal biomass produced on digestate

Algal biomass produced in AlgaeBioGas demo centre ponds is used for production of biogas. We will elaborate on this in the upcoming deliverable D3.7. Here we present possible alternative uses of algal biomass grown on digestate.

Since our biomass is grown on digestate, digestate could be subject to suitable pre-treatment, depending on the final use of biomass, especially if we are talking about biomass for food and feed which is under strict regulation in EU. Therefore, we made some laboratory scale test for possible pre-treatment of digestate, as described above. Here, we describe possible use of biomass grown on pre-treated digestate as well as non treated digestate. One of the options for using biomass grown on non-treated digestate is use of algal biomass as bio filling for bio-plastic, which was also tested with our biomass.

5.3.1 Use of biomass for bioplastics

One of the options for use of digestate is using it as nutrient source for production of algal biomass which is later used as source material for production of bioplastics. This concept is fairly new in contest of algal biomass, bust some tests have been done on laboratory scale. We tested algal biomass produced in demo centre as bio filling for composite plastic.

5.3.1.1 Overwiev

Every year, about 140 millions of tons of plastic are consumed worldwide, using approximately 150 millions of tons of fossil fuels and directly causing immense amounts of waste, which can take thousands of years to deteriorate. Bioplastic presents feasible alternative, since it is not based on fossil resources and can be bio-

degraded (Hempel et al. 2011). The global bioplastics market is thought to be growing at a rate of 20-25% per year. Bioplastics is generally made from renewable resources such as corn, sugars, potatoes, etc. and they are produced by a range of microorganisms (Arikan & Ozsoy 2015).

Algal biomass is a source of hydrosoluble polysaccharides (alginate, carrageenan, agarose) and contain up to 10% of cellulose in dry mass. Two main approaches in using algae in composites have been reported: first, use as fillers in order to decrease price and carbon footprint of polymer and utilise algae waste; second, as reinforcing fibres. Polyvinyl alcohol (PVA) composites were prepared with green alga *Ulva armoricana* and *Zostera marina*. PLA/Zostera composites were very brittle, the highest content of algae was 20 wt%. Algae as thermoplastics have been mentioned in international patents (Bulota & Budtova 2015).

Algal cellulose has high christallinity degree, which makes it good source for bioplastic. Little is known about natural fibres extracted from algae as reinforcement of biocomposites. Poly butylene succinat was reinforced with bleeched red algae and green algae *Ulva* was used with poly vinyl alcohol; best performance according to the tension tests and thermal degradation were achieved when concentration of algae was 30%. Tests done with cyanobacteria *Lyngbya* showed that this is a good candidate for natural fibre reinforcement of composite materials (Constante et al. 2015). *Chllorella* and *Spirulina* were used for blends of microalgal biomass and polyethylene, results showed varied degrees of compatibility. Chlorella exhibited higher bioplastic properties, but lower degree of compatibility with polyethylene as *Spirulina*. For commercial use of *Chlorella* bioplastic, blending is desired. In blends, *Spirulina* perfomed much better. *Chlorella* may benefit more significantly with addition of compatibilizers, resulting in better performance in compatibilized blends (Zeller et al. 2013).

Green, red and brown algae were used as filler with PLA in one of the studies which showed that chlorophyll starts depredating at 60°C, while hemicelluloses and cellulose starts depredating at 220°C and 315°C, respectively. Depending on structure of algae, different degradation is seen. The residue of inorganic matter in algae varies around 45-50%, due to high contents of salts and other impurities, which are seen as crystals on surface of algae. Presence of such “contaminants” is important as it is in direct contact with polymer matrix and hence affects matrix-filler interactions and induces chemical reactions in the matrix. Metals such as iron, zinc or copper can react with PLA upon heating and result in chain scission due to transesterification. Various elements, mostly metals, are present on surface of algae. The study showed most abundant elements on red algae are potassium, chlorine, sulphur sodium, calcium and nitrogen. Algal hydrophilicity and inorganic substances on surface resulted in poor adhesion between filler and matrix. The composites with red algae are slightly more thermally stable, compared to green and brown ones. Overall, the addition of algae flakes resulted in decrease of tensile strength, irrespectively to the algae type. For all composite formulations except green algae, the Young`s modulus at 40 wt% load reaches approx. the same value as that of neat PLA, i.e. around 2,6MPa; for green algae, Young modulus exceed that of neat PLA by 25%. Young`s modulus defines the relationship between stress (force per unit area) and strain (proportional deformation) in a material. Test with red, brown and green alga as filler for PLA showed, that except for green algae, all tensile mechanical characteristic of composite decreased with the increase of algal concentration. Larger particles resulted in slightly better mechanical properties. The results show that depending on the application, algae can be used as filler in thermoplastic composites up to at least 40 wt% loading (Bulota & Budtova 2015).

