CRANFIELD UNIVERSITY

Francesco Ometto

MICROALGAE TO ENERGY:
BIOMASS RECOVERY AND PRE-TREATMENTS OPTIMISATION
FOR BIOGAS PRODUCTION INTEGRATED WITH WASTEWATER
NUTRIENTS REMOVAL

School of Applied Sciences

PhD Thesis

Supervisors: Dr Raffaella Villa and Prof Bruce Jefferson
January 2014

CRANFIELD UNIVERSITY

School of Applied Sciences

PhD Thesis

Francesco Ometto

MICROALGAE TO ENERGY:
BIOMASS RECOVERY AND PRE-TREATMENTS OPTIMISATION
FOR BIOGAS PRODUCTION INTEGRATED WITH WASTEWATER
NUTRIENTS REMOVAL

Supervisors: Dr Raffaella Villa and Prof Bruce Jefferson
January 2014

This thesis is submitted in partial fulfilment of the requirements for
the degree of Doctor of Philosophy

ABSTRACT

The increasing concern about water quality and energy demand promotes the development of innovative and low-cost processes to improve the nutrient uptake and energy efficiency of existing wastewater treatments (WWT). In this context, the inclusion of a microalgae system (MAS) in the flowsheet of a WWT plant represents a sustainable alternative to conventional technologies, as it combines a low-cost nutrient uptake system with the production of biomass suitable for biofuel production. However, at present, the energy required to cultivate and process the algae cells is often too high to justify their use. The adoption of a low energy harvesting system and an efficient energy conversion process are the *sine qua non* requirements to guarantee the sustainability of the process.

In this thesis, current and innovative harvesting technologies for large scale applications have been reviewed to identify the optimal working conditions of each system and their link to the main characteristics of the algae suspension. In particular, the performance of the Ballasted Dissolved Air Flotation (BDAF) system was investigated using different algae and compared to the conventional Dissolved Air Flotation (DAF). BDAF was demonstrably a very viable harvesting method where the use of floating microspheres as ballasting agents allowed significant coagulant savings, reduced the level of energy dissipation within the flotation chamber, and lowered the overall carbon emissions and the process costs.

The use of microalgae as a feedstock for anaerobic digestion (AD), considered the most feasible process for algal biofuel production, results from their high energy content and potential in reducing greenhouse gas emissions. However, work published so far reports values of methane yield 30% to 70% lower than their potential theoretical value. This work investigated the effect of four biomass pre-treatment technologies (thermal, thermal hydrolysis, ultrasounds and enzymatic hydrolysis) on the microalgae cells, and their impact on the digestion process yields. For the first time, the impact of the specific mechanism of each pre-treatment on microalgae cell breakage was studied and linked to biogas production. Post-treatment analysis of the composition of the solubilised

biomass of two green algae, *Scenedesmus obliquus* and *Chlorella sorokiniana*, and the cyanobacteria *Artrhospira maxima,* revealed that thermal and ultrasonic pre-treatments are primarily responsible for cell wall deformation and breakages, while the enzymatic pre-treatment was the only one able to provide the complete cell wall solubilisation. With thermal treatments, maximum biogas improvement occurred at 165°C with *S. obliquus* yielding 268 ± 2 ml kg VS^{-1} (+ 208%) followed by *A. maxima* (+ 70%) and *C. sorokiniana* (+ 98%). The ultrasound pre-treatment produced 96% methane improvement with *S. obliquus*, 38% with *A. maxima* and 42% with *C. sorokiniana*. Notwithstanding these great results, enzymatic hydrolysis produced significantly higher amounts of methane, and, depending on the algae species, the methane production improved up to 12 times with yields ranging between 477 and 730 ml gVS^{-1}.

The data obtained in the initial part of the work was used to build the business case of a hypothetical WWT for the integration of a MAS in its flowsheet. The results showed that the layout of the process is algae specific and that this parameter, the choice of the algae species involved in the process, has a significant impact on the overall operational costs and carbon footprint of the system.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
AOM	Algogenic Organic Matter
ARB	Acetolysis Resistant Biopolymers
AS	Activated Sludge
AW	Aerated Wetland
BAME	Bacterial Acid Methyl Ester
BDAF	Ballasted Dissolved Air Flotation
BMP	Biochemical Methane Potential
BMT	Biochemical Methane Test
BOD	Biological Oxygen Demand
CCA	Culture Collection Algae
CFV	Cross Flow Velocity
CO_2e	Carbon Dioxide Equivalent
COD	Chemical Oxygen Demand
DAF	Dissolved Air Flotation
D_f	Fractal Dimension
DiAF	Disperse Air Flotation
DM	Dried Mass
DNA	Deoxyribonucleic acid
DOC	Dissolved Organic Carbon
DOF	Disperse Ozone Flotation
E_i	Energy Inputs
E_o	Energy Outputs
EPS	Extracellular Polymeric Substance
FAME	Fatty Acids Methyl Ester
HLR	Hydraulic Loading Rate
HRAP	High Rate Algae Ponds
HRT	Hydraulic Retention Time
IOMM	Iron Oxide Magnetic Microparticle
LCA	Life Cycle Analysis
MAS	Microalgae System
MBR	Membrane Reactor

MR	Membrane Reactor
NOM	Natural Organic Matter
OTU	Operational Taxonomy Unit
PBR	Photobioreactor
PLFA	Phospholipids Fatty Acid
PS	Primary Sludge
PUFA	Polyunsaturated Fatty Acid
RNA$_f$	Ribonucleic acid
SAF	Suspended Air Flotation
sCC	Soluble Carbohydrates Content
sCOD	Soluble Chemical Oxygen Demand
SED	Sedimentation
SLR	Solids Loading Rate
sPC	Soluble Proteins Content
STP	Standard Temperature and Pressure
T	Thermal
TAG	Triacylglycerols
TEM	Transmission Electron Microscope
TF	Trickling Filter
TFF	Tangential Flow Filtration
TH	Thermal Hydrolysis
TNC	Total Carbon and Nitrogen
TS	Total Solids
TSS	Total Suspended Solids
U	Ultrasounds
VFA	Volatile Fatty Acids
VS	Volatile Solids
VSS	Volatile Suspended Solids
wt.	Weight
WWA	Wastewater Algae
WWTP	Wastewater Treatment Plant

"Nothing in life is to be feared,
it is only to be understood."

Marie Curie (1867-1934)

Chapter 1

INTRODUCTION

1 INTRODUCTION

1.1 Project background

Algae are aquatic organisms, some of which are capable of converting sunlight and CO_2 into chemicals and energy through photosynthetic activities (Tommaselli, 2007). Worldwide, more than 30,000 different species have been isolated and classified according to their colour, size and shape, chemical characteristics, cell wall constituents and intracellular composition (John *et al.,* 2003; Mata *et al.,* 2010). Their dimension ranges from micrometres to metres, providing an obvious size division between micro-algae and macro-algae. The latter, commonly known as seaweed, are multicellular organisms similar to plants, growing predominantly in the marine environment, whereas micro-algae, or phytoplankton, include small unicellular organisms found in water representing the primary source of food for most aquatic fauna and causing seasonal algal blooms in rivers, lakes and ponds under eutrophic conditions.

Microalgae are commercially cultivated for the production of a number of different goods including human and animal food additives and biochemicals. However, algae biomass has the potential to deliver a wider range of products which include; bioenergy, biofuels and bio-based products (bio-plastics, bio-cosmetics, bio-solvents).

1.1.1 Microalgae biomass utilisation

Currently, despite the high number of algae species available in nature, only a few are mass cultivated, with a worldwide annual production exceeding 10,000 tons of dry mass (DM) (Brennan and Owende, 2010). More than 30% of these are used to meet the dietary requirements of a variety of animals such as fish, pets and farm animals. The remainder is used as food additives in the human diet or for the extraction of pigments, proteins, polyunsaturated fatty acids (PUFAs) and hydrocolloids (e.g. agar) for the production of cosmetics, pharmaceuticals and biopolymers (Spolaore *et al.,* 2006; Harun *et al.,* 2010).

Arthrospira sp., a blue-green alga (cyanobacteria), is the most largely produced microalga (3,000 ton y^{-1} as DM), followed by *Chlorella* sp. (2,000 ton y^{-1}), *Dunaliella salina* (1,200 ton y^{-1}) and *Haematococcus pluvialis* (classified as green-algae, 300 ton y^{-1}) (Brennan and Owende, 2010). *Arthrospira* sp., particularly enriched in

proteins (60 -70% DM), vitamins, antioxidants and fatty acids such as linoleic acid, is largely processed for human and animal health to treat renal failure, hyperlipidaemia and hypertension (Spolaore *et al.,* 2006). Used for akin applications, *Chlorella* sp. contains lutein, a pigment for eye health, and β-1,3-glucan, an active immune stimulator, a free radical scavenger and a reducer of blood lipids (Spolaore *et al.,* 2006; Harun *et al.,* 2010). *Dunaliella salina* and *Haematococcus pluvialis,* instead, are cultivated for the extraction of β-carotene and astaxanthin, respectively (Brennan and Owende, 2010). The former is an essential pigment source of vitamin A, vital for normal growth, immune system function and vision, while astaxanthin is a strong antioxidant fundamental in the aquaculture sector and for the production of pharmaceuticals and cosmetics.

The average market price for raw algae biomass used as a food additive into the aquaculture sector ranges between £1 and £8 per kgDM. On the contrary, the value of algae derivatives varies largely from a minimum of £170 per kilogram of products up to £1,000 when producing fine extracts such as omega-3 fatty acids (Pulz and Gross, 2004; Ip and Chen, 2005). This explains the increasing interest of the biochemical and pharmaceutical sectors in this biomass for the extraction of high valuable compounds, which represents, in the short term, the most profitable field for algae exploitation (Spolaore *et al.,* 2006; Brennan and Owende, 2010; Harun *et al.,* 2010).

1.1.2 Microalgae and biofuels

Biofuel production from microalgae biomass relies on the ability of microalgae to accumulate a high content of lipids, proteins and carbohydrates which can be easily converted into biofuels (Table 1.1). The higher production efficiency per hectare of cultivation (30 to 50 times) compared to other biomass such as sunflower, rapeseed and sugar cane, and the absence of lignin, a non-fermentable biomass component, make microalgae the optimal source for renewable energy production (Schenk *et al.,* 2008). Although the research community started to investigate the production of biodiesel, bioethanol, biohydrogen and biogas from algae more than 60 years ago (Borowitzka, 2013), the process is still not satisfactory or economic and, consequently, is not fully available on a commercial scale (Schenk *et al.,* 2008; Demirbas, 2010; Lee, 2011; Yang *et al.,* 2011; Zamolla *et al.,* 2011). According to different cost-benefit analyses and life-cycle assessments, the potential for

microalgae to become an important component of the future renewable energy strategies relies on the optimisation of the biomass production process together with the development of more advanced technologies to reduce the energy/cost inputs required to process the biomass to final production (Norsker *et al.,* 2011; Sturm and Lamer, 2011; Acién *et al.,* 2012; Jonker and Faaij, 2013; Slade and Bauen, 2013). For instance, the cost-production of algae-biofuels per unit of energy produced is estimated to range between £136 and £489 per GJ, against a gasoline/diesel production cost lower than £20 per GJ (Jonker and Faaij, 2013). Furthermore, while the current market price of conventional diesel is close to £0.46 – 0.52 per litre (West Texas, Dubai, Brent) and biodiesel from crops is sold at £0.36 – 0.40 3 l^{-1}, ethanol at £0.41 l^{-1} and hydrogen at £1.84 – 6.73 l^{-1} (Oncel, 2013), the costs for the only dry algal biomass production range from a minimum of £1.5 $kgDM^{-1}$ to almost £10 kg DM^{-1} depending on the cultivation process adopted (Norsker *et al.,* 2011; Slade and Bauen, 2013).

Currently, biodiesel and biogas are the most investigated and closest to market algal-derived biofuels, with a large number of pilot plant facilities in operation to establish process feasibility and asses new low-energy and low-costs technologies (Lee, 2011; Bahadar and Khan, 2013). Bioethanol and biohydrogen, instead, are at earlier stages of exploitation as they require a better understanding of the production process and the identification of optimal algae species or strains. For ethanol production, the process relies on the microalgae biomass' ability to accumulate starch which can be hydrolysed to glucose and fermented to ethanol using alcohol producing microorganisms (Melis and Happe, 2001; Eshaq *et al.,* 2010; Harun *et al.,* 2011; Miranda *et al.,* 2012; Liu *et al.,* 2013). Similarly, a better understanding of the direct and indirect photolysis mechanisms of production is required before considering large scale application for hydrogen production (Benemann, 2000; Oncel, 2013).

Table 1.1 Microalgae energy content compared to other biomass used for renewable energy production.

Biomass/Feedstock	Protein %	Lipid %	Carbohydrates %	Approx energy potential[a] MJ kg^{-1}	Approx electricity potential kWh kg^{-1}
Microalgae[b]					
Chlamydomonas reinharditii	48	21	17	18.65	5.22
Chlorella emersonii	32	29	41	22.73	6.36
C. emersonii (low N)	28	63	11	29.83	8.35
Chlorella vulgaris	51-58	14-22	12-17	19.15	5.36
C. vulgaris (low N)	7	40	55	24.79	6.94
Euglena gracilis	39-61	20-21	17-18	18.62	5.21
Scenedesmus obliquus	50-56	12-14	12-17	16.05	4.49
Spirulina maxima	60-71	6-7	13-16	15.88	4.45
Dunaliella salina	57	6	32	17.03	4.77
Sunflower[c]	-	50-54	-	19.24	5.39
Rapeseed[d]	18-20	43-45	-	19.51	5.46
Sugar cane[e]	-	-	63-75	11.2	3.13

[a]*using a conversion factor equal to 16 kJ g^{-1} proteins, 17 kJ g^{-1} carbohydrates and 37 kJ g^{-1} lipids (Atwater system: http://www.nutrientdataconf.org/PastConf/NDBC17/9-3_Stewart.pdf);* [b]*Heaven et al., 2010;* [c]*Robertson et al., 1978;* [d]*Lajolo et al., 1991;* [e]*Masarin et al., 2011; 'low N' = algae cultivated in low nitrogen media.*

1.1.2.1 Biodiesel

At present, biodiesel production depends on a series of steps where the lipid content of the algae biomass is initially extracted, and then processed and refined to the final product. Conventional extraction methods include solvent, supercritical fuel, ultrasound and oil press extraction, which often require a large amount of solvents and pre-dried biomass (Harun *et al.,* 2010; Singh and Gu, 2010). After extraction, the oil is treated to reduce viscosity and increase fluidity, and therefore turning it into commercial biodiesel (Rawat *et al.,* 2013). Commercially available methods include transesterification, catalysis and pyrolysis. Transesterification is the most commonly found technology at full-scale production, where the extracted lipids, triacylglycerols (TAGs), are mixed with alcohol to produce glycerol and fatty acid methyl esters (FAMEs), which are the main constituents of biodiesel (Meher *et al.,* 2006; Sharma and Singh, 2009; Mata *et al.,* 2010). Similarly, direct esterification achieves the same

lipid transformation using acids, bases or enzymes. In contrast, pyrolysis allows a direct conversion of the algal biomass to liquid fuel by heating it at high temperatures (350 - 700°C) in the absence of air. All three approaches are economically demanding and energy intensive, being the bottleneck for algae-biodiesel commercialisation. As a consequence, the research focuses on alternative systems such as *in situ* transesterification which allows biodiesel production from dry biomass processed with methanol and/or sulphuric acid at low/medium temperatures, e.g. 90 - 100°C (Johnson and Wen, 2009).

The quantity of biodiesel that can be produced from microalgae varies largely between species and will also depend on factors such as the efficiency of lipid extraction and the conversion yields. On average, from 1,000 tons of dry algae biomass, it is expected to obtain 180 ton of biodiesel at 30% lipid extraction efficiency and 60% conversion efficiency (Ventura *et al.,* 2013).

1.1.2.2 Biogas

Biogas, 60% CH_4, 30% CO_2, 5% N_2 and 2% H_2 on average, is produced by bacterial degradation of the algae biomass in the absence of oxygen through a four step process which includes hydrolysis, acidogenesis, acetogenesis and methanogenesis. Depending on the chemical composition of the specific algal species, the methane production can vary from 400 to 800 ml g^{-1} of volatile solids (VS), estimated using the Symons and Buswell equation (1933) which is based on the stoichiometric conversion of the organic matter in methane, carbon dioxide and ammonia (Heaven *et al.,* 2010). However, since the initial investigation of Golueke *et al.* (1957), the anaerobic digestion of algae under mesophilic conditions (35 - 38°C) has proved to be difficult. The presence of bacteria-resistant compounds causes low methane production (30 – 60% of the theoretical methane potential), which leaves the energy conversion process unsatisfactory (Sielve *et al.,* 2009; Mussgnug *et al.,* 2010).

To overcome this limitation and create a more efficient energy conversion process, a pre-treatment is usually recommended. Ultrasonic, high temperature, French press and enzymes are the most commonly used methods for biomass pre-treatments. Methane gas production of a mesophilic anaerobic digestion (AD) process with pre-treatments (Table 1.2) could lead to approximately 50% yield improvement (Chen and Oswald, 1998; Heerenklage *et al.,* 2010). These efficiencies are usually linked to

the algal characteristics. However, very often the energy required for the pre-treatment does not offset the additional energy produced in the process.

Another limitation for microalgae biomass digestion is its typical low carbon:nitrogen (C:N) ratio, close to 5:1 (Yen and Brune, 2007), compared to a conventional C:N ratio for AD being between 20:1 and 30:1 (Parkin and Owen, 1986). A low C:N ratio together with high ammoniac nitrogen concentration, derived from the high protein content, might produce an accumulation of volatile fatty acids (VFAs) inhibiting the whole digestion process. Although efficient digestion has been reported even at a low C:N ratio (Stroot *et al.,* 2001; Yen and Brune, 2007; Ehimen *et al.,* 2010), a number of different works suggested microalgae as an optimal feedstock for co-digesting carbon enrich biomass: for instance, changing from digesting 100% algae to a co-digestion with waste paper (40:60 algae:paper). Yen and Brune (2007) reported an increment on the methane production from 100 ml gVS^{-1} to 320 ml g VS^{-1}. Similar results were obtained using soybean oil and glycerin as a carbon enriched co-substrate of algae (Salerno *et al.,* 2009).

Despite the efficiency of the process, compared to the other algae-biofuels biogas production currently appears to be the most feasible energy conversion process (De Schamphelaire and Verstraete, 2009). The ability of treating low concentrated (2 - 5 % wt. total solids) wet biomass significantly reduced the energy inputs required by preliminary treatments such as harvesting and drying, improving the overall energy balance of the process.

Table 1.2 Microalgae pre-treatments for anaerobic digestion

Treatment	Parameters	Biogas improvement %	*References*
Ultrasound	20-1700 kHz 10-300 kWh m^{-3}	7-17	*González-Fernández et al., 2012* *Park et al., 2013*
French press	35-104 bar	27	*Heernklage et al., 2010*
Thermal	60-150 °C (NaOH, H_2SO_4, NH_4OH)	30-60	*Chen and Oswald, 1998* *Cho et al., 2013* *Alzate et al., 2012*
Enzymatic	Cellulose, Pectinase, Lipase,	50	*Ehimen et al., 2013* *Yin et al., 2010*

1.1.3 Microalga biomass production

Photoautotrophic microalgae require light, temperature, carbon dioxide (CO_2), nitrogen (N), phosphorus (P), and a number of micronutrients including vitamins to grow (Hu, 2007; Grobbelaar, 2007). Conventional outdoor cultivation systems rely upon the sun to guarantee enough light and temperature. As a consequence, they are subjected to seasonal weather changes which impact on the biomass production yields, doubling in spring and summer compared to autumn and winter (Arbib *et al.,* 2013; Garcia *et al.,* 2000). In addition, to enhance biomass production yields, CO_2 and nutrients need to be added to balance the low concentrations of these compounds in the atmosphere and in conventional fresh/marine water, respectively.

1.1.3.1 Cultivation systems

Microalgae are generally cultivated in outdoor in photobioreactors (PBRs), open ponds or using hybrid systems where biomass initially grown in a PBR is used as inoculum for the pond system (Rodolfi *et al.,* 2009; Brennan and Owende, 2010; Mata *et al.,* 2010; Williams and Laurens, 2010). Photo-bioreactors are typically designed as tubular or flat panel reactors, where the algae biomass (up to 2 kg m^{-3}) is maintained in suspension by a constant air flow (Tredici, 2007; Min *et al.,* 2010). Conversely, open ponds are designed as rectangular raceway channels where a continuous flow around the circuit is maintained by peddle wheels (Greenwell *et al.,* 2010; Huang *et al.,* 2010; Mata *et al.,* 2010). Compared to PBRs, open ponds are cheaper to construct and operate as the energy demand of paddle wheels ranges between 20 to 50 kWh ha^{-1}, compared to air pumps which require between 0.3 and 2 kWh m^{-3} to maintain turbulent flow along the PBR (Demirbas, 2010; Jorquera *et al.,* 2010; Mata *et al.,* 2010; Acién *et al.,* 2012; Jonker and Faaij, 2013). On the other hand, open ponds are more exposed to external contamination and atmospheric changes, limiting the biomass production to a maximum of 1 gDM l^{-1}, with average values between 0.5 and 0.6 gDM l^{-1} under optimal conditions (Cromar and Fallowfield, 1997; Tredici, 2007). Alternative systems, not yet used on a large scale, include biofilm solution where the biomass grows attached to the surfaces of supporting materials or is immobilised in a matrix (Lau *et al.,* 1994; Wei *et al.,* 2008; Zhang *et al.,* 2008; De-Basham and Basham, 2010).

31

1.1.3.2 Alternative source for nutrients and carbon dioxide

The combined costs of CO_2 and nutrients required to support algal growth have been estimated to contribute for more than 50% to the total biomass cost production (Slade and Bauen, 2013; Singh *et al.,* 2014). Hence, the identification of low cost or zero cost sources for these two main compounds can significantly reduce the costs of the biomass production.

For instance, fume gases from power plants or other energy-production facilities are a free source of CO_2 which can be directly uptaken (1.83 $kgCO_2$ $kgDM^{-1}$) by microalgae saving up to £4,500 per hectare of cultivation per year (Jonker and Faaij, 2013). However, this saving can only happen if the location of the plant is close to the algal facility or by offsetting the costs required for transferring the CO_2 onsite (Ventura *et al.,* 2013).

Similarly, the costs for pure nutrient supplements can be replaced by nutrient-rich wastewater such as municipal or industrial wastewater which contains all the nutrients and micronutrients required to support microalgae growth (Xin *et al.,* 2010; Sydney *et al.,* 2011; Singh *et al.,* 2014). In particular, municipal wastewater is enriched in NH_4^+, NO_3^- and PO_4^{2-} which are easily taken up by the algae (Grobbelaar, 2007). In addition, the use of wastewater as a free source of nutrients has the double advantage of contributing to the treatment of the wastewater.

Using treated municipal wastewater, with a starting ammonia concentration of close to 40 mg l^{-1}, García *et al.* (2000) reported more than 95% removal after 3 to 8 days of hydraulic retention time (HRT) using a mixture of algae species including *Dicyosphaerium pulchellum*, *Chlorella* sp., *Micratinium pusillum*, *Scenesdesmus armatus* and *S. acutus*, in an open pond system having a biomass concentration close to 0.35 gDM l^{-1} (3 - 4 mg l^{-1} as Chlorophyll *a*) in spring/summer seasons and 0.15 gDM l^{-1} (1 - 2 mg l^{-1} as Chlorophyll *a*) in autumn/winter seasons. Similarly, with inflow concentrations of 13 $mgNH_4$-N l^{-1} and 2 $mgPO_4$-P l^{-1}, Sydney *et al.* (2011) reported complete ammonia and phosphorus removal after 9 days HRT using *Chlorella* sp. which tripled its biomass from 0.15 to 0.51 gDM l^{-1}. *Scenedesmus* sp. achieved 98% ammonia and phosphorus removal after 5 days starting with even lower concentrations equal to 2.5 $mgNH_4$-N l^{-1} and 0.5 mgP l^{-1}, respectively (Xin *et al.,* 2010). Along with N and P uptake, different authors reported BOD and COD

removal between 40% and 50% when using primary effluents (Airbib *et al.,* 2013; Craggs *et al.,* 2012; Cromar and Fallowfield, 1997).

Despite the higher energy demand required by the system, PBR systems have been reported to achieve similar N and P uptake in significantly lower HRT (Arbib *et al.,* 2013, Doria *et al.,* 2012). For instance, when comparing the performance of open ponds to PBRs, Arbib *et al.* (2013) observed a 2-fold difference in the HRT with biomass concentration close to 0.2 gDM l^{-1} using ponds and 0.6 gDM l^{-1} in PBRs.

1.1.3.3 Harvesting

The contribution of the recovery step to the total algal biomass cost production has been estimated to be between 20% and 30% (Brennan and Owende, 2010). The small cell size (3 - 11 µm) of microalgae, their cell density similar to the water, the negative charge density, as well as the specific chemical composition of the whole algal suspension, are important parameters to be considered when selecting the harvesting process (Edzwald, 1993; Mata *et al.,* 2010; Christenson and Sims, 2011). All harvesting systems can be categorised into four main technological groups: sedimentation, flotation, filtration and centrifugation. The latter two technologies are one to ten times more energy demanding, and therefore more expensive, compared to sedimentation and flotation. Amongst all the flotation technologies, the Dissolved Air Flotation (DAF) system is considered one of the most efficient and low-energy on the market. The energy demand required by the system is about 0.3 kWh m^{-3}, against 0.3 - 8 kWh m^{-3} for centrifuge systems or pressure and vacuum filter (Molina Grima *et al.,* 2003). In the DAF system, dissolved micro-bubbles are trapped in the pre-flocculated algal biomass and the float conglomerates to the surface (Wiley *et al.,* 2009; Brennan and Owende, 2010; Williams and Laurens, 2010; Zamolla *et al.,* 2011). Furthermore, modification of the DAF system, such as PosiDAF and Ballasted Dissolved Air Flotation (BDAF), can achieve even higher energetical and financial savings. The PosiDAF process uses the same principle as the DAF system by modifying the charge of the bubble surfaces through the addition of polymers to the air saturator, which then aggregate with the algae cells (Henderson *et al.,* 2009). In the BDAF system, the air bubbles are replaced with low-density microspheres which, when added to the algae suspension prior to flocculation, become part of the algae floc driving flotation (Jarvis *et al.,* 2009). PosiDAF is more efficient in saving chemicals rather than energy, whereas the estimated energy demand of BDAF

ranges between 0.01 and 0.04 kWh m^{-3}, 60 to 80% lower than conventional DAF system (Jarvis *et al.,* 2009). Other emerging technologies such as bio-flocculation or dispersed ozone flotation are no-chemical and no-energy alternative systems, which could generate even higher savings (Cheng *et al.,* 2011; Salim *et al.,* 2011).

1.2 Project development

The work presented in this thesis was developed as part of the European project Advance Technologies for Water Resource Management (ATWARM) to investigate the feasibility of an integrated microalgae wastewater treatment (WWT) process for biogas production. In this context, the project brought together water companies including Anglian Water, Severn Trent Water, Scottish Water and Northern Ireland Water, as well as expertise from Cranfield University and other academic partners through the QUESTOR centre, the coordinator of the ATWARM project.

Two main research areas were identified as pivotal in the integrated system; microalgae harvesting and microalgal anaerobic digestion.

A low energy harvesting system, Ballasted Dissolved Air Flotation (BDAF), was investigated and compared to a conventional Dissolved Air Flotation (DAF) system using three different microalgae to assess the impact of the biomass characteristics on the process parameters.

In parallel, the recovered algae were tested for biogas production using a number of different pre-treatments which included thermal, thermal hydrolysis, ultrasound and enzymatic hydrolysis. All the pre-treatments were optimised for the three algal species to assess the impact of the biomass characteristics on the pre-treatment processes and on the digestion performances.

Finally, to establish the potential impact of the findings from the thesis, a series of case studies have been considered. In particular, the impact of different microalgae harvesting options and pre-treatments on the energy, carbon and economic balance of an integrated microalgae wastewater treatment has been estimated.

1.3 Aim and objectives

The main aim of the project was to optimise different harvesting technologies and anaerobic digestion (AD) pre-treatment processes using three different algae species, and to evaluate their impact on the energy balance of a wastewater treatment plant integrated with a microalgae system (MAS) for nutrient removal. It was hypothesised that an improvement in the biogas production of the microalgal cells in the AD process, combined with a low energy harvesting technology, has the potential to provide a self-sufficient energy wastewater treatment. A series of objectives were agreed:

1. To produce a state of the art review of low energy microalgal harvesting technologies.
2. To assess the impact of the characteristics of different microalgae on the efficiency of a low energy BDAF harvesting technology compared to the traditional DAF system using different harvesting conditions.
3. To estimate the potential biogas/methane production of different algae species under mesophilic anaerobic digestion conditions.
4. To investigate the effect of different pre-treatments (thermal, thermal hydrolysis, ultrasounds and enzymatic hydrolysis) on different microalgae species and the associated biogas improvements.
5. To evaluate the impact of DAF, BDAF and different AD pre-treatments on the energy balance and carbon footprint of an integrated microalgal wastewater treatment plant using the outcomes of objectives 2 and 4.

A conceptual diagram of the integrated microalgal wastewater treatment plant is reported in Figure 1.1, where the pre-defined objectives have been highlighted in different colours.
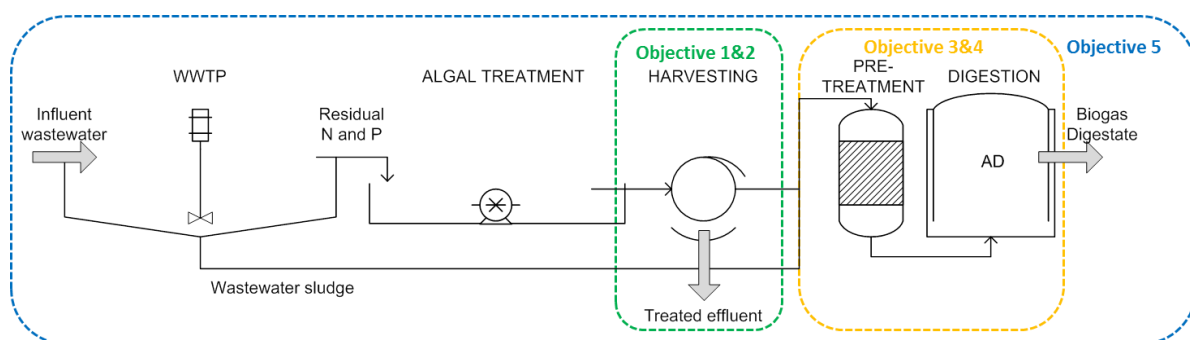
Figure 1.1 Integrated process for wastewater microalgae biogas production.

1.4 Thesis plan

This thesis is presented as a series of chapters formatted as papers for publication. All papers were written by Francesco Ometto, and edited by Dr Raffaella Villa (principal supervisor) and Prof Bruce Jefferson. All experimental work was designed, coordinated and completed by Francesco Ometto at Cranfield University (UK) with the contribution of a few other students over the years. The algae cultures were periodically subcultivated and monitored in collaboration with Rachel Whitton, Cranfield University. In Chapter 2, parts of the jar tests were completed by Carlo Pozza, University of Duisburg-Essen (DE), while preliminary work was performed in collaboration with Davide Pierobon, University of Padua (IT), and Marta Bortolotti, University of Verona (IT), during their internship. In Chapter 3, the bacteria community analysis was undertaken in collaboration with Robert Ferguson, Cranfield University. Parts of the ultrasound pre-treatments were performed in collaboration with Gerardo Quiroga, University of Oviedo (ES), and the preliminary work on enzymatic hydrolysis was completed by Pavel Psenicka, Czech University of Life Sciences Prague (CZ), as part of their internship.

Chapter 2, Harvesting Microalgae, focused on the separation process of the algae biomass from the growth media. Paper 2.1, entitled *Innovation on microalgae harvesting technologies for biofuels production: a review* by Ometto, F., Whitton, R., Jefferson, B. and Villa, R., to be submitted to Water Research, reviews the available harvesting microalgae technologies for large scale application, underlining the efficiency and the energy demand of different systems depending on the characteristics of the algae suspension. Paper 2.2 is entitled *The impact of replacing air bubbles with microspheres for the clarification of algae from low cell-density culture* by Ometto, F., Pozza, C., Whitton, R., Smyth, B., Gonzales Torres, A., Henderson, R.K., Jarvis, P., Jefferson, B. and Villa R., Water Research. This paper reports the experimental results relating to the comparison between a conventional harvesting technology, Dissolved Air Flotation (DAF) and the innovative low energy Ballasted Dissolved Air Flotation (BDAF) where floting microspheres replace air bubbles to allow algae separation.

Chapter 3, Anaerobic Digestion of Microalgae, focuses on biogas production from algal biomass. Paper 3.1, entitled *Adapting anaerobic digestion bacteria to algal*

biomass by Ometto, F., Ferguson, R., Whitton, R., Coulon, F., Jefferson, B. and Villa, R., to be submitted to Bioresource Technology, evaluates the potential of increasing anaerobic digestion efficiency adapting a bacteria community to microalgae. Paper 3.2 investigates the effect of different pre-treatments (thermal, ultrasound and enzymatic) of the algae on the biogas production. The title of this paper is *Impacts of microalgae pre-treatments for improved anaerobic digestion: thermal treatment, thermal hydrolysis, ultrasound and enzymatic hydrolysis by* Ometto, F., Quiroga, G., Psenicka, P., Whitton, R., Jefferson, B. and Villa, R., to be submitted to Water Research.

Chapter 4, Implication of the work, is an overall discussion which highlights the key findings of the previous chapters and then evaluates their practical implications using a business case for a hypothetical integrated algae wastewater treatment plant. The business case is based on the impact of different low-energy technologies for nutrient removal, including algae, to the flowsheet of a small treatment work (2000 p.e. capacity). The case, Paper 4.2, *Energy balance of an integrated microalgae wastewater treatment*, by Ometto, F., Jefferson, B. and Villa, R., compares the energy balance and the carbon emissions of different low-energy nutrient-removal technologies and algae, using the data obtained in Chapters 2 and 3.

Chapter 5, Conclusions and future work, summarises the key results and suggests recommendations for future investigations on the development of novel microalgae harvesting systems and anaerobic digestion pre-treatments.

A summary of the thesis plan is reported in Table 1.3.

Table 1.3 Thesis plan

Chapter	Paper	Objective addressed	Title	Journal	Status
2	2.1	1	Innovation of microalgae harvesting technology: a review.	Water Research	In preparation
	2.2	2	The impact of replacing air bubbles with microspheres for the clarification of algae from low cell-density culture	Water Research	Published
3	3.1	3	Adapting anaerobic digestion bacteria to algal biomass	Bioresource Technology	In preparation
	3.2	4	Impacts of microalgae pre-treatments for improved anaerobic digestion: thermal treatment, thermal hydrolysis, ultrasound and enzymatic hydrolysis	Water Research	In preparation
4	4.1	1, 2, 3, 4	Key observations	-	-
	4.2	5	Energy balance of an integrated microalgae wastewater treatment	Biomass and Bioenergy	In preparation
5	-	-	Conclusions and future works	-	-

1.5 References

Acién, F.G., Fernández, J.M., Magán, J.J., Molina, E., 2012. Production cost of a real microalgae production plant and strategies to reduce it. Biotechnol. Adv. 30, 1344-53.

Alzate, M.E., Munoz, R., Rogalla, F., Fdz-Polanco, F., Pérez-Elvira S.I., 2012. Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment. Bioresour. Technol. 123, 488-494.

Arbib, Z., Ruiz, J., Álvarez-Díaz, P., Garrido-Pérez, C., Barragan, J., Perales, J.A., 2013. Long term outdoor operation of a tubular airlift pilot photobioreactor and a high rate algal pond as tertiary treatment of urban wastewater. Ecol. Eng. 52, 143-153.

Bahadar, A., Bilal Khan, M., 2013. Progress in energy from microalgae: a review. Renew. Sustain. Energy Rev. 27, 128-148.

Benemann, J.R., 2000. Hydrogen production by microalgae 291-300.

Borowitzka, M.A., 2013. Energy from microalgae: a short history. Algae for Biofuels and Energy. 5, 1-15.

Brennan, L., Owende, P., 2010. Biofuels from microalgae: a review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sustain. Energy Rev. 14, 557-577.

Chen, P.H., Oswald, W.J., 1998. Thermochemical treatment for algal fermentation. Environ. Int. 8, 889-897.

Cheng, Y.L., Juang, Y.C., Liao, G.Y., Tsai, P.W., Ho, S.H., Yeh, K.L., Chen, C.Y., Chang, J.S., Liu, J.C., Chen, W.M., Lee, D.J., 2011. Harvesting of *Scenedesmus obliquus* FSP-3 using dispersed ozone flotation. Bioresour. Technol. 102, 82-7.

Cho, S., Park, S., Seon, J., Yu, J., Lee, T., 2013. Evaluation of thermal, ultrasonic and alkali pretreatments on mixed-microalgal biomass to enhance anaerobic methane production. Bioresour. Technol. 143, 330-336.

Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol. Adv. 29(6), 686-702.

Craggs, R., Sutherland, D., Campbell, H., 2012. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. J. Appl. Phycol. 24, 329-337.

Cromar, N.J., Fallowfield, H.J., 1997. Effect of nutrient loading and retention time on performance of high rate algal ponds. J. Appl. Phycol. 1, 301-309.

De Schamphelaire, L., Verstraete, W., 2009. Revival of the biological sunlight-to-biogas energy conversion system. Biotechnol. Bioeng. 103, 296-304.

De-Bashan, L.E., Bashan, Y., 2010. Immobilized microalgae for removing pollutants: review of practical aspects. Bioresour. Technol. 101, 1611-1627.

Demirbas, A., 2010. Use of algae as biofuel sources. Energy Conversion and Management. 51(12), 2738-2749.

Doria, E., Longoni, P., Scibilia, L., Iazzi, N., Cella, R., Nielsen, E., 2011. Isolation and characterization of a *Scenedesmus acutus* strain to be used for bioremediation of urban wastewater. J. Appl. Phycol. 24, 375-383.

Edzwald, J.K., 1993. Algae, bubbles, coagulants, and dissolved air flotation. Water Sci. and Technol. 27(10), 67-81.

Ehimen, E.A., Holm-Nielsen, J.B., Poulsen, M., Boelsmand, J.E., 2013. Influence of different pre-treatment routes on anaerobic digestion of a filamentous algae. Renew. Energ. 50, 476-480.

Eshaq, F.S., 2010. *Spirogyra* biomass a renewable source for biofuel (bioethanol) production. Int. J. Eng. Sci. 2, 7045-7054.

Garcia, J., 2000. High rate algal pond operating strategies for urban wastewater nitrogen removal. J. Appl. Phycol. 331-339.

Golueke, C.G., Oswald, W.J., Gotaas, H.B., 1957. Anaerobic digestion of algae. Appl. Microbiol. 5, 47-55.

González-Fernández, C., Sialve, B., Bernet, N., Steyer, J.P., 2012. Comparison of ultrasound and thermal pretreatment of *Scenedesmus* biomass on methane production. Bioresour. Technol. 110, 610-616.

Greenwell, H.C., Laurens, L.M.L., Shields, R.J., Lovitt, R.W., Flynn, K.J., 2010. Placing microalgae on the biofuels priority list: a review of the technological challenges. J. R. Soc. Interface. 7, 703-726.

Grobbelaar, 2007. Algal nutrition: In Richmond, A. (ed): Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science. 97-115 (2007).

Harun, R., Davidson, M., Doyle, M., Gopiraj, R., Danquah, M., Forde, G., 2011. Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility. Biomass Bioener. 35, 741-747.

Harun, R., Singh, M., Forde, G.M., Danquah, M.K., 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. Renew. Sustain. Energy Rev. 14, 1037-1047.

Heaven, S., Milledge, J., Zhang, Y., 2010. Comments on "Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable". Biotechnol. Adv. 29, 164-167

Heerenklage, J., Maxfield, T., Zapf, A., Adwiraah, H., Wieczorek, N., Koerner, I., 2010. Anaerobic digestion of microalgae: possibilities and limits. Proceeding of Venice 2010, Third International Symposium on Energy from Biomass and Waste, Venice, November 8-11, 2010.

Henderson, R.K., Parsons, S.A., Jefferson, B., 2009. The potential for using bubble modification chemicals in dissolved air for algae removal. Sep. Sci. Technol. 44, 1923-1940.

Hu, 2007. Environmental effects on cell composition: In Richmond, A. (ed): Handbook of microalgal culture: biotechnology and applied phycology, Blackwell Science. 83-93 (2007).

Huang, G., Chen, F., Wei, D., Zhang, X., Chen, G., 2010. Biodiesel production by microalgal biotechnology. Appl. Energy. 87, 38-46.

Ip, P.F., Chen, F., 2005. Production of astaxanthin by the green microalga *Chlorella zofingiensis* in the dark. Process Biochemistry. 40(2), 733-738.

Jarvis, P., Buckingham, P., Holden, B., Jefferson, B., 2009. Low energy ballasted flotation. Water Res. 43(14), 3427-3434.

John, D. M., Whitton, B. A., Brook, A. J. The freshwater algal flora of british isles: an identification guide to freshwater and terrestrial algae. Cambridge University Press (2003).

Johnson, M.B., Wen, Z., 2010. Development of an attached microalgal growth system for biofuel production. Appl. Microbiol. Biotechnol. 85, 525-534

Jonker, J.G.G., Faaij, A.P.C., 2013. Techno-economic assessment of micro-algae as feedstock for renewable bio-energy production. Appl. Energy 102, 461-475.

Jorquera, O., Kiperstok, A., Sales, E.A., Embiruçu, M., Ghirardi, M.L., 2010. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. Bioresour. Technol. 101, 1406-1413.

Lajolo, F.M., Lanfer Marquez, U.M., Filisetti-Cozzi, T.M.C.C., McGregort, D.I., 1991. Chemical composition and toxic compounds in rapeseed (*Brassicanapus, L.*) cultivars grown in Brazil. J. Agric. Food Chem. 39, 1933-1937.

Lau, P.S., Tam, N.F.Y., Wong, Y.S., 1995. Effect of algal density on nutrient removal from primary settled wastewater. Environ. Pollut. 89, 59-66.

Lee, D. H., 2011. Algal biodiesel economy and competition among bio-fuels. Bioresour. Technol. 102, 43-49.

Liu, C., Zheng, S., Xu, L., Wang, F., Guo, C., 2013. Algal oil extraction from wet biomass of *Botryococcus braunii* by 1,2-dimethoxyethane. Appl. Energy. 102, 971-974.

Masarin, F., Gurpilhares, D.B., Baffa, D.C., Barbosa, M.H., Carvalho, W., Ferraz, A., Milagres, A.M., 2011. Chemical composition and enzymatic digestibility of sugarcane clones selected for varied lignin content. Biotechnol. Biofuels 4, 55.

Mata, T.M., Martins, A. a., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. Renew. Sustain. Energy Rev. 14, 217-232.

Meher, L.C., Vidya, S.D., Naik, S.N., 2006. Technical aspects of biodiesel production by transesterification: a review. Renew. Sustain. Energy Rev. 10, 248-68.

Melis, A., Happe, T., 2001. Hydrogen production: green algae as a source of energy. Plant Physiol. 127, 740-748.

Min, M., Wang, L., Chen, Y., Li, Y., Chen, P., Ruan, R. 2009. Using wastewater for mass microalgae production as an energy crop. Proceeding of the Annual international meeting, American Society of Agricultural Engineers, New York, 2009.

Miranda, J.R., Passarinho, P.C., Gouveia, L., 2012. Bioethanol production from *Scenedesmus obliquus* sugars: the influence of photobioreactors and culture conditions on biomass production. Appl. Microbiol. Biotechnol. 96, 555-564.

Molina Grima, E., Belarbi, E.-H., Acién Fernández, F.G., Robles Medina, a, Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol. Adv. 20, 491-515.

Mussgnug, J.H., Klassen, V., Schlüter, a, Kruse, O., 2010. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. J. Biotechnol. 150, 51-56.

Norsker, N., Barbosa, M.J., Vermuë, M.H., Wijffels, R.H., 2011. Microalgal production: a close look at the economics, Biotechnol. Adv. 29, 24-27.

Oncel, S.S., 2013. Microalgae for a macroenergy world. Renew. Sustain. Energy Rev. 26, 241-264.

Park, K.Y., Kweon, J., Chantrasakdakul, P., Lee, K., Cha, H.Y., 2013. Anaerobic digestion of microalgae biomass with ultrasonic disintegration. Int. Biodeter. Biodegr. 85, 598-602.

Parkin, G.F., Owen, W.F., 1986. Fundamental of anaerobic-digestion of wastewater sludge. Journal of Environmental Engineering. 112, 867-920.

Pulz, O., Gross, W., 2004. Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol. 65, 635–48.

Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F., 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. Appl. Energy. 103, 444-467.

Robertson, J.A., Chapman, C.W., Wilson, R.L., 1978. Relation of days after flowering to chemical composition and physiological maturity of sunflower seed. J. of Am. Oil Chem. Soc. 55(2), 266-269

Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G., Tredici, M.R., 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol. Bioeng. 102, 100-12.

Salerno, M., Nurdogan, Y., Lundquist, T. J., 2009. Biogas production from algae biomass harvested at wastewater treatment ponds. Proceedings of Bioenergy Engineering Conference, Washington, October 11-14, 2009.

Salim, S., Bosma, R., Vermuë, M.H., Wijffels, R.H., 2011. Harvesting of microalgae by bio-flocculation. J. Appl. Phycol. 23, 849–855.

Schenk, P.M., Thomas Hall, S. R., Stephens, E., Marx, U. C., Mussgnug, J. H., Posten, C., Kruse, O., Hankamer, B., 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy Research, 1(1), 20-43.

Sharma, Y.C., Singh, B., 2009. Development of biodiesel: current scenario. Renew. Sustain. Energy Rev. 13, 1646-1651.

Sialve, B., Bernet, N., Bernard, O., 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol. Adv. 27, 409-416.

Singh, B., Guldhe, A., Rawat, I., Bux, F., 2014. Towards a sustainable approach for development of biodiesel from plant and microalgae. Renew. Sustain. Energy Rev. 29, 216-245.

Singh, J., Gu, S., 2010. Commercialization potential of microalgae for biofuels production. Renew. Sustain. Energy Rev. 14, 2596-2610.

Slade, R., Bauen, A., 2013. Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects. Biomass Bioener. 53, 29–38.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. J. Biosci. Bioeng. 101, 87-96.

Stroot, P.G., McMahon, K.D., Mackie, R.I., Raskin, L., 2001. Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions: digester performance. Water Res. 35, 1804-1816.

Sturm, B.S.M., Lamer, S.L., 2011. An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. Appl. Energy. 88, 3499-3506.

Sydney, E.B., da Silva, T.E., Tokarski, A., Novak, A.C., de Carvalho, J.C., Woiciecohwski, A.L., Larroche, C., Soccol, C.R., 2011. Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage. Appl. Energy. 88, 3291-3294.

Tomaselli, L., 2007. The microalgal cell. In Richmond, A. (ed): Handbook of microalgal culture: biotechnology and applied phycology, Blackwell Science. 178-214 (2007).

Tredici, 2007. Mass production of microalgae: In Richmond, A. (ed): Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science. 178-214 (2007).

Ventura, J.S., Yang, B., Lee, Y., Lee, K., Jahng, D., 2013. Life cycle analyses of CO_2, energy, and cost for four different routes of microalgal bioenergy conversion. Bioresour. Technol. 137, 302-310.

Wei, Q., Hu, Z., Li, G., Xiao, B., Sun, H., Tao, M., 2008. Removing nitrogen and phosphorus from simulated wastewater using algal biofilm technique. Front. Environ. Sci. Eng. China 2, 446-451.

Wiley, P.E., Brenneman, K.J., Jacobson, A.E., 2009. Improved algal harvesting using suspended air flotation. Water Environ. Res. 81, 702-708.

Williams, P.J.L.B., Laurens, L.M.L., 2010. Microalgae as biodiesel and biomass feedstocks: review and analysis of the biochemistry, energetics and economics. Energy and Environmental Science, 3(5), 554-590.

Xin, L., Hong-Ying, H., Jia, Y., 2010. Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, *Scenedesmus* sp. LX1, growing in secondary effluent. N. Biotechnol. 27, 59-63.

Yang, J., Xu, M., Zhang, X., Hu, Q., Sommerfeld, M., Chen, Y., 2011. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. Bioresour. Technol. 102, 159-65.

Yen, H.W., Brune, D.E., 2007. Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresourc. Technol. 98, 130-134.

Yin, L.J., Jiang, S.T., Pon, S.H., Lin, H.H., 2010. Hydrolysis of *Chlorella* by *Cellulomonas* sp. YJ5 Cellulases and its biofunctional properties. J. Food Sci. 75(9), 317-323.

Zamalloa, C., Vulsteke, E., Albrecht, J., Verstraete, W., 2011. The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. Bioresour. Technol. 102, 1149-1158.

Zhang, E., Wang, B., Wang, Q., Zhang, S., Zhao B., 2008. Ammonia-nitrogen and orthopshisphate removal by immobilized *Scenedesmus* sp. isolated from municipal wastewater for potential use in tertiary treatment. Bioresour. Technol. 99, 3787-3793.

Chapter 2
HARVESTING MICROALGAE

2 HARVESTING MICROALGAE

2.1 Innovation on microalgae harvesting technologies for biofuels production: a review

Francesco Ometto, Rachel Whitton, Bruce Jefferson and Raffaella Villa.

Cranfield University, Bedfordshire, UK;

Abstract

Biomass production cost is the main bottleneck for the large-scale commercialisation of microalgae biofuels. In particular, the harvesting step plays a key role in the process, affecting the characteristics of the separated biomass and hence their range of use. This chapter focuses on the main harvesting methods used in algae production (sedimentation, flotation, filtration and centrifugation), and in particular on innovative applications, to identify the most efficient and financially viable process for the production of algal biofuel. The identification of the most appropriate harvesting method was determined using the properties of individual algae species (size, shape, density and charge) and the properties of their post-growth suspension media (composition, algogenic organic matter, pH and zeta potential). This review links for the first time the biological aspects to the engineering parameters, and explains why, knowing the specific characteristics of the algae suspension to be treated, it will prevent the adoption of harvesting systems from being unable to guarantee high efficiencies or causing unexpected high operational costs and failures.

2.1.1 Introduction

Microalgae are a valuable biomass processed worldwide for the production of a number of goods, such as food additives, pharmaceutical compounds, biochemicals and biofuels. The production of these algae derivatives is the results of a number of energy intensive and cost demanding steps including (1) cultivation, (2) harvesting, (3) dewatering and (4) downstream processes to final products (Brennan and Owende, 2010). For highly valuable algae products such as pigments, Omega-3 Fatty Acids and bioactive compounds, such costs are offset through a high market price (£170 - £6,500 per kilogram of products) and lack of alternatives (Table 2.1). In contrast, algae biofuels are low value products which need to compete with the low price of other renewable or non-renewable fuels (Pulz and Gross, 2004; Ip and Chen, 2005; Harun *et al.,* 2010; Lee, 2011).

The recent analysis of Jonker and Faaij (2013) estimated that the cost of production of algae-biofuels ranges between £136 and £489 GJ^{-1} against gasoline/diesel costs ranging from £5 to £20 GJ^{-1}. Hence, there is a need to reduce algae-biofuels cost production to create a sustainable and economically competitive algae-biofuel market. Cost-benefit and life-cycle assessments have identified the optimisation of biomass cultivation and harvesting as two of the key steps for microalgae to become an important component of future energy production (Collet *et al.,* 2011; Harun *et al.,* 2011; Norsker *et al.,* 2011; Razon and Tan, 2011; Sturm and Lamer, 2011; Acién *et al.,* 2012;Jonker and Faaij, 2013; Slade and Bauen, 2013).

Cultivations for large scale production of algae-biofuel are currently limited to open pond systems (Tredici, 2007; Chen *et al.,* 2011; Craggs *et al.,* 2012), as they are more economical to construct and operate compared to closed photobioreactors (PBRs), although they require much a higher footprint (Grobbelaar, 2010; Christenson and Sims, 2011). For instance, the estimated annual energy demand required to produce 50 x10^3 ton of *Tetraselmis suecica* decreased from 153 to 8.7 GWh y^{-1} using raceway ponds rather than horizontal PBRs (Harun *et al.,* 2011). The cost production of raw biomass using raceway ponds has been estimated between £1.5 and £4.2 kg^{-1} of dry mass (DM) (Norsker *et al.,* 2011; Slade and Bauen, 2013). For efficient outdoor growth, algae cultures require nitrogen (N), phosphorus (P), micronutrients and carbon dioxide (CO_2) (Grobbelaar, 2007). Using waste CO_2 from power plants or other facilities and replacing pure nutrient supplements (7 – 10%

total production costs) with wastewater, which contains all the required components to support biomass growth (Singh *et al.,* 2014), has the potential to reduce the biomass production cost by 50% (Xin *et al.,* 2010; Zhang *et al.,* 2008; Zamalloa *et al.,* 2010; Craggs *et al.,* 2012) lowering the costs to £0.35 kgDM^{-1} (Slade and Bauen, 2013). Therefore, for biodiesel production, assuming a biomass oil content of 30% by weight (Chisti*,* 2007), the biomass cost to produce 1 litre of algae-biodiesel at 80% conversion efficiency (Xu *et al.,* 2006) will be close to £1.5 kg^{-1} (Chisti, 2007; Lee, 2011; Gallagher, 2011; Bahadar and Bilal Khan, 2013), whereas the current market price of conventional diesel as a final product (West Texas, Dubai, Brent) is close to £0.46 – 0.52 per litre (Slade and Bauen, 2013). Algae production costs can be further reduced by optimising the harvesting step, which is estimated to account for 20 - 30% of the total production cost (Gudin and Therpenier, 1986; Jonker and Faaij, 2013).

Microalgae harvesting technologies can be classified into four categories: (1) sedimentation, (2) flotation, (3) filtration and (4) centrifugation. According to Christenson and Sims (2011), on a representative number of large-scale microalgae cultivation facilities (open ponds), filtration (33%) is the most used harvesting method followed by flotation (22%), sedimentation (14%) and centrifugation (10%). While the energy demand relating to sedimentation processes is generally negligible, conventional flotation, filtration and centrifugation require energy inputs which can be as low as 0.1 - 0.5 kWh m^{-3} for flotation and membrane microfiltration, or higher than 5 kWh m^{-3} for centrifugation and vacuum filtration (Mohn, 1980; Shelef *et al.,* 1984; Uduman *et al.,* 2010). Despite the low operation-energy input, the economics of sedimentation and flotation systems is affected by the use of coagulants which impact the total production costs by more than 10% (Zamalloa *et al.,* 2010). In addition, the low concentration (1 - 5% wt. DM) of the separated biomass often requires additional concentration steps, which will further increase the overall costs of the process.

The mechanism of biomass recovery for the four identified harvesting systems has been described in a number of comprehensive reviews (Mohn, 1980; Shelef *et al.,* 1984; Molina Grima *et al.,* 2003; Uduman *et al.,* 2010; Christenson and Sims, 2011; Show *et al.,* 2012). The outcome of these works indicated that the optimal harvesting system will be determined (1) by the algae physical characteristics and (2) by the

downstream process requirements (e.g. solid content, cell integrity, dry or wet biomass). For instance, stable algal suspensions with negative zeta potential will have poor particle aggregation and hence chemical flocculation will not be a suitable separation process, whereas cultures with low density cells will not be suitable for sedimentation. Furthermore, the composition of the extracellular algogenic organic matter (AOM), which affects charge density, requires the adoption of preliminary destabilisation processes (e.g. chemical flocculation) to guarantee higher efficiencies of low energy harvesting systems such as sedimentation and flotation (Edzwald, 1993; Henderson *et al.,* 2010). On the other hand, while high valuable algae products require dry, intact and concentrated biomass to allow efficient extractions of biochemical components, low valuable algae derivates might not require the same level of concentration and dryness, which will allow the adoption of low energy harvesting technologies (Table 2.1). In particular, a large number of biofuel production processes, such as anaerobic digestion and alternative lipid/carbohydrate extraction methods, have been reported to efficiently process wet biomass at low solid contents (2 - 10 wt.% DM) achievable with low-energy, low-cost harvesting systems (Xu *et al.,* 2011; Frigon *et al.,* 2013; Liu *et al.,* 2013).

Over the last 20 years, a number of innovative separation technologies have been developed to combine high particle separation efficiency with low energy inputs. Particular efforts have been made to clarify the interaction between algae cells, AOM and separation efficiency to guarantee high cell recovery whilst maintaining low separation costs. Although the downstream process requirements remain a key parameter on the decision-making process, when selecting the harvesting solution, a preliminary screening of the technologies based on the biochemical composition of the algae suspension might have the potential to prevent unexpected harvesting failures and high operational costs.

The present work reviews current and innovative harvesting technologies for large scale application and links the optimal working conditions of each system to the main algae characteristics. In the first part, the characteristics of the microalgae suspension affecting cell separation are identified by distinguishing between cell properties and growth media characteristics. After which, the impact of these characteristics on different destabilisation processes and harvesting systems is used to provide a guide on optimal harvesting performances.

Table 2.1 Summary table of algae derivate valuable products and harvested biomass characteristics requirements

Parameters	Low market price products (£ 1-50 per kg)							High market price products (£ 170-37k per kg)													References
	Human nutrition	Animal nutrition	Cosmetics	Bio-diesel	Ethanol	Hydrogen	Methane	β-carotens	Astaxanthin	Phycobiliproteins	Lutein	Hydrocolloids	n-Butanol	Aceton	Propanediol	Linoleic acid	Arachidonic acid (AA)	Eicosapentaenoic acid (EPA)	Docosahexaenoic acid (DHA)	Others	
Algae																					
Aphanizomenon flos - aquae	•									•											Brennan and Owende, 2010; Oilgae[a];
Arthrospira sp.	•	•	•				•	•		•							•				Samson and LeDuy, 1986; Walker et al., 2005; Spolaore et al., 2006; Brennan and Owende, 2010;
Botryococcus braunii				•																	Frigon et al., 2013; Liu et al., 2013;
Chaetoceros gracilis				•														•			Wahlen et al., 2011; Oilgae[a];
Chlamydomonas sp.		•			•	•	•	•													Hirano et al., 1997; Hirayama et al., 1998; Frigon et al., 2013; Melis et al., 2000; Walker et al., 2005; Mussgnug et al., 2010; Oilgae[a];
Chlorella sp.	•	•	•	•	•		•	•		•											Hirano et al., 1997; Ueno et al., 1998; Ip and Chen, 2005; Spolaore et al, 2006; Converti et al., 2009; Frigon et al., 2013; Ometto et al., 2013; Oilgae[a];
Chlorococcum sp.				•	•																Harum et al., 2010; Halim et al., 2011;
Crypthecodinium cohnii	•																		•		Walker et al., 2005; Spolaore et al., 2006; Ganuza et al., 2008; Brennan and Owende, 2010;
Cryptonemia crenulata	•																				Spolaore et al., 2006;
Dunaliella sp.	•		•	•	•		•	•							•	•	•	•			Shirai et al., 1998; Walker et al., 2005; Brennan and Owende, 2010; Mussgnug et al., 2010; Tang et al., 2011; Oilgae[a];
Euglena gracilis							•														Mussgnug et al., 2010;
Haematococcus pluvialis	•			•			•		•												Spolaore et al., 2006; Razon and Tan, 2011; Brennan and Owende, 2010;
Isochrysis sp.; *Ulkenia*																			•		Brennan and Owende, 2010;
Laminaria	•		•																		Spolaore et al., 2006; Oilgae[a];
Microcystis aeruginosa				•																•	Oh et al., 2000;
Nannochloropsis sp.		•	•	•			•											•			Spolaore et al., 2006; Converti et al., 2009; Razon and Tan, 2011; Oilgae[a];
Neochloris oleoabundans				•																	Frigon et al., 2013;
Nitzschia																		•			Spolaore et al., 2006;
Pavlova viridis		•																•			Spolaore et al., 2006; Oilgae[a];
Phaeodactylum tricornutum		•					•											•			Spolaore et al., 2006; Zamalloa et al., 2012; Oilgae[a];
Phormidium																	•				Spolaore et al., 2006;
Porphyridium aeruginosa							•			•											Yang et al., 2011; Frigon et al., 2013; Oilgae[a];
Porphyra; Anabaena flos-aquae	•																				Spolaore et al., 2006;
Scenedesmus sp.				•	•	•	•			•											Yang et al., 2011; Miranda et al., 2012; Mandal and Mallik, 2012; Frigon et al., 2013; Ometto et al., 2013; Oilgae[a];
Schizochytrium																		•	•		Spolaore et al., 2006; Ganuza et al., 2008; Brennan and Owende, 2010; Oilgae[a];
Skeletonema		•																			Spolaore et al., 2006;
Thalassiosira		•																			Spolaore et al, 2006;
Tetraselmis suecica		•		•																	Spolaore et al., 2006; Wahlen et al., 2011;
Harvested biomass characteristics requirements[b]																					
Cells integrity	+++	+++	+++	+				+++				+++									Mohn, 1980; Shelef et al., 1984; Xu et al., 2011;
Final solid concentration	+++	++	+++	++				+++				+++									
Dry mass	++	+	+++	+				+++				+++									

[a]http://www.oilgae.com/ (accessed 17th June 2013); [b]'+++': high importance/absolute requirement; '++': medium importance/most recurrent requirement; '+': low importance/requirement related to the specific product.

