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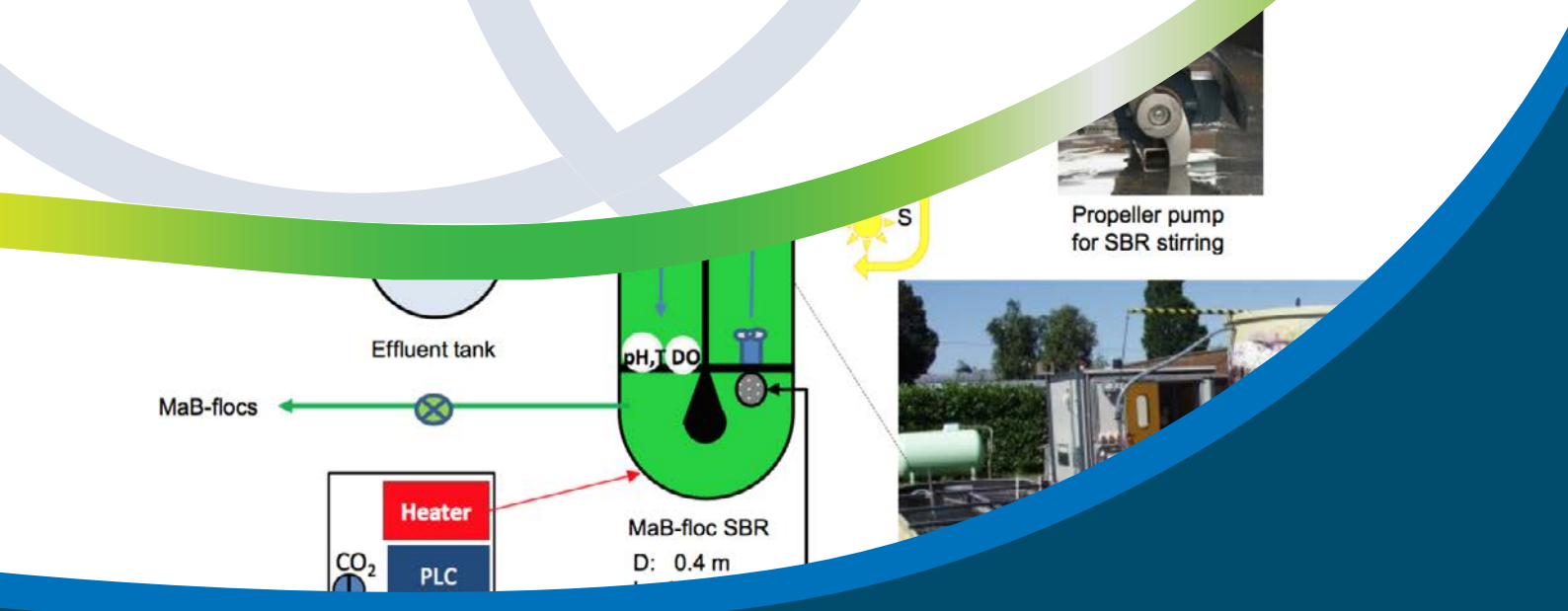
Best Practices for the Pilot-Scale Cultivation of Microalgae

Report WP1A6.01



Energetic Algae ('EnAlgae')

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Best Practices for the Pilot-Scale Cultivation of Microalgae

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Best Practices for the Pilot-Scale Cultivation of Microalgae

Executive Summary

This document is a compilation of Best Practice recommendations and considerations employed by the EnAlgae microalgal pilot facilities. EnAlgae was a four year Strategic Initiative of INTERREG IVB North West Europe programme. One of the outputs of the EnAlgae project was the development of an integrated network of pilot plants for growing microalgae. An important part of this activity was an exchange of information on optimal pilot operation both with respect to best practices and Standard Operating Procedures (SOPs; documented elsewhere).

Best Practices (including recommendations and considerations) are presented for siting a pilot plant; microalgae cultivation (including strain selection, preparation, maintenance and automation of production systems, nutrient sources and addition); harvesting microalgae biomass and biomass valorization. In addition, detailed technical descriptions of the different pilots and the hardware/software they use in operation have been provided. The document brings together Best Practices used by the microalgal partners to serve as a useful starting point for those new to pilot and commercial scale algal cultivation.

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Best Practices for the Pilot-Scale Cultivation of Microalgae

1.0 Introduction

1.1 Overview of microalgae cultivation and valorisation for energy production and other high-value products (biorefinery approach)

Owing to climate change, depletion of traditional fossil fuel reserves and increased demands for land and crops for human consumption, the application of algae for use in bioenergy production continues to receive worldwide attention. Due to high growth rates and productivity of biofuel pre-cursor molecules e.g. lipids and carbohydrates, microalgae potentially represent a valuable feedstock for renewable energy production (Davey *et al*., 2014; Scott *et al*., 2010). Microalgae can be cultivated on land unsuitable for agriculture and can utilise and remediate low-quality water, such as agricultural runoff, municipal, industrial or agricultural wastewaters, which are rich sources of nutrients for microalgal growth. Furthermore, the ability of these microorganisms to adapt (to a certain extent) to changing environments coupled with the capability to remediate liquid and gaseous wastes is an important positive feature for algal biomass utilisation. Subsequently, microalgal production is viewed by many as a potential paradigm shift from fossil based chemicals and fuels, to sustainable and renewable products and energy. However, currently the only way to offset the commercial and environmental costs of cultivating and processing algae for energy production would be to guarantee co-production of additional high quality end-products, often described as the biorefinery concept (Trivedi *et al*., 2015). To this end algal biotechnology is drawing increasing interest due to its potential as a source of valuable pharmaceuticals, pigments, carbohydrates, and other fine chemicals (Schlarb-Ridley and Parker 2013).

1.2 EnAlgae project and pilot plants

The Energetic Algae (EnAlgae) project (http://www.enalgae.eu/) is a four year programme that was approved by the EU financial instrument INTERREG IVB NWE Programme in March 2011. EnAlgae is an integrated network of algal pilot plants across NW Europe, which incorporates 19 partners and 14 observers across the seven EU Member states. Its overall aim is to reduce CO_2 emissions and dependency on unsustainable energy sources in NW Europe, through the development of sustainable technologies for algal biomass production, bioenergy and greenhouse gas mitigation, and taking these technologies from pilot facilities through to market-place products and services (EnAlgae, 2014). Six microalgal pilot plant demonstration facilities, each different from the other, were developed and rigorously tested as part of this project. These pilot plants reflect the diversity of microalgal production systems worldwide today and include photobioreactors, open-pond and raceway type systems designed to utilise gaseous and liquid waste-streams for the purposes of biomass production and utilisation for energy and additional products. The experience gathered from these pilot operations has been formulated into this document as a best practise guide for sustainable production of microalgae at pilot scale specific to the NW Europe region. A summary of these pilot plants can be seen in Table 1 with more detailed descriptions of each pilot plant found in the relevant appendices.

1.3 Scope of Best Practice report

Best practice (BP) is a technique that, through experience and research, has proven to reliably lead to a desired result. A commitment to using the best practices in any field is a commitment to using all the available knowledge and technology at one's disposal to ensure success. The aims of this best practice report are to describe considerations and best practice recommendations for the preparation, culture, harvesting and processing of microalgae biomass for the production of energy (and co-products where relevant) at a pilot scale in NW Europe. These recommendations draw on the diversity of considerable experience gathered from the different pilot operations within the EnAlgae consortium. Further, within the report, links are available to detailed protocols, techniques or methodology that were utilised by the different pilot plant operations within the EnAlgae consortium to achieve this aim. Other useful information including flow diagrams of operation and pilot schematics can be found throughout the report in addition to detailed information contained within the Appendices. Overall we envisage that this report will act as a valuable guide to pilot scale microalgae culture operators for the development of best practice management practices.

Table 1. Summary of the pilot plant operations within the EnAlgae project.

Pilot name	Pilot type	Key design elements/function	Detailed descriptions
P1-Swansea University Swansea, UK	Photobioreactor (PBR) facility: 1 × 400 L tubular horizontal PBR located in a temperature and light controlled laboratory 2 × 600 L tubular horizontal PBR 1 × 2000 L tubular vertical PBR in heated greenhouse. Portable Laboratory with PBR inside located at industrial site.	Various systems with flue gas utilisation and wastewater treatment; algal biomass production for high value products	Appendix 1.1
P2-Ghent University, Kortrijk, Belgium	Open raceway pond (28 m^2, approximately 10 m^3) stirred by propellers-wastewater treatment	Utilising local consortia of bioflocculating microalgae and bacteria (microalgal bacterial floc or MaB-floc) to treat various wastewaters at (agro-) industrial sites (Inagro and Alpro, BE)	Appendix 1.2
P3-InCrops Enterprise Hub, University of Cambridge, UK	Photobioreactors: small (100 mL to 1 L) to medium size (10 L to 150 L)	Pilot facility to investigate how by-products of drinking water purification can be used to grow algae such as *Phaeodactylum tricornutum*, *Chlorella vulgaris* and polar algae	Appendix 1.3
P4-Plymouth Marine Laboratory, Plymouth, UK	Photobioreactor	Large-scale facility coupled with emission stack from power station at industrial site (Boots Ltd., Nottingham, UK)	Appendix 1.4
P5-Hochschule für Technik und Wirtschaft des Saarlandes, htw saar Germany	Photobioreactor	Integrated algae production utilising dissolved nutrients in process water of marine recirculation aquaculture systems (RAS)	Appendix 1.5
P6-Wageningen UR/ACRRES, Netherlands	Open pond	The pilot consists of two open ponds (indoor and outdoor) of 250 m^2 that utilise waste streams from anaerobic digesters (CO_2 and excess heat) and LED light assisted pre-culture basins (1, 20 and 50 m^3). Main species grown are *Chlorella* spp. and *Scenedesmus* spp.	Appendix 1.6

EnAlgae Partners :

- Wageningen UR – ACRRES, Lelystad
- Cambridge University, Cambridge UK
- Plymouth Marine Laboratory, Plymouth, UK
- Ghent University, Kortrijk, Belgium
- Swansea University, Swansea, UK
- Htw saar Germany

2.0 Best practices for microalgae cultivation

2.1 Considerations for siting a pilot plant

Regardless of the subtle differences in application or indeed outputs of the different pilot systems developed within the EnAlgae project (which will become apparent throughout this Best Practice report), a number of initial common considerations can be highlighted regarding the choice of location for a pilot system. These considerations are worthy of note prior to pilot development and therefore have been described at the start of this report in Table 2. The following section describes best practices for the cultivation of microalgae within the developed pilot, both for microalgae monocultures cultured in synthetic growth medium or in diluted wastewater, and for mixed cultures grown in wastewater.

Table 2. List of considerations prior to siting and developing a pilot microalgal cultivation system in NW Europe.

Pilot aspect	Essential consideration	Desirable consideration
Location	• Large flat area with sufficient footprint for system and sundries. • If outside must have minimal shading from other structures. • Adequate electricity supply (potentially 2-phase). • If subject to variable temperatures a heating and cooling system may be required. • Adequate clean water supply. • Access to nutrient supply. For wastewater treatment systems the water must be of suitable composition (colour, nutrient concentration, nutrient ratios, toxic elements, suspended solids) and volume. • Access to CO_2 supply (flue gas, cylinders etc.) or wastewater which contains a suitable carbon source (e.g. bicarbonate, organic carbon). • Access to a drainage system that accepts treated wastewaters or adequate 'kill tanks'. • Ability to obtain planning permission and licensing for pilot development. • Proximity to skills base e.g. university or industrial end user. • Ready access to/for material inputs and maintenance contractors (pumps, metal framework, pipes/hosing, compressors, valves, environmental sensors, programmable logic controllers (PLC), data acquisition systems, electrical circuits, tanking, centrifuges, filtration systems etc.).	• If outside, preferably south-facing. • If open system, avoid locations with airborne contaminants (e.g. dust, pesticides, herbicides). • Close proximity to stock culture laboratory (if applicable). • Close proximity to maintenance staff or contractors. • General ease of access: roads and infrastructure. • Safe and easy access (e.g. enough space to walk around the reactors). • Access to low grade waste heat.
Inputs/costs	• Electricity for pumps, compressors, centrifuges etc. • Water consumption. • Supplemental lighting. • Nutrients (often not needed when using wastewater). • Labour/staff (process engineer, electrical engineers, external contractors, process engineers). • Transportation: breakdown and relocation of mobile systems. • Costs of growing/storing cultures/small inoculum.	• Skilled staff availability in cases of scientific research support. • Flue gas or other carbon source.
Regulation/ legislation	• Regulation and legislation associated with site boundaries including: use of gases, waste outputs including wastewater and gas emissions and use and disposal of hazardous chemicals. • Planning applications, planning permissions and permits may be required. • Staff should be trained in the use of hazardous chemicals and comply with the risk assessments and follow all relevant health and safety measurements. • In case of operation at an industrial site: contract with industrial partner regarding intellectual property, communication, safety, insurance, responsibilities, time period.	• In case of operation at an industrial site: additional insurance (e.g. fire, theft).

2.2 Selecting and maintaining microalgae and cyanobacteria for cultivation

Given the huge diversity of microalgae species and strains present in aquatic environments and indeed the number that are now available from the major service collections commercially (e.g. see CCAP; www.ccap.ac.uk), and the diversity of growth characteristics and metabolic biochemistry associated with these different species, the importance of selecting specific microalgae or cyanobacteria for cultivation should not be understated. In some cases these strains have been screened for specific attributes e.g. lipid profiles or growth characteristics. Where high quality data are available in the public domain e.g. Lang *et al*. (2011) and Slocombe *et al*. (2015), these can steer species, or even strain, selection. For pilot-scale cultivation the criteria for selecting microalgae is governed by several key factors including (but not limited to), the intended application of final biomass; the tolerance of the organism to the prevailing conditions of the cultivation system (e.g. temperature, light, salinity, flue-gas components); the potential growth rates and productivity of the strain(s) (Adesanya *et al*., 2014); and ease of harvesting. These considerations are often borne out of extensive experience with microalgae cultivation at pilot scale and will vary depending on local conditions. However, we can recommend several example strains of microalgae that have been tested to good effect within the EnAlgae project in the pilot scale systems, and have highlighted their various advantageous properties relevant to production at pilot scale (Table 3).