Further work on algal composites should focus on the improvement of adhesion between filler and matrix, which means choosing sufficient algae type and particle size in order to optimize composite mechanical performance (Bulota & Budtova 2015).

5.3.1.2 Algal biomass as bio-filling for composite plastic

One of the possible uses of algal biomass from our demo centre is for production of bio plastic. Therefore, we conducted series of test in order to test our biomass adequacy for bio plastic production.

Test were done for the AlgaeBioGas project by Ana Podgoršek at Polymer Technology College, Slovenj Gradec.

In the tests, microalgal biomass from AlgaeBioGas ponds was used, together with biomass of *Spirulina*, use of which was already described in the literature. We used algal biomass as biofillers for composite biodegradable plastic, made out of biodegradable polymer PLA (polylactic acid) and composite plastic made out of synthetic polymer POM (polyoxymethylene).

For composite plastic, two types of biofillers were used: mixed algal biomass from AlgaeBioGas ponds and monoculture of cyanobacteria *Spirulina*. For production of synthetic polymer, concentration of algae which showed the best results in composite plastic production was used.

1 Preparation of biofiller from algae

Wet algal biomass, shown on Figure 15, was collected from the pond and dryed on the sun (Figure 16). Dry biomass was ground (Figure 17) to ensure homogeneity of biofiller and with this, better distribution for injection molding.

Figure 15 Wet algal biomass

Figure 16 Dry algal biomass

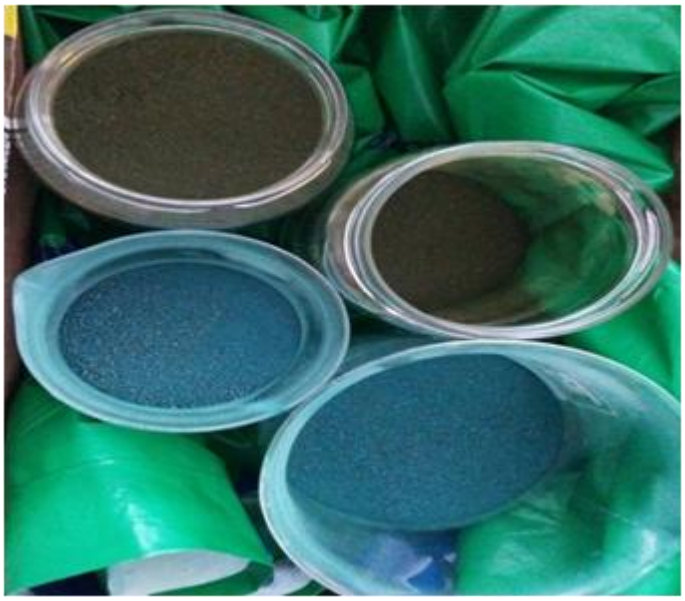

Figure 17 Ground algal biomass

2 Biomass analysis

Results show that *Spirulina* has higher degradation rate and higher temperature stability as mixture of algae from ABG ponds; degradation starts at 316°C. With heating up mixture of algae we recorded 86,85% decomposition of algal biomass, inorganic leftover was 13,15%. In case of *Spirulina*, smaller percentage of biomass was decomposed - 94,79%, 5,21% was inorganic leftover. Differences in the % of inorganic part are most likely due to higher heavy metal content in mixture of algae from AlgaeBioGas ponds.

Table 4 Heavy metals content in biomass from ABG ponds

Parameter	Unit	Measured value: mixed culture	Measured value: Spirulina	Method
Cu	mg/kg	56,64	8,1	SIST EN ISO 11885:2009
Zn	mg/kg	2109,89	72,08	SIST EN ISO 11885:2009
Cd	mg/kg	<1	<1	SIST EN ISO 11885:2009
Cr	mg/kg	67,56	<2	SIST EN ISO 11885:2009
Ni	mg/kg	43,01	<2	SIST EN ISO 11885:2009
Pb	mg/kg	8,63	<2	SIST EN ISO 11885:2009

With heating the mixture of algae from ABG ponds at 200°C, 11,41% ob biomass was lost and at 180°C 9,59% of biomass was lost. With heating from 40 to 180°C , 4,62% of biomass was lost and with heating from 40 to 180, 3,03% was lost.