2.1.2 Algae suspension characterisation

2.1.2.1 Microalgae cell properties: size, shape, density and charge

Typical dimensions for single cell microalgae such as *Chlorella* sp. *Dunaliella* sp., *Scenedesmus* sp. and *Mycrocystis* sp. at stationary growth phase range between 3 and 20 μm in diameter, while filamentous species, such as *Anabaena* sp., *Arthrospira* sp. and *Melosira* sp., can reach up to 100 μm length (Table 2.2). The shape of the algae depends on the species and varies from simple geometric scapes such as ellipses or spheres, to more complex structures such as a helix or disc (Canter-Lund and Lund, 1995; Tomaselli 2004). It is possible to distinguish between cells with a compact structure characterised by a rigid cell wall able to resist external compression forces, and cells with a more elastic configuration characterised by the presence of air vesicles in the membrane, making them more subjected to cell breakage under high pressure/turbulence conditions (Tomaselli, 2007; Bhave *et al.,* 2012; Purcell *et al.,* 2013).

Open pond cultures operate with biomass concentrations up to 1 kgDM m^{-3} with an average value of between 0.2 and 0.6 kgDM m^{-3}, while PBRs operate up to 2 kgDM m^{-3} (Cromar and Fallowfiels, 1997; Tredici, 2004; Min *et al.,* 2011). When in suspension, the algal cell surface has a negative electrical charge enabling stabilisation within the water body, in some species accentuated by the presence of flagella, causing repulsion forces between cells (Canter-Lund and Lund, 1995; Pietersen and Cloot, 1997). According to Pietersen and Cloot (1997), the average cell density value is equal to 1.02 g cm^{-3}, although it can be slightly lower for algae containing air bubbles within the membrane, which enable them to float.

These characteristics, small dimensions, non-flocculent nature and the low concentration, together with a cell density value similar to the water, are responsible for a slow cell sinking rate of 10^{-6} m s^{-1} which causes the natural sedimentation processes to take longer than a day or a week depending on the algae species (Benemann *et al.,* 1980; Shelef *et al.,* 1984; Granados *et al.,* 2012).

2.1.2.2 Growth media characterization: composition, AOM, pH and zeta potential

The characteristics of algal suspension changes during the growth cycle of the biomass (Pietersen and Cloot, 1997). Fresh water algae cultures are generally found at neutral pH values, while marine species are adapted to a stronger basic

environment (pH 8-9) due to the presence of salts in the water. The media composition is the most direct parameter affecting the overall characteristic of the algae suspension. For instance, low nitrogen concentrations increase the lipid and carbohydrate content of the biomass (cell wall and AOM composition) (Hu, 2007; Cheng *et al.,* 2011a), while the presence of multivalent cations, such as Mg^{2+} and Cl^{2+}, impact on the charge density increasing the negative surface zeta potential (Ozkan and Berberoglu, 2013). In addition, light intensity, temperature and mixing velocity and retention time contribute to enhancing algae growth and nutrient uptake (Hu, 2007). While growing, the algae modifies the composition of the media through the release of intracellular AOM that produces an increase in DOC (Pivokonsky *et al.,* 2006; Henderson *et al.,* 2008a; Li *et al.,* 2011). The AOM changes over time and is species specific. However, as reported by several authors the AOM characterisation (Kam and Gragory, 1999; Babel *et al.,* 2002; Wang *et al.,* 2006; Henderson *et al.,* 2008b; Li *et al.,* 2011; Ozkan and Berberoglu, 2013; Ometto *et al.,* 2014) of different algal species, *C. vulgaris, S. obliquus, A. maxima and M. aeruginosa,* shows some consistent characteristics:

- the AOM is composed primarily of proteins (hydrophobic) and polysaccharides (hydrophilic);
- in the absence of humic/fulvic acids, proteins are responsible for the hydrophobicity of the system;
- proteins and polysaccharides tend to complexify with iron and aluminum compounds limiting their nature of flocculant agents;
- an increase in protein:carbohydrate rate increases the hydrophobicity and reduces the charge density;
- the zeta potential ranges between -15 and -30 mV for fresh water algae at pH values of between 4 and 10;
- charge density values range between 0.002 and 0.6 peq $cell^{-1}$ and is affected by pH and the ionic strength of the medium. An increase in charge density is observed with increasing pH in fresh water media, while the opposite happens in the presence of high salt levels, which causes an increase in charge density with decreasing pH.

Table 2.2 Microalgae characteristics

Algae species	Picture	Classification	Growth condition	Scape	Particle size[a] (µm)	Surface area[a] (µm² cell⁻¹)	Charge density (peq cell⁻¹)	ZP (mV)	DOC (mg l⁻¹)	Worldwide production[b]	References
Chlorella vulgaris			JM	spherical	4.5φ	28	0.13 (pH 7.8)	-30.5 ± 1.2 (pH 7.8)	4.8 ± 0.6	2000 ton DM y⁻¹	*Ometto et al., 2014*
Chlorococcum sp.			BG-11	spherical	*9φ*	*254*	-	-10 (pH 7-8)	-	*na*	*Wu et al., 2012*
Chlorella zofingiensis		Green algae	BG-11	spherical	3.25	*33*	-	-13.2 3	51.7	*na*	*Zhang et al., 2012*
Scenedesmus obliquus			JM	spindle	6φ; 10l	49.5	0.05 (pH 7.5)	-34.6 ± 6.0 (pH 7.5)	3.8 ± 1.8	*na*	*Ometto et al., 2014*
Scenedesmus quadricauda			BB	spindle	*5φ; 10l*	*196*	-	-	11.8	*na*	*Pivokonsky et al., 2006*
Dunaliella salina			SW	spindle	*6φ; 10l*	*245*	-	-30 (pH >1)	18 ± 2.3	1200 ton DM y⁻¹	*Gimmler et al., 1991;*
Crypthecodinium cohnii		Red algae	SW	dinoflagellatta	18φ	1018	-	-	-	240 ton DHA y⁻¹	*Mendes et al., 2009;*
Arthrospira maxima			Zarrouk media	helical	4.5φ; 100l	3720	0.564 (pH 9.4)	-44.2 ± 7.8 (pH 9.4)	100 ± 0.7	3000 ton DM y⁻¹	*Ometto et al., 2014*
Microcystis aeruginosa		Cynobacteria	JM	spherical	5φ	95	0.0019 (pH 7)	-20 (pH 7)	18 ± 2.3	*na*	*Henderson et al., 2008a*
Anabaena flos-aqua			Zarrouk media	filamentous	*5φ*	*63*	-	-	16.8	500 ton DM y⁻¹	*Pivokonsky et al., 2006*
Melosira sp.			Diatom media	filamentous	18φ; 35l	5500	negligeble	-15 ± 7.8 (pH 9.4)	3.6 1	*na*	*Henderson et al., 2008a*
Nitzschia		Diatom	Diatom media	spindle	*5φ; 10l*	196	-	-28	-	*na*	*Konno, 1993*

[a]*where possible cells' dimension reported by the study were used to calculate the surface area, otherwise most likely dimension were estimated from pictures;* [b]*from Brennan and Owende, 2010;* [c]*Photos provided by Culture Collection Algae and Protozoa (CCAP) (Oban, Scotland);* [d]*Photos provided by La Molina (Hayward, CA); DM': Dried Mass; 'na': not available information. JM: Jaworski's Medium; BG-11: Blue-Green Medium; BB: Bold's Basal Medium; SW: Sea water.*

2.1.3 Destabilisation techniques

The efficiency of most microalgae harvesting systems can be improved by preliminary treatments, which aim to destabilise the suspension to enhance floc formation. Chemical flocculation and pH-induced flocculation are the most commonly used preliminary treatments though electric flocculation, bio-flocculation and ultrasonic aggregation are emerging alternatives (Table 2.3).

Table 2.3 Application of destabilisation processes

Destabilisation process	Efficiency		Main costs	Energy demand	Application
	small scale	large scale			
Chemical flocculation	high	high	chemicals	negligible	Sedimentation Flotation Filtration
pH induced flocculation	high	medium	chemicals	negligible	Sedimentation
Electric flocculation	high	low	electrodes	varies largely	Sedimentation Flotation
Bioflocculation	medium	low	*na*	negligible	Sedimentation
Ultrasounds	medium	*na*	*na*	intensive	Sedimentation Filtration

'na': not applicable

2.1.3.1 Chemical flocculation

Chemical flocculation enhances aggregation and improves floc formation by altering the surface properties of the algal cells reducing their inhibitory properties (Shelef *et al.,* 1984). Metal salts, such as aluminium chloride/sulphate and iron chloride/sulphate, or cationic polymers, e.g. Chitosan, Pestan and Zetag, are the most commonly used chemicals to help the formation of algae aggregates (Shelef *et al.,* 1984; Granados *et al.,* 2012). Compared to metal salts, cationic polymers have a significantly higher molecular weight and therefore require lower chemical dosages to produce similar removal yields (Molina Grima *et al.,* 2003). Depending on the separation technology adopted after flocculation, conventional metal salt dosages range between 10 and 200 mg per litre of solution, while cationic polymers are usually effective with less than 20 mg l^{-1} and rarely exceed 100 mg l^{-1} (Tables 2.4 and 2.5). Furthermore, they are less susceptible to protein and carbohydrate complexation than metal salts, suggesting a better performance at a high AOM

concentration (Molina Grima *et al.,* 2003; Pivokonsky *et al.,* 2006; Cheng *et al.,* 2011a). The average market price of aluminium and iron salts is between £0.3 and £2 kg^{-1}, compared to a price range of £1.5 to £90 kg^{-1} for cationic polymers (Cheng *et al.,* 2011a; Granados *et al.,* 2012). For this reason, in the last few years, the research has focused on innovative coagulants that combine high flocculation efficiency with low dosage and low cost.

For instance, Granados *et al.* (2012) reported a high flocculation efficiency (>90%) of *Muriellopsis* sp., using less than 40 mg l^{-1} flocculant, using a number of low cost (£0.8 – 4.5 kg^{-1}) polyelectrolytes, including cationic, non-ionic and ionic polymers, in addition to bentonites and activated carbon. Zheng *et al.* (2012), used poly ϒ-glutamic acid from *Bacillus subtilis* to aggregate *C. vulgaris* and *C. protothecoides* at doses close to 22 and 20 mg l^{-1}, respectively. Similarly, Oh *et al.* (2001) reported efficient bio-flocculant extraction from bacteria *Paenibacillus* sp.

The main disadvantage of chemical flocculation is related to the uncertainty of the optimal coagulant dosage required which varied between algae suspensions. Low cell concentration (< 0.5 mg l^{-1}), large particle sizes, high AOM concentrations, high charge density, as well as salinity concentrations higher than 5 g l^{-1} are often responsible for poor floc formation (Edzwald, 1993; Pieterse and Cloot, 1997; Knuckey *et al.,* 2006; Takaara *et al.,* 2007; Henderson *et al.,* 2010; Zheng *et al.,* 2012). In most cases, this is resolved by additional coagulant doses, although this increases the operational costs and the concentration of coagulant in the harvested biomass. For instance, marine algae tend to require between 5 to 10 times more coagulants than freshwater algae (Sukenik *et al.,* 1988).

Furthermore, overdosing impacts on the floc structure by modifying its morphology and increases the contamination of the separated biomass. For instance, using iron chloride (FeCl$_3$•6H$_2$O) for *Thalassiosira pseudonana* separation, Knuckey *et al.* (2006) reported a 10 fold density reduction of the algae in the floc by doubling the Fe^{3+} concentration from 250 to 500 µM.

Different authors have suggested using the AOM characteristics to estimate the efficiency of the coagulation step. It has been shown that for similar algal species, the protein:carbohydrate ratio is an indication of the coagulant dose required and that a high protein:carbohydrate ratio will generate a reduction in charge density and related chemical savings (Henderson *et al.,* 2012; Zhang *et al.,* 2012; Ometto *et al.,* 2014;). Practically, monitoring zeta potential values has been demonstrated to be an

efficient method to prevent poor chemical separation efficiency as optimal flocculation occurs between -10 mV and +10 mV (Henderson *et al.,* 2010; Ometto *et al.,* 2014).

2.1.3.2 pH induced flocculation

pH induced flocculation involves precipitation of magnesium hydroxide and calcium carbonate induced by a change in pH (Gregory and Duan, 2001). While precipitating, the crystalised salts bring together the algae cells (sweep flocculation) enhancing the sedimentation process. However, recent works suggested the presence of a more complex separation mechanism involving charge neutralization combined with salt precipitation (Yahi *et al.,* 1994; Vandamme *et al.,* 2012;). For instance, precipitating calcium phosphate and brucite have positive charges, which allow them to bind with the negatively charged algae in suspension (Vandamme *et al.,* 2012). As a consequence, the presence of Mg^{2+} and Cl^{2+} in the solution is essential to guarantee an efficient crystallisation and consequent floc formation (Yahi *et al.,* 1994). Accordingly, the advantages of such an approach are maximised in marine environments, as the background water counts sufficient concentration of Mg^{2+} and Cl^{2+} as opposed to a fresh water system where dosing is required (Semerjian and Ayoub, 2003).

Conventionally, the pH is increased by NaOH addition, however innovative applications suggest the potential use of carbon dioxide (CO_2) or aqueous ammonia (Spilling *et al.,* 2011; Chen *et al.,* 2012;). Using NaOH ($\sim$£ 0.08 kg^{-1}) with *C. vulgaris*, *Scenedesmus* sp. and *Chlorococcum* sp., Wu *et al.* (2012) reported an efficient algae floc formation when increasing the pH between 9 and 12 with an optimal magnesium ionic concentration of between 3 and 5 mg l^{-1}.

The adoption of CO_2 is based on the possibility to exploit the microalgae's photosynthetic ability to increase the pH above the threshold of floc formation by CO_2 uptake (Spilling *et al.,* 2011). This mechanism was successfully applied on *Phaeodactylum tricornutum* and *S. obliquus,* although it required more than 3 h to achieve optimal flocculation at pH values equal to 10.5 and 11.5 respectively.

Chen *et al.* (2012) investigated the potential of using ammonia to alter the pH and subsequently, after floc separation, converted the un-ionized ammonia to its ionic form by CO_2 injection to prevent the presence of toxic gaseous ammonia. The authors reported promising results using doses of up to 38 mmol ammonia with

Nannochloropsis oculata, *Dunaliella* HTBS and *C. sorokiniana* demonstrating the feasibility of the process and the ability of direct reuse of the clarified water.

2.1.3.3 Electric flocculation

During electrolysis a constant current applied between electrodes immersed in the algae solution generated positive metal polyvalent ions (Al^{3+}, Fe^{2+} or Fe^{3+}), attracting algae which lose their negative charge and bind together (Poeleman *et al.,* 1997; Duan and Gregory, 2003; Paersall *et al.,* 2010). In this process, the pH is a key parameter as high pH values support sweeping flocculation, while charge neutralisation occurs at low pH levels when ions are in their soluble state (Gao *et al.,* 2010; Vandamme *et al.,* 2012). Polyvalent ions tend to react with water and produce insoluble metal hydroxide responsible for sweep flocculation (Vandamme *et al.,* 2012).

Continuous applications are subjected to significant localised temperature variations near the electrodes which reduce the efficiency of the process (Alfafara *et al.,* 2002). Although the adoption of gentle mixing to guarantee a homogeneous distribution of the temperature during treatment was reported to be an efficient mitigation, it might interfere with the flow of the particles (Alfafara *et al.,* 2002).

The main advantage of this technology is the absence of high chemical concentrations in the separated biomass, however it can be energy intensive and cost demanding due to the need of electrode replacements (Poeleman *et al.,* 1997). Poeleman *et al.* (1997) reported an energy consumption equal to 0.33 kWh m^{-3} operating electrolysis for 75 min to a mixture of natural algae from a reservoir. Similar values were reported by Vandamme *et al.* (2011), who observed higher energy demand when treating the freshwater algae *C. vulgaris* (1 kWh m^{-3}) compared to the marine specie *P. tricornutum* (0.15 kWh m^{-3}) due to the lack of polyvalent ions already available in the solution. In accordance with Gao *et al.* (2010), higher current densities reduce the treatment time to below 30 minutes although higher energy inputs are required (2 kWh m^{-3}). While green algae responded well even at high currents (> 2 A), cyanobacteria are subjected to cell fragmentation causing high release of intracellular AOM (Gao *et al.,* 2010; Paersall *et al.,* 2010). Furthermore, when applied to marine algae cultures, the high salt concentrations subjected to currents higher than 0.6 A are responsible for sodium

hypochlorite formation causing irreversible degradation of the biomass (Gao *et al.,* 2010; Vandamme *et al.,* 2011).

2.1.3.4 Bio-flocculation

Bio-flocculation relies on the ability of specific algae species or bacteria to naturally aggregate under quiescent condition. While aggregating, auto-flocculating microorganisms (e.g. *Ettlia texensis, Ankistrodesmus falcatus*, *S. obliquus*) act as flocculating agents supporting floc formation, mixed with a non-flocculating culture (e.g *C.vulgaris, N. oleoambundas*) they have been observed to generate mixed culture floc causing high floc formation without the need of addition of chemical (Salim *et al.,* 2011; Salim *et al.,* 2012). However, when using bacteria as an auto-flocculating culture, the addition of a temporary source of organic carbon (e.g acetate, glucose or glycerine) in the algae culture is required to support bacteria growth. In addition, under nutrient limitation, the bacterial biomass releases extracellular polymers substances, which help floc formation (Lee *et al.,* 2009). Recently, the work of Zhang and Hu (2012) investigated the possibility of using filamentous fungal strains (*Aspergillus niger*) to generate co-pelletisation with algae, obtaining promising results.

The main advantage of this technology is the low energy input, estimated to be around 0.9 kWh per 10 ton of harvested dry mass, equal to 1 Wh m^{-3} assuming an initial biomass concentration of 1 g l^{-1} (Lee *et al.,* 2010). Depending on the downstream utilisation of the biomass, the process will be beneficial for the maintenance of the cell structure, although the separated biomass will be mixed with other algae, bacteria or fungi. For instance, where downstream cell wall breakage is required (e.g. chemical extraction), the adoption of cellulase-producing fungi will improve the degradation of cellulose enriched algae (Zhang and Hu, 2012).

2.1.3.5 Ultrasound

Ultrasound is a known method for microalgae cell disruption (Purcell *et al.,* 2013), however is has also been identified as an innovative harvesting technique by Bosma *et al.* (2003). Applying ultrasound to an algae suspension generates standing waves producing high pressure nodes and anti-nodes (low pressure) where algae can be concentrated or collected (Laurell *et al.,* 2007).

The use of high frequencies (2.1 MHz) and low power inputs (4 – 8 W) prevents stress of the cells which remained intact. Despite its potential application, the system exerts a very high energy demand of >1.4 kWh l^{-1}, and a need to control residual temperature (Bosma *et al.,* 2003).

However, low energy input ultrasound can be used as a pre-treatment to reduce the amount of flocculants required. For instance, Heng *et al.* (2009) reported a more efficient chemical flocculation (10 mg $FeCl_3$ l^{-1}) using ultrasound as a pre-treatment at lower frequencies (40 kHz) and higher power (40 – 80 W) for 30 seconds applied to a mixture of freshwater algae. Higher exposure times have been reported to result in cell breakage when treating algae containing gas vesicle in the cell wall (mixture of cyanobacteria), while input power of close to 120 W had a detrimental effect on the floc formation causing smaller flocs than at 40, 60 and 80 W (Zhang *et al.,* 2012). To illustrate, Zhang *et al.* (2012) used ultrasound with *Spirulina platensis* to reduce the floating ability of the alga by breaking the intracellular gas vesicles which efficient flocculation achieved at low coagulant doses (0.4 - 0.8 mg l^{-1} of polyaluminium chloride). The corresponding energy input required to treat 200 ml of algae for 5 seconds using a 50 W power was estimated as equal to 0.35 kWh m^{-3}.

2.1.4 Separation technologies

2.1.4.1 Sedimentation

2.1.4.1.1 Gravity sedimentation

Gravity sedimentation is a traditional solid-liquid separation process where algae are left to settle according to Stokes' Law (Shelef *et al.,* 1984). The small primary particle size of most algae results in a very low sedimentation rate, so pre-flocculation is commonly used to ensure settle rates of equal to or higher than 0.6 cm s^{-1} (Collet *et al.,* 2011), equivalent to a minimum hydraulic loading rate (HLR) of 0.14 m^3 m^{-2} h^{-1} (Metcalf and Eddy, 2003a). Conventional sedimentation systems (e.g. clarification tank or lamella type sedimentation tanks) achieve a final slurry concentration between 1 and 3% as total suspended solids (TSS), using less than 0.1 kWh m^{-3} (Yahi *et al.,* 1994; Uduman *et al.,* 2010). When higher solid concentrations are required, sedimentation can be adopted as a pre-concentration step combined with other technologies such as Dissolved Air Flotation (DAF) and centrifugation (Collet *et al.,* 2011). Compared to other harvesting systems, the absence of turbulent flows or high pressures guarantees the integrity of the microalgae structure, both internally (chloroplast) and externally (cell wall) (Chen *et al.,* 2012; Şirin *et al.,* 2012; Zheng *et al.,* 2012) and exerts a low energy demand (Shelef *et al.,* 1984; Schlesinger *et al.,* 2012).

Chemical flocculation is the most applied pre-flocculation technique achieving an efficient cell separation higher than 90% in 10 - 30 minutes settling time, depending on the chemical adopted and the environmental conditions (pH, charge density, cell concentration). Similarly, pH induced flocculation allows separation times of below 30 min, however using CO_2 or ammonia to alter the pH increasesthe settling time to up to 12 hours depending on the microalgae tested (Table 2.3). An even longer settling time is required for efficient separation by the bioflocculation and the autoflocculation system which limit their application at a larger scale (Salim *et al.,* 2012; Zhang and Hu, 2012). According to Lee *et al.* (2010), microbial flocculation/sedimentation applied to harvest 1 km^2 high rate algae pons (HRAP) having 5.8 days of cultivation time to reach the steady growth phase required three clarifiers in the series, having a HLR of 0.88 m^3 m^{-2} h^{-1} each (19 hours settling). Forcontext, such HLR are in the range typically seen in conventional secondary clarification units for wastewater treatment plants (0.66 and 1.16 m^3 m^{-2} h^{-1}), where

maximal sedimentation velocity is assumed as equal to 12 m s^{-1} (Metcalf and Eddy, 2003a).

Table 2.4 Gravity sedimentation performances under different working condition.

Treatment	Algae	Concentration	Efficiency (%)	pH	Settling time	CF	Coagulant agent	Dose	Scale	Reference
Post chemical flocculation	*Phaeodactylum tricornutum*	0.1 g l^{-1}	92 / 83 / 67	9.9 / 5.9 / 7.5	20 min	9.26 / 2.68 / 1.88	Chitosan / Al$_2$(SO$_4$)$_3$ / PAC	20 mg l^{-1} / 28 mg l^{-1} / 28 mg l^{-1}	B	*Sirin et al., 2011*
	Chlorella fusca / *Chlorella vulgaris* / *Scenedesmus* sp. / *Scenedesmus subspicatus*	0.5-2.5 g l^{-1}	>90	-	15min	37 / 36 / 38 / 37	Polyelectrolytes (ACTIPOL)	8 mg l^{-1} / 3 mg l^{-1} / 25 mg l^{-1} / 2 mg l^{-1}	B	*Granados et al., 2012*
	Botryococcus braunii	1 g l^{-1}	>90	11 / 9	10min	-	Al$_2$(SO$_4$)$_3$ / Pestan	200 mg l^{-1} / 100 mg l^{-1}	B	*Lee et al.,1998*
	Chlorella vulgaris / *Chlorella sorokiniana* / *Chlorella variabilis* / *Chlorella minutissima*	0.3 g l^{-1}	>90	8.5	30 min	-	Chitosan	69.6 mg l^{-1}	B	*Cheng et al., 2011b*
	Neocloris oleoabundans	0.5 g l^{-1}	<20 / 95 / <10	7.2	10 min	-	Fe$_2$(SO$_4$)$_3$ / Chitosan / Al$_2$(SO$_4$)$_3$	100 mg l^{-1}	B	*Beach et al., 2012*
	Chlorella protothecoides / *Chlorella vulgaris*	0.6 / 0.57	98 / 91	7.5	2 h / 2 h	29.8 / 20.5	ɣ-PGA	19.82 mg l^{-1} / 22.03 mg l^{-1}	B	*Zheng et al., 2012*
	Thalassiosira pseudonana (M)	3 x10^6 cells ml^{-1}	90	9.5	15 min	200-800	Magnafloc LT-25	0.5 mg l^{-1}	L	*Knuckey et al., 2006*
	Spirulina plantesis	3 x10^8 cells l^{-1}	90	8-9	30 min		PAC	2.4 mg l^{-1}	B	*Zhang et al., 2006*
Post pH induced flocculation	*Chlorella vulgaris* / *Scenedesmus* sp. / *Chlorococcum* sp. / *Phaeodactylum tricornutum* (M) / *Nannochloropsis oculata* (M)	0.68 g l^{-1} / 0.75 g l^{-1} / 0.77 g l^{-1} / 1.8 g l^{-1} / 1.6 g l^{-1}	>99 / >99 / >99 / >90 / >90	12 / 10.5 / 11.5 / 9 / 9.3	10 min	-	NaOH	-	B	*Wu et al., 2012*
	Chlorella vulgaris	0.5 g l^{-1}	>95	10.8 / 9.7	30min	50	NaOH / KOH / Ca(OH)$_2$ / Mg(OH)$_2$	9 mg g^{-1} DM / 12 mg g^{-1} DM / 18 mg g^{-1} DM / 27mg g^{-1} DM	B	*Vandamme et al., 2012*
	Phaeodactylum tricornutum / *Scenedesmus obliquus*	1.1 g l^{-1}	>90 / >70	11 / 11.5	30min	9	NaOH/CO$_2$	-	B	*Spilling et al., 2011*
	Chlorella sorokiniana / *Nannochloropsis oculata* (M) / *Dunaliella HTBS* (M)	- / - / -	50 / 93 / 91	10 / 10.7 / 10.8	12h / 3h / 3h	-	Ammonia	113.3 mmol / 57.31 mmol / 38.37 mmol	B	*Chen et al., 2012*
Bio-flocculation / Auto-flocculation	*Chlorella vulgaris*	0.5 - 0.6 g l^{-1}	30 / >50 / >45 / >60	-	3 h	-	*Scenedesmus obliqqus* / *Tetraselmis suecica* (M) / *Ankistrodesmus falcatus* / *Ettlia texensis*	Algae ratio 0.6-0.9	B	*Salim et al., 2012*
	Pleurochrysis carterae (M)	0.1 g l^{-1} / 0.5 g l^{-1}	>90	-	24 h	226	Microbe culture	-	B	*Lee et al., 2009*
	Chlorella vulgaris	3.3 x10^9 cells ml^{-1} / 2.6 x10^9 cells ml^{-1} / 3.9 x10^9 cells ml^{-1} / 2.8 x10^9 cells ml^{-1}	>80 / >99 / >93 / >99	5	24 h	-	*Aspergillus niger* / *Aspergillus flavus* / *Aerococcus viridans* / *Leucogyrophana arizonica*	-	B	*Zhang and Hu, 2012*
	Chlorella vulgaris	0.062 gl-1	83	11	10 min	-	Bioflocculant from *Paenibacillus* sp.	20 ml l^{-1}	B	*Oh et al., 2001*
	Scenedesmus obliqqus / *Tetraselmis suecica* (M) / *Ankistrodesmus falcatus*	-	>40 / >60 / >20	-	6 h	-	-	-	B	*Salim et al., 2011*
Electroflocculation	*Chlorella vulgaris* / *Phaeodactylum tricornutum* (M)	0.3-0.6 g l^{-1}	>90 / >80	8	30 min	-	Electrodes (Al) / Electrodes (Al)	- / -	B	*Vandamme et al., 2011*
	Chlorella sp.	0.2 g l^{-1} / 0.6 g l^{-1}	>95	-	10 min / 20 min	-	Electrodes (Al) 60V, 2A	-	B	*Pearsall et al., 2011*
	Algae mix	0.03-0.4 g l^{-1}	>99	8-9	10 min	-	Electrodes (Al)	-	B	*Azarian et al., 2007*
Ultrasounds	*Monodu subterraneus*	10^7 cells l^{-1}	85	7.8	3 sec		ultrasounds (4-8W)	-		*Bosma et al., 2003*
	Spirulina plantesis	3 x10^8 cells l^{-1}	>90	-	30 min	-	PAC*	10 mg l^{-1}	B	*Zhang et al., 2006*
	Algae mix	3 x10^7 cells l^{-1}	>80	8-8.1	30 min	-	FeCl$_3$*	0.8 mg l^{-1}	B	*Heng et al., 2009*

*CF: concentration factor; M: marine algae; B: bench scale; L: large scale; * ultrasounds were used as pre-treatment to improve chemical flocculation.*

2.1.4.1.2 Magnetic sedimentation

Magnetic separation is an induced sedimentation process where magnetite nanoparticles, or other magnetic polymers, are added to the algae suspension and mixed to allow flocculation (Liu *et al.,* 2009; Gao *et al.,* 2009; Xu *et al.,* 2011; Cerff *et al.,* 2012; Prochazkova *et al.,* 2013). Appling a magnetic field to the aggregated suspension results in complete clarification within a few minutes, leaving clarified water in one side and algae/magnetic particles in the other (Bitton *et al.,* 1975; Gao *et al.,* 2009). High cell separation has been observed with a number of different algae in both fresh water and marine cultivation environments (Table 2.5). For instance, *Botryococcus braunii* and *C. ellipsoidea* were successfully recovered (> 99%) in less than 3 minutes using a magnetite nanoparticle dose equal to 75 and 300 mg I^{-1} of solution, respectively (Xu *et al.,* 2011). Similarly, high separation efficiencies were observed using hydrophilic silica-coated magnetic particles on *Chlamydomonas reimhardtii, P. tricornutum, N. salina* in less than 5 minutes (Cerff *et al.,* 2012). Only 2 minutes were sufficient to settle 90% of *C. vulgaris* using microwave synthesised iron oxide magnetic micorparticles (IOMMs) (Prochazkova *et al.,* 2013). Using montomorillonite-Cu(II)/Fe(III) oxides particles, Gao *et al.* (2009) observed pH values higher than 6.5, lowering separation efficiency to below 80%, while the presence of Na^+ and Ca^{2+} optimized the performance. Depending on the downstream utilisation of the biomass, after separation, the magnetic material might need to be dissolved in a chemical solution (HCl, n-hexane, H_2SO_4) to allow pure microalgae cell recovery though membrane filtration (Xu *et al.,* 2011; Prochazkova *et al.,* 2013). The magnetic material can then be recycled and reused. Similarly to gravity sedimentation, when the magnetic material is added to the system, the solution required high mixing rates to allow optimal adsorption between particles. In most of the bench studies reviewed, a high shear rate was maintained for between 1 to 5 minutes prior to magnet (~ 0.5 T) activation. Compared to conventional sedimentation, magnetic separation significantly reduces the clarification time maintaining the energy requirements negligible. However, the costs involved with respect to the magnetic material production and post-harvesting separation limit the practical application of this technology (Xu *et al.,* 2011). To the best of our knowledge, no large or pilot scales for microalgae application are available.

Table 2.5 Magnetic sedimentation performances under different working conditions.

Technology	Algae	Concentration (mg l^{-1})	Efficiency (%)	pH	Separation time (min)	Additives	Dose*	Scale	References
Magnetic separation	*Chlorella vulgaris*	13.9	99	4	1-2	IOMM	0.8 g g^{-1}	B	*Prochazkova et al., 2013*
	Chlamydomonas reinhardtii	600-1500		8			0.07-0.8 g g^{-1}		
	Chlorella vulgaris	650		>8		MagSilica Fe$_3$O$_4$	0.03 g g^{-1}		
	Phaeodactylum tricornutum (M)	700-3500	>90	12	5-10		0.01-0.03 g g^{-1}	B	*Cerff et al., 2012*
	Nannochloropsis salina (M)	4200		8			0.03-0.2 g g^{-1}		
	Botryococcus braunii	1800	100	4	2-3	Fe$_3$O$_4$	0.04 g g^{-1}	B	*Xu et al., 2011*
	Chlorella ellipsoidea	800		7	2-4		0.4 g g^{-1}		
	Mycricysits aeruginosa	80	>94	6.8	20	Montmorillonite-Cu(II)/Fe(III) oxides	12.5 g g^{-1}	B	*Gao et al., 2009*
	Mycricysits aeruginosa	10^6 cells ml^{-1}	99	7	-	Chitosan Fe$_3$O$_4$	4 mg l^{-1} 1.6 mg l^{-1}	B	*Liu et al., 2009*
	Mix lake algae culture	10^5 cells ml^{-1}	>90	6.5	-	Fe$_3$O$_4$ Al$_2$(SO$_4$)$_3$	300 ug ml^{-1} 50 ug ml^{-1}	B	*Bitton et al., 1975*

*M: marine algae; B: bench scale; L: large scale; *grams or milligrams of additives per grams or liters of biomass.*

2.1.4.2 Flotation

Flotation is a solid-liquid separation mechanism where micro-bubbles, or low density microspheres, attached to the algal biomass float the algae floc to the surface allowing high cell recovery (Edzwald, 1993; Rawat *et al.,* 2013).

2.1.4.2.1 Dissolved Air Flotation (DAF)

Dissolved Air Flotation (DAF) is the most applied technique for flotation systems. In the DAF process, pressurised air is injected into a pre-flocculated algae solution where micro air bubbles (≤ 500 μm) bind with the algae aggregates reducing the net density and hence floating the aggregate to the surface (Edzwald, 1993). Compared to sedimentation where floc formation enhances sedimentation rate, with DAF optimal coagulant doses will provide floc the ability to resist the shear rate generated during saturated flow injection (Henderson *et al.,* 2010). According to the design parameter reported by Edzwald and Haarhoff (2011), the saturators operate with a recycle ratio of close to 10%, optimal pressure values between 350 and 600 kPa, with a typical value for microalgae harvesting equal to 450 kPa (Table 2.6), and HRT between 5 and 15 m^3 m^{-2} h^{-1}. Reported trials indicate very high separation efficiencies from 90% to 99%, despite the characteristic of the soluble matter which affects the coagulant dose. The only exception was the cyanobacteria *A. maxima,* which in two different cases reported poor separation due to high density floc

formation (Jarvis *et al.,* 2009; Ometto *et al.,* 2014). In terms of final solid concentration, DAF enables between 2 and 5% as TSS to be obtained depending on the desludging frequency (Rawat *et al.,* 2013).

The theoretical energy demand for a saturator is close to 0.02 kWh m^{-3} of treated water depending on operational conditions, however considering loss of energy and the energy required to pump the water into the saturator and allow flocculation, conventional energy usages range between 0.2 and 0.5 kWh m^{-3} of treated water (Molina Grima *et al.,* 2003). A number of recent innovations have reduced the energy demand through the development of a novel air flow device (micro flotation system) modifying a stream of continuous air flow into an oscillatory flow at a specific frequency using the Coanda effect (Hanotu *et al.,* 2012). Compared to DAF, this system enables a very fine bubble formation which optimises the flotation process and offers the potential for several orders of magnitude of reduction in energy demand. Similar to DAF, Dispersed Air Flotation (DiAF) was reported to be effective at harvesting some microalgae, however, due to the difficult control of air bubble size, does not find many applications (Liu *et al.,* 1999; Hanotu *et al.,* 2012).

2.1.4.2.2 Ballasted flotation

A different adaptation of the DAF system involves the replacement of air bubbles with microspheres to drive the flotation process. Similar to a ballasted sedimentation process where sands or micro particles are added to the system to improve sedimentation (Desjardins *et al.,* 2002), low density 40-100 µm glass microspheres (100 kg m^{-3}) are added into the flocculation tank to form stable aggregates with the algae cells and separate them without the need of the saturator (Jarvis *et al.,* 2009; Jarvis *et al.,* 2011). After the float is removed, the beads can be recovered through the use of a hydrocyclone. According to Ometto *et al.* (2014), compared to DAF, the so called Ballasted Dissolved Air Flotation (BDAF) allows high coagulant saving depending on the characteristic of the algae suspension. For instance, to harvest *C. vulgaris*, *A. maxima* (high salinity) and *S. obliquus* at pH 7 using BDAF, the author reported a reduction of aluminum sulphate equal to 45%, 25% and 17%, respectively, than with DAF. In addition to this economic saving, the BDAF required less than 40% of the energy required by DAF and can allow up to 60% carbon emission saving (Jarvis *et al.,* 2009; Ometto *et al.,* 2014). Furthermore, this system

has the potential to obtain an algae slurry with a TSS concentration of close to 10% (Ometto *et al.,* 2014). Nevertheless, the feasibility of the system still needs to be proven at a larger scale.

2.1.4.2.3 Coagulant free flotation

To reduce the use of coagulants in the concentrated biomass, a few studies reported promising results replacing the pre-coagulation system with the addition of polymers or surfactants, such as polyDADMAC or CTAB, into the saturator (Henderson *et al.,* 2009; Willey *et al.,* 2009). This generates positively charged bubbles that naturally bind with the algae cells in suspension enhancing flotation as in posiDAF and Suspended Air Flotation (SAF) systems. Despite the feasibility of the process, from the limited information available, large microalgae (e.g. *M. aeruginosa*) appeared to respond better than small single cells like *Scenedesmus* sp. and *Chlorella* sp., as they showed lower separation efficiencies than DAF (Henderson *et al.,* 2008c). In addition, Willey *et al.* (2009) reported the potential of a lower energy demand due to the higher air:solid (A:S) ratio achieved to obtain the same final solid concentration of 4.5% as TS. A bench scale system with an A:S ratio equal to 120:1 (HLR of 37 m^3 m^{-2} h^{-1} and solids loading rate (SLR) equal to 5.6 kg m^{-2} h^{-1}) required only 3 Wh m^{-3}, more than one order of magnitude less than DAF performed at 2:1 A:S ratio (HLR of 16 m^3 m^{-2} h^{-1} and SLR of 1.7 kg m^{-2} h^{-1}).

Ozone can be used as floating agents instead of air, without the need of a pre-flocculation step (Betzer *et al.,* 1980). Compared to the energy demand required by a saturator, ozone production is a low energy system as only 10 Wh are required to produce 1 g of ozone (Metcalf and Eddy, 2003b).

However, when using ozone, the algae biomass is subjected to high cell wall degradation which causes the release of internal AOM in the media, which can interfere on the turbidity and chlorophyll removal efficiency (Benoufella *et al.,* 1994; Ma *et al.,* 2006). Zhang *et al.* (2012) observed significant particle size reduction when treating a number of diatoms (15 - 20 μm long and 2 - 5 μm wide), including *Fragilaria* sp., *Navicula* sp. and *Diatoma* sp. Increasing the ozone dose from 0.5 to 2 mg l^{-1}, the authors observed an increasing amount of particles below 15 μm. High cell breakage was also detected by Ma *et al.* (2006), dosing 2 mg O_3 l^{-1} on *Oscillatoria amoena*. For these reasons, Cheng *et al.* (2010) suggested this harvesting method as an efficient option to combine harvesting and cell breakage

together. For instance, harvesting *C. vulgaris* with 0.005 - 0.03 mgO_3 $mgBiomass^{-1}$, the authors observed an increment of the C16:0 quantities available in the floated algae from 31% to 55%.

2.1.4.2.4 Electro flotation

Electro flotation uses oxygen and hydrogen bubbles produced at the anodes during electrolysis to drive the algae floc to the surface. Depending on the configuration of the system, the electrodes are placed horizontally at the bottom of the separation tank to ensure efficient distribution of the generated bubbles (Sandbank and Shelef, 1988), or vertically to attract the algae cells and then drive them to the surface along the electrode. The system reported complete separation of *B. braunii* after 30 min and was successfully applied to a large scale pond to separate mixed species of algae (Poelman *et al.,* 1997; Xu *et al.,* 2011). From the data reported by Poelman *et al.* (1997), 95% separation efficiency is achievable in 35 min for an algae flow of 1 m^3 h^{-1} using a 0.5 m^3 tank, which gives a HLR of 1 m^3 m^{-2} h^{-1} assuming a 0.5 m high tank. Compared to sedimentation application where high pH values are adopted, for flotation application, low pH levels are require to limit sweep flocculation mechanisms. Gao *et al.* (2010) reported optimal separation of *M. aeruginosa* adding Cl^- ions which reduce the separation time to below 20 min. However, chlorine generated during the process interacts with the microalgae causing severe cell demand and lysis.

In terms of energy, the demand of the process varied largely from 1.98 kWh m^{-3} to 0.33 kWh m^{-3} depending on the number of cathodes and anodes used, their surface area, the distance, and the specific voltage and current applied (Poelman *et al.,* 1997).

Table 2.6 Flotation separation performances under different working conditions.

Treatment	Algae	Concentration	Efficiency %	pH	Floting time (min)	Pressure	recycle ratio (%)	treatment	coagulant agent	dose	Scale	References
Air Flotation	*Mycricysits aeruginosa*	3×10^9 cell ml^{-1}	>80 (NTU) >73 (NTU)	8.0-8.4	5	-	10	chemical flocculation	PAC $Al_2(SO_4)_3$	37 mg l^{-1} 70 mg l^{-1}	B	*Yuheng et al., 2011*
	Chlorella sp.						10					
	Melosira	0.5-1 $\times 10^6$ cell ml^{-1}	>90	5.5	10		6	chemical flocculation	$Fe_2(SO_4)_3$	12.5 mg l^{-1}	B	*Jarvis et al., 2009*
	Mycricysits aeruginosa						10					
	Chlorella vulgaris	5×10^5 cell ml^{-1}	95							12.6 mg l^{-1}		
	Mycricysits aeruginosa	6×10^5 cell ml^{-1}	97	7	10	450 kPa	12	chemical flocculation	$Al_2(SO_4)_3$	3.78 mg l^{-1}	B	*Henderson et al., 2010*
	Melosira sp.	5×10^4 cell ml^{-1}	99							3.15 mg l^{-1}		
	Asterionella formosa	2×10^3 cell ml^{-1}	99							9.45 mg l^{-1}		
	Chlorella vulgaris	10^5 cell ml^{-1}	>90	6.5-6.7	10	483 kPa	6-12	chemical flocculation	$AlCl_3$	20 mg l^{-1}	L	*Edzwald, 1993*
	Cyclotella	5×10^4 cell ml^{-1}		6.3-6.5					$FeCl_3$	28 mg l^{-1}		
	Chlorella zofingiensis	8×10^7 cell ml^{-1}	>90	6.2	10	550 kPa	20	chemical flocculation	$Al_2(SO_4)_3$	63-633 mg l^{-1}		*Zhang et al., 2012*
	Scenedesmus obliquus	2×10^6 cell ml^{-1}	99							158 mg l^{-1}		
Dissolved Air Flotation (DAF)	*Chlorella vulgaris*	2×10^6 cell ml^{-1}	99	5	10	450 kPa	10	chemical flocculation	$Al_2(SO_4)_3$	13 mg l^{-1}	B	*Ometto et al., 2014*
	Arthrospira maxima	2×10^4 cell ml^{-1}	70-90							443 mg l^{-1}		
	Chlorella sp. / *Scenedesmus* sp.	131 mg l^{-1}	85	-	10	450 kPa	33	chemical flocculation	C-FLOC 60 (cationic polymer)	0.5 ml at 1% (per 100ml)	L	*Willey et al., 2009*
Disperse Air Flotation (DiAF)	*Chlorella* sp.	7×10^5 cell ml^{-1}	88-92	7-8	20	68-206 ml min^{-1}		chemical flocculation	$AlCl_3$ PAC CTAB Chitosan	50 mg l^{-1} 30 mg l^{-1} 40 mg l^{-1} 10 mg l^{-1}		*Liu et al., 1999*
Ballasted Dissolved Air Flotation (BDAF)	*Chlorella* *Melosira* *Mycricysits aeruginosa*	10^5 cell ml^{-1}	90	5.5	10		10	chemical flocculation	$Fe_2(SO_4)_3$ Glass beads (300 mg l^{-1})	12.5 mg l^{-1}	B	*Jarvis et al., 2009*
	Scenedesmus obliquus	2×10^6 cell ml^{-1}								95 mg l^{-1}		
	Chlorella vulgaris	2×10^6 cell ml^{-1}	99	5	10	450 kPa	10	chemical flocculation	$Al_2(SO_4)_3$ Glass beads (300 mg l^{-1})	13 mg l^{-1}	B	*Ometto et al., 2014*
	Arthrospira maxima	2×10^4 cell ml^{-1}								221 mg l^{-1}		

B: bench scale; L: large scale.

Table 2.6 Flotation separation performances under different working conditions (continued).

Treatment		Algae	Concentration	Efficiency %	pH	Floting time (min)	Flow characteristics		Pre-treatments			Scale	References
							Pressure	recycle ratio (%)	treatment	coagulant agent	dose		
Coagulant free flotation	Suspended Air Flotation (SAF)	*Chlorella* sp. / *Scenedesmus* sp.	131 mg l^{-1}	83	-	10	100 kPa	-	surfactant addition into the saturator	MicroFrothTM	2.5 ml l^{-1}	L	*Willey et al., 2009*
	PosiDAF	*Mycricysits aeruginosa*	7.5 x10^5cell ml^{-1}	63 95 >80	7	10	450 kPa	20	surfactant addition into the saturator	CTAB polyDADMAC Agefloc A50	0.0022-0.0040 meq l^{-1} 0.32-0.44 mg l^{-1} -	B	*Henderson et al., 2009*
		Chlorella vulgaris *Asterionella formosa* *Melosira sp*	1.7 x10^4cell ml^{-1} 6 x10^5cell ml^{-1} 1100 cell ml^{-1}	54 89 97	7	10	450 kPa	20	surfactant addition into the saturator	CTAB	0.005 meq l^{-1} 0.0008 meq l^{-1} 0.0005 meq l^{-1}	B	*Henderson et al., 2008c*
	Disperse Ozone Flotation (DOF)	*Scenedesmus obliqqus*	1.6 g l^{-1}	95 (NTU)	-	4	0.52 mg O_3 mg^{-1} algae; 0.65 kg cm^{-2}		-	-	-	B	*Cheng et al., 2011b*
		Chlorella vulgaris	640 (NTU)	98 (NTU)	6		0.24 mg O_3 mg^{-1} algae; 0.65 kg cm^{-3}		-	-	-	B	*Cheng et al., 2010*
		Microcystis aeruginosa	10^9 cell l^{-1}	>90	-	-	1.5-5 IO_3h^{-1}; 0.5 bar		-	-	-	B	*Benoufella et al., 1994*
Electroflotation		*Botryococcus braunii*	1.6 g l^{-1}	90-99	7-12	30	Electrodes (Al) 15V, 60A		-	-	-	B	*Xu et al., 2010*
		Mix algae	2-3.6 mg l^{-1}	99		35	Electrodes (Al) 18-85V, 1-4A		-	-	-	L	*Poelman et al., 1997*
		Microcystit sp.	350mg Ch(a) m^{-3}	99	8-9	10	Electrodes (Al) 60W dm^{-3}		-			B	*Alfafara et al., 2002*
		M. aeruginosa	15 x10^9 cell ml^{-1}	>90	8-9	20	Electrodes (Al) 30V,3 A		-	Cl^{-1}		B	*Gao et al., 2010*

B: bench scale; L: large scale.

2.1.4.3 Filtration

Conventional filtration is adopted to harvest large algae, such as *A. maxima*, *Scenedesmus* sp. and *Coelastrum* sp., using filter presses, diaphragm filters, vacuum bed filters, screen belt, vibrating screen and cylinder sieving machines (Mohn, 1980; Pretorius and Hensman, 1984;). Despite the high separation efficiency of the process, between 70% and 90%, these separation methods report energy demands between 3 and 5 kWh m^{-3} of suspension. Low energy alternatives rely on the efficiency of fine sand/silt bed filters and membrane reactor (MR) systems (Naghavi and Malone, 1986; Bhave *et al.,* 2012;). For highly concentrated algae solutions, sand filtration, largely applied in the drinking water sector, reported severe and rapid clogging which limits its utilisation leaving MR as the only feasible alternative (Naghavi and Malone, 1986; Esen *et al.,* 1991; Zhang *et al.,* 2010).

2.1.4.3.1 Membrane filtration

Modern membrane filtration systems are based on tangential flow filtration (TFF) and submerged filtration (Molina Grima *et al.,* 2003). Within the TFF system, the membrane is subjected to intensive cross flow velocity (CFV) causing biomass compaction on its surface. Submerged filtration instead uses lower pressures reducing the flux rate that can be applied. Despite the configuration adopted, the energy demand ranges between 0.3 and 1 kWh m^{-3} of suspension to obtain concentrated biomass of between 15% and 20% as TSS (Judd, 2006; Zhang *et al.,* 2010; Bhave *et al.,* 2012; Bilad *et al.,* 2012).

Depending on the nominal pore size of the membrane, it is possible to differentiate between microfiltration and ultrafiltration (Table 2.7). The first has an average pore size between of 0.04 and 1.5 µm while the second range between 1 to 100 kDa. Most of the algae has a cell size equal to or higher than 1 µm which suggests 0.5 µm as a conservative pore size diameter, however smaller sizes can retain other organisms such as protozoa or bacteria, and contribute to maintaining a high quality of the clarified liquid (Bhave *et al.,* 2012; Bilad *et al.,* 2012).

Fouling is the limiting factor of these applications and it is strongly affected by the presence of extracellular polymeric substances (EPS) which includes AOM (Babel *et al.,* 2002; Wicaksana *et al.,* 2012; Discart *et al.,* 2013; Discart *et al.,* 2014). Studying *Chlorella* sp., Babel *et al.* (2002) demonstrated the AOM released in solution during

storage of the biomass was the main cause of increasing fouling performance. An incremental increase in the extracellular AOM concentration from 0.01 to 0.03 mg C mg^{-1} cells increases the specific cake resistance by one order of magnitude from below 2 10^{11} m g^{-1} to more than 10^{12} m g^{-1}. After measuring the AOM composition, the authors detected a higher concentration of sugars than proteins, and suggested that the sugar content in the solution can be used as an index to predict cake resistance. Analysing the composition of fouled membranes, other authors verified a high presence of proteins and polysaccharides, confirming the importance of the extracellular AOM on the filtration efficiency (Wicaksana *et al.,* 2012). Furthermore, according to De Baerdemaeker *et al.* (2013), another important parameter affecting operation is the physical structure of the cell wall. In particular, the authors observed that rigid cell walls (e.g. *Chlorella* sp. and *Phaeodactylum* sp.) concentrate on the membrane surface without blocking the water passage (critical flux equal to 50 l m^{-2} h^{-1}) while cells with less mechanical resistance in the wall structure (e.g. *Isochrysis* sp. and *Pavlova lutheri*) can compress, leading to significantly reduced hydraulic passages (critical flux equal to ≤ 20 l m^{-2} h^{-1}) or even cause irreversible blockages. Similarly, Babel and Takizawa (2010) observed an initially low cake layer resistance when using rigid microalgae cells (e.g. 3 10^{12} m^{-1} for a pressure value of below 10 kPa), however as soon as membrane surface cell deposition occurs, the resistance increases exponentially due to the release of intracellular AOM under pressure which rapidly fills the gaps between cells and membranes (e.g. 6 10^{12} m^{-1} at pressure above 60 kPa).

In general, in the presence of high AOM concentration (>0.5 mg DOC l^{-1}), hydrophobic membranes, e.g polyvinylidene difluorure (PVDF) or polytetrafluoroethylene (PTFE) membrane, are more subjected to flux reduction than hydrophilic membranes e.g. polyethersulfone (PES), polyacrylonitrile (PAN) or cellulose ester (CE) membranes, as they tend to foul by adsorption of polysaccharides (Rossignol *et al.,* 1999; Hung and Liu, 2006). Between PVDF hollow fiber membranes and tubular ceramic membranes, the tubular system was reported to be more reliable for microalgae due to the higher resistance to backpulsing pressure (up to 100 bar) required to unblock the fouling membrane (Bhave *et al.,* 2012).

The use of coagulant can prevent fouling by reducing the fine particle fraction blocking the membrane (Danquah *et al.,* 2009; Bhave *et al.,* 2012; Lee *et al.,* 2012).

For instance, the addion of 100 mgChitosan l^{-1} in a suspension of *C. vulgaris* (2 gDM l^{-1}) increased the constant flux of the membrane (PTFE) from 6 l $m^{-2} h^{-1}$ to above 110 l $m^{-2} h^{-1}$ (Lee *et al.,* 2012). A few studies investigated the effects of ultrasounds (Rossignol *et al.,* 1999) and ozonation (Hung and Liu, 2006) pretreatments on the separation efficiency. In both cases, the idea was to reduce the particle size of the algae to limit blockages derived from small size particles. Although both systems reported efficient separation, the higher DOC content post-ultrasound treatment (due to cell breakages) increases the fouling resistance when using hydrophobic membranes.

Efficient alternatives apply a constant bubbling air flow (e.g. 1 l min^{-1}) during the filtration to maintain the membrane surface out of cells deposited (Wicaksana *et al.,* 2012). However, this increases the energy demand of the process by 0.23 kWh m^{-3}, as estimated for a full scale MBR treating municipal wastewater (Biland *et al.,* 2012).

Table 2.7 Membrane filtration performances under different working conditions.

Membrane filtration	Algae	Concentration mg l^{-1}	Efficiency %	pH	Final concentration	membrane		working condition			Pre-treatments			Scale	Reference
						material	pore size μm	TMP kPa	CFV m s^{-1}	constant flux l m^{-2} h^{-1}	treatment	additive	dose		
Microfiltration	*Chlorella sorokiniana*	14-29	-	-	-	PVDF / Anopore	0.17-0.38 / 0.10-0.14	5.5-8.5	0.1-0.24	50-100	-	-	-	B	*Wicaksana et al.,2012*
	Chlorella vulgaris / *Phaeodactylum tricornutum (M)*	410 / 230	>99	8.5	-	PVDF	0.008-0.036	0-35	-	20-40 / 30-50	-	-	-	B	*Bilad et al., 2012*
	Chlorella sp.	240	-	-	-	PVDF	0.45	6.45-86.43	-	-	-	-	-	B	*Babel and Takizawa., 2010*
	Arthrospira plantensis (M)	450	>90	9.5	-	organic membrane	0.1-1.5	1000	1	40-50	-	-	-	B	*Rossi et al., 2004*
	Chlorella sp.	4.8 x10^8 cells ml^{-1}		-		CA	1.2	50-150	0.13-4	100	-	-	-	B	*Ahmad et al., 2012*
	Chlorella vulgaris	140-340	-	-		PC	0.1-0.4	100-1000	-	-	-	-	-	B	*Discart et al., 2013*
	Nannochloropsis oculata (M)	1500-2000	99	7.5-8	>150 g l^{-1}	PVDF	0.1-0.2	60-100	< 1	240 283 458	- chemical flocculation	- FeCl$_3$ PAC	- 20 mgl^{-1} 50 mg l^{-1}	B	*Bhave et al., 2012*
	Chlorella vulgaris	2000	75-80 99	7	-	PFTE	0.91	100	-	6 110	- chemical flocculation	- chitosan	- 200 mg l^{-1}	B	*Lee et al., 2012*
	Tetraselma suecica (M)	420	>90	-	45-90		0.22	30	5 10^{-5}	20	-	-	-	B	*Danquah et al., 2009*
	Haslea ostrearia (M)	40	-	-	-	PVDF	0.1 0.2 0.4 0.8 1.5	50	2.5	140	-	-	-	B	*Rossignol et al., 1999*
	Nannochloropsis oculata	1490	-	-	-	PES-PVP PVC PVDF	0.1 0.4 0.4	-	-	40-50 50 50	-	-	-	B	*Baerdemaeker et al., 2013*
	Chlorella sp.	13.9	-	6	-	PVDF	0.22	60	0.43 0.84	10	ozonation	O$_3$	1mg l-1	B	*Hung and Liu, 2006*
Ultrafiltration	*Arthrospira plantensis (M)*	450	100	9.5	-	organic membrane	3-100kDa	1000	1	30-50	-	-	-	B	*Rossi et al., 2004*
	Chlorella vulgaris	300	-	-	-	PES	5kDa	10000	-	-	-	-	-	B	*Discart et al., 2013*
	Scenedesmus quadricauda	1004	>90	7-8.9	154 g l^{-1}	PVC	50kDa	34.5	0.17	30	-	-	-	B	*Zhang et al., 2010*
	Haslea ostrearia (M)	40	-	-	-	PES PAN PVDF	30kDa 40kDa 30kDa	20-50	2.5	140-150 100 80-100	-	-	-	B	*Rossignol et al., 1999*

M: marine algae; B: bench scale; TMP: transmembrane pressure; CFV: cross flow velocity.

2.1.4.4 Centrifuge

The density difference between algae and water enables the use of enhanced devices such as centrifuges which are common in sludge dewatering and enable high biomass concentrations of more than 20% total solids (TS) to be achieved (Mohn, 1980). Consequently, it is regarded as the most reliable approach and is common amongst the early schemes. Although conventional sedimentary centrifuges might have a discontinuous solid discharged system, they can operate in a continuous system showing a treatment capacity up to 400 l min^{-1} (Kothandaraman and Evans, 1972; Shelef *et al.,* 1984;). The biomass recovery efficiency depends on (1) the biomass settling rate, (2) the biomass residence time and (3) the biomass settling distance. The first parameter is determined according to Stokes' Law, while the residence time and the settling distance depend on the chosen flow rate and the specific centrifuge design, respectively (Molina Grima *et al.,* 2003). For disc-stack centrifuges, the settling distance ranges between 0.5 and 2 mm for centrifugal forces from 4000g to 15000g (Mohn, 1980).

Compared to the other harvesting technologies, it has the highest energy demand, ranging from 1 to 8 kWh m^{-3} of pond water, which limits its use for large scale applications of low cost products (Mohn, 1980; Sim *et al.,* 1988).

However, the recent economic analysis of Dassey and Theegala (2013) reconsidered the use of centrifuge application for algal biodiesel production. The authors observed that, for a 3000g system, increasing the flow rate from 1 l min^{-1} to more than 20 l min^{-1} (lower retention time) decreases the energy consumption from 20 to 0.8 kWh m^{-3}. Although this generates a reduction of the cell recovery efficiency from 94% to below 30%, it appears more economically convenient to process larger volumes at lower energy demands, than smaller volumes at higher energy demands. Similarly, Harun *et al.* (2011) reported the potential of using centrifugation in an integrated algal biogas and biodiesel production system. Against an estimated biogas production of 0.48 m^3 kg^{-1} dried *Tetraselmis suecica*, the energy demand to operate a raceway pond cultivation system with recovery of the biomass using a disk stack self-cleaning centrifuge was around 30% of the annual electricity output.

Although the purity of the biomass is guaranted, the high physical forces involved cause cell damage depending on the rotation speed and the cell's ability to resist compression. For instance, investigating the impact of the centrifuge separation on

the cell structure of different algae species subjected to acceleration factors between 1300g (lab-scale centrifuge) and 13000g (supercentrifuge), Heasman *et al.* (2000) reported apparent low cell damage for a number of green algae, while diatom and haptophyta were more exposed to cell damage. Independently, on the speed adopted, *T. chui*, *N. oculata*, *Rhodomonas salina* and *Pavlova lutheri* showed low cell damage (0 – 3%), while 12% of *Isochrysis* (T-Iso) and *Chaetoceros muelleri* cells were clearly damaged when using the supercentrifuge. As a consequence, centrifuges cause the release of intracellular AOM which could potentially reduce compound availability for downstream processes or compromise the biomass preservation.

2.1.5 Discussion and conclusions

The review of the scientific work on harvesting technologies undertaken in the last few decades has highlighted the continuous efforts to combine low-energy inputs with efficient separation systems. In particular, flotation techniques succeeded in reducing the overall energy requirements of the process maintaining high cell separation efficiencies and high HLR, preferrable for large-scale application (Figure 2.1 A). Most of the innovations in flotation processes are modifications of the conventional DAF system replacing the energy consuming air bubble injection (e.g. BDAF), or preventing or lowering the use of coagulants (e.g. PosiDAF, SAF, DOC). This has allowed the identification of energetically and economically efficient solutions depending on the downstream process requirements (Figure 2.2 B). For instance, compared to DAF, BDAF guarantees cell integrity and reduces chemical biomass contamination. On the contrary, PosiDAF, SAF and DOF produce coagulant-free concentrated biomass, although the impact of the treatments on the integrity of the cell structure will depend on the specific alga species treated.

The identification of new low-cost and equally efficient coagulants, instead, is the driving force for chemical flocculation/sedimentation processes, which remain the lowest energy demanding harvesting systems. The economy of the processes is strongly related to the destabilisation process adopted, making pH-induced sedimentation (when applicable) a cheap and valuable alternative to chemical sedimentation, reducing operational costs and limiting biomass contamination (Figure 2.1 B). However, compared to flotation technologies, sedimentation has a smaller HLR (Figure 2.1 A) and requires longer separation times, with the only exceptions being ultrasounds or electroflocculation (Table 2.8). In addition, the low biomass concentration of 1 - 2% TSS (wt.) in the overflow limits the feasibility of the process for large-scale application, especially when dry algae biomass is required. In this context, sedimentation finds efficient application as pre-concentration units of other more energy intensive systems such as DAF and centrifugation, reducing the overall energy demand of the process (Collet *et al.,* 2011; Zamalloa *et al.,* 2010; Sturm and Lamer, 2011; Jonker and Faaij 2013). For instance, according to the recent techno-economic assessment of Jonker and Faaij (2013), the adoption of a settling step prior to centrifugation allowed a reduction in harvesting costs of up to 80%.

