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ANAEROBIC DIGESTION FOR NUTRIENT RECYCLING IN INDUSTRIAL MICROALGAE CULTIVATION: EXPERIMENTS AND PROCESS SIMULATION

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To my family

Riassunto

Lo sfruttamento delle limitate scorte di combustibili fossili e le emissioni di gas serra dovute al loro utilizzo hanno spinto il mondo scientifico, negli ultimi anni, alla ricerca di nuove fonti rinnovabili di energia. Un'attenzione particolare è stata rivolta ai combustibili derivanti dalle biomasse. Tuttavia, i biocarburanti di prima e seconda generazione presentano numerosi svantaggi in termini di competizione con le risorse alimentari e di scarsa produttività rispetto agli ingenti fabbisogni energetici. Per tale ragione, la ricerca si è focalizzata sui biocarburanti di terza generazione, ovvero quelli prodotti a partire di microrganismi fotosintetici unicellulari chiamati microalghe. Allo stato attuale, però, la produzione industriale di combustibili derivati da queste ultime è ancora troppo costosa, e li rende non competitivi sul mercato con quelli da fonti fossili.
Un contributo notevole ai costi per la produzione di biomassa microalgale è dato dall'apporto dei nutrienti, in particolar modo azoto e fosforo, forniti tramite fertilizzanti di vario tipo. D'altra parte, dopo l'estrazione dei lipidi dalle microalghe per la produzione dei biocarburanti, la biomassa residua trattiene praticamente il 100% di questi elementi, che possono essere quindi recuperati e riciclati alla coltura. La digestione anaerobica della biomassa estratta sembra una delle più promettenti tra le varie tecniche di riciclo dei nutrienti, e costituisce infatti l'argomento principale di questo studio.

Nella parte sperimentale del lavoro, la biomassa della specie microalgale *Chlorella vulgaris* (fornito da NEOALGA™), dopo un'estrazione dei lipidi con solvente, è stata utilizzata come substrato per la digestione anaerobica su scala di laboratorio. Tramite i cosiddetti test di BMP (*biochemical methane potential*), si è innanzitutto valutata la produttività di metano della biomassa algale estratta, che è risultata essere di circa 150 Nml/(g di solidi volatili). Inoltre, l'effluente del processo di digestione (il "digestato"), è stato impiegato, dopo centrifuga per separare la frazione solida, come fonte di nutrienti per la coltivazione delle microalghe. Di tale liquido è stata effettuata l'analisi elementare, per poter confrontare il suo contenuto in macro e micronutrienti con quello di un mezzo di coltura sintetico, il BG11. Dall'analisi si è potuta evidenziare una netta carenza di fosforo e di zolfo, mentre l'azoto (presente nel digestato in forma ammoniacale) e i micronutrienti sono risultati in concentrazione superiore rispetto al BG11. Il fosforo, durante il processo di digestione, infatti, precipita nella forma di sali insolubili ($Mg_3(PO_4)_2$ o $Ca_3(PO_4)_2$), mentre lo zolfo è principalmente perso nel biogas come acido solfidrico. Il digestato liquido, dunque, è stato opportunamente diluito per raggiungere la stessa concentrazione di azoto del mezzo sintetico, ed è stato adoperato come mezzo di coltura, in tre modalità: tal quale, con il solo supplemento di $H_2PO_4^-$ e con

l'aggiunta di $H_2PO_4^-$ e SO_4^{2-}. I risultati sono stati comparati con quelli ottenuti in BG11 (controllo), in cui però l'azoto è stato fornito in forma ammoniacale, invece che di nitrato, per riprodurre il digestato. È risultato che l'aggiunta contemporanea di P e di S è stata necessaria e sufficiente a raggiungere la stessa concentrazione cellulare finale rispetto al controllo, confermando la presenza di tutti gli altri micronutrienti essenziali. Si è cercato, tramite diversi trattamenti sul digestato a monte della separazione del particolato (acidificazione, aggiunta di EDTA o di bicarbonato di sodio), di solubilizzare una maggiore frazione di fosfato nella frazione liquida, allo scopo di renderla disponibile per l'assimilazione da parte delle microalghe. Il trattamento più efficace si è ottenuto facendo reagire l'effluente di digestione con $NaHCO_3$. Tuttavia, il digestato è risultato essere disomogeneo nel suo contenuto e nella sua granulometria di solido, cosa che ha influenzato fortemente le rese di estrazione del fosforo. La frazione acquosa del digestato pretrattato è stata utilizzata diluita 1:2 e utilizzata per un'ulteriore coltura microalgale, con la sola aggiunta di zolfo. Si è così misurata una concentrazione cellulare finale pari a circa la metà rispetto al controllo, mentre la velocità di crescita ha presentato un decremento del 25% circa. Poiché il fosforo disponibile nel mezzo di coltura era solo lievemente inferiore a quello presente nel BG11 standard, si suppone che questa crescita inferiore sia da imputare ad una carenza di micronutrienti legata in qualche modo al trattamento con bicarbonato di sodio.

Il processo integrato di produzione della biomassa e digestione anaerobica della stessa è stato simulato in Aspen Plus, con lo scopo di comprendere le possibilità di riciclo dei nutrienti al variare di diverse variabili operative. Innanzitutto, il fotobioreattore è stato simulato come un reattore PFR per poter confrontare i dati ottenuti dalla simulazione con quelli nelle colture batch, in termini di consumo dei nutrienti, pH e produttività di biomassa. Il modello cinetico di Monod con più substrati limitanti è stato implementato tramite un programma Fortran collegato al simulatore. Un caso base è stato discusso per valutare i bilanci di massa di azoto, fosforo e acqua nel processo, e per poter individuare le maggiori perdite e le possibilità di riciclo. È emersa l'importanza di riciclare la maggior quantità possibile di acqua a valle delle sezioni di separazione/concentrazione della biomassa (sedimentatore e centrifuga). Questo riciclo, infatti, non solo riduce il *water footprint* del processo, ma anche le necessità di un approvvigionamento esterno di N e P, dato che quell'acqua contiene gran parte dei nutrienti forniti (e non assimilati nella biomassa). Inoltre, si è potuto osservare che la digestione anaerobica, con le sue potenzialità attuali, è in grado di apportare notevoli riduzioni nelle necessità di fornitura di azoto come fertilizzante (fino all'80% in meno). Purtroppo, però, a causa della formazione dei precipitati gran parte del fosforo viene perso e deve essere reintegrato fresco dall'esterno. Un'analisi di sensitività è stata effettuata al variare delle portate di nutrienti forniti, del tempo di permanenza nel reattore, della biodegradabilità

dell’alga nel digestore anaerobico e della frazione di fosforo recuperabile nella frazione liquida del digestato. Le portate di nutrienti ottimali, forniti in maniera stechiometrica rispetto alla composizione dell’alga, sono risultate quelle appena sufficienti ai fini di evitare la limitazione, senza un ulteriore eccesso (causa di maggiori perdite). È stato selezionato un tempo di permanenza ottimale pari a circa 1 giorno, in quanto valori inferiori diminuiscono la produttività algale, mentre valori superiori comportano maggiori volumi, costi di impianto e occupazione di area. Si è visto che aumentare la biodegradabilità dell’alga nel digestore (migliorabile tramite pretrattamenti chimico-fisici a monte) ha effetti positivi sul recupero dell’azoto, ma è pressoché ininfluente su quello del fosforo, se questo viene per la maggior parte perso nel solido. Invece, il riciclo di P migliora notevolmente con la biodegradabilità se ne viene recuperata una frazione accettabile nella fase acquosa dell’effluente. Infatti, la percentuale di recupero del fosforo nel liquido è la variabile che più influisce sulla possibilità di riciclo e sulle perdite di nutriente. Ciò mette in evidenza la vitale importanza che assume la ricerca di nuovi ed efficienti metodi di solubilizzazione del fosforo. Infine, si è verificato che l’assorbimento della CO_2 a monte del reattore non è influenzato negativamente dalla presenza degli altri nutrienti, e che, allo stesso tempo, l’ammoniaca non è eccessivamente volatilizzata per colpa dello stripping da parte della corrente gassosa. Ciò consente di alimentare la parte di carbonio sotto forma liquida (soluzione di carbonato) piuttosto che gassosa, riducendo notevolmente i costi di funzionamento del processo.

Abstract

This study assesses anaerobic digestion as a technique for nutrient recycling for the cultivation of microalgae. Microalgal biomass after lipid extraction was used as substrate for a lab scale anaerobic digestion. The digestate was centrifuged and the liquid fraction was analyzed in its elemental composition. Microalgal cultivation using this liquid as nutrient source was investigated. Several techniques were tested to extract phosphorus from the solid phase to the liquid one. The pretreated digestate was diluted and used as a culture medium with no P external supply. Process simulation was used to compare the growth results obtained in a PFR reactor with Monod's kinetic model with batch experiments. Furthermore, the integrated production/anaerobic digestion process was analyzed in terms of mass balances of nitrogen, phosphorus and water. A sensitivity analysis was eventually performed to highlight the operating variables which are more relevant in order to maximize both water and nutrient recycling.

Contents

Introduction

The growing concern about fossil fuels depletion and greenhouse gases (GHG) emissions has led to the research of more economically and environmentally sustainable alternative energy sources. Fuels derived from biomass represent a promising option; the focus is now on third generation biofuels (produced from photosynthetic microorganisms called microalgae) which are able to overcome the disadvantages of first and second generation ones.

However, the sustainability and the feasibility of the production of microalgae at the industrial scale has to overcome numerous challenges. The most relevant one is nutrient supply, nitrogen and phosphorus above all. The use of external fertilizers is in fact too expensive and unsustainable for a large-scale algal production, to be able to satisfy the massive biofuel requirements needed to replace fossil fuels. A solution to this problem consists in nutrient recycling. Several techniques can be exploited in order to recover nitrogen and phosphorus from the residual algal biomass, after lipid extraction; in fact, these compounds are not assimilated in the oleaginous fraction of the microalgae.

In this study, anaerobic digestion is analyzed as it is one of the most interesting alternatives; also, it can be used to improve the energy balance of the whole integrated process, thanks to biogas production.

The thesis is composed of four chapters. In the first one, the general topic of microalgal biofuels is addressed with respect to production steps, possibilities, pros and cons, limits to industrial application. Microalgal cultivation and nutrient supply to satisfy the requirements for growth are then discussed. The available techniques for nutrient recycling are listed, focusing on anaerobic digestion (AD). Eventually, the availability of N and P in AD effluent and the possibility to recycle the liquid fraction as a cultivation medium are evaluated. Chapter 2 deals with the materials and methods for the experimental part of this work. An overview is made about the cultivation process, including algal species, culture systems, analytical procedures to monitor algal growth, nutrients consumption and lipid extraction from the biomass. The experimental setup, as well as the measurement methods and the calculations to evaluate the biochemical methane potential (BMP) of the algal biomass residues are described. The techniques used to solubilize P from the solid fraction of the digestate into the liquid one, in order to make it available for microalgal cultivation, are reviewed.

The experimental results are reported in the 3rd chapter. Algal biomass residues are characterized in their BMP, which is compared with theoretical values. The liquid effluent of the digestion is used as a nutrient source for cultivation, with or without external macronutrient supplies, and algal growth is compared to the control culture in a standard

synthetic medium, for different cases. Several methods of phosphorus solubilisation are discussed, and one of them is applied to treat the digestate prior to a further microalgal cultivation.
Chapter 4 contains all the details about the process simulation of the production and anaerobic digestion of microalgal species *Chlorella vulgaris*, which was performed by Aspen Plus™. The setup is developed in all its steps, including models, stoichiometry, kinetics, flowsheet building and calculations algorithms. Two base cases are discussed, one with the reaction section only and one with the integrated production/anaerobic flowsheet. A sensitivity analysis is performed to evaluate the effect of a number of variables on algal productivity, nutrients loss and external makeup requirements. Eventually, an absorber unit is simulated to assess the interference between CO_2 dissolution in the aqueous medium and the presence of nutrients in the liquid phase of the reactor inlet.

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Chapter 1

Biofuels from microalgae and nutrient recycling: state of art

In this chapter a general overview is made about microalgae-based biofuels, with their possibilities, advantages, drawbacks, limits to industrial application. The biodiesel production process is briefly described in all its phases. Then, particular attention is paid to microalgal cultivation and to nutrients requirement for growth, as these represent a huge contribution to the production cost, as well as the most relevant constraint to large scale commercialization of algal biodiesel. Several techniques for nutrient recycling are described, focusing on anaerobic digestion (AD). Nutrients availability in AD effluent (especially N and P) and the suitability of the liquid fraction to be recycled and to sustain algal growth are eventually discussed.

1.1 Biofuels: why are they interesting?

Fossil fuels are a finite resource produced naturally from the fossilization process of the organic material of living organisms over millions of years. They fulfil most of world's energy demands, including electricity generation, fuel for transportation and heating. Global energy demands are expected to increase by 35% in the next two decades, due to a rise in population and to economic growth of developing countries. To meet rising energy demands, the great consumption of fossil fuels is depleting available resources and increasing energy prices (Chaudry *et al.*, 2015). The high rate of petrofuel use is also responsible for increasing atmospheric CO_2, which is a major greenhouse gas (GHG) and one of the main causes of global warming. Furthermore, the accumulation of CO_2 in the atmosphere is responsible for other issues, such as ocean acidification, with consequent loss of marine biodiversity (Mata *et. al*, 2010). To meet future energy demand without damaging the environment, fossil fuels should be replaced with some alternative energy sources that are environmentally friendly and sustainable. Apart from solar, wind and geothermal energies, biomass resources can be exploited to generate electricity, and also to produce liquid and gaseous renewable fuels. Biofuels have the potential to be an interesting alternative to the depleting fossil fuels, as they are able to reduce carbon emissions and solve part of the problems concerning the increasing energy requirements (Chaudry *et al.*, 2015).

First generation biofuels, produced from food crops (such as corn and sugarcane), evidenced notable economic, environmental and political concern, as they compete (in terms of arable land and freshwater use) with human and animal food production (Chaudry *et al.*, 2015). Second generation biofuels produced from non-edible crops (such as perennial grasses and

Jatropha) and agricultural wastes do not directly compete with the food supply. Most targeted energy crops can also utilize low quality land, which is not suitable for food crops. Second generation biofuels have the potential to contribute to solutions for problems related to energy; however, they are not sufficient to entirely fulfil rising energy demands (Chaudry *et al.*, 2015). Dedicated energy crops for these biofuels are facing many challenges (such as high cost, low energy density, high water and nutrient requirements) and are still in the research and development stage. Third generation biofuels, produced from microalgae, are now becoming the focus of research as the possible renewable energy source for the future, being able to overcome the disadvantages of first and second generation ones (Alam *et al.*, 2015). Microalgae can provide several types of renewable biofuels, such as methane, biodiesel, and bio-hydrogen. There is a number of advantages for producing biofuel from algae, which are listed in section §1.3. Unfortunately, the commercial production of microalgae biofuel is not economically sustainable and competitive with fossil fuels yet. A recent estimate suggests a final microalgal biodiesel production cost in the range of 0.42–0.97 $/l (raceway pond with an assumed biomass productivity ≥30 g m^{-2} d^{-1} and an oil content of 50% by weight in the biomass) (Chisti, 2013). Algal biomass with an oil content of 40% w/w should be produced at a cost of no more than $0.25/kg, to compete with petroleum; the actual cost appears to be at least 10 times greater so far. Under optimized conditions, it may be reduced to 0.68$/kg, which it is still 3-fold greater than required (Chisti, 2013). However, the goal of a large scale production of third generation biofuels is under research and development, to overcome its numerous challenges.