Heating up *Spirulina* to 200°C and 180°c showed 8,21% and 6,66% loss of mass, respectively. With heating from 40 to 200°C and 40 to 180, 4,38% and 2,45% biomass was degraded, respectively.

The results show, that mixture of algae from ABG ponds is less heat resistant than *Spirulina*. Heating from 40 to 200°C, similar share from mixture and *Spirulina* was degraded: 4,38% and 4,62%, respectively.

Based on thermogravimetric analysis we decided to use temperature of 200 °C for injection moulding.

3 Extruding of biomass

Ground algal biomass was added to biopolymer PLA in different proportions:

95 : 5 (PLA : biomass)

90 : 10 (PLA : biomass)

Figure 18 Granulate with 10% Spirulina

Figure 19 Granulate with 5% Spirulina

Figure 20 Granulate with 10% biomass from ABG ponds

Figure 21 Granulat with 5% biomass from ABG ponds

4 Granule injection moulding

Granule injection moulding was done by Krauss Maffei CX 50-18' Blue power machine at temperature 175°C. Figure 22 and Figure 23 show plastics made out of PLA and 10 and 5% *Spirulina*, respectively. Figure 24 and Figure 25 shows PLA with 10 and 5 % of mixture of algae from ABG ponds, respectively.

Figure 22 PLA with 10% Spirulina

Figure 23 PLA with 5% Spirulina

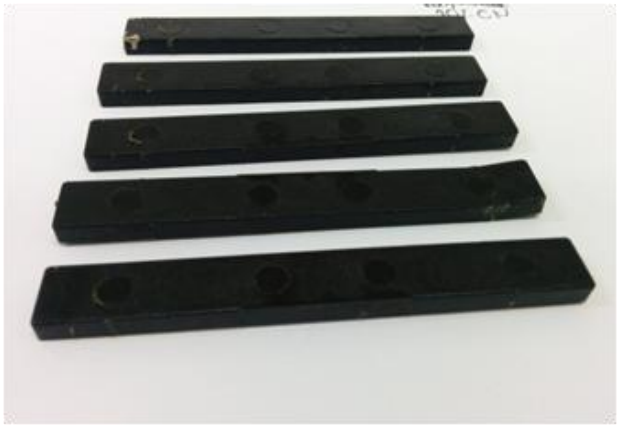

Figure 24 PLA with 10% biomass from ABG ponds

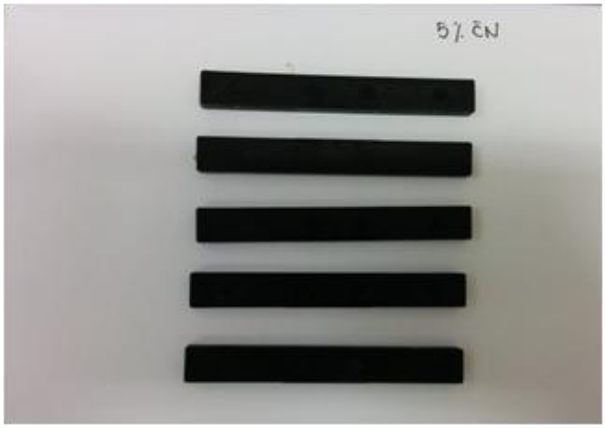

Figure 25 PLA with 5% ob biomass from ABG ponds

5 Thermogravimetric analysis

Test showed that plastic made with *Spirulina* is less temperature resistant than plastic made with mixture of algae from ABG ponds.

6 Bending properties

Test revealed that adding algal biomass to the material does not harden the material. *Spirulina* turned out to be more compatible with PLA than mixture of algae from ABG ponds, although plastic with 5% added mixture of algal biomass has better bending strength than plastic made with *Spirulina*.

7 Summmary of the results

With addition of algae from ABG ponds or *Spirulina*, degradation temperature is lowered. Adding algae does not affect E-module. With addition of algae from ABG ponds bending strength is lower, on the contrary, with *Spirulina* bending strength first decreases and then increases. This shows *Spirulina* compatibility with PLA matrix, when *Spirulina* concentration is 5-10%. For algae from ABG ponds, bending strength is higher at 5% of algae in comparison with 10% *Spirulina*, making algae from ABG pond better, regarding bending strength.

Deflection at maximal bending strength was best at 5% mixture from ABG ponds, meaning the mixture is suitable for use at the highest tested loads.