Low biomass contamination and cell breakage can be achieved with optimised membrane filtration (MF), which allows high biomass concentration using medium energy demand (Figure 2.1). However, the small HLR (≤ 0.1) combined with the high dependence of the separation efficiency on the membrane characteristics (pore size and material) and the algae suspension (AOM and cells wall rigidity) limit the use of MF in large-scale systems. Similarly, despite the high separation efficiency, the low AOM dependence and the guaranteed biomass purity, centrifuges remain too energy demanding to meet algae biofuel production requirements (Figure 2.1 A).

A

B
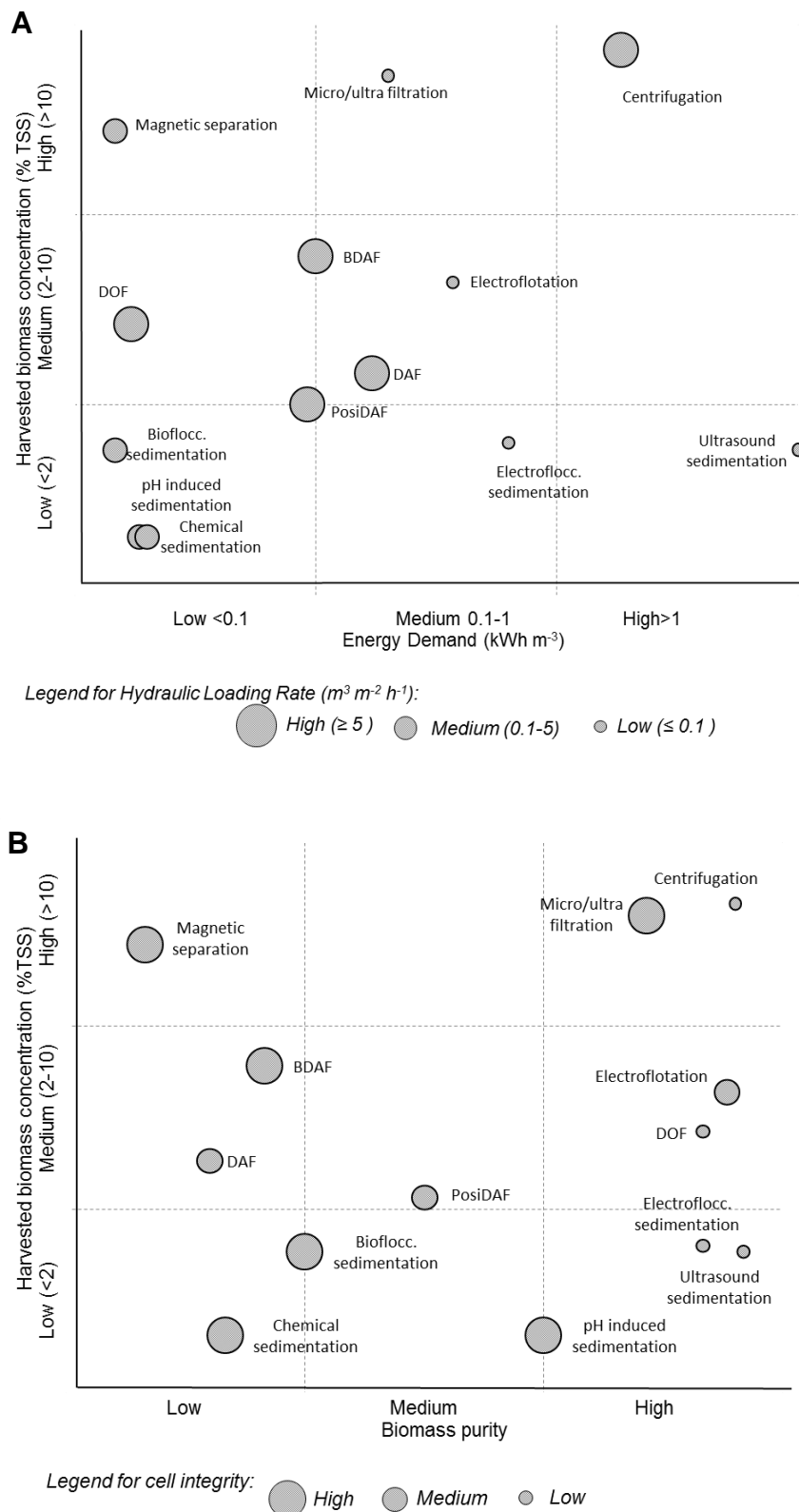
Figure 2.1 Comparison of the harvesting technologies reviewed in this chapter based on (A) operational impact and (B) quality of the separated biomass against final biomass concentration.

Table 2.8 Summary table of the microalgae harvesting technologies reviewed in this paper.

Separation technology	Separation efficienty[a] (%)	Energy demand (kWh m^{-3})	Chemical demand[a] (g m^{-3})	Separation time[a] (min)	Main affecting parameters	Practical limitations
Sedimentation						
chemical flocculation	high (90 - 99)	negligeble/low (0-0.1)[b]	vaires largely (2-200)	vaires largely (10-2h)	high salinity/high AOM/low cells concentration	operational costs are subjected to the price of the cuagulants
pH induced flocculation	medium (80 - 90)	negligeble/low (0-0.1)[b]	limited (4-15)	vaires largely (10-12h)	low salinity/low cells concentration	not efficient with fresh water suspention
electrofloccualtion	medium (80 - 90)	low/medium high (0.15-1)[c]	null	fast/medium fast (10-30)	low salinity/low cells concentration	not efficient with fresh water suspention/ electrodes need periodical replacemnt
bioflocculation	low (70 - 80)	negligeble/low (0-0.1)[d]	null	vaires largely (10-24h)	low cells concentration	the using bacteria cultures required a source of organic carbon
ultrasound	high (90 - 99)	very high[e]	null	medium fast (~ 30)	low cells concentration	energy intensive/cooling system required/pilot demostration required
Magnetic separation	high (90 - 99)	negligebled	limited (0.1-12)	very fast/fast (1-20)	low cells concentration	production and reuse of magnetic material/pilot demostration required
Flotation						
DAF	high (90 - 99)	low/medium high (0.2-0.5)[f]	vaires largely (2-200)	fast (5-20)	high salinity/high AOM/cells size	operational costs are subjected to the price of the cuagulants
BDAF	high (90 - 99)	low (0.03-0.1)[g]	vaires largely (2-100)	fast (~ 10)	high salinity	recover of micrsospheres/ pilot demostration required
PosiDAF/SAF	medium (80 - 90)	negligeble/low (0.003)[h]	very limited (4-15)	very fast (~ 4)	cells size	pilot demostration required
DOF	medium (80 - 90)	neglegible/low (0.02)[i]	null	fast (~ 11)	cells resistance	pilot demostration require
Electroflottation	medium (80 - 90)	low/medium high (0.15-1)[c]	null	fast (7-12)	low salinity	not efficient with fresh water suspention/ electrodes need periodical replacement
Membrane filtration						
microfiltration/ ultrafiltration	high (90 - 99)	medium/ medium high (0.3-0.7)[j]	null/vaires largely (0-100)	fast/very fast	high AOM/filamentous algae/ high biomass concentration	additional costs for reducing fouling, cleaning and replace membranes
Centrifugation	high (90 - 99)	medium high/ high (0.8-8)[kl]	null/limited	very fast	filamentous algae	low efficiency at low energy input/ energy intensive

[a]*according to table provided in this paper;* [b]*Uduman et al., 2010;* [c]*Vandamme et al., 2011;* [d] *Lee et al., 2010;* [e]*Bosma et al., 2003;* [f]*Molina Grima et al., 2003;* [g]*Jarvis et al., 2009;* [h]*Willey et al., 2009;* [i]*Metcalf and Eddy, 2003b;* [j]*Bhave et al., 2012;* [k]*Dassey and Theegala, 2013;* [l]*Mohn, 1980;*

Overall, the present review identified a number of feasible low-energy and low-cost microalgae harvesting solutions which have the potential to significantly contribute to the reduction of the energy and production costs of microalgal biomass, as far as the characteristics of the algae suspension respect the requirements of the process.

Although some of the savings achieved by the novel systems have not yet been demonstrated at full-scale, their current understanding and applications are promising. One of the most significant findings emerging from this work is the importance of the specific characteristics of the algae suspension, identified as the principal factor affecting separation efficiency and operational performances across all technologies. This, together with the downstream process requirements and the physical properties (size, shape, density and charge) of the specific algae, is an important factor in the decision-making process when choosing the most suitable harvesting technology. Poor or superficial understanding of the characteristics of the algae suspension to harvest might result in unexpected failures and low cell recovery, which, in turn, will increase operational costs and energy demand

2.1.6 Acknowledgments

2.1.7 References

Acién, F.G., Fernández, J.M., Magán, J.J., Molina, E., 2012. Production cost of a real microalgae production plant and strategies to reduce it. Biotechnol. Adv. 30, 1344-53.

Ahmad, A.L., Mat Yasin, N.H., Derek, C.J.C., Lim, J.K., 2012. Crossflow microfiltration of microalgae biomass for biofuel production. Desalination. 302, 65-70.

Alfafara, C.G, Nakano, K., Nomura, N., Igarashi, T., Matsumura, M., 2002. Operating and scale-up factors for the electrolytic removal of algae from eutrophied lakewater. J. Chem. Technol. Biotechnol. 77(8), 871-876.

Azarian, G.H., Mesdaghinia, A.R., Vaezi, F., Nabizadeh, R., Nematollahi, D., 2007. Algae removal by electro-coagulation process, application for treatment of the effluent from an industrial wastewater treatment plant. Iranian J. Publ. Health. 36(4), 57-64.

Babel, S., Takizawa, S., 2010. Microfiltration membrane fouling and cake behavior during algal filtration. Desalination. 261, 46-51.

Babel, S., Takizawa, S., Ozaki, H., 2002. Factors affecting seasonal variation of membrane filtration resistance caused by *Chlorella* algae. Water Res. 36, 1193-1202.

Baerdemaeker, T.D., Lemmens, B., Dotremont, C., Fret, J., Roef, L., Goiris, K., Diels, L., 2013. Benchmark study on algae harvesting with backwashable submerged flat panel membranes. Bioresour. Technol. 129, 582-591.

Bahadar, A., Bilal Khan, M., 2013. Progress in energy from microalgae: a review. Renew. Sustain. Energy Rev. 27, 128-148.

Beach, E.S., Eckelman, M.J., Cui, Z., Brentner, L., Zimmerman, J.B., 2012. Preferential technological and life cycle environmental performance of chitosan flocculation for harvesting of the green algae *Neochloris oleoabundans*. Bioresour. Technol. 121, 445-449.

Benemann, J.R., Koopman, B.C. Weissman, J.R., Eisenberg, D.M. Goebel, R.P., 1980. Development of microalgae harvesting and high rate pond technologies in California.

In Shelef, G., Soeder, C.J. (eds): Algae biomass, Elsevier, Amsterdam. 457-528 (1980).

Benoufella, F., Laplanche, A., Boisdon, V., Bourbigot, M.M., 1994. Elimination of *Microcystis* cyanobacteria (blue green algae) by an ozoflotation process: a pilot plant study. Wat. Sci. Tech. 30(8), 245-257.

Betzer, N., Argaman, Y., Kott, Y., 1980. Effluent treatment and algae recovery by ozone-induced flotation. Water Res. 14, 1003-1009.

Bhave, R., Kuritz, T., Powell, L., Adcock, D., 2012. Membrane-based energy efficient dewatering of microalgae in biofuels production and recovery of value added co-products. Environ. Sci. Technol. 46, 5599-606.

Bilad, M.R., Vandamme, D., Foubert, I., Muylaert, K., Vankelecom, I.F.J., 2012. Harvesting microalgal biomass using submerged microfiltration membranes. Bioresour. Technol. 111, 343-352.

Bitton, G., Fox, J.L., Strickland, H.G., 1975. Removal of algae from Florida lakes by magnetic filtration. Appl. Environ. Microbiol. 30(6), 905-908.

Bosma, R., van Spronsen, W.A., Tramper, J., Wijffels, R.H., 2003. Ultrasound, a new separation technique to harvest microalgae. J. Appl. Phycol. 15, 143-154.

Brennan, L., Owende, P., 2010. Biofuels from microalgae: a review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sustain. Energy Rev. 14, 557-577.

Canter-Lund, H., Lund, J.W.G., Freshwater algae: their microscopic world explored. Biopress. Bristol, England(1995).

Cerff, M., Morweiser, M., Dillschneider, R., Michel, A., Menzel, K., Posten, C., 2012. Harvesting fresh water and marine algae by magnetic separation: Screening of separation parameters and high gradient magnetic filtration. Bioresour. Technol. 118, 289-295.

Chen, C.-Y., Yeh, K.-L., Aisyah, R., Lee, D.-J., Chang, J.-S., 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour. Technol. 102, 71-81.

Chen, F., Liu, Z., Li, D., Liu, C., Zheng, P., Chen, S., 2012. Using ammonia for algae harvesting and as nutrient in subsequent cultures. Bioresour. Technol. 121, 298-303.

Cheng, Y., Juang, Y., Liao, G., Ho, S., Yeh, K., Chen, C., Chang, J., Liu, J., Lee, D., 2010. Dispersed ozone flotation of *Chlorella vulgaris*. Bioresour. Technol. 101, 9092-9096.

Cheng, Y., Juang, Y., Liao, G., Tsai, P., Ho, S., Yeh, K., Chen, C., Chang, J., Liu, J.,

Chen, W., Lee, D., 2011b. Harvesting of *Scenedesmus obliquus* FSP-3 using dispersed ozone flotation. Bioresour. Technol. 102, 82-87.

Cheng, Y.-S., Zheng, Y., Labavitch, J.M., VanderGheynst, J.S., 2011a. The impact of cell wall carbohydrate composition on the chitosan flocculation of *Chlorella*. Process Biochem. 46, 1927-1933.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294-306.

Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol. Adv. 29 (6), 686-702.

Collet, P., Hélias, A., Lardon, L., Ras, M., Goy, R.-A., Steyer, J.-P., 2011. Life-cycle assessment of microalgae culture coupled to biogas production. Bioresour. Technol. 102, 207-14.

Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P., Del Borghi, M., 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. Chemical Engineering and Processing: Process Intensification. 48, 1146-1151.

Craggs, R., Sutherland, D., Campbell, H., 2012. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. J. Appl. Phycol. 24, 329-337.

Cromar, N.J., Fallowfield, H.J., 1997. Effect of nutrient loading and retention time on performance of high rate algal ponds. J. Appl. Phycol. 1, 301-309.

Danquah, M.K., Ang, L., Uduman, N., Moheimani, N., Forde, G.M., 2009. Dewatering of microalgal culture for biodiesel production: exploring polymer flocculation and tangential flow filtration. J. Chem. Technol. Biotechnol. 84, 1078-1083.

Dassey, A.J., Theegala, C.S., 2013. Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications. Bioresour. Technol. 128, 241-245.

Desjardins, C., Koudjonou, B., Desjardinis, R., 2002. Laboratory study of ballasted flocculation. Water Res. 36 (3), 744-754.

Discart, V., Bilad, M.R., Marbelia, L., Vankelecom, I.F.J., 2014. Impact of changes in broth composition of *Chlorella vulgaris* cultivation in a membrane photobioreactor (MPBR) with permeate recycle. Bioresour. Technol. 152, 321-328.

Discart, V., Bilad, M.R., Vandamme, D., Foubert, I., Muylaert, K., Vankelecom, I.F.J., 2013. Role of transparent exopolymeric particles in membrane fouling: *Chlorella vulgaris* broth filtration, Bioresour. Technol. 129, 18-25.

Duan, J., Gregory, J., 2003. Coagulation by hydrolysing metal salts. Adv Colloid Interface Sci. 100-102, 475-502.

Edzwald, J.K., 1993. Algae, bubbles, coagulants, and dissolved air flotation. Water Sci. Technol. 27 (10), 67-81.

Edzwald, J.K., Haarhoff, J., 2011. Air saturation. In: Dissolved Air Flotation for water clarification, pp. 3.1-3.36. J. Am. Water Works Assoc. (2011).

Esen, I.I., Puskas,K., Banat, I.M., Al-Daher, R., 1991. Algae removal by sand filtration and reuse of filter material. Waste Management. 11, 59-65.

Frigon, J.-C., Matteau-Lebrun, F., Hamani Abdou, R., McGinn, P.J., O'Leary, S.J.B., Guiot, S.R., 2013. Screening microalgae strains for their productivity in methane following anaerobic digestion. Appl. Energy 108, 100-107.

Gallagher, B.J., 2011. The economics of producing biodiesel from algae. Renewable Energy. 36, 158-162.

Ganuza, E., Benítez-Santana, T., Atalah, E., Vega-Orellana, O., Ganga, R., Izquierdo, M.S., 2008. *Crypthecodinium cohnii* and *Schizochytrium* sp. as potential substitutes to fisheries-derived oils from seabream (*Sparus aurata*) microdiets. Aquaculture. 277 109-116.

Gao, S., Du, M., Tian, J., Yang, Jianyu, Yang, Jixian, Ma, F., Nan, J., 2010. Effects of chloride ions on electro-coagulation-flotation process with aluminum electrodes for algae removal. J. Hazard. Mater. 182, 827-34.

Gao, Z., Peng, X., Zhang, H., Luan, Z., Fan, B., 2009. Montmorillonite–Cu(II)/Fe(III) oxides magnetic material for removal of cyanobacterial *Microcystis aeruginosa* and its regeneration. Desalination. 247, 337-345.

Gimmler, H., Schieder, M., Kowalski, M., Zimmermann, U., Pick, U., 1991. *Dunaliella acidophila*: an algae with a positive zeta potential at its optimal pH for growth. Plant, Cell Environ. 14, 261-269.

Granados, M.R., Acien, F.G., Gomez, C., Fernandez-Sevilla, J.M., Molina Grima, E., 2012. Evaluation of flocculants for the recovery of freshwater microalgae. Bioresourc. Technol. 118, 102-110.

Gregory, J., Duan, J., 2001. Hydrolyzing metal salts as coagulants. Pure Appl. Chem. 73, 2017-2026.

Grobbelaar, 2007. Algal nutrition: In Richmond, A. (ed): Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science. 97-115 (2007).

Gudin, C., Therpenier, C., 1986. Bioconversion of solar energy into organic chemicals by microalgae. Adv Biotechnol Processes. 6, 73-110.

Halim, R., Danquah, M.K., Webley, P.A., 2012. Extraction of oil from microalgae for biodiesel production: a review. Biotechnol. Adv. 30, 709-732.

Hanotu, J., Bandulasena, H.C.H., Zimmerman, W.B., 2012. Microflotation performance for algal separation. Biotechnol. Bioeng. 109, 1663-73.

Harun, R., Davidson, M., Doyle, M., Gopiraj, R., Danquah, M., Forde, G., 2011. Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility. Biomass Bioen. 35, 741-747.

Harun, R., Singh, M., Forde, G.M., Danquah, M.K., 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. Renew. Sustain. Energy Rev. 14, 1037-1047.

Heasman, H., Diemar, J., O'Connor, W., Sushames, T., Foulkes, L., 2000. Development of extended shel-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs, a summary. Aquaculture Research. 31, 637-659.

Henderson, R.K., Baker, A., Parsons, S.A., Jefferson, B., 2008a. Characterization of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. Water Res. 42(13), 3435-3445.

Henderson, R.K., Parsons, S.A., Jefferson, B., 2008b. Successful removal of algae through the control of zeta potential. Separ. Sci. Technol. 43(7), 1653-1666.

Henderson, R.K., Parsons, S. A., Jefferson, B., 2008c. Surfactants as bubble surface modifiers in the flotation of algae: dissolved air flotation that utilizes a chemically modified bubble surface. Environ. Sci. Technol. 42(13), 4883-4888.

Henderson, R.K., Parsons, S.A., Jefferson, B., 2009. The potential for using bubble modification chemicals in dissolved air flotation for algae removal. . Separ. Sci. Technol. 44,1923-1940.

Henderson, R.K., Parsons, S.A., Jefferson, B., 2010. The impact of differing cell and algogenic organic matter (AOM) characteristics on the coagulation and flotation of algae. Water Res. 44(12), 3617-3624.

Heng, L., Jun, N., Wen-jie, H., Guibai, L., 2009. Algae removal by ultrasonic irradiation–coagulation. Desalination. 239, 191-197.

Hirayama, S., Ueda, R., Ogushi, Y., Hirano, A., Samejima, Y., Hon-Nami, K., Kunito, S., 1998. Ethanol production from carbon dioxide by fermentative microalgae. Anonymous Studies in Surface Science and Catalysis. 114, 657-660.

Hirano, A., Ueda, R., Hirayama, S., Ogushi, Y., 1997. CO_2 fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation, Energy. 22, 137-142.

Hu, 2007. Environmental effects on cell composition: In Richmond, A. (ed): Handbook of microalgal culture: biotechnology and applied phycology, Blackwell Science. 83-93 (2007).

Hung, M.T., Liu, J.C., 2006. Microfiltration for separation of green algae from water. Colloids and Surfaces B: Biointerfaces. 51, 157-164.

Ip, P.F., Chen, F., 2005. Production of astaxanthin by the green microalga *Chlorella zofingiensis* in the dark. Process Biochemistry. 40(2), 733-738.

Jarvis, P., Buckingham, P., Holden, B., Jefferson, B., 2009. Low energy ballasted flotation. Water Res. 43(14), 3427-3434.

Jarvis, P., Martin, J., Winspear, T. and Jefferson, B., 2011. Ballasted flotation with glass microspheres for removal of natural organic matter. Separ. Sci. Technol. 46(16), 2489-2495.

Jonker, J.G.G., Faaij, a. P.C., 2013. Techno-economic assessment of micro-algae as feedstock for renewable bio-energy production. Appl. Energy 102, 461-475.

Judd, S., 2006. The MBR book. Elsevier, Oxford (2006).

Kam, S., Gregory, J., 1999. Charge determination of synthetic cationic polyelectrolytes by colloid titration. Colloids Surf. 159(1), 165-179.

Knuckey, R.M., Brown, M.R., Robert, R., Frampton, D.M.F., 2006. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacult. Eng. 35(3), 300-313.

Konno, H., 1993. Settling and coagulation of slender type diatoms. Wat. Sci. Tech. 27(11), 231-240.

Kothandaraman, V., Evans, R., 1972. Removal of algae from waste stabilization pond effluents: a state of the art. Illinois State Water Survey, circular 108. http://www.isws.uiuc.edu/pubdoc/C/ISWSC-108.pdf (accessed 8 June 2012).

Laurell, T., Petersson, F. and Nilsson, A., 2007. Chip integrated strategies for acoustic separation and manipulation of cells and particles. Chem. Soc. Rev. 36(3), 492-506.

Lee, A.K., Lewis, D.M., Ashman, P.J., 2009. Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. J. Appl. Phycol. 21, 559-567.

Lee, A.K., Lewis, D.M., Ashman, P.J., 2010. Energy requirements and economic analysis

of a full-scale microbial flocculation system for microalgal harvesting. Chem. Eng. Res. Design. 88, 988-996.

Lee, D.H., 2011. Algal biodiesel economy and competition among bio-fuels. Bioresour. Technol. 102, 43-49.

Lee, D., Chen, G., Chang, Y., Lee, K., 2012. Harvesting of chitosan coagulated *Chlorella vulgaris* using cyclic membrane filtration-cleaning. Journal of the Taiwan Institute of Chemical Engineers. 43, 948-952.

Lee, S.J., Kim, S., Kim, J., Kwon, G., Yoon, B., Oh, H., 1998. Effects of harvesting method and growth stage on the flocculation of the green alga *Botryococcus braunii*. Lett. Appl. Microbiol. 27, 14-18.

Li, W.W., Sheng, G.-P., Liu, X.-W., Yu, H.-Q., 2011. Recent advances in the separators for microbial fuel cells. Bioresour. Technol. 102, 244-252.

Liu, C., Zheng, S., Xu, L., Wang, F., Guo, C., 2013. Algal oil extraction from wet biomass of *Botryococcus braunii* by 1,2-dimethoxyethane. Appl. Energy. 102, 971-974.

Liu, D., Li, F., Zhang, B., 2009. Removal of algal blooms in freshwater using magnetic polymer. Water Sci. Technol. 59, 1085-91.

Liu, J.C., Chen, Y.M., Ju, Y.H., 1999. Separation of algal cells from water by column flotation. Sep. Sci. Technol. 34, 2259-2272.

Ma, J., Fang, J.Y., Wang, L.N., Guo, J., Chen, Z.L., 2006. Effect of preozonation on characteristics of algae cells and algae-derived organic matter (AOM) with respect to their removal by coagulation. Water Sci. Technol. Water Supply. 6(4), 145-152.

Melis, A., Zhang, L., Forestier, M., Ghirardi, M.L., Seibert M., 2000. Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii.* Plant. Physiology. 122, 127-135.

Mendes, A., Reis, A., Vasconcelos, R., Guerra, P., Lopes da Silva, T., 2009. *Crypthecodinium cohnii* with emphasis on DHA production: a review. J. Appl. Phycol. 21, 199-214.

Metcalf & Eddy, Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003a. Wastewater engineering, treatment and reuse. McGraw Hill Education. 361-389, (2003).

Metcalf & Eddy, Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003b. Wastewater engineering, treatment and reuse. McGraw Hill Education. 1289, (2003).

Min, M., Wang, L., Chen, Y., Li, Y., Chen, P., Ruan, R. 2009. Using wastewater for mass microalgae production as an energy crop. Proceeding of the Annual international

meeting. American Society of Agricultural Engineers, New York, 2009.

Mohn, F.H., 1980. Experiences and strategies in the recovery of biomass from mass cultures of microalgae. In Shelef, G., Soeder, C.J. (eds): Algae biomass, Elsevier, Amsterdam. 547-571 (1980).

Mohn, F.H., 1988. Harvesting of micro-algal biomass. In Borowitzka, M.A., Borowitzka, L.J. (eds): Micro-algal biotechnology. Cambridge University Press, Cambridge. 395-414 (1988).

Molina Grima, E., Belarbi, E.H., Acién Fernández, F.G., Robles Medina, a, Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol. Adv. 20, 491-515.

Mussgnug, J.H., Klassen, V., Schlüter, a, Kruse, O., 2010. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. J. Biotechnol. 150, 51-56.

Naghavi, B., Malone, R.F., 1986. Algae removal by fine sand/silt filtration. Water Res. 20(3), 377-383.

Norsker, N., Barbosa, M.J., Vermuë, M.H., Wijffels, R.H., 2011. Microalgal production: a close look at the economics. Biotechnol. Adv. 29, 24-27.

Oh, H., Lee, S.J., Park, M., Kim, Hee-sik, Kim, Hyoung-chin, Yoon, J., Kwon, G., Yoon, B., 2001. Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus* sp . AM49. Biotechnol. Lett. 23(15), 1229-1234.

Oilgae, http://www.oilgae.com/ (accessed 17 June 2013).

Ometto, F., Pozza, C., Whitton, R., Smith, B., Torres, A.G., Henderson, R.K., Jarvis, P., Jefferson, B., Villa R., 2014. The impact of replacing air bubbles with microspheres for the clarification of algae from low cell-density culture. Water Res. 53, 168-179 (Current Thesis, Paper 2.2.)

Ometto, F., Whitton, R., Coulon, F., Jefferson, B., Villa R., 2013. Improving the energy balance of an integrated microalgal wastewater treatment process, Waste and Biomass Valorization. DOI: 10.1007/s12649-013-9230-2 (Current Thesis, Appendix E).

Ozkan, A., Berberoglu, H., 2013. Physico-chemical surface properties of microalgae. Colloids Surf. B. Biointerfaces 112, 287-93.

Pearsall, R., Connelly, R., Fountain, M., Hearn, C., Werst, M., Hebner, R., Kelley, E., 2011. Electrically dewatering microalgae. IEEE Trans. Dielectr. Electr. Insul. 18, 1578-1583

Pieterse, A.J., Cloot, A., 1997. Algal cells and coagulation, flocculation and sedimentation

processes. Water Sci. and Technol. 36(4), 111-118.

Pivokonsky, M., Kloucek, O., Pivokonska. L., 2006. Evaluation of the production, composition and aluminium and iron complexation of algogenic organic matter. Water Res. 40(2), 3045-3052.

Poelman, E., De Pauw, N., Jeurissen, B., 1997. Potential of electrolytic flocculation for recovery of micro-algae. Resour. Conserv. Recycling. 19, 1-10.

Pretorius, W.A., Hensman, L.C., 1984. The selective cultivation of easy harvestable algae using crossflow microscreening. Water Sci. Technol. 17, 791–802.

Prochazkova, G., Safarik, I., Branyik, T., 2013. Harvesting microalgae with microwave synthesized magnetic microparticles. Bioresour. Technol. 130, 472-477.

Pulz, O., Gross, W., 2004. Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol. 65, 635-648.

Purcell, D., Parsons, S.A., Jefferson, B., 2013. The influence of ultrasounds frequency and power on the algal species *Microcysis aeruginosa*, *Aphanizomenon flos-aquae*, *Scenedesmus subspicatus* and *Melosira* sp.. Environmental Technol. 34(17), 2477-2490.

Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F., 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. Appl. Energy. 103, 444-467.

Razon, L.F., Tan, R.R., 2011. Net energy analysis of the production of biodiesel and biogas from the microalgae: *Haematococcus pluvialis* and *Nannochloropsis*. Appl. Energy 88, 3507-3514.

Rossi, N., Jaouen, P., Legentilhomme, P., Petit, I., 2004. Harvesting of cyanobacterium *Arthrospira platensis* using organic filtration membranes. Food Bioprod. Process. 82, 244-250.

Rossignol, N., Vandanjon, L., Jaouen, P., Quéméneur, F., 1999. Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultrafiltration. Aquacult. Eng. 20, 191-208.

Salim, S., Bosma, R., Vermuë, M.H., Wijffels, R.H., 2011. Harvesting of microalgae by bio-flocculation. J. Appl. Phycol. 23, 849-855.

Salim, S., Vermuë, M.H., Wijffels, R.H., 2012. Ratio between autoflocculating and target microalgae affects the energy-efficient harvesting by bio-flocculation. Bioresour. Technol. 118, 49-55.

Samson, R., LeDuy, A., 1986. Detailed study of anaerobic digestion of *Spirulina maxima*

algal biomass. Biotechnol. Bioeng. 28, 1014-1023.

Sandbank, E., Shelef, G., 1988. Harvesting of algae from high-rate ponds by flocculation-flotation. In Mara, D.D., Marcecos do Monte, M.H. (eds): Waste stabilization ponds. Pergamon Press, Oxford. 257-264 (1988).

Schlesinger, A., Eisenstadt, D., Bar-Gil, A., Carmely, H., Einbinder, S., Gressel, J., 2012. Inexpensive non-toxic flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production. Biotechnol. Adv. 30, 1023-1030.

Semerjian, L., Ayoub, G.M., 2003. High-pH–magnesium coagulation–flocculation in wastewater treatment. Adv. Environ. Res. 7, 389-403.

Shelef, G., Sukenik, A., Green, M., 1984. Microalgae harvesting and processing : a literature review. SERI/STR-231-2396; Technion Research and Development Foundation Ltd.: Haifa, Israel.

Show, K., Lee, D., Chang, J., 2013. Algal biomass dehydration. Bioresour. Technol. 135, 720-729.

Sim, T.S., Goh, A., Becker, E.W., 1988. Comparison of centrifugation, dissolved air flotation and drum filtration techniques for harvesting sewage-grown algae. Biomass. 16, 51-62.

Singh, A., Nigam, P.S., Murphy, J.D., 2011. Renewable fuels from algae: an answer to debatable land based fuels. Bioresour. Technol. 102, 10-6.

Şirin, S., Trobajo, R., Ibanez, C., Salvadó, J., 2012. Harvesting the microalgae *Phaeodactylum tricornutum* with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. J. Appl. Phycol. 24, 1067-1080.

Shirai, F., Kunii, K., Sata, C., Teramoto, Y., Mizuki, E., Murao, S., Nakayama, S., 1998. Cultivation of microalgae in the solution from the desalting process of soy sauce waste treatment and utilization of algal biomass for ethanol fermentation. World J. Microbiol. Biotechnol. 14, 839-843.

Slade, R., Bauen, A., 2013. Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. Biomass Bioen. 53, 29-38.

Spilling, K., Seppälä, J., Tamminen, T., 2011. Inducing autoflocculation in the diatom *Phaeodactylum tricornutum* through CO_2 regulation. J. Appl. Phycol. 23, 959–966.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. J. Biosci. Bioeng. 101, 87–96.

Sturm, B.S.M., Lamer, S.L., 2011. An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. Appl. Energy. 88, 3499-3506.

Sukenik, A., Bilanovic, D., Shelef, G., 1988. Flocculation of microalgae in brackish and sea waters. Biomass 15, 187-199.

Takaara, T., Sano, D., Konno, H., Omura, T., 2007. Cellular proteins of *Microcystis aeruginosa* inhibiting coagulation with polyaluminum chloride. Water Res. 41, 1653-1658.

Tang, H., Abunasser, N., Garcia, M.E.D., Chen, M., Simon Ng, K.Y., Salley, S.O., 2011. Potential of microalgae oil from Dunaliella tertiolecta as a feedstock for biodiesel, Appl. Energy. 88, 3324-3330.

Tomaselli, L., 2007. The microalgal cell. In Richmond, A. (ed): Handbook of microalgal culture: biotechnology and applied phycology, Blackwell Science. 178-214 (2007).

Tredici, 2007. Mass production of microalgae: In Richmond, A. (ed): Handbook of microalgal culture: biotechnology and applied phycology, Blackwell Science. 178-214 (2007).

Uduman, N., Qi, Y., Danquah, M.K., Forde, G.M., Hoadley, A., 2010. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. J. Renew. Sustain. Energy 2, 012701.

Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B., Muylaert, K., 2012. Flocculation of *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical implications. Bioresour. Technol. 105, 114-9.

Vandamme, D., Pontes, S.C.V., Goiris, K., Foubert, I., Pinoy, L.J.J., Muylaert, K., 2011. Evaluation of electro-coagulation-flocculation for harvesting marine and freshwater microalgae. Biotechnol. Bioeng. 108, 2320-2329.

Wang, Y., Hubbe, M.A., Sezaki, T., Wang, X., Rojas, O.J., Argyropoulos, D.S., 2006. The role of polyampholyte charge density on its interactions with cellulose. Nordic Pulp Pap. Res. J., 21(5), 158-165.

Wahlen, B.D., Willis, R.M., Seefeldt, L.C., 2011. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. Bioresour. Technol. 102, 2724-2730.

Walker, T.L., Purton, S., Becker, D.K., Collet, C., 2005. Microalgae as bioreactors. Plant Cell Rep. 24, 629-41.

Wicaksana, F., Fane, A.G., Pongpairoj, P., Field, R., 2012. Microfiltration of algae (*Chlorella sorokiniana*): critical flux, fouling and transmission. J. Membr. Sci. 387-388, 83-92.

Wiley, P.E., Brenneman, K.J., Jacobson, A.E., 2009. Improved algal harvesting using

suspended air flotation. Water Environ. Res. 81, 702-708.

Wu, Z., Zhu, Y., Huang, W., Zhang, C., Li, T., Zhang, Y., Li, A., 2012. Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. Bioresour. Technol. 110, 496-502.

Xin, L., Hong-Ying, H., Jia, Y., 2010. Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, *Scenedesmus* sp. LX1, growing in secondary effluent. N. Biotechnol. 27, 59-63.

Xu, L., Guo, C., Wang, F., Zheng, S., Liu, C., 2011. A simple and rapid harvesting method for microalgae by in situ magnetic separation. Bioresour. Technol. 102, 10047-10051.

Yahi, H., Elmaleh, S., Coma, J., 1994. Algal flocculation-sedimentation by pH increase in a continuous reactor. Wat. Sci. Tech. 30(8), 259-267.

Yang, J., Xu, M., Zhang, X., Hu, Q., Sommerfeld, M. and Chen, Y., 2011. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. Bioresour. Technol. 102, 159-165.

Yuheng, W., Shengguang, Z., Na, L., Yixin, Y., 2011. Influences of various aluminum coagulants on algae foc structure, strength and flotation effect. Procedia Environmental Sciences. 8, 75-80.

Zamalloa, C., Vulsteke, E., Albrecht, J., Verstraete, W., 2011. The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. Bioresour. Technol. 102, 1149-1158.

Zhang, E., Wang, B., Wang, Q., Zhang, S., Zhao B., 2008. Ammonia-nitrogen and orthopshisphate removal by immobilized *Scenedesmus* sp. isolated from municipal wastewater for potential use in tertiary treatment. Bioresour. Technol. 99, 3787-3793.

Zhang, G., Wang, B., Zhang, P., Wang, L., Wang, H., 2006. Removal of algae by sonication-coagulation. J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. 41, 1379-1390.

Zhang, J., Hu, B., 2012. A novel method to harvest microalgae via co-culture of filamentous fungi to form cell pellets. Bioresour. Technol. 114, 529-535.

Zhang, X., Amendola, P., Hewson, J.C., Sommerfeld, M., Hu, Q., 2012. Influence of growth phase on harvesting of *Chlorella zofingiensis* by dissolved air flotation. Bioresour. Technol. 116, 477-484.

Zhang, X., Hu, Q., Sommerfeld, M., Puruhito, E., Chen, Y., 2010. Harvesting algal biomass for biofuels using ultrafiltration membranes. Bioresour. Technol. 101, 5297-5304.

Zheng, H., Gao, Z., Yin, J., Tang, X., Ji, X., Huang, H., 2012. Harvesting of microalgae by

flocculation with poly (γ-glutamic acid). Bioresour. Technol. 112, 212-220.

Zheng, H., Yin, J., Gao, Z., Huang, H., Ji, X., Dou, C., 2011. Disruption of *Chlorella vulgaris* cells for the release of biodiesel-producing lipids: a comparison of grinding, ultrasonication, bead milling, enzymatic lysis, and microwaves. Appl. Biochem. Biotechnol. 164, 1215-1224.

2.2 The impact of replacing air bubbles with microspheres for the clarification of algae from low cell-density culture

Francesco Ometto[i], Carlo Pozza[ii], Rachel Whitton[i], Beatrice Smyth[iii], Andrea Gonzalez Torres[iv], Rita K. Henderson[iv], Peter Jarvis[i], Bruce Jefferson[i] and Raffaella Villa[i].

[i] Cranfield University, Bedfordshire, UK;

[ii] University of Duisburg-Essen, Essen, DE;

[iii] Queen's University, Belfast, UK;

[iv] The University of New South Wales, Sydney, AU;

Abstract

Dissolved Air Flotation (DAF) is a well-known coagulation-flotation system applied at large scale for microalgae harvesting. Compared to conventional harvesting technologies DAF allows high cell recovery at lower energy demand. By replacing microbubbles with microspheres, the innovative Ballasted Dissolved Air Flotation (BDAF) technique has been reported to achieve the same algae cell removal efficiency, while saving up to 80 % of the energy required for the conventional DAF unit. Using three different algae cultures (*Scenedesmus obliquus*, *Chlorella vulgaris* and *Arthrospira maxima*), the present work investigated the practical, economic and environmental advantages of the BDAF system compared to the DAF system. 99% cells separation was achieved with both systems, nevertheless, the BDAF technology allowed up to 95% coagulant reduction depending on the algae species and the pH conditions adopted. In terms of floc structure and strength, the inclusion of microspheres in the algae floc generated a looser aggregate, showing a more compact structure within single cell alga, than large and filamentous cells. Overall, BDAF appeared to be a more reliable and sustainable harvesting system than DAF, and was found to be less algal strain specific and more sustainable as it allowed equal cells recovery reducing energy inputs, coagulant demand and carbon emissions.

Keywords: microalgae harvesting; dissolved air flotation; ballasted flotation; floc structure; carbon footprint;

2.2.1 Introduction

Algae harvesting optimisation is a fundamental need for the feasibility of third generation biofuels (biodiesel, bioethanol, biohydrogen and biogas from microalgae) (Lee, 2011). This is most apparent in the cases where algae are grown in wastewater to provide a dual benefit of nutrient removal and biofuel generation. A reduction of the energy and costs associated with this process has the potential to make algae-biofuels more economically competitive in the market (Molina Grima *et al.,* 2003). Furthermore, as carbon emissions are becoming an important factor in decision making in the water/energy sector (OFWAT, 2010), more sustainable technologies are required to provide environmental benefits often measured in terms of reduced carbon footprint.

Centrifuges, membrane filtration and flocculation-flotation units are the common harvesting systems applied in large scale culture (Christenson and Sims, 2011). While the energy demand for centrifuges or pressure and vacuum filters ranges between 1 and 8 kWh m^{-3} of treated water, flocculation-flotation configurations require lower energy inputs (0.1 and 0.5 kWh m^{-3}) which has seen an increase in research related to flocculation-flotation systems in recent years (Molina Grima *et al.,* 2003; Rawat *et al.,* 2013). In the Dissolved Air Flotation (DAF) system, micro-bubbles attached to the pre-flocculated algal biomass, float the algae floc to the surface allowing high cell recovery (Edzwald, 1993; Rawat *et al.,* 2013) (Figure 2.2 A). The efficiency of this process, as in all flocculation-flotation treatments, relies on floc formation which is affected by particle morphology, suspension characteristics and coagulant properties (Pieterse and Cloot, 1997). In particular, the extracellular algogenic organic matter (AOM) of the suspension plays a key role in coagulant demand and floc structure and strength (Henderson *et al.,* 2010; Li *et al.,* 2011). AOM is composed predominantly of carbohydrates (hydrophilic) and proteins (hydrophobic) and has a negative charge (≤ -15 mV) depending on the algal strain and its growth phase (Henderson *et al.,* 2008a). Optimal coagulant doses allow floc formation to be able to resist the shear rate generated during saturated flow injection (450-600 kPa) and have been observed to occur at zeta potential values close to +/- 0 mV where the coagulant is responsible for particle charge neutralisation (Henderson *et al.,* 2008a).

A modified DAF system, Ballasted Dissolved Air Flotation (BDAF), has been reported to achieve the same removal efficiency while saving from 60% to 80% of the energy demand, and related CO_2 emissions, compared to conventional flotation units (Jarvis *et al.,* 2009). Unlike traditional ballasting techniques where high density granular additives (e.g.

microsand) are used to improve sedimentation efficiency (Desjardins *et al.,* 2002), BDAF uses low-density microspheres to support flotation (Figure 2.2 B). Microspheres are added into the system during the rapid mix stage in the same way as conventional ballasting agents, and then incorporated into the floc matrix to drive the flotation process, replacing the use of microbubbles (WO/2006/008474 and US Patent 6890431). Once the algae-bead floc has been harvested, the microspheres can be separated from the algal biomass and recycled into the system. The effect of low density glass microsphere addition on the pre-flocculation process was first investigated by Jarvis *et al.* (2009), who identified an optimal glass beads concentration close to 300 mg l^{-1} for harvesting an algae cells suspension of 10^6 cells ml^{-1}. Although the author reported a floc size reduction due to the beads addition, the effect of the physical (cells size and shape) and chemical (soluble content) algae characteristics on the strength and structure of the ballasted algae floc was not investigated. In addition, as the beads' presence allowed less turbulent flotation mechanisms (no saturated flow injection) compared to DAF, there is the potential to identify different optimal coagulation conditions that might generate additional advantages on top of the energy saving.

The present work investigates the performance of the BDAF technology applied to three different algae (*S. obliquus*, *C. vulgaris* and *A. maxima*) compared to the conventional DAF. Optimal flocculation-flotation conditions were identified in terms of pH and coagulant dose depending on the specific cell morphology and AOM composition of each alga biomass. The impacts of microspheres inclusion into the algal biomass were assessed to compare floc characteristic between the two technologies. In addition, life cycle analysis (LCA) was applied to both DAF and BDAF harvesting option to investigate practical, economic and environmental benefits while moving from one system to the other.
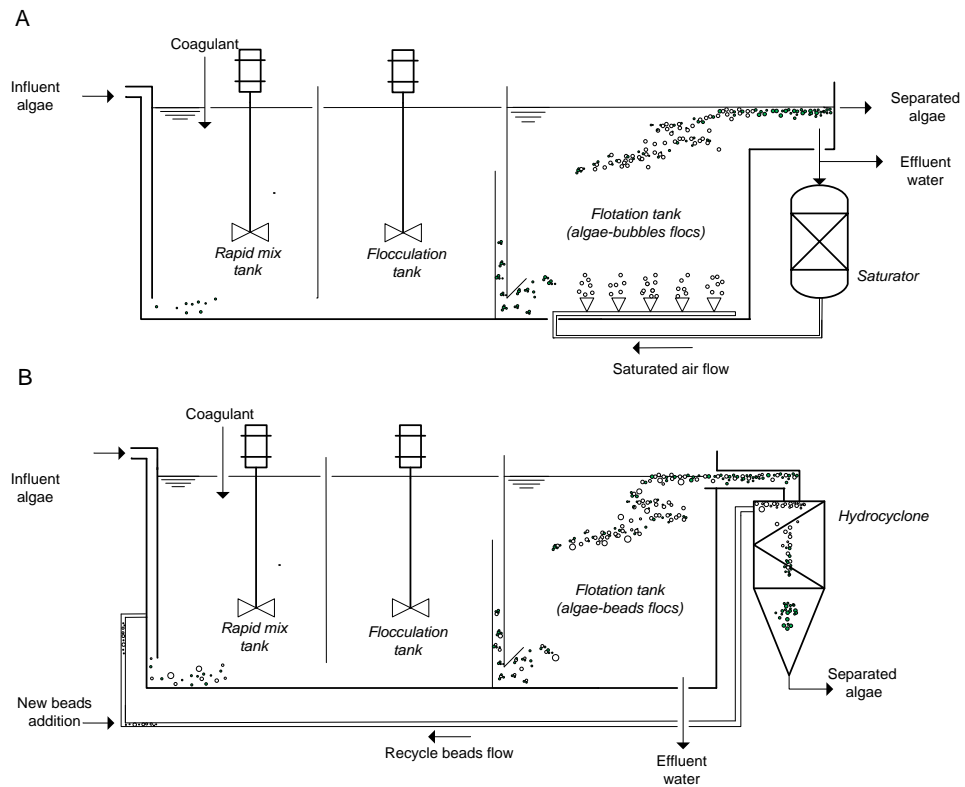
Figure 2.2 Schematic representation of Dissolved Air Flotation (A) and Ballasted Dissolved Air Flotation (B) systems

2.2.2 Materials and Methods

Algal harvesting batch tests were performed at the stationary growth phase (maximum yield) where the cell morphology is homogeneous and the AOM has the greatest effect on the coagulation (Appendix A). First, the algae cultures were characterised for cell morphology and the AOM composition. Secondly, optimal coagulation conditions were identified in terms of pH, coagulant dose, residual cells, turbidity and zeta potential. Subsequently, the characteristics of the optimal algae floc were investigated for size, strength and fractal dimension.

2.2.2.1 Algal culture

The two green algae, *S. obliquus* (276/42) and *C. vulgaris* (211/BK), and the blue-green alga *A. maxima* (1475/9) commonly known as *Spirulina*, were obtained from the Culture Collection for Algae and Protozoa (CCAP) (Oban, UK). All algae were cultivated in glass tanks illuminated with two fluorescent light tubes, Sun-glo 20 W and Arcadia 18 W. *S. obliquus* and *C. vulgaris* were grown at 18ºC in Jaworski media (50 litres) under constant illumination and mixed using an aquarium pump. *A.maxima* was grown in Zarrouk media (25 litres) at 28 ºC and 16/8 hours light/dark cycle, with daily mixing by hand.

2.2.2.2 Algae suspension and AOM characterisation

Cell counting was performed manually using a light microscope with a haemocytometer or a Sedge-wick Rafter as appropriate. Algogenic organic matter (AOM) was characterised and extracted at the stationary growth phase after centrifugation and filtration (1µm) according to the methods described by Henderson *et al.* (2008a). Samples were characterised for protein content, carbohydrate content and dissolved organic carbon (DOC). Bovine serum albumin (BSA) and glucose were used for calibration of protein and carbohydrate content respectively and read at 750 nm (BSA) and 480 nm (glucose) absorbance using a Jenway 6505 UV/Vis spectrophotometer. A Shimadzu TOC-5000A (Malvern, UK) was used for DOC analysis. Charge density of the algal suspension was measured through zeta potential analysis (Malvern Zetasizer 2000HAS, Malvern, UK) by the addition of an increasing dose of PolyDADMAC (Sigma Chemicals, UK) with a defined charge density value equal to 6.2 meq g^{-1} (Sharp *et al.,* 2006). Total suspended solids (TSS) were measured according to standard methods (APHA).

2.2.2.3 Harvesting performance

Jar test experiments were carried out using an EC Engineering DBT6 DAF jar tester (Alberta, CND). Separate experiments were carried out in duplicate at pH 5, 7 and 9, using aluminium sulphate ($Al_2(SO_4)_3$) as the coagulant. The DAF and BDAF tests were performed according to Henderson *et al.* (2008b) and Jarvis *et al.* (2009), respectively. Briefly, 1 litre of algal suspension was rapidly mixed for 2 to 3 minutes at 200 rpm, while varying coagulant doses were added and the pH was adjusted using a 0.1 M HCl (5 M in the case of *A. maxima*) and 0.1 M NaOH solution. Slow mixing (30 rpm) was then maintained for 15 minutes (flocculation period). Within the DAF system, air saturated deionised water buffered with 0.5 mM $NaHCO_3$ and 1.8 mM NaCl was supplied at 450 kPa and an equivalent recycle ratio of 10%. In the BDAF system, 300 mg l^{-1} of low-density glass beads (100 kg m^{-3}) obtained from Trelleborg Emerson and Cuming Inc. (Mansfield, USA) were added to the system prior to coagulant addition. Algae flocs were then allowed to float for 10 minutes. Clarified samples were taken from the vessel base and characterised for residual cells and zeta potential as previously described. Turbidity was measured using a HACH 2100N Turbidimeter (Düsseldorf, DE). Residual aluminium concentration was measured using a Perkin Elmer AAnalyst800 atomic absorption spectrometer (Waltham, USA).

2.2.2.4 Floc size and breakage

Jar testing of both systems was completed under verified optimum conditions of pH and coagulant dose. To create a growth and breakage floc profile, the algal particle size distribution was measured every minute using a Malvern Mastersizer2000 (Malvern, UK). A peristaltic pump was used to maintain a constant flow of 1.5 l h^{-1} from the jar, through the laser diffraction unit and back into the jar. The suspension was rapidly mixed (200 rpm) for 2 minutes while the coagulant dose and pH were adjusted. Flocculation conditions were then maintained at 30 rpm for 15 minutes. Subsequently, the mixing speed was adjusted to 30 rpm, 50 rpm, 75 rpm, 100 rpm, 150 rpm and 200 rpm, equivalent to mean velocity gradient (G) values of 7.4 s^{-1}, 15.9 s^{-1}, 29.3 s^{-1}, 45.2 s^{-1}, 82.9 s^{-1}, 128 s^{-1}, as determined using a conversion equation provided by the Mastersizer supplier, for an additional 15 minutes. Fractal dimension values (D_f) were obtained from a log - log plot of scattering intensity versus wave number considering the gradient of the straight line. Light energy intensity values were converted to raw scattering intensity using the software provided by the Mastersizer supplier.

2.2.2.5 Life cycle assessment

Life cycle assessment (LCA) was carried out for hypothetical full scale DAF and BDAF systems having a treatment capacity (Q) of 20,000 $m^3 d^{-1}$, to compare the energy demand, carbon footprint and costs of the two systems. As the focus of this study is the comparison of the two harvesting options (DAF and BDAF), the up- and downstream processes are excluded from the analysis as they are assumed to be the same in both cases and therefore have no effect on a comparative analysis. Calculations were performed for (1) operational carbon emissions, based on the operational inputs (electricity, coagulant and glass beads) required to harvest the algae biomasses, and (2) embodied carbon emissions, based on the two major construction differences between the two systems: the saturator for DAF, and the hydrocyclone for BDAF. Carbon emissions are calculated in terms of carbon dioxide equivalent (CO_2e), a measure of the total global warming potential of greenhouse gases, using standard carbon coefficient conversion factors (Table 2.9) and $kgCO_2e\ d^{-1}$ as the functional unit is $kgCO_2e\ d^{-1}$. The cost assessment was completed using available data on average market prices and information from personal communication with different suppliers.

The saturator design was assessed according to the guidelines and parameters reported by Edzwald and Haarhoff (2012) for a volumetric air requirement equal to 7 ml l^{-1}: packed saturator operating at 500 kPa (saturation gauge pressure), 55 l $m^{-2}\ s^{-1}$ (mass hydraulic loading), 9.95% recycle ratio (Q_r/Q), packing depth of 1400 mm and associated energy consumption, out of any secondary losses, equal to 0.013 kWh m^{-3} of raw water flow (fresh water). Equation 2.1 was used for the determination of the mass (M) of a cylindrical vessel with hemispherical ends made of stainless steel A36

$$M = 2\pi r^2 (r + w) P \frac{\rho}{\delta} \qquad (2.1)$$

where *r* is the saturator radius, *w* the packing depth, *P* the gauge pressure, ρ the steel density (7800 kg m^{-3}) and δ the steel ultimate tensile strength (400Mpa). Christy®Pak polypropylene pall rings (38 mm, 51 kg m^{-3}, 140 $m^2\ m^{-3}$) were considered as packing material.

Conventional hydrocyclones operate at a pressure value between 50 and 300 kPa (Jun *et al.,* 2009) and have a total mass of stainless steel or cast iron, according to available commercial information, between 8 and 45 kg for a design capacity between 10 and 25 m^3

h^{-1}. For the purpose of the LCA, a 150 kPa pressure drop (high efficient sand separator), 18 kg mass (stainless steel) and a treatment capacity equal to 0.02 Q, were assumed. The energy demand for pumping water into both saturator and hydrocyclone units were estimated in terms of water-energy according to Equation 2.2

$$Pw = \frac{Q\,\rho\,g\,h}{\eta\,3.60\,10^6} \qquad (2.2)$$

where Pw is the required daily power (kW), Q the water flow rate (m^3 d^{-1}), ρ the fluid density (1000 kg m^{-3}), g gravity (9.81 m s^{-2}), h the pressure drop (m head) and η the pump efficiency (70%).

The coagulant (aluminium sulphate, $Al_2(SO_4)_3$) dosage was calculated from the optimal doses determined in the present work. For the glass bead demand, assuming a treatment capacity of 850 m^3 hr^{-1} and optimal bead concentration of 300 mg l^{-1}, 255 kg hr^{-1} of beads were required. However, when the system is in steady state 242 kg hr^{-1} of beads were recycled inside the system (95% bead recycling efficiency), with 13 kg hr^{-1} of new beads needed as 13 kg hr^{-1} (1.5 g m^{-3}) of beads remain in the harvested biomass.

Table 2.9 Carbon factors

Carbon factors	Units	Value	References
Stainless steel	kgCO$_2$e kg^{-1}	3.23	
Cast iron[a]	kgCO$_2$e kg^{-1}	1.520[a]	
Polypropylene[b]	kgCO$_2$e kg^{-1}	2.334[b]	UM, 2011
Aluminium sulphate[c]	kgCO$_2$e kg^{-1}	0.493[c]	
Glass beads[d]	kgCO$_2$e kg^{-1}	0.900[d]	Hammond and Jones, 2011
Electricity[e]	kgCO$_2$e kWh^{-1}	0.484[e]	DEFRA/DECC, 2013

[a]*The emission factor for cast iron is for the product at the factory gate;* [b]*The emission factor is for polypropylene fibres;* [c]*The emission factor for aluminium sulphate is for the product in powder form at the factory gate;* [d]*The emissions factor is for UK primary glass (cradle-to-gate). Emissions from beads loss were excluded from the analysis as they had no significant impact on the overall balance;* [e]*The emissions factor is for UK electricity and accounts for emissions from generation as well as for losses in transmission and distribution.*

2.2.3 Results and Discussion

2.2.3.1 Algal suspension characteristic

Key differences between the three algae were observed in terms of their surface area, AOM concentration and composition as well as charge density (Table 2.10). To illustrate, the surface area of *S. obliquus* was approximately double that of *C. vulgaris* at 49.5 and 28.3 μm^2 cell^{-1}, respectively. Compared to these two single cells algae, the filamentous *A. maxima* presented a significantly higher surface area with an average value close to 3720 μm^2 cell^{-1}. In terms of AOM, *S. obliquus* and *C. vulgaris* showed comparable DOC concentrations, while *A. maxima* reported higher values. For instance, the carbohydrate content of the two green algae ranged between 4 and 5 mg l^{-1}, as glucose, while it was seven to ten times higher in *A. maxima* (38.18 ± 2.62 mg l^{-1} as glucose). In contrast, the protein content was more consistent across the three algae, with *S. obliquus* at 6.31 ± 1.40 mg l^{-1}, *C. vulgaris* at 1.80 ± 0.09 mg l^{-1} and *A. maxima* at 5.24 ± 0.1 mg l^{-1} as BSA. At stationary growth phase the pH value of the algae suspensions was close to 7.5, 7.8 and 9.7 for *S. obliquus*, *C. vulgaris* and *A. maxima*, respectively. The related charge density measurement was equal to 1 ± 0.06, 4.6 ± 0.22 and 0.15 ± 0.02 peq μm^{-2} for the same algae with zeta potential values equal to or more negative than -30 mV. As the pH value was adjusted to the desired condition, the zeta potential, as well as the AOM concentration, reported little adjustments while clear pH-dependence was observed with charge density measurements. Growing in salt-free media, the charge density of green algae increased with the pH (Wang *et al.,* 2006), from 0.85 to 1.31 peq μm^{-2}, and from 2.76 to 5.52 peq μm^{-2}, at pH 5 and pH 9, for *S. obliquus* and *C. vulgaris,* respectively. In contrast, *A. maxima,* cultivated in a strong base solution, showed higher charge density values at lower pH (0.28 ± 0.02 peq μm^{-2} at pH and 70.31 ± 0.01 peq μm^{-2} at pH 5), congruent with polymeric hydrolysis reducing the charge of the functional groups (Kam and Gragory, 1999).

Overall, the three algae showed clear physical and chemical differences, which, according to previous investigations have the potential to impact on the specific harvesting performance (Zhang *et al.* 2012; Henderson *et al.,* 2010). To compare, in terms of proteins:carbohydrates ratio, our results (Table 2.10) are in the same range as those reported by Henderson *et al.,* (2010) for equal or similar algae, like *C. vulgaris* (0.4), *Microsystis aeruginosa* (0.6), *Asterionella formosa* (0.2) and *Melosira* sp. (0.2). As reported by the authors, between close algae strains, a higher proteins:carbohydrates ratio

suggests a lower coagulant demand, having higher hydrophobicity and consequent lower charge density. Similarly, larger cells required higher coagulant doses as well as concentrated suspensions and growth media with a salinity concentration higher than 5 g l^{-1} (Pieterse and Cloot, 1997; Knuckey *et al.,* 2006). Hence, from our analysis (Table 2.10), *A. maxima* is expected to require the highest coagulant dose compared to *S. obliquus* and *C. vulgaris,* having the largest surface area, high charge density and high salinity growth media. Between the two green algae, the higher proteins:carbohydrates ratio and the lower charge density of *S. obliquus*, suggest this alga will require less coagulant than *C. vulgaris*. However, the larger surface area and the higher proteins content might have a detrimental effect on the flocculation process (Henderson *et al.,* 2010) leaving *C. vulgaris* with the lowest coagulant demand.

Table 2.10 Characterisation of microlgal suspention used in jar tests. Algae samples were taken at the stationary growth phase and diluted with deionised water, buffered with 0.5 mM NaHCO$_3$ and 1.8 mM NaCl, to reach the reported concentration.