Table 3. Microalgae species utilised at pilot plants within the EnAlgae project for potential energy production.

Species used	Desired characteristics	Energy focus	Other attributes/ considerations	Used in Pilot
Chlorella spp. (green algae) *Scenedesmus* spp. (green algae) *Nannochloropsis* spp. (Eustigmataceae) *Phaeodactylum* spp.(diatom) *Chlamydomonas* spp. (green algae)	• High biomass productivity. • High oil content.	• Biodiesel (see Griffiths and Harrison, 2009).	• Tolerant to different liquid waste streams (municipal, agricultural, aquaculture). • Tolerate fluctuating nutrients. • Readily available from culture collections.	P1, P3, P6,
Phaeodactylum tricornutum (marine diatom) *Tetraselmis suecica* (marine flagellate) *Isochrysis galbana* (marine flagellate) *Dunaliella salina* (halophile green algae) *Pavlova lutheri* (marine flagellate)	• Grow in brackish / marine conditions.	• Biodiesel (see Griffiths and Harrison, 2009). • Anaerobic digestion.	• Source of omega 3 and 6 oils.	P1, P3
Chlorogloeopsis spp. (cyanobacteria)	• High biomass productivity. • Thermotolerant	• Hydrothermal liquefaction (HTL; Biller *et al*., 2012).	• Autoflocculating for harvesting. • Tolerant to chemicals in flue-gas emissions within the screened loading rate. • Synthesizes bioactives (Llewellyn *et al*., 2011).	P4

In microalgal culture operations where the composition of the biomass is largely uncontrollable or not critical, e.g. the bioremediation of localised effluents, one strategy is to use microbial consortia containing microalgae harvested from the relevant wastewater at the site. These can then be used as an initial inoculum, as these species are already adapted to grow on these waste streams. However in certain reactor types, such as heavily mixed ponds, the reactor environment differs from the location where the inoculum was collected. Therefore an adapted microbial consortium will naturally develop and may vary markedly from the initial microbial composition of the inoculum (Van Den Hende, 2014). An obvious advantage of this approach is that since a local microbial consortia is collected from the relevant environment, maintenance of microalgae cultures at a laboratory scale is often not required. The disadvantage of this approach is that the dominant microalgae species of the final biomass can be highly variable from the initial inoculum, and to date this species selection cannot be predicted via modelling.

Regardless of strain(s) chosen for pilot scale monoculture cultivation, there is often a requirement to maintain them at a laboratory scale for the purposes of up-scaling for cultivation in the pilot reactor system. Indeed the maintenance of stable starter cultures for a specific purpose, e.g. the production of a valuable metabolite, is a fundamental prerequisite for the sustainability of any biotechnological process, algal or otherwise. This requirement for strain maintenance and culture at different scales (0.001–100 L) is dictated to a large extent by the limitation on the minimum volume of actively growing algae culture needed to initiate a new culture. For most applications we recommend for inoculation 1–10 % v/v when scaling up to the pilot system and detailed consideration needs to be given with respect to the need for both master stock cultures and working stock cultures. We recommend that wherever possible dedicated, trained personnel familiar with microalgae cultivation, are utilised for the purpose of stock culture maintenance and preservation. Although the maintenance or sub-culturing practices of strains is largely dependent on species; we generally recommend that in the short term (weekly or fortnightly) strains are sub-cultured in appropriate liquid media to ensure a rapidly growing healthy inoculum is available for immediate use. For medium term storage (6 months) we recommend cultures are also maintained on solid media preferably under relatively low light conditions (PAR < 25 µmol m^2 s^{-1}), and for long-term storage strains are held as cryopreserved master stocks if technically possible. Further details of strain sub-culture and maintenance can be found in the Scottish Association for Marine Science report on "Criteria that should be considered, and options available, for best practice maintenance of micro-algal strains associated with the EnAlgae project" and a series of SOPs on best practice of the management and maintenance of master stock-cultures by serial transfer and cryopreservation.

2.3 Preparation and inoculation of pilot systems

In all cases of 'closed' pilot systems i.e. photobioreactors used within the EnAlgae project, a process of system preparation was taken as an important step to ensure minimal microbial contamination and ensure quality and consistency of biomass and associated products. This usually involved the pre-treatment of the bioreactor system with a biocide (or 1 % bleach [sodium hypochlorite] solution) followed by subsequent cleansing. In open raceway ponds which utilise microalgae consortia to treat wastewater, the systems do not need to be disinfected prior to inoculation, akin to other wastewater treatment systems. Once the system has been cleansed it is ready to fill with the relevant growth media and/or add nutrients to the system prior to inoculation (or indeed throughout culture). As a general recommendation for closed reactor systems, nutrient stock solutions should be either directly sterilised to remove microbial contamination, and/or the final growth media should be sterilised wherever possible (usually filtered through 0.2 µm porosity filters or via autoclave methods).

The inoculation of closed systems typically requires 1–10 % of pilot system volume of inoculum to ensure a successful healthy growing culture and therefore scale-up of cultures from smaller stock culture volumes is imperative. For open wastewater systems that are inoculated with locally growing microbial populations, culturing of the inoculum in laboratory scale reactors prior to pilot scale cannot be recommended, as indoor and outdoor cultures in the same wastewater may greatly differ in community structure (Van Den Hende *et al*., 2014a) The variable solar radiation experienced in NW Europe can become a critical factor during inoculation of pilot systems. Intense sunlight may cause photoinhibition and even cell death in dilute cultures of microalgae that are formed during inoculation. Therefore, we recommend shading of the PBR be considered in the early 'lag-phase' of culture growth after inoculation, especially during spring and summer, until cells resume growth. Ideally if the bioreactor system is modular, one module should be inoculated initially, and microalgae cells should be redistributed to the remaining modules once actively growing.

2.4 Control parameters during culturing: monitoring and automation

It is generally important to monitor and control several factors during pilot operation and microalgal production in order to maintain high biomass productivity and ensure acceptable yields and quality of biomass (and associated products) for valorisation. The number and type of parameters that we recommend are monitored and controlled and will vary between pilot types and research aims, depending on the type of installation and the main purpose of the algae growing system (e.g. algae biomass or water remediation). A summary of these parameters used at each pilot in the EnAlgae project; why they are measured; frequency of measurement; and general considerations and recommendations and links to detailed example Standard Operating Procedures (SOPs; see EnAlgae report entitled 'Standard Operating Procedures for Analytical Methods and Data Collection in Support of Pilot-Scale Cultivation of Microalgae') describing measurements themselves, can be found in Table 4. Furthermore, these parameters have been categorised into *essential* and *desirable* to reflect their relative importance and requirement in general pilot operations, in recognition that the availability of local resources such as staff and infrastructure will dictate the number of parameters that can practically be measured.

Table 4. Recommendation parameters that should be monitored and controlled during pilot scale microalgal production.

Parameters	Frequency	Automation used*	Purpose	Considerations/recommendations
Essential				
pH	10 min average, hourly, daily	Yes (P1, P2, P5, P6)	• Used as an indirect measurement of dissolved CO_2. Used to control automated CO_2 injection and maintain neutral pH status for adequate microalgal photosynthesis and growth. Used as an indirect indicator of photosynthetic activity. In the case of wastewater treatment: comparison with effluent discharge norms.	• Manual measurement simple and requires basic equipment (see SOP 1.2). If automated, requires weekly re-calibration or *in situ* calibrated pH probes. • As pH depends on the temperature and ideally must be temperature compensated, a temperature probe should be included in/with the pH sensor.
Light (radiation)	10 min average, hourly	Yes (P1, P2, P5)	• Light is required for microalgae photosynthesis and growth therefore a useful indicator of growth potential in the system.	• The large temporal and regional variability in this parameter across NW Europe will dictate productivity. • Measured using Photosynthetic Active Radiation (PAR) meters (see SOP 1.4) using the standard units $\mu mol\ m^{-2}\ s^{-1}$. • For outdoor facilities local light radiation data can be gathered from the nearest meteorological station (usually via the internet) as indicator of prevailing light conditions. • In summer used to control temperature in greenhouse and cooling of PBR with water.
Temperature	10 min average, hourly, daily	Yes (P1, P2, P5, P6)	• Growth of microalgae temperature dependent and therefore governs productivity.	• Typical tolerable temperature range 16–27°C for most microalgae used in NW Europe. • Desirable to use external heat sources in combination with automated temperature control during autumn/winter periods to maintain productivity. • Manual measurement simple and requires basic equipment, i.e. temperature sensor. • If automated, requires calibrated probes *in situ* (see SOP 1.1).
Biomass	Daily/twice a week	Yes (P1, P2)	• A parameter measured to monitor growth efficiency and productivity of the system. Used to determine the harvest volume.	• Cell numbers can be enumerated using microscopy. Accurate when calibration method is rigorous. Not suitable for filamentous, multicellular or flocculating strains (see SOP 3.3). • Light absorbance measurements (optical density) automated. They must be calibrated with dry weight of the microalgae in use (non-linear calibration; see SOP 3.2). The method is not feasible for cultures with variable composition and is not recommended in the case of coloured or turbid wastewater. • Gravimetric measurements (dry weight and ash-free dry weight; or TSS and VSS in wastewater treatment) are a simple and very accurate indicator of total biomass density, but time consuming and there is an additive effect of contaminating microorganisms (see SOP 3.1). • Chlorophyll-a is a good indicator of microalgae growth but must be carefully calibrated to species and conditions (see SOP 3.4). • Total organic carbon (TOC) is an accurate measurement of biomass and can be measured using a spectrophotometer and test kits or specialist equipment (direct TOC/TIC analyser) but is time-consuming if not using kits and there is an additive effect of contaminating microorganisms and dissolved organic carbon (see SOP 4.6).

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Parameters	Frequency	Automation used*	Purpose	Considerations/recommendations
Nutrients e.g. nitrate (NO_3^-), ammonia (NH_4^+); phosphate (PO_4^{3-})	Daily-weekly	Yes (P2)	• Used to monitor nutrient removal by microalgae or microbial consortia in all system types (including wastewater) or by other processes (ammonia volatilisation, etc.), and determine nutrient supplementation requirements where relevant. • Used to compare with the effluent discharge norms (in case of wastewater treatment or discharged growth media).	• Most measurements are based on colorimetric assays (requires spectrophotometer) and use of manual test kits (see SOP 2.1–2.6). • There are many nitrate probes on the market, however the cheaper ones are not reliable and give very variable results. • Total nitrogen and phosphorus can be measured if information regarding the chemical form of nutrients is not required (see SOP 2.4 and 2.6), and should also be measured in case of effluent discharge into water bodies.
Desirable				
Microalgae predators	Weekly	No	• Can cause a sudden crash in system if numbers escalate rapidly, especially in systems open to the environment e.g. ponds, wastewater raceways.	• Weekly microscopic examination and enumeration of predators e.g. rotifers advised especially in open pond systems.
Biomass nitrogen and phosphorus content	Weekly	No	• This ideally should be measured in conjunction with total carbon in the biomass and nutrients in the media to ascertain nutrient uptake and utilisation by microalgae biomass and physiological status.	• The elemental analyses of both N and P in biomass are complex and require specialist equipment and training (see SOP 4.6)
Total Inorganic Carbon (TIC)	Weekly/2 per week	Yes (P2)	• Used to monitor inorganic carbon concentration in the growth medium/wastewater and as a potential indicator of photosynthetic activity.	• Manual measurement simple but requires spectrophotometer (see SOP 2.8).
Dissolved oxygen (DO)	Hourly/ every 10 minutes	Yes (P1, P2)	• Used as an indirect measurement of photosynthetic activity (or mechanical aeration by injection of oxygen-rich flue gas). In the case of wastewater treatment: to determine aerobic, anoxic or anaerobic conditions of the reactor and link with nutrient removal processes, especially for BOD_5 and nitrogen species (e.g. nitrification, denitrification, etc.). As supersaturated DO concentrations in the reactor can be toxic for certain microalgae species, DO should be monitored for these species.	• Manual measurement simple and requires DO sensor (see SOP 2.9). If automated, requires calibrated DO probe *in situ*. As DO depends on the temperature, a temperature probe should be included in the DO sensor.
Water consumption	Daily	Yes (P2)	• Relevant parameter for cost calculation.	
Electricity consumption	Daily	Yes (P2)	• Relevant parameter for cost calculation.	
Heat consumption	Daily	No	• This gives information regarding heat usage (and excess loss) in the system.	

**For a description of the hardware and software for automation please see Appendix 2.0. Note that all pilots measured the above parameters regardless of automation. A major constraint of submersed DO probes is biofouling on the optical surfaces of the sensor or biofouling at the inside wall of the PBR at the position of an external sensor. If possible, optical surfaces should be cleaned regularly. Alternatively, correction factors can be obtained from parallel manual determinations of DO in a spectrophotometer. These measurements should be taken at the same time each day.*

2.5 Bioremediation with microalgae / continuous production systems

If, in addition to microalgae biomass production, another primary objective of the pilot facility is to remediate wastewater streams, and proof of the remediation is essential for pilot performance criteria, then a separate set of parameters can additionally be measured to support remediation criteria. A summary of these parameters, why they are measured, frequency of measurement, different forms of the same measurement, general considerations and recommendations, and links to detailed example Standard Operating Procedures (SOPs) describing measurements themselves, can be found in Table 5.

Table 5. Parameters that should be monitored to assess wastewater treatment by microalgae (or microalgae-containing microbial consortia).