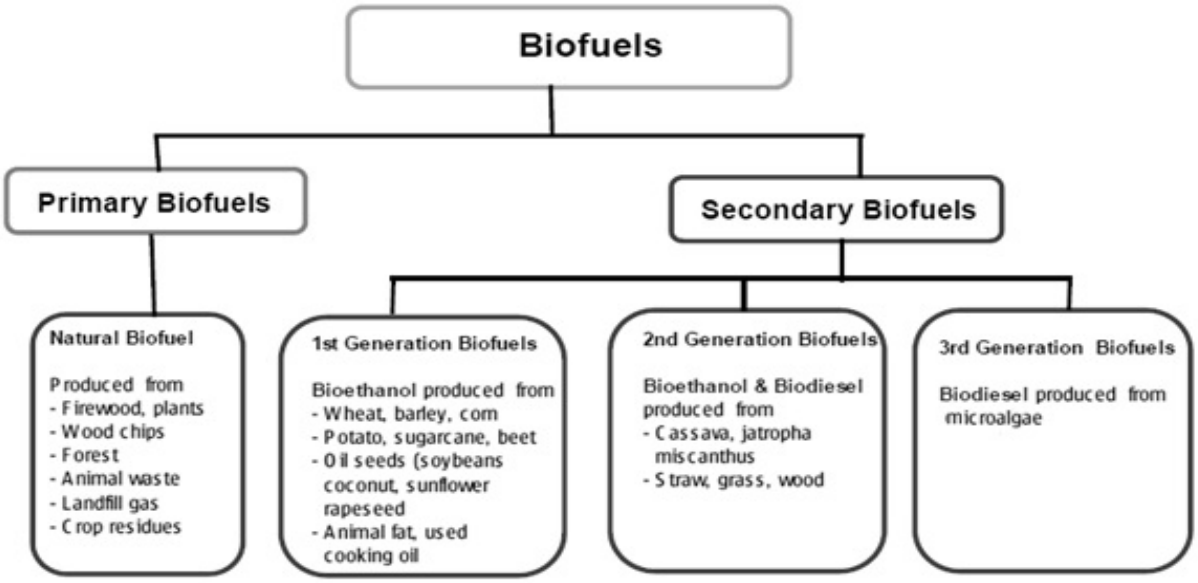

Figure 1.1 *Biofuels classification according to their source (Alam et al., 2015).*

1.2 What are microalgae?

Microalgae are eukaryotic photosynthetic organisms, which are naturally found in fresh water and marine environment. They can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure; they can be unicellular or live in colonies. There are more than 50,000 species of microalgae, with a size ranging from 1 to 10 µm (Mata *et al.*, 2010). Microalgae are present in the biogeochemical cycle in a similar way to plants, as they absorb atmospheric CO_2 and fix it into polysaccharides, proteins, and other biological molecules needed to build and maintain cellular structures; the energy for these processes is provided by photosynthesis. Like most terrestrial plants, aside from CO_2, their growth requires light, water, macronutrients as nitrogen (N), phosphorus (P), and other micronutrients (in smaller quantities) (Alam *et al.*, 2015). Microalgae produce fatty acids and lipids (such as triacylglycerides or TAGs) by metabolism and form structures where these substances are stored and then used as a source of energy when necessary.

Table 1.1 *Lipid content and productivities of different microalgae species (adapted from Mata et al., 2010).*

Marine and freshwater microalgae species	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/day)	Volumetric productivity of biomass (g/L/day)	Areal productivity of biomass (g/m²/day)
Botryococcus braunii	25.0–75.0	–	0.02	3.0
Chlorella emersonii	25.0–63.0	10.3–50.0	0.036–0.041	0.91–0.97
Chlorella protothecoides	14.6–57.8	1214	2.00–7.70	–
Chlorella sorokiniana	19.0–22.0	44.7	0.23–1.47	–
Chlorella vulgaris	5.0–58.0	11.2–40.0	0.02–0.20	0.57–0.95
Chlorella sp.	10.0–48.0	42.1	0.02–2.5	1.61–16.47/25
Chlorella pyrenoidosa	2.0	–	2.90–3.64	72.5/130
Chlorella	18.0–57.0	18.7	–	3.50–13.90
Chlorococcum sp.	19.3	53.7	0.28	–
Dunaliella salina	6.0–25.0	116.0	0.22–0.34	1.6–3.5/20–38
Dunaliella primolecta	23.1	–	0.09	14
Dunaliella tertiolecta	16.7–71.0	–	0.12	–
Dunaliella sp.	17.5–67.0	33.5	–	–
Nannochloris sp.	20.0–56.0	60.9–76.5	0.17–0.51	–
Nannochloropsis oculata	22.7–29.7	84.0–142.0	0.37–0.48	–
Nannochloropsis sp.	12.0–53.0	37.6–90.0	0.17–1.43	1.9–5.3
Neochloris oleoabundans	29.0–65.0	90.0–134.0	–	–
Nitzschia sp.	16.0–47.0			8.8–21.6
Pavlova salina	30.9	49.4	0.16	–
Pavlova lutheri	35.5	40.2	0.14	–
Scenedesmus obliquus	11.0–55.0	–	0.004–0.74	–
Scenedesmus quadricauda	1.9–18.4	35.1	0.19	–
Spirulina platensis	4.0–16.6	–	0.06–4.3	1.5–14.5/24–51
Spirulina maxima	4.0–9.0	–	0.21–0.25	25
Thalassiosira pseudonana	20.6	17.4	0.08	–
Tetraselmis suecica	8.5–23.0	27.0–36.4	0.12–0.32	19
Tetraselmis sp.	12.6–14.7	43.4	0.30	–

The biochemical composition (and, in particular, lipid content) of microalgae varies according the species (see Table 1.1) and the culture conditions, such as light, temperature, pH, salinity and nutrients. Lipid concentration can be increased by up to 80% above natural levels, by optimizing growth determining factors (Alam *et al.*, 2015).

1.3 Advantages of microalgae-based biofuel

Microalgae represent an interesting feedstock for biofuels as they have the following advantages (Rawat *et al.*, 2013; Mata *et al.*, 2010):

- significantly lower land requirement, estimated 10% of the land needed to produce the same amount of biodiesel from oil bearing crops; furthermore, non-arable land can be exploited for cultivations;
- great lipid storage capacity and oil yield; these lipids possess a high level of saturation (making microalgae a more suitable feedstock for biodiesel);
- higher photosynthetic efficiency, resulting in greater lipid content;
- high growth rates and short generation times; some microalgae can double their biomasses within 24 hours and the shortest doubling time during their growth is around 3.5 hours;
- very short harvesting life and growth throughout the whole year (no seasonality as terrestrial crops): this allows multiple and continuous harvesting of biomass;
- less freshwater requirement for cultivation than terrestrial plants, as they can grow in wastewaters, brackish or salty waters (this is the case of marine species);
- growth of microalgae can effectively remove phosphates, ammonium and nitrates from wastewater; this is an ideal substrate for the cultivation of microalgae for biofuels production while the culture acts as a tertiary treatment for wastewater;
- some microalgae produce valuable by-products (pigments, biopolymers, omega-3, carotenoids and antioxidant substances for commercial or nutraceutical purpose);
- lipids derived from microalgal oil are carbon neutral fuels due to the photosynthetic fixation of atmospheric carbon dioxide or CO_2 sequestration from industrial exhaust and flue gases, thereby reducing emissions of this major greenhouse gas.

1.4 Biodiesel production process

Various conversion methods including transesterification, fermentation, pyrolysis, liquefaction and anaerobic digestion are used to produce biodiesel, bioethanol, bio-oil and methane from the main components of microalgae. Among possible products, biodiesel seems to be one of the most interesting alternatives. Biodiesel is a mixture of fatty acid alkyl esters obtained by transesterification of vegetable oils or animal fats. These lipid feedstocks are composed by 90–98% (weight) of triglycerides and small amounts of mono and diglycerides, free fatty acids (1–5%), residuals of phospholipids, phosphatides, carotenes, tocopherols, sulphur compounds, and traces of water (Mata *et al.*, 2010). In the context of large scale microalgal cultivation, the process configuration is defined as the combination of economic viability, upstream and downstream processing (Rawat *et al.*, 2013).

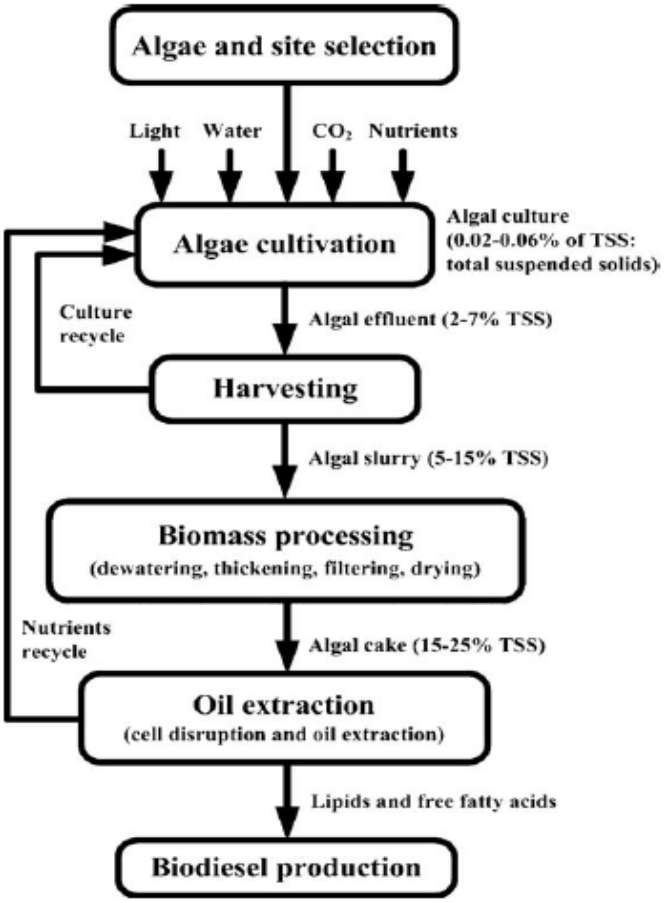

Figure 1.2 *Main steps for microalgal biomass and biodiesel production (Mata et al., 2010)*

Fig. 1.2 shows a scheme of the most important steps to produce algal biodiesel; after strain selection, the design and implementation of a cultivation system for microalgae growth is performed; subsequent operations include biomass harvesting, processing and oil extraction to supply the biodiesel production unit (Mata *et al.*, 2010).

1.4.1 Algae selection and cultivation techniques

During strain selection for microalgal cultivation, several factors must be taken into account (Mata *et al.*, 2010):

- growth rate (biomass per unit time per unit volume);
- lipid content (amount and distribution of fatty acids and triglycerides);
- resistance to environmental conditions changes (temperature, nutrients input, light, competition from other microalgal species or microorganisms);
- nutrients availability;
- ease of biomass separation and processing;
- possibility of obtaining other valuable chemicals;

- the fact that local microalgal species have a competitive advantage under the local geographical, climatic, and ecological conditions;
- data available on strains of interest.

Microalgae can grow in three metabolic pathways:

- photoautotrophically, i.e. using light as sole energy source; it is converted to chemical energy through photosynthetic reactions, in which CO_2, carbonate or bicarbonate is used as inorganic substrate to produce organic compounds;
- heterotrophically, i.e. only organic compounds (e.g. glucose, acetate, glycerol) represent carbon and energy source;
- mixotrophically, i.e. performing photosynthesis as the main energy source, though both organic compounds and CO_2 are used.

Among these, the predominant method commonly used for microalgal cultivation is photoautotrophic cultivation, though some species, such as *Chlorella vulgaris* and *Spirulina platensis* can grow well in all these conditions (Mata *et al.*, 2010).

There are several factors influencing algal growth (Mata *et al.*, 2010), such as:

- abiotic factors: light (quality and quantity), temperature, nutrient concentration, O_2, CO_2, pH, salinity and toxic chemicals;
- biotic factors: pathogens (bacteria, fungi, viruses) and competition by other algae;
- operational factors such as mixing, shear stress on cells, dilution rate, depth, harvest frequency, and addition of bicarbonate.

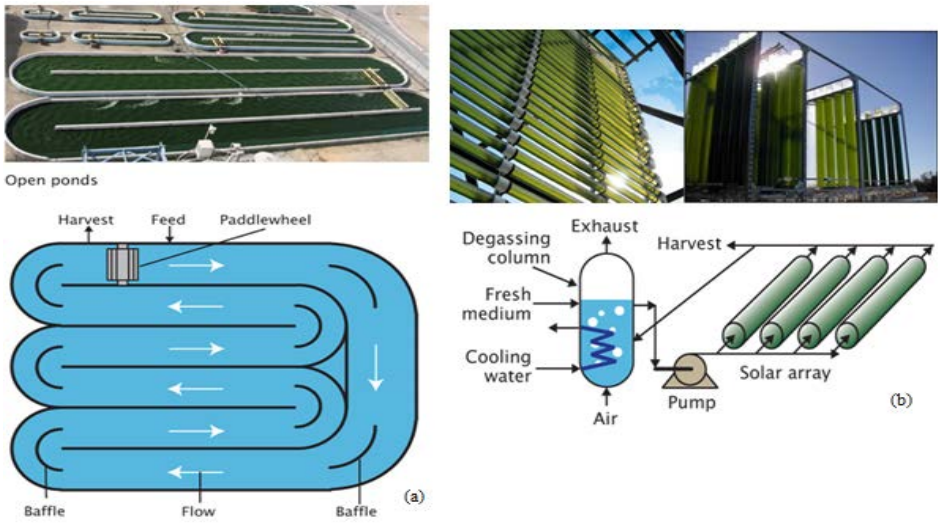

Figure 1.3 *Photo/schematic representation of a raceway pond (a) and tubular PBRs (b) (Johnson et al., 2009).*

Microalgae cultivation can be performed in open-culture systems (lakes, raceway ponds) or in highly controlled closed-culture systems called photobioreactors, or PBRs (Fig. 1.3) (Chisti, 2007). Raceway ponds are closed loop recirculation channels, (≈0.3 m deep), where flow is guided by a paddlewheel and baffles contribute to mixing. PBRs are made of a degassing zone (to remove excess oxygen) and a solar collector; this is made of glass or plastic and is

oriented and built in several different geometries (vertical/horizontal, tubular array, flat plate, etc...), all with the purpose to maximize sunlight capture. A comparison of the two systems is made in Table 1.2.

Table 1.2 *A comparison of open and closed culture systems for microalgae (Mata et al., 2010).*

Culture systems for microalgae	Closed systems (PBRs)	Open systems (Ponds)
Contamination control	Easy	Difficult
Contamination risk	Reduced	High
Sterility	Achievable	None
Process control	Easy	Difficult
Species control	Easy	Difficult
Mixing	Uniform	Very poor
Operation regime	Batch or semi-continuous	Batch or semi-continuous
Space required	A matter of productivity	PBRs ~ Ponds
Area/volume ratio	High (20–200 m^{-1})	Low (5–10 m^{-1})
Population (algal cell) density	High	Low
Investment	High	Low
Operation costs	High	Low
Capital/operating costs ponds	Ponds 3–10 times lower cost	PBRs > Ponds
Light utilization efficiency	High	Poor
Temperature control	More uniform temperature	Difficult
Productivity	3–5 times more productive	Low
Water losses	Depends upon cooling design	PBRs ~ Ponds
Hydrodynamic stress on algae	Low–high	Very low
Evaporation of growth medium	Low	High
Gas transfer control	High	Low
CO_2 losses	Depends on pH, alkalinity, etc.	PBRs ~ Ponds
O_2 inhibition	Greater problem in PBRs	PBRs > Ponds
Biomass concentration	3–5 times in PBRs	PBRs > Ponds
Scale-up	Difficult	Difficult

1.4.2 Downstream processing

The first step after the cultivation unit is to separate the microalgal biomass from water and to recover it for downstream processing. Currently, there are several methods to harvest microalgae from the dilute broth, such as: bulk harvesting, to separate microalgae from suspension (such as gravity sedimentation, flocculation and flotation); thickening, to concentrate the microalgae slurry after bulk harvesting (such as centrifugation and filtration) (Lam & Lee, 2012). Most harvesting systems employ a 2-stage dewatering process, where stage 1 increases the algae concentration from 0.05-0.5% (0.5-5 g/l) to 1–2% by mass, while stage 2 takes the biomass concentration to ~20%. This operation contributes for 20-30% of the cost of the entire process. Table 1.3 shows the pros and cons of various harvesting methods (Rawat *et al.*, 2013). With no chemical addition, the recovery of nutrient-rich water to the growth chamber is possible and advantageous in terms of sustainability of the whole biodiesel production process. Dewatered algae are then dried, milled into fine powder and pretreated to disrupt cellular structure.

Table 1.3 *Pros and cons of techniques used for harvesting microalgal biomass (Rawat et al., 2013).*

Technique	Pros	Cons
Filtration	Low cost, water reuse	Slow, membrane fouling and clogging, limited volume, cell damage
Centrifugation	Rapid, easy, efficient	Very high energy input
Gravity sedimentation	Low cost, potential for water recycling	Slow process, product deterioration, separation depends on cell density
Chemical flocculation	Low cost, low cell damage	Biomass toxicity, no water reuse, inefficient, potential to remove lipids, produces large quantity of sludgethat increases the difficulty to dehydrate the biomass
Dissolved air flotation (DAF)	Low cost, easy application at large scale	Needs flocculants, water reuse and product extraction may be negatively affected
Bio-flocculation	High efficiency, no damage to cells	No water reuse, higher energy input than other flocculants
Electrolytic flocculation	High efficiency	High energy input (up to 16KWh/kg biomass), increased temp may damage system, fouling of cathodes
Cross-flow membrane	Water reuse, removal of pathogens, protozoa	Membrane fouling, requirement for frequent use
Submerged membrane microfiltration	Low cost, less shear stress, less membrane fouling than conventional cross-flow	Membrane fouling, scale up potentially has problems

Lipids can be extracted from dried algal biomass using different chemical and physical operations. Chemical solvents are the most common method, as they have high selectivity and solubility towards lipids (Lam & Lee, 2012). The disadvantages are mostly related to their toxicity, safety issues and price. Solvents such as n-hexane, methanol, ethanol and mixed methanol–chloroform (2:1 v/v) are effective to extract microalgae lipids, but the efficiency is highly dependent on algal strains (Lam & Lee, 2012). Cell disruption enhances solvent diffusion and improves lipid recovery rate. Some techniques to disrupt microalgal cell wall are autoclaving, chemical lysis, bead-beating, high pressure homogenization, ultrasonication, microwaving, osmotic shock (Lam & Lee, 2012). In organic solvent extraction, water and solvent are removed using liquid–liquid separation methods, such as evaporation, vacuum distillation, or solvent adsorption. Supercritical CO_2 offers many advantages (Halim *et al.*, 2012): no toxicity; low temperature; high diffusivity and low surface tension, allowing penetration of smaller pores; easy separation of CO_2 at ambient temperature after extraction (by pressure release) (Lam & Lee, 2012; Halim *et al.*, 2012). However, it needs high pressure, with the related costs and safety issues. Lipids that are removed from microalgae biomass are now ready to be converted to biodiesel. Transesterification is a multiple step reaction, including 3 reversible steps in series, where triglycerides are converted to diglycerides, then to monoglycerides; these react with an alcohol (usually methanol, which is the cheapest one), to form esters (biodiesel) and glycerol (by-product) (Mata *et al.*, 2010).