	Parameter	*S. obliquus*	*C. vulgaris*	*A. maxima*
Algae suspension	Concentration (cells ml^{-1})	$2 \times 10^6 \pm 1 \times 10^5$	$2 \times 10^6 \pm 1 \times 10^5$	$2 \times 10^4 \pm 1 \times 10^3$
	Particle shape	spindle	spherical	filament
	Particle size[a] (µm)	6 w; 10.5 l	4.5 Ø	4.5 Ø; 300 l
	Surface area[a] (µm^2 cell^{-1})	49.5	28.3	3719.9
	Solids (mg TSS l^{-1})	174 ± 14	112 ± 14	117 ± 19
	Turbidity (NTU[b])	124 ± 5	40 ± 8	105 ± 10
	pH	7.5 ± 0.2	7.8 ± 0.1	9.7 ± 0.2
	Zeta Potential (mV)	-34.6 ± 6.0	-30.5 ± 1.2	-44.2 ± 7.8
	Charge density (peq cell^{-1})	0.050 ± 0.003	0.130 ± 0.006	0.564 ± 0.061
AOM	DOC (mg l^{-1})	3.81 ± 1.81	4.77 ± 0.59	100.5 ± 0.70
	Proteins:DOC ratio	1.91 ± 0.76	0.40 ± 0.33	0.39 ± 0.04
	Carbohydrates:DOC ratio	1.69 ± 0.77	0.92 ± 0.25	0.05 ± 0.01
	Poteins:Carbohydrates ratio	1.24 ± 0.06	0.43 ± 0.16	0.13 ± 0.01

[a]*average value;* [b]*Nephelometric Turbidity Unit.*

2.2.3.2 Algal removal and coagulant demand

High algal cell recovery (≥ 99%) was achieved using both harvesting systems. However, significant differences for optimal coagulant dose and zeta potential were observed in relation to the algal strain and the pH value used (Figure 2.3). To illustrate, within the DAF system (Figure 2.3 – left column), *S. obliquus* showed complete removal at increasing coagulant demand for pH 5, pH 7 and pH 9 at zeta potential values lower than 15mV, 8mV and -10mV, respectively. Similarly, *C. vulgaris* coagulant demand increased with the pH, but in all experiments optimal removal occurred at neutral or negative zeta potential values. Despite the same range of zeta potential observed with the green algae, *A. maxima* showed different behaviour. In DAF conditions, algae separation predominately accrued by sedimentation instead of a flotation mechanism evidenced through visual observation of the flocs being too heavy to be lifted by the air bubbles injected at the tests air to solids ratio used. Hence, after an initial flotation they started to settle, suggesting DAF to be inappropriate for high density flocs (Jarvis *et al.,* 2009). At the highest pH condition, the zeta potential value slightly changed with increasing coagulant doses, remaining close to the initial value of -38 mV which is congruent with dissociation models for coagulants demonstrating that aluminium is in its precipitated hydroxide form at pH 9. At pH 7, complete clarification was obtained at -15 mV, while at pH 5 the optimal zeta potential value was close to 0 mV.

Separation trials performed under a BDAF set up achieved the same cell recovery observed within the DAF experiment but at lower coagulant doses (Figure 2.3 – right column). For instance, coagulant reductions of 40%, 14% and 22% were observed in the case of *S. obliquus* when operated at pH 5, 7 and 9 respectively. In contrast, no significant saving was observed with *C. vulgaris* at pH 5, while 45% and 75% reduction was obtained at pH 7 and pH 9, respectively. *A. maxima* showed removal efficiency below 80% only at pH 9. At pH 7 and pH 5, complete removal was obtained at zeta potential values close to -32 mV and -26 mV, respectively. Coagulant savings reached 95% at pH 5, while remaining between 25% and 30% for the other two conditions.

Overall, DAF was effective only on *S. obliquus* and *C. vulgaris* separation, while BDAF enables an efficient flotation for both green algae and the filamentous cyanobacteria. Both systems reported poor separation (< 80%) at low coagulant doses (< optimal dose), however, with ballasting agents the low efficiency was more due to uncompleted algae-beads floc formation rather than floc breakage as observed during DAF separation consistent with the lower levels of energy dissipation encountered in BDAF (Jarvis *et al.,*

2011). In all experimental conditions, optimum cell recovery occurred at pH 5 and the coagulant demand increased with the pH in all algae suspensions. As organic particles, the optimal coagulation condition of microalgae requires a low pH level (~pH 5-6) where charge neutralisation mechanisms are dominant (Stumm and Morgan, 1962). Similar behaviour was reported in the work of Henderson *et al.* (2010) where harvesting *C. vulgaris* at pH 7 required four times more coagulant than at pH 5. In agreement with previous work, conventional flotation achieved complete algal separation as the zeta potential approached neutral or positive values (Henderson *et al.* 2008b); however, in the ballasted flotation experiments, the same removal occurred at more negative zeta potential values as a consequence of the reduced coagulant dosage.

Aluminium sulphate was an effective coagulant and residual aluminium concentration in all clarified samples was always equal to or less than 1 mg l^{-1}. In terms of optimal coagulant demand, the experimental results confirmed initial considerations (section 2.2.3.1) based on cell characteristics and AOM composition and showing values within the range of similar freshwater microalgae (Henderson *et al.,* 2010; Molina Grima *et al.,* 2003; Edzwald, 1993). *A. maxima* required the highest amount of coagulant ($\geq$ 60 mg Al l^{-1}, at pH 5, DAF), followed by *S. obliquus* (25 mg Al l^{-1}, at pH 5, DAF) and *C. vulgaris* (2 mg Al l^{-1}, at pH 5, DAF). Between the two green algae, the larger surface area and the higher proteins contents responsible for protein complexation with aluminium, justify the higher coagulant dosage observed in *S. obliquus* compared to *C. vulgaris* despite the lower charge density (Pivokonsky *et al.,* 2006; Henderson *et al.,* 2010).

According to our observations, the BDAF has the potential to generate a more concentrated final algae paste than DAF. After conventional flotation of *S. obliquus* the solid content of the concentrated biomass was between 1% and 2% in term of TSS (biomass concentrated in the top 10 ml of the 1l jar) which is within expected values (Rawat *et al.,* 2013). For the same final volume, the TSS percentage obtained using BDAF was close to 5% due to the presence of the glass beads. Assuming an efficient post flotation beads separation by using a hydrocyclone with 20% (vol.) underflow (Jianghua *et al.,* 2009), the estimated final TSS content would range from 5% to 10%.
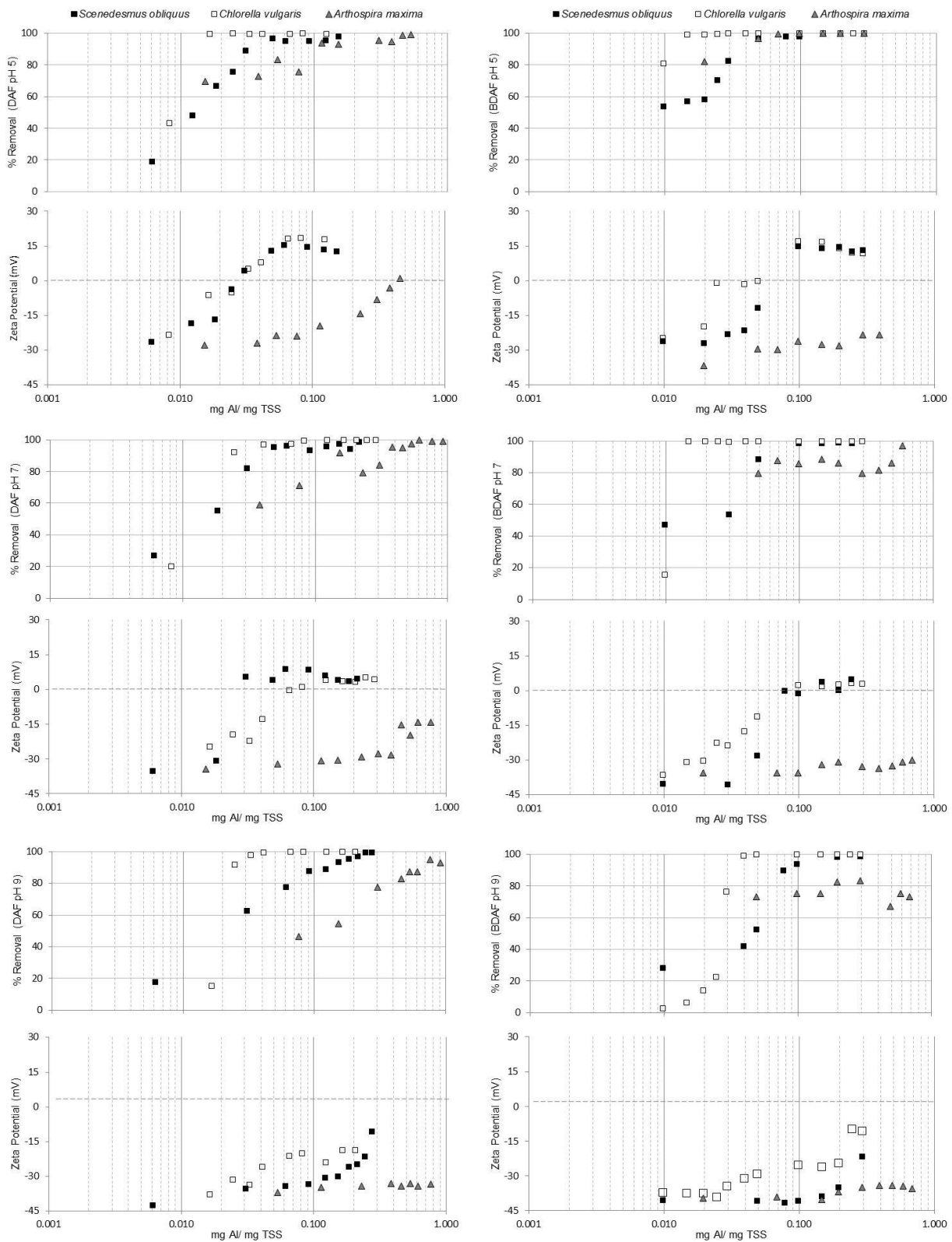
Figure 2.3 Dose response curve and corresponding zeta potential values for *S. obliquus*, *C. vulgaris* and *A. maxima*, for DAF (right column) and BDAF (left column) system at pH 5 (A), pH 7 (B) and pH 9 (C).

2.2.3.3 Floc growth and strength profiles

Floc comparison was made based on equivalent media volumetric diameter (d_{50}). Comparing the steady state floc size achieved during chemical (pre DAF) and ballasted flocculation, *S. obliquus* did not show significant differences as the d_{50} remained close to 130.3 ± 11.2 µm. In contrast, *C. vulgaris* reported floc size reduction, from 223.6 ± 14.5 µm to 113.8 ± 13.2 µm, while *A. maxima* floc size grew from 103.3 ± 16.5 µm to 146.3 ± 14.6 µm. Floc size reduction due to the addition of microspheres was first reported by Jarvis *et al.* (2009), who linked this to a consequent reduction of the floc strength compared to conventional aggregates. However, the peculiarity of *A. maxima* (filamentous algae) suggests that the physical property of the cell has a key role in the algae-bead floc formation/structure, as observed in conventional algal aggregation (Pieterse and Cloot, 1997). Despite the flocculation condition investigated, *S. obliquus* and *C. vulgaris* achieved steady state floc size after 9 to 12 minutes, while *A. maxima* required less than 4 minutes (Appendix C). Accordingly, the *C. vulgaris* floc growth rate ranged between 10 µm min^{-1} and 60 µm min^{-1} during the first 5 minutes and between 1 µm min^{-1} and 10 µm min^{-1} afterwards until steady state was reached. No flocculation time-lag was observed in any of the experiments, suggesting that an optimal coagulant dose was provided (Clasen *et al.,* 2000). During the first 2 minutes at a high shear rate (while coagulant was added and the pH level adjusted), an instant floc formation was observed for *S. obliquus* and *A. maxima*. Subsequently, the d_{50} value gradually decreased to a steady state floc size.

Using the steady state floc size as an indication of the floc strength (Jarvis *et al.,* 2005), *C. vulgaris* formed the strongest floc, followed by *S. obliquus* and *A. maxima* in the DAF system. Conversely, in the ballasted system, the sequence was reversed with the strongest floc associated to *A. maxima,* followed by *S. obliquus* and *C. vulgaris.* Extending the analysis to the flocs behaviours when exposed to an increasing shear rate (Figure 2.4), the chemically induced floc of the two green algae showed a clear resistance of up to 16 G (50 rpm). As the shear rate increased, the degradation rates increased. The same correlation was observed using microspheres, but no floc breakage resistance was detected at low shear rates confirming a more fragile floc.
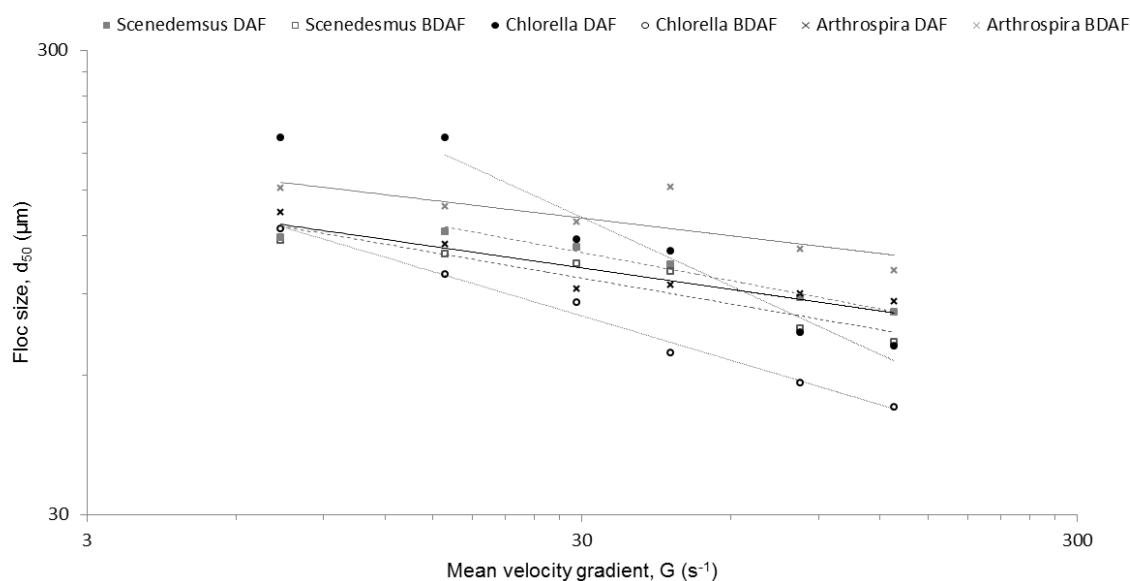
Figure 2.4 Log log plot of steady state floc size vs increasing shear rate plotted as G, with and without microspheres addition

A more detailed analysis of the floc strength between the two systems was possible comparing the floc strength coefficient (log C) and the floc strength constant (γ), as well as the floc response to increased shear rate exposure. The floc strength coefficient and constant were extrapolated from Figure 2.4 as described by Jarvis *et al.* (2005). The first, represented by the y-axis intercept, gives an indication of the floc strength. The second, the gradient of the slope, reveals information on floc breakage mechanisms: floc fragmentation for γ values close to 0.5 and floc erosion for values between 1 and 2 (Li *et al.,* 2006). Compared to the conventional flotation system, the addition of glass beads did not change the strength coefficient of *S. obliquus* (Table 2.11). However, it generated an 18% reduction with *C. vulgaris* and 3% improvement with *A. maxima.* This confirmed previous observations based on steady state floc size, as the beads addition affects the most *C. vulgaris*' floc strength and reinforced *A. maxima.* The calculated floc strength's constant values indicated fragmentation as the main floc breakage mechanism in both systems, which is consistent with other observations on similar freshwater algae (Table 2.11). Furthermore, exposed to an increase shear rate, the d_{50} showed clear reduction from values higher than 100 µm to lower sizes as a result of floc fragmentation (Figure 2.5). However, as observed with similar algae (Henderson *et al.,* 2006), the two green algae showed some evidence of an erosion mechanism after 15 minutes of exposure to an increased shear rate, with a small increment in the volume of particle sizes between 4 and

111

6 µm (Figure 2.5 A and C). Furthermore, at ballasted conditions, *C. vulgaris* reported a small increment of large particle sizes (450-650 µm) suggesting floc re-structuring during the breakage (Figure 2.5 D). This reinforced the possibility that high post-separation beads recovery can be achieved as the algae-beads floc break, enabling beads recycling and algae concentrations at the same time. Efficient bead separation was observed at bench scale at a high shear rate (200 rpm) for all three algae tested. However, the ratio between clearly separated and algae-linked beads was not determined.

Table 2.11 Comparison between floc strength constants, floc strength coefficients and floc size reduction of different microalgae under different flocculation conditions.

Particles	Experiment condition			Floc strength			References
	Coagulant	Dose (mg l^{-1})	pH	log G	ɣ	%[a]	
Unballasted							
Scenedesmus obliquus	Al	40	5	2.34	0.20	38	
Chlorella vulgaris	Al	10	5	2.84	0.49	72	*Current work*[b]
Arthrospira maxima	Al	134	5	2.23	0.15	8	
Chlorella vulgaris	Al	-	5	3.82	0.89	89	*Henderson et al., 2006*
Microcystis aeruginosa	Al	0.7	6	2.95	0.26	70	*Gonzalez Torres and Henderson, 2013*[c]
	Al	1	7	3.09	0.39	78	
	Fe	3	6	3.39	0.39	73	
	Fe	3	7	3.42	0.39	63	
	Al	5	6	3.12	0.33	55	
	Al	4	7	2.95	0.21	52	
	Fe	20	6	2.98	0.02	9	
	Fe	50	7	3.10	0.13	32	
NOM	Al	-	5.5	2.81	0.21	50	*Henderson et al., 2006*
NOM	Fe	8	4.5	-	-	66	*Jarvis et al., 2009*
Ballasted							
Scenedesmus obliquus	Al	30	5	2.34	0.23	44	
Chlorella vulgaris	Al	6	5	2.37	0.33	51	*Current work*[b]
Arthrospira maxima	Al	77	5	2.30	0.13	22	
NOM	Fe	8	4.5	-	-	75	*Jarvis et al., 2009*

[a]*Floc size reduction compared to original size (steady state) after 15 minutes at shear rate of 200 rpm;* [b]*Differences with previously reported optimal coagulant doses are related to differences in the AOM composition of batches of algae used in the two experiments at different times;* [c]*Gonzalez Torres, A., Henderson, R.K., (2013). Personal communication, University of New South Wales, Australia*
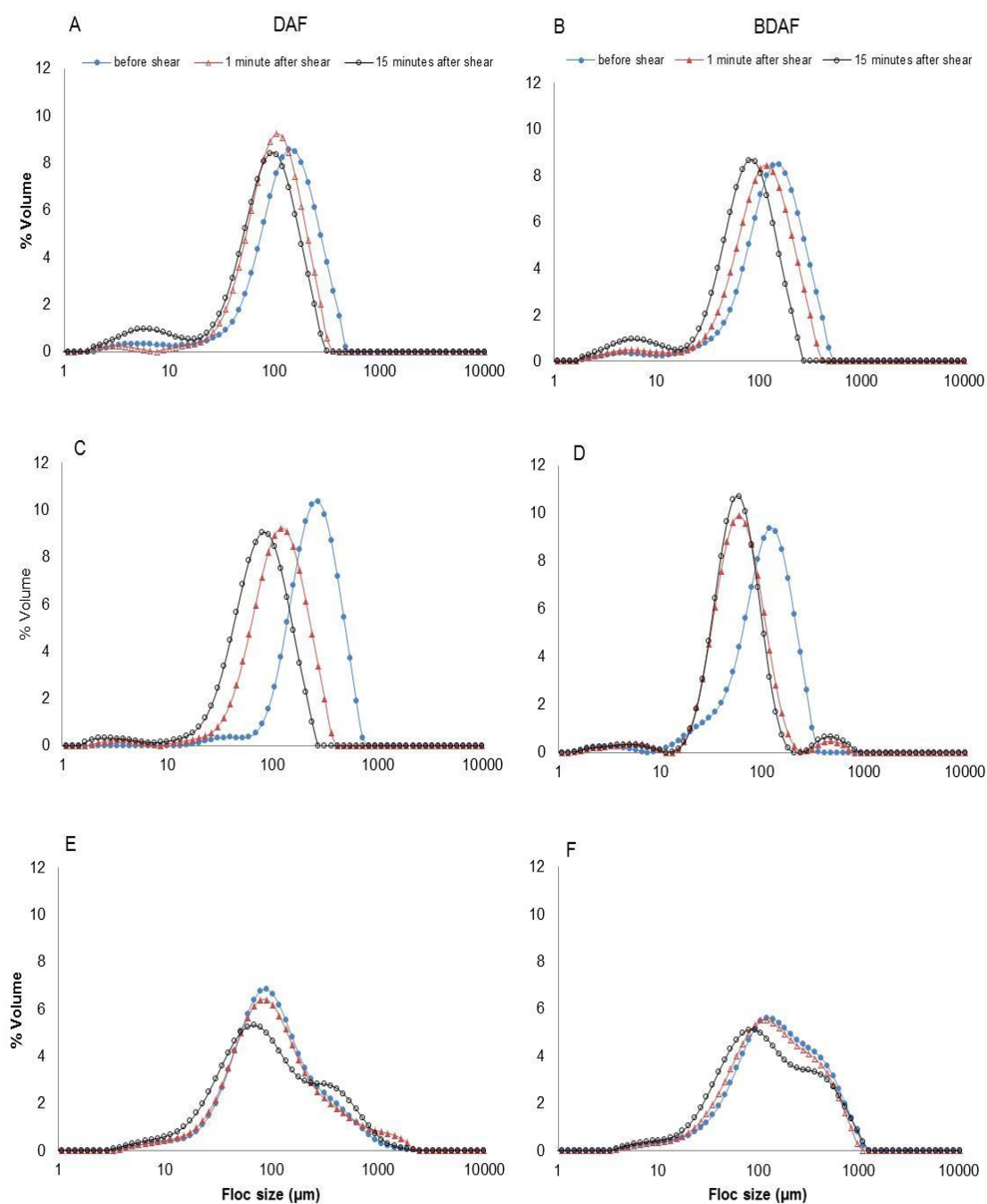
Figure 2.5 Floc breakage profile for DAF (left column) and BDAF (right column) system, of *S. obliquus* (A and B), *C. vulgaris* (C and D) and *A. maxima* (E and F), before and after exposure to a shear rate of 200 rpm.

A microscopic analysis of the algae flocs confirmed that beads interact differently with unicellular and filamentous algae (Figure 2.6). In the first case, the algae cells were distributed around the bead's surface (Figures 2.6, A2 and B2) creating a compact structure (Jarvis *et al.,* 2009). In contrast, filamentous algae tend to create a more structured agglomeration where microspheres are in a pivoted position (Figure 2.6, C2). These observations are confirmed by fractal dimension (D_f) analysis (Figure 2.8). When moving from the DAF to BDAF systems, the D_f increases from 2.18 ± 0.05 to 2.25 ± 0.03 and from 2.43 ± 0.02 to 2.55 ± 0.04 for *S. obliquus* and *C. vulgaris*, respectively, suggesting a similar compact structure. In contrast D_f decreased from 2.45 ± 0.08 to 2.25 ± 0.04 in the case of *A. maxima* endorsing the change in structure (from a compact aggregation to a more open one). Exposed to increasing shear rate, *S. obliquus* showed D_f reduction to 2 ± 0.01 and 2.04 ± 0.04 with and without beads, respectively, from 100 rpm and afterwards (Figure 2.7). Similarly, the fractal dimension of *A. maxima* decreased with the shear rate. However, resistance to shear rate of or less than 100 rpm was observed only without the presence of beads. In contrast, despite the flocculation condition provided, *C. vulgaris* D_f increased with the shear rate. Visual observations of samples exposed to a high shear rate (200 rpm for 15 minutes) showed an algal re-suspension associated with the presence of a clear glass bead layer on the surface. This supports the algae-bead floc breakage and suggests that the re-structuring mechanism observed within *C. vulgaris* might affect only the algae cells as microspheres float on the surface allowing successful beads recovery.
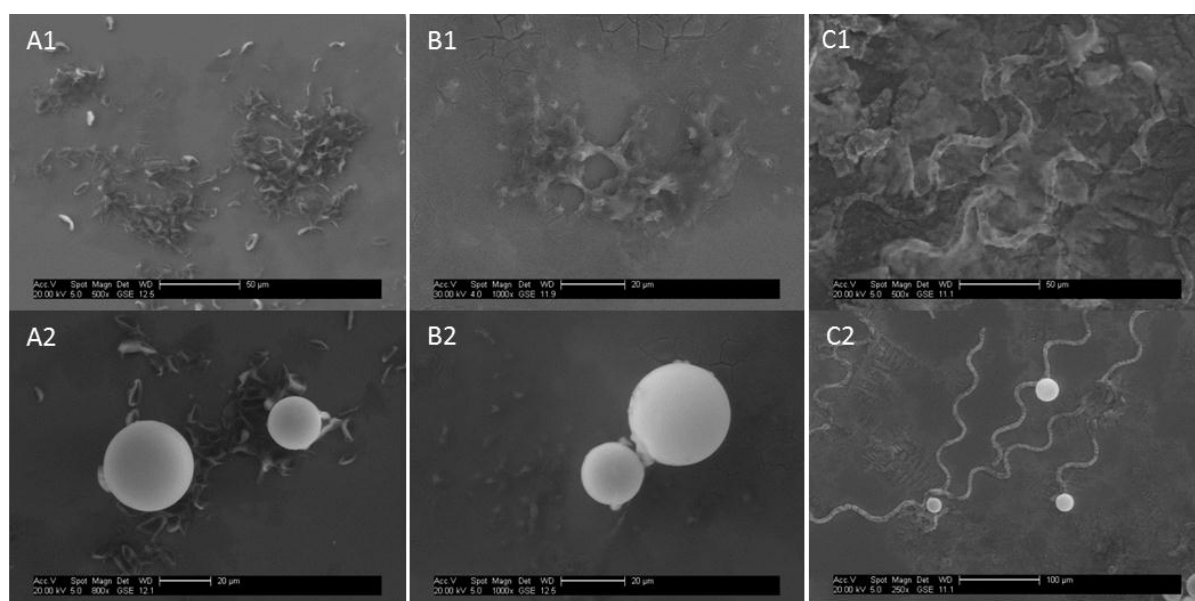
Figure 2.6 Algae floc images ESEM Fei XL30 for *S. obliquus* (A), *C. vulgaris* (B) and *A. maxima* (C) in conventional flocculation condition (1) and in ballasted condition (2).
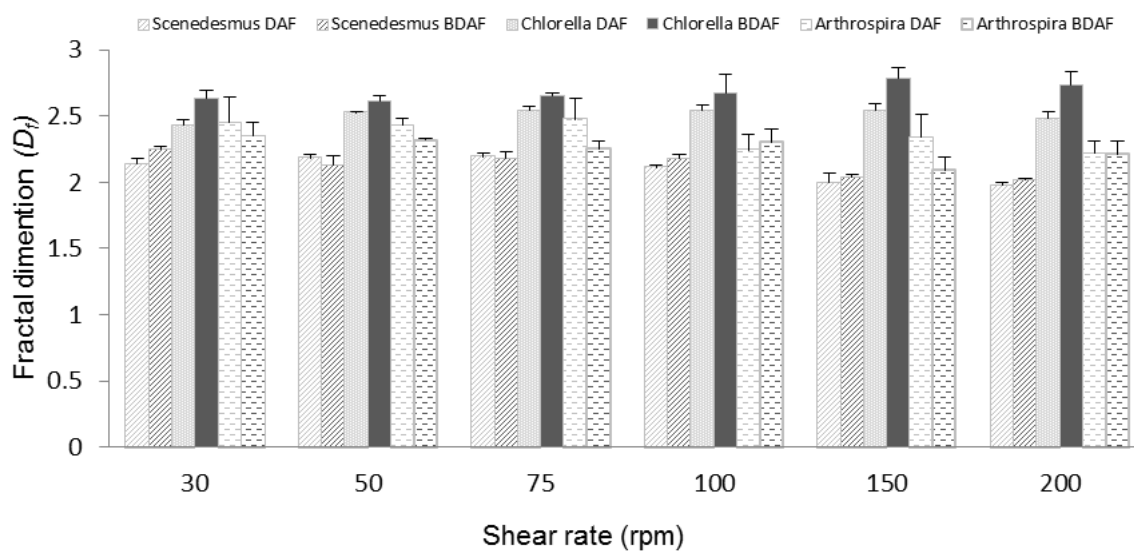
Figure 2.7 Fractal breakage profile for DAF (light grey) and BDAF (dark grey) system, of *S. obliquus*, *C. vulgaris* and *A. maxima* exposed for 15 minutes at increasing shear rates

.

2.2.3.4 Life cycle assessment

The results from the LCA show that, compared to the traditional DAF technology, the adoption of BDAF allows up to 33%, 58% and 44% carbon emission saving for *S. obliquus*, *C. vulgaris* and *A. maxima,* respectively (Table 2.12). Most of the carbon savings come from the lower coagulant demand associated with the system as the coagulant dosage was always the main contributing factor to operational carbon emissions (Figure 2.8). In terms of embodied carbon, the BDAF technology allows a saving close to 300 kg CO_2e, corresponding to a 40% reduction compared to the DAF system. The contributions of the saturator and the hydrocyclone to the total embodied carbon were nearly equal. However, significant differences were observed between the embodied carbon of the two associated pumps. As the hydrocyclone works at lower pressure and treats 5 times less effluent than the saturator, the BDAF unit required a smaller pump which generated a 66% CO_2e reduction; however, this saving was partially outweighed by the glass beads addition with more than 200 kg of CO_2e (50% of the total BDAF embodied carbon).

From the economical perspective, the high price of glass beads (£70 - 80 kg^{-1}) limited the cost reduction. For instance, on a 10 year bases, the conventional life time of a pump (Skongaard and Nielsen, 2004), *S. obliquus* and *A. maxima* reported economic benefits equal to £1.2 and £18.4 million, while *C. vulgaris* was more economically harvested using the DAF unit, because of the lower coagulant demand required compared to the other algae. Overall, compared to the conventional DAF system, the BDAF technology was found to be more sustainable in terms of carbon footprint and to offer significant economic savings depending on the algae biomass used and the price of the flouting ballasting agent.

Table 2.12 Carbon footprint and cost analysis of the DAF and BDAF systems.

Inputs	Materials	Carbon emissions[a]		Costs	
		DAF	BDAF	DAF	BDAF
Embodied		$kg\ CO_2\ e$		$£_{capital\ costs}$	
Saturator	Stainless steel[b]	48	-	15000[c]	-
Packing material	Polypropylene[d]	119	-	800[e]	
Hydrocyclone	Stainless steel[f]	-	58	-	3000[c]
Beads	Glass[g]	-	230	-	15300[h]
Pump[i]	Cast iron	623	208	7500	1500
Subtotal		*790*	*496*	*23300*	*19800*
Saving			*295*		*3500*
Operational		$kg\ CO_2e\ d^{-1}$		$£\ d^{-1}$	
Energy[j]	Electricity	317	12	92	3
Coagulant *(S. obliquus)*		2485	1864	4032	3024
Coagulant *(C. vulgaris)*	Aluminium sulphate[k]	621	373	1008	605
Coagulant *(A. maxima)*		8324	4783	13507	7762
Beads	Glass[g]	-	12	-	780[h]
Saving (10 year life time)		$t\ CO_2e$		*millions £*	
S. obliquus			3340		1.2
C. vulgaris			1979		-1.1
A. maxima			13996		18.4

[a]*calculated applying the carbon factors reported in table 1;* [b]*15 kg of stainless steel calculated from equation 1;* [c]*average market price for full operating unit (online search);* [d]*51 kg polypropylene according to the information provided by Christy®Catalytics for Christy Pak 1 Polypropylene Pall Rings;* [e]*personal communication from supplier Christy®Catalytics;* [f]*18 kg as reported in the material and methods (section 2.2.2.5);* [g]*calculations based on the information reported in the material and method (section 2.2.2.5);* [h]*personal communication from supplier Trelleborg Offshore, Boston;* [i]*carbon emissions based on 410 kg and 137 kg of cast iron DAF and BDAF respectively according to commercial information provided by Grundfos CAPS (costs were also provided);* [j]*electricity costs of £0.14 kWh^{-1} (average UK value);* [k]*carbon emissions based on data reported in table 3, while costs are based on average market prices (Granados et al., 2012).*
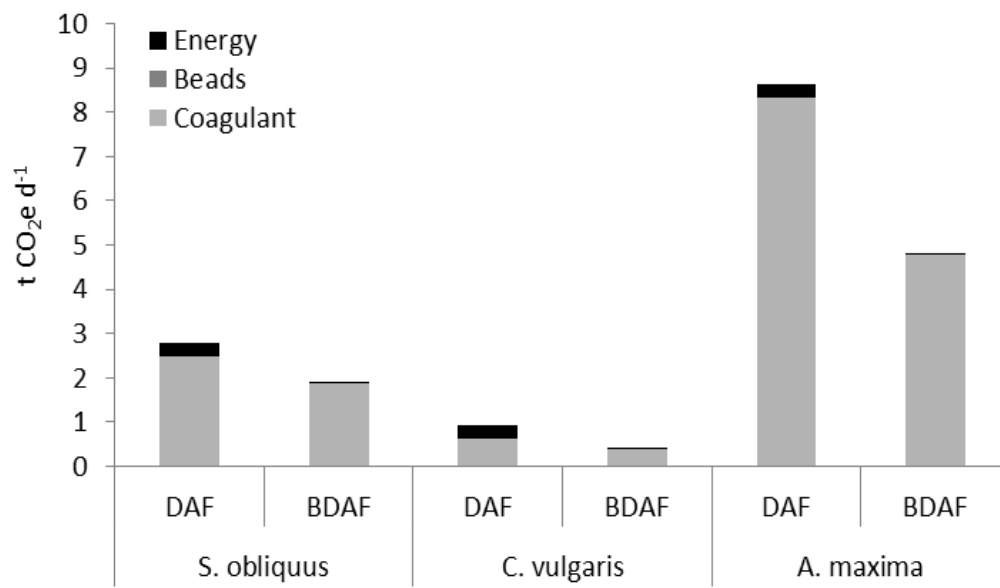
Figure 2.8 Operational carbon footprint comparisons between DAF and BDAF for the three algae *S. obliquus, C. vulgaris* and *A. maxima.*

2.2.4 Conclusions

Ballasted Dissolved Air Flotation (BDAF) was demonstrated to be a more feasible and sustainable microalgae harvesting option compared to the conventional flotation technology. The adoption of floating microspheres as ballasting agents (1) allowed significant coagulant saving, (2) showed a more reliable technology benefitting from a reduced level of energy dissipation within the flotation chamber, and (3) lowered the overall carbon emissions and (4) the process costs. The comparison between the conventional and the ballasted floc structure and strength revealed that the algae-beads aggregation was more affected by the cell's morphology than the AOM. Single cell algae formed compact and strong algae-bead flocs, while filamentous species resulted in a more expanded and inferior structure. However, the AOM composition was confirmed to be a key parameter for the determination of optimal flocculation conditions, as it affected the coagulant demand in both DAF and BDAF technologies. Further research focused on the microspheres recovery process is required to optimise hydrocyclone configuration in order to guarantee beads recovery and energy/costs savings.

2.2.5 Acknowledgments

2.2.6 References

Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol. Adv. 29 (6), 686-702.

Clasen, J., Mischke, U., Drikas, M., Chow, C., 2000. An improved method for detecting electrophoretic mobility of algae during the destabilisation process of flocculation: flocculant demand of different species and the impact of DOC. Journal of Water Supply: Research and Technology - AQUA 49 (2), 89-101.

DEFRA and DECC, 2013. Greenhouse gas conversion factor repository. Department for Environment, Food and Rural Affairs and Department of Energy and Climate Change. http://www.ukconversionfactorscarbonsmart.co.uk/ (accessed 17 June 2013).

Desjardins, C., Koudjonou, B., Desjardinis, R., 2002. Laboratory study of ballasted flocculation. Water Res. 36(3), 744-754.

Edzwald, J.K., 1993. Algae, bubbles, coagulants, and dissolved air flotation. Water Sci. Technol. 27(10), 67-81.

Edzwald, J.K., Haarhoff, J., 2012. Air saturation. In: Dissolved Air Flotation For Water Clarification. 3.1-3.36. J. Am. Water Works Assoc.

Granados, M.R., Acien, F.G., Gomez, C., Fernandez-Sevilla, J.M., Molina Grima, E., 2012. Evaluation of flocculants for the recovery of freshwater microalgae. Bioresourc. Technol. 118, 102-110.

Hammond, G., Jones, C., 2011. Inventory of carbon and energy, version 2.0. Sustainable Energy Research Team (SERT), University of Bath. http://www.bath.ac.uk/mech-eng/sert/embodied/ (accessed 25 July 2011).

Henderson, R.K., Baker, A., Parsons, S.A., Jefferson, B., 2008a. Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. Water Res. 42(13), 3435-3445.

Henderson, R.K., Parsons, S.A., Jefferson, B., 2008b. Successful removal of algae through the control of zeta potential. Separ. Sci. Technol. 43(7), 1653-1666.

Henderson, R.K., Parsons, S.A., Jefferson, B., 2010. The impact of differing cell and algogenic organic matter (AOM) characteristics on the coagulation and flotation of algae. Water Res. 44(12), 3617–3624.

Henderson, R.K., Sharp, E., Jarvis, P, Parsons, S.A., Jefferson, B., 2006. Identifying the linkage between particle characteristics and understanding coagulation performance. Water Sci. Technol.: Water Supply. 6(1), 31-38.

Jarvis, P., Buckingham, P., Holden, B., Jefferson, B., 2009. Low energy ballasted flotation. Water Res. 43(14), 3427-3434.

Jarvis, P., Jefferson, B., Gregory, J., Parsons, S.A., 2005. A review of floc strength and breakage. Water Res. 35(14), 3121-3137.

Jarvis, P., Martin, J., Winspear, T. and Jefferson, B., 2011. Ballasted flotation with glass microspheres for removal of natural organic matter. Separ. Sci. Technol. 46(16), 2489-2495.

Jianghua, Y., Qitao, Y., Kim, Y., 2009. Performance analysis of a hydrodynamic separator for treating particulate pollutants in highway rainfall runoff. Environ. Eng. Res. 14(4), 262-269.

Jun, H., Lian-suo, A., Zhi-quan, W., 2009. Study on application and operation optimization of hydrocyclone for solid-liquid separation in power plant. Proceeding of the World Congress on Engineering and Computer Science (WCECS) San Francisco, USA, 1.

Kam, S., Gregory, J., 1999. Charge determination of synthetic cationic polyelectrolytes by colloid titration. Colloids Surf. 159(1), 165-179.

Knuckey, R. M., Brown, M. R., Robert, R., Frampton, D. M. F., 2006. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacult. Eng. 35(3), 300-313.

Lee, D.H., 2011. Algal biodiesel economy and competition among bio-fuels. Bioresourc.Technol. 102(1), 43-49.

Li, L., Gao, N., Deng, Y., Yao, J., Zhang, K., 2012. Characterization of intracellular and extracellular algae organic matters (AOM) of *Microcystic aeruginosa* and

formation of AOM-associated disinfection byproducts and odor and taste compounds. Water Res.46(4), 1233-1240.

Li, T., Zhu, Z., Wang, D., Yao, C., Tang, H., 2006. Characterization of floc size, strength and structure under various coagulation mechanisms. Powder Technol. 168 (2), 104-110.

Molina Grima, E., Berlarbi, E.H., Acién Fernández, F.G., Robles Medina, A., Chisti, Y., 2003. Recovery of microalgae biomass and metabolites: process options and economics. Biotechnol. Adv. 20(7), 491-515.

OFWAT, 2010. Playing our part: how can we cut greenhouse gas emissions in the water and sewerage sectors? The Water Services Regulation Authority. http://www.ofwat.gov.uk/sustainability/climatechange/prs_web_1007emissions (accessed 19 July 2012).

Pieterse, A.J., Cloot, A., 1997. Algal cells and coagulation, flocculation and sedimentation processes. Water Sci. and Technol. 36(4), 111-118.

Pivokonsky, M., Kloucek, O., Pivokonska. L., 2006. Evaluation of the production, composition and aluminium and iron complexation of algogenic organic matter. Water Res. 40 (2), 3045-3052.

Rawat, I., Kumar, R.R., Mutanda, T., Bux, F., 2013. Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. Appl. Energy 103, 444-467.

Sharp, E.L., Jarvis, P., Parsons, S.A., Jefferson, B., 2006. Impact of fractional character on the coagulation of NOM. Colloids Surface A. 286 (1), 104-111.

Skongaard, A., Nielsen, C.B., 2004. Pump handbook. Grundfos Managmnent http://net.grundfos.com/doc/webnet/mining/_downloads/pump-handbook.pdf (accessed 30 September 2013).

Stumm, W., Morgan, J.J., 1962. Chemical aspects of coagulation. J. Amer. Water Works Assoc. 54 (8), 971-994. Wang, Y., Hubbe, M.A., Sezaki, T., Wang, X., Rojas, O. J., Argyropoulos, D. S., 2006. The role of polyampholyte charge density on its interactions with cellulose. Nordic Pulp and Paper Research Journal. 21(5), 158-165.

UM, 2011. CCaLC© manual, version 2.0, carbon calculations over the life cycle of industrial activities. The University of Manchester. http://www.ccalc.org.uk/ (accessed 17 August 2011).

Wang, Y., Hubbe, M.A., Sezaki, T., Wang, X., Rojas, O.J., Argyropoulos, D.S., 2006. The role of polyampholyte charge density on its interactions with cellulose. Nordic Pulp Pap. Res. J., 21(5), 158-165.

Zhang, X, Amendola, P., Hewson, J.C., Sommerfeld, M., Hu, Q., 2012. Influence of growth phase on harvesting of *Chlorella zofingienis* by dissolved air flotation, Bioresourc. Technol. 116, 477-484.

Chapter 3

ANAEROBIC DIGESTION OF MICROALGAE

3 ANAEROBIC DIGESTION OF MICROALGAE

3.1 Adapting anaerobic digestion bacteria to algal biomass

Francesco Ometto, Robert M. W. Ferguson, Rachel Whitton, Frédéric Coulon, Bruce Jefferson and Raffaella Villa

Cranfield University, Bedfordshire (UK)

Abstract

The adaptation of a microbial community of an anaerobic digestion (AD) to a resilient substrate, such as microalgae biomass, has the potential to improve the digestion efficiencies and hence the process energy balance. Previous works in the 50s showed limited benefits in microalgal digestion due to the presence of a high quantity of residual undigested cells in the sludge, reflecting the resistance to bacteria degradation of this biomass. However, the knowledge at the time did not allow for a detailed characterisation of the bacterial community and the impact of this feedstock on the process. In this work, the microbial community of an AD reactor was adapted over time to digest single-strain microalgae grown on synthetic media and mixed-strain algae biomass from a wastewater treatment plant. The adapted community showed the ability to process the algal feedstock efficiently without any change in reactor performance. Phospholipids fatty acid (PLFA) and 454-Pyrosequencing analysis revealed a significant structural change in the adapted systems when compared to the original bacterial community.

These results demonstrated the ability of a conventional AD bacteria population to adapt to algal biomass digestion, resulting in a more specialised bacterial community which has the potential to generate a more efficient process. However, this does not necessary imply higher biogas productions, which are linked to the specific chemical characteristics of the algal biomass (e.g to their biomethane potential).

Keywords: cell wall breakage, thermal hydrolysis, ultrasounds, enzymes, energy balance.

3.1.1 Introduction

Interest in the use of microalgae as a feedstock for anaerobic digestion (AD) arises from the potential high energy content enclosed in their biomass and the associated potential in reducing greenhouse gas emissions (Schenk *et al.,* 2008). The theoretical methane yield of different algae species ranges from 450 to 800 ml g VS^{-1} (Heaven *et al.,* 2011), 1-2 times higher than other fermentable biomass feedstock (Nallathambi *et al.,* 1997). However, all the digestion work published in the literature reports values of methane yield from 30 to 70% lower than their potential theoretical value (Table 3.1). Detailed investigations on the microalgae cell composition revealed the presence of cell-specific biopolymers in the wall (sporopollenin and algaenan). These biopolymers create thick layers with cellulose and hemicellulose making microalgae cells resistant to bacterial degradation (Abo-Shady *et al.,* 1993) and hence reduce fermentation yields. Thus far, research has mainly focused on the optimisation of microalgae pre-treatments to improve cell wall degradation and solubilisation (Alzate *et al.,* 2012; González-Fernández *et al.,* 2012). However, the energy and additional costs required by these pre-treatments are only balanced by the profits created by the final product of the process, and this is not often the case for AD and the additional methane yields (Passos *et al.,* 2013).

A potential alternative to the pre-treatment processes is the adaptation of the bacterial population to the microalgae feedstock (Golueke *et al.,* 1957; Supaphol *et al.,* 2011). When digesting or co-digesting specific biomass, the characteristic of the microbial community plays an important role on the overall digestion performance, and it is considered to be one of the factors responsible for low biogas yields (Demirel and Scherer, 2008; Supaphol *et al.,* 2011). A similar approach is very often used for feedstock containing recalcitrant substrates.

In their first investigation on algal biomass digestion, Golueke *et al.* (1957) reported to have adapted a microbial population to digest 100% algae feedstock (composed of *Scenedesmus* sp. and *Chlorella* sp.) by gradually increasing the percentage of algal material in the feed over time. Pure algal biomass produced between 200 and 300 ml gVS^{-1} as methane after 30 days at 35ºC, comparable to other recently reported values (Table 3.1). The microscopic analysis of the digested material revealed a large proportion of intact algae cells; for that reason, the authors concluded that conventional mesophilic digestion conditions were not able to

maximise algal biomass digestion. To our knowledge, no other specific attempts have been made to adapt the AD microbial community to efficiently digest microalgae. Despite the contribution of the original investigation (Golueke *et al.,* 1957), the scientific and technological knowledge of the time did not allow a detailed investigation of the microbial population to ascertain if the limited digestion process was related to the specific microbial activity, the surrounding inhibiting conditions or solely to the algal biomass characteristics. This understanding has particular relevance when waste algal biomass from a eutrophic environment is used as additional feedstock for existing AD plants (Allen *et al.*, 2013), which can accept the additional organic loading if the performance of the reactor can be maintained.

The current work aims to address this knowledge gap by adapting a select microbial community able to degrade microalgal biomass in a mesophilic environment and to examine the changes in the community structure caused by this adaptation. The microbial community structure was determined using phospholipids fatty acid (PLFA) and 454-pyrosequencing analysis before, during and after the course of the experiment to follow microbial temporal changes and to determine adaptation and digestion efficiency improvement. Semi-continuous sludge digesters were fed with a gradual increase of algae biomass to reach a final ratio between primary sludge (PS) and algae of 20:80, from an initial condition of 100% PS. Two different algae biomass were used: (1) cultivated microalgae from culture collection and (2) natural wastewater alga biomass recovered from the University wastewater treatment plant. Adapted and non-adapted communities were then characterised and used as inoculum for a BMP analysis of three different algae biomass to assess their bacterial activity.

Table 3.1 Microalgae methane yield comparison between theoretical and experimental data.

Algae	Theoretical	Experimental		References
	ml CH$_4$ gVS^{-1}	ml CH$_4$ gVS^{-1}	%^{b}	
Chlorella vulgaris	544-569	350-372	61-68	*Frigon et al., 2013; Ras et al., 2011*
Chlorella sorokiniana	-	279-287	-	*Frigon et al., 2013*
Chlorella sp.	-	290-328	-	*Frigon et al., 2013; Zamalloa et al., 2012*
Scenedesmus obliquus	531-536	279-287	68-70	*Frigon et al., 2013; Zamalloa et al., 2012*
Arthrospira maxima	483-484	250-340	52-70	*Samson and LeDuy, 1982*
Arthrospira platensis	481-500	285-301	-	*Mussgnug et al., 2010*
Dunaliella salina	471	307-339	57-62	*Mussgnug et al., 2010*
Chlamydomonas reinhardtii	579	382-392	65-67	*Mussgnug et al., 2010*
Euglena Gracilis	555-558	299-303	53-54	*Mussgnug et al., 2010*
Botryococcus braunii	-	320-366	-	*Frigon et al., 2013*
Nannochloropsis gaditana	-	224-232	-	*Frigon et al., 2013*
Scenedesmus sp., *Chlorella* sp.	-	230-260	40-48	*Golueke et al., 1957*
Scenedesmus sp., *Chlorella* sp.	-	143	25-29	*Yen and Bume, 2007*
Chlamydomonas sp., *Nitzschia*, *Chlorella* sp., *Scenedesmus* sp.	-	156	27-29	*Passos et al, 2013*
Chlamydomonas sp. , *Scenedesmus* sp., unkown	-	387-407	66-77	*Alzate et al., 2012*

^{a}*Heaven et al., 2010;* ^{b}*percentage production compared to theoretical values;*

3.1.2 Material and methods

3.1.2.1 Algae biomass

The green algae *Scenedesmus obliquus* (276/42) and *Chlorella sorokiniana* (211/8K), and the cyanobacteria *Arthrospira maxima* (1475/9) were obtained from the Culture Collection for Algae and Protozoa (CCAP), (Oban, UK). *S. obliquus* and *C. sorokiniana* were cultivated in 50 L of Jaworski media under constant illumination and mixing at 20ºC, while the *A. maxima* was cultivated in 25 L of Zarrouk media at 26ºC, under a 16/8 light/dark cycle and daily manual mixing (Ometto *et al.,* 2014). Culture collection algae (CCA) were harvested in their stationary growth phase (15-20 days) using a laboratory centrifuge to reach a volatile solid content of 15 ± 2 g l^{-1} (Appendix A). The wastewater algal biomass (WWA) was obtained from Cranfield University wastewater treatment plant (WWTP) during a seasonal algae bloom on the secondary sedimentation tank. It was characterised by a mixture of non-identified single cells and filamentous microalgae species, and by the presence of the macrophyta *Lemna* sp.. The biomass was collected using a sieve, homogenised with a blender and diluted with onsite effluent water to a final volitele solid (VS) concentration of 24 ± 2 g l^{-1}. All biomass was maintained at 4ºC until utilisation.

3.1.2.2 Adaptation procedure

Digested wastewater sludge (28 ± 2 gVS l^{-1}) obtained from a local treatment work was used as the starting inoculum to digest two different substrates: a mixture of culture collection algae (70% *S. obliquus* and 30% *C. sorokiniana*) having a VS concentration equal to 15 ± 2 g l^{-1} and the wastewater algae described in section 3.1.2.1. PS, obtained from a local WWTP, with a VS concentration of 30 ± 2 g l^{-1} was used as a substrate in the control reactors. To limit the introduction of new bacteria into the system, PS was autoclaved (30 min at 121 ºC) before utilisation. The experiment was performed in triplicate using 2 L glass bottles placed in a water bath at 38ºC and equipped with the appropriate apparatus for gas collection and feed injection/sampling inlet (Figure 3.1). The digesters were set up and operated as follows: At zero time, each digester was filled with 300 ml of inoculum and 1500 ml of PS. Every two days, 200 ml of sample was removed from each digester and replaced with 200 ml of fresh substrate, with a loading rate of between 2 and 3 gVS$_{add}$ d^{-1} depending on the substrate characteristics. For the first 11 days (1.2

hydraulic retention time), all bottles were fed with PS to allow optimal digestion conditions. After that, PS was used as feed in the control, while the CCA and WWA digestors were fed with the following mixture of PS and CA or WWA: 80% PS and 20% algae in volume (80/20), between day 12 and day 28, 50/50 from day 30 to day 48 and 20/80 from day 50. Biogas measurements were converted to standard temperature and pressure (STP), and the methane content was detected using a Servomex 1440 gas analyser (Crowborough, UK). Solid content, pH, Alkalinity, COD and sCOD were measured according to standard methods (APHA). Digestate composition in terms of total carbon and nitrogen (TCN) was determined according to standard method ISO 10694:1995 using a TCN Vario III Elementar Analyser (Isoprime, DE) on freeze-dried samples. Statistical analysis (ANOVA) of biogas production was carried out in http://www.r-project.org/, with significance accepted at a *p* value equal to 0.05.
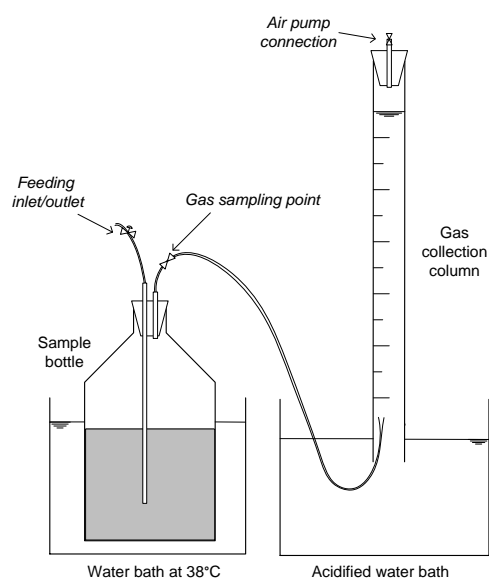
Figure 3.1 Anaerobic digestion semi-continuous reactor.

3.1.2.3 Volatile Fatty Acids (VFA)

Digestate (40 ml) was centrifuged at 5000 g for 5 min and the supernatant was syringe filtered at 0.45 µm (Millipore, DE). 5 µl of 97% sulphuric acid was added to the sample to prevent acid degradation and stored at – 20ºC until analysis. VFA analysis was performed on 100 µl samples using a HPLC (535 Kontron, Bio-TEK, UK) equipped with UV detector (210 nm) and a Bio-Rad fermentation column (Cat 125-0115, 300 x 7.8 mm) maintained at 65ºC. The mobile phase was 0.001 M

sulphuric acid in HPLC grade water with a flow rate of 0.8 ml min^{-1}. Acetic, propionic, n-butyric, and iso-butyric acids were quantified using an external multilevel calibration ranging from 0.1 g l^{-1} to 5 g l^{-1}. The error in the repeatability of measurements for each acid was 0.6%, 0.77%, 0.72%, and 1.13%, respectively.

3.1.2.4 Phospholipids Fatty Acids (PLFAs)

Total lipids were extracted from 40 g aliquot of freeze-dried digestate using a modified version of the Bligh-Dyer technique as described by Frostegård *et al.* (2011). Briefly, dried fatty acid methyl esters (FAMEs) were resuspended in 0.2 ml of Hexane (Sigma, UK) and analysed by gas chromatography equipped with a flame ionisation detector (GC-FID Agilent Technologies 6890N) (Pankhurst *et al.,* 2012). FAMEs were identified by comparison of retention times with the 26 bacterial acid methyl ester (BAME) standards (SUPELCO, Sigma, UK). 24.44 µg ml^{-1} of Nonadecanoic acid methyl ester (Sigma, UK), used as an internal standard, was added to each sample after the solid phase extraction. The taxonomic affiliations were undertaken in accordance with the data reported by Londry *et al.* (2004) and Ferguson (2013). Gram-positive bacteria were represented by the series of iso and anteiso branch saturated PLFA. Gram-negative bacteria were represented by cyclopropane, hydroxyl and monounsaturated PLFA. The 16:0 straight chain PLFA was previously identified as an ubiquitous bacterial marker (Piotrowska-Seget and Mrozik, 2003). The PLFA 18:2w9cis and 18:1w7trans were used as markers for Clostridia. Bacterial biomass was converted into a number of cell equivalents using a conversion factor of 5.9 x 10^{10} cells per µmol of PLFA (Kieft *et al.,* 1994).

3.1.2.5 454-Pyrosequencing analysis and bioinformatics

DNA was extracted from 200 mg of the sample using a MoBio Power Soil kit (MO BIO Laboratories, Inc, UK). The quality of the extracted DNA was assessed on 0.8% agarose gels. Phusion high fidelity polymerase (Biolabs, New England, UK) was used for the amplification of different 16S rRNA gene fragments as described in Ferguson, 2013. The sequence data was processed using the CloVR-16S 1.0 pipeline (http://clovr.org/) following the method described by White *et al.* (2011). Briefly, the Qiime script "plit_libraries.py" (http://qiime.org) and the Mothur script "unique.seqs" were used to remove poor quality sequences and to cluster unique sequences, respectively. Putative chimeras were identified against the "16S rRNA

gold database" from isolated representative sequences, using default parameters and were excluded from further analysis. Following a preliminary sequence grouping, classification and allignament using the Qiime workflow "pick_otus_throigh_otu_table.py", the sequences were clustered into operational taxonomic units (OTUs) with a 97% nucleotide sequence identity threshold for all readings, using the Qiime script "pick_otus.py". The representative sequences of each cluster were then classified at Phylum, Class, Order, and Family (0.5 confidence threshold) using the Ribosomal Database Project Bayesian classifier (http://rdp.cme.msu.edu/) with the script "assign_taxonomy.py". The results are presented as the number of sequences assigned to OTUs identified at the respective taxonomic levels. Weighted Unifrac was carried out with Qiime and was clustered using the function "hclust" in r (http://www.R-project.org/).

3.1.2.6 Biochemical Methane Potential (BMP)

Anaerobic digestion batch experiments were performed according to the method described by Angelidaki *et al.* (2009). The three adapted digestates (PS, FWA and WWA) collected at day 65 from the 2 l digestors were used as inoculum, while concentrated *S. obliquus* was used as substrate. 100 ml serum bottles were filled with 40 ml of inoculum/substrate ($VS_{inoculum}$:$VS_{substrate}$ ratio equal to 2) and 20 ml of nutrient solution (Appendix B). The pH was adjusted to 7 ± 0.2 using 1 M NaOH and 1M HCl solution. Samples were flushed with N_2 gas, sealed with a PTFE crimp cap, and placed at 38ºC under constant agitation (150 rpm). Biogas production was determined at days 2, 5, 8, 12, 16 and 21. Measured data was converted to STP and corrected by subtracting the average blank control's (inoculum + nutrients) biogas volumes from each test digester's biogas volume. All experiments were performed in triplicate.

3.1.3 Results

3.1.3.1 Adaptation experiment: digestion performances

Despite the different yields observed for the three systems, the trends in biogas production were similar in all the reactors, showing stable production from day 18 (2 HRT) (Figure 3.2 A). The control (100% PS) yielded between 200 and 288 ml $kgVS_{add}^{-1}$ as biogas, at 80% methane. Similar values were observed in the WWA reactors, where the increasing amount of waste algae biomass in the feedstock did not affect the digestion performances. On the contrary, compared to the control, the addition of CCA caused a 30% decrease in the biogas production showing between 130 and 150 ml $kgVS_{add}^{-1}$ (60 – 70% methane). Statistical analysis confirmed the similarities between the control and WWA reactors ($p > 0.05$), and the significant differences of those compared to CCA ($p < 0.05$).

The VS content decreased in all the reactors from an initial concentration of 30 g l^{-1} to 12 g l^{-1} from day 29 (Figure 3.2 C), showing a higher VS reduction in the control ($62 \pm 1\%$) compared to the CCA ($53 \pm 5\%$) and WWA ($41 \pm 5\%$). This is in agreement with the observations of Golueke *et al.* (1957) and our microscopic analysis of the samples, which identified the presence of intact post-digestion algae cells, suggesting limited digestion (Figure 3.3). The pH values (Figure 3.2 D) remained constant in all reactors for the entire duration of the experiment (pH 7.5 ± 0.3). Conversely, from an initial value close to 3700 mg $CaCO_3$ l^{-1} at day 18, the alkalinity decreased until day 65 by 18%, 28% and 46% for the control, WWA and CCA sample, respectively (Figure 3.2 D).