Parameter	Purpose	Considerations/recommendations
Electrical Conductivity (EC)	• An indication of the total ionized constituents and the salinity.	• To compare the effluent EC with the effluent discharge norm. • Manual measurement with EC sensor (see SOP 1.3).
Turbidity	• Indication of the water clarity.	• To compare the influent and effluent turbidity; indirect measurement of suspended solids in the influent and effluent. • To determine the turbidity removal efficiency and rate during treatment. • Very easy to measure, but needs special equipment (see SOP 3.2).
Biological Oxygen Demand (BOD_5)	• The amount of dissolved oxygen needed by aerobic microorganisms to break down organic carbon in 5 days.	• To compare the effluent BOD_5 with the effluent discharge norm. • To determine the BOD_5 removal efficiency and rate during treatment (see SOP 2.10). • BOD_5 is based on microbial oxygen consumption in typical gastight BOD_5 bottles. Takes 5 days to measure and needs specific equipment.
Chemical Oxygen Demand (COD)	• The chemical oxygen demand is a test used to indirectly determine the organic content of water (based on the oxygen needed to oxidise the organic matter).	• To compare the effluent COD with the effluent discharge norm in wastewater treatment systems. • To determine the COD removal rate and efficiency during treatment (see SOP 2.11). • COD is based on an colorimetric assay (requires spectrophotometer).
Total Carbon (TC), Total Organic Carbon (TOC) and Total Inorganic Carbon (TIC)	• The total amount of carbon, organic carbon and inorganic carbon.	• To determine the TIC removal during treatment, as it is an indicator of the interplay of carbon based processes such as photosynthesis (TIC removal), carbonate precipitation (TIC removal), and respiration (TIC addition) and CO_2 dissolving of flue gas injection (TIC addition). • Manual measurement is straightforward, but requires a spectrophotometer and test kit or a TOC/TIC/TC analyser (see SOP 2.8).
Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)	• Can be used to determine the biomass productivity and the harvesting volumes, and to determine the VSS/TSS ratio as indicator of the organic and inorganic content of microalgae-bacteria consortia.	• Gravimetric measurement is a simple very accurate indicator of total biomass density, but it is time consuming and there is an additive effect of contaminating microorganisms (see SOP 3.1).
Nutrients e.g. nitrate (NO_3^-), ammonia (NH_4^+), phosphate (PO_4^{3-})	• Used to monitor nutrient removal by microalgae or microbial consortia in all system types (including wastewater) or by other processes (ammonia volatilisation, etc.), and determine nutrient supplementation requirements where relevant. Used to compare with the effluent discharge norms (in case of wastewater treatment or discharged growth media).	• Most measurements are based on colorimetric assays (requires spectrophotometer) and use of manual test kits (see SOP 2.1–2.6). • Total nitrogen and phosphorus can be measured if information regarding the chemical form of the nutrient is not required (see SOP 2.4 and 2.6), and should also be measured in case of effluent discharge into water bodies.

Furthermore, since the numbers of parameters to be measured during microalgae cultivation are numerous, it is desirable and recommended to have at least some measurements automated *in situ*. This has the advantage of reducing the labour requirements for parameter measurement, permitting greater frequency of measurement, and, with appropriate feedback control systems in place, permits more rigorous control of conditions within the production system e.g. the use of pH measurement to control CO_2 addition to cultures. As detailed in Table 6 a number of measurement parameters were automated at the EnAlgae pilot plants. Examples of software control systems used in the automation of these pilots can be found in Appendix 2.0. However, since this list is not exclusive and a number of appropriate software control systems are commercially available, a list of generic recommendations on the desirable characteristics of such systems (other than low cost and high reliability) has been generated based on experience gained from the EnAlgae pilots which is described in Table 6.

Other parameters that pertain to characterisation of the microalgae biomass in terms of proximate and chemical composition have been described in the biomass valorisation section.

Table 6. General considerations and recommendations for automation and control system of pilot plants.

Aspect	Considerations and recommendations
Hardware	• Parts readily available from electronics suppliers. • Colour coding or labelling: circuit diagram to reflect this. • Circuitry should be housed securely in a cabinet located away from drains and splashes. • Emergency stop buttons should be easily and prominently located e.g. to stop rotors.
Software	• User friendly visual interface. • Programming language that is widely used so that users can be easily trained (not essential). • Ease of maintenance-reduced reliance on service providers.
System control	• Ability to switch between fully automated and manual over-ride. • Ability to separate parameter control and modules (photobioreactor units) if required e.g. run at different speeds.
Real time analysis	• Visual representation of the operation modus (e.g. flue gas addition, period of a Sequencing Batch Reactor cycle) and measured parameters e.g. graph of light levels. • Ability to display visuals on a number of devices for remote monitoring is very important.
Data collection	• Sufficient memory capacity for data storage and back-up of data. • Ability to export as CSV/MS Excel. • Ability to define measurement intervals (minimum down to minute intervals). • Should allow for detection and removal of instrument noise through signal averaging.

2.6 Sourcing nutrients (carbon, nitrogen and phosphorus) for cultivation

Generally, the elemental composition of microalgae is C: N: P of 106: 15: 1 (atomic basis) or 50: 7: 1 (weight basis). The broader proximate composition of microalgae comprises lipids (ca. 7–23%), carbohydrates (ca. 5–32%), and proteins (ca. 6–52%), where chemical composition is dependent on the species and culture conditions (Becker, 2007; Griffiths and Harrison, 2009; Singh and Gu, 2010). Subsequently microalgae need a supply of macronutrients containing carbon, nitrogen and phosphorus and indeed other micronutrients such as magnesium and iron, in order to sustain growth. Therefore it is essential that any microalgal cultivation system at pilot scale cater for these nutrient demands in order to sustain acceptable levels of biomass productivity for downstream processing. Many different nutrient sources were used within the EnAlgae project to cultivate microalgae at pilot scale. Based on these observations, an overall recommendation when sourcing nutrients for microalgal growth is, where possible, to choose a low-cost sustainable source available locally to the pilot facility. Although this may result in additional costs at the front-end of pilot development e.g. the use of specialised infrastructure to capture CO_2 from flue gases, these can potentially be offset in comparison to the ongoing costs of sourcing nutrients from further afield. Within the EnAlgae project, there was an absolute requirement for additional carbon (usually CO_2 in photoautotrophic culture) for all of the microalgae pilot cultivation systems, including for some wastewater bioremediation systems, namely MaB-floc raceway ponds treating aquaculture wastewater (Van Den Hende *et al.*, 2014a) and food industry conventional activated sludge (CAS) effluent (Van Den Hende *et al.*, 2015b), but not for food industry up flow anaerobic sludge blanket (UASB) effluent (Van Den Hende *et al.*, 2015b). Examples of the various sources of CO_2 used along with associated considerations for each source can be found in Table 7. Aside from the wastewater remediation MaB-floc pilot (P2), other essential nutrients were additionally supplied to the EnAlgae pilots during microalgae cultivation, and have also been summarised in Table 7.

Table 7. Diversity of nutrient sources utilised at different microalgal pilot systems within the EnAlgae project.

Nutrient	Source	Considerations and recommendations
Carbon CO_2, bicarbonate, carbonate, organic carbon	Coke-oven flue gas (P1). Wood-burner flue gas (P1). Flue gas from Combined Heat and Power (CHP; P6). Synthetic flue gas from biogas combustion (5% CO_2; P2).	• Other toxic gases (NO_x, SO_x) must be monitored. • High CO_2 (needs to be adjusted to <20%). • High temperature (80°C) needs to be cooled to 20°C. • CO_2 variable-must monitor pH as indirect measurement (see SOP 1.2). • Low flue gas flow rates (<0.0001 vvm) in case of only pH adjustment by flue gas injection for aquaculture and food industry wastewater (P2).
	Power-station flue-gas (P4).	• Advantage of high CO_2. • High temperature, high flow rates. Specialised infrastructure-high capital investment required. • Location dependent e.g. power station.
	Compressed air (P3). CO_2 cylinder (P5, P3). Carbon present in wastewater (P2). *In situ* production of CO_2 via organic carbon oxidation (potentially at P2).	• Low CO_2 but relatively cheap. Not sufficient for high density cultures (>1 g L^{-1} dw). • Relatively cheap if added on demand (pH control required). Use either pre-mixed 5% CO_2 in air or 100% CO_2 mixed with air on site (care must be taken with asphyxiation risks). • No additional costs. • No additional costs.
Nitrogen and phosphorus Nitrate (NO_3^-), ammonia (NH_4^+); phosphate (PO_4^{3-})	Agricultural waste stream (dairy manure and anaerobic digestate; P1). Aquaculture waste stream (P1, P2). Food industry waste streams (P2). Process water of zero-exchange Recirculation Aquaculture Systems (RAS) with clear water technology (P5). Brine solution* (P3). Artificial fertilizers (potassium nitrate and potassium phosphate).	• Required pre-treatment/processing (acid treatment pH<3; diafiltration; see Gerrardo *et al*., 2013); solids removal in settling tanks at P2). • High variability in N and P-monitoring and supplementation sometimes required, although not for some pilots e.g. P2. • Continuous supply of N and P, integration in RAS requires high degree of automation. • Nitrate rich but high salt and low in phosphorus. • More expensive than waste streams, but no contamination with organic matter particles that interferes with light interception and algae growth.
Minerals and vitamins Iron, manganese, zinc, copper and molybdenum	Salt complexes (P5). Wastewaters (P2).	• Chelates are often used for optimal availability of micronutrients. Otherwise they will precipitate with other ions (e.g. phosphate salts) or they will oxidise (e.g. iron). • Depending on the wastewater type, minerals and vitamins need to be added, e.g. especially iron should be monitored.

**Derived from local water purification process (see Appendix A1.3)*

When using wastewater remediation as a strategy to source nutrients and cultivate microalgae, we recommend the wastewater itself must first be screened to confer its level of essential nutrients and pathogens and thus suitability for microalgae production, as the chemical composition of wastewaters can be dynamic and subject to fluctuation over different temporal scales. Furthermore, for most wastewaters the screening and settling of large particles in a primary treatment step is recommended prior to microalgae cultivation (Van Den Hende, 2014). Moreover, given the unforeseen variability in wastewater composition over relatively short timescales, we recommend that the levels of each nutrient be routinely measured (and subsequently supplemented to optimum levels if necessary) throughout the cultivation process. The analysis of these nutrient parameters (total inorganic carbon, total organic carbon, total carbon, total nitrogen, ammonia, nitrate, nitrite, total phosphorus, phosphate and heavy metals) are described in the SOPs described in EnAlgae report 'Standard Operating Procedures for Analytical Methods and Data Collection in Support of Pilot-Scale Cultivation of Microalgae', and recommended in Tables 4 and 5.

The optimum levels of nutrients in any system are governed by a large number of factors including but not limited to microalgae species; prevailing environmental conditions (light/temperature); the nature of the pilot system etc. For laboratory scale experiments different media are prescribed in the literature (e.g. BG-11, Basal Bold

Medium). However, these media aim to prevent suboptimal nutrient availability. For larger scale installations sometimes lower nutrient levels are used borne out of experience which is often case-specific. Therefore definitive values cannot be recommended here. However, threshold levels for nutrients and several generic media descriptions used at the pilot operations to culture microalgae are described in Appendix 3.0. For water remediation the algae grow on the nutrients present in the wastewater. When nutrient concentrations are decreased to levels permitted to be discharged, the algae or MaB-flocs are harvested and the clean water is discharged.

In contrast to wastewater treatment, cultivation of microalgae with process water of RAS builds on the stability of the primary process (fish/crustacean production). For welfare and health, dissolved wastes, which are the nutrients for the microalgae, are controlled regularly (3 times a week) in such facilities. Despite daily fluctuations of nutrient concentrations due to the feeding and physiology of fish, a constant flow of dissolved nutrients is delivered to the algae. The N/P ratio of the process water fits that of microalgae. Integration of a stable algal growth system can not only replace denitrification and phosphate precipitation, two loss-processes in RAS, but will recover the nutrients in the biomass harvested. Remediation of process water with microalgae is based upon model-predicted dimensioning of the algae growth facility and requires automated process control. See Appendices.

3.0 Best practices for harvesting microalgal biomass

3.1 Harvesting microalgae biomass

The harvesting and de-watering of microalgae biomass is recognised as one of the most critical factors for the development of microalgal operations from pilot plant to commercial scale, as the obvious need to process large volumes of water to produce a concentrated biomass (and associated co-product) can result in high production costs unless carefully considered and appropriately engineered. A comprehensive review of the harvesting techniques employed by the pilot plants within the EnAlgae project has already been published (Gerardo *et al*., 2015) and we would recommend the reader review this document carefully before choosing a harvesting system for their pilot operation. However, the various harvesting techniques and associated advantages and disadvantages have been summarised in Table 8. The overall recommendation with regards to harvesting is to employ strategies that minimise costs but at the same time produce a product with desirable water content for further processing/utilisation. In most cases we would recommend a primary sedimentation step of the microalgae biomass (with or without the use of flocculants) to produce an initial slurry of 1–10% w/v solids followed by further processing using the dewatering techniques mentioned in Table 8 to produce a concentrated biomass (>15–40% solids). However, the primary sedimentation step is only useful when the settling velocity of the microalgae is relatively high. If that is not the case, preliminary membrane concentration to ca. 15% w/v solids followed by further concentration is recommended.

Table 8. Summary of harvesting approaches utilised at the different pilot plants within the EnAlgae project.