None of the mixtures with algae works as strengthening agent, but this feature could be improved with additives.

Glass transition temperature decreases, enabling lower temperature for processing and lower degradation level of matrix and algae during processing.

Crystallinity of pure PLA is 50%, melting point is 153°C. Crystallinity while using 5% mixture of ABG ponds, increases to 86%, melting point is lower for 4°C. In the case of 10% mixture from ABG ponds, crystallinity lowers to 39,61% and melting point is lower for 1°C. Mixture of algae from ABG ponds works as nucleating agent, increasing crystallinity. With higher crystallinity, probability for cracks after cold crystallinity is lower. Lowering the melting point temperature lowers processing temperature and consequently lower energy consumption.

Maximal concentration of mixture of algae from ABG ponds used for plastic is 5-10%, whereas max concentration from *Spirulina* is above 10%.

One of the options, considering high salinity in the ponds and digestate, might be growing *Spirulina* as a main culture, if biomass should be used for bio plastic production.

The highest concentration of algal biomass used in extrusion was 10%. At concentration of more than 10% extrusion was not possible, due to the crumbling of composite.

5.3.2 Biomass for fish feed

Microalgae feeds are used in aquaculture, mainly for production of larvae and juvenile shell and finfish. Most commonly used alga is *Spirulina* for fish and shrimp feed, another popular algae is *Hematococcus* with pigment astaxhantin, which gives salmon flesh reddish colour.

Due to high demand for fish products and consequently higher prices for fish meal, used as fish feed, other sources for aquaculture feed are being explored. Microalgae represent suitable replacement for fish meals, since they have high protein content, good nutritional value and are easy to cultivate also in areas unsuitable for plants. Currently, microalgae are used in aquaculture as food additives, fish meal, oil replacement, colouring of salmonoids, enhancers of nutritional value of zooplankton fed to fish larvae and fry. Positive effect of using microalgae in aquaculture are: weight gain, increased TAG and protein deposition in muscle, improved resistance to disease, improved taste and consistency of feed, increased omega-3 fatty acid content, increased rate of growth of aquatic species due to better digestibility. After all, microalgae are natural food for aquatic organisms. Microalgae are utilized in aquaculture as live feeds for molluscs, crustaceans, some fish species and for zooplankton used in aquaculture food chains. Most frequently used species of microalgae in aquaculture are *Chlorella, Tetraselmis, Isochrystis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema* and *Thalassiosira* genera. To be suitable for aquaculture, microalgae strain must be easy to culture, non-toxic, have high nutritional value with correct cell size and shape and digestible cell wall to make nutrients available. Protein and vitamin content is a major factor in determining nutritional value of microalgae (Guedes et al. 2015). Addition of microalgae to larval fish culture tanks confers a number of benefits such as perverting bumping against walls of the tanks, enhancing predation on zooplankton, nutritional value of zooplankton and improving larval digestive and immune functions (Anon 2012).

We wanted to use produced biomass from ABG ponds for fish feed, but unfortunately the biomass produced at the time of the test had too high levels of heavy metals, namely zinc and cadmium (see Table 4). Therefore, said biomass was not used for further testing, but we made some test with *Spirulina*, which shows that using biomass for fish feed is an option, we only have to establish the right biomass. Knowing the capacity of certain algal species for taking up heavy metals, one of the options would be heaving one algal pond as pre-treatment stage and leading the pre-treated waste water/digestate to the main pond, where algal biomass grown would be sufficient for use as fish feed.

5.3.3 Biomass for food

It seems that producing food from waste is not a particularly sensible path. But Spirulina farming producing food grade Spirulina is facing a legislative problem when the producers want to have Eco certified or Organic certified product. Up to

a year ago use of natural rock Chilean saltpeter (sodium nitrate) was generally approved for Eco-certified or Organically certified production of Spirulina, but this changed and Spirulina growers have to find alternative was to obtain Eco-certified source of (nitrogen rich) nutrients. Anaerobic digestion of (ecologically grown) plants seem to be a viable source. Indeed some of the Spirulina manufacturers have already turned to such sources, but most of them use sources that are a waste of energy (plant perfusions or similar technologies).

It seems that digestate from agricultural biogas plant that does not use any waste substrates would be a suitable source of ecologically certified nitrogen. This would also be used to produce energy instead of wasting it. But this approach is facing the same general challenges as our treatment of digestate: sub-optimal color, unbalanced nutrient structure, potential contents of heavy metals, variability in digestate composition and similar.