Although the specific chemical composition of the three different substrates was unknown, the similarities between PS and WWA suggested a more similar biomass composition compared to the CCA. According to the calculation of Heaven *et al.* (2011), the theoretical methane production of *S. obliquus* and *Chlorella* sp. ranges between 450 and 530 ml $kgVS_{add}^{-1}$, a lower value to the one accepted for conventional PS, e.g. 500 – 700 ml $kgVS_{add}^{-1}$ (Metcalf and Eddy, 2003). Therefore, a lower yield in the CCA sample was to be expected in processing a feedstock with a lower energetic content compared to the control. However, while this can explain the digestion performance at the end of the experiment when 80% of the substrate is made of algae, the low production between day 18 and day 31 is more likely to reflect some inhibiting factors affecting the overall digestion performance as the

amount of algae biomass introduced in the system is marginal (20%) compared to PS.
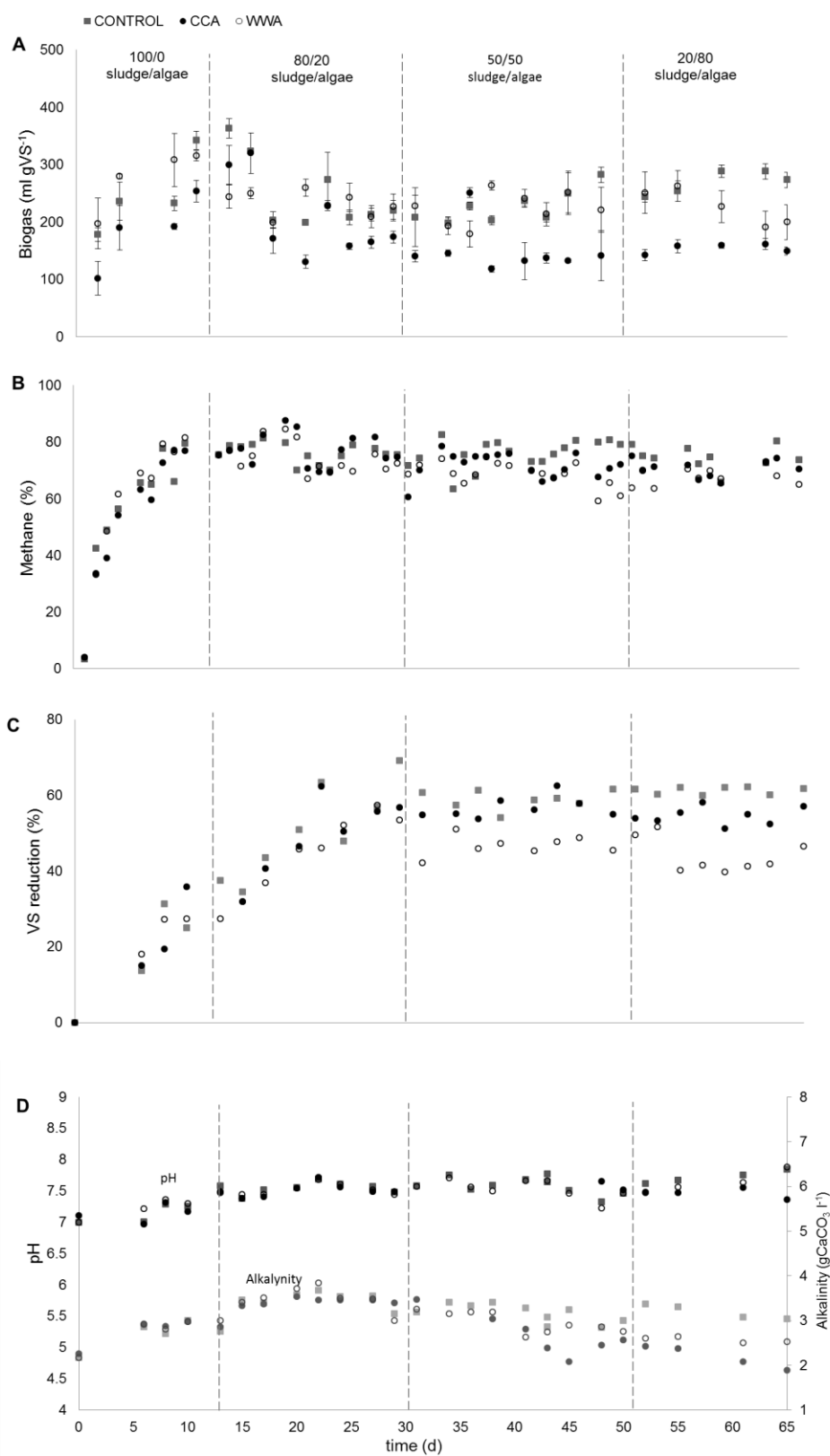
Figure 3.2 Adaptation experiment results: (A) biogas production, (B) methane content, (C) volatile solid concentration and (D) pH and alkalinity over time.
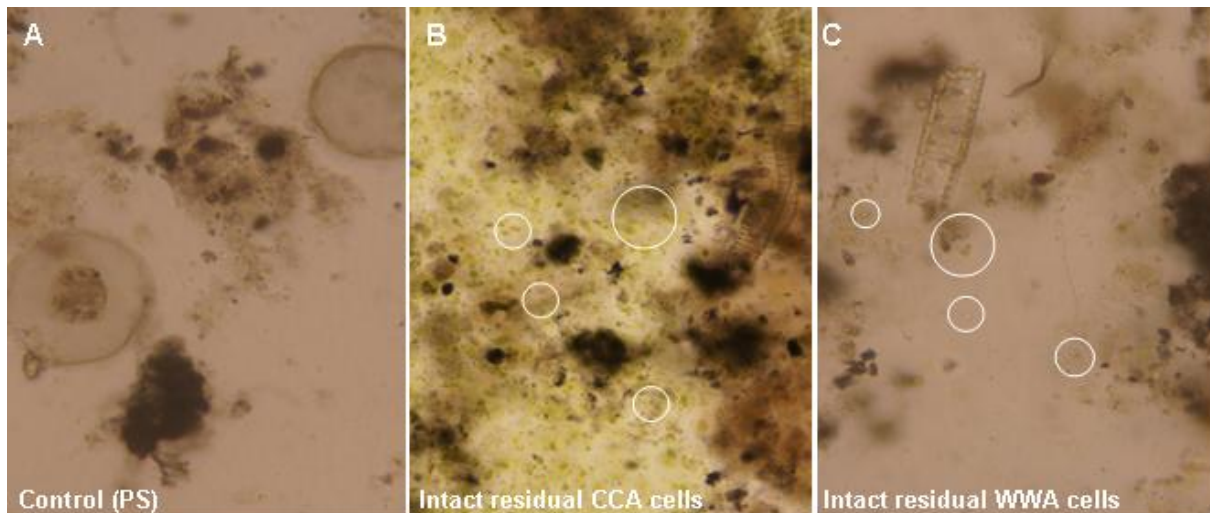
Figure 3.3 Residual post digestion algae cells (Light microscope x20).

3.1.3.2 Changes in the microbial community

PLFA analysis was used to quantify changes in the bacterial community. The increasing amount of algal biomass addition in the digesters significantly affected the original bacteria community (wastewater sludge). The initial addition of algae to the feed caused a significant decrease in bacterial biomass, calculated from PLFA analysis, in both CCA and WWA samples reporting biomass values an order of magnitude lower than the control (Figure 3.4). Further increases in the proportion of algae in the feed to 50/50 on day 30 resulted in further decreases in bacterial biomass only when digesting WWA. However, at day 56, the bacterial biomass returned to the intial value of 3×10^8 cells kgVS^{-1}. This suggests that, although the biomass was partially able to recover and sustain the same level of biogas production as the control, a change in community had occurred (Schwarzenauer and Illmer, 2012).
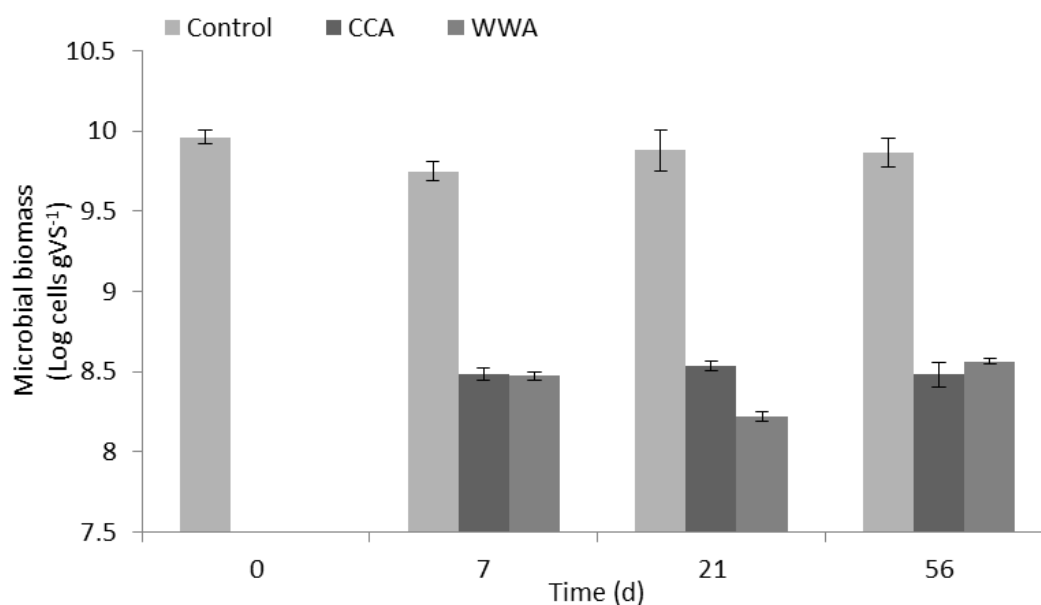
Figure 3.4 Available microbial biomass into the system. Average value on triplicate sample, error bars denote standard deviation.

The dominant PLFAs in the control were 14:0, 15:0i, 15:0ai, 16:1w7c, 16:0, 18:1w9c, 18:1w9t and 18:0. This indicates that the bacterial community was dominated by low GC G+, *Clostridium, Bacillus* and *Actinobacteria* (Figure 3.5). These PLFAs remained stable in the control for the duration of the trial. The addition of algae to the feed produced an increase in the 18:1w9trans PLFA of between 20% and 120% on day 21; this was accompanied by a corresponding decline in the PLFA 18:1w9cis. This caused a change in the cis-trans ratio which is a known response to starvation stress in PLFA fingerprints. This indicates that the VS introduced by PS/Algae mixture was not fully available to the bacterial community, resulting in starvation and the decrease in biomass reported in Figure 3.4 (Frostegård *et al.,* 2011).

There were changes in the structure of the bacterial lipid fingerprint also with 16:0, 15:0i, 15:0ai, and16:1w7c and 18:0 PLFA not present on day 21 in both CCA and WWA and reappearing on day 56 in the CCA samples only. All of these PLFA are markers for low GC G+, *Clostridium, Bacillus* and *Actinobacteria*. This indicates either a shift in the community structure within these groups and/or change in function of the bacterial community. By day 56, the 18:1w9cis PLFA reappeared with a increment of 18% compared to the control, indicating that the community was no longer starved and it had adapted to digesting the algal feed. While in the CCA samples, the specific PLFAs reappearing indicates a temporary change/stress in the

bacteria community being able to recover and return to similar initial condition, their complete disappearance in the WWA suggests a significant change of the bacteria population.
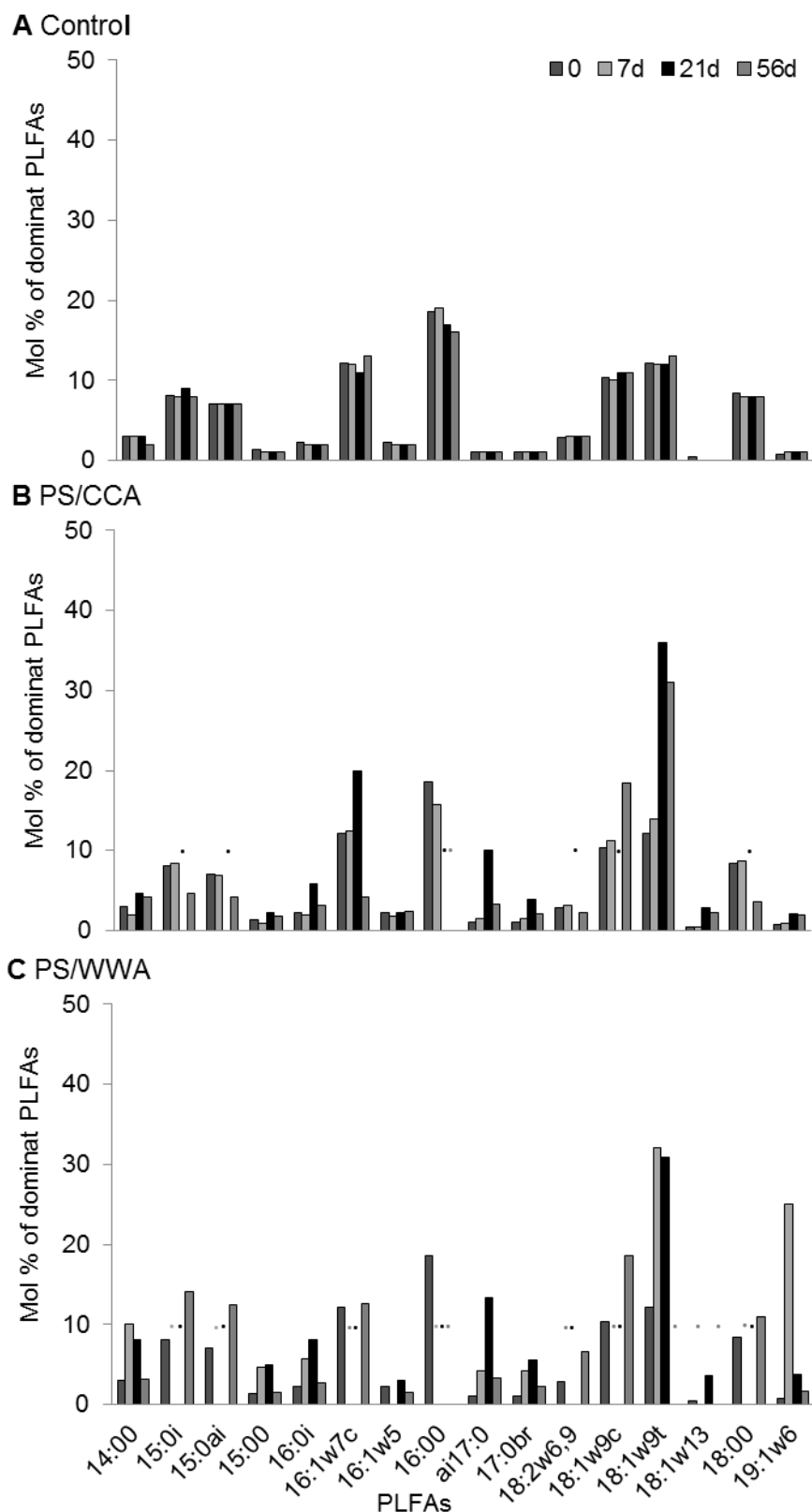
Figure 3.5 Mol % of dominant PLFA (> 5%). Dots highlight the PLFA available in the control experiment (A) that disappear at day 21 when digesting CCA (B) and WWA (C) to reappeared or not at day 56.

3.1.3.3 454-Pyrosequencing analysis.

454-Pyrosequencing was carried out to determine the detailed structure of the bacterial communities after the adaptation period on day 65. 2347 sequences were retrieved from the three samples; > 98% of the sequences were of the target length of between 350 and 450 base pairs, indicating the recovery of good quality sequences.

The WWA had double the number of operational taxonomic units (OTUs) compared to the control; 195 vs 77 (Table 3.2). Both algal treatments showed higher diversity (Shannon index $H' = -\sum_{(i=1)}^{s} p_i\ log(p_i)$) compared to the control starting point. However, the evenness of the diversity (Pielou's evenness $J' = H'/H'_{max}$ where $H'_{max}=log(S)$) was lower for both algae treatments (Table 3.2). Lower evenness is a sign of higher specialisation of the adapted bacterial communities as a result of the bacterial selection process. This has implications for AD operators as bacterial communities with lower evenness are known to be less resilient to stress and these digesters may, therefore, be more prone to poor performance when process conditions change, such as feedstock composition and concentration (Wittebolle *et al.*, 2009; Werner *et al.,* 2012)

Table 3.2 Structure comparison between adapted bacterial communities.

Treatment	Control (PS)	CCA	WWA
OTUs	77	103	195
Diversity (*H'*)	3.12	3.76	4.02
Evenness (*J*)	0.72	0.68	0.59

UNIFRAC analysis showed that all three treatments had distinct microbial communities with very little similarity between treatments. Although the algal treatments were marginally more similar to each other than to the control, they were still > 35% dissimilar, showing that the different algal feedstock had different effects on the bacteria. The structures of the bacterial communities are displayed in Figure 3.6. The control treatment had > 25% unknown bacterial OTUs and was dominated by *Clostridia, Bacteroidetes, and Proteobacteria* which is consistent with other

studies of bacterial communities in AD (Schlüter *et al.,* 2008; Kröber *et al.,* 2009; Lee *et al.,* 2012; Werner *et al.,* 2012; Ferguson, 2013).

The digestion of CCA resulted in high numbers of OTUs related to candidate phylum OP10, recently classified as *Armatimonadetes*. The dominant *Clostridia* orders were also different to the control with dominance of *Thermoanaerobacteriles* and *Erysipelotrichales* (in contrast to *Clostridiales)*. An increase in OP10 was not replicated with the WWA treatment, however, the previously undetected order, *Thermotogae* made up > 20% of OTUs. There were also OTUs of photosynthetic bacteria from the *Phylum Cyanobacteria*. The light available to the microbial population in the reactor is limited, therefore it is very likely that these bacteria were present in the algae feed. This suggests that some of the bacterial diversity observed in the WWA sample came from the algal feed which added a heterogeneous mixture of algae species and aquatic species into the digester capable of surving in anaerobic conditions. This explains the higher number (double) of OTUs detected in this sample in comparison with the control and CCA treatment, where this effect was minimised by the single algal culture used. The presence of these OTUs from the algal cultivation stage may have been significant in the improved performance in comparison to the CCA treatment, as these OTUs will have originated from an ecosystem where algae was the main carbon source.

Figure 3.6 Proportion of OTUs determined by 454-pyrosequencing in the Control. Rings show Phylum, Class and Order from inner to outer ring.
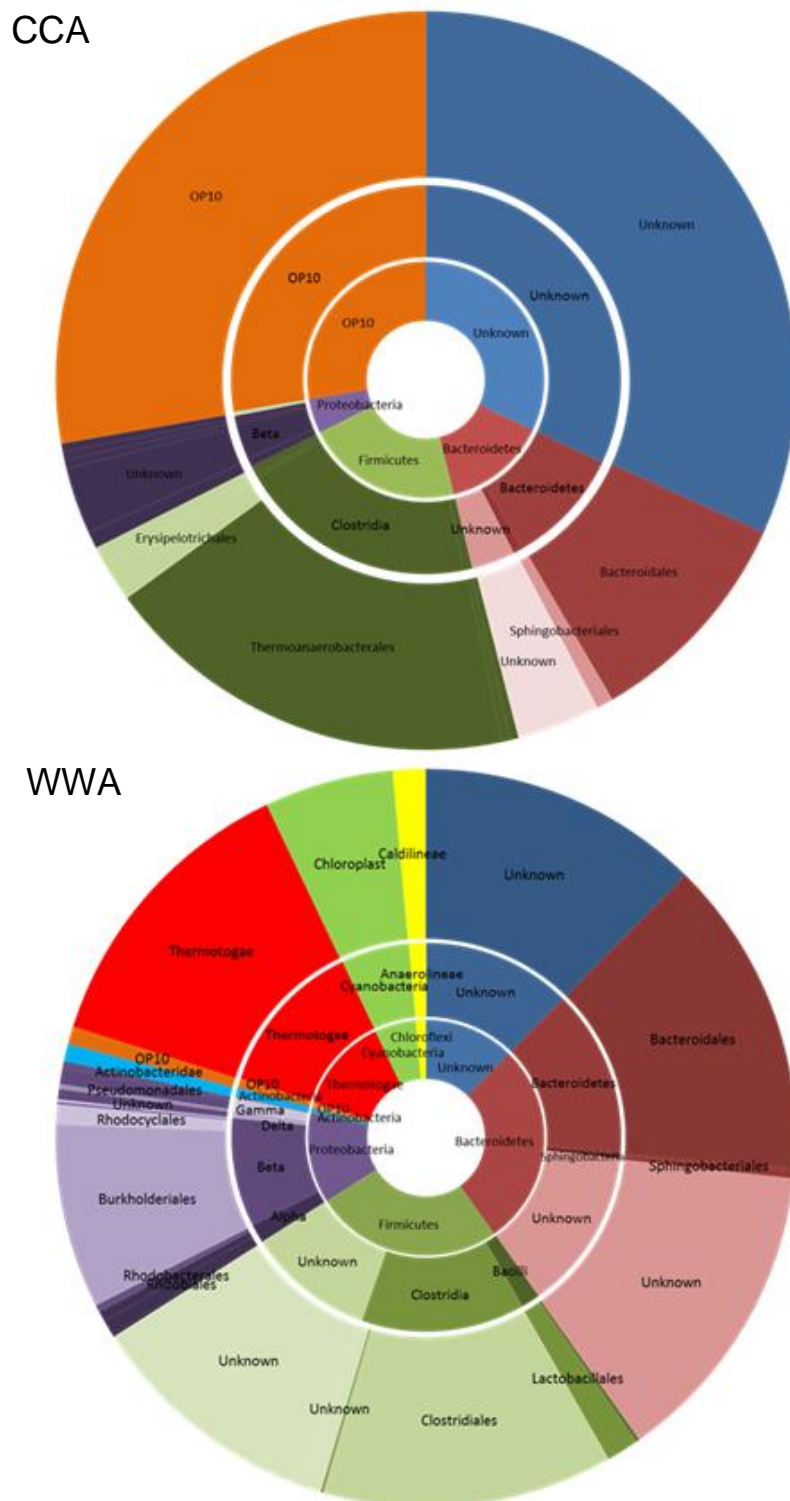
Figure 3.7 Proportion of OTUs determined by 454-pyrosequencing, in the culture collection algae (CCA) and wastewater algae (WWA). Rings show Phylum, Class and Order from inner to outer ring.

3.1.3.4 Adapted digestate characterisation

To evaluate the quality of the adapted seeds and their ability to digest specific algae biomass, at day 65 all three digestates were characterised in terms of pH, alkalinity, VFA and C:N ratio, and used as inoculum for the BMP test of three different microalgae: *S. obliquus*, *C. sorokiniana* and *A. maxima*. For all considered parameters, the control (digested PS) and the WWA digestate showed small differences reflecting the similar performances observed during the adaptation experiment (Figure 3.2). Conversely, CCA showed lower alkalinity, C:N ratio and total VFA concentration. However, despite the differences between the three samples, all parameters compared well to conventional sets of data for optimal AD with the only exception being the C:N ratio (Table 3.3). While, theoretically, the optimal C:N ratio for AD ranges between 20:1 and 30:1 (Parkin and Owen, 1986), sewage sludge is efficiently digested at a C:N ratio lower than 16:1 (Stroot *et al.,* 2001). Using algae biomass, Ehimen *et al.* (2010) reported an optimal digestion of residual *Chlorella* biomass (2% VS) preliminary processed for lipid extraction, applying a C:N ratio from 10 to 15. A different study showed high methane yields (0.57 m3 l^{-1} d^{-1}) from a mixture of *Scenedesmus* sp. and *Chlorella* sp. having a C:N ratio of 6.7 and a VFAs content close to 5000 mg l^{-1} (Yen and Brune, 2007).

Table 3.3 Characterisation of digestate

Digestate	pH	Alcalinity mg $CaCO_3$ l^{-1}	Total VFA mg l^{-1}	C:N
Control	7.8	3040 ± 200	1.58 ± 0.30	7.36 ± 0.02
CCA	7.4	1884 ± 107	0.87 ± 0.34	5.74 ± 0.08
WWA	7.7	2595 ± 274	1.29 ± 0.36	7.92 ± 0.03

The BMP of *S. obliquus* using the three different inoculum showed similar ($p > 0.05$) cumulative methane production, equal to 236 ± 23 ml gVS_{add}^{-1} and comparable with those obtained in similar batch digestion conditions reported in Table 3.1 (Table 3.4). Similar behaviour was observed digesting *A. maxima,* while the three inoculums performed differently with *C. sorokiniana* ($p < 0.05$), with the PS-seed yielding 20% and 35% more than the CCA-adapted and the WWA-adapted, respectively.

However, taking into account the different amounts of biomass available in the different systems, estimated from the PLFAs analysis, the bacterial activity in the three substrates was between 4 to 14 times higher when using algae-adapted communities than the PS-adapted (Table 3.4). This suggests that even if the increment in the cumulative biogas production per kg VS added was undetectable, the algae-adapted bacteria were more active on digesting algae than the non-adapted. Indeed, a lower amount of adapted organisms produced as much biogas as the higher quantity of non-adapted bacteria. The small scale of the test did not allow the determination of the VS reduction; however, this is likely to be similar between the three different communities.

Table 3.4 BMP analysis bacterial activity estimation

Algae	Inoculum	K_d (d^{-1})	Biogas $(ml\ gVS^{-1})$	Methane $(ml\ gVS^{-1})$	Bacteria activity* $(mlCH_4\ nmolPLFA^{-1})$
	WWS seed	0.25	534 ± 9	258 ± 27	0.06
S. obliquus	CCA seed	0.20	508 ± 52	226 ± 17	0.23
	WWA seed	0.16	457 ± 42	244 ± 14	0.30
	WWS seed	0.13	420 ± 41	191 ± 8	0.04
C. sorokiniana	CCA seed	0.12	354 ± 16	154 ± 11	0.17
	WWA seed	0.11	252 ±28	123 ± 21	0.15
	WWS seed	0.14	310 ± 31	123± 17	0.03
A. maxima	CCA seed	0.10	317 ± 13	120 ± 9	0.13
	WWA seed	0.16	305 ± 18	119 ± 16	0.15

methane production per unit of bacterial biomass.

3.1.4 Conclusion

The present study, designed to determine the capability of a conventional anaerobic microbial community to digest algal biomass, demonstrated that:

- the inclusion of algal biomass into the feed of a conventional AD process caused a shock to the endogenous bacterial community and a negative impact on the digester yields;
- the impact of the algal biomass on the digester yields will depend on the algae methane content in comparison to the original feed and it will be greater when this difference is high;
- acclimation with algae biomass caused a structural change in the original AD bacterial community, not only a metabolic adaptation of the endogenous microorganisms;
- compared to a conventional bacterial community, the adapted microorganisms were more efficient at digesting algae showing higher yields per unit of bacterial biomass;

These results demonstrated the ability of a conventional AD bacteria population to adapt to algal biomass digestion, resulting in a more specialised bacterial community which has the potential to generate a more efficient process. However this does not necessarily imply higher biogas productions, which are linked to the specific chemical characteristics of the algal biomass (e.g to the biomethane potential).

This confirmed (1) the limited digestibility of raw algae biomass and (2) the need for preliminary treatment of the algae cells to allow higher yields.

An implication of this work relates to the use of seasonal waste algae biomass from natural environment as a valuable feedstock for existing AD plants. Adapted digesters, processing constantly small amounts of algae, have the potential to accept higher seasonal loading without causing detrimental effects on the overall performances of the reactor.

3.1.5 Acknowledgments

3.1.6 References

Abo-Shady. A.M., Mohamed, Y.A., Lasheen, T., 1993. Chemical composition of the cell wall in some green algae species. Biol. Plantarum. 35, 629-632.

Allen, E., Browne, J., Hynes, S., Murphy, J.D., 2013. The potential of algae blooms to produce renewable gaseous fuel. Waste Manage. 33(11), 2425-2433.

Alzate, M.E., Munoz, R., Rogalla, F., Fdz-Polanco, F., Pérez-Elvira S.I., 2012. Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment. Bioresour. Technol. 123, 488-494.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., van Lier J.B., 2009. Defining the biomethane potential (BMP) of solid organic waste and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59, 927-934.

Demirel B, Scherer, P., 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. Rev. Environ. Sci. Biotechnol. 7, 173-190.

Ehimen, E.A., Sun, Z.F., Carrington C.G., Birch, E.J. and Eaton-Rye, J.J., 2011. Anaerobic digestion of microalgae residues resulting from the biodiesel production process. Appl. Energ.88(10), 3454-3463.

Ferguson, R.M.W., 2013. Feedstock influence on the process parameters and the microbial community in anaerobic digestion. Ph.D. thesis, Cranfield University, UK (2013).

Frigon, J.C., Matteau-Lebrun, F., Adou, R.H., McGinn, P.J., O'Leary, S.J.B., Guiot, S.R., 2013. Screening microalgae strains for their productivity in methane following anaerobic digestion. Appl. Energ. 108, 100-107.

Frostegård, A., A. Tunlid, E. Baath, 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43(8), 1621-1625.

Golueke, C.G., Oswald, W.J., Gotaas, H.B., 1957. Anaerobic digestion of algae. Appl. Biotechnol. 5, 47-55.

González-Fernández, C., Sialve, B., Bernet, N., Steyer, J.P., 2012. Impact of microalgae characteristics on their conversion to biofuels. Part II Focus on biomethane production. Biofuels Bioprod. Bioref. 6, 205-218.

Heaven, S., Milledge, J., Zhang, Y., 2011. Comments on anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol. Advanc. 29(1), 164-167.

Kieft, T.L., Ringelberg, D.B., White, D.C., 1994. Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Appl. Environ. Microb. 60(9), 3292-3299.

Kröber, M., Bekel, T., Diaz, N.N., Goesmann, A., Jaenicke, S., Krause, L., Miller, D., K. Runte, J., Viehöver, P., Pühler A., Schlüter, A. 2009. Phylogenetic characterization of a biogas plant microbial community integrating clone library 16S-rDNA sequences and metagenome sequence data obtained by 454-pyrosequencing. J. Biotechnol.142(1), 38-49.

Lee, S.H., Kang, H.J., Lee, Y.H., Lee, T.J., Han, K., Choi, Y., Park, H.D., 2012. Monitoring bacterial community structure and variability in time scale in full-scale anaerobic digesters. J. Environ Monit. 14(7), 1893-1905.

Londry, K.L., Jahnke L.L., Des Marais, D.J., 2004. Stable carbon isotope ratios of lipid biomarkers of sulfate-reducing bacteria. Appl. Environ. Microb. 70(2), 745-751.

Mussgnug, J.H., Klassen, V., Schlüter, A., Kruse, O., 2010. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. J. Biotechnol. 150, 51-56.

Nallathambi, G.V., 1997. Anaerobic digestion of biomass for methane production: a review. Biomass Bioen. 13, 83-114.

Ometto, F., Pozza, C., Whitton, R., Smith, B., Torres, A.G., Henderson, R.K., Jarvis, P., Jefferson, B. and Villa R., 2014. The impact of replacing air bubbles with microspheres for the clarification of algae from low cell-density culture. Water Re. 53, 168-179. (Current Thesis, Paper 2.2).

Pankhurst, L. J., Whitby, C., Pawlett, M., Larcombe, L.D., McKew, B., Deacon, L.J., Morgan, S.L., Villa, R., Drew, G.H., Tyrrel, S.,. Pollard, S.J.T, Coulon, F., 2012. Temporal and spatial changes in the microbial bioaerosol communities in green-waste composting. FEMS Microbiology Ecol. 79(1), 229-239.

Parkin, G.F., Owen, W.F., 1986. Fundamental of anaerobic-digestion of wastewater sludge. Journal of Environmental Engineering. 112, 867-920.

Passos, F., Garćia, J., Ferrer, I., 2013. Impact of low temperature pretreatment on the anaerobic digestion of microalgal biomass. Bioresour. Technol. 138, 79-86.

Piotrowska-Seget, Z., Mrozik, A., 2003. Signature lipid biomarker (SLB) analysis in determining changes in community structure of soil microorganisms. Pol. J Environ. Stud. 12(6), 669-675.

Samson, R., LeDuy, A., 1982. Biogas production from anaerobic digestion of *Spirulina maxima* algal biomass. Biotechnol. Bioeng. 24(8), 1919-1924.

Schenk, P.M., Thomas Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O. and Hankamer, B., 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy Research. 1(1), 20-43.

Schlüter, A., Bekel, T., Diaz, N.N., Dondrup, M., Eichenlaub, R., Gartemann, K.H., Krahn, I., Krause, L., Krömeke, H., Kruse, O., Mussgnug, J.H., Neuweger, H., Niehaus, K., Pühler, A., Runte, K.J., Szczepanowski, R., Tauch, A., Tilker, A., Viehöver P., Goesmann A., 2008. The metagenome of a biogas-producing microbial community of a production-scale biogas plant fermenter analysed by the 454-pyrosequencing technology. J Biotechn. 136(1-2), 77-90.

Schwarzenauer, T., Illmer, P., 2012. PLFA profiles for microbial community monitoring in anaerobic digestion. Folia Microbiol. 57(4), 331-333.

Song, Y.C., Kwon, S.J., Woo, J.H., 2004. Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic and thermophilic digestion of sewage sludge. Water Res. 38, 1653-1662.

Stroot, P.G., McMahon, K.D., Mackie, R.I., Raskin, L., 2001. Anaerobic codigestion of municipal solid waste and biosolids under various mixing condition. I. Digestr performance. Water Res. 35, 1804-1816.

Supaphol, S., Jenkins, S.N., Intomo, P., Waite, I.S., O'Donnell, G.A., 2011. Microbial community dynamics in mesophilic anaerobic co-digestion of mixed waste. Bioresour. Tehcnol. 102, 4021-4027.

Ras, M., Lardon, L., Bruno, S., Bernet, N., Steyer, J., 2011. Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*, Bioresour. Technol. 102, 200-206.

Wang, Y. Zhang, Y., Wang, J., Meng, L., 2009. Effect of volatile fatty acid concentrations on methane yield and methanogenic activity. Biomass Bioenerg. 33, 848-853.

Werner, J.J., Knights, D., Garcia, M.L., Scalfone, N.B., Smith, S., Yarasheski, K., Cummings, T.A., Beers, A.R., Knight, R., Angenent, L.T., 2012. Bacterial community structures are unique and resilient in full-scale bioenergy systems. Proceedings of the National Academy of Sciences of the United States of America. 108(10), 4158-4163.

White, J.R., Arze, C., Matalk, M., Team, T.C., Angiuoli, S., Fricke, F.W., 2011. CloVR-16S: phylogenetic microbial community composition analysis based on 16S ribosomal RNA amplicon sequencing - standard operating procedure, version1.0. Nature Procedings. DOI:10.1038/npre.2011.5888.1

Wittebolle, L., Marzorati, M., Clement, L., Balloi, A., Daffonchio, D., Heylen, K., De Vos, P., Verstraete, W., Boon, N., 2009. Initial community evenness favours functionality under selective stress. Nature. 458(7238), 623-626.

Yen, H.W., Brune, D.E., 2007. Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresour. Technol. 98(1), 130-134.

Zamalloa, C., Boon, N., Verstraete, W., 2012. Anaerobic digestibility of *Scenedesmus obliquus* and *Phaedactylum tricornutum* under mesophilic and thermophilic conditions. Appl. Energ. 92, 733-738.

3.2 Impacts of microalgae pre-treatments for improved anaerobic digestion: thermal treatment, thermal hydrolysis, ultrasound, enzymatic hydrolysis.

Francesco Ometto[i], Gerardo Quiroga[ii], Pavel Psenǐckǎ[iii], Rachel Whitton[i], Bruce Jefferson[i] and Raffaella Villa[i].

[i] Cranfield University, Bedfordshire (UK)

[ii] University of Oviedo, Oviedo (ES)

[iii] Czech University of Life Sciences Prague, Prague (CZ)

Abstract

Anaerobic digestion (AD) of microalgae is principally inhibited by the chemical composition of algae cell walls containing cellulose and acetolysis resistant biopolymers (ARB) able to resist bacterial degradation. The adoption of pre-treatments such as thermal, thermal hydrolysis, ultrasound and enzymatic hydrolysis have the potential to break the cell wall, partially degrade these biopolymers and release the intracellular algogenic organic matter (AOM) removing the inhibitory components and enhancing biogas yields. This work investigated the effect of four different pre-treatments on the microalgae cells, and their impact on the quantity of soluble biomass released in the media and thus on the digestion process yields. The analysis of the composition of the soluble COD released and of the TEM images of the cells showed two main degradative actions associated with the processes: (1) cell wall damage with the release of intracellular AOM (thermal, thermal hydrolysis and ultrasound) and (2) degradation of the cell wall constituents with the release of intracellular AOM and the solubilisation of the cell wall biopolymers (enzymatic hydrolysis). As a consequence of this, enzymatic hydrolysis showed the greatest biogas yield increments (> 270%) followed by thermal hydrolysis (60 – 100%) and ultrasounds (30 – 60%) depending on the algae species

Keywords: microalgae, cell wall degradation, pre-treatments, anaerobic digestion, enzymes, thermal hydrolysis, ultrasounds.

3.2.1 Introduction

Microalgae are currently considered as the best biomass for the production of renewable energy. Their high energy content, fast growth rate and the ability to adapt to a number of different environments give them the potential to meet all energetic, economic and environmental requirements for sustainable energy production (Mata *et al.,* 2010; González-Fernández *et al.,* 2012a). However, the overall energy balance of the conversion process may still be uneconomic as a result of the sequence of energy intensive steps required to cultivate, harvest and pre-treat the biomass (Acién *et al.,* 2012; Slade and Bauen, 2013). Of the currently available biomass to energy technologies, such as gasification, thermochemical liquefaction, direct combustion and anaerobic digestion (AD), AD provides the most feasible process for large scale application which, depending on the chemical composition, has the potential to yield up to 800 mlCH$_4$ gVS^{-1} (Heaven *et al.,* 2011). One of the reasons is that it does not require high concentrated and/or pre-dried biomass which reduces the energy inputs required to produce the feedstock (Pragya *et al.,* 2013).

However, some microalgae species have the ability to resist microbial degradation, achieving significantly lower methane yields than expected (Golueke *et al.,* 1957; Ometto *et al.,* 2014). Detailed investigations on microalgae structure and chemical composition identified the cell wall as the main limiting factor to microbial degradation (Atkinson *et al.,* 1972; Burczyk *et al.,* 1999). In particular, cellulose and acetolysis resistant biopolymers (ARB), such as sporopollenin and algaenan, provide microalgae cells with the strength and thickness to resist bacterial degradation. During AD, limited cell wall degradation will affect the amount of intracellular algogenic organic matter (AOM) released in the media and, therefore, methane production.

High energy (thermal and ultrasound) and low energy (mechanical and biological) pre-treatments can be used to: (1) degrade the cell wall, (2) release AOM and hence (3) enhance methane production (Alzate *et al.,* 2012; González-Fernández *et al.,* 2012b; Cho *et al.,* 2013a). For example, a mixture of microalgae pre-treated at 110°C, 140°C and 170°C for 15 minutes produced increases in soluble COD which yielded methane increments of 19%, 33% and 46% respectively from an initial value of about 270 ml gVS^{-1} (Alzate *et al.,* 2012). Similar increases to methane production

have been obtained at lower temperatures i.e. 55°C (+ 11%), 75°C (+ 21%), 95°C (+ 39%), using a longer treatment time (5 hours) (Passos *et al.,* 2013).

When using ultrasound, the required specific energy input depends on the physical characteristics of the algae cell, e.g. shape, size and intracellular structure (Purcell *et al.,* 2013). For example, when the filamentous algae *Microspora* sp. was treated at 57 MJ kg^{-1}, a 60% increase in total COD solubilisation and 22% in methane production was achieved (Alzate *et al.,* 2012). Higher energy inputs were required to process single cell algae such as *Scenedesmus* sp. (130 MJ kg^{-1}) and *Chlorella* sp. (1600 MJ kg^{-1}) to double the methane production from 164 to 306 ml gVS^{-1} and from 250 to 450 ml gVS^{-1}, respectively (González-Fernández *et al.,* 2012b; Park *et al.,* 2013).

Very often, however, the additional methane yield does not offset the pre-treatment energy requirements leading to an overall negative energy balance (Cho *et al.,* 2013a; Passos *et al.,* 2013). Low energy pre-treatments are preferred as they are more likely to achieve a more balanced process. Mechanical pre-treatments, such as quartz sand grinding under wet or dry conditions, have shown limited benefits applied to *Chlorella* sp. for lipid extraction (Zheng *et al.,* 2011). In contrast, the same authors reported that biological pre-treatments using enzymatic additions like cellulases proved to be more successful. In agreement to this work, Yin *et al.* (2010) observed that the addition of cellulases to *C. sorokiniana* enhanced cell wall degradation and produced increases in the release of proteins, peptides and sugars of 25, 6 and 8 times, respectively, after three hours at 50°C. Similarly, the addition of cellulases to *C. vulgaris* produced 60% and 85% hydrolysis yields, after 24 h and 72 h treatment (Cho *et al.,* 2013b). To the best of our knowledge, only the work of Ehimen *et al.* (2013) investigated the effect of this treatment on AD, reporting an increase in methane production of 40% pre-treating *Rhizoclonium* biomass (filamentous cladophorales) with an enzymatic mixture containing protease, α-amylase, xylanase, lipase and cellulose.

The fact that pre-treatments increase the soluble COD fraction is a clear indication of intracellular AOM release, caused by a stress to the microalgae cells which can result in higher digestion efficiency. Although different studies have compared the digestibility improvements of the algae biomass subjected to different pre-treatments (Alzate *et al.,* 2012; González-Fernández *et al.,* 2012c; Cho *et al.,* 2013a; Ehimen *et*

al., 2013), so far their effects on the structure of the microalgal cells have not been fully investigated. Attempts to quantify physical cell breakage efficiency have been made using light microscopy, particle size distribution and dual-fluorescence analysis (González-Fernández *et al.,* 2012b; Purcell *et al.,* 2013), without a real understanding of the degradation mechanisms.

Batch anaerobic digestion experiments were used to assess the effect of different pre-treatments (thermal, thermal hydrolysis, ultrasound and enzymatic hydrolysis) on the methane production of three microalgae species (*Scenedesmus obliquus*, *Chlorella sorokiniana* and *Arthrospira maxima*) commonly found in wastewater treatments plants. The three algae, chosen for their differences in cell wall structure and composition, were tested under different operating conditions and optimised for maximum soluble COD release, and hence biogas production, with each process. In addition, this paper provides the first insight into the degradation mechanism of physical and biological pre-treatments on the algal cell.

3.2.2 Materials and methods

3.2.2.1 Analytical methods

The algae biomass was characterised for solids content and soluble matter composition (COD, proteins and carbohydrates) before and after each treatment. COD and solid content were measured in duplicate using standard methods (APHA). Soluble matter was obtained after centrifugation at 60,000 rpm for three minutes and syringe filtration (0.45 µm). The soluble protein (sP) and soluble carbohydrate (sC) contents were quantified using the methods described by Frølund *et al.* (1995) and Dubois *et al.* (1956). Protein content was measured at 750 nm as bovine serum albumin (BSA) equivalent (Sigma-Aldrich, UK), while carbohydrate content was measured at 480 nm as glucose equivalent (Sigma-Aldrich, UK). The lipid content, defined as other compounds including lipids, was estimated by subtracting the protein and carbohydrate sCOD equivalent from the total sCOD, 1.25 gO_2 $gBSA^{-1}$ and 1.07 gO_2 $gGlucose^{-1}$, respectively. A statistical study on the sCOD set of data was performed using a Scheirer-Ray-Hare test with a significant acceptance at a *p* value equal to 0.05. The ratio between volatile suspended solids (VSS) and total volatile solids (VS) was adopted as the solubilisation index.

Visual inspection of the algae cells was performed using an optical microscope and TEM analysis. For TEM sample preparation, concentrated cell paste was washed with a series of rinses (sodium cacodylate buffer) and fixatives (Gluaraldehyde, Osmium tetroxide and Uranyl acetate), dehydrated with Ethanol and Propylene oxide and embedded in Araldite CY 212 as described by Audrey *et al.* (1998). All analyses were carried out in triplicate.

3.2.2.2 Algal cultures

All algae cultures were obtained from the Culture Collection for Algae and Protozoa (CCAP), (Oban, UK). *S. obliquus* (276/42) and *C. vulgaris* (211/BK) were grown on Jaworski media at 18°C under constant illumination while *A. maxima* (1475/9) was grown on Zarrouk media at 28°C using a 16/8 hours light/dark cycle (Ometto *et al.,* 2014). The algae biomass was collected during the stationary growth phase (Appendix A) and concentrated to 20 ± 2 g l^{-1} as total solids (TS) by a combination of sedimentation and centrifugation. Samples were stored at 4°C, for a maximum of 7 days, before pre-treatment.

3.2.2.3 Pre-treatments condition

3.2.2.3.1 Thermal and Thermal Hydrolysis

Thermal (T) and thermal hydrolysis (TH) treatments were undertaken using a Baskerville autoclave and steam generator WON15827 (Manchester, UK). The unit is composed of two connected pressure vessels: a reactor vessel and a steam generator. For thermal treatments, an aliquot of concentrated algal biomass (200 ml) was placed in the reactor vessel, heated and maintained at the required temperature for 30 minutes. For thermal hydrolysis treatments, steam was first generated in the steam generator vessel and then injected into the reactor unit containing the same amount of algae biomass, pre-heated at 70⁰C to limit condensation effect.

Five temperatures were tested using both configurations: (1) 105°C, (2) 120°C, (3) 145°C, (4) 155°C and (5) 165°C, with associated saturated pressure close to 1, 2, 3, 5 and 7 bar. The final volume of the sample was measured and used for further calculations.

3.2.2.3.2 Ultrasound

Five specific energy inputs (E_i) were applied to each sample using a Hielscher Ultrasound UP400S (Teltow, DE): (U1) 0.35, (U2) 3.5, (U3) 10, (U4) 20 and (U5) 35 MJ kg TS^{-1}. Between 250 and 500 ml of concentrated algae was placed in a glass beaker and then placed in an ice bath to limit ultrasonically derived temperature increases. The power input was fixed at 100W (24 kHz) and the fixed energy input was achieved with a consequential exposition time of 50 sec (U1), 5 min (U2), 8 min (U3), 10 min (U4) and an additional 10 min (U5), respectively. Minor deviations from these values were observed due to equipment sensitivity and different TS in the initial samples according to Equation 3.1 (Alzate *et al.,* 2012):

$$E_i = \frac{P \times t}{V \times TS} \qquad (3.1)$$

where *P* represents the power (Watt), *t* the exposure time (seconds), *V* the sample volume (millilitres) and TS the total solid concentration (g l^{-1}). At the end of each treatment time, 100 ml of sample was collected and used for analysis.

3.2.2.3.3 Enzymatic

Five commercial enzymes (E1, E2, E3, E4, E5), with different specific activities, were tested under optimal environmental conditions according to the information provided by the suppliers (Table 3.5). Preliminary tests were undertaken to identify the optimal enzymatic concentration. Between 5 to 10 ml of algae was centrifuged, re-suspended in a pH 6 buffer solution (0.1M Na_2HPO_2 and 0.1M NaH_2PO_4) to reach 2% TS, and incubated with the enzymes for 24 h at 50°C using increasing enzyme concentrations (25, 50, 150, 250, and 350 u ml^{-1}). The released soluble content was measured as reported in section 3.2.2.1.

Table 3.5 List of enzymes and their characteristics.

Enzyme	Commercial name	Composition	Supplier
E1	DepolTM 40L	Cellulase 1,200 u g^{-1} + 800 u g^{-1} Endogalactouronase	Biocatalysts Ltd, UK
E2	LipomodTM 957	Esterase 3,600 u g^{-1} + Protease 90 u g^{-1}	Biocatalysts Ltd, UK
E3	DepolTM 220L	Alpha amylase 25,000 u g^{-1}	Biocatalysts Ltd, UK
E4	Pectinase P2611	Pectinase 3,800 u g^{-1}	Sigma Co Ltd, UK
E5	LipomodTM 166P	Esterase 5,220 u g^{-1}	Biocatalysts Ltd, UK

3.2.2.4 Batch anaerobic digestion test

Anaerobic digestion batch experiments were performed using a modified method of Angelidaki *et al.* (2009) as previously described in Ometto *et al.* (2013). Briefly, tests with untreated and treated algae biomass (substrate) were conducted in a 1:1 (substrate:inoculum) volatile solid (VS) ratio, using digested sludge obtained from a local wastewater treatment plant as inoculum (Appendix B). Samples were flushed with N_2 gas, sealed with a PTFE crimp cap, and placed at 38°C under constant agitation (150 rpm). Biogas production was determined every two to three days until no significant gas production was detected, for a maximum of 35 days. Data was converted to standard temperature and pressure (STP). The biogas volume produced by the test substrate was corrected by subtracting the average blank controls production (inoculum + nutrients). Methane content was detected using a

Servomex 1440 gas analyser (Crowborough, UK). For the digestion of enzymatically pre-treated algae, using E1, E2 and a 1:1 (E1:E2) mixture at 150 u ml^{-1}, separate blank tests were carried out to quantify the effect of the enzymatic addition to the inoculum digestion. All experiments were conducted in triplicate and cellulose was used as an external control to verify inoculum activity over time.

3.2.2.5 Energy balance

The ratio between energy input and energy output (E_i/E_o) of each pre-treatment condition was considered as an indication of the energy balance. Values lower or equal to 1 represent positive and neutral balance, respectively (Passos *et al.,* 2013). The energy output (E_o = kJ gVS^{-1}) was measured on the net increase methane content (ΔP) expressed in ml CH$_4$ gVS^{-1}, multiplied by the methane heating value (ξ = 35,800 kJ mCH$_4$$^{-3}$) as reported in Equation 3.2:

$$E_o = \frac{\Delta P \times \xi}{10^6}$$
(3.2)

The energy input (E_i = kJ gVS^{-1}) was estimated using Equation 3.1 (section 2.2.2) for ultrasound treatment, and Equation 3.3 (Passos *et al.,* 2013) for thermal and enzymatic treatments, where the main energy input was related to the heat required to raise the biomass from the initial temperature (T_0) to the pre-treatment temperature (T_p). T_0 was assumed equal to the ambient temperature (20 °C) while T_p was equal to 50°C, 105°C, 120°C, 145°C, 155°C or 165°C. For specific density (ρ) and specific heat (γ) values, microalgal suspension was assumed equal to water, 1 g ml^{-1} and 4.18 x10^{-3} kJ g^{-1} °C^{-1}, respectively. Heat losses were assumed to be negligible, while heat recovery efficiency (ɸ) was assumed equal to 85%.

$$E_i = \frac{\rho \times V \times \gamma \times \left(T_p - T_o\right) \times (1 - \varphi)}{VS}$$
(3.3)

3.2.3 Results and Discussion

3.2.3.1 Pre-treatments optimisation

3.2.3.1.1 Thermal and Thermal Hydrolysis

During thermal (T) and thermal hydrolysis (TH) pre-treatments, the biomass was subjected in both cases to a combined thermal and pressure increase, causing cell degradation and an incremental release of the sCOD fraction (Figure 3.8). Preliminary investigations demonstrated that the range of pressures reached during the experiments (from 1 to 7 bar) alone were too low to affect the microalgae cell structure (data not shown). It was therefore assumed that the temperature was the main regulator mechanism for biomass solubilisation in both systems (Valo *et al.,* 2004; Cho *et al.,* 2013a).

After thermal hydrolysis (TH), the amount of sCOD released by *S. obliquus* and *C. sorokiniana* increased with the temperature to a maximum of 508 mg gTS^{-1} (22 fold) and 400 mg gTS^{-1} (5.4 fold), respectively. This was translated in a VSS/VS ratio decrease, from an initial value close to 1, to 0.58 ± 0.02 with both algae corresponding to a 40% biomass solubilisation (Table 3.6). Similarly, Cho *et al.* (2013a) obtained a 5.5 fold sCOD increase after autoclaving a mixture of *Scenedesmus* sp. and *Chlorella* sp. for 30 min at 120°C, while Keymer *et al.* (2013) obtained a 10 fold sCOD increase at 170°C using a mixture of natural algae, confirming comparable treatment efficiency. In contrast, the cyanobacteria *A. maxima* released a constant amount of sCOD between 105°C and 155°C with a VSS/VS ratio between 0.71 and 0.78 across the range. At 165°C the sCOD concentration doubled to 116 mg gTS^{-1} in both pre-treatments (Figure 3.8), reaching a 65% solubilisation, higher than the one obtained with the single cell algae.

At temperatures lower than 150°C, each algal species released similar amounts of sCOD in the two pre-treatments, confirming our initial hypothesis that identified the temperaure as the main degradation regulator. At temperatures higher than 150°C, the two green algae released significantly more sCOD with the TH treatment than with the thermal treatment, whereas the sCOD increase with the filamentous cyanobacteria was again similar for both pre-treatments. This suggests that, for single cell algae characterised by the presence of cellulose and ARB, the rapid change of temperature/pressure caused by steam injection was only effective at pressures higher than 4 bar.

Floc formation due to cell wall breakage was observed with all three microalgae; *S. obliquus* and *C. sorokiniana* produced significant floc formations only at temperatures higher than 145°C, while *A. maxima* started to form aggregates at 105°C. Post-treatment floc formation due to exopolymers and intracellular compounds released during the treatment is an indication of cell wall breakage (González-Fernández *et al.,* 2012b). At 165 °C, *A. maxima* showed a homogenous mixture which, together with high sCOD values and low VSS/VS ratios, suggests an almost complete biomass solubilisation. These results show that, in addition to the temperature of the treatment, the algal species and characteristics are equally important when using thermal pre-treatments. Cellulose-free cells, such as cyanobacteria, achieve significantly higher cell damage at lower temperatures while ARB-enriched cells, such as green algae, require more than 165°C to achieve complete solubilisation.
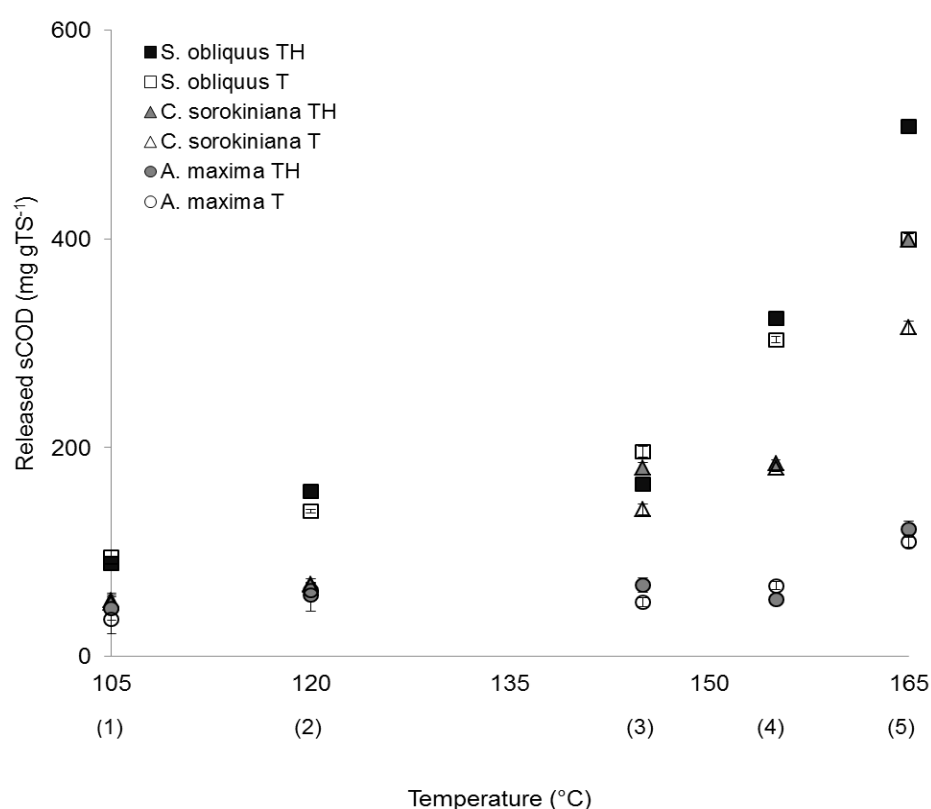

Figure 3.8 Thermal (T) and Thermal Hydrolysis (TH) solubilisation. Released soluble COD at increasing treatment temperatures (p<0.05).

3.2.3.1.2 Ultrasounds

When exposed to increasing energy with ultrasounds, all three algae released additional sCOD. The two green algae *S. obliquus* and *C. sorokiniana* showed a linear correlation between the energy input and the sCOD released (Figure 3.9). The highest sCOD increase occurred at 35 MJ kg TS^{-1} (U5), 10 fold for *S. obliquus* (346 mg gTS^{-1}) and 5 fold for *C. sorokiniana* (166 mg gTS^{-1}). However, both algae reported a limited solid solubilisation (VSS/VS), close to 20% (Table 3.6). In contrast, *A. maxima* reached 82% solubilisation at a lower energy input of 10 MJ kgTS^{-1} (U3) showing small differences when subjected to higher energy treatments. Due to the lack of cellulose and ARB components, this confirmed that, similar to what was observed for the thermal treatments, *A. maxima* can be degraded more easily than the two green algae. Furthermore, compared to *Scenedesmus* sp. and *Chlorella* sp., *Arthrospira* sp. is characterised by the presence of septa and air vesicles on the cell wall structure making this alga particularly sensitive to the localised high pressures produced by ultrasonic treatment (Purcell *et al.,* 2013).

In agreement with our results, when treating different mixtures of algae biomass, Alzate *et al.* (2012) reported different energy demands to achieve equal COD solubilisation for filamentous and single cells algae. *Microspora* sp., a filamentous algae, was efficiently treated using 50% less energy (~25 MJ kg^{-1}) than single cells algae *Acutodesmus obliquus*, *Oocystis* sp. and *Nitzschia* sp. Limited impact on the cell wall structure of single cell microalgae, despite the high energy used, was also reported by González-Fernández *et al.* (2012a) who observed similar particle size distribution when treating *S. obliquus* at energy inputs ranging from 35.5 MJ kg^{-1} (equal to U5) to 129 MJ kg^{-1}. Our results suggest that ultrasound produced more structural damage on the cells of *A. maxima* whereas most of the sCOD released by the green algae was the result of intracellular AOM escaping the cell boundaries.
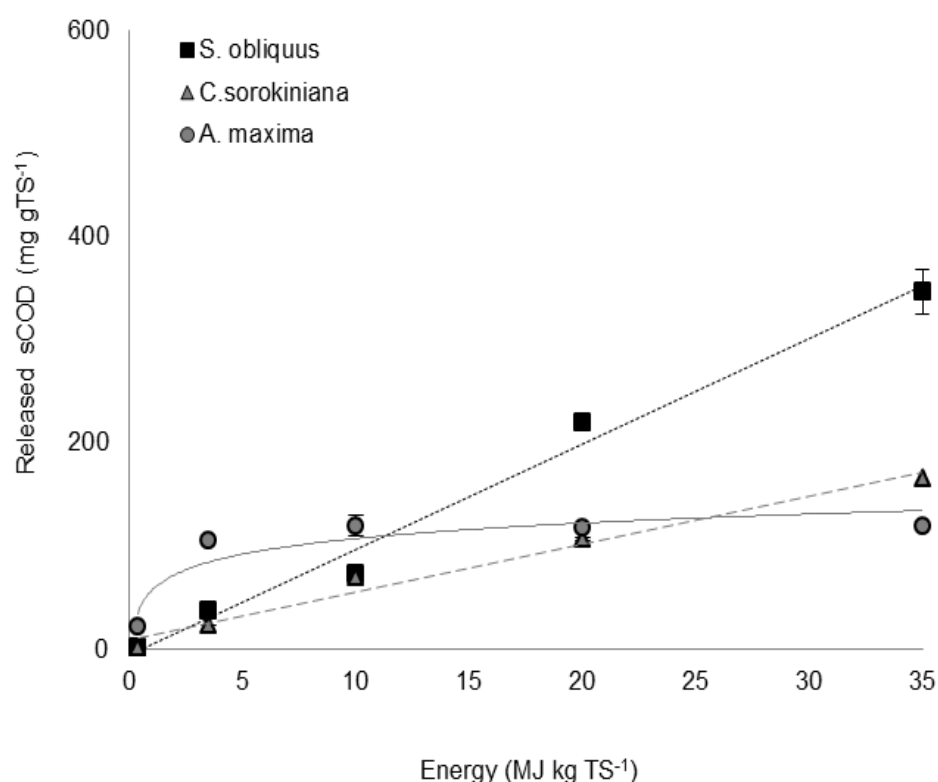
Figure 3.9 Ultrasound solubilisation. Released soluble COD after exposure to increasing amount of energy ($p < 0.05$).

3.2.3.1.3 Enzymatic hydrolysis

Enzymatic hydrolysis was performed using single enzymes and mixed enzymatic preparations. During these pre-treatments, the action of low temperature thermal treatment (50°C for 24 hours) was combined with the catalytic activity of the enzymes. Compared to single enzymes, mixtures of enzymes released more than double the sCOD (Figure 3.10). The cellulase and pectinase mix (E1) and the esterase and protease mix (E2) were the most effective catalysts for all three algae followed by the single enzyme esterase (E5). The α-amylase (E3) was particularly active on *C. sorokiniana*, whereas pectinase (E4) mainly degraded *S. obliquus* suggesting a more selective action for these two enzymes being cell-algal and cell-wall component specific.

A consistent dose of between 150 and 250 u ml^{-1} was required to maximise the sCOD released irrespective of the enzymes used (Figure 3.10). In particular, using E2 *S. obliquus* required 250 u ml^{-1} to release up to 360 mg gTS^{-1} as sCOD, while

with *C. sorokiniana* and *A. maxima* maximum hydrolysis occurred with 150 u ml^{-1} releasing 389 and 434 mg gTS^{-1}, respectively. Similar dosages were applied by Yin *et al.* (2010) using *Chlorella* sp., suggesting optimal enzymatic additions for microalgae with a low (10% w/w) and high (20% w/w) solid concentration close to 150 u ml^{-1}. Although it was not possible to measure the VSS/VS ratio, due to the influence of the enzymatic addition on the VS, the amount of sCOD released by the enzymes was similar to the amount released by the thermal treatment (TH165), suggesting a comparable solid solubilisation (35 – 45%).