Harvesting technique	Concept	Advantages	Disadvantages	Used at pilot
Sedimentation of bioflocculant microalgal bacterial flocs (MaB-flocs)	Gravitational settling of MaB-flocs.	• Capital and operating costs low (settling tank only). • No flocculants needed, so no cost or chemical contamination of the microalgal biomass or the treated wastewater.	• Space requirement is lower compared to natural sedimentation as Hydraulic Retention Time (HRT) is only 1 hour. • Microalgal biomass also contains bacteria.	P2
Natural sedimentation	Gravitational settling of suspended cells.	• Capital and operating costs low.	• Space requirements often high. • Slow technique-biomass deterioration issues. • Not applicable for small single celled species (e.g. *Nannochloropsis* spp.).	P4, P6
Flocculation followed by gravity sedimentation	Induced coagulation of cells by various methods (chemical, autoflocculation; physical and physico-chemical.	• Easy to implement and comparatively inexpensive.	• Flocculant undesirable contaminant in biomass. • High variability leading to inefficiency and unreliability. • Reuse of return water can give problems when flocculants are used due to the presence of residues of the flocculant.	P4, P6
Centrifugation	Utilises centrifugal forces to increase the rate of sedimentation.	• Can be applied universally to all microalgae species. • Quick processing time. • Good reproducibility. • Produce product with low water content.	• Energy intensive. • Specialised equipment required-increased capital and maintenance cost. • Generates high gravitational and shear forces that can damage harvested cells. • Difficult to centrifuge cells with high lipid contents.	P1, P3, P6
Flotation in fresh water	Induce flotation of microalgal cells using very fine gas bubbles with oscillating injector.	• Useful when microalgal cells exhibit natural flotation characteristics.	• Cells must be hydrophobic-often requires addition of surfactants or coagulants. • Energy costs relatively high.	P6
Flotation in brackish and marine systems	Venturi injectors or air/ozone dispergers.	• Foam formation is enhanced by low levels of ozone.	• Low energy costs, applicable to marine species only.	P5
Filtration	Uses solid-liquid separation with a semi-permeable filter acting as barrier.	• Very efficient. • Range of filtration types to suit application-versatile. • Moderate to very low energy needs, depending on pore size.	• Requires specialised apparatus-increased capital and maintenance cost.	P1, P2, P4, P6

4.0 Biomass valorisation

Although the original focus of the EnAlgae project was the utilisation of microalgae biomass for energy production, a range of possible uses of the final microalgal biomass and indeed the chemicals it contains were studied by several, if not all, of the pilot facilities. This was driven by the reality that the utilisation of microalgae biomass for energy purposes alone is not currently economically feasible or indeed scalable. It should be stressed that these valorisation studies were early stage and experimental, subsequently the recommendation for best practices in this context is constrained. However, what is summarised here are descriptions of techniques used to evaluate microalgae biomass for biogas production and for potentially useful chemicals when exploring biorefinery options; examples of biomass valorisation studied (with associated considerations and recommendations); and some of the techniques investigated for downstream processing of biomass (with associated considerations and recommendations).

4.1. Bioenergy

MaB-flocs and microalgae are not a mature feedstock for anaerobic digestion (AD; Wiezcorek *et al*., 2015), thus, their biochemical methane potential (BMP) needs to be determined experimentally. Reported experimental BMPs of untreated microalgal biomass or MaB-flocs range between 50 and 510 NL CH_4 kg^{-1} VS, or 45–440 NL CH_4 kg^{-1} TS, with an AD efficiency of 26–79% (Mehrabadi *et al*., 2015; Van Den Hende *et al*., 2015a; Bohutskyi *et al*., 2014; Passos *et al*., 2014; Ward *et al*., 2014). Short batch or longer time-consuming continuous reactors are used to assess the BMP of novel substrates. As inoculum digestate is collected, it is recommended to allow this inoculum to adapt to this novel substrate prior to performing batch experiments, as the inoculum type effects the BMP (Wieczorek *et al*., 2015). The optimal substrate: inoculum ratio depends on the biomass type, but for microalgal biomasses good results have been obtained with ratios of 0.2–0.5 g volatile solids (VS) substrate: g VS inoculum (Wieczorek *et al*., 2015). More details on best practices on determination of BMP of biomasses were presented earlier, for example in the standard VDI 4630 (2006). Based on the theoretical BMP calculated from the COD (Table 9) and on the experimental BMP, the biomass to methane conversion efficiency can be calculated. To increase the BMP of microalgal biomass, pretreatment steps may be applied prior to digestion e.g. mechanical (sonication) or thermal processing. Next to the technical potential, assessing the economic potential is of major importance, as to date this is the major bottleneck with anaerobic digestion of microalgae and MaB-flocs (Van Den Hende *et al*., 2015).

Table 9. Summary of measurements used by the EnAlgae pilots to evaluate the biochemical methane potential (BMP) of microalgal biomass or MaB-flocs.

Parameter	Measurement technique	Purpose	Considerations / recommendations
COD (Chemical Oxygen Demand)	Colourimetric	• The chemical oxygen demand is a test used to indirectly determine the organic content of biomass based on the oxygen needed to oxidise the organic matter and is used to determine the theoretical BMP of the biomass, that is 0.350 NL CH_4 g^{-1} COD.	• COD is based on a colorimetric assay (requires spectrophotometer; see SOP 2.11).
BMP (Biochemical Methane Potential)	Batch assays	• To determine the BMP, that is the standardised volume of CH_4 of biomass.	• Batch assays consist of gastight reactors and a set-up to collect the produced biogas, e.g. eudiometers (see SOP 5.1).
Biogas sampling and composition	Gas chromatography (GC-TCD)	• To determine the CH_4 and CO_2 composition of the produced biogas.	• Care should be taken during storage of biogas samples (see SOP 5.2).

4.2. Chemicals

4.2.1. Assessing microalgae biomass for valuable chemicals

One of the advantages of utilising algal biomass for fuel production is the abundance of other saleable products, which can be recovered prior to using the biomass for energy production. Indeed, it is generally recommended that multiple potential products are evaluated from microalgae biomass in order to maximise commercial potential and sustainability of biomass production. Microalgae are widely used as feedstock for traditional applications in cosmetics, pharmacy and nutrition sectors and a variety of bioactive substances, such as carotenoids, polysaccharides and β-carotene can be derived from the biomass (Shlarb-Ridley and Parker, 2013). Such products are marketed as tablets, capsules and liquids (containing purified high value molecules such as fatty acids, pigments and stable isotope biochemicals), and cosmetics found in face and skin care products, such as anti-aging cream, refreshing or regenerant care products, emollient and anti-irritant in peelers (Samarakoon and Jeon 2012; Koller *et al*., 2012). Another growing market is the application of microalgae in animal feeds for poultry and aquaculture, due in part to the rising costs of protein worldwide and which encourages the use of microalgae as alternative and renewable ingredients as feeds (Becker 2007).

Table 10. Summary of measurements used by the EnAlgae pilots to evaluate microalgae biomass chemical composition for potential valorisation strategies.

Biochemical	Measurement Technique	Purpose	Considerations
Lipids (including fatty acids)	Extraction and gravimetric analyses (see SOP 4.3).	• Lipid component of biomass utilised for energy production (biodiesel).	• Requires organic solvents for extraction from biomass. • Simple procedure.
	Fourier Transform Infrared Spectrophotometry (FTIR).		• No solvent extraction necessary- use whole biomass. • Specialist equipment and training required.
	Extraction and fatty acid analyses (see SOP 4.7).	• Permits determination of individual fatty acid composition including nutritionally important omega-3 fatty acids. Also useful for determining biodiesel suitability of oil (fatty acid composition dependent).	• Requires organic solvents for extraction from biomass and a further processing step (derivatisation; trans-esterification) to produce Fatty Acid Methyl Esters (FAME). • Specialist equipment (e.g. GC-MS or GC-FID) and training required.
Protein	Acid extraction and colorimetric determination (see SOP 4.1).	• Protein component of biomass is an important consideration for animal feed utilisation.	• Simple procedure requiring basic equipment but requires handling of acids at high temperatures.
Carbohydrate	Acid hydrolysis and colorimetric assay (see SOP 4.2).	• Indicative of sugar content of biomass.	• Simple but time consuming. • Hazardous chemicals required.
Carotenoids	Extraction and colorimetric assay/chromatography (see SOP 4.5).	• Potential high-value component of biomass and used to indicate algal metabolism e.g. during light adaptation.	• Requires use of organic solvents. • Specialist equipment required e.g. spectrophotometer and High Performance Liquid Chromatography (HPLC).
Phycobilins	Extraction and colorimetric assay/fluorescence meter / chromatography.	• C-phycocyanin, A-phycocyanin and phycoerythrin are high-value compounds present in cyanobacteria (Sarada *et al*., 1999).	• Determination via a spectrophotometer is easy, but pigments are light- and temperature-sensitive, and protocols are species-dependent. • Market value strongly depends on the purity of the extracts. • Specialist equipment required, e.g. spectrophotometer, fluorescence meter (specific excitation/emission spectra) and HPLC.

In the broadest context the biochemicals that were measured in microalgae biomass as part of the EnAlgae project included proteins, carbohydrates and lipids but specific chemicals including carotenoids and some fatty acids were also evaluated in different biomass types from the different EnAlgae pilot plants. Subsequently a number of detailed protocols (SOPs) pertaining to the biochemical analyses of microalgal biomass have been generated and can be recommended. Table 10 represents a summary of the biochemicals measured in the different microalgae biomass types with links to the appropriate SOPs that can be found, amongst others, in a separate report (EnAlgae report 'Standard Operating Procedures for Analytical Methods and Data Collection in Support of Pilot-Scale Cultivation of Microalgae).

4.2.2. Uses of microalgae biomass and processing of biomass for chemical recovery

The potential uses of microalgal biomass that were evaluated at the research level by the different pilot plants included recovery of chemicals from the biomass for potential incorporation in health-care products; utilisation of the whole biomass as a precursor for energy production (hydrothermal liquefaction, biodiesel and biochemical methane); use in animal/aqua feeds or use as fertiliser. However, a general recommendation where possible would be to explore combinations of all approaches in order to maximise potential financial return from the biomass in order to make the process of biomass production commercially feasible i.e. a biorefinery approach. A further recommendation would be to carefully consider the limitations that the type of pilot production system may have on the potential utilisation of the biomass produced. For example, there may be restrictive legislation regarding biomass grown on waste effluent nutrient sources and its incorporation into animal feeds and subsequently the human food chain or indeed its associated chemicals into health-care or pharmaceutical products. This will be governed largely by local government legislation and rulings and should be thoroughly reviewed before exploring application of microalgae biomass. Current regulatory issues limit the use of wastewater-grown microalgae or MaB-flocs in feed in EU Member States since the addition of wastewater-grown algae in animal feed is subject to tight EU regulations (Van Den Hende *et al*., 2014c). The inclusion of wastewater-grown microalgae or MaB-flocs in animal feed is restricted in Europe by regulation EC No.767/2009 which also restricts the use of faeces and urine including that of fish (aquaculture) in feed. Therefore, microalgae or MaB-flocs grown on aquaculture wastewater should be proven to be free of faeces and urine before they can be included in feed. The above restriction only entails entering the feed market, but does not restrict the use of wastewater-grown microalgae or MaB-flocs in feed at the wastewater treatment site itself. For example, aquaculture wastewater-grown MaB-flocs could be used by the aquaculture farmer as feed ingredient if these MaB-flocs are produced at the aquaculture farm.

The applications of microalgae biomass that were explored by the pilot plants in the EnAlgae project can be seen in Table 11. It is recognised that the recovery of additional products from microalgae biomass aside from using the material for energy production is crucial to develop a commercially valuable production platform. This will often require the implementation of processing steps to yield these valuable co-materials from the biomass. Cell disruption is a key unit operation in the recovery of the intracellular products from microalgae. Typical cell disruption methods include bead mill, high pressure homogenisation, sonication, microwave, freeze thawing, osmotic shock, supercritical fluid extraction and several chemical and enzymatic methods (Molina Grima *et al*., 2003; Yusuf 2007; Doucha and Lívanský 2008; Balasundaram *et al*., 2009; Pasquet *et al*., 2011). Again, this was only investigated at a research level by the different pilot operations and thus the presentation of best practice procedures is constrained. For example, the process of bead milling biomass generated from P4 (composed of the cyanobacteria *Chlorogloeopsis fritschii*) was extensively investigated (Balasundaram *et al*., 2012). Other methods of cellular disruption included the Constant Systems LTD 2.2 Kw disrupter (Daventry, Northants, UK) used by P1 (Swansea University). This system compresses the harvested culture, then releases the pressure resulting in a large pressure change which then forces the culture, at ultrasonic speeds, onto a metal plate to smash the remaining un-disrupted cells. However, in this case what should be considered is that this process is energetically demanding and the initial capital outlay is high. Of course, when adding the whole biomass to feed or food products the cell disruption process can be skipped and the biomass processing will then be restricted to drying and subsequent milling.

Table 11. Example applications of biomass investigated at the different pilot plants.

Microalgae biomass	Application examined	Comments/considerations
Chlorogloeopsis (cyanobacteria) P4	• Energy (Hydrothermal Liquefaction [HTL]). • Healthcare products (chemical extracts).	• HTL particularly suitable for resulting biomass with high water content and not dependent on chemical precursor synthesis e.g. lipids for biodiesel. • The product from P4 was shown to be useful biomass for HTL (Biller *et al.*, 2012).
MaB-flocs P2	• Energy (methane-anaerobic digestion). • Aquafeed (shrimp feed). • Slow-release fertiliser (for greenhouse tomato). • Colour pigments (e.g. phycocyanin and phycoerythrin) and other high-value phytochemicals (e.g. neophytadiene).	• Use of aquaculture wastewater-grown microalgae-bacteria (MaB) flocs for methane production is not recommended as it is inefficient with low economic value and AD reactor scaling (Van Den Hende *et al.*, 2015a and submitted). • Low inclusion leads to increased shrimp pigmentation (added value), but European regulations are prohibitive for use of wastewater-derived biomass in the feed market (Van Den Hende *et al.*, 2014c). • Use of aquaculture wastewater-grown MaB-flocs as fertilizer for greenhouse tomatoes increased the carotenoid and sugar level in tomato fruits, but high calcium content of MaB-flocs can lead to imbalance of nutrients (Coppens *et al.*, submitted). • For colour pigment, only proof-of-principle of one wastewater type obtained so far (CAS effluent food industry; Van Den Hende *et al.*, 2015).
Nannochloropsis salina P1, P5	• Green water and live feed in hatchery.	• No European regulations pertaining to the use of process water in aquafeed production (Appendix 1.5).
***Chlorella* and *Scenedesmus* spp.** P1 and P6	• Animal feed.	• Biomass has to meet regulations for feed security (GMP+; https://www.gmpplus.org). • Biomass should be free of harmful microbes e.g. *Salmonella*, entero bacteria, *Escherichia coli* etc. • Pre-treatment of digestate/manure nutrient sources to pilot may be necessary e.g. to remove pathogenic organisms.
Mixed algal consortium P1	• Energy (methane-anaerobic digestion (AD).	• Algal biomass grown on AD municipal waste based media, provided methane production comparable to other feedstocks.

Note: none of the proposed products were utilised for commercial purposes and were only studied at laboratory scale.