1.5 Microalgal growth

In a batch culture, five algal growth phases can be recognized (Mata *et al.*, 2010) (Fig. 1.4): (1) the lag phase; (2) the exponential growth phase, with the maximum growth rate under the specific conditions; (3) the declining growth phase; (4) the stationary growth phase; (5) decline or death phase. The dashed curve shows the nutrients depletion during all phases. The lag phase corresponds to the period of physiological acclimation of cell metabolism to new nutrient or culture conditions.

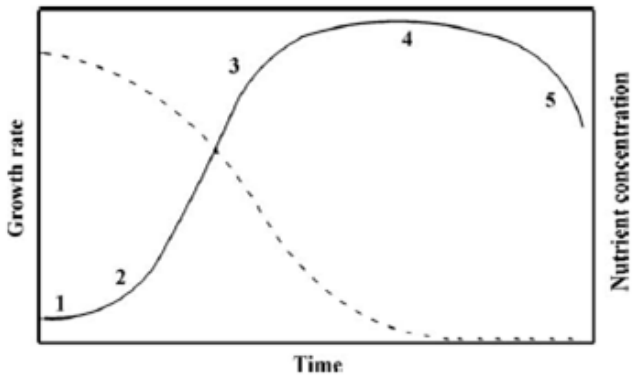

Figure 1.4 *Schematic representation of algal growth in batch culture (solid line) and nutrients concentration (dashed line) (Mata et al., 2010)*

For instance, growth lag is observed when shade-adapted cells are exposed to higher light intensities. During this phase, a little increase of cell density may occur. During exponential phase, microalgae start to grow as a function of time according to an exponential function. Declining growth phase is characterized by the reduction of the cell division rate due to physical or chemical limiting factors (nutrients, light, pH, carbon dioxide, etc.). In the fourth stage, the limiting factors and the growth rate are balanced, maintaining a constant cell density. During the last phase, the culture conditions (depletion of a nutrient, overheating, pH disturbance or contamination) are against sustainable growth, and the cell density starts to decrease. To achieve high microalgal production rates, the cultures should be maintained in the exponential phase of growth. In continuous cultures, for instance, fresh medium is continuously added to microalgae, which allows permanent exponential growth cultivation, by setting a proper residence time.

1.6 Carbon supply

Photosynthesis is a complex process through which light energy and inorganic carbon is converted into organic matter. Carbon contributes to all organic compounds, and is the main microalgal biomass element. It can range between 17.5 and 65% on dry weight (according to species and culture conditions), but most species contain about 50% of carbon (Markou *et al.*, 2014). Carbon is mainly taken up by photosynthetic microorganisms in its inorganic form of CO_2, which is dissolved in the aquatic environment. The amount of available carbon dioxide is a major limitation for large scale cultivations (Chisti, 2013), because in the normal atmosphere (0.040% v/v), growth is limited by CO_2 mass transfer. Stoichiometrically, to produce 1 ton of algal biomass requires at least 1.83 tons of carbon dioxide (Chisti, 2007). Potentially, CO_2 emitted by coal-fired power stations or by the cement industry can be used to grow algae. Subsequently, CO_2 has to be provided actively to the culture. The main ways to supply CO_2 are: (1) pumping air (which can be enriched in CO_2); (2) pumping concentrated CO_2; (3) bicarbonate salts (Chisti, 2013). A high mass flux of CO_2 to the liquid phase could

lead to medium acidification; ideally, the rate of CO_2 sparged in the culture should match the rate of its assimilation by the microalgae, with simultaneous pH adjustment (Markou *et al.*, 2014). Bicarbonates can only be applied on species which tolerate high pH and high ionic strength. However, their use could be efficient because they are more soluble than CO_2 (>90 g/l at 25°C for $NaHCO_3$) and lead to a higher lipid accumulation, which is attractive for biodiesel production (Markou *et al.*, 2014).

1.7 Nitrogen supply

Nitrogen is the second most abundant element in microalgal biomass, and its content ranges from 1% up to 14% (typically 5-10%) of dry weight. It is a constituent of essential biochemical compounds for the biomass, such as nucleic acids (DNA, RNA), amino acids (proteins) and pigments (chlorophylls, phycocyanin). It can be taken up in inorganic form of NO_3^-, NO_2^-, NO, NH_4^+ and in some cases N_2, but also in organic form, like urea or amino acids (Markou *et al.*, 2014). Microalgae have a very high protein content (30-60%) when compared to terrestrial plants, therefore the nitrogen requirements are higher (Lam & Lee, 2012). For example, to produce 1 kg of oil from microalgae, 0.29-0.37 kg of N-fertilizer are necessary, against the 0.14 kg needed for rapeseed or 0.12 kg for sunflower (Lam & Lee, 2012). The need of nitrogen fertilizer represents not only a major cost for microalgae cultivation, but also a relevant indirect input of energy and a source of CO_2 and other GHG. Several N sources that are available and exploitable for algal cultivation are now described.

1.7.1 Inorganic nitrogen

Nitrates (NO_3^-) are the most commonly used mineral nitrogen form for microalgae and cyanobacteria cultivation on synthetic media. The most frequently used salts are $NaNO_3$ and KNO_3. Nitrate does not display toxic effects to cells, and microalgae can tolerate concentrations of up to 100 mmol/l; however, it was observed that the growth is negatively affected when the concentration of nitrate is increased (Markou *et al.*, 2014).

Nitric oxide (NO) could be considered as an interesting nitrogen form mainly when flue gases are used. It has a very low solubility in the cultivation medium, and this is the rate-limiting factor to supply NO to microalgal cultures. Nitric oxide, however, is a free radical and high intracellular concentrations will have detrimental effects; the degree of tolerance to NO is species-dependent (Markou *et al.*, 2014).

Nitrite (NO_2^-) is frequently found in natural environments as an intermediate product of the nitrification process (oxidation of ammonia to nitrate). However, nitrite is also an intracellular intermediate of the nitrogen metabolism; it is the product of reduction of nitrate to nitrite by the nitrate reductase, then it is reduced further to ammonium through nitrite reductase. CO_2 seems to be necessary for nitrite assimilation, as it favors the action of nitrite reductase

(Markou 2014). Although nitrite can be taken up and used as N source, it is toxic at high concentrations. Ammonia/ammonium is the preferred nitrogen source for microalgae/cyanobacteria because its uptake and assimilation consumes less energy compared to the other nitrogen sources (Markou *et al.*, 2014). However, the microalgal biomass production or the growth rate using ammonia/ammonium as nitrogen source is similar to that when nitrate is used, or even lower (Markou *et al.*, 2014). Ammonia is a volatile molecule, but, unlike CO_2, its solubility in water is very high (about 35% w/w at 25 °C). When ammonia is dissolved in water, it reacts with H_2O molecules to form a buffer system of ammonia/ammonium:

$$NH_4^+ + OH^- \overset{pK=9.25}{\longleftrightarrow} NH_3 + H_2O \qquad (1.1)$$

The equilibrium between the forms of ammonium (ionized form) and free ammonia (the unionized gaseous form in solution) depends mainly on pH (Fig 1.7).

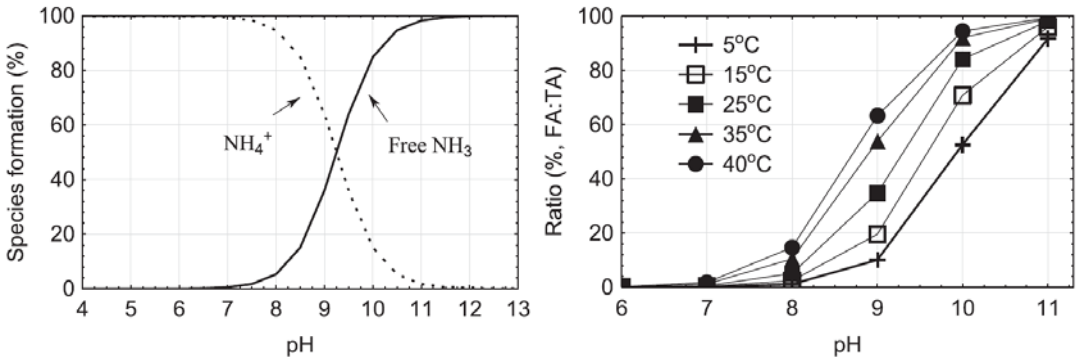

Figure 1.7 *Effect of pH and temperature on the concentration of free ammonia and the ratio of free ammonia (FA) to total ammonia (TA) (Markou et al., 2014)*

At pH>9.25 (= pK at 25 °C), free ammonia (NH_3) is the dominant species. Temperature has also a significant effect on the ammonia/ammonium species equilibrium; the pK value decreases as temperature increases, which means that free ammonia starts to be dominant at lower pH values with increasing temperatures (Fig. 1.7). A serious constraint when using ammonia/ammonium is the potential toxicity.

Free ammonia has detrimental effect on microalgae in relatively low concentrations (2 mmol/l), while ammonium ion is non-toxic (Markou *et al.*, 2014). The degree of toxicity is however species dependent. Ammonia toxicity should be taken into consideration when wastewaters rich in ammonia are used as the cultivation medium. Some solutions to this problem include: regulating pH value and keep it lower than the pK of the equilibrium; diluting the wastewater to avoid an inhibitory concentration of NH_3; fed-batch cultivation mode, where NH_3 is added gradually to the medium (Markou *et al.*, 2014). The assimilation of different nitrogen forms influences the pH of the culture medium. If ammonium is provided as the N source, the pH may drop due to the release of H^+ during assimilation, while

the pH will increase due to the release of OH^- when nitrate is applied. Another drawback of using ammonia as nitrogen source is its loss from the cultivation media due to volatilization, especially at higher pH values (Markou *et al.*, 2014).

1.7.3 Organic nitrogen

Microalgae can utilize nitrogen from organic forms such as urea and some amino acids, which are transported actively into the cells and are metabolized intracellularly (Markou *et al.*, 2014). The most relevant exploitable organic nitrogen source for microalgae cultivation is urea, which is hydrolyzed to ammonia and carbonic acid, which can both be assimilated, by microalgae and cyanobacteria. Many researchers reported that urea has a positive influence in the growth of some species, such as in *Spirulina platensis* and *Chlorella sp.* (Markou *et al.*, 2014), whose growth rates are equal or higher compared to cultures using other nitrogen sources. Microalgae are also able to use nitrogen from amino acids, in autotrophic and heterotrophic cultivation. However, this capability is species dependent and growth rates vary significant between the microalgae and the amino acid used (Markou *et al.*, 2014). Various wastewaters derived from the livestock or food processing sector could be used as organic N source, but the presence of bacteria in the culture which convert the organic N to inorganic one seems to be necessary (Markou *et al.*, 2014).

1.8 Phosphorus supply

Phosphorus is another important nutrient for microalgal growth and its biomass content varies from 0.05% up to 3.3% (Markou *et al.*, 2014). Phosphorus is present in several organic molecules that are essential to metabolism, such as nucleic acids (RNA and DNA), membrane phospholipids and ATP. Unlike carbon and nitrogen nutrients, which are renewable, phosphorus is derived from fossil phosphate-rocks, which are non-renewable so that their reserves are expected to be depleted in the future (Markou *et al.*, 2014). There are various types of fertilizers that could be used as P source for microalgae cultivation (e.g. potassium, sodium and ammonium phosphates or superphosphates), but all of them are produced using phosphate-rock as feedstock. In natural environments and wastewaters, P is present in various forms such as orthophosphate, polyphosphate, pyrophosphate, metaphosphate and their organic forms. It is well known that phosphorus is taken up by the cells in the orthophosphate form (Markou *et al.*, 2014). However, other inorganic and organic phosphorus forms (mainly dissolved organic phosphorus, or DOP, but also insoluble phosphorus compounds) can also be used by microalgae. The capability of DOP to be taken up depends on the chemical composition of microalgae. However, most of the DOP compounds cannot be directly assimilated by microalgae and have first to be mineralized (Markou *et al.*, 2014). Inorganic P forms other than orthophosphate have first to be converted to orthophosphate to be suitable

for uptake by microalgae. This is accomplished by the action of various phosphatase enzymes. Orthophosphate forms speciation follows the equilibria of dissociation of phosphoric acid according to the solution pH, as shown in Fig. 1.8:

$$H_3PO_4 + H_2O \overset{pK_1 = 1.90}{\longleftrightarrow} H_2PO_4^- + H_3O^+ \quad (1.2.1)$$

$$H_2PO_4^- + H_2O \overset{pK_2 = 6.25}{\longleftrightarrow} HPO_4^{2-} + H_3O^+ \quad (1.2.2)$$

$$HPO_4^{2-} + H_2O \overset{pK_3 = 12.92}{\longleftrightarrow} PO_4^{3-} + H_3O^+ \quad (1.2.3)$$

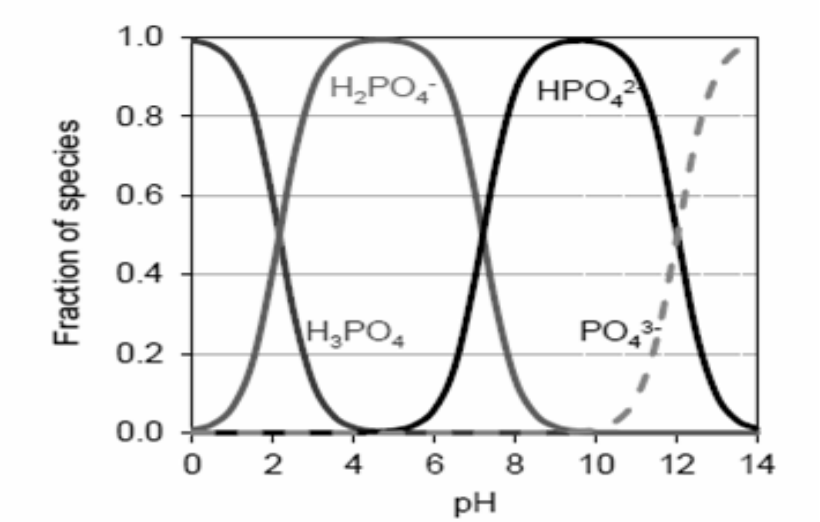

Figure 1.8 *Effect of pH on orthophosphates speciation (adapted from Businelli, 2007).*

In commonly used culture conditions, $H_2PO_4^-$ and HPO_4^{2-} are the two predominant orthophosphate ion forms, and can both be actively assimilated by microalgal cells.

The uptake rate of phosphorus is affected by the cell condition and by several environmental factors, such as available energy (light), pH, temperature, salinity/ionic strength of the cultivation medium and available ions such as K^+, Na^+ or Mg^{2+}. Microalgae and cyanobacteria may accumulate intracellular phosphorus reserves as polyphosphate granules, which can be used as a P source when phosphate is depleted in the surrounding medium. This behavior is known as *luxury uptake* and is observed in microalgae as well as in cyanobacteria (Markou *et al.*, 2014). The capability to store excess phosphorus can be exploited for removal of phosphorus from wastewaters. However, in cultures with synthetic fertilizers, luxury uptake should be avoided in order to maximize the biomass yield per mass of nutrient fed. At high pH, due to photosynthesis and the alkalization of the cultivation medium, polyvalent cations, such as calcium (Ca^{2+}) and magnesium (Mg^{2+}) may precipitate with phosphates, and this may reduce the availability of phosphorus (Markou *et al.*, 2014), especially in wastewaters with high content of divalent cations.

1.9 Sulphur, potassium and micronutrients

For an uninhibited microalgal growth, the cultivation medium has to contain several other nutrients (micronutrients) besides carbon, nitrogen and phosphorus (macronutrients). Essential micronutrients are K, Mg, S, Ca, Na, Cl, Fe, Zn, Cu, Mo, Mn, B and Co.