As for the other pre-treatments, the performance of the enzymes is linked to the cell wall composition of the different algae. The main components of *Scenedesmus* sp. and *Chlorella* sp. cell wall are sugars (24 – 74%), uronic acid (4 – 24%), proteins (2 – 11%), glucosamine (0 – 15%), in addition to cellulose and hemicellulose (Blumreisinger *et al.,* 1983). On the contrary, the cell wall of *A. maxima*, a cellulose-free microalga, is composed of murein (peptidoglycan) layers covered by a coat of lipopolysaccharide (Tomaselli, 2007). In agreement with previous investigations, cellulases performed well with all three algae (Yin *et al.,* 2010; Fu *et al.,* 2010; Harun *et al.,* 2011; Zheng *et al.,* 2011). However, in our work, the mix of protease and esterase released higher amounts of sCOD than those previously reported (Sander and Murthy, 2009; Ehimen *et al.,* 2013). This suggests that for an effective enzymatic hydrolysis of the microalgae wall, it is necessary to take into account the protein and polysaccharide component as well as the cellulose component. The visual observation of the pre-treated algal biomass revealed a significant change in colour from dark green to dark brown with no formation of algal floc. This suggests that with the enzymatic hydrolysis, different breakage mechanisms produced the release of sCOD compared to the thermal and ultrasound pre-treatments.
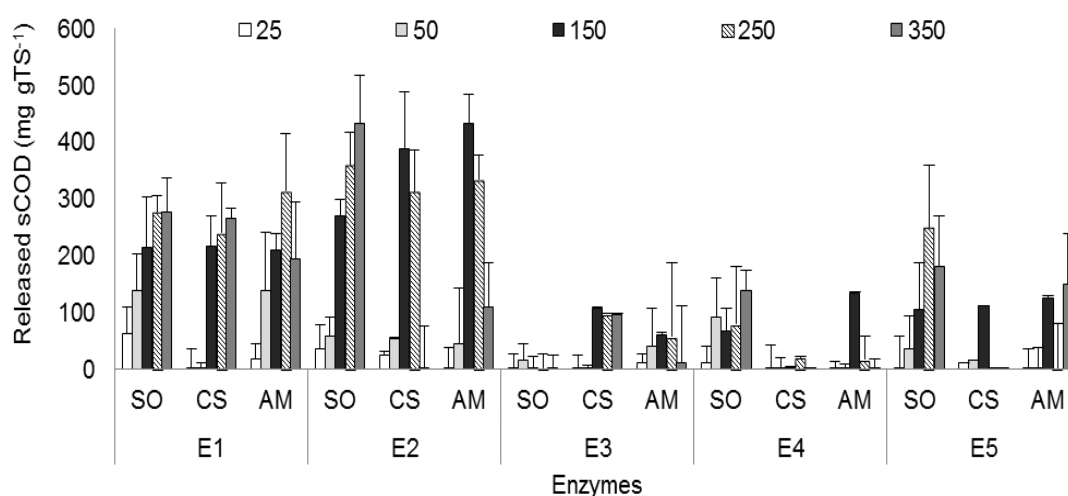
Figure 3.10 Enzymatic hydrolysis. Released soluble COD released (out of sCOD released by control sample incubated without any enzymes) with different enzymes (E1-E5) at increasing dosage (25, 50, 150, 250, 350 u ml^{-1}) for *S. obliquus* (SO), *C. sorokiniana* (CS) and *A. maxima* (AM) (p<0.05).

3.2.3.2 Treatment comparison: efficiency and cell wall breaking mechanisms

The composition of the AOM released by thermal hydrolysis at 165ºC (TH5) and ultrasonic pre-treatment at 35 MJ kg TS^{-1} (U5) was compared to the enzymatic one (average value between E1 and E2 at 150 u ml^{-1}). The amount of soluble proteins (sP), carbohydrates (sC) and other compounds, including lipids (sL), released by the algal biomass changed significantly with each treatment and for each of the algal species investigated (Figure 3.11). The differences in composition of the soluble fraction indicate different mechanisms of action between the three pre-treatment processes tested. For single cell algae such as *S. obliquus* and *C. sorokinian,a* most of the protein and lipid content of the biomass belongs to the intracellular AOM, while carbohydrates are the main constituent of the cell wall (Blumreisinger *et al.,* 1983; Heaven *et al.,* 2011). Consequently, releases of organics that are high in protein and lipid content suggest an efficient release of the AOM and cell wall breakage, whereas high sugar concentrations suggest efficient degradation and solubilisation of the cell wall constituents. Accordingly, the differences in composition can be used as a diagnostic indicator of the principle mechanism of action.

Thermal hydrolysis was the most effective pre-treatment in releasing proteins, followed by ultrasounds and enzymes. To illustrate, in the case of *S. obliquus*, the

protein content released by thermal hydrolysis (80% of sCOD) was equal to 331 mg gTS^{-1} as BSA, 2.8 and 4.2 times higher than with ultrasound and enzymes, respectively. Similarly, *C. sorokiniana* (77% of sCOD) reported a 4.5 fold increase in protein content compared to ultrasounds and 1.2 fold increase compared to enzymatic hydrolysis. No significant differences were observed with *A. maxima* across the three systems with an average value close to 50 mgBSA gTS^{-1} (~50% of sCOD after TH and U, 20% after enzymatic hydrolysis).

Ultrasounds released the highest amount of other/lipids, equal to 46% and 31% of the total sCOD for *S. obliquus* and *C. sorokiniana*, respectively, followed by enzymatic and thermal hydrolysis. When using *A. maxima,* the enzymatic hydrolysis was more effective than ultrasound, producing more than 70% of the total released sCOD as lipid-related compounds. Consequently, both ultrasound and thermal hydrolysis resulted in cell wall breakages and the associated release of AOM.

In contrast, enzymatic hydrolysis was the most effective pre-treatment in releasing carbohydrates followed by TH and ultrasound, and so operated by cell wall degradation rather than direct breakage. In particular, with *S. obliquus*, enzymatic hydrolysis (38% of sCOD) increased the soluble carbohydrate concentration by 1.7 times more than TH, and 4.5 times more than ultrasound. Similarly, with *C. sorokiniana*, the sC concentration increased to 42 mg gTS^{-1} as glucose with ultrasound, and to 65 and 95 mg gTS^{-1} after TH and enzymes, respectively. *A. maxima* showed similar low concentrations after each treatment (5% of sCOD as an average value).

A. maxima has a different distribution of proteins, carbohydrates and lipids between the AOM and the membrane (Heaven *et al.,* 2011; Tomaselli, 2007). A high lipid content suggests membrane degradation and solubilisation, whereas high protein and carbohydrate contents indicate the release of intracellular AOM. Hence, enzymes seem to act preferentially on cell wall components, while thermal and ultrasound treatments produce a release of AOM due to structural cell wall deterioration.
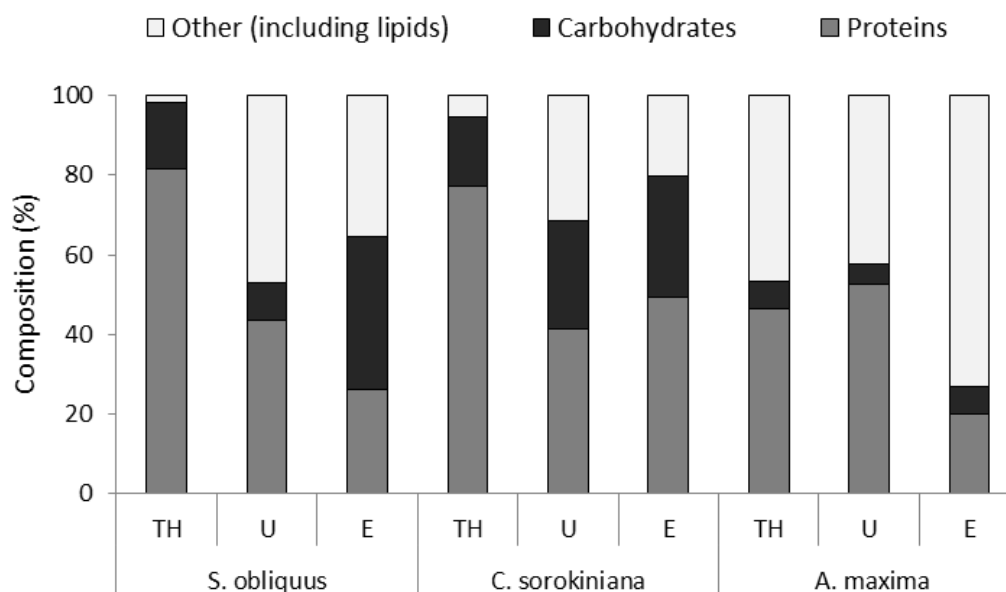
Figure 3.11 Comparison between soluble content released by thermal hydrolysis (TH), ultrasound (U) and enzymatic (E) treatments on algae biomass.

The difference in action mechanisms was confirmed by microscopic analysis of untreated and treated green algae cells (Figure 3.12). The TEM images of untreated cells clearly showed all main microalgae cell components, such as the cell wall, the nucleus and thylakoids filling most of the cytoplasm (Figure 3.12, SO1 and CV1). Thermal hydrolysis expanded and partially disaggregated the cell wall structure causing the release of internal AOM in the media. This is clearly shown in Figures 3.12 SO2 and CV2 by the loss of cell turgidity and the appearance of empty/clear areas inside the cells boundaries. As previously reported by Choi *et al.* (2011), ultrasound treatment caused a loss of external cell boundaries (small black points surrounding the cell wall) and release of AOM into the media (Figures 3.12, SO3 and CV3). Loss of external cell boundaries and cell turgidity were less evident after enzymatic hydrolysis, despite the high amount of AOM released (clear spaces inside the cells boundaries and high measured sCOD). The less distorted cell structure suggests a more specific degradation and solubilisation of the cell wall components (e.g. mono-, di-saccharides) (Figure 3.12, SO4 and CV4). For instance, investigating the sugar composition of the material released by enzymatic hydrolysis of *Chlorella* sp., Rodrigues and da Silva Bon (2011) were able to ascertain the cell wall composition of different algae strains demonstrating specific enzymatic activities on cell wall.
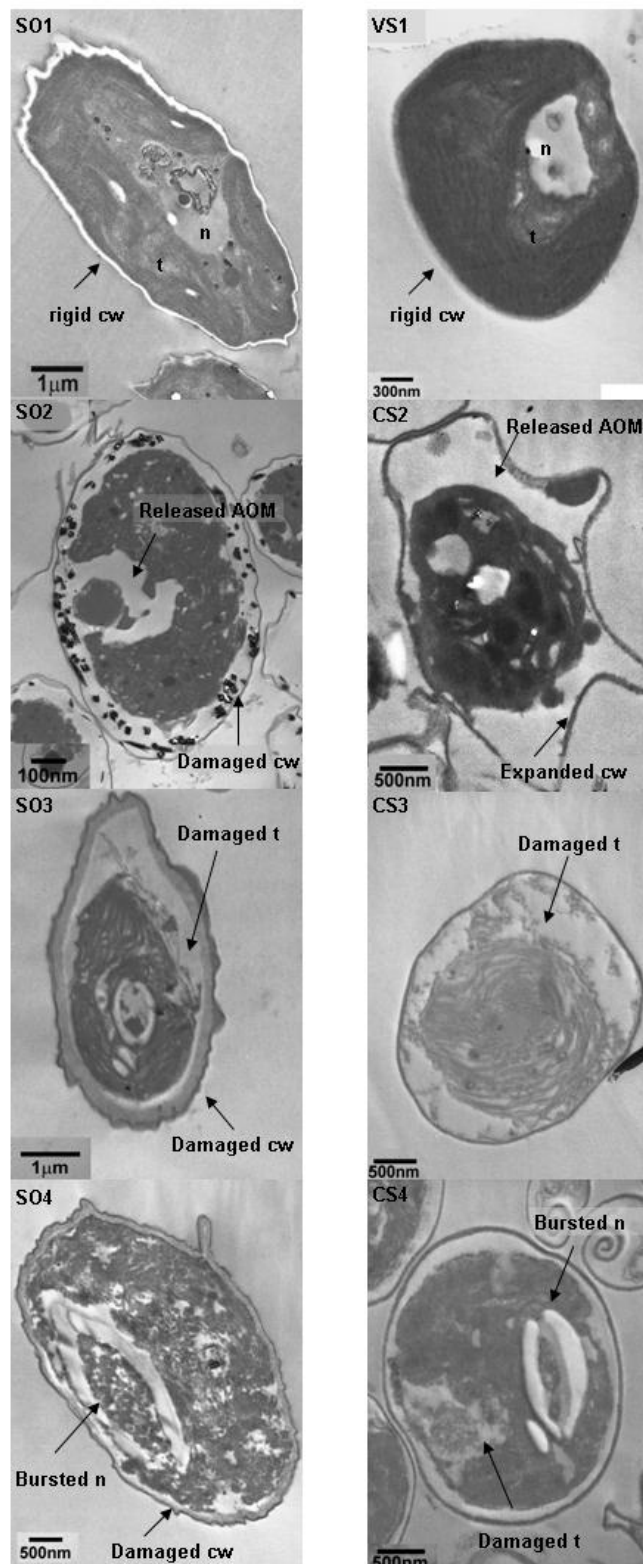
Figure 3.12 TEM picture of *S. obliquus* (SO) and *C. sorokiniana* (CS), untreated (1) and after thermal hydrolysis (2), ultrasound (3) and enzymatic (4) treatment. Nomenclatures: cw= cell wall; n= nucleus, t= thylakoids.

3.2.3.3 Pre-treatment effects on energy recovery

3.2.3.3.1 Anaerobic digestion batch test

Anaerobic digestion of untreated microalgae biomass produced up to 88 ± 2, 118 ± 5 and 60 ± 6 ml kg VS^{-1} as methane, for *S. obliquus*, *C. sorokiniana* and *A. maxima*, respectively. All pre-treated biomass showed digestion improvements with the only exception of *A. maxima* when treated at 105°C and 120°C (Table 3.6). With thermal treatments, the maximum methane improvement occurred at 165°C with *S. obliquus* yielding 268 ± 2 ml kg VS^{-1} (+ 208%) followed by *A. maxima* (+ 70%) and *C. sorokiniana* (+ 98%). Compared to the thermal treatments, the ultrasound produced 96% methane improvement with *S. obliquus*, 38% with *A. maxima* and 42% with *C. sorokiniana*. Using similar digestion conditions, Alzate *et al.* (2012) reported 55% methane production improvement, from 198 ± 9 to 307 ± 9 ml gVS^{-1}, digesting a mixture of natural algae pre-treated at 170°C. When treated with ultrasound (10 - 57 MJ kg^{-1}), the same algal mixture showed a methane yield increment of between 6 and 13%, confirming that thermal treatments can be more effective than ultrasounds in enhancing microalgae digestibility.

Compared to thermal treatments, enzymatic hydrolysis produced significantly higher amounts of methane. Depending on the algae species when using E1 and E2, the methane production improved up to 12 times with yields ranging between 477 and 730 ml gVS^{-1}. Using a 1:1 (E1:E2) enzymatic mixture, further increases were achieved at a 16 fold increase in methane production increments with *A. maxima*, 6.7 folds with *S. obliquus* and 3.5 folds with *C. sorokiniana*.

Similar results were observed when digesting PS undergoing the same pre-treatments. The highest methane increase, equal to 174%, was obtained after enzymatic hydrolysis (E1:E2), while thermal hydrolysis (TH5) and ultrasound (U5) produced improvements of 111% and 45%, respectively.

To our knowledge, a direct comparison on AD production of similar enzymatically treated microalgae has never been published. However, the work of Donoso-Bravo and Fdz-Polanco (2013) on enzymatic treatment of sewage sludge enriched with grease trap waste compares well to our results with E1 (585 ml gVS^{-1}). Using lipases as main hydrolytic agents, the authors measured a methane production of 500 ml gVS^{-1} equal to a 130% increase.

Although enzymes are sensitive to pH and temperature changes, once inside the AD reactor, they are likely to contribute to the overall digestion process, firstly by supporting the hydrolysis activity, and secondly by contributing to the biogas production as an additional substrate (proteins). For instance, while the batch digestion of the inoculum showed a biogas production equal to 50 ± 5 ml gVS^{-1}, the inoculum with the enzymes produced between 100 and 150 ml gVS^{-1} biogas, depending on the enzymes.

In agreement with previous investigation on AD feedstock pre-treatments, digestion improvements were proportional to the COD solubilisation achieved (Carrère *et al.,* 2010). However, similar amounts of sCOD and VS released by the same algae produced different methane yields when pre-treated with different processes (Figure 3.13). For instance, with *C. sorokiniana*, sCOD concentration of 200 mg gVS^{-1} produced between 150 and 200 ml gVS^{-1} of methane after thermal or ultrasound pre-treatment (Figure 3.13 A) and 400 ml gVS^{-1} after enzymatic hydrolysis (Figure 3.13 B). Similar results were observed with S. obliquus and *A. maxima* at 450 mg sCOD and 1300 mg sCOD, respectively (Figure 3.13).

These results mirror those reported in Section 3.2.3.2 for the action mechanism of the pre-treatments. The higher yields obtained after enzymatic hydrolysis were the direct consequence of efficient cell wall biochemical degradation which enabled (1) removal of the limiting factors affecting the AD process (cellulose and ARB being able to resist bacteria degradation) and, depending on the algae species, (2) release of a higher amount of energetically valuable components such as sugars and lipids. In contrast, the high intracellular AOM released after thermal and ultrasonic treatments was the result of cell wall breakage only which did not allow the solubilisation of bacterial resistant or inhibitory compounds.
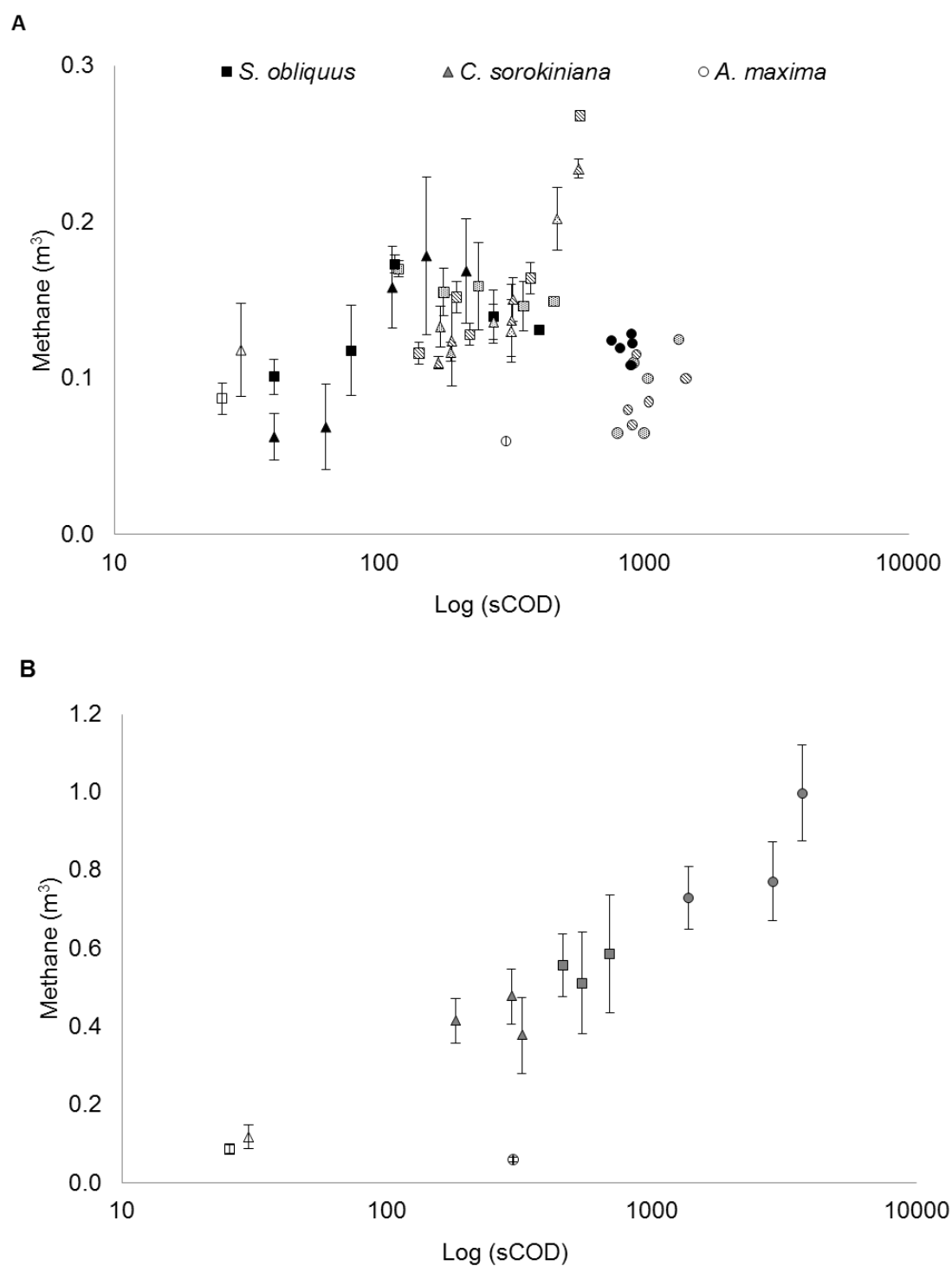
Figure 3.13 Methane productions per available sCOD post (A) physical pre-treatments including thermal (dotted markers), thermal hydrolysis (lined markers) and ultrasound (full markers), and (B) enzymatic hydrolysis. Empty markers represent untreated biomass.

Table 3.6 Summary table: biogas production pre and post treatment (mean±SD) of all tested biomass.

Treatment	Scenedesmus obliquus					Chlorella sorokiniana					Arthrospira maxima					Primary Sludge				
	VSS/VS	Biogas (ml/gVS$_{add}$)	Increase[a] (%)	CH$_4$[b] (%)	E_i/E_0	VSS/VS	Biogas (ml/gVS$_{add}$)	Increase[a] (%)	CH$_4$[b] (%)	E_i/E_0	VSS/VS	Biogas (ml/gVS$_{add}$)	Increase[a] (%)	CH$_4$[c] (%)	E_i/E_0	VSS/VS	Biogas (ml/gVS$_{add}$)	Increase[a] (%)	CH$_4$[b] (%)	E_i/E_0
Control[d]	0.95	265±10	-	60	-	0.96	273±37	-	62	-	1.16	185±20	-	-	-	0.82	480±38	-	68	-
T1	0.83	270±11	10	60	3.09	0.87	297±27	9	55	4.73	0.78	129±20	-11	-	-	0.55	484±91	38	63	**0.49**
T2	0.87	359±14	35	60	**1.33**	0.96	310±68	14	66	11.12	0.79	127±24	-12	-	-	0.45	451±45	29	66	**0.55**
T3	0.78	337±39	27	65	**1.58**	0.61	356±16	30	58	5.96	0.78	219±06	51	-	27.64	0.55	440±78	26	66	**0.96**
T4	0.83	370±35	40	63	2.06	0.54	313±54	15	60	6.14	0.78	175±14	21	-	78.06	0.55	412±96	18	61	**1.15**
T5	0.6	381±14	44	68	2.11	0.63	393±50	44	69	**1.67**	0.37	250±5	72	-	21.80	0.56	567±65	62	66	**0.66**
TH1	0.85	331±85	25	60	2.51	0.79	301±11	10	57	6.54	0.77	157±09	8	-	212.97	0.55	415±64	19	65	**0.81**
TH2	0.82	340±14	28	67	**1.39**	0.86	298±20	9	58	10.01	0.71	138±12	-5	-	-	0.39	507±74	45	65	**0.59**
TH3	0.75	313±23	18	63	2.69	0.72	324±53	19	60	8.34	0.78	173±18	19	-	85.42	0.55	567±57	62	66	**0.54**
TH4	0.89	404±6	52	60	**1.60**	0.53	315±41	15	64	3.86	0.77	235±9	62	-	24.16	0.55	510±82	46	63	**0.53**
TH5	0.58	548±2	107	70	**0.75**	0.57	461±4	69	69	**1.22**	0.35	200±40	38	-	43.60	0.58	640±3	83	68	**0.46**
U1	0.99	266±27	2	64	1.21	0.95	249±12	2	62	5.43	0.75	206±3	33	-	1.88	0.97	675±50	29	70	0.26
U2	0.99	299±24	14	60	4.18	0.88	252±40	3	65	10.86	0.49	199±21	28	-	22.22	0.82	654±23	24	69	4.11
U3	0.89	333±16	27	73	3.83	0.89	320±25	31	66	3.17	0.18	203±22	31	-	58.19	0.82	520±37	9	70	55.42
U4	0.90	292±12	8	63	13.21	0.90	368±12	34	64	5.26	0.15	180±5	16	-	214.87	0.80	553±84	5	68	14.64
U5	0.89	307±15	18	65	27.85	0.83	375±35	53	65	10.06	0.13	214±18	38	-	162.94	0.73	616±17	18	69	13.06
E1[e]	-	1425±224	403	63	**0.06**[h]	-	1158±116	236	46	**0.07**[h]	-	1461±173	630	-	**0.04**[h]	-	975±84	174	62	**0.08**[h]
E2[f]	-	1065±201	273	58	**0.07**[h]	-	868±253	227	56	**0.10**[h]	-	1545±201	672	-	**0.04**[h]	-	609±54	71	62	**0.10**[h]
E1:E2[g]	-	1669±63	485	63	**0.06**[h]	-	1292±148	387	60	**0.09**[h]	-	1996±254	898	-	**0.03**[h]	-	886±268	150	68	**0.08**[h]

[a]*compared to the control batch experiment performed with every new batch of algae;* [b]*methane content at the end of the batch digestion;* [c]*not directly measured over time and assumed equal to 60%;* [d]*average value between all control experiment performed;* [e]*inoculum+enzymes biogas production equal to 102 ± 19 ml gVS^{-1};* [f]*inoculum+enzymes biogas production equal to 121 ± 23 ml gVS^{-1};* [g]*inoculum+enzymes biogas production equal to 124 ± 22 ml gVS^{-1};* [h]*energy to produce the enzymes was exclude from the calculation;*

3.2.3.3.2 Energy aspects

In most of the tested conditions, the excess methane production was not sufficient to balance the energy required to pre-treat the biomass. Data plotted on a log-log scale chart (Ei/Eo ratio vs biogas increment) shows three clusters for the three main different pre-treatments (Figure 3.14). The enzymatic pre-treatment results are located at the bottom right part of the graph representing the most energetically balanced conditions, as the biogas production over balanced the energy input (+15 kJ kgVS^{-1}). The central part of the diagram is predominantly populated by thermally treated biomass and distributed across the neutral energy balance line (log Ei/Eo = 1). Higher energy inputs (higher temperatures), were responsible for higher methane yields and a more positive energy balance. Results relating to the ultrasound are located on the top left part of the diagram, with an average net energy balance of close to -30 MJ kg VS^{-1}. In this case, low specific energy inputs were more energetically efficient even with methane increments lower than 50%. To compare, the results reported by Cho *et al.* (2013a) on *Scenedesmus* sp. and *Chlorella* sp. biomass pre-treated with ultrasound (39 - 234 MJ kg VS^{-1}) and thermal treatment fit the clusters of Figure 3.14, confirming the different energy impacts of the two pre-treatments. When applied to wastewater sludge, thermal treatment showed more positive energy balances than treated microalgae, while ultrasounds and enzymatic hydrolysis provided similar results. The more negative energy balance of *A. maxima* when subjected to thermal pre-treatments was related to the low biogas yields measured in the batch tests. This was mainly due to the release of lower energy content compounds compared to those released after ultrasounds and enzymatic hydrolysis.
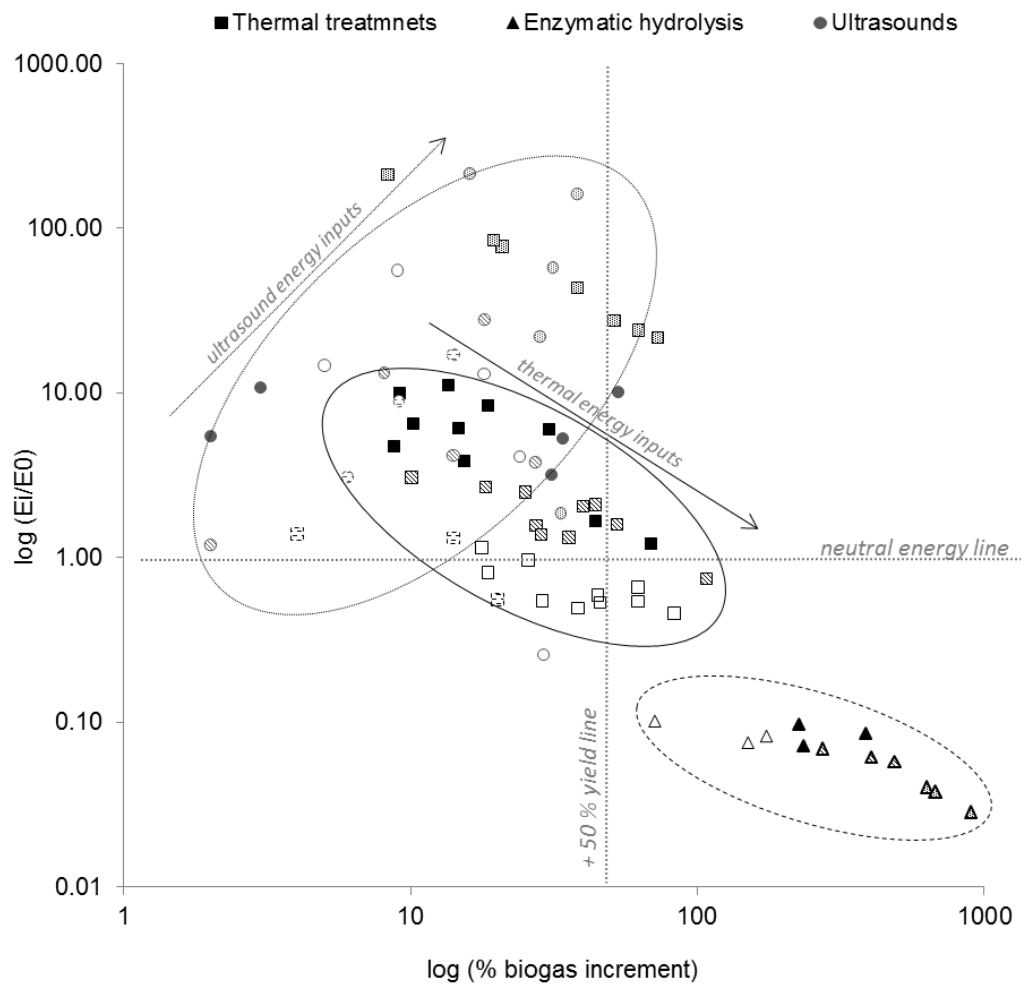
Figure 3.14 Energy balance of each treated biomass at related percentage biogas increment: *S. obliquus* (lined markers), *C. sorokiniana* (full markers), *A. maxima* (dotted markers) and primary sludge (empty markers); fragmented markers line (Cho *et al.,* 2013)

3.2.4 Conclusions

The pre-treatment process used to degrade microalgae cells and the algae species involved in the process have a substantial impact on the efficiency of the digestion of microalgae biomass. Although each of the pre-treatments analysed improved the soluble fraction of the biomass by reducing its VS content, only the enzymatic hydrolysis enabled the solubilisation of the cell wall constituents. This allowed a higher biogas production compared to thermal and ultrasound pre-treatments responsible for physical cell wall degradation (deformation and breakage). In particular, ARB-enriched algae (e.g. *Chlorella* sp. and *Scenedesmus* sp.) required more energy intensive pre-treatments (higher temperatures, higher specific energy and higher enzymatic dosages) than cellulose free algae (e.g. *Arthrospira* sp.).

Although the current study is based on small batch experiments, the findings suggest the key role of pre-treatments is in the optimisation of biogas production from microalgae. Of the methods currently used by the industry to pre-treat organic waste and sludge, ultrasounds produced the most energetically imbalanced process while high temperature thermal hydrolysis and enzymatic hydrolysis were the most energetically efficient. Further investigation and experimentation into the effect of enzymatic additions on AD is strongly recommended to validate the very positive impact on the process and their costs/feasibility for large scale applications.

3.2.5 Acknowledgements

3.2.6 References

Acién, F.G., Fernández, J.M., Magán, J.J., Molina, E., 2012. Production cost of a real microalgae production plant and strategies to reduce it. Biotechnol. Adv. 30, 1344-1353.

Alzate, M.E., Munoz, R., Rogalla, F., Fdz-Polanco, F., Pérez-Elvira S.I., 2012. Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment. Bioresour. Technol. 123, 488-494.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., van Lier J.B., 2009. Defining the biomethane potential (BMP) of solid organic waste and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59, 927-934.

Atkinson, A.W.J., Gunning, B.E.S., John, P. C. L., 1972. Sporopollenin in the cell wall of Chlorella and other algae: Ultrastructure, chemistry, and incorporation of 14C-acetate, studied in synchronous cultures. Planta, 107(1), 1-32.

Audrey M., Glauert Peter R. Lewis, 1998. Biological specimen preparation for transmission electron microscopy: practical methods in electron microscopy. Portland Press (1998).

Blumreisinger, M., Meindl, D., Loos, E., 1983. Cell wall composition of chlorococcal algae. Phytochemistry. 22(7), 1603-1604.

Burczyk, J., Śmietana, B., Termińska-Pabis, K., Zych, M., Kowaloski, P., 1999. Comparison of nitrogen content amino acid composition and glucosamine content of cell walls of various chlorococcales algae. Phytochemistry. 51, 491-497.

Carrère, H., Dumas, C., Battimelli, A., Delgenès, J.P., Steyer, J.P., Ferrer, I., 2010. Pretreatments methods to improve sludge anaerobic degradability: a review. J. Hazard, Materi. 183, 1-15.

Cho, H.S., Oh, Y.K., Park, S.C., Lee, J.W., Park., J.Y., 2013b. Effects of enzymatic hydrolysis on lipid extraction from *Chlorella vulgaris*. Renew. Energ. 54, 156-160.

Cho, S., Park, S., Seon, J., Yu, J., Lee, T., 2013a. Evaluation of thermal, ultrasonic and alkali pretreatments on mixed-microalgal biomass to enhance anaerobic methane production. Bioresour. Technol. 143, 330-336.

Choi, J.A., Hwang, J.H., Dempsey, B.A., Abou-Shanab, R.L.A., Min, B., Song, H., Lee, D.S., Kim, J.R., Cho, Y., Hongi, S., Jeon, B.H., 2011. Enhancement of fermentative bioenergy (ethanol/hydrogen) production using ultrasonication of *Scenedesmus obliquus* YSW15 cultivated in swine wastewater effluent. Energy Environ. Sci. 4, 3513-3520.

Donoso-Bravo, A., Fdz-Polanco, M., 2013. Anaerobic co-digestion of sewage sludge and grease trap: assessment of enzyme addition. Process Biochem. 48, 936-940.

Dubois, M., Gille, K. A., Hamilton, J. K., Rebers, P. A., Smith, F., 1956. Colorimetric method for determination of sugar and related substances. Analytical Chemistry. 28(3), 350-356.

Ehimen, E.A., Holm-Nielsen, J.B., Poulsen, M., Boelsmand, J.E., 2013. Influence of different pre-treatment routes on anaerobic digestion of a filamentous algae. Renew. Energ. 50, 476-480.

Frølund, B., Palmgren, R., Keiding, K. and Nielsen, P.H., 1995. Extraction of extracellularpolymers from activated sludge using a cation exchange resin. Water Res. 30, 1749-1758.

Fu, C., Hung, T., Chen, J., Su, C., Wu, W., 2010. Hydrolysis of microalgae cell walls for production of reducing sugar and lipid extraction. Bioresour. Technol. 101, 8750-8754.

Golueke, C.G., Oswald, W.J., Gotaas, H.B., 1957. Anaerobic digestion of algae. Appl. Biotechnol. 5, 47-55.

Gonzalez-Fernandez, C., Sialve, B., Bernet, N., Steyer, J.P., 2012a. Impact of microalgae characteristics on their conversion to biofuels. Part I Focus on cultivation and biofuel production. Biofuels Bioprod. Bioref. 6, 105-113.

González-Fernández, C., Sialve, B., Bernet, N., Steyer, J.P., 2012b. Comparison of ultrasound and thermal pretreatment of *Scenedesmus* biomass on methane production. Bioresour. Technol. 110, 610-616.

González-Fernández, C., Sialve, B., Bernet, N., Steyer, J.P., 2012c. Thermal pretreatment to improve methane production of *Scenedesmus* biomass. Biomass Bioenerg. 40, 105-111.

Harun, R., Danquah, M.K., 2011. Enzymatic hydrolysis of microalgal biomass for bioethanol production. Chem. Eng. J. 168, 1079–1084.

Heaven, S., Milledge, J., Zhang, Y., 2011. Comments on Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol. Adv. 29(1), 164-167.

Keymer, P., Ruffell, I., Pratt, S., Lant, P., 2013. High pressure thermal hydrolysis to increase the methane yield during anaerobic digestion of microalgae. Bioresour. Technol. 131, 128-133.

Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. Renew. Sust. Energ. Rev. 14, 217-232.

Ometto, F., Pozza, C., Whitton, R., Smith, B., Torres, A.G., Henderson, R.K., Jarvis, P., Jefferson, B., Villa R., 2014. The impact of replacing air bubbles with microspheres for the clarification of algae from low cell-density culture. Water Res. 53, 168-179. (Current Thesis, Paper 2.2).

Ometto, F., Whitton, R., Coulon, F., Jefferson, B., Villa R., 2013. Improving the energy balance of an integrated microalgal wastewater treatment process, Waste and Biomass Valorization. DOI: 10.1007/s12649-013-9230-2. (Current Thesis, Appendix E).

Park, K.Y., Kweon, J., Chantrasakdakul, P., Lee, K., Cha, H.Y., 2013. Anaerobic digestion of microalgae biomass with ultrasonic disintegration. Int. Biodeter. Biodegr. 85, 598–602.

Passos, F. Garcia, J., Ferrer, I., 2013. Impact of low temperature pretreatment on the anaerobic digestion of microalgal biomass. Bioresour. Technol. 138, 79-86.

Pragya, N., Pandey, K.K., Sahoo, P.K., 2013. A review on harvesting, oil extraction and biofuels production technologies from microalgae. Renew. Sustain. Energ. Rev. 24, 159-171.

Purcell, D., Parsons, S.A., Jefferson, B., 2013. The influence of ultrasounds frequency and power on the algal species *Microcysis aeruginosa*, *Aphanizomenon flos-aquae, Scenedesmus subspicatus* and *Melosira* sp.. Environmental Technol. 34(17), 2477-2490.

Rodrigues, M.A., Pinto da Silva Bon, E., 2011. Evaluation of *Chlorella* (Chlorophyta) as source of fermentable sugars via cell wall enzymatic hydrolysis. Enzymes Research. doi:10.4061/2011/405603.

Sander, K., Murthy, G.S., 2009. Enzymatic degradation of microalgal cell walls, American Society of Agricultural and Biological Engineers (ASABE). Paper n. 1035636.

Slade, R., Bauen, A., 2013. Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. Biomass Bioenerg. 53, 29-38.

Suslick, K.S., 1990. Sonochemistry. Science. 247, 1439-1445.

Tomaselli, L., 2007. The microalgal cell. In Richmond, A. (ed): Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science. 178-214 (2007).

Valo, A., Carrère, H., Delgenès, J.P., 2004. Thermal, chemical and thermo-chemical pre-treatment of waste activated sludge for anaerobic digestion. J. Chem. Technol. Biot. 79, 1197-1203.

Yin, L.J., Jiang, S.T., Pon, S.H., Lin, H.H., 2010. Hydrolysis of *Chlorella* by *Cellulomonas* sp. YJ5 Cellulases and its biofunctional properties. J. Food Sci. 75(9), 317-323.

Zheng, H., Yin, J., Gao, Z., Huang, H., Ji, X., Dou, C., 2011. Disruption of Chlorella vulgaris cells for the release of biodiesel-producing lipids: a comparison of grinding, ultrasonication, bead milling, enzymatic lysis, and microwaves. Appl. Biochem. Biotechnol. 164, 1215-1224.

Chapter 4

IMPLICATIONS OF THE WORK

4 IMPLICATIONS OF THE WORK

4.1 Key observations

The key observations from the work presented in this thesis are:

1. The characteristics of the whole microalgae suspension in terms of cell size, shape and concentration, and extracellular algogenic organic matter (AOM) play a fundamental role on the cell recovery efficiency, limiting the number of applicable harvesting solutions for large-scale cultivation plants. Viable harvesting solutions include the innovative Ballasted Dissolved Air Flotation (BDAF) and low energy sedimentation systems (Paper 2.1, section 2.1.5).

2. With all three algae, optimal doses were observed at zeta potential values close to zero. Lower dosage was always linked to low pH values and low protein:carbohydrate ratio in the algae suspension (Paper 2.1, Section 2.1.2 and 2.2, Section 2.2.3.2).

3. Ballasted dissolved air flotation (BDAF) was identified as a feasible harvesting system reducing chemical demand by 14 - 95% depending on the algae suspension, carbon emission by 33 – 58% and energy demand by 60 – 80% compared to the use of traditional DAF (Paper 2.2, Sections 2.2.3.1 and 2.2.3.4).

4. The microbial community within an anaerobic digester will adapt to the presence of algae, enabling it to be used as a feedstock material without the need for pre-treatments (Paper 3.1, Section 3.1.3.4).

5. The impact of pre-treatment on algae is species specific due to differences in the cell wall composition of each algae (Paper 3.2, Section 3.2.3.2).

6. The composition and quantity of released soluble COD post pre-treatment is an effective parameter to indicate the potential increase in biogas production (Paper 3.2, Section 3.2.3.3).

7. Enzymatic hydrolysis is the most effective and energetically efficient pre-treatment able to achieve complete algae digestion. Thermal hydrolysis and ultrasounds also provided a high release of soluble COD, however the biomass degradation was often not able to generate enough additional biogas to offset the energy input required by the pre-treatment (Paper 3.2, Section 3.2.3.3.2).

4.2 Energy neutral microalgae wastewater nutrient removal strategy

4.2.1 Introduction

Overall, the consideration of the findings outlined in the thesis concern the impact of choices in terms of algal species, harvesting technologies and pre-treatments on the overall energy balance of an integrated microalgae wastewater treatment.

The most pertinent application with which to assess such impacts relates to the use of algal based wastewater treatment to upgrade the treatment capability of existing small sewage works (2,000 – 5,000 p.e.). Treatment flowsheets for such applications are traditionally based around low energy, relatively passive, low maintenance technologies for the removal of BOD and suspended solids with some degree of nitrification. An illustrative example flowsheet would include a primary sedimentation tank followed by a trickling filter and a final sedimentation tank (or humus tank) that will meet a 20/30 BOD/SS standard (Figure 4.1). Future legislation is expected to require substantial improvement in the removal of ammonia and phosphorus with the possibility of discharge consents as low as 1 mg l^{-1} (Vale, 2013). Traditional upgrading options to meet such a standard would commonly involve rebuilding the sewage works based on high energy processes such as activated sludge or membrane bioreactors with chemical dosing for phosphorus removal (Metcalf and Eddy, 2007; Shi, 2011). This deviates from the preference at such scales for low energy, low maintenance systems and so provides an opportunity for the development of alternative technology options. At present, this is most commonly manifested in the form of aerated wetlands that enable tight ammonia standards without excessive demand on energy or maintenance (Butterworth *et al.,* 2013). However, micro algae systems (MAS) differentiate from the other possible options as the nutrient removal process occurs as a consequence of controlled algal growth thus providing a source of additional anaerobic digestion feed material and hence energy production. Consequently, the use of MAS offers the potential to treat sewage effluent in small works down to low nutrient concentrations on a net energy neutral basis and hence maintain the overarching philosophy of the original sites.

To establish the potential impact of differences in algal species, harvesting approach and pre-treatment options on the efficacy of MAS, a series of implementation scenarios have been considered. All are based around a small treatment works (2,000 p.e.) reflecting the most likely application scale for wastewater microalgae systems. The analysis only considers the upgrade components in each case to establish the relative suitability of each upgrade option. In total, three upgrade options have been considered based on either the most likely low impact option currently used (Case A: aerated wetland) or the use of MAS after primary sedimentation (Case B – high biomass production) or as a tertiary treatment option (Case C – lower biomass production) (Figure 4.1). Additionally, two algal reactor configurations are considered based on high rate algal ponds (HRAP) and photobioreactors (PBR) to reflect current discussions on preferred technology (Whitton *et al.*, 2013).
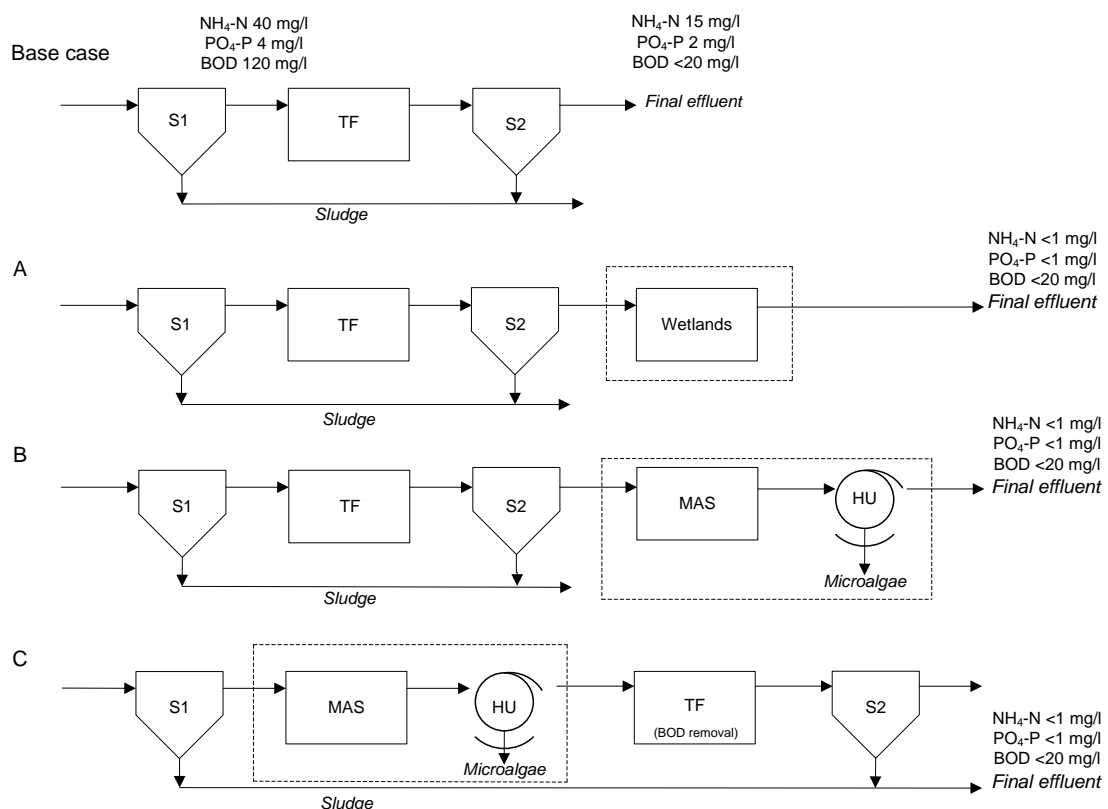

Figure 4.1 Business case scenarios. TF: trickling filter: MAS: microalgae system; HU: harvesting unit; WTS: wetlands; S1: primary sedimentation; S2: secondary sedimentation.

4.2.2 Materials and methods

4.2.2.1 Design parameters

All cases have been designed to meet the new standard with validation of design decisions based on information from existing literature and the results obtained in the current thesis. For instance, aerated wetlands as tertiary treatments (Case A) have been shown to meet a 10 mgTN l^{-1} (Austin and Nivala, 2009) and allow phosphorus concentration below 0.25 mgPO$_4$-P l^{-1} (Leader *et al.,* 2005; Ko *et al.,* 2004). Equivalent evidence for the MAS systems (Cases B and C) indicates an ability to meet both ammonia and phosphorus removal targets in both scenarios. For instance, previous studies have demonstrated the ability of MAS to reduce initial concentrations of 13 mgNH$_4$-N l^{-1} and 2 mgPO$_4$-P l^{-1} to below the detection limit based on a 7 to 9 days retention time resulting in an algal biomass production of 0.2 - 0.4 gDM l^{-1} (Sydney *et al.*, 2011). Similarly, use of an open pond in Scotland enabled effluent NH$_4$-N concentration of between 1 and 0.5 mg l^{-1} and PO$_4$-P between 0.9 and 0.03 mg l^{-1} with a 7 day hydraulic retention time (HRT) using primary effluent with a harvested biomass of between 0.3 and 0.4 gDM l^{-1}, depending on the specific HRT and season (Cromar and Falloefield, 1997). Accordingly, it is common to operate open ponds under different HRTs between warm and cold seasons to guarantee constant N and P removal (Craggs *et al.,* 2012; Cromar and Falloefield, 1997).

The specific design parameters adopted for each scenario are reported in Table 4.1. The nutrient uptake achieved by growing microalgae (Case B and Case C) was evaluated using two different growth-systems: a high rate algae pond (HRAP) and a close photobioreactor (PBR) to reflect current design discussions on new systems. Three different algae species were used for this study: *Scenedesmus obliquus* (90% VS), *Chlorella vulgaris* (90% VS) and *Arthrospira maxima* (70% VS). Although, *A. maxima* requires different conditions (additional salts, higher temperatures and pH values), we have assumed that the composition of the base-case effluent will allow the growth of all three algae, so that we can consider the impact of a filamentous algae species compared to

single cells (*S. obliquus* and *C. vulgaris*). The algal biomass concentration achieved at the end of the treatment was assumed to be equal to 200 mgDM l^{-1} and 400 mgDM l^{-1} in Case B and Case C, respectively, using HRAPs, and equal to 300 mgDM l^{-1} and 600 mgDM l^{-1} for the same cases when using PBRs (Olguín *et al.,* 1997; Garcia *et al.,* 2000; Baliga and Powers, 2010;.Craggs *et al.,* 2012). To guarantee constant nutrient uptake, the MAS was operated at longer HRTs during the cold seasons compared to spring and summer (Garcia *et al.,* 2000) and the target removal based on data from existing literature on an example effluent quality from the base case (Metcalf and Eddy, 2007).

Table 4.1 Main design parameter and assumption

Design parameters	Value	Notes	Reference
Trickling filter			
Inflow	336 m^3 d^{-1}	Water availability of 210 l d^{-1} p.e.$^{-1}$	*Metcalf and Eddy, 2007*
Dimension	84 m^2	HLR of 4 m^3 m^{-2} d^{-1}	*Metcalf and Eddy, 2007*
Primary effluent	335 m^3 d^{-1}	Assuming dry solid production of 70 kg m^{-3} at 6 % TS.	*Metcalf and Eddy, 2007*
Final effluent	333 m^3 d^{-1}	Assuming dry solid production of 60 kg m^{-3} at 1.5 % TS.	*Metcalf and Eddy, 2007*
Solids production	74 kgVS d^{-1}	2.4 m^3 d^{-1} at 5 % VS content assumed equal for all scenarios.	
Energy demand	0.252 kWh m^{-3}	Applied to Base case: average energy demand of the whole treatment plant .	*Shi, 2011*
Aerated wetland			
Dimension	3423 m^2	Considering an inflow of 10.3 m^2 m^{-3}.	*Austin and Nivala, 2009*
Biomass production	negligible	Occasional biomass from cuts of the vegetation and operational maintenance was excluded from the analysis	*Ko et al., 2004*
Energy demand	0.49 kWh m^{-3}	Aerated wetland	*Austin and Nivala, 2009*
High rate pond			
HRT	7 - 10 d	7d HRT from Oct. to Mar. and 10d HRT from Apr. to Sep.	*Garcia et al., 2000*
Dimension	8340 m^2	0.4 m depth for 10d HRT for Case C (primary effluent).	
	8379 m^2	0.4 m depth for 10d HRT for Case B (secondary effluent).	
Biomass production	22 - 57 kgVS d^{-1}	Seasonal variation of the biomass was taken into account considering 200 mg l^{-1} as maximal production between May and July with 50% reduction from Nov. to Feb. and 25% reduction in the remaining months. VS/TS ratio equal to 0.9 for *S. obliquus* and *C. vulgaris*, and 0.7 for *A. maxima*.	*Olguín et al., 1997; Garcia et al., 2000; Baliga and Powers, 2010; Craggs et al., 2012;*
	33- 85 kgVS d^{-1}	Seasonal variation of the biomass was taken into account considering 300 mg l^{-1} as maximal production between May and July with 50% reduction from Nov. to Feb. and 25% reduction in the remaining months. VS/TS ratio equal to 0.9 for *S. obliquus* and *C. vulgaris*, and 0.7 for *A. maxima*.	
Energy demand	25 kWh ha^{-1} d^{-1}	Mixing using paddle wheels.	*Jonker and Faaij, 2013*
Photobioreactor			
HRT	3 - 5 d	3d HRT from Oct. to Mar. and 5d HRT from Apr. to Sep., considering 1/2 HRT required compare to HRAP.	*Arbib et al., 2013*
Dimension	6000 m^2	Tubes with 0.3m diameter, 400 m long, organised in four tuber per lines having a distance of 1 meter between one to another.	*Acién et al., 2012*
Biomass production	44 - 114 kgVS d^{-1}	Seasonal variation of the biomass was taken into account considering 400 mg l^{-1} as maximal production between May and July with 50% reduction from Nov. to Feb. and 25% reduction in the remaining months. VS/TS ratio equal to 0.9 for *S. obliquus* and *C. vulgaris*, and 0.7 for *A. maxima*.	*Arbib et al., 2013; Bahadar and Khan, 2013.*
	66 - 128 kgVS d^{-1}	Seasonal variation of the biomass was taken into account considering 600 mg l^{-1} as maximal production between May and July with 50% reduction from Nov. to Feb. and 25% reduction in the remaining months. VS/TS ratio equal to 0.9 for *S. obliquus* and *C. vulgaris*, and 0.7 for *A. maxima*.	
Energy demand	0.3 kWh m^{-3}	Mixing using turbulent air flow. In the literature, value varies largely from 0.08 up to 2.5 kWh m^{-3}.	*Norsker et al., 2012*

The energy and carbon impact of the harvesting step was evaluated using three different technologies: Dissolved Air Flotation (DAF), Ballasted Dissolved Air Flotation (BDAF) and sedimentation (SED) to benchmark against systems where self flocculation occurs (Collet *et al.,* 2011; Zamalloa *et al.,* 2011). In the sedimentation case, additional thickening is required which is assumed to be achieved through the use of a floatation thickener. In all harvesting configurations, cell recovery efficiency was assumed to be equal to 95% with the solid content of the harvested biomass varying between each system based on the specific overflow applied (Table 4.2). For the reasons reported in Paper 2.2, DAF was excluded from the analysis when using *A. maxima*.

Table 4.2 Harvesting design parameter and assumption

Design parameters	Value	Notes	Reference
Dissolved Air Flotation (DAF)			
Chemical demand	10 - 40 mgAl l^{-1}	10 mg l^{-1} of suspended *C. vulgaris,* and 40 mg l^{-1} of suspended *S. obliquus*. Coagulant demand was estimated to increased to 30 and 60 mg l^{-1}, respectively, for the same three algae, when considering combine sedimenadation and DAF to achieve same final biomass concentration.	Current Thesis, Paper 2.2
Overflow solids concentration	3 % TS	Average value reported in the literature considering 95 % separation	*Rawat et al., 2013*
Energy demand	0.3 kWh m^{-3}	Average value reported in the literature.	*Molina Grima et al., 2003*
Ballasted Dissolved Air Flotation (BDAF)			
Chemical demand	6 - 77 mgAl l^{-1}	6 mg l^{-1} of suspended *C. vulgaris*, 30 mg l^{-1} of suspended *S. obliquus* and 77 mg l^{-1} of suspended *A. maxima*.	Current Thesis, Paper 2.2
Glass microspheres demand	1 - 5 kg d^{-1}	300 mg l^{-1} are required to harvest the biomass; 99 % beads recovery with a treatment capacity of 14 m^3 $h^{-1.}$	Current Thesis, Paper 2.2
Overflow solids concentration	5 % TS	Average estimated value considering 95 % separation efficiency.	Current Thesis, Paper 2.2
Energy demand	0.05 kWh m^{-3}	Average estimated value reported in the literature.	*Jarvis et al., 2009*
Sedimentation			
Overflow solids concentration	1 % TS	Average value reported in the literature considering 95 % separation	*Yahi et al., 1995*
Energy demand	0.02 kWh m^{-3}	Average value reported in the literature.	*Yahi et al., 1995*

4.2.2.2 Energy balance

The energy balance of each scenario refers to the net energy demand between the electricity used by the nutrient removal process and ancillary equipment (harvest and pre-treatment) and the electricity generated by anaerobic digestion of the additional algal biomass. The methane yield of the wastewater sludge produced by the base case was assumed constant in all scenarios at 800 ml

kgVS$_{add}$$^{-1}$ (Shi, 2011), whereas the methane content of the different algal species was varied according to the data in Chapter 3 complimented with data from the literature. Untreated algae biomass was assumed to yield approximately 30% of the theoretical methane production (Heaven *et al.*, 2011), whereas the adoption of thermal (thermal hydrolysis at 165°C) and enzymatic (8x10^6 u kgTS^{-1} at 50°C) pre-treatments produced up to 60% and 90%, respectively. In Case B, the theoretical methane value of *S. obliquus, C. vulgaris* and *A. maxima* was assumed equal to 534 ml kgVS$_{add}$$^{-1}$, 652 ml kgVS$_{add}$$^{-1}$ and 482 ml kgVS$_{add}$$^{-1}$, respectively. The same values were applied to Case C with the exception of *C. vulgaris* which was assumed to have a lower methane content equal to 534 ml kgVS$_{add}$$^{-1}$ (Heaven *et al.,* 2011). The higher amount of available nitrogen when cultivating *Chlorella* sp. in primary effluent instead of secondary causes a change in the composition of the biomass, reducing the amount of lipid content and therefore theoretical methane potential (Sialve *et al.,* 2009). The electricity generated with AD was calculated assuming an 80% biomass digestion efficiency, and a methane energy conversion of 9.7 kWh m^{-3} at 30% efficiency (Ometto *et al.,* 2013). While the energy demand by enzymatic hydrolysis was expected to be offset by the heat generated by the CHP system, thermal hydrolysis requires additional energy input estimated at approximately 20% of the produced biogas (Pérez-Elvira and Fdz-Polanco, 2012).

4.2.2.3 Operational cost and carbon footprints

Operating costs and carbon footprints were calculated in British Pound Sterling (£) and carbon dioxide equivalent (CO_2e), respectively, applying standard conversion factors (Table 4.3). The estimation included the net electricity required to run the MAS or aerated wetland, the amount of chemicals, glass microspheres (for BDAF harvesting unit) and enzymes used, and the transportation of the additional algal biomass from the WWTP to the AD plant. According to the National Non-Food Crops Centre (http://www.biogas-info.co.uk/index.php/ad-map.html), in England there are 213 AD plants, which gives a density value of 0.002 AD plant per squared kilometer. Assuming the *base case* is located in the middle of a 500 km^2 area, the return trip for the

transportation of the sludge/biomass was assumed equal to 50 km. The number of trucks required was calculated considering the volume of sludge and biomass produced by each specific scenario configuration according to Table 4.3.

Table 4.3 Carbon and costs factors adopted for the analysis

	Value	Unit	Notes	*Reference*
Carbon factors				
Electricity	0.484	$kgCO_2e\ kWh^{-1}$	UK electricity generation considering losses in transmission and distribution.	*DEFRA/DECC, 2013*
Aluminium sulphate	0.493	$kgCO_2e\ kg^{-1}$	In powder form at the factory gate.	*UM, 2011*
Sodium bicarbonate	1	$kgCO_2e\ kg^{-1}$	In powder form at the factory gate.	*UM, 2011*
Microspheres	0.9	$kgCO_2e\ kg^{-1}$	UK primary glass production (cradle-to-gate).	*Hammond and Jones, 2011*
Enzymes	4	$kgCO_2e\ kg^{-1}$	Average value of five different emission factors of different enzymes including protease, glucoamylase and alpha-amylase.	*Nielsen et al., 2007*
Transport	0.722	$kgCO_2e\ km^{-2}$	15t diesel truck at average loading value.	*DEFRA/DECC, 2013*
Loading	0.5	$kgCO_2e\ m^{-3}$	considering diesel emission factor of 0.0869 $kgCO_2e\ MJ^{-1}$.	*Personal communication: Beatrice Smyth, Northern Ireland Water, Belfast (2013)*
Costs				
Electricity	0.14	$£\ kWh^{-1}$	Average UK value.	
Aluminium sulphate	800	$£\ ton^{-1}$	Average market prices.	*Granados et al., 2012*
Sodium bicarbonate	90	$£\ ton^{-1}$	Average market prices for metal salts.	*http://www.alibaba.com/showroom/sodium-bicarbonate-price.html*
Microspheres	10000	$£\ ton^{-1}$	16 % of the original market price for small quantity 60£ kg^{-1}.	*Personal communication: Will Ricci, Trelleborg Offshore, Boston (2013)*
Enzymes	1000	$£\ ton^{-1}$	Average market price for powder enzymes at 200000 U gTS^{-1} equal to 30g $kgTS^{-1}$ algae.	*http://www.alibaba.com/product-gs/1537723257/Industrial_Enzyme_Alkaline_Protease.html?s=p*
Transport	1	$£\ km^{-1}$	Considering a 10 m^3 truck.	*http://www.ooida.com/EducationTools/Tools/costpermile.asp*
Loading	60	$£\ h^{-1}$	Average UK operator costs, considering 0.8 h for loading/unloading per truck.	

4.2.3 Results

4.2.3.1 Energy balance

The energy produced from anaerobic digestion of the collected sludge from the base case (BC) was sufficient to exceed that required for operation generating a net surplus of 4.2 MWh y^{-1}. As the production occurs at centralised sites the generated energy is unavailable for direct use on the site but does demonstrate the overall energy sustainability of using low energy treatment technologies on a catchment basis. Inclusion of an aerated wetland (AW) to upgrade the treatment capacity of the works increased energy demand by 59.6 MWh y^{-1} resulting in a net deficit of 55.4 MWh y^{-1}.