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Appendix 1.1: Pilot plant 1 (Swansea University)

This pilot plant comprises a 400 L tubular horizontal photobioreactor (PBR; Varicon Aqua Solution, Malvern Worcestershire, UK) located in an environmentally controlled (temperature, light) greenhouse alongside two 600 L PBRs of same design (Fig. A1.1.1). Alongside this is a purpose built 2000 L tubular vertical PBR (Fig. A1.1.2) combined with a flue gas facility (wood pellet burner) to supply CO_2 for microalgae growth. All PBRs can be operated in continuous mode, with automated nutrients and a chemically sterile water supply. The online sensors installed in the systems measure pH, temperature, oxygen and CO_2. Furthermore, the purpose built Algal Growth Laboratory (AGL) consists of a series of PBRs (10–80 L; Fig. A1.1.3) which utilise flue gas from a coke oven as a source of CO_2 and heat to produce algal biomass. The AGL is situated at Tata Steel (Swansea, Port Talbot, UK). All of these facilities are operated together with the support of a microalgal preparation laboratory housed at the University, where another series of PBRs (20–80 L) are used for maintaining and producing microalgae inocula for the larger pilot operations.

The main purposes of these facility operations were focused on wastewater (dairy and fish farm, AD waste) and flue gas remediation, production of algal biomass for high value products development and light and nutrient optimisation of different algal species.

Figure A1.1.1. Horizontal and vertical tubular PBR (Varicon Aqua Solutions, Malvern Worcestershire, UK) housed in the environmentally controlled greenhouse at Swansea University (P1).

Figure A1.1.2. Vertical tubular 2000 L PBR housed in the greenhouse. This system is fed CO_2 by a purpose built wood pellet burner.

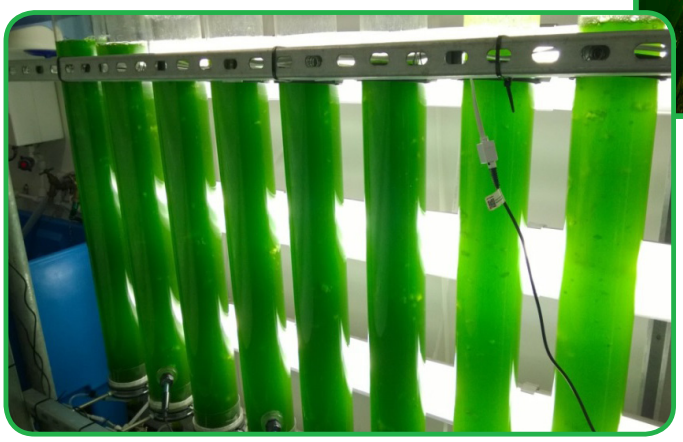

Figure A1.1.3. The 100 mm diameter, 10 L tubular PBRs inside the AGL based at Tata Steel (Swansea, UK).

Appendix 1.2: Pilot plant 2 (Ghent University)

Pilot Plant 2
Ghent University
Aquaculture Practice Center of Inagro, Roeselare, Belgium (2012–2013);
Alpro, Wevelgem, Belgium (2014–2015)

This pilot system is designed specifically for the treatment of various wastewaters and is based on the concept of microalgal bacterial floc sequencing batch reactors (MaB-floc SBRs; Van Den Hende, 2011b adapted from Gutzeit *et al*., 2006). MaB-flocs are aggregations of a natural microbial consortium (microalgae and bacteria) species which directly grow as flocs on nutrient rich wastewater. Post wastewater treatment these MaB-flocs are designed to sediment quickly under gravity during the night in the SBR, resulting in a biomass-free supernatant which can be discharged (Fig. A1.2.1). The EnAlgae facilities at the Ghent University include one indoor MaB-floc SBR of 400 L (constructed by Ghent University, Campus Kortrijk; Fig. A1.2.2) and one outdoor pilot-scale MaB-floc SBR facility of 28 m^2 (constructed by two Belgian SMEs: Bebouwen and Bewaren nv and CATAEL bvba; Fig. A1.2.3). Although the MaB-floc SBR system is not designed as a flue gas bioremediation technology, flue gas is injected in the MaB-floc SBR to maintain an optimal reactor pH. This flue gas injection was needed during treatment of low-strength wastewater, such as wastewater from pikeperch aquaculture (Van Den Hende *et al*, 2014a) and CAS effluent of the food industry (Van Den Hende *et al*., 2015b), but was not needed for high strength wastewater, such as UASB effluent of the food industry (Van Den Hende *et al*., 2014a; 2015b). The harvested MaB-floc biomass of this pilot facility was screened for its technical potential as feedstock for biochemical methane production (Van Den Hende *et al*., 2015a), pigment-enhancing shrimp feed (Van Den Hende *et al*., 2014) and organic slow-release fertilizer (Coppens *et al*., 2015). The harvested MaB-floc biomass of this pilot facility operated at Alpro was screened for its technical potential as feedstock for biochemical methane production (Van Den Hende *et al*., 2014) after extraction of high value compounds such as phycocyanin and phycoerythrin (Van Den Hende *et al*., submitted).

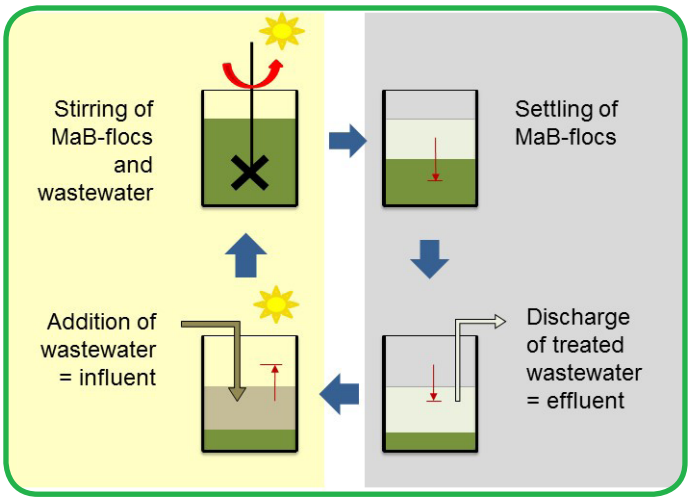

Figure A1.2.1. Operation phases of a MaB-floc SBR during the daytime (yellow) and night time (grey)

As well as SBR operation, the MaB-floc SBR system possesses several other unique attributes (adapted from Gerardo *et al*., 2015). The MaB-flocs inoculum is collected outdoors, so it is not necessary to maintain axenic cultures of micro-organisms at a laboratory scale. Domination of MaB-flocs by one microalgae species is possible for certain wastewaters (Van Den Hende *et al*., 2014) and the operation as SBR may result in a feast and famine management (nutrient stress) of the MaB-floc community which may result in accumulation of lipids, carbohydrates and pigments in microalgae (Van Den Hende, 2014). Furthermore, MaB-flocs settle at night in the reactor without the addition of flocculants avoiding costly algae harvesting steps (see section 3.0 on harvesting) and the settled MaB-floc slurry can be dewatered in a filter press with relatively large pore size. For example, sieving at 150–250 µm resulted in a MaB-floc cake of up to 43±8% dry matter and a harvesting efficiency of 98.8±0.9% total suspended solids (TSS) (Van Den Hende *et al*., 2014). Nutrient-poor wastewater can be treated in a MaB-floc SBR with a high MaB-floc biomass density without the need for membranes (perfusion reactor). Indeed, because MaB-flocs can be easily separated from the wastewater in the reactor, the hydraulic retention time and sludge retention time can be decoupled, akin to conventional activated sludge systems (Tchobanoglous, 2003). For example, a hydraulic retention time for wastewater treatment of 4 days can be combined with a MaB-floc retention time above 20 days and a minimum MaB-floc density of 0.500 g total suspended solids (TSS) L^{-1} (Van Den Hende *et al*., 2014).

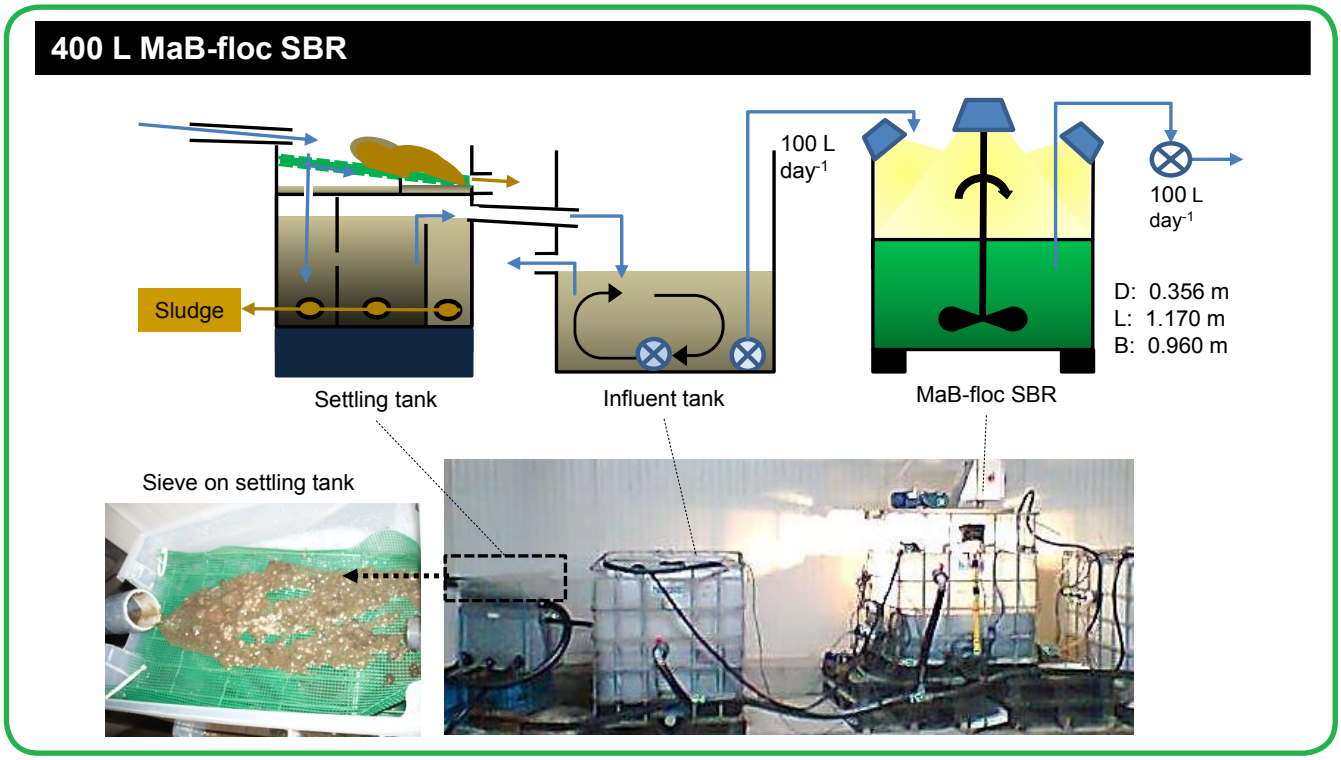

Figure A1.2.2. Indoor pre-pilot-scale MaB-floc SBR facility (adapted from Van Den Hende et al., 2014a).

Additional information:

- Hardware and pilot construction: Lode Bourez, SME Bebouwen & Bewaren nv, Hertsberge, Belgium, info@bebouwenenbewaren.be
- Software and automation: Nikolas Taelman, CATAEL bvba, Bellegem, Belgium, info@catael.be
- Pilot operation: Sofie Van Den Hende, Ghent University, Kortrijk, Belgium, sofie.vandenhende@ugent.be; sofie_vdhende@yahoo.com

In contrast to SBR operation of non-photosynthetic microorganisms, the reactor phases need to be aligned with the diurnal variation of light since sunlight is needed for the photosynthetic aeration by microalgae. Furthermore, the MaB-floc settling phase should take place at night. If MaB-floc settling takes place during daytime, MaB-flocs may start floating due to gas bubbles formed by excessive photosynthetic oxygen production (Van Den Hende, 2014). This would then result in unwanted MaB-floc withdrawal in the subsequent effluent withdrawal phase. The reactor is not stirred during night time to allow MaB-floc settling and to minimise stirring costs. However, in case of excessive gas bubble formation (e.g. CO_2) during night time, the reactor should be stirred for a few short periods to remove these gas bubbles and consequently enhance MaB-floc settling (Van Den Hende, 2014). The effluent withdrawal takes place at the end of the night or early in the morning. During daytime excessive oxygen production may result in MaB-floc floating (Van Den Hende *et al*., 2014). To maintain aerobic conditions, influent can be added after a period of photosynthetic aeration (Van Den Hende, 2014).

The pre-pilot facility was initially constructed for the treatment of pike perch culture wastewater (Van Den Hende, 2014) and consists of a wastewater pre-treatment system (settling tank), an influent collection tank, a lighted MaB-floc reactor and an effluent collection tank (Fig. A1.2.2), as described in more detail by Van Den Hende *et al*. (2014a). The MaB-floc reactor has an effective volume of 400 L consists of a plastic open tank illuminated by one 500W halogen lamp and ten 20W halogen lamps. The reactor is operated indoors without heating. A centrifugal pump is used for influent feeding controlled by a level regulator. A peristaltic pump is used for daily effluent withdrawal after the MaB-floc settling phase at night. An overhead stirrer is used for mixing. No mechanical aeration of the reactor is performed. Wastewater is continuously fed to an influent tank after sieving (4 mm porosity) and subsequent settling. In case of aquaculture wastewater, fish feed particles and faeces need to be removed regularly from the sieve and settling compartments. The buffer tank can be mixed with a water pump to avoid sludge accumulation.