Sulfur is important for microalgae to thrive and its content in the biomass is 0.15-1.6%. It is a component of amino acids and of sulfolipids that are part of the lipid bilayer of the cell membranes. It is a constituent of vitamins, regulatory compounds, and secondary metabolites. Sulfur can be found in many forms; the S demands of microalgae are fulfilled mainly by the assimilation of sulfate (SO_4^{2-}) while other forms such as sulfide are toxic (Markou *et al.*, 2014). Sulfate is available in various fertilizers and can be provided as $MgSO_4$, $(NH_4)_2SO_4$, K_2SO_4, $(NH_4)_2SO_4$ e $MgSO_4$. Some industrial wastewaters are also a source for sulfates, such as those derived from paper milling, food processing and distillery. Potassium content in some microalgae ranges from 1.2% to 1.5%; it plays an important biological role, being an activator for a number of enzymes involved in photosynthesis and respiration; it affects protein and carbohydrate synthesis and regulates the osmotic potential of cells (Markou *et al.*, 2014). Magnesium is another essential element for microalgal biomass production. Its content in microalgae is between 0.35% and 0.7%. It participates in vital cell processes such as ATP reactions for carbon fixation and is an activator for several major enzymes. It is a constituent of the photosynthetic apparatus and in particular of the chlorophylls. Magnesium in aqueous solutions is mainly present as Mg^{2+}; when pH is high, Mg^{2+} may precipitate as phosphate or hydroxide. Most non-synthetic media which are used for algal cultivation (such as wastewaters, anaerobic digestion effluents or the aqueous phase from hydrothermal liquefaction) are deficient in this element, which has to be added separately. Calcium is a further important constituent of cell walls. It also affects the cell division and is a secondary messenger determining the overall morphogenesis. Calcium content in microalgal biomass varies from 0.2% to 1.4% (Markou *et al.*, 2014). High concentrations in the cultivation medium at high pH results with the formation of $CaCO_3$ and other calcium salts which precipitate, thus decreasing the alkalinity of the medium and the concentration of some minerals, such as phosphorus.

Iron is one of the most significant trace elements required by microalgae; its content ranges between 0.05 and 0.2%. It is involved in fundamental enzymatic processes such as oxygen metabolism, electron transfer, nitrogen assimilation, and DNA, RNA and chlorophyll synthesis (Markou *et al.*, 2014).

1.10 The importance of nutrient recycling

Due to the amounts requires, nutrient use can account for up to half of costs and energy input in microalgal cultivation (Xia & Murphy, 2016). For instance, if an empirical formula for microalgae is assumed to be $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$, the production of 1 ton of alga requires 45 kg of N and 4 kg of P (Zhang *et al.*, 2014; Chisti, 2007). The production of 1 l of biodiesel requires between 0.23 and 1.55 kg of N and 29-145 g of P depending on the cultivation conditions (Rösch *et al.*, 2012).

The supply of nutrients is expected to severely limit the extent to which the production of biofuels from microalgae can be sustainably expanded. In particular, phosphorus is a finite resource, as it is present as phosphate rock only; concerning N, almost as much N fertilizer can be produced as desired, but doing so will require more fossil energy. Fixing atmospheric nitrogen by the Haber–Bosch process, as currently used, requires a huge amount of energy, so that nearly 1.2% of global energy consumption goes to producing N fertilizers for agriculture. The existing supply systems of N and P fertilizers for agriculture is thus insufficient to sustain any significant scale production of algal biomass for extraction of oils (Chisti, 2013). The competition of organic fertilizers with food growers and the increasing price of fossil fuels (which is directly linked with fertilizers cost) could lead to a significant rise in nutrient costs. On the other hand, the production of fertilizers requires energy and releases carbon dioxide. The whole point of attempting to switch to biofuels is to reduce carbon emissions: therefore, producing more fertilizer through conventional technology is counterproductive. Making algae independent of an external supply of nitrogen is essential for their sustainable production. All this suggests that N and P must be recovered from the algal biomass once the oil has been extracted and, somehow, recycled (Chisti, 2013). As the oil contains relatively little N and P, most of the nutrients should be found in the spent biomass. Another solution would be to use organic compounds which are present in wastewater. Aquaculture systems involving microalgae cultivation in wastewater seems to be quite promising, as it combines two advantages: 1) providing nutrients for algal growth; 2) biological cleaning and eutrophication prevention (Mata *et al.*, 2010). However, this method still has drawbacks such as: low productivity; possibility of contamination by competing microorganisms, inhibition by other compounds present in wastewater (ammonia, nitrite, heavy metals, organic acids, etc…), especially if it comes from agricultural/industrial applications (Markou *et al.*, 2014). The feasibility and sustainability of a large scale production of microalgal biofuels strongly depend on the possibility of reducing external nutrient supply by fertilizers, especially concerning N and P.

This fundamental objective could be achieved through nutrient recycling. Two main approaches can be considered in respect to this. The first one consists in recycling the culture medium after harvesting the biomass, and supplement nutrients only to compensate for their

consumption/loss; this would also help cutting the high freshwater requirements and costs involved in a large scale biofuel production.

According to a second strategy, the nutrients in the residual biomass after lipid extraction should be somehow recovered. The output of the extraction process are the isolated lipid (algal oil) and lipid-extracted algae biomass residue, or algal cake. Algal cake is mainly composed of carbohydrates and proteins, and nearly 100% of N and P in the microalgae are retained in it. Several technologies have been studied to recover these nutrients from the spent biomass, and the most relevant ones are be divided in two categories: hydrothermal processes and anaerobic digestion.

1.11 Hydrothermal processes

Table 1.4 *Summary of all hydrothermal technologies, with their conditions, products, main advantages and disadvantages. References: [a]= Lòpez Barreiro et al. (2015); [b]=Heilmann et al. (2010);[c]=Du et al. (2012); [d]=Barbera et al. (2016); [e]=Biller et al. (2012).*

Process	Conditions	Products	Pros	Cons
HTG [a]	T=400-700°C; P=25-30 MPa Supercritical water With or without catalyst	Gas (CO_2, CO, CH_4, H_2, C_2-C_3 compounds). Nutrient-rich aqueous phase. High T, low algae concentrations, long residence times and some catalysts (e.g. Ru) enhance gas yields.	High efficiency, use of direct wet biomass, lower temperature needed for gas formation than dry gasification.	High energy (high T) and capital costs (high T, materials); product value is not competitive.
HTC [a] [b] [c]	T≈200°C P<2MPa τ≈30 min Slurry reactor	Insoluble solid (hydrochar). Nutrient-rich aqueous phase. T and τ residence time influence solid fraction yield, lipid yield and nutrient release in liquid phase	Mildest conditions; high yield, easy-filterable biochar with lots of uses; algae grown in hydrolysate have higher lipid content, higher C and lower N than in BG11 (favorable for biofuel production)	Low value of the solid product makes process not very competitive
FH [d]	Continuous-flow reactor T=200–300°C P=20 MPa τ=6-10 s	Low-N, high-C solid that retains the lipid fraction; liquid hydrolysate that contains up to 66% of the initial N content, plus other inorganic elements. The product composition depends on T/residence time.	No inhibitory compounds, continuous operation, non-perishable solid in which lipids are more concentrated and easily extractable; algal growth in hydrolysate is comparable with synthetic medium.	Strict control of residence time is required, as it strongly influences product yields/composition and selectivity
HTL [a] [e]	T=280-370°C P=10-25 MPa With or without catalysis	Biocrude for further refining (yield=27-47%, depending on T and feedstock composition), gas (CO, CO_2 and H_2), solid, aqueous phase. T and residence time influence solid and gas yields. Recovery in liquid phase: 15-56% N, 20-30% P	Solid residue (high in N, low in C) used as a fertilizer or bio-char. Recycle to cultivation of gaseous phase (CO_2). Energy recovery through combustion of char and biocrude (high heating value). Water phase is very high in all nutrients for algal growth.	Aqueous phase contains toxic compounds such as phenols, fatty acids, nitrogen heterocycles and Ni; heavy dilution is needed to avoid inhibition

Hydrothermal processes, as summarized in Table 1.4, include hydrothermal gasification (HTG), hydrothermal liquefaction (HTL), hydrothermal carbonization (HTC) and flash hydrolysis (FH). All of these processes convert biomass to a liquid, solid or gaseous product by contact with an aqueous medium, at high temperature and pressures (200-600°C and 5-40 MPa). During the operation, the polymeric structures in the biomass (such as proteins and polysaccharides) are hydrolyzed, leading to the formation of a large spectrum of products. Biomass harvesting and drying represent a significant contribution to the production cost of biofuels from microalgae: these "wet" processes possess the great energetic advantage of avoiding a previous drying of the microalgal cake, as they work with biomass suspensions in aqueous phase. An aqueous by-product which is rich in nutrients (N, P, C, Fe, Ca, Mg, K, Na) is always formed; it can be recycled back for microalgal cultivation as a nutrient source, strongly enhancing economic and environmental sustainability of the whole process in terms of freshwater and nutrient requirements.

1.12 Anaerobic digestion (AD)

Anaerobic digestion is one of the most promising among the different nutrient recycling technologies, thus it has been widely investigated for microalgae production. During AD, microorganisms derive energy and grow by metabolizing organic material in an oxygen-free environment; they convert organic carbon to biogas, a combination of mostly methane (60%) and carbon dioxide (40%). Anaerobic microbiological decomposition can be subdivided into four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The acidogenic bacteria excrete enzymes for biopolymer hydrolysis and convert soluble organic compounds (such as amino acids, sugars and free fatty acids) to volatile fatty acids (VFAs) and alcohols. These are then converted by acetogenic bacteria into acetic acid or hydrogen and carbon dioxide. Methanogenic bacteria then use acetic acid or hydrogen and carbon dioxide to produce methane. The effluent from the digester, which is a suspension called digestate, is rich in nutrients and may be used for nutrient recycling (Zhang *et al.*, 2014). In particular, its liquid fraction after solid–liquid separation is an excellent candidate for direct recycling in the microalgae cultivation system, due to the low solids and high nutrient content. On the other hand, the solid fraction inhibits light penetration in the pond and thus cannot be recycled. However, as it contains nutrients and carbon, it can potentially be used as a soil amendment. Moreover, the energetic value of the produced methane can improve the energetic balance of the microalgae to biofuel process (Sialve *et al.*, 2009). A further synergistic benefit of integrating anaerobic digestion with algal biofuel program is the ability to use microalgal cultures to increase methane content of the biogas. The concentration of carbon dioxide in the biogas from anaerobically digested microalgae is in the range of 30 to 50% (Ward *et al.*, 2014). From an energy recovery perspective, the CH_4/CO_2 mass ratio needs to be above unity,

indicating that a gas purification step is required for microalgae derived biogas. Additionally, the biogas could be recycled back to the aquatic culture: due to the solubility of methane (low) and carbon dioxide (high), uptake of CO_2 by microalgae is significant leaving high concentrations of CH_4 in the gas after the purification step (Ward *et al.*, 2014). N and P fed into the digester are typically retained at 100% in the digestate (Zhang *et al.*, 2014). During the degradation of organic compounds, a portion of organic N will be mineralized into inorganic nitrogen forms, such as ammonia, which are readily available for uptake by microalgae. Studies of mineralization rates for digested microalgae biomass report ranges from 25% to 69% of total N input. Mineralized N mostly stays in the liquid fraction of the digestate, while organic N usually remains in the solid fraction. Unfortunately, the majority of P (60%–86%) was shown to remain in the solid fraction as well (Zhang *et al.*, 2014). Because a solid–liquid separation process is required for nutrient recycling in the microalgae cultivation system, understanding the nutrients retention in each fraction and their form (organic or inorganic) is quite important. Incorporating of anaerobic digestion in microalgae biofuel production and bio-refinery operations will increase the cost effectiveness of the process, helping it to become economically feasible and environmentally sustainable (Ward *et al.*, 2014). Fig. 1.9 illustrates the conceptual implementation of anaerobic digestion into the algal production processes (Ward *et al.*, 2014).

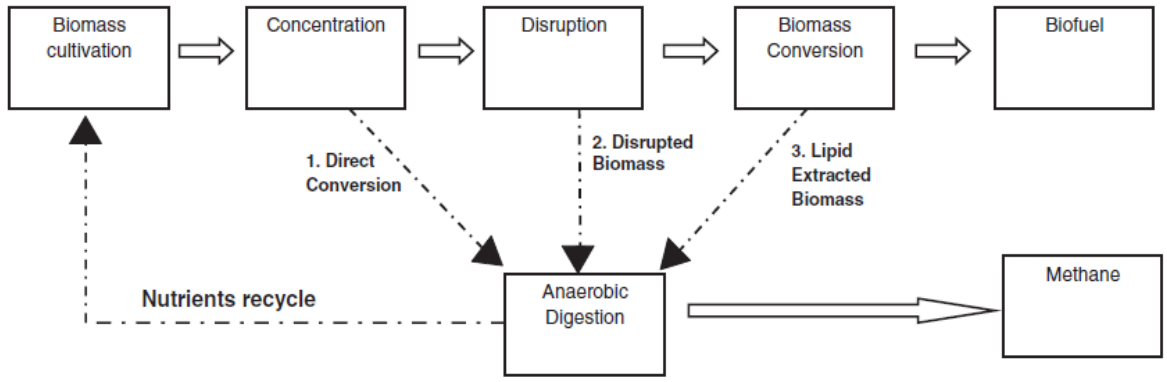

Figure 1.9 *Possibilities to incorporate anaerobic digestion in the microalgal biofuel production process.*

Pathway 1 could be used in a wastewater process where the cell wall is degradable by bacterial activity within the digester. The second pathway illustrates the anaerobic digestion of biomass after cell wall disruption is done prior to conversion. The third one is the traditional biodiesel practice, where lipid is extracted and residual algal biomass is converted to biogas by anaerobic digestion and methane fermentation. However, lipids are an attractive substrate for anaerobic digestion and have a higher theoretical methane potential compared to proteins and carbohydrates (Ward *et al.*, 2014). It has been suggested that the conversion of microalgal biomass to methane rich biogas is energetically more favorable than lipids removal from microalgae biomass where the total lipid content is lower than 40% (Ward *et al.*, 2014). On the other hand, the removal of lipids from microalgae biomass for liquid biofuel

production prior to AD of the residual microalgae biomass can be beneficial to anaerobic digestion processes, as they can cause inhibition (long chain fatty acids can interfere with methanogenic bacteria by adsorption on their walls or membranes). Up to 20% lipid content showed no inhibitory effect; in the case of a higher content (>30%), even if it is economically disadvantageous, it is necessary to extract lipids in order to avoid inhibition in the AD process. Note that lipid extraction methods used on microalgal biomass can affect its digestibility. For example, the mixture chloroform/methanol is considered an excellent solvent for extraction (Ramos-Tercero *et al.*, 2014); but, unfortunately, biochemical methane potential (BMP) tests on lipid-extracted biomass showed strong inhibition of methanogenic bacteria, and only CO_2 was produced, because of the toxic effect of residual chlorinated solvent. Butanol, ethanol, hexane and methanol have been shown to have no detrimental effects on anaerobic digestion when residual solvents are removed by heating (Ward *et al.*, 2014).

1.12.1 Theoretical methane potential from AD

When the C, H, O and N composition of a sludge or generic substrate is known, a stoichiometric relationship (known as Buswell formula) can be used to estimate the theoretical gas composition on a percent molar basis (Sialve *et al.*, 2009):

$$C_cH_hO_oN_n + \left(\frac{4c-h-2o-3n}{4}\right)H_2O \rightarrow \left(\frac{4c+h-2o-3n}{8}\right)CH_4 + \left(\frac{4c-h+2o+3n}{8}\right)CO_2 + nNH_3 \quad (1.3)$$

where *c*, *h*, *o* and *n* are the carbon, hydrogen, oxygen and nitrogen molar composition respectively. The methane yield can be estimated as:

$$CH_4\ yield\ \left(\frac{l}{g\ VS}\right) = B_0 = \frac{1}{8}\left(\frac{4c+h-2o-3n}{12c+h+16o+14n}\right) * V_m \quad (1.4)$$

where V_m is the molar volume of methane, which is 22.14 l at 0 °C and 1 atm (normal conditions) and VS stands for volatile solids. The volumetric ratio R_G of methane to carbon dioxide can be computed in this way:

$$R_G = \frac{4-m}{4+m}, \quad m = \frac{-h+2o+3n}{c} \quad (1.5)$$

where *m* is defined as the average carbon oxidation state in the substrate.

The biogas composition depends also on the amount of CO_2 which is dissolved in the liquid phase through the carbonate system, and is therefore strongly related to pH. The ammonia production yield in the digester can be evaluated using the equation:

$$Y_{N-NH_3}\left(\frac{mg}{gVS}\right) = \frac{17 * 1000 * n}{12c + h + 16o + 14n} \quad (1.6)$$

Note that the volatile solids in these equations do not represent the whole amount of volatile solids present in the biomass, but only the degraded one. The experimental yields will certainly be lower than theoretical values because microalgal cells biodegradability must be taken into account. Values of degradability from 50 to 75% are reported in literature (Zhao *et al.*, 2014). Table 1.6 shows specific methane yields for carbohydrates, lipids and proteins; it confirms how greater methane potential is linked to higher lipid content.