The use of MAS resulted in additional energy production to a maximum of 43 MWh y^{-1} which occurred when either *S.obliquus* or *C. vulgaris* was grown in primary effluent using a photo-bioreactor, harvested by BDAF and pre-treated with enzymatic hydrolysis. However, the generated energy was insufficient to offset the average energy demand of a PBR at 145 MWh y^{-1} and was just in excess of the minimum reported energy demand of a PBR at 39 MWh y^{-1} (Figure 4.2 B and Appendix D). In contrast, the lower energy demand required to operate HARP, 6.5 MWh y^{-1}, resulted in more cases exceeding the energy demand with examples for all three algal species considered (Figure 4.2 A). Comparison across these cases revealed that use of traditional DAF and not including a pre-treatment step prevented the production of an excess amount of energy. Additionally, in only one case thermal pre-treatment was able to generate a net production (use of BDAF and growth in a HARP using primary effluent). Consequently, the results indicate that net energy production across the MAS can only occur if a low energy harvest technology such as BDAF and enzymatic hydrolysis pre-treatment is used.
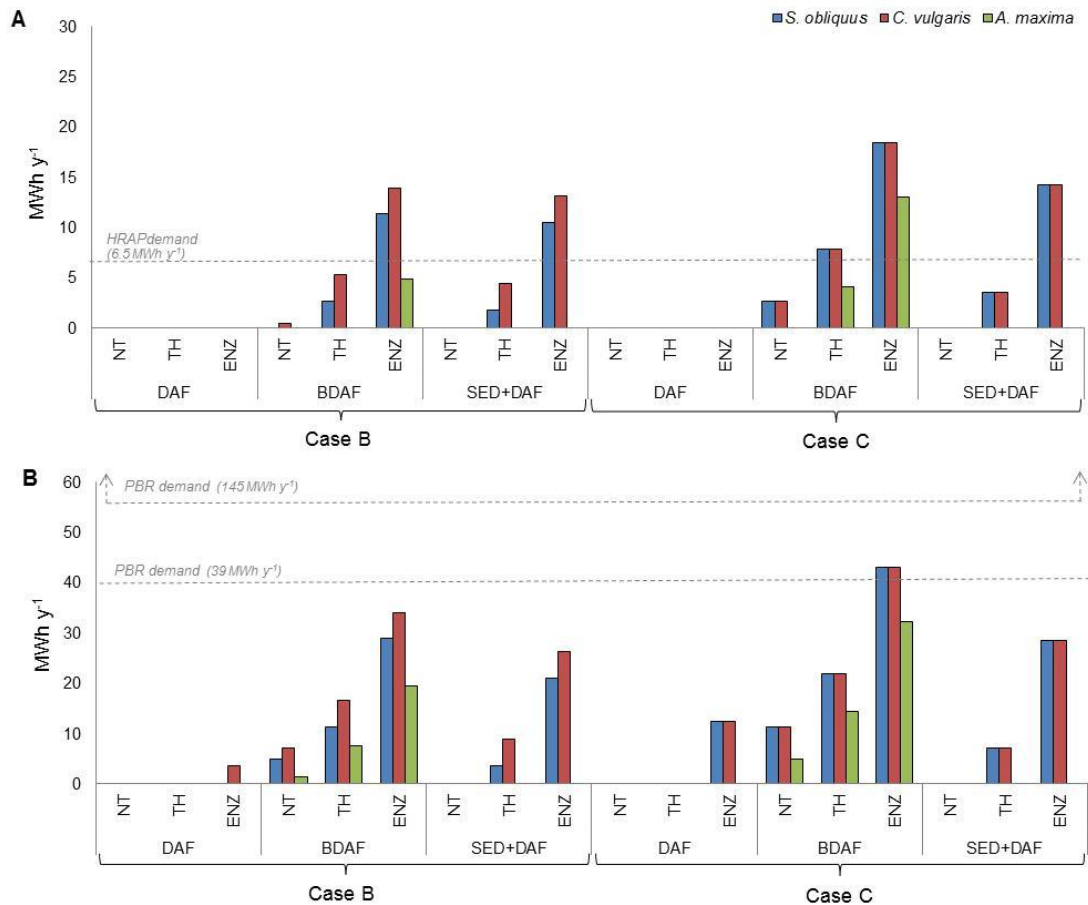
Figure 4.2 Available energy for nutrients removal (net electricity produced from the algae, subtracted of the energy required for harvesting and pre-treatment) using two different cultivation systems (A) high rate algae pond and (B) photobioreactors. NT: non pre-treated; TH: thermal hydrolysis; ENZ: enzymatic hydrolysis.

Consequently, the use of MAS resulted in a net reduction in energy deficit in all cases compared to the aerated wetland (Case A) with a few scenarios resulting in an overall net energy generation of between 2.2 and 12.8 MWh y^{-1}. Therefore, if appropriate choices are made with respect to algal species, harvest technology and pre-treatment, then energy neutral nutrient polishing is possible. Analyses across all the scenarios revealed that energy surplus was possible in cases where enzymatic hydrolysis was coupled with BDAF (Figure 4.3). Inclusion of BDAF as the harvest step significantly reduced the overall net energy deficit in all cases with a maximum deficit of 7.1 MWh y^{-1} representing a

minimum reduction of 88% compared to the aerated wetland. In comparison use of traditional DAF resulted in a reduction in the net energy deficit of between 39% and 7% compared to the aerated wetland demonstrating that MAS provides benefits even when utilising established harvest options. The choice of algal species had a relatively minor impact when choosing between the two single cell algae with the *C. vulgaris* reducing the net deficit slightly compared to *S. obliquus.* The minor difference observed due to algal species is congruent with the fact that the impact is more prominently observed in relation to chemical dose requirements during harvest. This is expected to have an insignificant impact on energy but becomes more important during carbon emission and cost analysis (Figure 4.3 and 4.4). In contrast, comparison with the filamentous algae indicated a significant difference where the reduced biogas productivity of the *A. maxima* reduced the net benefit of MAS and resulted in only one scenario where a net energy surplus was generated (BDAF, enzymatic hydrolysis and growth in a HARP using primary effluent) (Figure 4.3 C). The choice of pre-treatment options had a significant impact on the overall net energy balance with the maximum difference between options observed between no treatment and enzymatic hydrolysis at 16 MWh y^{-1}. Thermal hydrolysis was positioned in between the other two options with a reduction from 8 to 10 MWh y^{-1} in comparison with enzymatic hydrolysis.
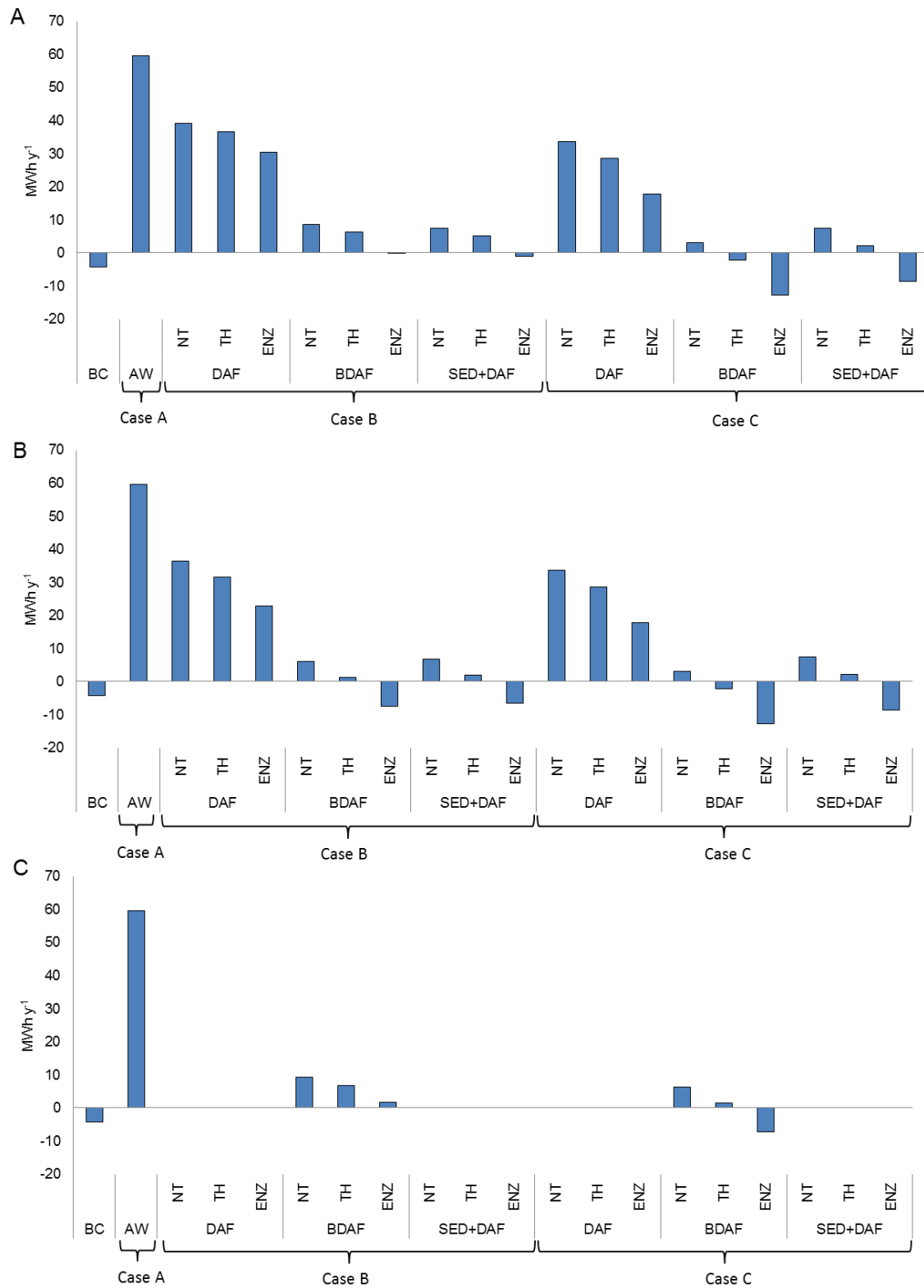
Figure 4.3 Additional energy demand for (A) *S. obliquus*; (B) *C. vulgaris* and (C) *A. maxima* compared to the base case (BC) and aerated wetland (AW), based on using a HARP - negative numbers represent a surplus of electricity.

4.2.3.2 Operational carbon footprint

The inclusion of an aerated wetland increased the operational carbon of the base case by 28.85 tCO_2eq y^{-1} as a consequence of the additional electricity demand for delivering the required oxygen (Figure 4.4). In contrast, the additional carbon footprint associated with the electricity demand was lower in all MAS systems reaching a maximum of 62% and a minimum of 0.15% of the aerated wetland case. However, in all MAS systems additional operational carbon footprint was required in terms of the transport of the additional biomass and the coagulating chemicals for the DAF plant and further requirements based on the replacement of lost glass beads used in the BDAF configurations and enzymes where enzymatic hydrolysis was used. Between the two green algae, the additional carbon footprint was most pronounced in the case of *S. obliquus* due to its higher coagulant dose requirements as a consequence of the elevated protein content in the excreted AOM (Paper 2.2, Section 2.2.3.1). The impact of this made the total operational carbon footprint exceed that of the aerated wetland when traditional DAF was used as the harvest technology. To illustrate, the additional carbon footprint associated with coagulant use was 13.6 tCO_2eq y^{-1} for *S. obliquus,* and 6.4 tCO_2eq y^{-1} for *C. vulgaris* representing 46% and 16.8% of the total footprint. In the latter case, the reduced chemical use resulted in an overall lower carbon footprint associated with MAS even when DAF was used. A significant further reduction was observed when low energy harvesting as employed with, for instance, the total operational carbon footprint ranging between 2.1 and 14.5 tCO_2eq y^{-1}. Component analysis of the BDAF cases with *C. vulgaris* revealed the enzymes to represent the single biggest component, contributing up to 65% of the total footprint. In contrast, the very high coagulant demands associated with harvest of filamentous algae *A. maxima* exerted the largest impact on the total carbon footprint contributing up to 99% of the total and a maximum footprint of 30.8 tCO_2eq y^{-1} associated with use of BDAF and no pre-treatment (Figure 4.4 C). Overall, a much greater impact was observed in relation to the selection of algae species compared to the analysis of energy reflecting the significant impact that species selection is known to have on coagulation (Henderson *et al.,* 2010; Ometto *et al.,* 2014).
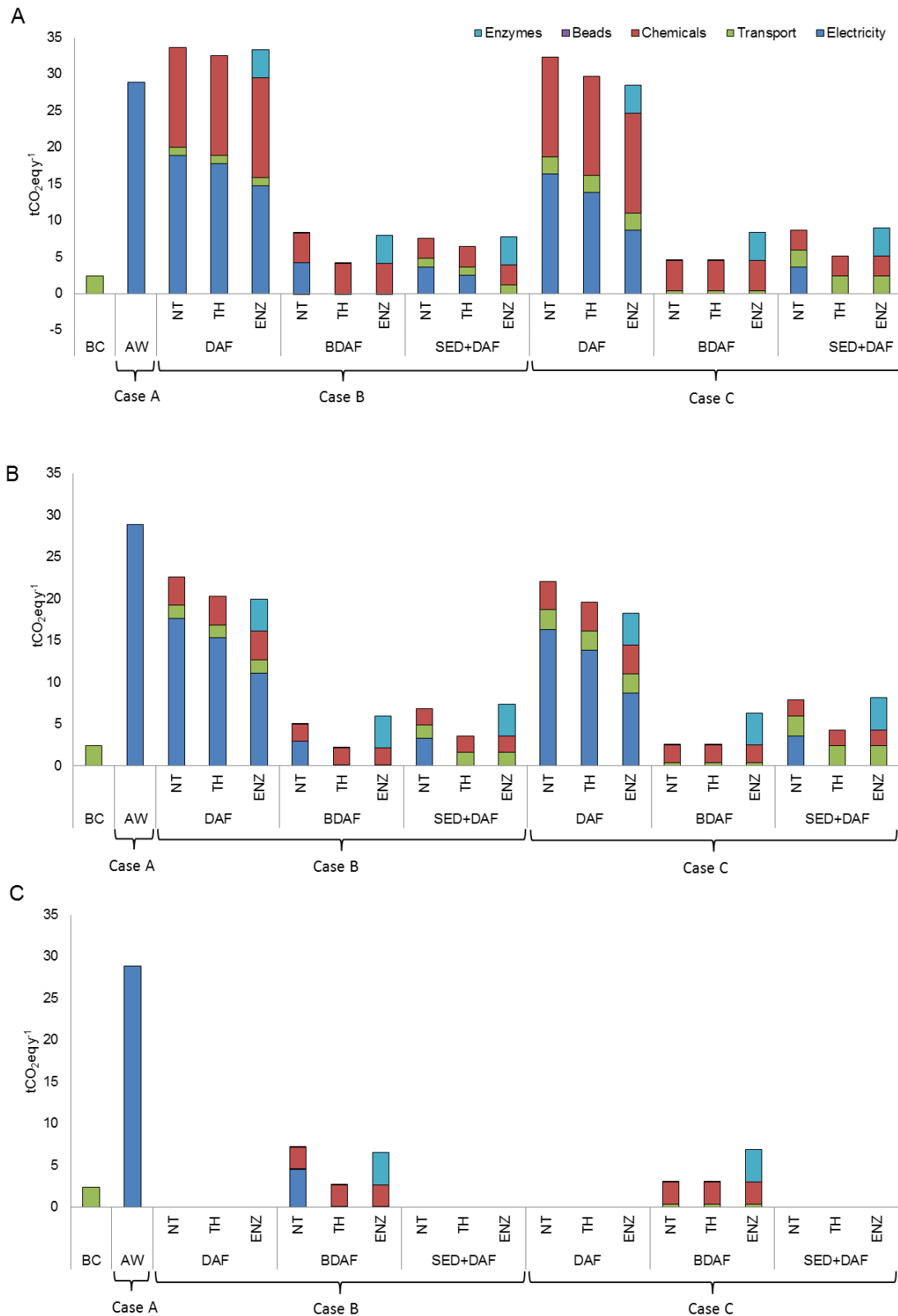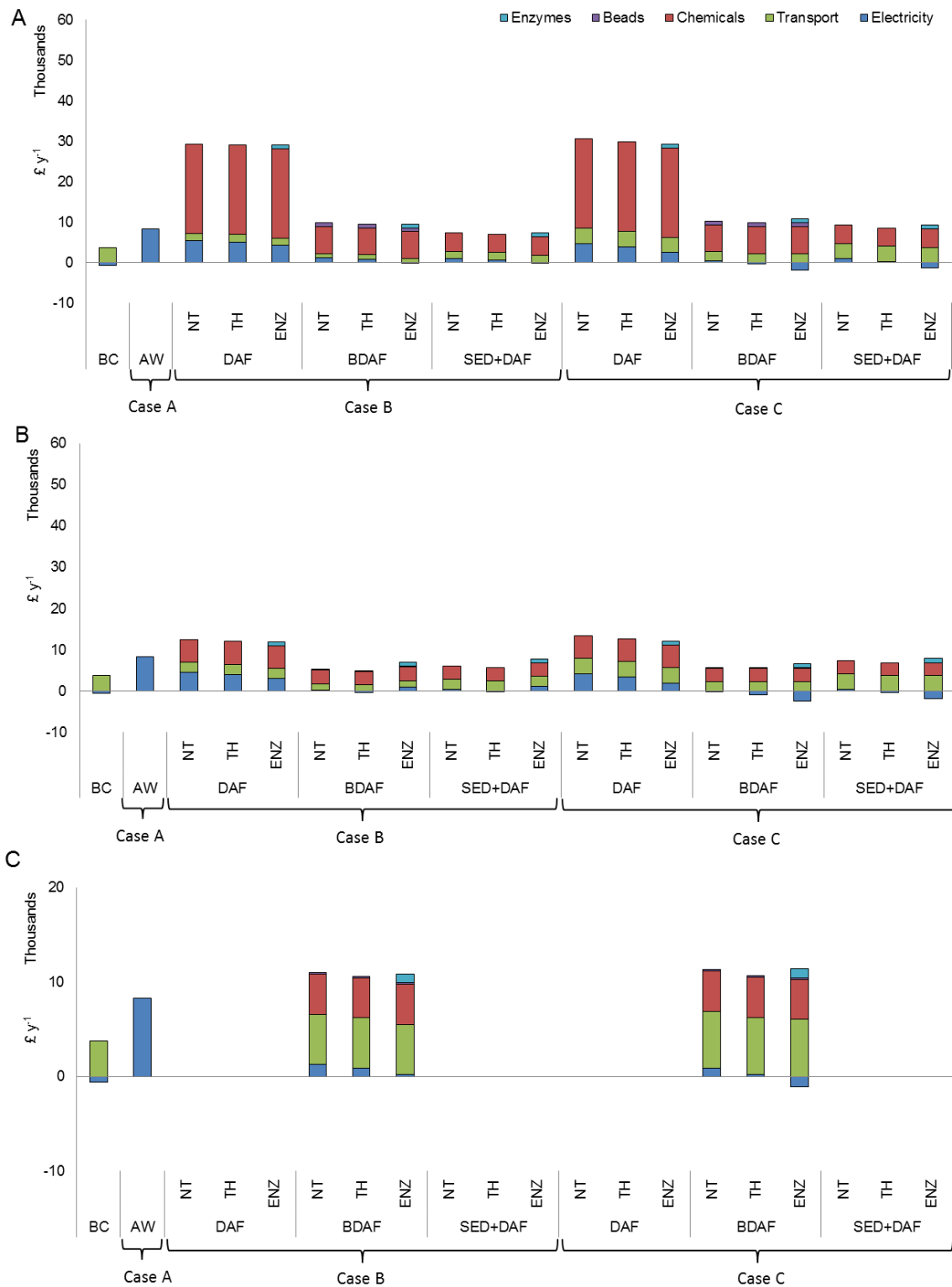
197

Figure 4.4 Additional operational carbon emission for (A) *S. obliquus*, (B) *C. vulgaris* and (C) *A. maxima,* compared to the base case (BC) and the aerated wetland (AW), based on using a HARP.

4.2.3.3 Operational costs

The total additional opex associated with the aerated wetland is significantly lower than all MAS cases for *S. obliquus* and *A. maxima* reflecting the high coagulant demands exerted by these two algal species. To illustrate, the aerated wetland resulted in an increased opex of £8,352 y^{-1} compared to an opex range for *S. obliquus* of between £9,838 y^{-1} and £30,599 y^{-1} corresponding to treatment costs of 6.8 p m^{-3}, 7.9 p m^{-3} and 24.7 p m^{-3} respectively. This compares to typical operating costs associated with activated sludge processes of 7 p m^{-3} (Shi, 2011) and a recent reported cost of 14 p m^{-3} to implement reactive media in constructed wetland for phosphorus removal (Jefferson, 2013). In comparison, when *C. vulgaris* is considered, the lower coagulant requirement significantly reduces the total additional opex such that treatment costs vary between 3.5 p.m^{-3} and 10.1 p m^{-3} for BDAF and DAF configurations respectively. Correspondingly, MAS utilising appropriate algae and harvest technology can deliver a total treatment opex lower than that of the aerated wetland. The impact of pre-treatment options on opex was relatively minor compared to the impacts associated with selection of algal species and harvest technology. To illustrate, the maximum difference in annual opex associated to pre-treatment choice was £2,468. This compares to a maximum calculated annual income of £2,397 associated to additional energy production equivalent to 1.9 p m^{-3} for the BDAF, enzymatic hydrolysis treatment in primary effluent configuration (Case C).

Figure 4.5 Additional operational costs for (A) *S. obliquus*, (B) *C. vulgaris* and (C) *A. maxima,* compared to the base case (BC) and the aerated wetland (AW), based on using a HARP - negative number represent the energy surplus sold to the market .

4.2.4 Discussion and conclusions

It was originally hypothesised that the inclusion of a microalgae system (MAS) on a wastewater treatment flow sheet could provide a route for energy-neutral nutrient removal systems if low energy harvesting technologies were adopted and an efficient energy conversion of the algae biomass was guaranteed. From the analysis of the different scenarios, MAS proved to be a viable alternative compared to conventional upgrade technologies such as aerated wetlands as applied to small municipal WWTs.

To produce an integrated energy neutral MAS, it is necessary to combine low energy harvesting processes with efficient AD pre-treatments. In particular, the case scenarios, which included BDAF for harvesting microalgae and enzymatic hydrolysis for the pre-treatment, generated a surplus of energy compared to the base case and confirmed the original hypothesis (Table 4.4). Further, in comparison to the aerated wetland, significant enhancement of the energy associated to nutrient polishing could be achieved with a net difference of between 58 and 72.4 MWh y^{-1}. However, current aerated wetland systems are undergoing energy optimisation as they known to use around 2.5 Wh person^{-1}, a value similar to the energy demand of activated sludge on a per population basis (Pearce, 2013). Consequently, the difference is likely to be reduced with anticipated savings of up to 50% in the total energy demand expected with the next generation of aerated wetlands. However, irrespective of efficiency savings all alternatives to MAS will exert an additional energy demand on the system and so will neither exceed the potential of MAS due the options ability to produce energy from the waste algal biomass.

Accordingly, the main advantage of MAS is the waste algae biomass generated representing a valuable feedstock for AD. Hence, future technology developments within MAS that increase productivity or reduce associated energy use will further enhance the suitability of the technology. To illustrate, the adoption of cultivation system such as PBRs allow higher biomass productivity, limit cross infection by unwanted algae species or microorganisms preventing loss of biomass, and so should be preferred (Day *et al.,* 2012). However, as previously observed, these technologies are currently too energy

demanding to allow excess of energy production such that currently, HRAP are the only feasible MAS configuration (Slade and Bauen, 2013). Further, the above analysis has been based on day light irradiation and seasonal weather variations associated with the UK limiting the algae biomass productivity (Cromar and Falloefield, 1997; Roleda *et al.,* 2013). A higher or lower irradiation over the year will generate a higher and lower amount of energy available for nutrient removal as defined in section 1.1.3.1. In addition, the location of the MAS near sources of spare heat and CO_2 such as a CHP system, will allow the use of these two resources able to support the algal growth and mitigate adverse weather condition such as low temperatures (Ventura *et al.,* 2013). For instance, an increase in biomass concentration at the harvesting point from 200 gDM m^{-3} to 240 gDM m^{-3} (+ 20%) during the summer season (pick value) will allow an additional algal energy output of 31 %, 29 % and 45 % for *S. obliquus, C. vulgaris* and *A. maxima*, respectively, when considering BDAF as the harvesting technique and enzymatic hydrolysis as the AD pre-treatment in Case B (Figure 4.6). This will allow even *A. maxima,* primarily unable to balance the energy requirement of the HRAP in (Figure 4.2), to generate an energy surplus equal to 50 kWh y^{-1}. Conversely, a reduction of the algae biomass will reduce the overall energetic benefits depending on the algae species. In Case B, *S. obliquus* will be able to guarantee an energetically self-sufficient HRAP with a 28% biomass reduction, while, the higher energy content of *C. vulgaris* allows an higher biomass reduction of 37% before the energy balance ceases to be beneficial. Similar behaviour was observed in Case C with *Scenedesmus* sp. and *Chlorella* sp. Were able to resist a 48% reduction in biomass, compared to 33% when cultivating *A. maxima*.
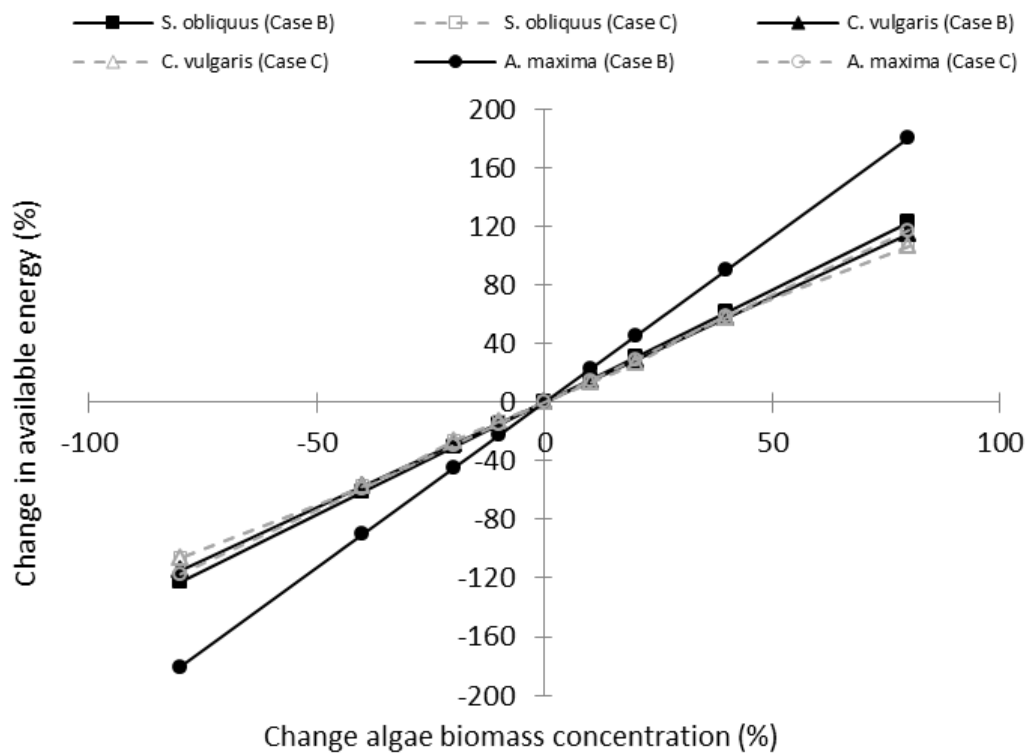
Figure 4.6 Impact of algae biomass variation on available energy for nutrient removal considering the optimal case scenario (BDAF + Enzymatic hydrolysis).

Translation of the above analysis to more amenable climates for algal growth indicates that the overall balance becomes significantly enhanced. To illustrate, assuming a *S. obliquus* biomass concentration equal to 500 mg l^{-1} is applied all year round, a maximum surplus energy generation of 17.2 MWh y^{-1} could be achieved when using BDAF for harvesting and enzymatic hydrolysis as the pre-treatment to AD (an improvement of 5.5 MWh y^{-1} compared to the best case described in the current analysis). Consequently, where sufficient land is available and the climate appropriate, MAS can be implemented at larger wastewater treatment plants contributing to both nutrient removal and the overall energy balance (Ometto *et al.,* 2013).

While the energy balance of the system is more affected by the technologies adopted to process the algae and the amount of generated biomass, the specific algae species primarily impact on coagulant doses and related operational carbon emissions and opex. In the present analysis the coagulant demand was fixed for each alga according to the experimental observations

reported in Chapter 2 (standard growth medium, stationary growth phase). However, at full scale the specific growth stage at the harvesting point will be defined by the HRT adopted to meet the prefixed nutrients concentration in the final effluent. Therefore, the specific characteristic of algae biomass, such as AOM, charge density and cells concentration are expected to vary over time affecting the optimal coagulant demand (Appendix A, Figure A.3). Monitoring the zeta potential will help maintain a high harvesting efficiency (Henderson *et al.,* 2008; Ometto *et al.,* 2014) but will impact the overall balances through a potential increase in chemical use. To illustrate, a 10% increase in coagulant demand when harvesting *C. vulgaris, S. obliquus,* and *A. maxima* (Case B, BDAF follow by enzymatic hydrolysis) will increase the annual operational costs by £330 (+ 5%), £1,700 (+ 9%) and £4,250 (+ 9%), respectively (Figure 4.7 A). Correspondingly, *C. vulgaris* appears the most robust choice of algae as the impact in changing coagulant demand has the least impact. For instance, a positive economic balance can be maintained until a 40% increase in coagulant dose when comparing the contribution of the overall system with aerated wetlands (Table 4.4). In addition, the higher energy output of this specific algal species when cultivated under low nitrogen concentration (Case B), limits the impact of the coagulant demand on the opex (Figure 4.7 A). Conversely, with *S. obliquus* a 60% reduction of the coagulant dose is required in order to be more favourable than the aerated wetland solution, while the cultivation of *A. maxima* represents, in all cases, the most expensive scenario (Table 4.4).

In term of carbon footprint, an additional 0.21, 1 and 3.2 tCO_2eq y^{-1}, for *C. vulgaris, S. obliquus,* and *A. maxima,* respectively, was observed when considering a 10% increase in coagulant demand irrespective of the treatment configuration adopted (Figure 4.7 B). When comparing the overall carbon emission of the MAS with the aerated wetlands (Case A), both green algae maintain environmental benefits up to an 80% increase in coagulant demand. In contrast, the significantly higher coagulant dosage required by *A. maxima* results in an higher total carbon emission than the aerated wetland unless the coagulant demand is reduced by 15%.
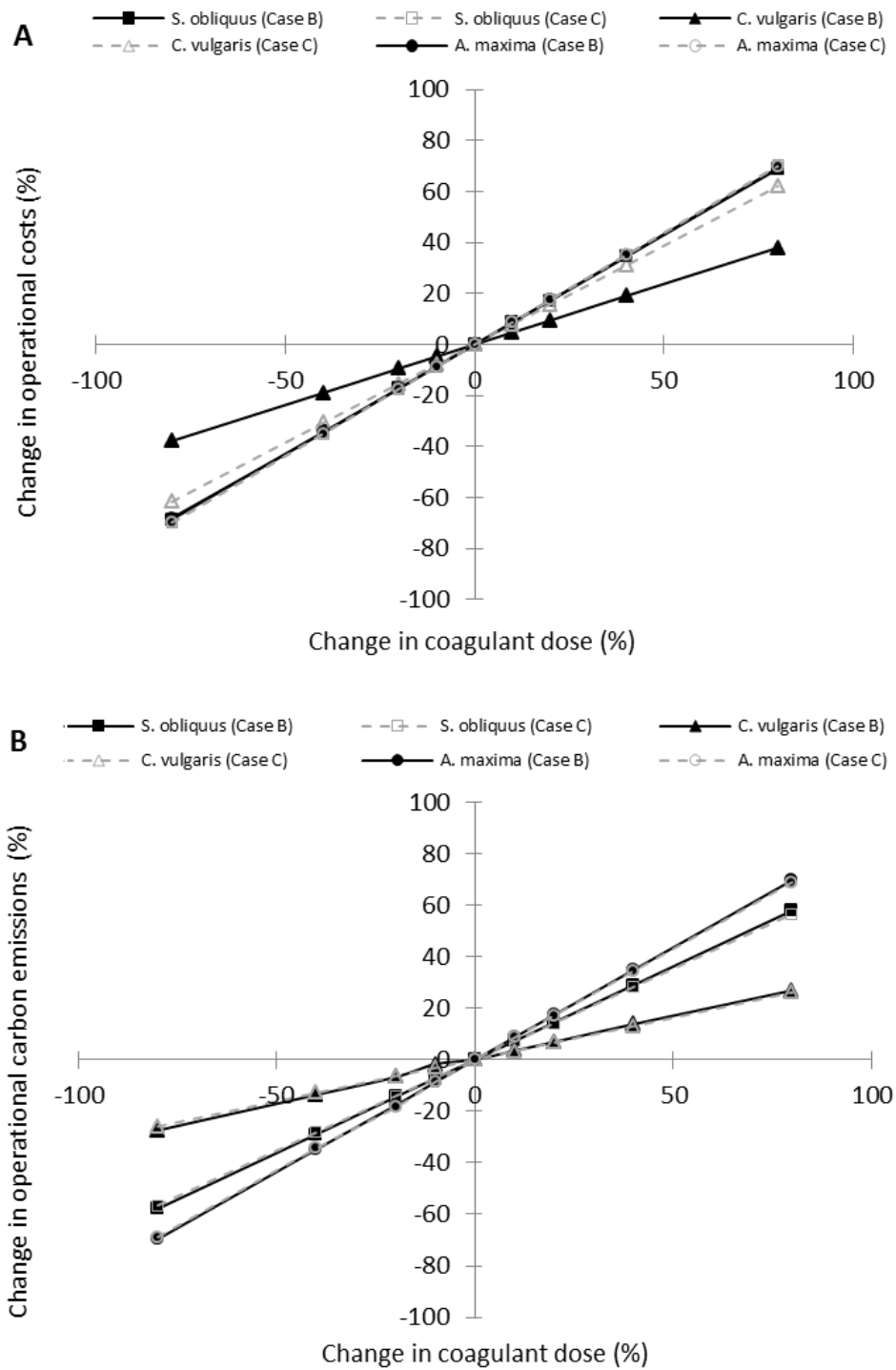
Figure 4.7 Impact of coagulant demand variation on (A) operational costs and (B) operational carbon emissions of the optimal case scenario identified (BDAF + Enzymatic hydrolysis).

Overall, the adoption of appropriate MAS enables the net generation of energy to be maintained at small works even when meeting tighter nutrient discharge consents and so fits with the current philosophy on small works (Vale, 2013). In contrast, even the most effective MAS option required an increase in opex of 234% and an increase in carbon footprint of 362% compared to the base case. Nevertheless, despite the environmental impact, applying a carbon emissions price of £4.94 per tCO_2 (Ares, 2013), the economic impact of the carbon footprint is negligible compared to the overall operational costs (Table 4.4). In all cases, MAS provided benefits compared to the aerated wetland, demonstrating the overall suitability of the technology option for upgrading small works.

Ultimately, consideration of the impact of design choices on the efficacy of MAS shows that harvest technology, pre-treatment options and algal reactor configuration principally influence the overall energy balance. In contrast, the impact of choice with regards to algal species is most predominantly observed in terms of the carbon footprint and the opex where the specific algae growth conditions will significantly impact on the overall outcomes of the system.

Table 4.4 Comparison between optimal energy balances considering the contribution of the overall system.

Scenario	Net energy consumption	Operational emissions	Carbon emission costs	Operational costs
	MWh y^{-1}	tCO$_2$eq y^{-1}	£ y^{-1}	£ y^{-1}
Base Case	-4.3	2.4	11.9	3,200
Case A	55.4	29.2	144.9	11,600
Case B (HRAP)[a]				
S. obliquus	-9.2	16.5	81.5	22,500
C. vulgaris	-11.7	8.4	41.5	10,200
A. maxima	-2.6	32.5	160.5	52,400
Case C (HRAP)[a]				
S. obliquus	-17	16.8	82.9	22,100
C. vulgaris	-17	8.7	43	7,500
A. maxima	-11.6	32.8	162	51,930

[a]*algae using BDAF as harvesting system and enzymatic hydrolysis as pre-treated; negative number represent surplus of energy; [a] economics saving when using MAS compared to aerated wetlands (Case A), applying a carbon emissions allowances of £ 4.94 per tCO$_2$ (Ares, 2013)*

4.2.5 References

Acién, F.G., Fernández, J.M., Magán, J.J., Molina, E., 2012. Production cost of a real microalgae production plant and strategies to reduce it. Biotechnol. Adv. 30, 1344-53.

Arbib, Z., Ruiz, J., Álvarez-Díaz, P., Garrido-Pérez, C., Barragan, J., Perales, J.A., 2013. Long term outdoor operation of a tubular airlift pilot photobioreactor and a high rate algal pond as tertiary treatment of urban wastewater. Ecol. Eng. 52, 143-153.

Ares, A. 2013. Carbon Price Floor. Library House of Commons, Standard Notes SN/SC/5927. www.parliament.uk/briefing-papers/sn05927.pdf (accessed 16 January 2014).

Austin, D., Nivala, J., 2009. Energy requirements for nitrification and biological nitrogen removal in engineered wetlands. Ecol. Eng. 35, 184-192.

Bahadar, A., Bilal Khan, M., 2013. Progress in energy from microalgae: A review. Renew. Sustain. Energy Rev. 27, 128-148.

Baliga, R., Powers, S.E., 2010. Sustainable Algae Biodiesel Production in Cold Climates. Int. J. Chem. Eng. 2010, 1-13.

Butterworth, E., Dotro, G., Jones, M., Richards, A., Onunkwo, P., Narroway, Y., Jefferson, B., 2013. Effect of artificial aeration on tertiary nitrification in a full-scale subsurface horizontal flow constructed wetland. Ecol. Eng. 54, 236-244.

Collet, P., Hélias Arnaud, A., Lardon, L., Ras, M., Goy, R., Steyer, J. P., 2011. Life-cycle assessment of microalgae culture coupled to biogas production. Bioresour. Technol. 102(1), 207-214.

Craggs, R., Sutherland, D., Campbell, H., 2012. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. J. Appl. Phycol. 24, 329-337.

Cromar, N.J., Fallowfield, H.J., 1997. Effect of nutrient loading and retention time on performance of high rate algal ponds. J. Appl. Phycol. 1, 301-309.

Day, J.G., Slocombe, S.P., Stanley, M.S., 2012. Overcoming biological constraints to enable the exploitation of microalgae for biofuels, Bioresour. Technol. 109, 245-251.

DEFRA and DECC, 2013. Greenhouse gas conversion factor repository. Department for Environment, Food and Rural Affairs and Department ofEnergy and Climate Change. http://www.ukconversionfactorscarbonsmart.co.uk/ (accessed 17 June 2013).

Garcia, J., 2000. High rate algal pond operating strategies for urban wastewater nitrogen removal. J. Appl. Phycol. 331-339.

Granados, M.R., Acien, F.G., Gomez, C., Fernandez-Sevilla, J.M., Molina Grima, E., 2012. Evaluation of flocculants for the recovery of freshwater microalgae. Bioresourc. Technol. 118, 102-110.

Hammond, G., Jones, C., 2011. Inventory of carbon and energy, version 2.0. Sustainable Energy Research Team (SERT), University of Bath. http://www.bath.ac.uk/mech-eng/sert/embodied/ (accessed 25 July 2011).

Heaven, S., Milledge, J., Zhang, Y., 2010. Comments on "Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable". Biotechnol. Adv. 29, 164-7.

Henderson, R. K., Parsons, S. A., Jefferson, B., 2008. Successful removal of algae through the control of zeta potential. Separ. Sci. Technol. 43(7), 1653-1666.

Henderson, R., Parsons, S. A., Jefferson, B., 2010. The impact of differing cell and algogenic organic matter (AOM) characteristics on the coagulation and flotation of algae. Water Res. 44(12), 3617-3624.

Jarvis, P., Buckingham, P., Holden, B., Jefferson, B., 2009. Low energy ballasted flotation. Water Res. 43 (14), 3427-3434.

Jefferson, B. 2013. Personal communication, Cranfield Waster Science Institute (CWSI), Cranfield University, United Kingdom.

Jonker, J.G.G., Faaij, a. P.C., 2013. Techno-economic assessment of micro-algae as feedstock for renewable bio-energy production. Appl. Energy 102, 461-475.

Ko, J., Day, J.W., Lane, R.R., Day, J.N., 2004. A comparative evaluation of money-based and energy-based cost–benefit analyses of tertiary municipal wastewater treatment using forested wetlands vs. sand filtration in Louisiana, Ecol. Econ. 49, 331-347.

Leader, J.W., Reddy, K.R., Wilkie, a C., 2005. Optimization of low-cost phosphorus removal from wastewater using co-treatments with constructed wetlands. Water Sci. Technol. 51, 283-90.

Metcalf & Eddy, Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2007. Wastewater engineering, treatment and reuse. McGraw Hill Education. (2007).

Molina Grima, E., Belarbi, E.-H., Acién Fernández, F.G., Robles Medina, a, Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol. Adv. 20, 491-515.

Nielsen, P.H., Oxenbøll, K.M., Wenzel, H., 2007. Cradle-to-gate environmental assessment of enzyme products produced industrially in Denmark by Novozymes A/S. Int J LCA, 12(6), 432-438.

Norsker, N., Barbosa, M.J., Vermuë, M.H., Wijffels, R.H., 2011. Microalgal production — A close look at the economics, Biotechnol. Adv. 29, 24-27.

Olguín, E.,J., Galicia, S., Camacho, R., Mercado, G., Pérez, T.J., 1997. Production of Spirulina sp. in sea water supplemented with anaerobic effluents in outdoor raceways under temperate climatic conditions. Appl. Microbiol. Biotechnol. 48, 242-247.

Ometto, F., Pozza, C., Whitton, R., Smith, B., Torres, A.G., Henderson, R.K., Jarvis, P., Jefferson, B., Villa R., 2014. The impact of replacing air bubbles with microspheres for the clarification of algae from low cell-density culture. Water Res. 53, 168-179. (Current Thesis, Paper 2.2).

Ometto, F., Whitton, R., Coulon, F., Jefferson, B., Villa R., 2013. Improving the energy balance of an integrated microalgal wastewater treatment process, Waste and Biomass Valorization (Appendix E).

Pearce, P. 2013. Sustainable processes to meet low ammonia consents: the small works challenge. 2nd Conference in low energy wastewater treatment systems, 24th September 2013, Solihul, UK.

Pérez-Elvira, S.I., Fdz-Polanco, F., 2012. Continuous thermal hydrolysis and anaerobic digestion of sludge. Energy integration study. Water Sci. Technol. 65, 1839-46.

Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F., 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production, Appl. Energy. 103, 444-467.

Roleda, M.Y., Slocombe, S.P., Leakey, R.J.G., Day, J.G., Bell, E.M., Stanley, M.S., 2013. Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy, Bioresour. Technol. 129, 439-449.

Shi, C.Y., 2011 Mass flow and energy efficiency of municipal wastewater treatment plants. IWA Publishing, London (2011).

Sialve, B., Bernet, N., Bernard, O., 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol. Adv. 27, 409-416.

Slade, R., Bauen, A., 2013. Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects. Biomass Bioener. 53, 29-38.

Sydney, E.B., da Silva, T.E., Tokarski, A., Novak, A.C., de Carvalho, J.C., Woiciecohwski, A.L., Larroche, C., Soccol, C.R., 2011. Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage, Appl. Energy. 88, 3291-3294.

UM, 2011. CCaLC© manual, version 2.0, carbon calculations over the life cycle of industrial activities. The University of Manchester. http://www.ccalc.org.uk/ (accessed 17 August 2011).

Vale, P., 2013. Sustainable phosphorus removal. In: 2nd national conference on Low energy wastewater treatment, 24th September 2013, Solihul, UK.

Ventura, J.S., Yang, B., Lee, Y., Lee, K., Jahng, D., 2013. Life cycle analyses of CO_2, energy, and cost for four different routes of microalgal bioenergy conversion. Bioresour. Technol. 137, 302-310.

Whitton, R., Villa, R., Jefferson, B. 2013. Wastewater treatment using immobilised algal biomass. In: International Algal Conference on Innovative

Sequestration and Biological Production, 10th – 11th June 2013, Lolland, DK.

Yahi, H., Elmaleh, S., Coma, J., 1994. Algal flocculation-sedimentation by pH increase in a continuous reactor. Wat. Sci. Tech. 30(8), 259-267.

Zamalloa, C., Vulsteke, E., Albrecht, J., Verstraete W., 2011. The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. Bioresour. Technol. 102, 1149-1158.

Chapter 5

CONCLUSIONS AND FUTURE WORKS

5 CONCLUSIONS AND FUTURE WORKS

5.1 Conclusions

The major conclusion of this work was that microalgae represents a viable alternative for wastewater nutrients removal systems, improving the energy balance of a small integrated microalgae wastewater treatment when combined with low cost harvesting technologies and high efficient AD pre-treatments such as thermal or enzymatic hydrolysis. However, the feasibility of the system will depend also on the algae species used for the process.

Specific conclusions were as follows:

- A review of the innovation on microalgae low energy harvesting technologies showed that the specific characteristics of the algae suspension, such as spent growth media (which includes of algogenic organic matter) and cell's physical characteristics, determine the range of technologies which can guarantee efficient separation and low cost processes (Paper 2.1; Objective 1). The work also suggests that the quality of the recovered algal biomass will depend on the harvesting system and that the market price of the final algae products will also help to determine the most appropriate process. Hence, when selecting the harvesting technology for specific applications, it is fundamental to know in detail the characteristics of the algal biomass and the economical/energy targets to achieve.

- The low-energy harvesting system used in Paper 2.2, Ballasted Dissolved Air Flotation (BDAF), was demonstrated to be a low-cost and more sustainable alternative to the original Dissolved Air Flotation (DAF) process, suitable for low cost algae derived products such as biofuels. In addition to the energy savings, the adoption of floating microspheres in place of air micro-bubbles was able to reduce coagulant demand (up to 95% depending on the algae species), generating carbon and economic saving (Paper 2.1; Objective 2). The cost of the process could be further decreased once the separation and recovery method of the glass

microspheres will be optimised. Similarly to the coagulant demand, the bead separation process will be linked to the algae species treated and to the strength of the algae-bead floc formed by the algae. The floc structure obtained using low concentrations of coagulant is different when treating single cells or filamentous algae. The latter in particular showed a more expanded and loose structure, suggesting lower energy requirements for the bead recovery system.

- Algae biomass can be used as anaerobic digestion (AD) feedstock, and their degradation yields can be improved by adapting the microbial community of the digester. Indeed, algae harvested from the wastewater treatment plant will be associated to a microbial community adapted to use the algae cells and their degradation products. As suggested in Paper 2.1, the endogenous microbial community added to the digester with the wastewater algae produced a positive impact on the composition of the AD microbial community and the reactor performance (Paper 2.1; Objective 2 and 3). Hence, when digesting untreated algae, it is recommended to provide an adaptation period to the AD system using step-wise additions and after that maintain a constant amount of algae in the feedstock. This finding is of particular interest for processing seasonal algae waste from eutrophic environments which can therefore be treated by existing facilities without compromising their performances.

- The stoichiometric biogas potential of microalgae can only be achieved by pre-treating the biomass. As for the harvesting process, optimal pre-treatment conditions are algae species specific (Paper 3.2; Objective 4). In particular, higher energy intensive processes need to be used when dealing with single cell algae containing acetolysis resistant polymers (ARB). As a consequence, when designing new microalgae cultivation systems for biogas or biofuel production, the pre-treatment unit needs to take into account the specific characteristics of the algae biomass to allow efficient cell degradation. Conversely, when integrating a microalgae nutrient removal process on an existing WWT plant with on-

site sludge pre-treatments, the algae biomass could be selected to maximise the impact of the existing pre-treatment unit. Amongst the pre-treatments investigated in Paper 3.2, enzymatic hydrolysis showed the highest levels of cell solubilisation, producing the most positive energy balance. This suggests that the breakage of the cell wall membrane obtained using physical pre-treatments is not sufficient to guarantee complete microalgae digestion which required solubilisation of the bacterial resistant compounds affecting digestion.

- The inclusion of the microalgae system (MAS) as an upgrade option for small sewage works enables delivery of stringent effluent nutrient discharge concentrations whilst generating surplus of energy (Paper 4.2; Objective 5). The utilisation of a low-energy harvesting unit (e.g. BDAF), combined with an efficient low-energy pre-treatment (e.g. enzymatic hydrolysis) showed the potential to more than offset the energy demand, generating additional energy saving. This saving can be used to balance the low biomass availability expected when processing final wastewater effluent, where the low nutrient content limits the growth of excess biomass.

- Compared to alternative conventional low–energy wastewater treatments such as aerated wetlands, the MAS was demonstrated to be more economic and sustainable, reducing operational costs and carbon emissions (Paper 4.2; Objective 5).

- The impact of design choices on the efficacy of MAS demonstrated that harvest technology, pre-treatment options and algal reactor configuration principally influenced the overall energy balance. In contrast, the impact of algal species selection is most predominantly observed in terms of the carbon footprint and the opex.

5.2 Future works

In the course of this project, a few areas for further research have been identified. These are listed below:

- Chapter 2 showed that the AOM composition and the physical characteristics of the microalgae cells have a large impact on the harvesting process costs and hence on the whole production cost of algae biomass. Additional algae species need to be characterised to provide a broader picture of the influence of different algae and their AOM composition on specific harvesting technologies. All the thesis work was carried out using algae cultivated indoor on synthetic media to allow a fair comparison of the different methods investigated. A follow-on investigation on the impact of wastewater on the AOM composition and cell characteristics of algae grown in environmental conditions will allow validatation of the results into more practical applications.

- A large- and pilot-scale BDAF harvesting system using algae grown in wastewater is required to validate the energy and financial savings identified in the current thesis. In particular, the microsphere separation process needs to be set-up to guarantee efficient algae–bead separation without compromising the integrity of the algae cells. In addition, as for the previous point, other algae species and/or mixed communities need to be tested to verify the performance of the system in environmental conditions.

- The glass microspheres (ballasting agents) represent the major costs for the BDAF harvesting system. Substitution of those with a more economical material, such as biopolymers, has the potential to make this technology more competitive on the market. However, different materials will impact differently on the algae-bead floc formation and the coagulant dosages which will need to be addressed by further experimental work.

- Pilot- and full-scale facilities for AD of microalgae biomass are still limited. A pilot-scale study will confirm the overall feasibility of the

process. In addition, the behaviour of the algae biomass when pre-treated and/or co-digested with wastewater sludge or other biomass, or when using a mix of different algae species needs to be addressed with further research.

- The results reported in Paper 3.2 demonstrated the use of enzymes as an efficient low energy pre-treatment to enhance the methane production from microalgae biomass. The outcomes of this work are very promising and, together with the current research interest in this area, suggest the need for additional investigations of the enzymatic hydrolysis using different enzymes. For example, the adoption of enzymes working at low temperatures could allow their direct application in the reactor without the need of a separate pre-treatment step of the biomass. In addition, the impact of the enzymes on the AD microbial community, as well as on digestion parameters such as volatile fatty acids, alkalinity and pH, need to be investigated.

APPENDICES

APPENDICES

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

Appendix A - Microalgae growth curve

Growth curves of *Scenedesmus obliquus, Chlorella vulgaris* and *Arthrospira maxima*. Cell counting was performed manually using a light microscope with a haemocytometer for S*. obliquus* and *Chlorella* sp. or a Sedgewick Rafter for *A. maxima*. All algal biomass was harvested at the stationary growth phase (maximum yield) where the cell morphology is homogeneous and the AOM has the greatest effect on the coagulation.
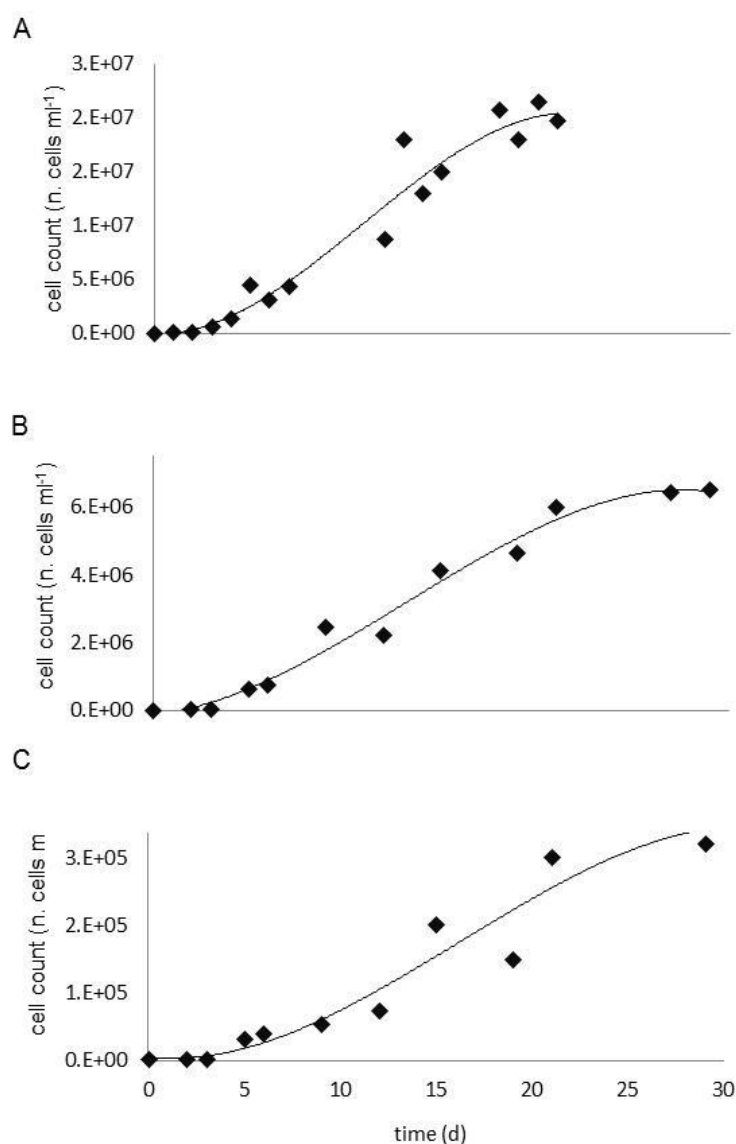
Figure A.1 Microalgae grow curve. *Chlorella vulgaris* (A), *Scenedesmus obliquus* (B) cultivated in Jaworski's medium and *Arthrospira maxima* (C) cultivated in Zarrouk's medium.

225

A.1 Jaworski's medium

In 1 litre, 1 ml of stock solution: 4 g/200ml $Ca(NO_3)_2$ $4H_2O$; 2.48 g/200ml KH_2PO_4; 10g/200ml $MgSO_4$ $7H_2O$; 3.18 g/200ml $NaHCO_3$; 0.45 g/200ml EDTAFeNa and EDTANa$_2$; 0.496 g/200ml H_3BO_3 with 0.278 g/200ml $MnCl_2$ $4H_2O$ and 0.2 g/200ml $(NH_4)_6Mo_7O_{24}$ $4H_2O$; 0.008 g/200ml Cyanocobalamin with Thiamine HCl and Biotin; 26 g/200ml $NaNO_3$; 7.2 g/200ml Na_2HPO_4 $12H_2O$.

A.2 Zarrouk medium

In 1 litre: 18.0 g NaHCO3, 2.5 g NaNO3, 0.5 g K2HPO4, 1.0 g K_2SO_4, 1.0 g NaCl, 0.04 g $CaCl_2$, 0.08 g Na_2EDTA, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$ and 1.0 ml trace elements (TE). TE: 2.86 g H_3BO_3, 0.02 g $(NH_4)_6Mo_7O_{24}$, 1.8 g $MnCl_2 \cdot 4H_2O$, 0.08 g Cu_2SO_4, 0.22 g $ZnSO_4 \cdot 7H_2O$, all in 1 litre).

A.3 Algogenic Organic Matter (AOM)

In Figure A.2 the composition of the AOM released at exponential (EXP) and stationary (ST) growth phase of different algae species is compared. The proteins:carbohydrates ratio affects the hydrophobicity of the overall algae suspension, and therefore the charge density and coagulant demand (Chapter 2).
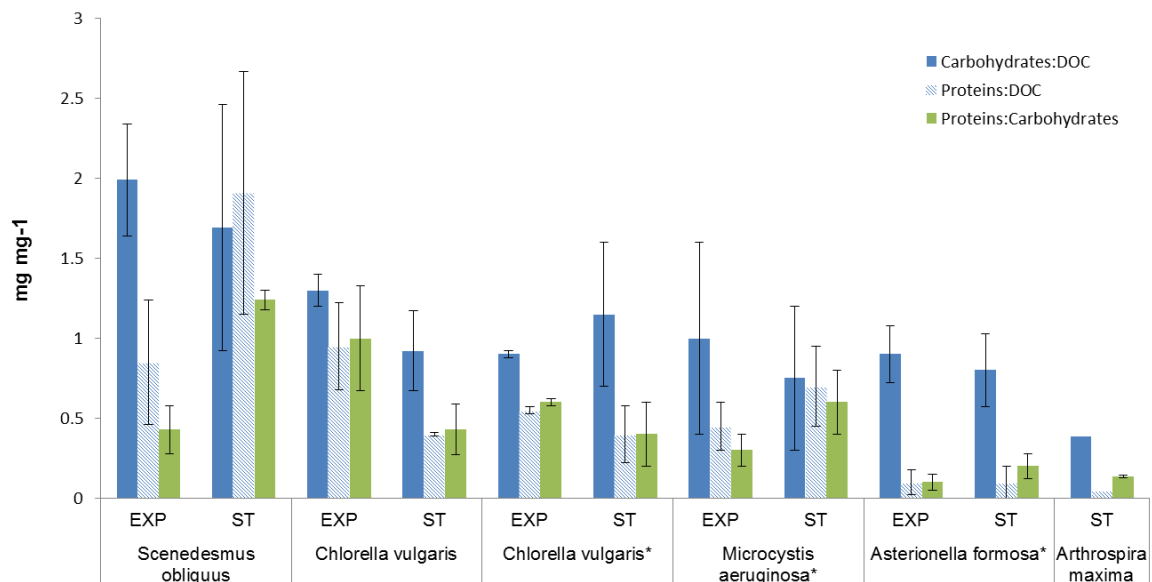

Figure A.2 AOM concentration in different algae species. *EXP = exponential growth phase; ST = stationary growth phase. *Henderson et al., 2010.*

A.4 References

Henderson, R., Parsons, S. A., Jefferson, B., 2010. The impact of differing cell and algogenic organic matter (AOM) characteristics on the coagulation and flotation of algae. Water Res. 44(12), 3617-3624.

Appendix B - Preliminary experiments of anaerobic digestion

B.1 Experiment 1

Correlation between small (100 ml serum bottle placed in an incubator at 38°C with constant shaking) and large (1000 ml duran bottle placed at 38°C in a water bath and shake manually once a day) batch digestion experiments using a number of different samples including microalgae (mixture of *Scenedesmus obliquus* and *Chlorella* sp.), digestate from four AD plants and cellulose.
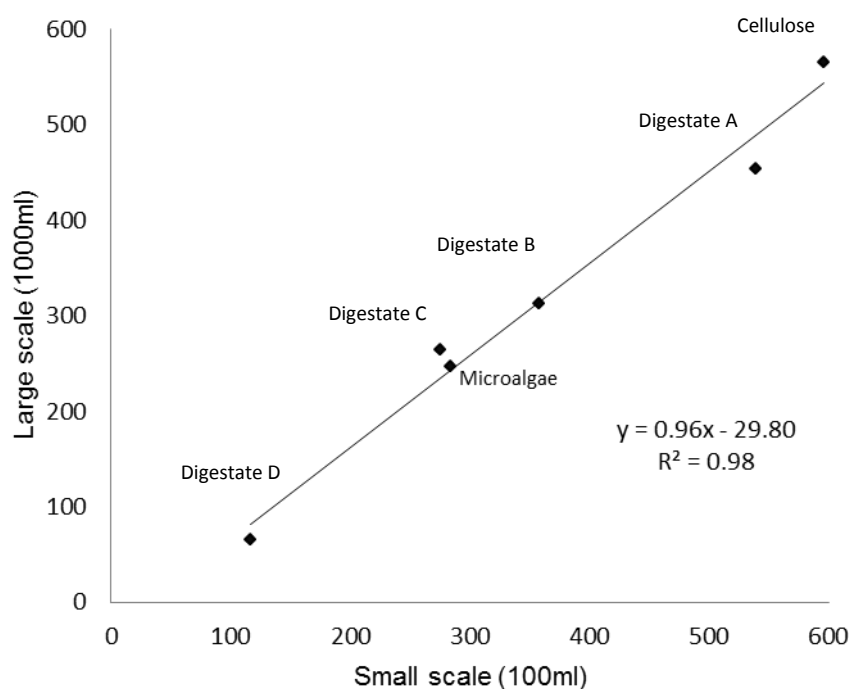
Figure B.1 Biogas production (ml gVS_{add}^{-1}) using large scale and small scale batch system.

B.2 Experiment 2

Batch anaerobic digestion of cultivated algae biomass under different $VS_{inoculum}$:$VS_{substrate}$ ratio (VSr) using *Scenedesmus obliquus*, *Chlorella sorokiniana*, *Arthrospira maxima*, Primary sludge (PS) and a mixture of PS and waste activated sludge (WAS).

Table B.1 Biogas production of different substrates.