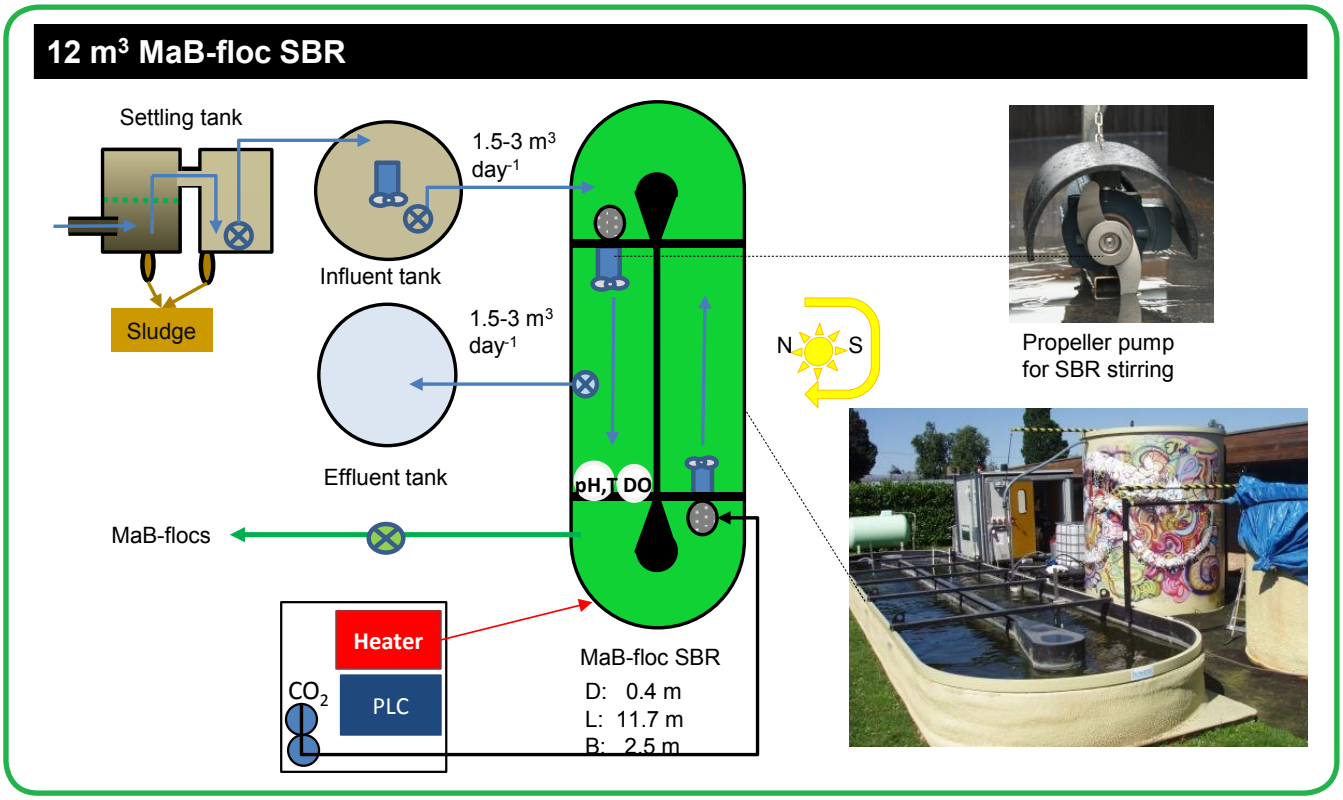

Figure A1.2.3. Mobile outdoor pilot-scale MaB-floc SBR facility (Van Den Hende, 2014a).

The facility consists of influent and effluent tanks, a raceway pond, a container, harvesting tanks, a heating system, flue gas injection system, PLC automation and measurement probes, as described more in detail by Van Den Hende *et al*. (2014a). The working volume of this MaB-floc raceway pond can be altered from 10 to 14 m^3. To enhance start-up during winter, the reactor can be heated via a system consisting of warm water tubes and a gas boiler. Two propeller pumps stir the raceway. Effluent is withdrawn with a submerged pump. The outdoor influent buffer tank can be discontinuously stirred with a propeller pump to avoid sludge accumulation while wasting excess influent.

If needed, wastewater is mechanically pre-treated in an indoor settling tank. The first compartment of this settling tank removes particles larger than 1.2 mm by a vertical sieving screen. The second compartment removes settling and floating sludge. From here the water flows to an indoor buffer tank and is pumped to an outdoor influent buffer tank (HRT of all tanks was a maximum of 3 days).

Investing in Opportunities
This project has received European Regional Development Funding through INTERREG IV B.
INTERREG IVB

Appendix 1.3: Pilot plant 3 (InCrops Enterprise Hub)

Pilot Plant 3

InCrops Enterprise Hub

University of East Anglia, UK in collaboration with the Department of Plant Sciences, University of Cambridge, UK.
http://www.incropsproject.co.uk/

http://www.bioenergy.cam.ac.uk/abc-directory/microalgal-growth-facility

http://www.bioenergy.cam.ac.uk/abc-directory/algal-innovation-centre-cambridge

Location: Cambridge University Botanic Garden, University of Cambridge, UK

Contact: Dr Matthew Davey, Department of Plant Sciences, University of Cambridge, mpd39@cam.ac.uk

This pilot plant has a range of facilities to study algal growth and physiology. These are split between laboratory based studies using up to 1 L flasks in controlled environment growth shakers and semi-natural environment reactors based inside a polytunnel where there are 5 to 10 litre plastic bag 'sock' reactors, 10 L upright tube reactors with aeration systems and a 300 L tubular semi-closed bioreactor system (see Fig. A1.3.1).

There is also access to six Infors Multitron controlled environment growth shakers for small scale (well plate, 100 mL to 1 L flasks) experiments and culture maintenance. Many of the units have experimental LED lighting for improved energy usage during algal growth. There are static incubator units for culture stocks and maintenance and a cold room facility for polar species experiments. For the 10 L socks and tubes, this is a simple growth system that uses polyethylene layflat tubing as disposable reactor bags or reusable tubes with aeration and sampling port holes. These are filled with either 5 or 10 L of growth medium and algal inoculums and can be aerated with air or a mix of air and CO_2 and samples can be taken directly from them using a tube and syringe set up.

The bespoke tube design was manufactured in collaboration with Anaero Ltd (Cambridge UK). The second large-scale bioreactor is a 300 L horizontal tubular system, designed by Steve Skill and similar in design to the Plymouth Marine Laboratory pilot plant. The system is controlled by Adept control software (Adept Technology, Chavanod, France) and can be fully automated. There are options for heating the cultures and for measurements of optical density (OD), temperature, light, pH and nitrate. Current research focus concerns utilisation of high-nitrate (> 4g L^{-1}) waste product (brine) from a local drinking water company (Cambridge Water, Cambridge, UK) as a source of nitrate for algal growth.

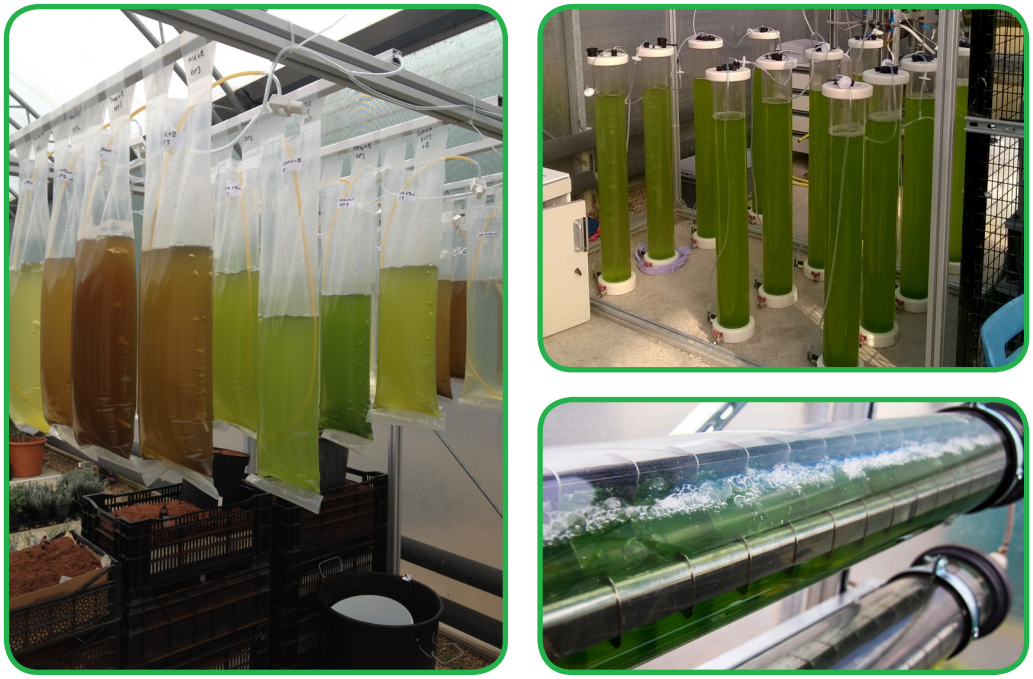

Figure A1.3.1. Different PBRs utilised at the Cambridge pilot facility

(a) 5 to 10 L plastic bag reactors with aeration system (b) 10 L reusable tube reactors (c) 300 L tubular semi-closed bioreactor system of same design as PML pilot (P4).

Appendix 1.4: Pilot plant 4 (Plymouth Marine Laboratory)

Pilot Plant 4
Plymouth Marine Laboratory
Boots Ltd Industrial site, Nottingham, UK

This pilot plant consists of a low energy 16,000 L PBR (Skill, 2010) housed in a polytunnel and directly coupled to a power plant emission stack (Boots Ltd., Nottingham, UK; Fig. A1.4.1). Hot flue gases (containing CO_2) are delivered from the power plant to the PBR utilising the residual pressure (0.005 Bar) at the base of the flue stack and then is ducted directly into the PBR modules. The PBR design (Fig. A1.4.2) contacts the hot flue gases without gas compression (an internal rotor is used to "scoop" air into the liquid growth medium) leading to lower energy utilisation and flue gas residence time in the PBR can be varied by restricting the incoming emissions (Fig. A1.4.3). Minimising energy input into all the process steps is a major consideration within this pilot plant with both flue gas delivery and PBR operation requiring only 20–70 W m^{-3}. Targets of PBR design include bioremediation of the flue gas stream from a combined cycle power station; low energy utilisation; multiple flue gas contacting carbon capture system; freshwater algal species robustness; low energy biomass harvesting and downstream processing. The PBR system was used to propagate a thermophilic freshwater cyanobacteria (*Chlorogloeopsis fritschii*) with a focus on production of bioactive extracts coupled with hydrothermal liquefaction (HTL) of whole biomass for energy production. Nutrients recovered from HTL were proven suitable for further use to support microalgal biomass production.

Figure A1.4.1. (a)16,000 L PBR showing the flue gas delivery duct from the power plant (picture taken pre-covering with polytunnel) (b) PBR interior inside polytunnel (Skill, 2010).

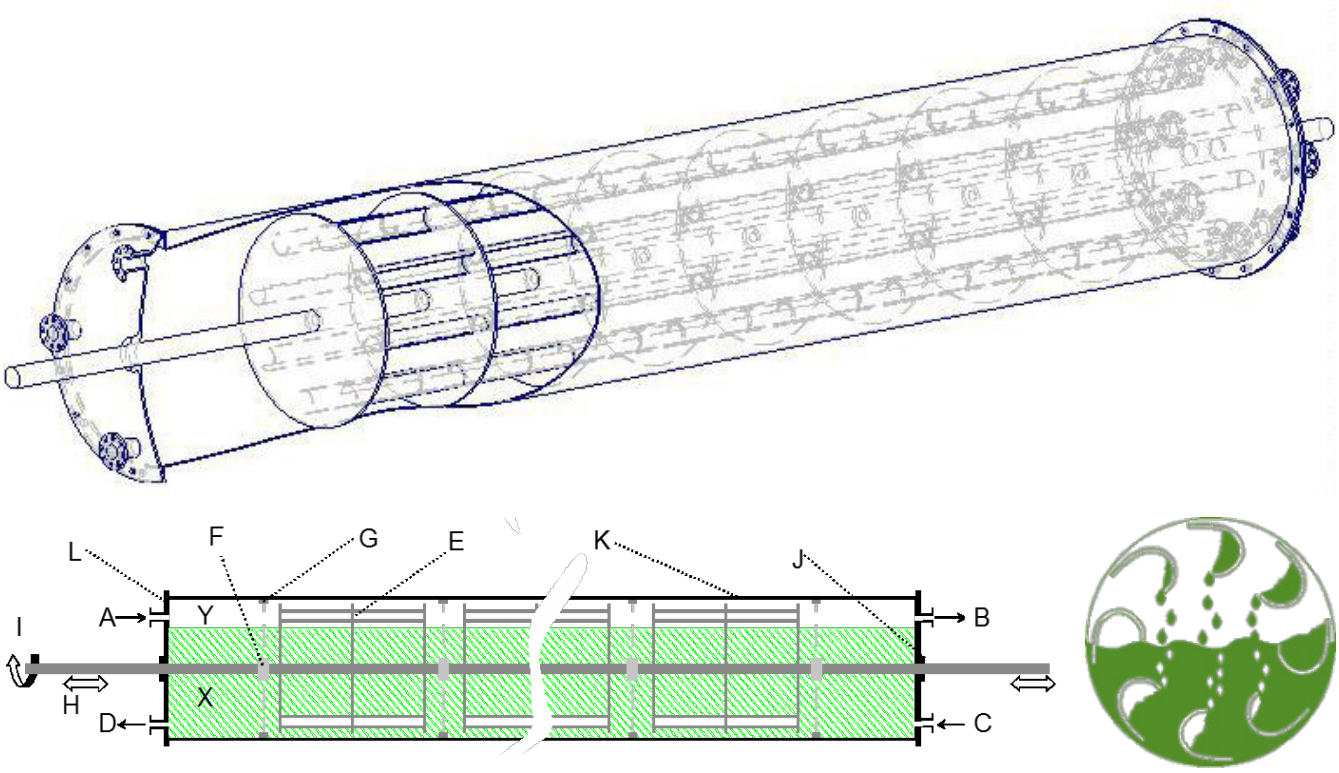

Figure A1.4.2. (i) Cut-away drawing of a PBR module at pilot plant 4. (ii) Schematic of PBR and internal rotor system.