Table 1.6 *Specific methane yield for 3 types of organic compounds (Sialve et al., 2009)*

Substrate	Composition	l CH_4/gVS
Proteins	$C_6H_{13.1}O_1N_{0.6}$	0.851
Lipids	$C_{57}H_{104}O_6$	1.014
Carbohydrates	$(C_6H_{10}O_5)_n$	0.415

For the specific case of proteins, the formula was calculated with the average composition in amino acids weighted by their frequency in *Chlorella vulgaris* (Sialve *et al.*, 2009). The species that can reach higher lipid content (e.g. *C. vulgaris*) have a higher methane yield. In the case where lipids are extracted from algae before digestion, the potential methane yield is lower while the released ammonium is higher (Table 1.7).

Table 1.7 *Estimation of the theoretical methane potential and theoretical ammonia release (in brackets, the same estimations in case of residual biomass after lipid extraction)*

Species	Proteins (%)	Lipids (%)	Carbohydrates (%)	Calorific value (kJ/g)	CH_4 (L CH_4 g VS^{-1})	N–NH_3 (mg g VS^{-1})
C. vulgaris	29	18	51	18	0.64 (0.56)	27.0 (32.9)
C. emersonii	28	63	11	29	0.92 (0.76)	26.1 (70.5)
C. protothecoides	38	11	52	19	0.65 (0.60)	34.5 (39.8)

It is obvious that experimental methane production will be lower to theoretical ones, whether whole algae or lipid-extracted biomass is digested. For example, several authors report that 147-403 mlCH_4/gVS are produced from the digestion of whole *C. vulgaris* (with different loading rates and retention times) (Zhao *et al.*, 2014; Polakovičová *et al.*, 2012; Ward *et al.*, 2014; Ras *et al.*, 2011).

1.12.2 AD parameters and operative variables

The AD process can take place at three different temperature ranges: psychrophilic (below 25°C), mesophilic (25°C – 45°C), and thermophilic (45°C – 70°C). Generally, an increased temperature has a positive effect on the metabolic rate of the microorganisms, so the process runs faster and has higher productivity.

However, many disadvantages have been identified when increasing temperature: decreased stability, low-quality effluent, increased toxicity and susceptibility to environmental conditions, larger investments, poor methanogenesis and higher net energy input to keep the fermenter isothermal (Mao *et al.*, 2015). Although mesophilic systems exhibit better process stability and higher richness in bacteria, they have lower methane yields and suffer from poor

biodegradability and nutrient imbalance. Therefore, the optimal conditions for AD would be thermophilic hydrolysis/acidogenesis and mesophilic methanogenesis, which is consistent with a two-phase anaerobic digestion process (Mao *et al.*, 2015). Mesophilia appears to be the optimal condition in one-stage processes (Sialve *et al.*, 2009). In general, AD microorganisms are very sensitive to temperature changes, which affect hydrogen/ methane production, and the decomposition of organic materials.

The operational pH affects the digestive progress and products directly. The ideal pH range for AD of microalgae has been reported to be 6.8–7.4 (Kwietniewska & Tys, 2014). Methanogenic bacteria are extremely sensitive to pH fluctuations and prefer a pH value around 7.0 as the growth rate of methanogens is greatly reduced below pH 6.6. Acid-forming bacteria are less sensitive and tolerate pH in range of 4.0–8.5, but the optimal pH for hydrolysis and acidogenesis is between 5.5 and 6.5 (Kwietniewska & Tys, 2014). Therefore, once again, it would be convenient to perform hydrolysis/acidification and acetogenesis/methanogenesis processes in two separate stages. Before entering the digester, biomass must be concentrated; systems used to digest solid waste are classified according to the percentage of Total Solids (TS) in the waste stream:

1. 10-25% TS: low solids anaerobic digestion (wet fermentation);
2. >30% TS: high solids anaerobic digestion (dry fermentation).

It has been reported that the highest methane production rates occur at 60-80% humidity of the substrate entering the digester (Kwietniewska & Tys, 2014).

Substrate to inoculum ratio, also called F/M (food to microrganisms) ratio, is expressed in this case as (gVS microalgal biomass)/(gVS sludge). This has been identified as a key factor of methane productivity from algal biomass (Kwietniewska & Tys, 2014). It was reported that cumulative methane yield was the highest when F/M was equal to 0.5. (Kwietniewska & Tys, 2014; Ramos-Tercero *et al.*, 2014). Organic loading rate (OLR) represents the amount of volatile solids, or of chemical oxygen demand, fed into a digester per day per unit volume under continuous feeding (gVS/l/day or gCOD/l/day), or simply per unit volume (gVS/l or gCOD/l) in batch operation or BMP tests. At increasing OLR, the biogas yield increases to an extent, but, above the optimal OLR value, VS degradation and methane production decline because of overloading (Kwietniewska & Tys, 2014). Adding daily a large volume of new material may result in changes in the digester's environment and temporarily inhibits bacterial activity during the early stages of fermentation. Optimum OLR depends on the type/composition of algal substrate. Another operating variable is the retention time, i.e. the time required to complete the degradation of organic matter. It is associated with the microbial growth rate and depends on operating temperature, OLR and substrate composition

(Kwietniewska & Tys, 2014). It is possible to distinguish the solid retention time (SRT), i.e. the average time that bacteria (solids) spend in a digester, and hydraulic retention time (HRT) which is defined by the following equation:

$$HRT = \frac{V}{Q} = \frac{biological\ reaction\ volume\ in\ digester}{influent\ flow\ rate} \quad (1.7)$$

An average HRT of 15–30 days is required to treat waste under mesophilic conditions (a bit shorter for a thermophilic plant). Decreasing the HRT usually leads to VFA accumulation, while a longer HRT than optimal one results in insufficient utilization of digester components. For algal biomass, an HRT below 10 days results in low methane productivity (Kwietniewska & Tys, 2014). The two types of retention time must be sufficiently high to avoid microorganisms washout from the reactor, and also to avoid limitation by hydrolysis (which is generally the limiting step of the overall conversion of complex substrates to methane). When the process is operated at low loading rate and high hydraulic retention time, methane yield is constant and maximal (Sialve *et al.*, 2009).

1.12.3 Problems with microalgae as a substrate for AD

The effluent from anaerobic digestion of microalgal biomass surely represents an interesting alternative to synthetic growth media such as BG11, while biogas could help closing the energy balance and enhance economic feasibility of the biofuel production process. However, there are still significant operating problems concerning this process, such as substrate concentration, biodegradability, and low C/N ratio of microalgal biomass. Concentrating or harvesting it prior to digestion is a fundamental challenge to the financial viability of the whole process, and is a major disadvantage compared to hydrothermal treatments (Ward *et al.*, 2014). Low volatile solids (VS) rate is due to the small concentration of microalgae biomass. De Schamphelaire and Verstraete (2009) concluded that a concentrating step would be necessary for optimal performance of the AD step, to avoid insufficiency of digestible substrate and washout of the anaerobic bacteria community. Due to the high expenses associated with harvesting and dewatering steps, new cost efficient laboratory and pilot scale technologies are under development, such as membrane reactors as digesters, better combinations of settling and centrifugation, coagulants/flocculants) (Ward *et al.*, 2014).

The degradation of the cell wall is strongly correlated to the amount of gas produced during anaerobic digestion. Up to 60% of the untreated microalgae biomass added to the anaerobic digester will remain undigested due to the cell wall remaining intact throughout the digestion process. Microalgal cells are known in fact to be able to effectively resist bacterial attack and to remain intact also after leaving a digester with a 30-day hydraulic retention time (Ward *et al.*, 2014). A higher gas production is linked to the microalgae species that had either no cell wall or a cell wall made of protein, while gas yield decreases for microalgal species that have

a carbohydrate-based cell wall containing hemicellulose. In this case, a pretreatment step is needed to disrupt the cell wall and increase bacterial hydrolysis before addition to the anaerobic digester.

Several combination of pretreatments have been tested (mechanical, physical, thermo-chemical, enzymatic). For example, thermochemical ones to destroy cell walls combining the use of NaOH and temperature above the thermal limit of the microalgal species have been performed. All tests including pretreated biomass produced better results than untreated control comparisons. Ultrasonic pretreatments are also efficient, ensuring similar results to thermochemical ones in shorter times. Another available technology is high pressure thermal hydrolysis (HPTH) (Ward *et al.*, 2014). HPTH processes heat substrate to approximately 160 °C at a pressure of approximately 6 bars. After these conditions have been maintained for 20–30 min the pressure is reduced in a flash drum where the cells break and release the cell contents. Some authors reported an extraction method using a Soxhlet apparatus with hexane to extract the lipid, that increased the biomethane potential of the microalgae biomass (Ward *et al.*, 2014). When both lipid extraction and HPTH were combined a digestibility of the lipid extracted and HPTH microalgae biomass of +110% was recorded compared to untreated one. This process is energy intensive but energy balances demonstrate that HPTH coupled with anaerobic digestion can be energy-positive due to the higher methane potential from the substrate.

Another issue with using microalgae as a substrate of AD is the low carbon/nitrogen (C/N) ratio which characterizes algal biomass. Literature data (Ward *et al.*, 2014) showed that it varies from 4.16 to 7.82: these low values are due to its richness in nitrogen and proteins. When the C/N ratio is below 20, an imbalance occurs between carbon and nitrogen requirements for the anaerobic bacterial community or consortia, leading to relevant nitrogen release in the form of ammonia during digestion (up to 7000 mg/l). Ammonia can become inhibitory to methanogenic bacteria and results in VFA accumulation within the digester (Kwietniewska & Tys, 2014). The inhibition level of VFA for AD has been reported to be around 6000 mg/l. A remedy to overcome problematically low C/N ratios is co-digestion (Ward *et al.*, 2014): microalgae have been co-digested with other waste streams, such as manure, paper, corn stalks, glycerol (which is a by-product of lipid transesterification) to increase the C/N ratio. Better methane yields and less inhibitory effects from ammonia have been reported with this approach. The optimum C/N ratio was found to be between 20 and 30 (Ward *et al.*, 2014).

1.12.4 Inhibition

Inhibition is a major problem concerning AD operation; it is mainly caused by ammonia, salinity in the marine species medium and hydrogen sulphide (H_2S). The high nitrogen

content in microalgae (especially with high biomass concentration in the AD influent) can lead to significant accumulation of ammonia-nitrogen during anaerobic digestion. An increase in pH or temperature can be very harmful to the bacterial community as the equilibrium shifts to the more toxic unionized form of ammonia-nitrogen NH_3-N. Free ammonia is the main responsible for inhibition, and its fraction at equilibrium in can be calculated by (Uggetti *et al.*, 2014):

$$\frac{[NH_3]}{[NH_4^+] + [NH_3]} = \left(1 + \frac{10^{-pH}}{10^{-\left(0{,}0901821 + \frac{2729{,}92}{T\,(K)}\right)}}\right) - 1 \qquad (1.8)$$

Ammonia gas within the digester is inhibitory at a much lower concentration than the aqueous ionized form of ammonium–nitrogen. N-NH_4^+ is toxic at high levels and has a moderately inhibitive effect from 1500–3000 mg/L. Above 3000 mg/L a strong inhibitive effect is associated with N-NH_4^+, which can lead to a drop in gas production.

A value of 80 mg/l of N-NH_3 has been found to be the minimum inhibitory level, though a wide range of inhibiting ammonia concentrations has been identified, spanning from 1.7 to 14 g/L (Montingelli *et al.*, 2015). Inhibiting thresholds depend on various factors such as acclimation, nature of substrate and inoculum, operating conditions (Sialve *et al.*, 2009). Ammonia toxicity affects methanogens in two ways: (1) the NH_4^+ ion inhibits the methane-synthesizing enzyme directly, and (2) the hydrophobic NH_3 molecule diffuses passively through the cell, causing proton imbalance and/or K deficiency (Ward *et al.*, 2014). Marine microalgae, such as *Tetraselmis spp.* and *Nannochloropsis spp.*, are grown in a saline environment, with a high NaCl content (0.5-1 M). High salinity levels have been shown to be inhibitory as they can cause bacterial cells to dehydrate due to increased osmotic pressure. Alkali and alkaline earth metal ions (such as K^+, Ca^{2+} and Mg^{2+}) are needed in very low concentrations for cellular metabolism in bacteria, and higher concentrations can be extremely toxic to methanogenic bacteria. Na^+ is the most dangerous ion: it is required for the metabolism of anaerobic microflora in a range from 0.002 to 0.004 M, but above 0.14 M it becomes strongly inhibitory. However, it has been proved feasible to use salt-adapted microorganisms capable of tolerating high salinities (Sialve *et al.*, 2009).

Freshwater microalgal biomass contains low levels of sulfureted amino acids and their digestion releases lower amounts of hydrogen sulfide than other types of substrates (Ward *et al.*, 2014). Sulphur compounds, such as sulfate, can act as electron acceptors for sulfate reducing bacteria that convert organic compounds in an anaerobic reactor, and produce hydrogen sulfide gas. Gaseous H_2S is corrosive and can damage machinery, such as gas engine power generators, and piping. Except for sulfide, sulfur compounds are not harmful to anaerobic bacteria unless at high concentrations. Sulfide is needed for cellular metabolism in low concentrations by bacteria, but concentrations higher than 200 mg/L become extremely

toxic to methanogens (Ward *et al.*, 2014). H_2S is harmful because it diffuses into the cytoplasm through the cell membranes: it may form disulfide cross-links between polypeptide chains, denaturating the proteins. Unionized sulfide is much more toxic than ionized sulfide (S_2^-). The speciation between the two compounds is also dependent of temperature and pH (Ward *et al.*, 2014). Sulfate reducing bacteria (SRB) compete with methanogenic bacteria for acetate and hydrogen. The SRB have a higher affinity for acetate than methanogens, prevailing on them under low acetate concentrations. This competitive inhibition results in the shift from methane generation to sulfate reduction. Sulfate reducers and methanogens are very competitive at COD/SO_4 ratios of 1.7 to 2.7. An increase of this ratio favors methanogens, while a decrease is favorable to SRB. (Ward *et al.*, 2014).

1.12.5 Nutrient availability in AD effluent

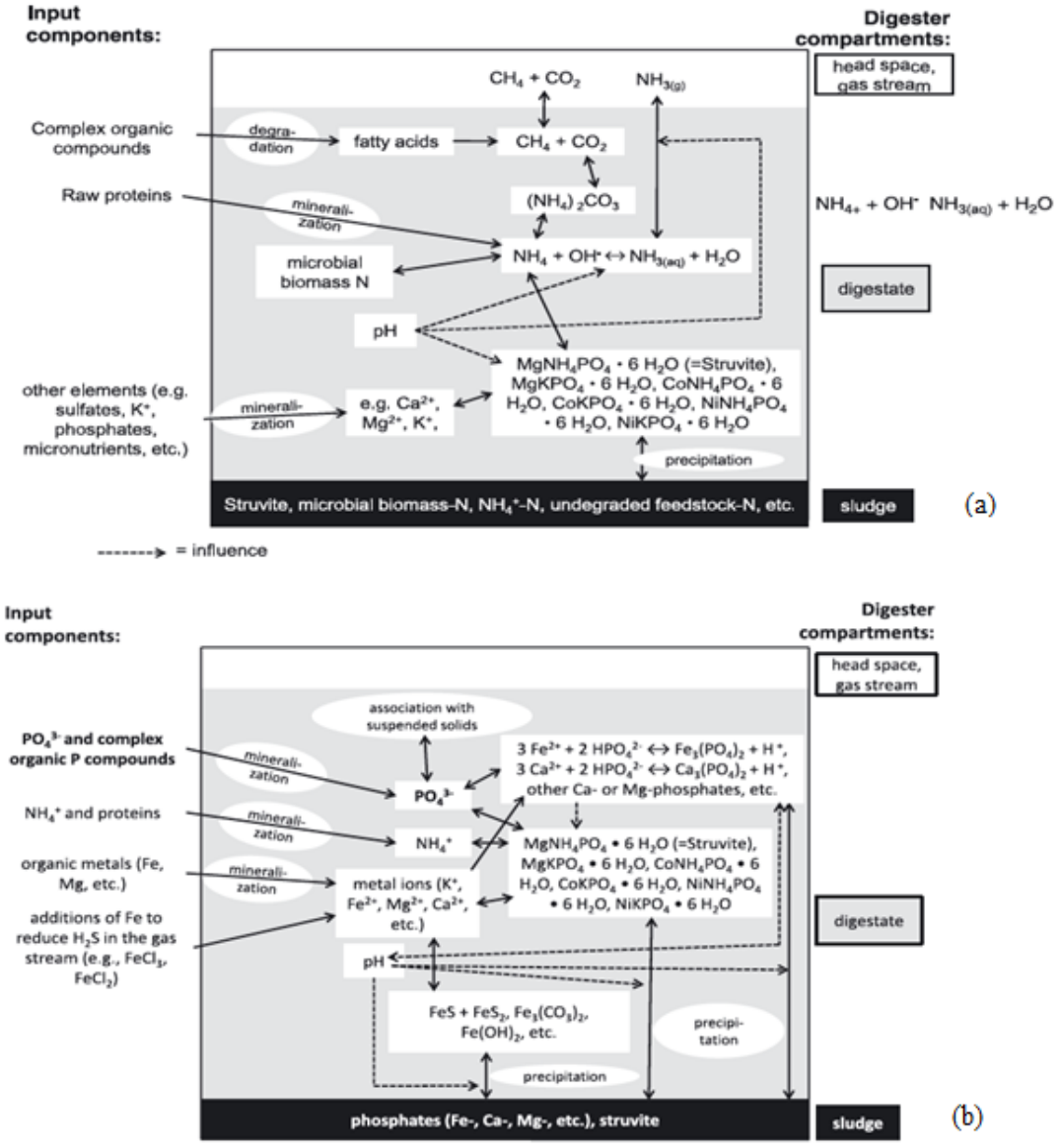

Figure 1.10 *Nitrogen (a) and phosphorus (b) turnover in anaerobic digesters (Möller & Müller, 2012).*