In our lab scale testing of Spirulina growth on biogas digestate (prior to start of this project) we obtained Spirulina growth rates similar to growth in ordinary Spirulina growth media when we used appropriately filtered and diluted digestate. At AlgaeBioGas demonstration centre we did not repeat these tests as the digestate source is food waste and it would be impossible to eco-certify such nutrient source.

We have set-up a Spirulina growth tank in the AlgaeBioGas greenhouse when we want to perform such tests on combination of digestate and artificial media. The aim of this testing in 2016 will be determination of methods for digestate purification before it can be technically used for Spirulina growth (it will never be legally appropriate for such use due to waste nature of the substrate). We intend to replicate the same procedure at the partner's site in Tuscany, Italy when we are first setting-up the ordinary Spirulina farm (planned May 2016). They have a biogas plant running exclusively on (ecologically grown) agricultural crops; digestate from that plant will be a good candidate to be used as Eco-certified Spirulina nutrient.

6 References

Anon, 2012. International Aqua Feed. , 15, p.56. Available at: http://www.aquafeed.co.uk/.

Arikan, E.B. & Ozsoy, H.D., 2015. A Review: Investigation of Bioplastics. , 9, pp.188–192.

Bulota, M. & Budtova, T., 2015. PLA/algae composites: morphology and mechanical properties. *Composites Part A: Applied Science and Manufacturing*, 73(0), pp.109–115. Available at: http://www.sciencedirect.com/science/article/pii/S1359835X15000809.

Constante, A. et al., 2015. Utilization of algae blooms as a source of natural fibers for biocomposite materials: Study of morphology and mechanical performance of Lyngbya fibers. *Algal Research*, 12, pp.412–420. Available at: http://linkinghub.elsevier.com/retrieve/pii/S2211926415300795.

Fu, F. & Wang, Q., 2011. Removal of heavy metal ions from wastewaters: a review. *Journal of environmental management*, 92(3), pp.407–18. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/21138785 [Accessed January 21, 2014].

Fujii, K. et al., 2010. Potential use of the astaxanthin-producing microalga, *Monoraphidium* sp. GK12, as a functional aquafeed for prawns. *Journal of Applied Phycology*, 22(3), pp.363–369. Available at: http://link.springer.com/10.1007/s10811-009-9468-z.

Guedes, A.C., Sousa-pinto, I. & Malcata, F.X., 2015. *Application of Microalgae Protein to Aquafeed*,

He, J. & Chen, J.P., 2014. A comprehensive review on biosorption of heavy metals by algal biomass: Materials, performances, chemistry, and modeling simulation tools. *Bioresource Technology*, 160, pp.67–78. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0960852414000935.

Hempel, F. et al., 2011. Microalgae as bioreactors for bioplastic production. *Microbial cell factories*, 10(1), p.81. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3214846&tool=pmcentrez&rendertype=abstract.

Holbrook, G.P. et al., 2014. Use of the microalga *Monoraphidium* sp. grown in wastewater as a feedstock for biodiesel: Cultivation and fuel characteristics. *Applied Energy*, 131, pp.386–393. Available at: http://dx.doi.org/10.1016/j.apenergy.2014.06.043.

Ikehata K., et al., 2006. Degradation of Aqueous Pharmaceuticals by Ozonation and Advanced Oxidation Processes: A Review, Ozone: Science & Engineering, 28:6, 353-414, DOI: 10.1080/01919510600985937. Available at: http://www.tandfonline.com/doi/pdf/10.1080/01919510600985937

Liu, Z. & Liu, Y., 2015. Synergistic Integration of Electrocoagulation and Algal cultivation to Treat Liquid Anaerobic Digestion Effluent and Accumulate Algal Biomass. *Process Biochemistry*. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1359511315301148.

Romera, E. et al., 2007. Comparative study of biosorption of heavy metals using different types of algae. *Bioresource Technology*, 98(17), pp.3344–3353.

Sheets, J.P. et al., 2015. Beyond land application: Emerging technologies for the treatment and reuse of anaerobically digested agricultural and food waste. *Waste Management*, 44, pp.94–115. Available at: http://www.sciencedirect.com/science/article/pii/S0956053X15300532.

Trivedi, J. et al., 2015. Algae based biorefinery—How to make sense? *Renewable and Sustainable Energy Reviews*, 47, pp.295–307. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1364032115002051.

Zeller, M.A. et al., 2013. Bioplastics and their thermoplastic blends from *Spirulina* and *Chlorella* microalgae. *Journal of Applied Polymer Science*, 130, pp.3263–3275.