A = inlet port for low density fluid phase Y; B = outlet port for treated/resultant fluid phase Y; C = inlet port for liquid phase X; D = outlet port for liquid phase X. E = bucket or scoop assembly attached to rotor; F, G = bearing assembly to support rotor within the transparent shell K; I = rotation of the rotor assembly within the shell; J = mechanical seal and bearings attached to the end plates L. Fluid phase Y examples include carbon dioxide containing off gases from fossil fuel combustion, cement kilns, fermentation processes, biogas from anaerobic digestion, life support systems etc.; Liquid phase X = suspension of photosynthetic microorganisms or photosynthetic tissue culture. This phase can contain solid particles to aid internal wall cleaning (e.g. 5 mm polyethylene beads); K = shell construction materials such as polyethylene, polypropylene, polyurethane, polycarbonate, polyvinylpyrrolidone, polyvinylchloride, polystyrene, poly(ethylene terephthalate), poly(ethylene naphthalate), poly(l,4-cyclohexane dimethylene terephthalate), polyolefin, polybutylene, polyacrylate and polyvinlyidene chloride, per-fluoro plastics, PTFE, PET, soda glass, borosilicate glass, quartz glass. The shell material does not have to be rigid. The 'containment' shell may also consist of a thin walled polyethylene type bag which is supported by a rigid support frame creating a substantially cylindrical profile.

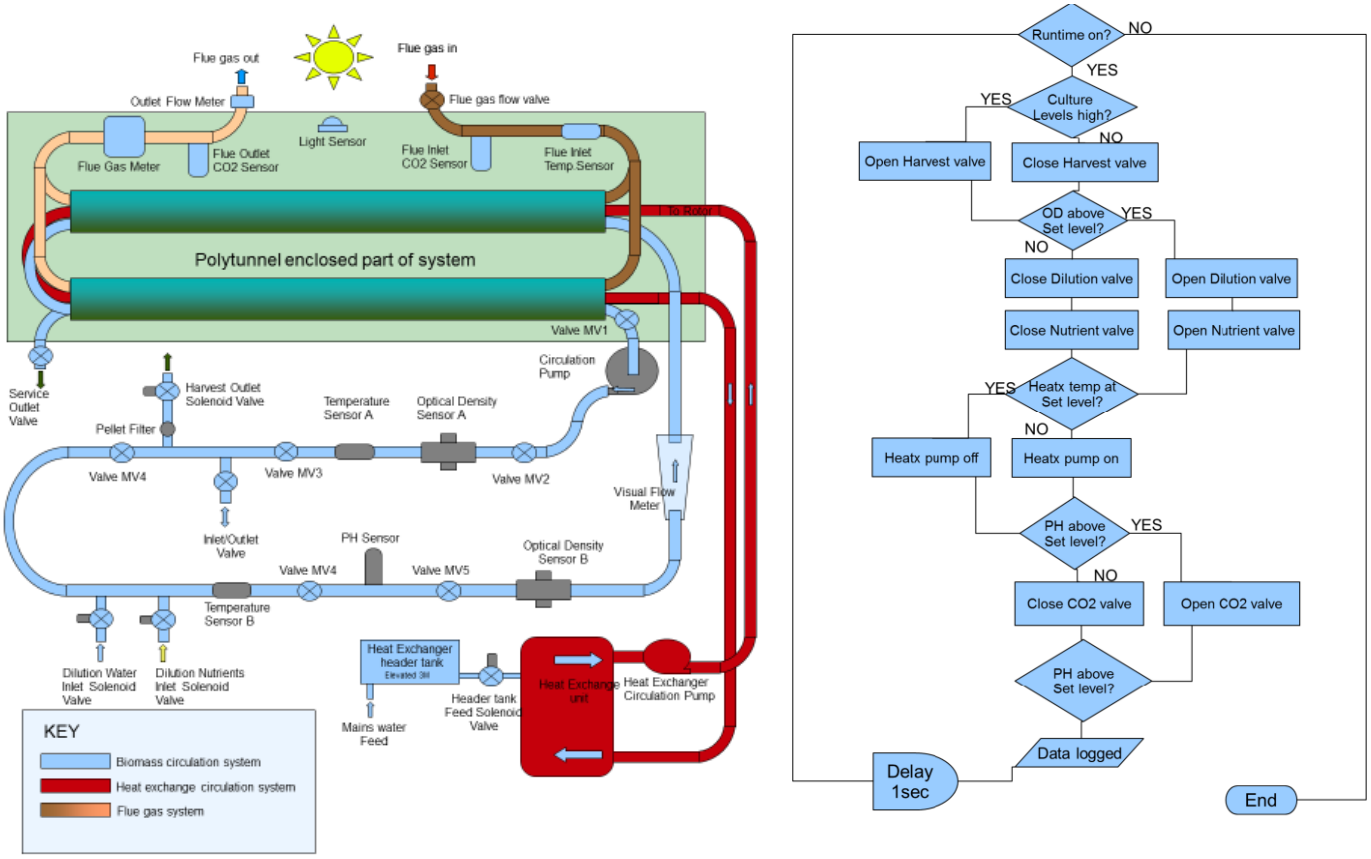

Figure A1.4.3. (a) Diagram depicting the layout of the circulation system, sensors and control valves (b) Flow chart of normal operation loop used by the automated control system.

Appendix 1.5: Pilot plant 5 (htw saar)

Pilot Plant 5

htw saar

Hochschule für Technik und Wirtschaft des Saarlandes, Germany

This pilot plant consists of three flat panel airlift PBRs located in a greenhouse (Fig. A1.5.1). The algae receive nutrient-rich process water of a RAS for marine fish. Depending on the nutrient concentration in the process water of the RAS, nutrients are supplied in a fed-batch mode (nutrient concentration > 150 mg NL^{-1}) or in a continuous stream of process water (lower nutrient concentrations; Fig. A1.5.2). Temperature and pH in the panels are continuously monitored during operation. The signals are used for process control operated by a programmable logic controller (SIMATEC ET 200S, Siemens). Assimilation of CO_2 by algal photosynthesis increases the pH signaling demand for extra CO_2. At a set point the PLC opens a solenoid valve to allow CO_2 injection. PBR temperature is adjusted by automated spraying of water on the PBR surface (sunny side) with foggers for efficient evaporative cooling. Again the PLC opens a solenoid valve to allow water to pass if cooling is required (see SOP automation). The greenhouse itself receives sufficient irradiation by sunlight during spring, summer, and autumn for continuous microalgae production. However, from late October to the end of February, LED panels are used to enhance microalgae productivity.

The process water of the RAS for marine fish is continuously treated in the primary water purification system. Dissolved and particulate waste streams from fish production are separated; nitrifying bacteria in a biofilter quickly convert ammonia excreted by fish into nitrate (see Fig. 1.5.3 for more details). Nitrate, phosphate and CO_2 dissolved in the clear process water serve as nutrients for microalgae and are recycled by harvesting the algal biomass. Optimising biomass productivity thus also optimises bioremediation in a coupled system.

Microalgae grow by cell division. The specific growth rate *k* of a microalgae and the carrying capacity of the growth system determine the yield of the system and the removal of nutrients from process water by microalgae. The coupling of the RAS and PBR production units requires knowledge about the productivity and well defined interfaces between fish production and algae production units

Figure A1.5.1. Image of the flat panel airlift PBR system at Pilot 5 (Subitec GmbH, Germany).

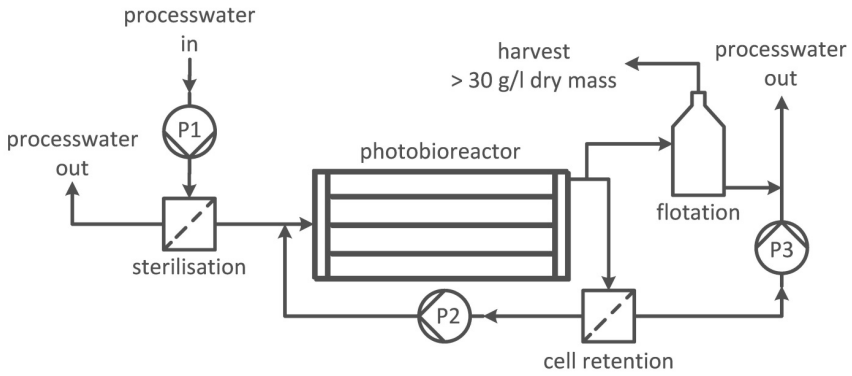

Figure A1.5.2. Schematic of the integrated RAS-PBR system.

The RAS process water, which carries the nutrients for biomass production, is supplied through pump P1. This process water is filter-sterilized to remove predators (zooplankton grazers) and prevent bacterial contamination using a ultrafiltration cartridge which provides a high filtration rate (up to 300 L h^{-1}) through a large surface area (2.1 m^2) in a small volume (ca. 700 ml). Excess process water leaves the PBR via the cell retention unit, consisting of a PVDF filter membrane (3.3 m^2). The gear pump (P3) is controlled by a level sensor and maintains a slight vacuum on a PVDF filter membrane to drive the filtration process and send back the clear permeate to the RAS. Pump P2 ensures a continuous retentate flow towards the PBR (inner flow cycle). The harvesting mode is controlled by optical density (OD). If OD exceeds a set point, pump P3 is halted. A slightly increased hydrostatic pressure builds up and allows the fluid containing the microalgae to leave the system towards the flotation unit. The ozone enhanced dispersed air flotation unit concentrates the biomass (harvest) and feeds back the remaining process water into the RAS. Flotation allows an up-concentration of biomass from 3 g L^{-1} to 40 g L^{-1} dry ash-free biomass. The harvesting pathway is maintained until the OD in the inner cycle has decreased to a lower set-point. Then pump P3 resumes removal of excess process via the cell retention unit.

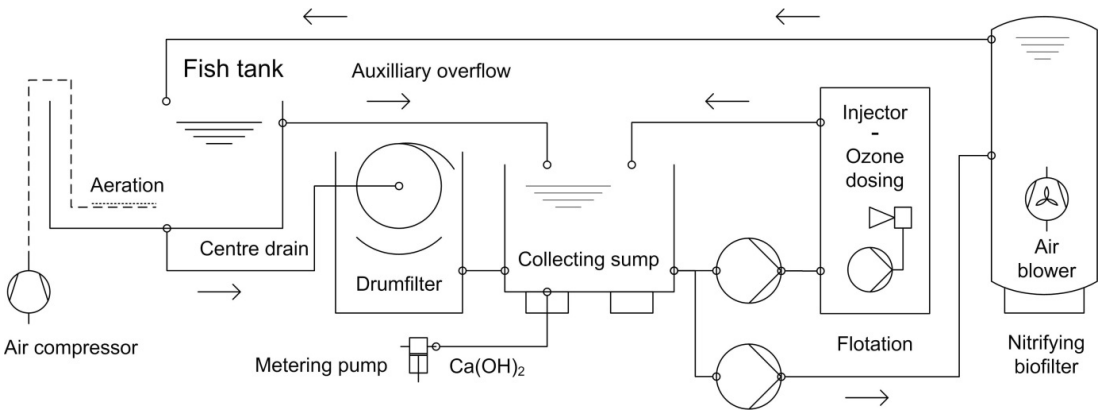

Figure A1.5.3. Schematic of the zero-discharge RAS system at htw saar.

The RAS has a fish tank volume of 7 m^3 and a total system volume of 10 m^3. The fish tank is 5 x 2 x 0.7 m in length, width, and depth. Process water discharges via the centre drain of the fish tank, passes a screen (40 µm, drum filter) and enters into a collecting tank (sump). For safety reasons an auxiliary overflow has been mounted at the water surface. This water was directly discharged into the collecting tank. Two pumps deliver the water from the collecting tank towards the nitrifying biofilter and the flotation unit. Flotation (protein skimmer) was used for polishing the water by removing particles (<1 µm) including bacteria. The process is enhanced by dosing with ozone. Particle control and hygiene in the RAS are the main purposes of this flotation. The water effluent from the flotation is fed back into the collecting tank. The nitrifying biofilter brings the process water back into the fish tank. Twice the volume of the primary production tank is treated by the water purification system per hour (see also Orellana et al., 2014). Since the efficiency of the system would dilute the microalgae system if directly integrated, the microalgae production unit is located in a side stream (Fig. A.1.5.2). In- and out-flow of the integrated algae cultivation unit are connected to the collecting tank. The process water transferred to the algae cultivation unit should be transparent and without discoloration and it should carry negligible amounts of dissolved and particulate organic matter. The back-flow from the algae cultivation system should end in the immediate vicinity of the in-flow of the protein skimmer of the main RAS to remove undesirable residual turbidity from the algae harvesting process.

Appendix 1.6: Pilot plant 6 (UR/ACCRES)

The microalgae open pond installations at the ACRRES pilot site were designed to maximize the use of locally available waste products such as waste heat, CO_2 (flue gas) and nutrients in digestate. The pilot consists of installations for anaerobic co-digestion (two tanks of 500 m^3 each) combined with a Combined Heat and Power (CHP) unit of 123 kW to produce electricity; maize refinery; and a microalgae production placed near a mixed dairy-arable farm (Fig. A1.6.1).

The algae growing facilities consist of two algae ponds of 250 m^2 each (one outdoor and one indoor). Both ponds utilise excess heat and flue gas (CO_2) from the CHP unit. Both the outdoor as well as the indoor pond are constructed with an earth wall that is covered with black plastic (polypropylene). The pond allows for a maximum water table depth of 80 cm. The average water area of both ponds is 250 m^2. The water in the ponds is stirred with a propeller mixer of 0.9 kW. Infrastructure was constructed to transport the flue gas and the cooling water from the CHP to the open ponds. Air or flue gas is sparged into the culture via perforated tubes at the bottom of the pond, using a blower that requires 2.7 kW. The flue gas addition is regulated based on the pH level of the ponds. The water in the ponds is heated with the cooling water of the CHP that is pumped through tubes at the bottom of each of the ponds. The use of digestate as nutrient source is not yet realised. Firstly, more research is required with regard to pre-treatment of the digestate as the dark colour and the presence of solids interact negatively with the algae production. Until now nutrient supply has been achieved with chemical fertilisers. Nutrient supply is achieved via two small pumps that regularly insert solved nutrients in the ponds. Two tanks with solved nutrients are used, one with nitrogen and magnesium and one with phosphorus and micronutrients. Temperature and pH are monitored continuously in order to regulate the flue gas and heat addition. With regard to inoculating the ponds, two pre-culture tanks have been installed of 1 and 20 m^3, respectively, in order to stepwise increase the culture volume. The growth of algae in these tanks is supported by underwater LED lamps (400–700 nm).