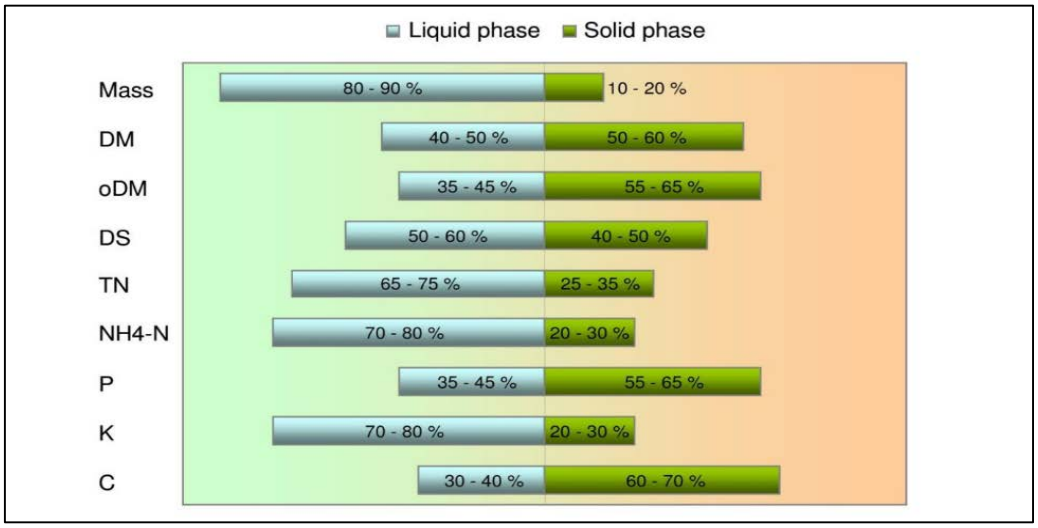

Figure 1.11 *Ranges of distribution of digestate components in the solid and liquid fractions (http://www.bioenergy.org.nz/documents/resource/TG08-the-production-and-use-of-digestate-as-fertiliser.pdf).*

Complex organic N compounds are mineralized to ammonium in the digester. A part of the N-NH_4^+ is used by the digesting microorganisms for growth. Further processes are formation of struvite and ammonium carbonate, while traces are volatilized in the biogas stream as ammonia (<1%) (Fig. 1.10a).

The solubility of P and micronutrients is strongly influenced by pH. Struvite (magnesium ammonium phosphate) is a phosphate mineral with formula $NH_4MgPO_4 \cdot 6H_2O$, and is commonly used as an agricultural fertilizer. Raising the pH moves the chemical equilibrium towards the formation of phosphate ($HPO_4^{2-} \rightarrow PO_4^{3-}$), with the subsequent precipitation as calcium or magnesium phosphate (Fig. 1.10b). Simultaneously, the binding form of other elements (such as Fe) may also be influenced by AD, affecting P turnover and precipitation (Möller & Müller, 2012). The soluble P fraction decreases substantially during AD. Significant losses have been observed for Ca, Mg, and Mn as they partially crystallize out as phosphates and carbonates. Therefore, digestates contain only trace amounts of Ca^{2+}, Mg^{2+}, and inorganic P in solution (Möller & Müller, 2012). Mineralization of N, P, and Mg combined with a substantial increase of the pH can enhance the formation and crystallization of struvite. This process can be used to remove N and P from manures to reduce P and N loadings, but it is something to avoid if the purpose is to use liquid fraction of digestate for nutrient recycling. Many ionic species (e.g. Ca^{2+}, K^+, CO_2^{3-}) can influence struvite formation by reacting with its component ions. Furthermore, the degradation of organic matter forms sulfate (SO_4^{2-}), which, in the absence of O_2, reacts with H^+ to form H_2S and other molecules, increasing the digester pH (Möller & Müller, 2012). This causes a strong decrease of sulfate concentration and an increase of sulfide and C-bonded S concentrations, metal-sulfide precipitation and sulfur volatilization. Protein-rich feedstocks increase H_2S content in biogas, as S is introduced mainly as a constituent of amino acids. However, sulphate is the available

form for microalgae uptake, and the formation of H_2S and sulphides decreases its amount (apart from leading to inhibition). Thus, some methods have been studied to reoxidize sulphide to reutilisable sulfate, as microaeration and sulphur oxidizing bacteria (Pokorna & Zabranska, 2015). Separation of digestates creates two products, a liquid and a solid material. Most of the total P is allocated to the solid phase (Fig. 1.11). As shown in Table 1.8, the liquid phase is characterized by low dry matter and P concentration and high N and K contents. A total of 45–80% of the N in the liquid phase is present as ammonium (Möller & Müller, 2012). Phosphorus loss via precipitation in the solid phase of the digestate represent a major issue concerning nutrient recycling. In order to be able to recover P from the digestate and recycle it back to microalgal cultivation, it is essential to solubilize it as much as possible in the aqueous phase; several strategies for this purpose have been proposed (see section §1.13).

Table 1.8 *Digestate characteristics after solid-liquid separation. (Möller & Müller, 2012). DM= Dry Matter; FM= Fresh Matter; ?=No data found/no data available*

	Liquid fraction of digestates	Solid fraction of digestates
DM (%)	4.5–6.6	19.3–24.7
Organic DM (% DM)		40–86
Total N (% DM)	7.7–9.2	2.2–3.0
Total N (kg Mg^{-1} FM)	4.0–5.1	4.6–6.5
Total NH_4^+ (kg Mg^{-1} FM)	1.8–3.0	2.6–2.7
NH_4^+ share on total N (%)	40–80	26.0–49.4
Total C (% DM)	48.0	39.6–40.0
C:N ratio	3.7–4.8	11.2–19.3
Total P (% DM)	0.4–0.7	1.9
Total P (kg Mg^{-1} FM)	0.7–1.0	2.0–2.5
Total K (% DM)	3.9	3.6
Total K (kg Mg^{-1} FM)	3.5–5.2	3.4–4.8
Total Mg (% DM)	?	0.2–0.4
pH	7.9	8.5

1.12.6 Microalgal growth in digestate

Table 1.9 shows the main characteristics of the liquid digestate derived from wet AD of microalgae. According to the values reported, pH falls into the optimal range for freshwater microalgae (6-8) and alkaliphilic ones (8.5-10). The digestate contains most of the nutrients that are necessary for algal growth: nitrogen, phosphorus (which are mostly in the ammonium and phosphate forms, respectively) and other micronutrients (Xia & Murphy, 2016). Ward *et al.* (2014) reports digestate nutrient values of 2940 mg/l of ammonia-nitrogen, 390 mgl/l of total P and 320 mg/l of potassium. However, high turbidity and ammonia content in the digestate could hinder algal growth (other compounds are present in concentrations which are well below the inhibiting ones). The first can be reduced by removing suspended materials through filtration, sedimentation or centrifugation (Xia & Murphy, 2016). Ammonia nitrogen levels in digestate are usually very high (1000-3000 mg/l), but initial dilution could solve this problem (while reducing turbidity at the same time).

Table 1.9 *Typical characteristics of liquid digestate (adapted from Xia & Murphy, 2016).*

Characteristic	Range	Characteristic	Range
pH	6.7-9.2	Cobalt (Co), mg/l	0.02-0.04
COD, mg/l	210-6900	Copper (Cu), mg/l	0.09-21.4
Total inorganic carbon (TIC), mg/l	939-1353	Iron (Fe), mg/l	0.9-65
Total Nitrogen (TN), mg/l	139-3456	Lead (Pb), mg/l	0.03-2.8
Percentage of ammonia nitrogen (TAN/TN)	65-98%	Magnesium (Mg), mg/l	3-659
Total Phosphorus (TP), mg/l	7-381	Manganese (Mn), mg/l	0.1-17
Percentage of Phosphate (PO_4-P/TP)	82-90%	Molybdenum (Mo), mg/l	<1.8
Aluminum (Al), mg/l	0.1-34	Nickel (Ni), mg/l	<1.4
Boron (B), mg/l	0.9-4	Potassium (K), mg/l	102-2707
Cadmium (Cd), mg/l	<1	Silicon (Si), mg/l	26-72
Calcium (Ca), mg/l	65-1044	Sodium (Na), mg/l	126-709
Chlorine (Cl), mg/l	160-438	Sulphur (S), mg/l	111-115
Chromium (Cr), mg/l	<1.2	Zinc (Zn), mg/l	0.9-13

The optimal N/P mass ratio for microalgal growth is suggested to be around 7 (Xia & Murphy, 2016). In some cases, an insufficient phosphorus supply can be a limiting factor in liquid digestate applied to microalgal cultivation. The growth rate of microalgae almost doubles through the addition of phosphate to liquid digestate (Xia & Murphy, 2016). Similarly, the C/N ratio of microalgae is in the range 4–8, indicating that the carbon sources in digestate may be much lower than required. Both external inorganic sources (e.g. CO_2, bicarbonate) and organic carbon sources (e.g. VFAs, sugars) can be effectively provided to enhance microalgal growth (Xia & Murphy, 2016). Moreover, liquid digestate is contaminated and sterilization prior to cultivation is not economically convenient for mass production. Selection of a strain with resistance or non-susceptibility to biological contamination would be a key issue for large scale cultivation in the digestate (Xia & Murphy, 2016).

Alternatively, control of environmental parameters (e.g., light, temperature) and operational ones (e.g., hydraulic and biomass retention, nutrient supply, pH) is a cost-effective strategy to reduce biological contamination in these microalgal systems. However, biomass productivities and concentrations (dry weight) of microalgae cultivated in liquid digestate are in the ranges of 0.03–0.67 g/l/day and 0.4–4.8 g/l. These values are comparable with or slightly higher than those of photoautotrophic cultivation in synthetic medium (Xia &

Murphy, 2016). Uggetti *et al.* (2014) reported that growth rates of *Scenedesmus sp.* vary in the range 0.04-0.09 day^{-1}, according to initial substrate/inoculum ratio in the culture and ammonia concentration. This study confirms that digestate is an effective substrate for microalgal growth promoting biomass production up to 2.6 gTSS/l. It is suggested that microalgae concentration in the medium, more than the digestate turbidity, is responsible of preventing light diffusion: consequently, this self-shading phenomenon reduces algal growth rate. Another factor affecting the initial growth rate is the initial ammonia concentration. The different NH_4^+ concentrations of digestate applied in this experiment (from 50 to 260 mg/l) resulted in initial NH_3 concentrations ranging from 2 to 34 mg/l. When the initial ammonia concentration was increased from 2 to 9 mg/l, the growth rate decreased, on average, by 18%. Besides, a further rise from 9 to 34 mg/l was responsible for 77% reduction of the growth rate. Although ammonia is an excellent source of nitrogen for microalgal growth, free ammonia is toxic to most strains of microalgae due to its uncoupling effect on photosynthetic processes in isolated chloroplasts. However, in order to control ammonia inhibition, its content may be reduced by diluting digester effluents (Xia & Murphy, 2016).

1.13 Phosphorus solubilisation techniques in the AD effluent

The soluble phosphorus concentrations in the liquid fraction of digestate usually are too low for economically convenient recovery (Mehta & Batstone, 2013). Strategies to improve P solubilisation and recovery during/after the AD include addition of chemicals, lowering the pH during AD, digestate acidification, modifying the operating conditions (Mehta & Batstone, 2013). One of the most studied strategies for phosphorus extraction, either from digestate or from sewage sludge ash), is acid treatment. In the experiment carried out by Mehta & Batstone (2013), less than 5-10% of the total P, Ca and Mg was in soluble form in the digestate, in which, instead, most of the N and K remained soluble. A bioavailability test (citric acid extraction) showed that P, Ca and Mg in the digestate were totally available. Solubilisation of P, Ca and Mg started below a threshold of pH 5.5, and reached a plateau at pH=4.5 and no significant release of nutrients occurred with further acid addition. It indicates that these nutrients were released from organic matter during digestion, and then wereprecipitated after bonding with Ca and Mg (to form insoluble inorganic compounds) or adsorbed on solid surfaces in the digestate. These effects reduce the feasibility of post-digestion recovery of the nutrients. Zhang *et al.* (2010) showed how the more acid was added, more phosphorus (as well as more Ca^{2+}) was mobilized into the liquid phase as a result of pH decrease (from 7.8 to 3.8) and consequential phosphates dissolution. In other experiments, where phosphorus had to be leached from incinerated sewage sludge ash (ISSA), up to 91% of the original P was recovered (depending on extraction time, acid load/concentration, acid type, liquid-to-solid ratio) (Gea *et al.*, 2014, Donatello *et al.*, 2010). Another approach to this

issue is the use of additives capable of sequestering metallic ions, which precipitate as phosphates. An example is represented by chelating agents such as EDTA, which could be used alone or in combination with acidification. EDTA has the ability to form complexes with metallic ions such as Ca^{2+} and Mg^{2+} that would instead precipitate as phosphates, leading to soluble phosphate ion release in the aqueous phase:

$$Ca_3(PO_4)_2 + 3[EDTA]^{4-}_{(aq)} \leftrightarrow 3[EDTA - Ca]^{2-}_{(aq)} + 2PO_4^{3-} \quad (1.9)$$

Zhang et al (2010) managed to release 91% of the P in their digestate by adding EDTA up to 70 mmol/l concentration. Unfortunately, EDTA is expensive and cannot be used in large scale applications (Latif *et al.*, 2015).

Some operating and reactor design solutions have been proposed to enhance P solubilisation. An example is represented by anaerobic membrane bioreactors (AMBR). They have short hydraulic retention times but maintain high solids retention time to produce more biomass. As a consequence, the solid retained in the reactor can eventually be hydrolyzed to release nutrients to the solution over the residence time period. Dissolved nutrients can be continuously removed from the membrane permeate through appropriate nutrient recovery techniques: this continuous nutrient removal would reduce in-reactor precipitation and improve P recovery up to 99% (Mehta & Batstone, 2013). Another possible solution is the application of a two-stage AD process (one with hydrolysis/acidification and one with methanogenesis). It has been shown that the acidic conditions in the first stage favor solubilisation and mobilization of nutrients from the solid biomass to the leachate, making them more accessible for removal. Latif *et al.* (2015) proposed instead to perform the whole digestion process at acidic pH (<5.7). This led to a significant increase in phosphorus release compared to neutral pH (up to 3.6 times), related to the dissolution of Ca-P and Mg-P compounds under acidic conditions. Unfortunately, at the same time, a relevant decrease in methane yield was observed (-33%), mainly due to a reduced hydrolysis of particulate organic matter. As additives and acidification could somehow hinder one or more biochemical phases in AD by reducing microbial activity, it seems to be more effective to act directly on the effluent.

1.14 Aim of the thesis

The aim of this work is to evaluate the possibility to quantitatively recycle nutrients in the cultivation of a microalgal species (*Chlorella vulgaris*). To this purpose, this species will be cultivated in the liquid effluent obtained from the anaerobic digestion of the lipid-extracted biomass of the same species in order to reduce external nitrogen and phosphorus supplies, and possibly close nutrients balance through their recycling from the spent biomass. Moreover, biochemical methane potential test of the lipid-extracted biomass will be also performed in

order to evaluate the biogas production (to close also the energy balance of the whole process). Based on experimental results, the economic feasibility of an industrial production of biofuels derived from microalgae (whose cost is still too high and not competitive with fossil fuels), as well as their environmental sustainability will be evaluated. Growth rate, final biomass concentration and nutrient consumption of cultures in digestate will be compared with control cultivation in synthetic medium. The possibility to perform treatments to the whole digestate, in order to increase the availability of soluble phosphorus in its liquid fraction will be assessed. The reduction of nutrient requirement for microalgal culture through anaerobic digestion will also be evaluated in a process simulation perspective, by using Aspen Plus®.

Chapter 2
Experimental materials and methods

In this chapter the materials and methods necessary to set up the experimental work are described. First, some details concerning algal cultivation, such as the species of microalgae, the growth systems and the medium, are listed. Then, the analytical techniques to characterize algal growth and nutrient consumption are briefly explained. Furthermore, the setup of a laboratory scale anaerobic digestion system and the methods and calculations needed to monitor biogas production are reported. Eventually, a short note about the procedures to extract phosphorus from the solid phase of the digestion effluent into in the aqueous one is provided.