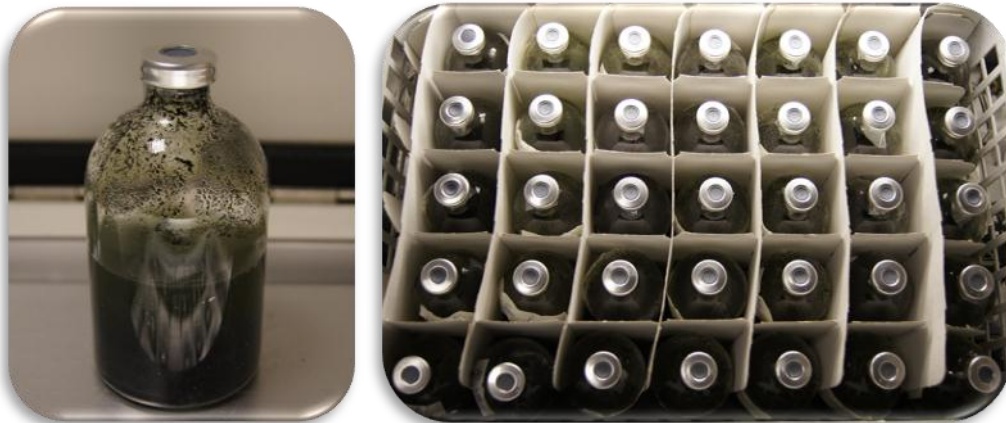| Substrate	VSr 2:1			VSr 1:1			VSr 1:2		
	K_h	Biogas	CH_4	K_h	Biogas	CH_4	K_h	Biogas	CH_4
	(d^{-1})	(ml gVS^{-1})	(%)	(d^{-1})	(ml gVS^{-1})	(%)	(d^{-1})	(ml gVS^{-1})	(%)
S. obliquus	0.26	563±112	61	0.08	335±10	58	0.05	317±85	43
C. sorokiniana	0.27	425±38	67	0.07	298±03	60	0.06	322±64	54
A. maxima	0.14	310±30	62	0.12	145±06	-	-	-	-
PS	0.15	614±14	82	0.09	350±62	83	0.08	278±03	83
PS/WAS 80/20	0.12	286±48	70	0.13	330±86	85	0.10	234±7	75
PS/WAS 60/40	0.25	540±2	83	0.11	463±73	81	0.08	324±104	80

Figure B.2 Small scale anaerobic digestion apparatus

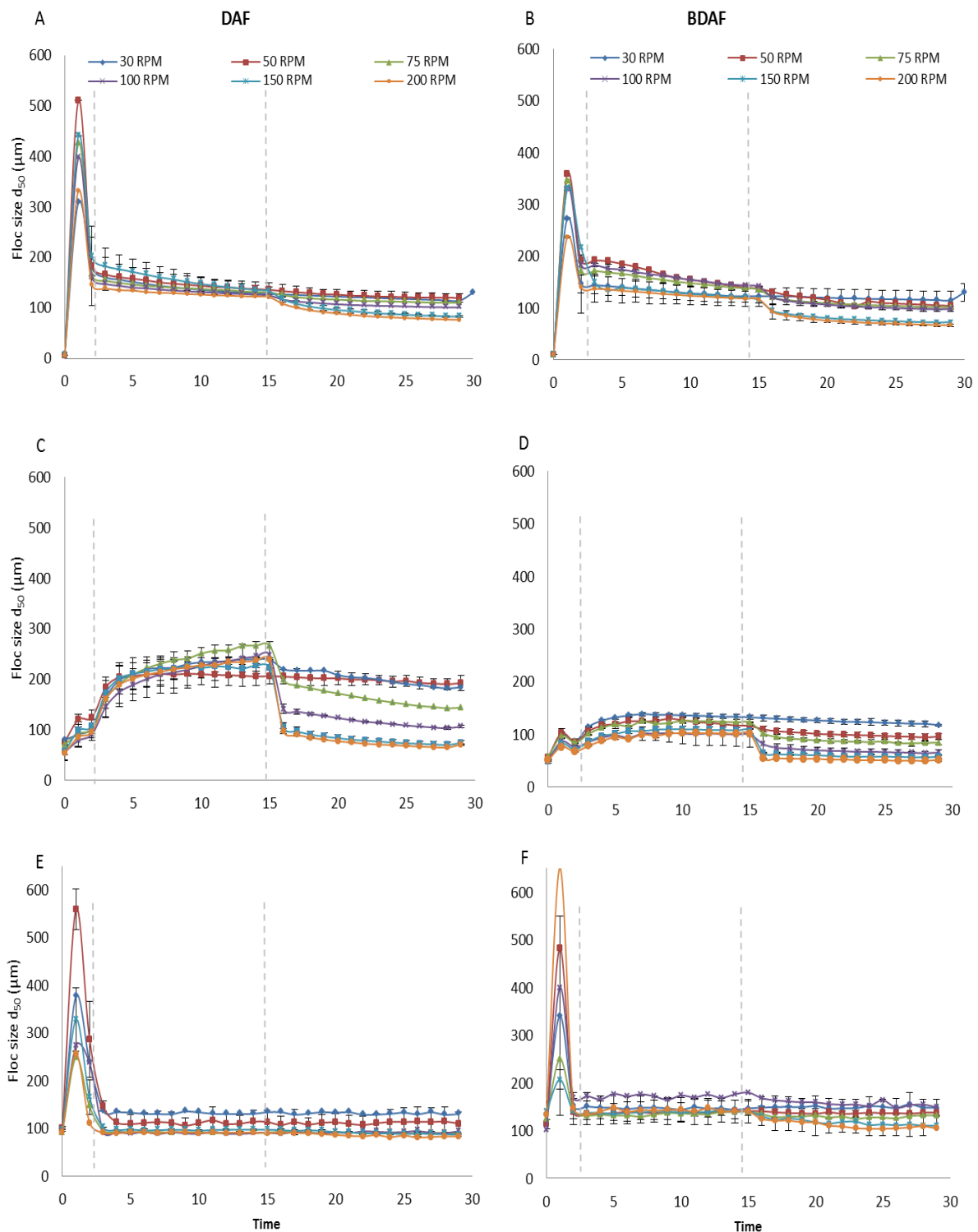# Appendix C - Paper 2.2 supporting information

Figure C.1 Floc growth and breakage profile for DAF (left column) and BDAF (right column) system, of *S. obliquus* (A and B), *C. vulgaris* (C and D) and *A. maxima* (E and F) at increasing shear rate

Appendix D - Paper 4.2 supporting information

Figure D.1 Additional energy demand for (A) *S. obliquus*; (B) *C. vulgaris* and (C) *A. maxima* compared to the base case (BC) and aerated wetland (AW), based on using a PBR - negative numbers represent a surplus of electricity.

Figure D.2 Additional operational carbon emission for (A) *S. obliquus*, (B) *C. vulgaris* and (C) *A. maxima,* compared to the base case (BC) and the aerated wetland (AW), based on using a PBR.
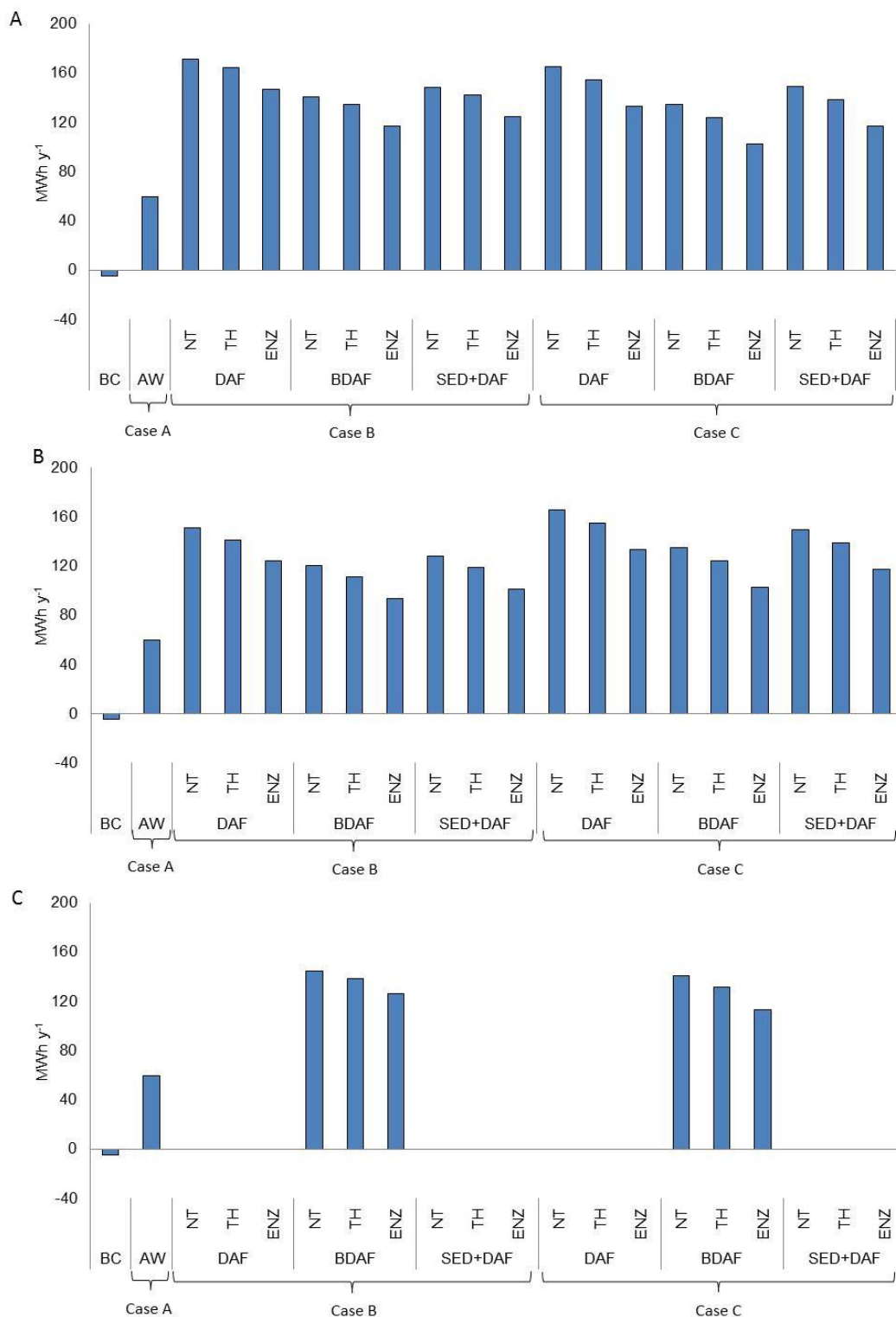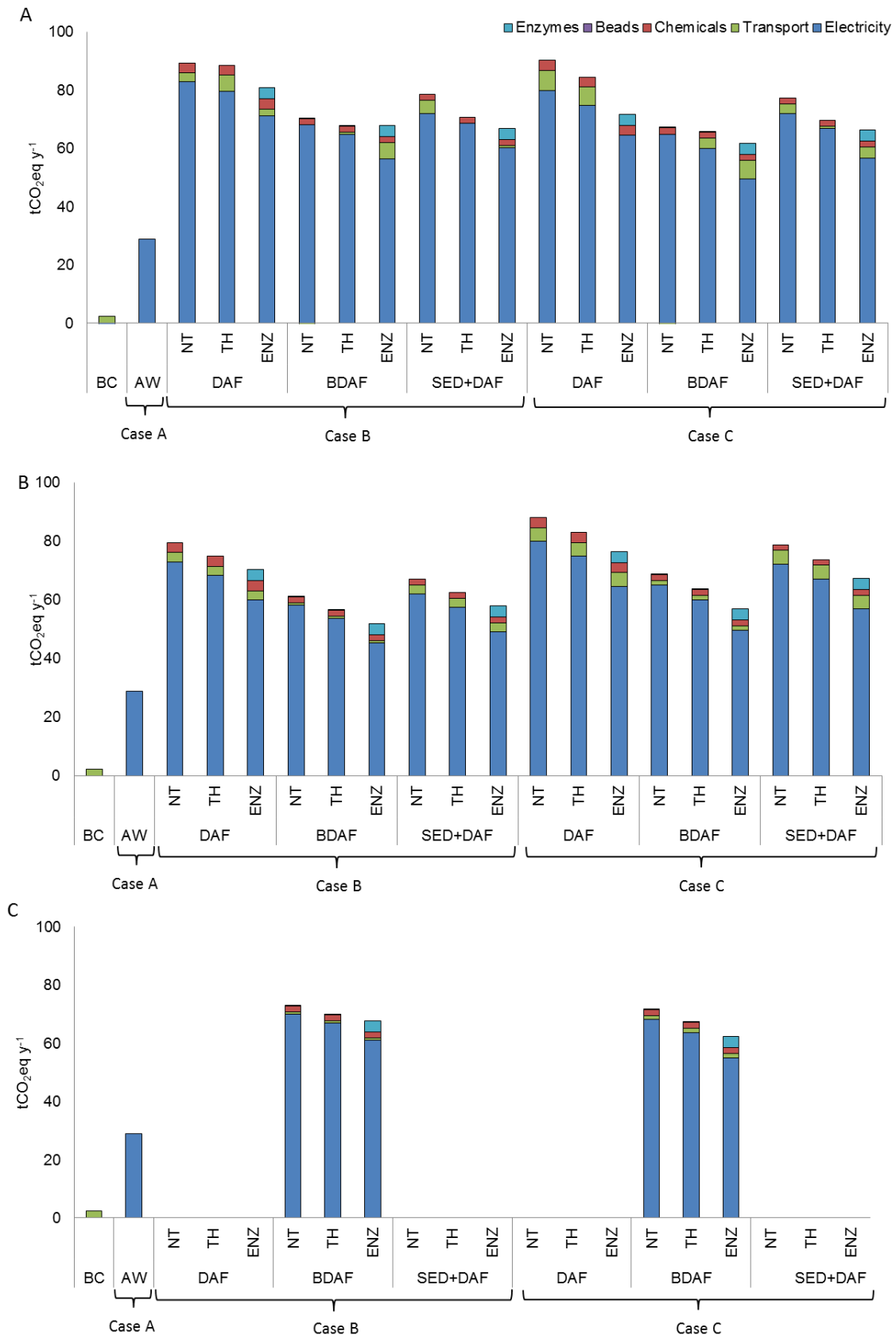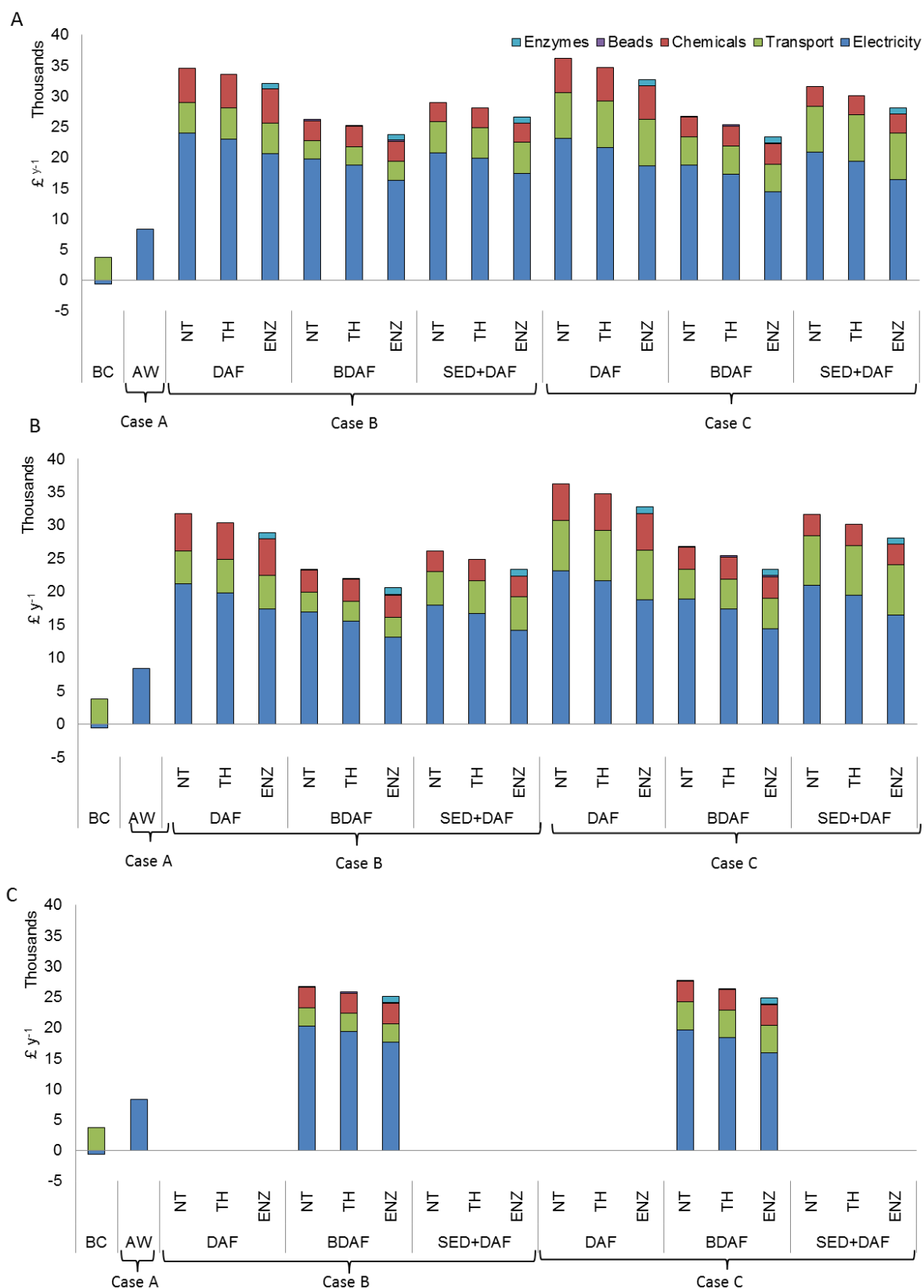
Figure D.3 Additional operational carbon emission for (A) *S. obliquus*, (B) *C. vulgaris* and (C) *A. maxima,* compared to the base case (BC) and the aerated wetland (AW), based on using a PBR.

Appendix E - Improving the energy balance of an integrated microalgal wastewatert treatment process

Francesco Ometto, Rachel Whitton, Frédéric Coulon, Bruce Jefferson and Raffaella Villa.

Cranfield University, Bedfordshire, UK

Abstract

The inclusion of a microalgal system in a wastewater treatment flowsheet for residual nutrient uptake can be justified by processing the waste biomass for energy recovery. Low energy harvesting technologies and pre-treatment of the algal biomass are required to improve the overall energy balance of this integrated system. *Scenedesmus obliquus* and *Chlorella* sp., achieving nitrogen and phosphorus removal rates higher than 90%, were used to compare cells recovery efficiency and energy requirements of two energy efficient harvesting systems: Dissolved Air Flotation (DAF) and Ballasted Dissolved Air Flotation (BDAF). In addition, thermal hydrolysis was used as a pre-treatment to improve biogas production during anaerobic digestion. The energy required for both systems was then considered to estimate the daily energy demand and efficiency of two microalgae wastewater treatment plants with a capacity of 25,000 and 230,000 p.e., respectively. Overall, a high algal cells recovery efficiency (99%) was achieved using low energy demand (0.04 kWh m^{-3} for BDAF) and a coagulant dose reduction between 42 and 50% depending on the algal strain. Anaerobic digestion of pre-treated *S. obliquus* showed a 3-fold increase in methane yield. Compared to a traditional activated sludge process, the additional tertiary microalgal treatment generates an integrated process potentially able to achieve up to 76% energy efficiency.

E.1.1 Introduction

Utilisation of algae as part of a nutrient removal strategy within wastewater treatment enables relatively passive polishing of residual nitrogen and phosphorus in reactors with residence times ranging between 2 - 4 days (Martinez *et al.,* 2000; Xin *et al.,* 2010). For instance, batch reactors operated over a 2-day cycle time containing *Chlorella vulgaris* and *Scenedesmus obliquus* resulted in 80 and 96% removal of ammonia respectively (Ruiz-Marin *et al.,* 2010). Extending residence times to 15 days with *S. obliquus* has demonstrated the capability to reach effluent concentration as low as 0.01 mg l^{-1} total phosphorus (TP) (Xin *et al.,* 2010) indicating potential for small works to meet very low discharge consents as long as sufficient land is available. In addition to nutrient removal, microalgae can acts as CO_2 sequestration agent at rates of around 1.8 $kgCO_2$ $kg_{biomass}^{-1}$ and so have the potential to be integrated into biogas upgrade loops as a means of CO_2 disposal.

A range of reactor configurations have been considered including algal ponds, photo-bioreactors, immobilisation and attached systems (Demirbas *et al.,* 2010; Christenson *et al.,* 2011; Pittman *et al.,* 2011). Photo-bioreactors are typically used when high value products are generated from the algae biomass where concentrations in the reactors can reach up to 2 kg m^{-3} (Min *et al.,* 2011; Tredici 2007). In the Case of wastewater treatment the majority of systems are based on algal ponds where biomass concentration remains below 1 kg m^{-3} with average values between 0.2 and 0.6 kg m^{-3} (Cromar and Fallowfield 1997; Tredici 2007). In either configuration two additional requirements must be met for them to be integrated into a wastewater treatment flowsheet. Firstly, the algae must be separated from the water phase prior to discharge and secondly the algae must be used/disposed of. In the wastewater context, anaerobic digestion of the collected biomass appears the most sensible option as the quantities are generally quite small and the AD assets already exist. In such cases an interesting opportunity presents itself whereby the energy required to operate the algae reactors may be offset by the additional energy produced through digestion of the used algal biomass. Examination of the requirements for integration of algae reactors into a standard wastewater flowsheet reveals two key components: (i) the need for a low energy cell recovery system to reduce energy requirement for biomass harvesting and (ii) the

need to maximum biogas production from algae through pre-treatment of the algal cells.

Typical separation processes used for algae harvest include centrifuges or pressure and vacuum filters with associated energy demands ranging between 0.3 and 8 kWh m^{-3} (Molina Grima *et al.,* 2003). At large scale, low energy systems (< 0.3 kWh m^{-3}) such as chemical flocculation, bio-flocculation or autoflocculation, are considered efficient pre-concentration technologies which can reduce operation costs when combine with traditional harvesting system (Beach *et al.,* 2012; Salim *et al.,* 2012) The main alternative to those is the use of dissolved air flotation (DAF). The system generates micro bubbles of air which attach to algae cells and allow them to float (Edzwald, 1993). Generation of the bubble is through released of a supersaturated water solution akin to beer production and has an energy associated with it of around 0.3 kWh m^{-3} (Jarvis *et al.,* 2009). Recent innovations in the technology have replaced the produced air with glass beads in a process called ballasted dissolved air flotation (BDAF) were the beads can be recycled enabling reduction in energy of 60 – 80% compared to traditional DAF systems (Jarvis *et al.,* 2009). Anaerobic digestion of algae in traditional mesophilic digesters yields between 30% and 50% of the potential theoretical values (Golueke *et al.,* 1957; Mussgnug *et al.,* 2010; Heaven *et al.,* 2011) Higher efficiencies has been reported for thermophilic conditions or when co-digesting algae with other biomass (Yen and Brune 2007; Zamalloa *et al.,* 2012). In all cases, the hardness of the cell wall seems to represent the main inhibitor factor (Golueke *et al.,* 1957; Gonzalez-Fernandez *et al.,* 2012a). The cell wall of green algae is mainly composed of sugars (24 – 74%), such as glucose, mannose and galactose, forming cellulose and hemicellulose with biopolymers (e.g. sporopollenin, algaenan) which are responsible of the thickness and the resistance of the cells to bacteria degradation (Abo-Shady *et al.,* 1993; Gonzalez-Fernandez *et al.,* 2012b). In order to overcome this limitation, a range of pre-treatment methods such as ultrasound, high temperature, French press and enzymes have been used to improve algae digestion and biomethane yields (Alzate *et al.,* 2012; Gonzalez-Fernandez *et al.,* 2012b; Ehime *et al.,* 2013). In relation to wastewater treatment, one of the most commonly used pre-treatment processes is the thermal hydrolysis (STOWA, 2006; Shi, 2011). The process works by applying a combination of temperature (150 – 170 °C) and pressure (6 - 8 bar), which breaks down the physical structure of all the organic material including algae.

Linking together the innovative approaches outlined here potentially improves the opportunity to be more sustainable and energetically balanced in relation to nutrient removal. The current paper considers this by evaluating the impact of inclusion of these technologies in a wastewater flowsheet containing an algal reactor for nutrient polishing (Figure 4.6). In particular the work compares Dissolved Air Flotation and Ballasted Dissolved Air Flotation for algae collection and the effect of a thermal hydrolysis pre-treatment on algal cell disruption and digestion yields using *S. obliquus* and *Chlorella* sp*.* The two technologies were combined in different scenarios to estimate the energy demand and the energy efficiency at two different scales of operation: 25,000 and 230,000 p.e., respectively.
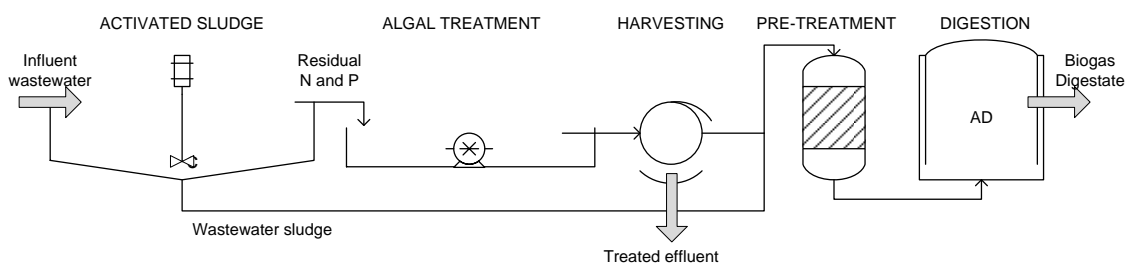
Figure E.1 Schematic diagram of an integrated microalgae wastewater treatment process

E.1.2 Materials and methods

Algal culture

Experiments were conducted on two single cell green microalgae species: *S. obliquus* (276/42) and *Chlorella* sp. (211/BK) which were obtained from the Culture Collection for Algae and Protozoa (Oban, UK) and cultivated in Jaworski Media (Henderson *et al.,* 2009). Algal growth was characterised using cell counting with soluble protein content (sPC) and soluble carbohydrate content (sCC) measured according to the methods described in Henderson *et al.* (2009). Solids content, chemical oxygen demand (COD) and soluble COD (sCOD) were measured according to APHA standard methods (Greenberg *et al.,* 1992).

Microalgae harvesting

Jar tests experiments (1l) were undertaken using an EC Engineering DBT6 DAF jar tester (Alberta, CND). The DAF and BDAF tests were performed according to

Henderson *et al.* (2009) and Jarvis *et al.* (2009), respectively. Biomass concentration of $5 \times 10^6 \pm 10^5$ cells ml^{-1} was used for the different testing condition. The pH was adjusted to 7 using a 0.1 M HCl and 0.1 M NaOH solution. 300 mg l^{-1} of low-density glass beads between 40 and 100 µm with a density of 100 kg m^{-3}, from Trelleborg Emerson and Cuming Inc. (Mansfiled, USA) were used after a pre-flotation test to eliminate non-floating beads (Jarvis *et al.,* 2009), Aluminium sulphate ($Al_2(SO_4)_3$) was used as coagulant. The clarified samples were analysed for residual cell content. All analyses were carried out in duplicate.

Thermal hydrolysis treatment

Thermal hydrolysis of the algal biomass was achieved using a Baskerville autoclave and steam generator WON15827 (Manchester, UK). The unit is composed of two connected pressure vessels. Steam generated at 165°C and 8 bar was flash-injected for 30 min into the reaction vessel where concentrated algae, 200 ml at 2.0 ± 0.5 g TS l^{-1}, were maintained at 90°C. Cell counting, solid content, COD, sCOD, sCP, sPC were measured in duplicate before and after treatment.

BioMethane Test (BMT)

The biomethane production was determined using a modified method of Angelidaki *et al.* (2009) Digested sludge seed (inoculum) was obtained from a local WWTP and incubated at 38°C for 2-3 weeks to eliminate any residual activity. Seed and pre-concentrated algal biomass were mixed to obtain a volatile solids (VS) ratio of 1:1 ($VS_{seed}:VS_{algae}$). 20 ml of the mix was then transferred to a 100 ml serum bottle. The pH was adjusted to a value of 7 using a 1 M NaOH solution and the bottles were filled with 40 ml of nutrient solution (Angelidaki *et al.,* 2009) to a final volume of 60 ml leaving a head space of 40 ml. All tests were flushed with N_2 gas, sealed with a PTFE crimp cap, and then placed into a shaking incubator at 38°C and 150 rpm. Biogas production and composition were determined at day 2, 5, 8, 12, 16, 21 and 25. The methane content was measured using a Servomex 1440 gas analyser (Crowborough, UK). All tests were conducted in triplicate using treated or untreated algae.

Energy efficiency evaluation

The daily energy demand and efficiency of an integrated wastewater plant involving an activated sludge (AS) system followed by a microalgal raceway pond and an on-site anaerobic digestion (AD) was evaluated using the data reported by Shi (2011) and by Zamalloa *et al.* (2011). The efficiency of the WWTP is defined as the percentage amount of energy produced compare to the total energy demand. The treatment capacity and energy requirement of the two plant sizes considered are shown in Table 4.3. Six different scenarios with different configuration were considered including:

1. Activated Sludge and Anaerobic Digestion (AS+AD)

2. Activated Sludge, Algal Pond, DAF harvesting system and Anaerobic Digestion (AS+Pond+DAF+AD)

3. Activated Sludge, Algal Pond, BDAF harvesting system and Anaerobic Digestion (AS+Pond+BDAF+AD)

4. Activated Sludge and Anaerobic Digestion with a Pre-Treatment step (AS+Pre-treat.+AD)

5. Activated Sludge, Algal Pond, DAF harvesting system and Anaerobic Digestion with a Pre-Treatment step (AS+Pond+DAF+Pre-treat.+AD)

6. Activated Sludge, Algal Pond, BDAF harvesting system and Anaerobic Digestion with a Pre-Treatment step (AS+ Pond+BDAF+Pre-treat.+AD).

Harvesting energy demand values used were equivalent to 0.3 kW m^{-3} and 0.04 kW m^{-3} for DAF and BDAF system, respectively (Jarvis *et al.,* 2009). The energy generated by the wastewater sludge digestion was back calculated from the assumed energy efficiency (Table E.1). Additional energy from algal digestion was estimated using the methane yields reported in the present work, applying a methane energy conversion of 9.7 kWh m^{-3} and 30% efficiency (Shi, 2011).

Table E.1 Integrated WWTP parameter design

Traditional WWTP (AS+AD)	TP25K	TP230K
Capacity	25000 p.e.	230000 p.e.
Influent$_a$	4200 m^3 d^{-1}	38640 m^3 d^{-1}
Energy demand (AS)$_b$	0.6 kWh m^{-3}	0.45 kWh m^{-3}
Energy efficiency without AD pre-treatment$_c$	25%	35%
Energy efficiency with AD pre-treatment$_c$	40%	60%
Algal treatment	TP25K	TP230K
Pond dimension$_d$	8.4 ha	77.3 ha
Biomass production (VS)$_e$	1.06 ton d^{-1}	9.74 ton d^{-1}
Energy demand (cultivation)$_f$	32.5 kWh ha^{-1} d^{-1}	32.5 kWh ha^{-1} d^{-1}

[a]water availability of 210 l d^{-1} p.e. and a recovery coefficient of 0.8; [b]electricity consumption (Shi, 2011; [c] assuming a thermal hydrolysis energy demand of 30 Wh pe^{-1} d^{-1}(STOWA, 2006); [d]raceway pond with 4 d HRT (Xin et al., 2010) and 0.2 m depth (Zamalloa et al., 2011); [e]biomass concentration of 280 g VS m^{-3} and an harvesting recovery coefficient of 0.9 for both S. obliquus and Chlorella sp.; [f]electricity consumption of a low level mixing system (paddle wheel) to guarantee a velocity 15 cm s^{-1} (Zamalloa et al., 2011).

E.1.3 Results and discussion

Harvesting technologies

Full algal cells recovery (> 99 %) was achieved using both harvesting systems: BDAF confirming the potential to use a lower energy alternative to traditional DAF. The associated energy saving of using BDAF as opposed to DAF was estimated at 0.26 kW m^{-3} resulting in an overall reduction in energy of 0.98 MWh d^{-1} at the small scale and 9.04 MWh d^{-1} at the larger scale (Table E.2). An additional benefit of using the BDAF configuration was observed in association to chemical usage with a 40% reduction in metal coagulant use at the operating pH of 7 with *S. obliquus* and 50% lower with *Chlorella* sp. (Table 4.4). The difference in coagulant demand between *S. obliquus* and *Chlorella* sp. relates to differences in the AOM (Algogenic Organic Matter) composition for the two algae (Henderson *et al.,* 2010) with the reduced charge density associated with the AOM produced from *Chlorella* sp. requiring less coagulant for optimal removal. It was estimated that the BDAF allows coagulant saving up to 100 g Al$_2$(SO$_4$)$_3$ m^{-3} of influent water depending on the algal species.

This reduction could represent an economic saving of € 525 d^{-1} at small scale, and € 4057 d^{-1} at larger scale, based on the current average market price (Granados *et al.,* 2012) of the aluminium salts.

Table E.2 Cells recovery, energy input and coagulant dose required (mean ± SD) for DAF and BDAF*.*

	Cell recovery %	Coagulant dose mg Al l^{-1}	Energy input* kW m^{-3}	Coagulant dose mg Al l^{-1}	Energy input* kW m^{-3}
		DAF		BDAF	
S. obliquus	99	40 ± 14	0.3	23 ± 9	0.04
Chlorella sp.	99	8 ± 2	0.3	4 ± 1	0.04

* According with Jarvis *et al.* (2009).

Thermal hydrolysis of the algal biomass

Thermal hydrolysis has a significant impact on the properties of the algal biomass of both species. To illustrate, in the case of *S. obliquus* the ratio between volatile suspended solids and volatile solids (VSS/VS) of the concentrated biomass decreased from 0.8 ± 0.2 to 0.5 ± 0.2 as a result of pre-treatment. Whereas, in the case of *Chlorella* sp*.* the VSS/VS ratio decreased from 1 ± 0.2 before treatment to 0.8 ± 0.1 after treatment indicating a greater resistance to the impact of elevated temperatures and pressures. Microscopic analysis supported the observation of a difference in impact due to species selection based on the percentage of cells disrupted which decreased from 70 % in the case of *S. obliquus* to less than 50 % in the case of *Chlorella* sp. In addition, both algae showed post treatment aggregates as a consequence of releasing high amount of intracellular molecules which suggest that cells wall was disrupted (González-Fernández *et al.,* 2012). The impact of these differences in terms of the released organic material were most noticed in terms of proteins where application of the pre-treatment step increase the level of soluble proteins from 25 mg l^{-1} to 8149 mg l^{-1} in the case of *S. obliquus* compared to an increase from 27 mg l^{-1} to 2722 mg l^{-1} in the case of *Chlorella* sp. A smaller level of difference was observed as a function of algal type in the case of soluble COD, which increased by 7528 mg l^{-1} and 5306 mg l^{-1} for *S. obliquus and Chlorella* sp. respectively. Whereas more similar changes in soluble carbohydrates were observed at 2018 mg l^{-1} for *S. obliquus* and at 2137 mg l^{-1} for *Chlorella* sp.

The impact of this greater release of soluble material in the case of *S. obliquus* is observed in relation to the BMT (anaerobic digestion for 25 days at 38°C) where application of a pre-treatment step increased the methane yield from 0.13 ± 0.02 m³ kg⁻¹ VS$_{add}$ to 0.32 ± 0.05 m³ kg⁻¹ VS$_{add}$. This value is closer to the range of the theoretical methane content ($0.53 - 0.54$ m³ kg⁻¹ VS$_{add}$) as calculated by Heaven *et al.,* (2011). Untreated *Scenedesmus* biomass was reported to yield between 0.12 and 0.18 m³ kg⁻¹ VS$_{add}$ (Mussgnug *et al.,* 2010; González-Fernández *et al.,* 2012b). Our results compare favourably to the one reported by Gonzalez-Fernandez *et al.* (2012b) who obtained a methane production of 0.22 m³ kg⁻¹ VS$_{add}$ (133 dm³ kg⁻¹ COD$_{in}$) after thermal treatment at 90 °C for 3h. Similarly, Alzate *et al.* (2012) achieved a final methane production of 0.36 and 0.40 m³ kg⁻¹ VS$_{add}$, closer to our values, digesting a mixed culture (10 gTS kg⁻¹, 20 % *Scenedesmus* sp.) after treatment at 140 °C (1.2 bar) and 170°C (6.4 bar), respectively. The equivalent trial with *Chlorella* sp. generated only a small change in methane yield, from 0.10 ± 0.01 to 0.15 ± 0.01 m³ kg⁻¹ VS$_{add}$, suggesting lower impact of the combined heat and pressure treatment on the cell wall. Theoretical methane conversion values for this algae range between 0.45 and 0.57 m³ kg⁻¹ VS$_{add}$ (Heaven *et al.,* 2011). Different authors (Mussgnug *et al.,* 2010; Ras *et al.,* 2011) reported higher methane yields than the one reported in this paper digesting untreated *Chlorella* biomass (0.15 - 0.35 m³ kg⁻¹ VS$_{add}$). However, our biogas yields were similar to the one reported in literature and increased from 0.29 ± 0.01 to 0.49 ± 0.03 m³ kg⁻¹ VS$_{add}$ after the pre-treatment (Figure E.3). The lower methane yields obtained suggest a potential inhibition of the methanogenesis process, probably related to the chemical composition of the algal biomass. Moreover, the thermal pre-treatment of *Chlorella* sp. released a similar amount of carbohydrates and COD, but less proteins then *S. obliquus.* These differences have generated different C:N ratios in the two systems which, as reported by other authors (González-Fernández *et al.,* 2012b), could have affected the overall biogas composition. This is confirmed by the methane content in the biogas which decreased from 60 % to 51 % after pre-treatment whit *Chlorella* sp. (Figure E.3b), while increased from 46 % to 73 % whit *S. obliquus* (Figure E.2b).

Microscopic observations of the digested samples showed no residual intact cells only for pre-treated *S. obliquus* (Figure E.4b). In all the other cases, residual algal biomass was identified in the residual solids after digestion (Figure E.4a, E.4c and

E.4d) indicating the pre-treatment had not sufficiently enhanced digestion of *Chlorella* sp. The results presented here are in agreement with Valo *et al.,* (2004), who demonstrated that thermal hydrolysis pre-treatment of a specific biomass (waste activated sludge) resulted in enhanced biogas production and methane yield due to a reduction of the solids content and a parallel increase of organic compounds released. However, the current work identifies that in the specific case of microalgae the impact is likely to be highly related to a given algal species. The differences are likely to be due to the thickness and composition of the cell wall, which is known to vary between species (Mussgnug *et al.,* 2010; González-Fernández *et al.,* 2012a).
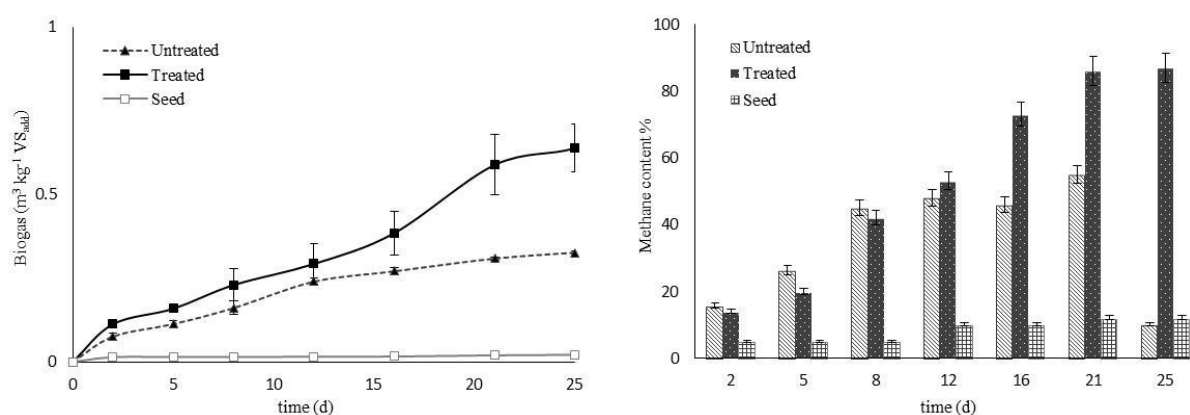

Figure E.2 *S. obliquus* BMT cumulative biogas production (a) and percentage methane content (b) of treated and untreated algal biomass at 38°C.
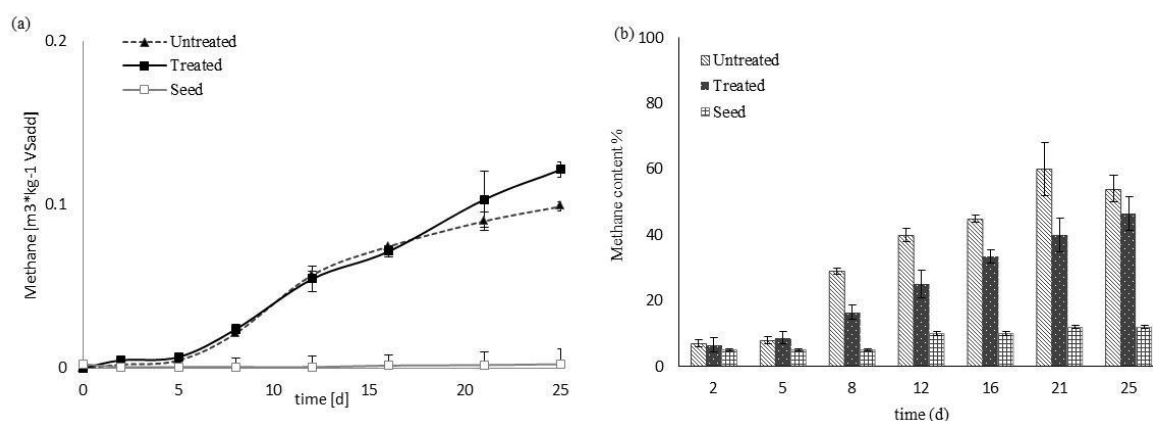

Figure E.3 *Chlorella sp.* BMT cumulative biogas production (a) and percentage methane content (b) of treated and untreated algal biomass at 38°C.
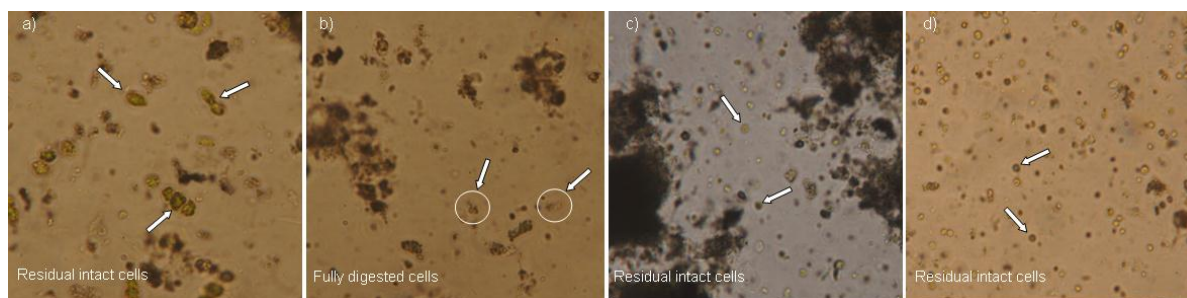
Figure E.4 Microscope analysis of digested sample (optical microscope x40); a*) S. obliquus untreated,* b) *S. obliquus treated,* c) *Chlorella* sp*.* untreated, d) *Chlorella* sp*.* treated.

Energy balance

Anaerobic digestion of the collected sludge generates 25% and 35% of the total energy demand required to run the works for the control case (scenario1, no algae, no pre-treatment) for the small and the large scale respectively (Table E.3). The remaining difference demonstrates the importance of sludge imports on the overall energy balance on operating sites. Generating additional solids for anaerobic digestion through the algal reactors, a possible alternative to sludge imports, (scenario 2) resulted in an increase of the overall net energy demand of the works by 61 and 95% for the small and large cases for both algal types. The increase was a result of the energy required to operate the pond and DAF units not being offset by the increased energy production. Adoption of the innovative BDAF process (scenario 3) reduced this impact with an increase in net energy demand of only 9 and 14.5%. These levels are similar to those of other tertiary nutrient removal processes which suggest algal reactors may be suitable for use on an energy basis even with low biogas yields. For instance, the energy values related to alternative tertiary treatments, such as wetlands (0 – 0.21 kWh m^{-3}) (Austin and Nivala, 2009) always required additional energy demand and do not produce a valuable feedstock.

Inclusion of a sludge pre-treatment device in the non-algal case (scenario 4) resulted in an increase in energy production of 0.68 MWh d^{-1} at the smaller scale and 8.48 MWh d^{-1} at the larger scale. The additional energy production resulted in an increase in the net energy balance across the works whereby the site produced 40% and 60% of the total demand at the small and large scale respectively. The increased energy production from inclusion algae into the pre-treated sludge mix (scenario5) enabled a greater proportion of the increased energy demand from inclusion of the pond and

the DAF unit to be met at both scales. To illustrate, inclusion of an algae nutrient process decreased the overall energy efficiency of the works to 36% at the smaller scale using *Chlorella* sp., a decrease of 4% compared to the pre-treated sludge only Case (Figure E.5, scenario 4). At the larger scale, the energy efficiency decreased by 12% to a total value of 48% of the works demand due to the limited impact of the pre-treatment on the algal methane production. In comparison, *S. obliquus*, which showed higher energy production after pre-treatment, reported a 4% energy efficiency improvement at small scale. However, at large scale the overall efficiency decreased from 60% to 57%. Switching to the BDAF unit for harvest, changed the balance significantly. In the Case of the small works an increase in net energy demand of 0.12 MWh d^{-1}, compare with the control case, was observed only considering *Chlorella* sp., although this included the entire energy demand of the pre-treatment unit and so generated the lowest energy option in total. This was further magnified at the larger scale where the increased energy generation from the pre-treated algal biomass more than offset the energy demand of the pond, BDAF and pre-treatment units leading to a net energy gain of 4.48 MWh d^{-1} for *S. obliquus* and 1.08 MWh d^{-1} for *Chlorella* sp. In this case the energy production from biomass generated on site (sludge and algae) was able to meet 76% and 64% of the total demand for energy, which represents an increase of 16 and 4% over the sludge only case (scenario 4)

.

Table E.3 Energy demand and efficiency of different integrated WWTPs configurations for both treatment plants and algal strain

			Energy$_a$ (MWh d^{-1})							
			Energy demand				*S. obliquus*		*Chlorella* sp.	
Scenarios description			Activated Sludge	Algae Pond	Algae Harvest	Sludge/Algae Pre-treat.	AD Energy recovery	Net energy consumption	AD Energy recovery	Net energy consumption
TP 25K	1	AS+AD	2.52				-0.63$_b$	1.89$_b$	-0.63$_b$	1.89$_b$
	2	AS+Pond+DAF+AD	2.52	0.27	1.13		-0.88	3.04	-0.88	3.04
	3	AS+Pond+BDFA+AD	2.52	0.27	0.15		-0.88	2.06	-0.88	2.06
	4	AS+Pre-treat.+AD	2.52			0.75	-1.31$_b$	1.96$_b$	-1.31$_b$	1.96$_b$
	5	AS+Pond+DAF+Pre-treat.+AD	2.52	0.27	1.13	0.75	-2.05	2.62	-1.68	2.99
	6	AS+Pond+BDAF+Pre-treat.+AD	2.52	0.27	0.15	0.75	-2.05	1.64	-1.68	2.01
TP 230K	1	AS+AD	17.39				-6.09$_b$	11.30$_b$	-6.09$_b$	11.30$_b$
	2	AS+Pond+DAF+AD	17.39	2.51	10.43		-8.35	21.98	-9.65	21.98
	3	AS+Pond+BDFA+AD	17.39	2.51	1.39		-8.35	12.94	-9.65	12.94
	4	AS+Pre-treat.+AD	17.39			6.90	-14.57$_b$	9.72$_b$	-14.57$_b$	9.72$_b$
	5	AS+Pond+DAF+Pre-treat.+AD	17.39	2.51	10.43	6.90	-21.37	15.86	-20.24	19.26
	6	AS+Pond+BDAF+Pre-treat.+AD	17.39	2.51	1.39	6.90	-21.37	6.82	-20.24	10.22

a) positive numbers represent electricity consumption values while negative numbers show electricity produced; b) the value reported for scenarios 1 and 4 represent energy generated from wastewater sludge digestion. In all the other scenarios the value shows the energy generated from algae/sludge co digestion by adding the two estimated energy values.

Overall, the results demonstrate that when appropriate choices are made around the ancillary equipment then the use of algae for nutrient removal can represent a viable source of energy production and hence provide an energy neutral nutrient removal strategy. Critical to this is the use of pre-treatment to ensure the inclusion of algae in the anaerobic digestion generates sufficient biogas to justify its inclusion. In such Case algae could be viewed as an appropriate alternative to co-digestion of imported non-sewage sludge wastes. The importance of this is that it avoids logistic and regulatory barriers and it enhances biogas production in digesters meant for sewage sludge processing. However, pre-treatment alone is insufficient as the energy demand of traditional technologies for algal separation is likely to be too high to justify the approach. In such case the significance of BDAF system becomes more important as it lowers the total energy demand by 1 MWh d^{-1} at small scale and 9 MWh d^{-1} at larger scale compared to the traditional DAF system. Ultimately both components are required to enhance the potential for inclusion of algae as a nutrient removal process.

The impact of which algae are used within the nutrient removal process was demonstrated in this study by looking at two similar single cell green algae both of which are commonly used in algal biomass production. In the current case an 8-9% difference was seen on the overall balance as a function of species with *Chlorella* sp. generating less energy than *S. obliquus.* The difference is thought to occur due to the combination of the strong species–specific wall structure found within *Chlorella* sp*.* (Syrett and Thomas, 1973; González-Fernández *et al.,* 2012a) and the differences within the AOM generated and released after pre-treatment, effecting the final biogas composition. Given that the structure of the two algae strains is reasonably similar it is reasonable to assume that when using other algae species significantly different outcomes may occur. Common algae species found in the UK include filamentous strains of green, diatoms and blue-greens all of which have examples of appendages and mobility associated to them (Henderson *et al.,* 2008a). Previous work on separation of algae has shown that such differences can have a significant impact on the chemical and energy requirements for harvesting (Henderson *et al.,* 2008b; Henderson *et al.,*

2010). In addition previous studies on different algae have also shown species-specific outcomes in relation to the impact of pre-treatment and the biogas production achieved (Mussgnug *et al.,* 2010; Alzate *et al.,* 2012; Ehime *et al.,* 2013). Importantly the selection of the most appropriate algae species for enhanced nutrient removal from wastewater in temperate climates remains unclear and highlights that understanding the overall impact of the use of algae cannot be determined without knowledge of the species involved.
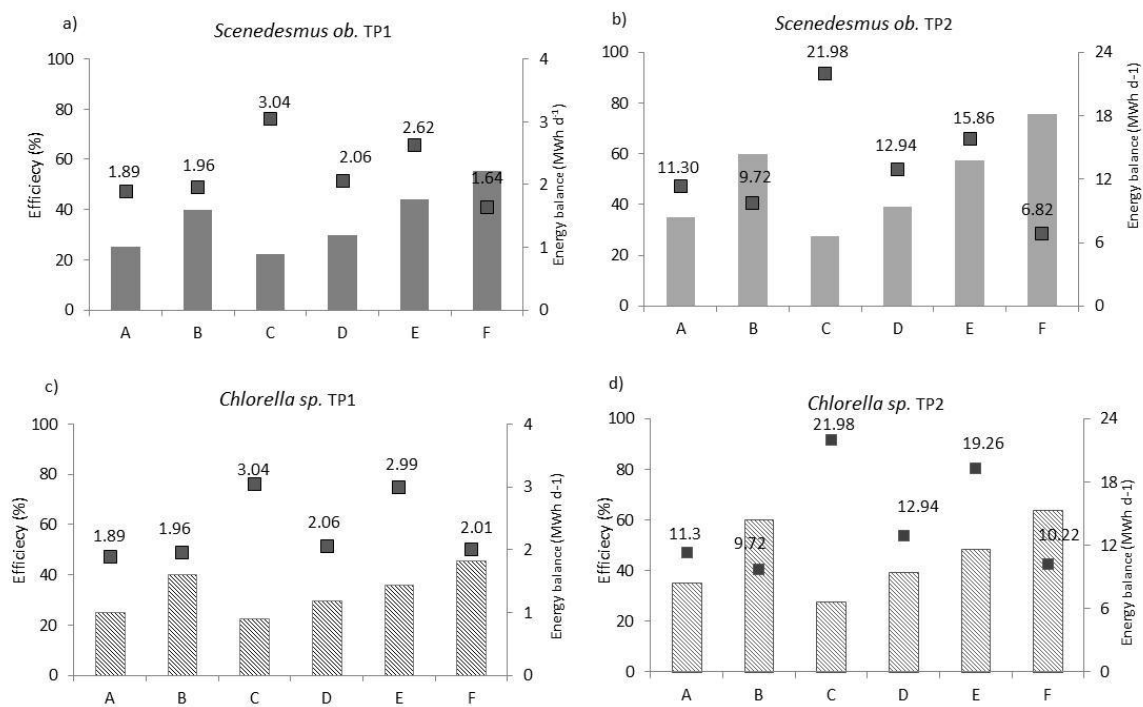

Figure E.5 Plant efficiency (column) and Energy balance (square) of the scenarios: *S. obliquus* in TP 25K (a) and TP 230K (b); *Chlorella* sp. in TP 25K (c) and TP 230K (d).

E.1.4 Conclusions

The adoption of low energy harvest and algal biomass pre-treatment has been shown to have a significant impact on the overall suitability of using algae for nutrient removal in wastewater treatment. The BDAF process reduced the overall energy requirements between 30 % and 40 % depending on the plant size. Thermal hydrolysis pre-treatment allowed a complete utilisation of the included *S. obliquus* cells under mesophilic temperatures maximising the potential energy gain from inclusion of the biomass. The combination of the two technologies demonstrated the possibility of achieving high-energy efficiency (76 %) and a more sustainable WWTP. Adoption of the approach needs knowledge of the specific species involved in the removal process as different strains of algae require different pre-treatment conditions and will be able to release different amount of energy and this remains a key research challenge going forward.

E.1.5 Acknowledgment

E.1.6 References

Abo-Shady. A.M., Mohamed, Y.A., Lasheen, T., 1993. Chemical composition of the cell wall in some green algae species. Biol. Plantarum. 35, 629-632.

Alzate, M.E., Munoz, R., Rogalla, F., Fdz-Polanco, F., Pérez-Elvira S.I., 2012. Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment. Bioresourc. Technol. 123, 488-494.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., van Lier J.B.: 2009. Defining the

biomethane potential (BMP) of solid organic waste and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59, 927-934.

Austin, D., Nivala, J., 2009. Energy requirement for nitrification and biological nitrogen removal in engineered wetlands. Ecol. Eng. 35, 184-192

Beach, E.S., Eckelman, M.J., Cui, Z., Brentner, L., Zimmerman, J.B., 2012. Preferential technological and life cycle environmental performance of chitosan flocculation for harvesting of the green algae *Neochloris oleoabundans*. Bioresourc. Technol. 121, 445-449.

Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol. Adv. 29, 686-702.

Cromar, N.J., Fallowfield H.J. 1997. Effect of nutrient loading and retention time on performance of high rate algal ponds. J. Appl. Phycology 9, 301-309.

Demirbas, A., 2010. Use of algae as biofuel sources. Energy Convers. Manage. 51, 2738-2749.

STOWA, Dutch Foundation for Applied Water Research, 2006. Cambi process sheets http://www.stowa-selectedtechnologies.nl/Sheets/Cambi.Process.html (accessed 18 May 2012).

Ehime, E.A., Holm-Nielsen, J.B., Poulsen, M., Boelsmand, J.E., 2013. Influence of different pre-treatment routes on the anaerobic digestion of a filamentous algae. Renew. Energ. 50, 476-480.

Edzwald, J.K., 1993. Algae, bubbles, coagulants, and dissolved air flotation. Wat. Sci. Techol. 27, 67-81.

Golueke, C.G., Oswald, W.J., Gotaas, H.B., 1957. Anaerobic digestion of algae. Appl. Biotechnol. 5, 47-55.

Gonzalez-Fernandez, C., Sialve, B., Bernet, N., Steyer, J.P., 2012a. Impact of microalgae characteristics on their conversion to biofueld. Part II Focus on biomethane production. Biofuels Bioprod. Bioref. 6, 205-218.

González-Fernández, C., Sialve, B., Bernet, N., Steyer, J.P., 2012b.. Thermal pretreatment to improve methane production of *Scenedesmus* biomass. Biomass Bioenerg. 40, 105-111.

Granados, M.R., Acien, F.G., Gomez, C., Fernandez-Sevilla, J.M., Molina Grima, E., 2012. Evaluation of flocculants for the recovery of freshwater microalgae. Bioresour. Technol. 118, 102-110.

Greenberg, A.E., Clesceri, L.S., Eaton, A.D., 1992. Standard methods for the examination of water and wastewater. APHA

Heaven, S., Milledge, J., Zhang, Y., 2011. Comments on Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnology Advances 29, 164 -167.

Henderson, R.K., Chips, M., Cornwell, N., Hitchins, P., Holden, B., Hurley, S., Parsons, S.A., Wetherill, A., Jefferson, B., 2008a. Experiences of algae in UK waters: a treatment perspective. Water and Environ. J. 22, 184-188.

Henderson, R.K., Parsons, S.A., Jefferson, B., 2008b. The impact of variable algal functionality on treatment. Water Res. 42, 1827-1845.

Henderson, R.K., Parsons, S.A., Jefferson, B., 2009. The potential for using bubble modification chemicals in dissolved air flotation for algae removal. Sep. Sci. Technol. 44, 1923-1940.

Henderson, R.K., Parsons, S.A., Jefferson, B., 2010. The impact of differing cell and algogenic organic matter (AOM) characteristics on the coagulation and flotation of algae. Water Res. 44, 3617-3624.

Jarvis, P., Buckingham, P., Holden, B., Jefferson, B., 2009. Low energy ballasted flotation. Water Res. 43, 3427-3434.

Martínez, M.E., Sánchez, S., Jiménez, J.M., El Yousfi, E., Munoz, L., 2000. Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. Bioresourc. Technol. 73, 263-272.

Min, M., Wang, L., Li, Y., Mohr, M.J., Hu, B., Zhou, W., Chen, P., Ruan, R., 2011. Cultivating *Chlorella* sp. in pilot-scale photobioreactor using centrate wastewater for microalgae biomass production and wastewater nutrient removal. Appl. Biochem. Biotechnol. 165, 123-137.

Molina Grima, E., Belarbi, E.H., Acién Fernández, F.G., Robles Medina, A., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process option and economics. Biotechnol. Adv. 20, 491-515.

Mussgnug, J.H., Klassen, V., Schlüter, A., Kruse, O., 2010. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. J. Biotechnol. 150, 51-56.

Pittman, J.K., Dean, A.P., Osundeko, O., 2011. The potential of sustainable algal biofuel production using wastewater resources. Bioresourc. Technol. 102, 17-25.

Ras, M., Laurent, L., Sialve, B., Bernet, N., Steyer, J.P., 2011. Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*. Bioresourc. Technol. 102, 200-206.

Ruiz-Marin, A., Mendoza-Espinosa, L.G., Stephenson, T.. 2010. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresourc. Technol. 101, 58-64.

Salim, S., Vermuë, M.H., Wijffels, R.H., 2012. Ratio between autoflocculating and target microalgae affets the energy-efficient harvesting by bio-flocculation. Bioresourc. Technol. 118, 49-55.

Shi, C.Y., 2011 Mass flow and energy efficiency of municipal wastewater treatment plants. IWA Publishing, London (2011).

Sialve, B., Bernet, N., Bernard, O., 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol. Adv. 27, 409-416.

Strum, B.S.M., Lamer, S.L., 2011. An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. Appl. Energ. 88, 3499-3506.

Syrett, P.J., Thomas, E.M., 1973.The assay of nitrate reductase in whole cells of *Chlorella*: strain differences and the effect of cell walls. New Phytol. 72, 1307-1310.

Tredici, M.R., 2007. Mass production of microalgae: photobioreactors. In Richmond, A. (ed): Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science. 178-214 (2007).

Valo, A., Carrère, H., Delgenès, J.P., 2004. Thermal, chemical and thermo-chemical pre-treatment of waste activated sludge for anaerobic digestion. J. Chem. Technol. Biotechnol. 79, 1197-1203.

Xin, L., Hong-Ying, H., Jia, Y., 2010. Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, *Scenedesmus sp.* LX1, growing in secondary effluent. New Biotechnol.. 27, 59-63.

Yen, H.W., Brune, D.E., 2007. Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresourc. Technol. 98, 130-134.

Zamalloa, C., Vulsteke, E., Albrecht, J., Verstraete W., 2011. The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. Bioresour. Technol. 102, 1149-1158.