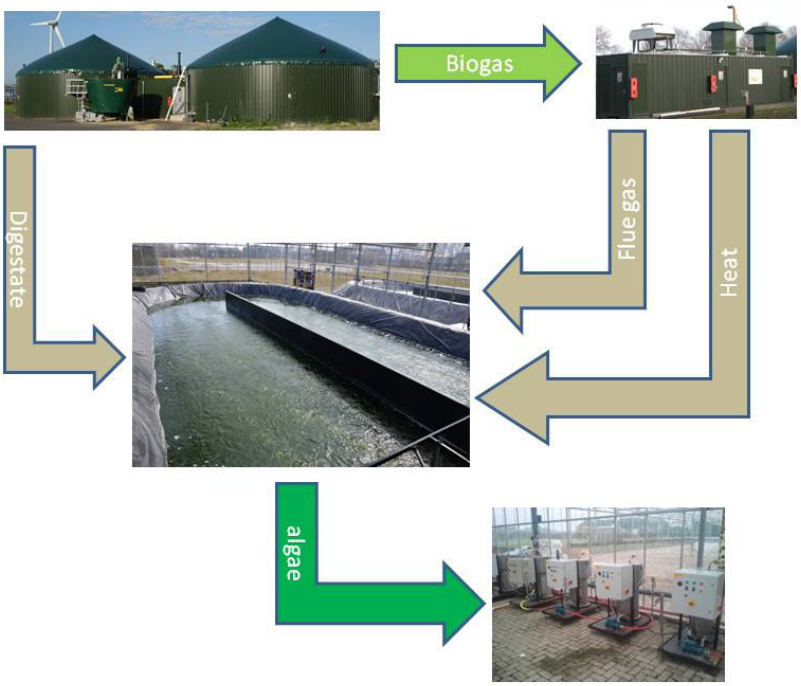

Figure A1.6.1. Flowchart of open pond system at the ACRRES pilot site (the use of digestate as nutrient source is not yet realised).

Appendix 2.0

Automation equipment used at P4

Software

- LabVIEW System Design Software – National Instruments. www.ni.com
- DAQFACTORY HMI / SCADA SOFTWARE –Azeotech Inc. – www.azeotech.com
- Windmill data acquisition software – Windmill Software Ltd www.windmill.co.uk

Hardware

- Labjack – www.labjack.com
- National Instruments – www.ni.com
- Advantech Automation – www.advantech.com

The automation of pilot facility P2 was built by the Belgian SME CATAEL bvba specifically for the EnAlgae project. This consisted of three items: (1) the field (e.g. sensors), (2) steering (PLC and laptop) and (3) remote control (Fig. A2.1). A PLC (Phoenix) which had a standard Ethernet/Profinet interface for connection with a network and communication with a laptop was chosen. A webpage was developed and installed on a laptop to visualise and steer the pilot operation modus, such as pump flow rates, temperature, etc. (visual interface; Fig. A2.2). Pilot operators were able to switch from fully automated to manual override. Programming and hardware configuration of the PLC was done based on PC WorX software according to the IEC 61131 standard for PLC programming. Furthermore, CATAEL installed a SQL server on the laptop and developed an Excel-based tool to collect data of parameters measured in the reactor (pH, temperature, dissolved oxygen, reactor liquor level), outdoors (light intensity, temperature, rain) and of pilot compounds (influent pump flow rate, effluent pump flow rate, propeller mixing pump rates, energy consumption, flue gas flow rate). For remote monitoring and data mining, pilot operators used the software Teamviewer. This remote control is of major importance since the pilot facility was located on an industrial site and not at the university campus. An automated cell phone text messaging warned the pilot operators in case of emergency situations (e.g. reactor level to low).

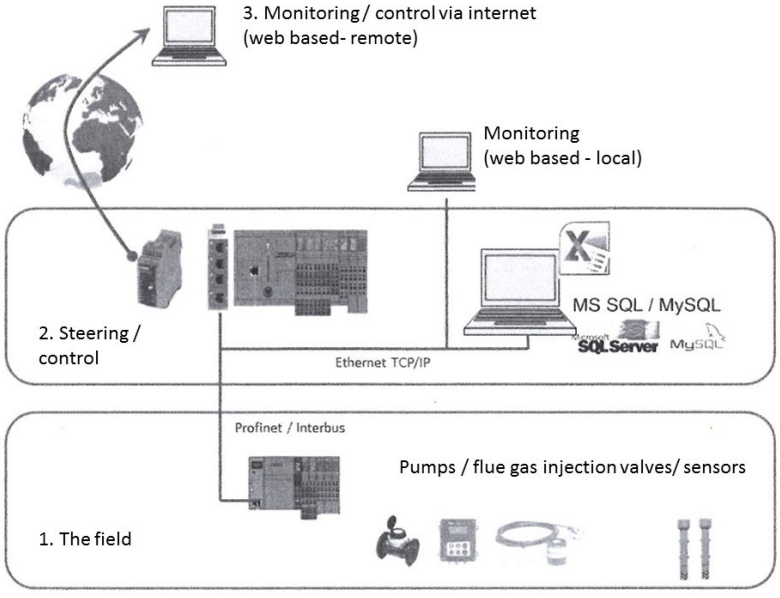

Figure A2.1. Overview of the automation configuration of P2 (picture is courtesy of CATAEL bvba)

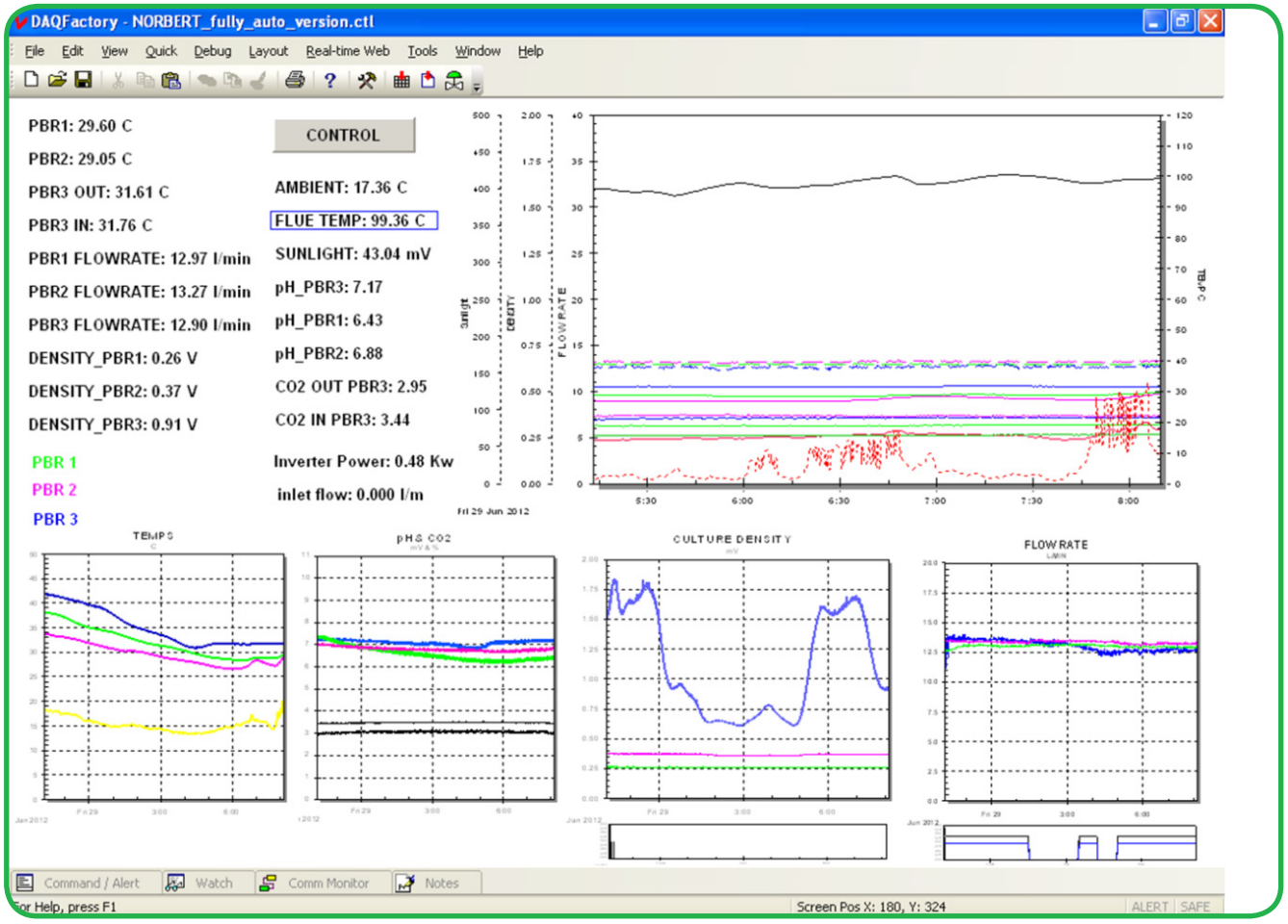

Figure A2.2. Screenshot of DaqFactory data interface

Appendix 3.0

Table A3.1. Levels for nitrogen and phosphorus in the growth medium for the different pilots.

Pilot	Media	Nitrogen			Phosphorus	
		Total N (mg L^{-1})	NO_3^- (mg L^{-1})	NH_4^+ (mg L^{-1})	Total P (mg L^{-1})	PO_4^{3-} (mg L^{-1})
P1	f/2		54.7			3.4
	Agriculture waste		0	15.9		6.5
	AD municipal waste		0	24.3		10.4
P2						
P3	BBM	124	547	0	49.9	153.3
	f/2	12.4	54.7	0	1.12	3.4
P4			1500	0		40
P5						
P6		30–40	30–40		4–5	4–5

Table A3.2. Thresholds levels for nutrients used in open pond systems at pilot P6.

Nutrient	Threshold level mg L^{-1}
Nitrogen	30–40
Phosphorus	4–5
Magnesium	7
Fe	0.2
Manganese	0.07
Molybdenum	0.07
Zinc	0.02

Examples of media for the culture of a range of microalgae species

The Culture Collection of Algae and Protozoa (CCAP) website (http://www.ccap.ac.uk/pdfrecipes.htm) details many of the major media recipes for maintaining different microalgae cultures. However, please note that sometimes very different media can be named under the same acronym, e.g. “HSM” is Horse Serum Media under CCAP and Sueoka High Salt Media under Chlamy.org http://www.chlamy.org/Sueoka.html. The latter (Sueoka High Salt Media) is the common growth media for saline tolerant microalgae. Caution must therefore be taken in deciding what is the correct media for your facility and experiments. Some of the common media utilised by the different pilot operations can be found in Table A3.3 below:

Table A3.3. Examples of culture media types to maintain different microalgal species at the various pilot plants.

Pilot	Microalgae species	Media used
P1	*Chlorella vulgaris*, *Scenedesmus quadricauda*, *Porphyridium purpureum*, *Nannochloropsis oculata*, *Nannochlorpsis oceanica*, *Tetraselmis suecica*, *Isochrysis galbana*, *Chaetoceros muelerii*, *Phaeodactylum tricornutum*	f/2
P2	MaB-flocs (dominance of *Ulothrix* or *Klebsormidium* spp.)	Wastewater pikeperch aquaculture
	MaB-flocs (dominance of *Desmodesmus* spp.)	UASB effluent food industry
	MaB-flocs (dominance of *Aphanothece* or *Aphanocapsa* spp.)	CAS effluent food industry
P3	*Phaeodactylum tricornutum*	f/2
	Chlorella vulgaris	3N-BBM+V
P4	*Chlorogloeopsis* spp.	BG11
P5		
P6	*Chlorella* and *Scenedesmus* spp.	Combination of available mineral fertilisers as used in agriculture (potassium nitrate, potassium phosphate, magnesium sulphate, mix of micronutrients).

Glossary

AD	Anaerobic Digestion
AGL	Algal Growth Laboratory
BG-11	Blue-Green Basal Bold Medium
BMP	Biochemical Methane Potential
BOD	Biological Oxygen Demand
BP	Best Practice
CAS	Conventional Activated Sludge
CCAP	Culture Collection of Algae and Protozoa (http://www.ccap.ac.uk)
CHP	Combined Heat and Power
COD	Chemical Oxygen Demand
CSV	Comma Separated Values
DO	Dissolved Oxygen
EC	Electrical Conductivity
FAME	Fatty Acid Methyl Esters
FTIR	Fourier Transform Infrared Spectrophotometry
GC-FID	Gas Chromatography – Flame Ionization Detector
GC-MS	Gas Chromatography – Mass Spectrometry
GC-TCD	Gas Chromatography with Thermal Conductivity Detector
GMP	Good Manufacturing Practice
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
HTL	Hydrothermal liquefaction
LED	Light Emitting Diode
MaB-floc	Microalgal-bacterial floc
OD	Optical Density
PAR	Photosynthetically Active Radiation
PBR	Photobioreactor
PC	Phycocyanine
PE	Phycoerythrine
PET	Polyethylene terephthalate
PLC	Programmable Logic Controllers
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene fluoride
RAS	Recirculation Aquaculture Systems
SBR	Sequencing Batch Reactor
SOP	Standard Operating Procedures
TC	Total Carbon
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon
TSS	Total Suspended Solids
UASB	Up-flow Anaerobic Sludge Blanket
VS	Volatile Solids
VSS	Volatile Suspended Solids

Investing in Opportunities

This project has received European Regional Development Funding through INTERREG IVB.

INTERREG IVB

EnAlgae is a four-year Strategic Initiative of the INTERREG IVB North West Europe programme. It brings together 19 partners and 14 observers across 7 EU Member States with the aim of developing sustainable technologies for algal biomass production.

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This report has been produced by Swansea University, lead partner of the EnAlgae project.
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