2.1 Algal species and growth systems

The microalgal species which was used to study the growth in digestate is *Chlorella vulgaris*, a small, spherical algae whose size is 5-10 µm. This species is more resistant and less susceptible to a non axenic medium (such as digestate) than other ones; it is also characterized by high growth rates (Mata *et al.*, 2013). For preinocula and batch growth curves, 250 ml Drechsel glass bottles were used, and approximately 1 l/h of CO_2-enriched air (5% v/v) was supplied to each culture (Fig.2.1). The CO_2 % in air and the air flowrate were regulated by two flow meters.

Figure 2.1 *A) Drechsel bottle B) Bottles connected to the feed of CO_2-enriched air.*

The gases were filtered (microfilter with 0.5 m of porosity) and bubbled through the microalgal suspensions. To avoid sedimentation, every culture was continuously mixed by a stirring magnet, placed at the bottom of the reactor. Light was supplied by neon fluorescent

lamps, placed horizontally in front of the cultivation bottles. Light intensity was measured through a photoradiometer (Delta OHM HD 2102.1), which quantifies the PAR (photosynthetically active radiation, 400-700 nm). The bottles are placed in a thermostated incubator that guarantees the optimal temperature for the chosen algal strain (27÷28°C). Each batch experiment started with an initial microalgae inoculation of OD_{750} = 0.2, which corresponds to a cell concentration of about $2*10^6$ cells/mL.

2.2 Cultivation medium for control

For all control experiments, BG11 medium was used. Through NH_4Cl addition, BG11 was modified so that N was supplied in the form of ammonium NH_4^+, in order to simulate the composition of the digestate, keeping an equivalent concentration of 247 mg/L N. BG11 composition is reported in Table 2.1. The culture medium and all the materials were sterilized in an autoclave at 121 °C for 20 min in order to prevent any contamination.

Table 2.1 *Composition of modified BG11.*

Component	Concentration	Unit of measure
Na_2MG EDTA	1.00	mg/l
Ferric ammonium citrate	6.00	mg/l
Citric acid·H_2O	6.00 ·	mg/l
$CaCl_2$·$2H_2O$	36.0	mg/l
$MgSO_4$ ·$7H_2O$	75.0	mg/l
K_2HPO_4	30.5	mg/l
H_3BO_3	2.86	mg/l
$MnCl_2$ ·$4H_2O$	1.81	mg/l
$ZnSO_4$·$7H_2O$	0.222	mg/l
$CuSO_4$·$5H_2O$	0.079	mg/l
$COCl_2$ · $6H_2O$	0.050	mg/l
$NaMoO_4$ ·$2H_2O$	0.391	mg/l
Na_2CO_3	20.0	mg/l
Hepes 1M pH 8	$1.00 \cdot 10^{-2}$	mmol/l
NH_4Cl	0.943	g/l

2.3 Algal growth monitoring

Algal growth was monitored daily, by measuring the optical density (OD) at 750 nm with a UV–visible UV 500 double beam spectrophotometer (from Spectronic Unicam, UK). In addition, cell concentration was monitored using a Bürker Counting Chamber (HBG, Germany). These two methods are explained in detail later. Specific growth rate constants of batch experiments were calculated as the slope of the linear regression of the logarithm of

cellular concentration, during the exponential phase of growth. pH was measured daily with a pH-meter (HI 9124, HANNA Instruments) and was always kept in the optimal range 7.3÷7.7 by adding a NaOH solution.

2.3.1 OD

For *C. vulgaris*, the optical density (OD) of the culture, also called absorbance, is directly proportional to the concentration of microalgal cells in the culture. The measurement is performed at a fixed wavelength of 750 nm. This value, in fact, is out of the range of absorbance of pigments (chlorophyll and carotenoids) by the cells, so light attenuation is due to scattering only: suspended cells cause a deflection of light, which is directly proportional to cell concentration. The linear relationship between absorbance and cell concentration is valid in a range of absorbance between 0.1 and 1; therefore, if the sample is too concentrated, it must be properly diluted to fit this range. Before the measurement, it is necessary to prepare two cuvettes with the culture medium as blanks, to set the zero (to remove the contribution of the medium itself to the total OD). Then, one of them is used as a reference to the sample during the OD measurement.

2.3.2 Cell count

Cell concentration can be directly measured at the optical microscope thanks to Bürker chamber. This is composed of a support containing 2 cells with a depth of 0.1 mm each, above which the slide is fixed. The cells are divided into 9 squares with 1 mm sides, which are separated by a triple line (Fig.2.2) .

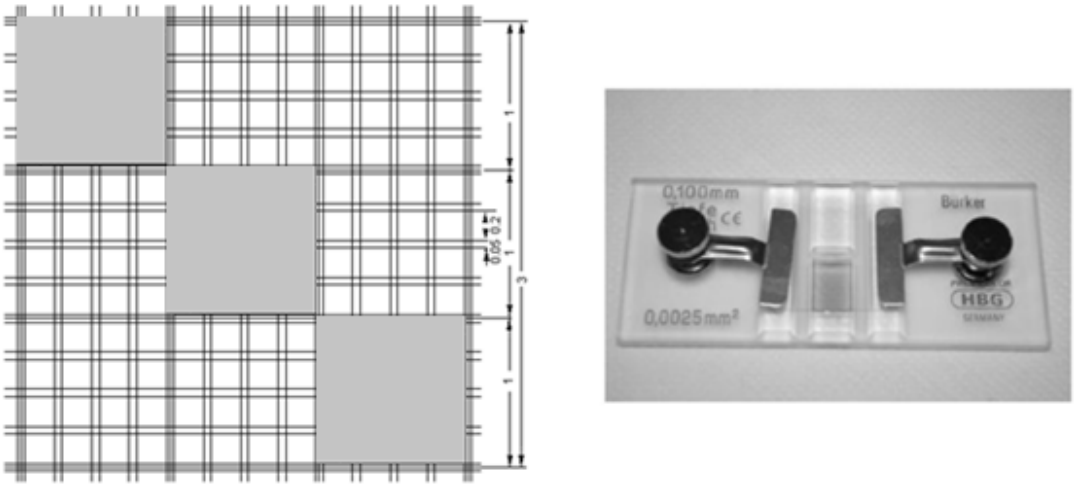

Figure 2.2 *Schematic representation (on the left) and photo (on the right) of a Bürker counting chamber.*

Each of these bigger squares (representing a volume of 0.1 μl) is divided into 16 smaller squares, with a surface of 1/25 mm^2, delimited by a double line. The following procedure is adopted:

- the sample is properly diluted, in order to have 20÷100 cells per bigger square;
- the aqueous cell suspension is taken up with the pipette and injected into one of the two cells of the chamber, in order to fill it completely;
- the slide and its support are inserted under the microscope; the cells in the 3 squares in diagonal position (the ones that are colored in Fig.2.2) are counted, considering the most external line on the top and on the left as borders;
- cell concentration (*N*) is then calculated (in cells/ml) the following way:

$$N = n_{mean} * f_d * 10^4 \quad (2.1)$$

where n_{mean} is the average number of cells counted in the diagonal squares, f_d is the dilution factor and 10^4 is due to the fact that each bigger square has a volume equal to 0.1 μl.

2.3.3 Dry weight

The dry weight (DW) of microalgal biomass represents the weight concentration of dry biomass expressed in g/L. First, a known volume of culture ($V_{culture}$) is withdrawn, which depends on the cell concentration. To separate the bulk water from the biomass, nitrocellulose filters with pore size of 0.2 μm are used. These filters are first dried up in oven for 10 min at 107°C to eliminate the absorbed humidity, then they are weighed on the microscale (Atilon Acculab Sartorius Group) to measure the tare (*DWt*). The phase separation takes place by suction of the liquid volume of culture through the filter, performed by a vacuum flask. The filter, on which wet biomass is deposited, is kept in the oven for 2 h at 107°C to eliminate microalgal intracellular water. Then, the gross weight (*DWg*) of the filter is measured. The dry weight of the sample is then calculated in the following way:

$$DW = \frac{DWg - DWt}{V_{culture}} \quad (2.2)$$

2.4 Nutrient consumption

N, P and S concentrations in the culture medium were measured using standard test kits, at initial and final points for batch experiments in the case of N and P, and throughout the whole growth process in the case of S. A sample of culture was filtered with a syringe and a 0.2 nm syringe-filter to measure the dissolved nutrients only.

2.4.1 Ammonia-nitrogen

Ammonia nitrogen (N-NH_4^+) was measured with HYDROCHECK SPECTRATEST (Reasol), by colorimetric reaction with Nessler reagent (potassium tetraiodomercurate) in alkaline conditions:

$$2K_2(HgI_4) + NH_3 + KOH \rightarrow (Hg_2ONH_2)I + 7KI + 2H_2O \qquad (2.3)$$

Ammonium ions are first converted to ammonia by KOH. This reaction leads to a yellow/orange color of the sample. Absorbance measurement is then performed at 445 nm, with a reacting volume of 5 ml, after 5 min at least from the beginning of the reaction. The calibration line (Fig. 2.3) was determined by measuring standard solutions at known concentration of ammonia (supplied as NH_4Cl). Prior to reaction, the samples have to be diluted to reach a NH_3 concentration in the range covered by this calibration line.
The ammonia concentration in mg/l is obtained from the following equation:

$$[NH_3] = 9.8089 * Abs_{445} - 0.2607 \qquad (2.4)$$

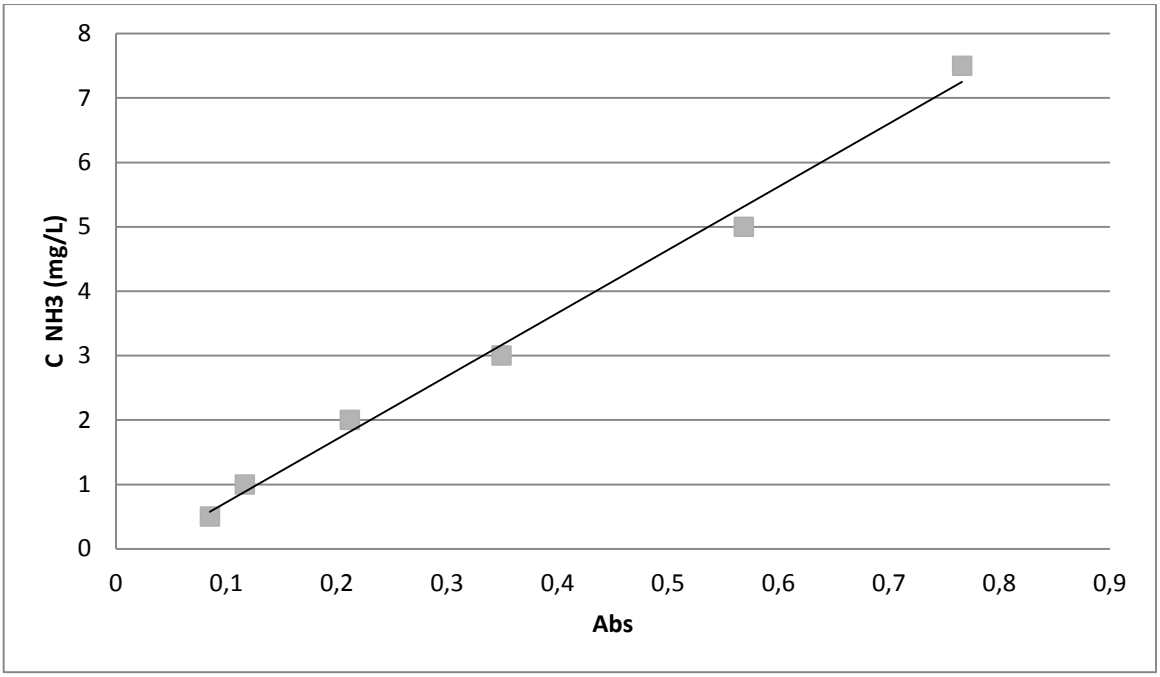

Figure 2.3 *Calibration line for ammonia measurement (with a regression coefficient R^2 = 0.9932).*

2.4.2 Orthophosphates

Orthophosphate phosphorus (PO_4–P) is measured with the molybdate/ascorbic acid method. The reaction mechanisms involves the formation of a dying complex formed by orthophosphate ion, ammonium molybdate and potassium antimonyl tartratein acid

environment. This complex is then reduced by the ascorbic acid, leading to a blue color of the sample, whose intensity is proportional to phosphate concentration. The reactant has a limited stability (3-4 h), so it has to be prepared before each analysis, with the composition reported in Table 2.2.

Table 2.2 *Composition of the reagent for orthophosphate measurement.*

Component	**Concentration in the reactant**
Sulphuric acid	2.5 N
Potassium antimonyl tartrate	0.136 g/l
Ascorbic acid	10.8 g/l
Ammonium molybdate	6 g/l

The final reaction volume is 2.5 ml, and 0.250 ml of reactant are added to each sample. Reaction time is 10 min and the absorbance measurement is performed at a wavelength equal to λ=705 nm. Orthophosphate quantification is achieved through a calibration line, which was built by measuring standard solutions at known concentration of $H_2PO_4^-$ (Fig. 2.4).

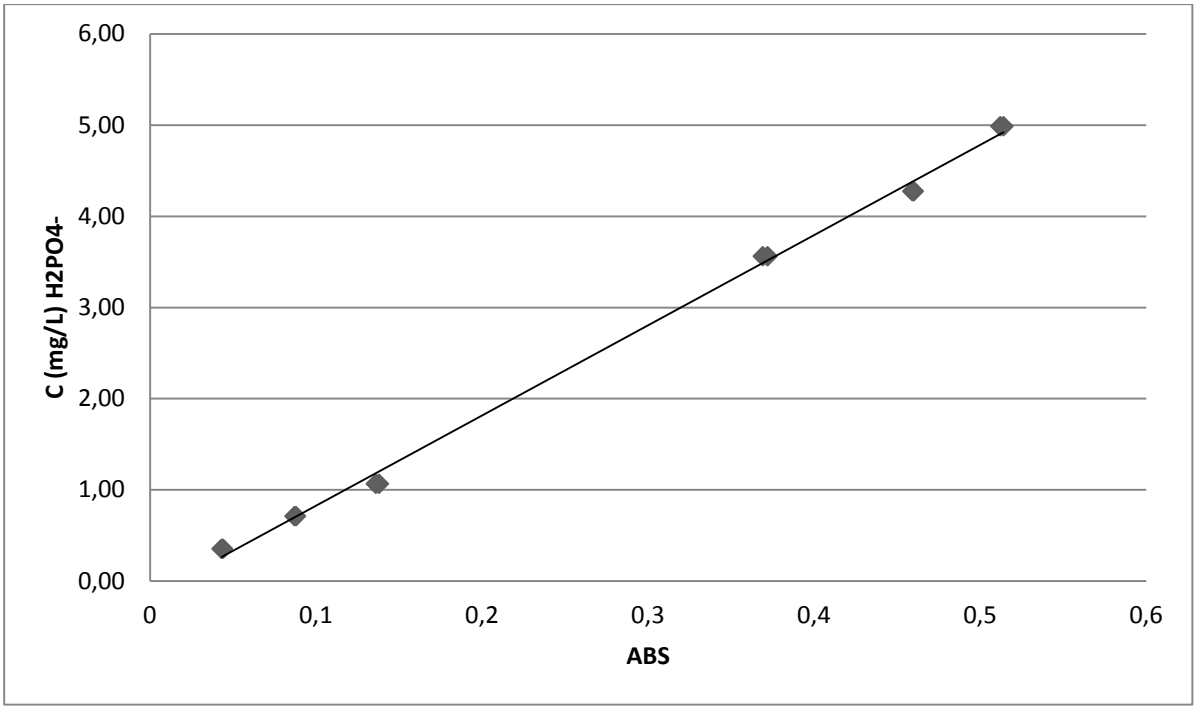

Figure 2.4 *Calibration line for orthophosphate measurement (with a regression coefficient R^2 = 0.9428).*

The orthophosphate concentration, expressed in mg/L of $H_2PO_4^-$, is obtained from the following equation:

$$[H_2PO_4^-] = 9.7916 * Abs_{705} - \ 0.3555 \qquad (2.5).$$

2.4.3 Sulphates

This method utilizes the quantitative formation of insoluble barium sulfate in polyethylene glycol. The absorbance measured at 600 nm is proportional to sulfate level in the sample. The reacting mixture is composed of two reactants; a powder ("Reagent B") has to be dissolved into a liquid ("Reagent A"), with a concentration of 95 mg/ml. This mixture has to be prepared fresh each time and used within 1 hour; 100 µl of this solution is needed for each sample, and the absorbance in the spectrophotometer can be measured 5 minutes after the beginning of the reaction.

The sulphate concentration, expressed in mg/l of SO_4^{2-}, is calculated from equation 2.6, and the calibration line (obtained with standard solutions at known concentrations of SO_4^{2-}) is shown in Fig. 2.5:

$$SO_4^{2-} = 157.28 * Abs_{600} + \ 4.715 \qquad (2.6)$$

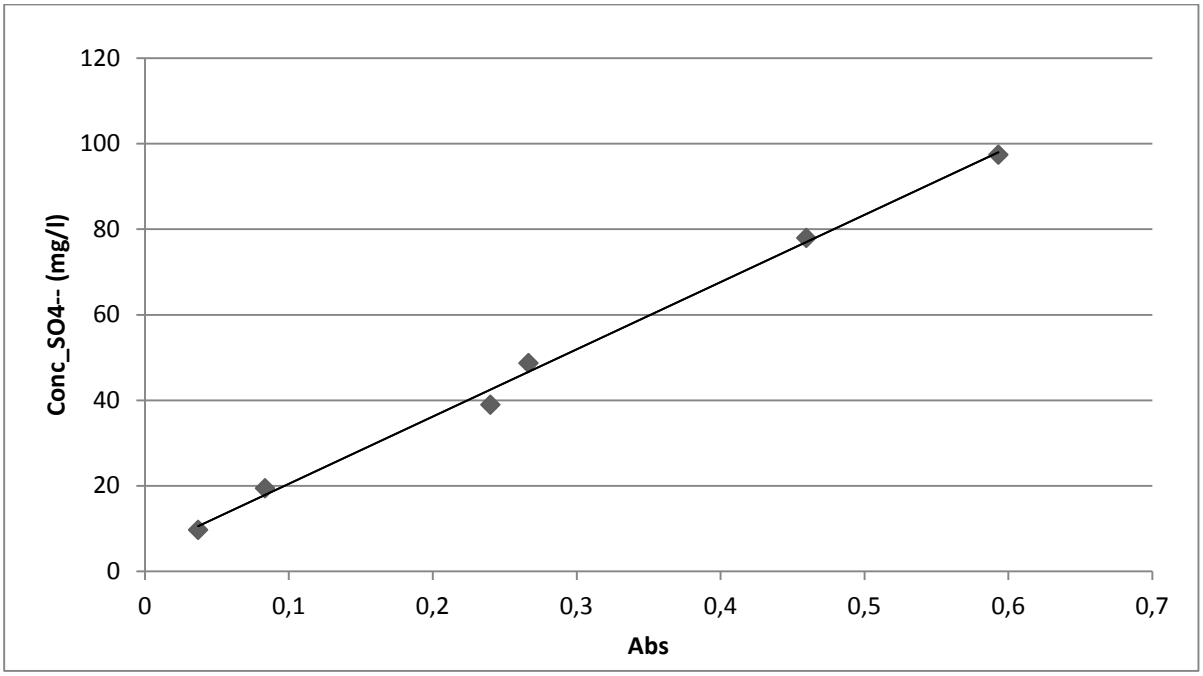

Figure 2.5 *Calibration line for sulphate measurement(with a regression coefficient R^2 = 0.9963).*

2.5 Lipid extraction

Pre-concentrated and pre-dried *Chlorella vulgaris* algal biomass (provided by NEOALGAE™) was used for lipid extraction. A sample of about 4 g of biomass was

prepared by using mortar and pestle to disrupt cell walls, then the material was kept in an oven at T≈103°C for 2 h to eliminate the residual humidity. The dry powder was placed on a paper thimble which was then located into a Soxhlet apparatus, typically used for solvent extraction. The Soxhlet extractor shown in Fig. 2.6 is characterized by three parts: a lower chamber with the solvent, an upper chamber with the solid material and a condenser with cold water as a coolant. The lower chamber is heated to evaporate the solvent that passes to the upper chamber through an external tube called “distillation arm”. The condenser is composed of two concentric tubes, where cold water flows externally to condense the solvent that drips down to the upper chamber. Inside this chamber there is the thimble containing the solid material. The thimble acts as a filter so that the solute (lipids) can pass through it but the residual biomass does not. The upper chamber is then filled with solvent that soaks the material and extracts the solute. From the bottom, a little tube, the “siphon side arm”, connects the upper chamber with the lower one. Therefore the solvent fills the upper chamber until the pressure inside the siphon arm goes down, and swallows up the solvent (plus the extract) back again to the lower chamber. The process goes on until all the solute is extracted.
The solvent used for extraction was a mixture of ethanol and hexane in a 2.5:1 volumetric ratio. The operation was carried out overnight at 110°C. Then, a rotary evaporator was used to remove the solvent from the extract. This apparatus, represented in Fig. 2.6, is characterized by two chambers (the evaporation flask and the collecting flask), a condenser and a water bath.

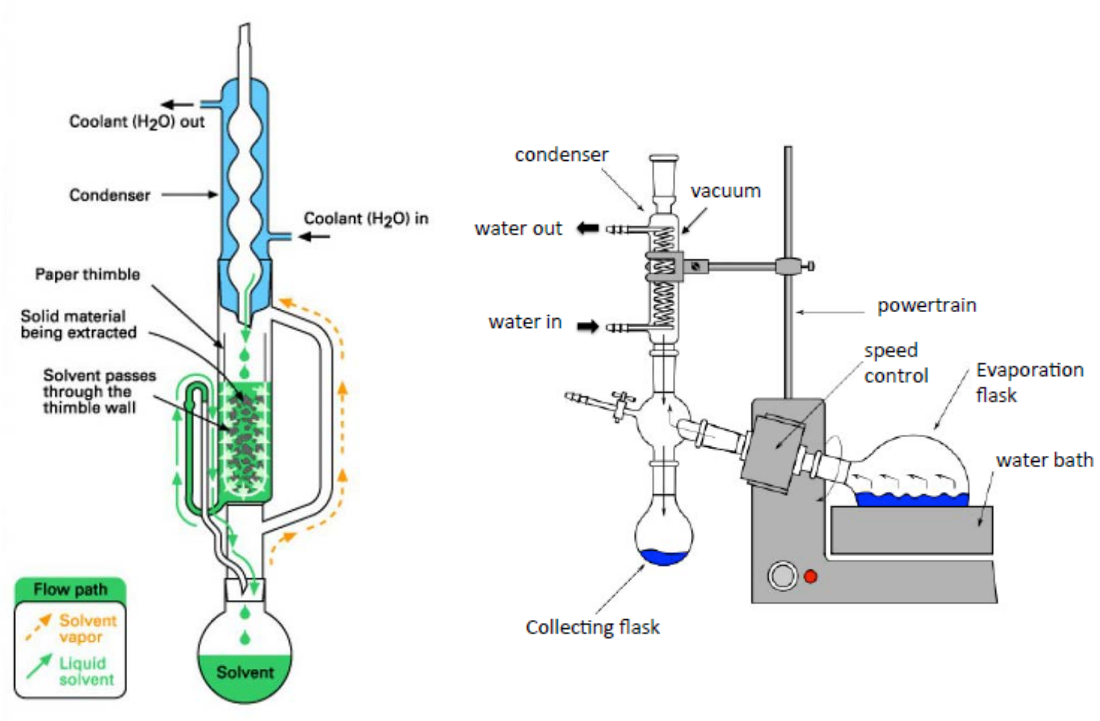

Figure 2.6 *Schematic representation of a Soxhlet apparatus (on the left) and of a rotary evaporator (on the right).*

The evaporating flask, with the mixture that has to be separated, is placed in a water bath and rotates with a velocity set manually by the speed controller. The water bath is heated so that the solvent can evaporate leaving the solute in the flask. The gas goes then inside the condenser, where cold water flows inside a serpentine, and returns to the liquid state, dripping inside the collecting flask. A vacuum pump is used to lower the pressure inside the evaporation system. The water bath was kept at 45°C. The process lasts until all the solvent was evaporated, so that only the algal lipids remained in the evaporation flask. At this point, the oil can be measured gravimetrically to calculate the extraction yield as the ratio of the amount of oil over the initial mass of microalgae (weighed before the experiment).

2.6 Biochemical methane potential (BMP) tests

Lipid-extracted algal biomass (LEA) was used as the substrate for anaerobic digestion for two purposes:

- BMP (Biochemical Methane Potential) calculation, in order to evaluate biogas and methane production from residual biomass;
- recovery of the liquid fraction of the digestate as culture medium.

Biogas production experiments were carried out using, as inoculum, the sludge collected from an anaerobic digester of sewage sludge from a municipal wastewater treatment plant (Ca'Nordio) located in Padova, Italy. Both biomass and anaerobic sludge were previously analyzed to evaluate their content in Total Solids (TS) and Volatile Solids (VS). For the former, the sample was left in the oven at 105°C for 24 h: humidity is evaporated, so that the remainder material represents the amount of total solids. Then, the same sample is inserted in a muffle furnace at 550°C for 4 h and what remains represents the NVS (non-volatile solids). The weight difference between TS and NVS is equal to the VS. The procedure is summarized in Fig. 2.7. The biomass/inoculum ratio (or F/M, food to microorganisms) used for BMP tests was 0.5 (gVS microalgae)/(gVS sludge) (Ramos-Tercero *et al.*, 2014). To respect this condition, 6 bottles with a volume of 500 ml were filled with 250 ml of sludge and the corresponding amount of algal biomass calculated using TS/VS analysis results (head space volume V_{head}= 300 ml). In addition, blank tests with the inoculum alone were prepared, in double, to measure the quantity of biogas produced only by the activated sludge itself. The experiments were planned as shown in Table 2.3. Once closed, the bottles were flushed with N_2, replacing the air in the head space, in order to guarantee anaerobic conditions. They were then inserted in a thermostatic bath at 35±1°C.

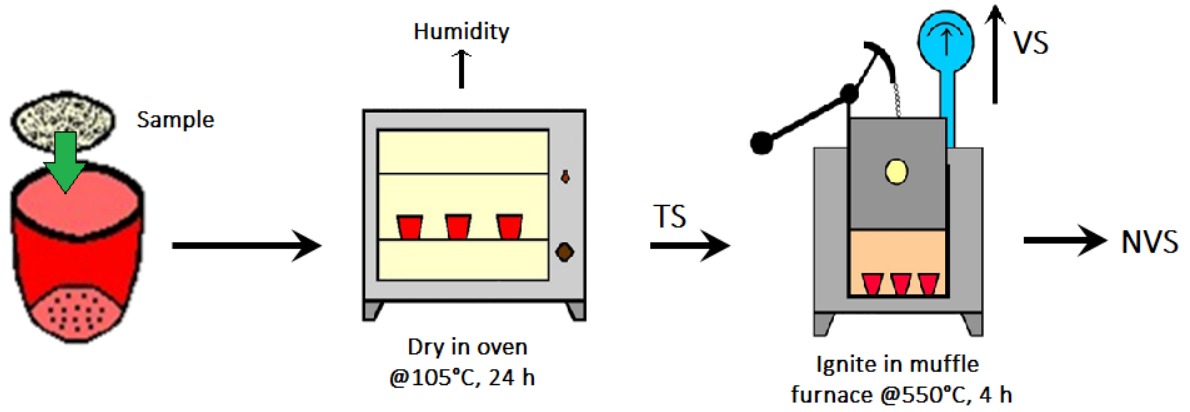

Figure 2.7 *Schematic representation of the procedure for volatile solids (VS) and total solids (TS) measurement.*

Table 2.3 *Experimental plan for anaerobic digestion phase*

Bottle #	Purpose	Digestate post-treatments
2 X	Blank tests (inoculum only)	*None*
1 X	Analysis of solid and liquid products	*None*
2 X	Subsequent growth in digestate	
1 X	Analysis of solid and liquid products	*Yes, to increase phosphorus solubilisation*
2 X	Subsequent growth in digestate	

The experiment covered a period of 40 days, during which the following variables were measured:

- the volume of biogas produced, through water displacement method;
- CO_2 and methane concentrations in the gas remaining in the head space of the bottles through a portable composition analyzer, or landfill gas analyzer (LFG20-ADC Gas Analysis Ltd).

According to the water displacement method (shown in Fig. 2.8) the excessive pressure in the reactor due to biogas production moves an equal quantity of liquid to a second bottle. The volume of the liquid moved, and, accordingly, the volume of biogas produced, is measured in a graduated cylinder. The liquid used in measurements was an acidified (pH<3) and saline (NaCl 25 %) solution in order to avoid the dissolution of methane and carbon dioxide into the liquid.

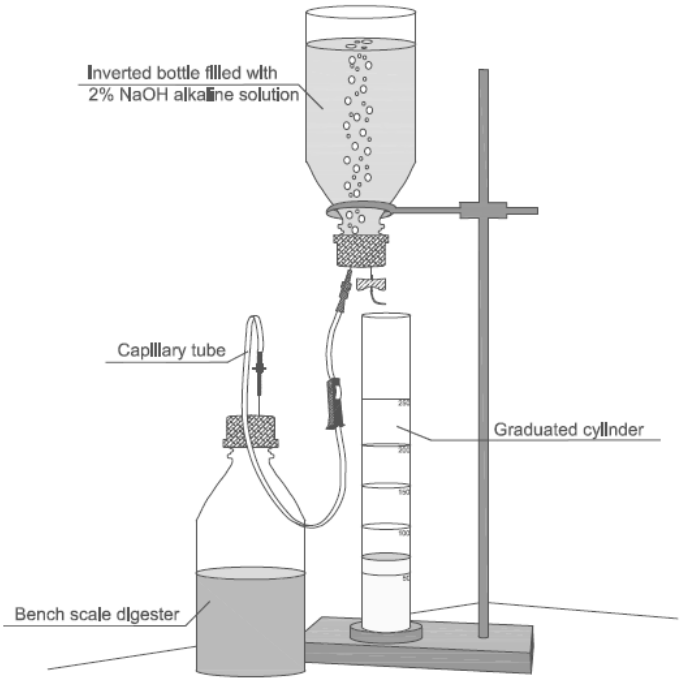

Figure 2.8 *Schematic representation of water displacement method for biogas volume measurement.*

The gas analyzer is based on an infrared technology to measure CO_2 and CH_4 concentration and an electrochemical sensor for O_2 concentration. The LFG analyzer was calibrated with air before every measurement.

It must be taken into account that CO_2 and CH_4 mix themselves both in the head space volume and in the biogas that has already been produced. Hence, to have the net gas production between times t-1 and t, the amount of gas measured at the t-1 has to be subtracted. In fact, during the progress of the experiment, the head space doesn't contain N_2 only, but also an increasing amounts of CO_2 and CH_4. Thus, the following formulas have been used in the calculations:

$$V_{CO_2}^{(1)} = \left(V_{biogas}^{(1)} + V_{head}^{(1)}\right) * \frac{\%\, CO_2^{(1)}}{100} \tag{2.7}$$

$$V_{CH_4}^{(1)} = \left(V_{biogas}^{(1)} + V_{head}^{(1)}\right) * \frac{\%\, CH_4^{(1)}}{100} \tag{2.8}$$

for the 1st measurement, while:

$$V_{CO_2}^{(t)} = \left(V_{biogas}^{(t)} + V_{head}\right) * \frac{\%\, CO_2^{(t)}}{100} - V_{head} * \frac{\%\, CO_2^{(t-1)}}{100} \tag{2.9}$$

$$V_{CH_4}^{(t)} = \left(V_{biogas}^{(t)} + V_{head}\right) * \frac{\%\, CH_4^{(t)}}{100} - V_{head} * \frac{\%\, CH_4^{(t-1)}}{100} \tag{2.10}$$

for all the other ones. All the separate quantities were summed up to calculate the total cumulative amount of CO_2 and CH_4 produced. Then, the following variables have been calculated to obtain the final result:

1. the net CH_4/CO_2 production, by subtracting the gas amount produced in the blank bottles to eliminate the contribution of the sludge itself:

$$V_{i,net}^{(t)} = V_i^{(t)} - V_{i,blank}^{(t)} \tag{2.11}$$

2. the normal CH_4/CO_2 production, to refer to normal conditions (0°C, 1atm):

$$V_{i,norm}^{(t)} = V_{i,net}^{(t)} * \frac{273{,}15\ °C}{308{,}15°C} = V_{i,net}^{(t)} * 0.886 \tag{2.12}$$

3. the specific CH_4/CO_2 production, to calculate the BMP in terms of gas produced per gVS of microalgal biomass consumed:

$$V_{i,norm,spec}^{(t)} = \frac{V_{i,norm}^{(t)}}{gVS\ biomass} = \frac{V_{i,norm}^{(t)}}{g\ biomass * \left(\frac{\%VS}{100}\right)} \tag{2.13}$$

The BMP curves obtained in this way were then averaged between all 6 bottles to obtain the final BMP diagram. The methane fraction in the produced biogas was calculated as well. At the end of the digestion process, the content of the 6 bottles was split, according to Table 2.3:

- half was immediately centrifuged to separate the liquid phase to be analyzed and recovered as culture medium. This aqueous phase was sent to Chelab S.r.l. for a composition analysis, in order to compare the amount of nutrients with standard BG11 medium and assess some possible macro- or micro-nutrient lacks;
- the other half was pretreated in different ways in attempt to dissolve more phosphorus in the liquid phase.

2.7 Digestate treatments

2.7.1 Acidification and EDTA addition

The phosphorus extraction process was carried out by treating the raw digestate, before separating the liquid from the solid phase by centrifugation, with different concentrations of sulphuric acid (H_2SO_4) to reach different pH values. Then, the mixture was left in a magnetically stirred beaker for 2 h at room temperature, and it was filtered with qualitative filtering paper disks to eliminate the solid particulate. The filtration process was carried out with the help of a flask connected to a vacuum pump. The filtered liquid was treated with NaOH to neutralize the acid effect. After alkaline treatment, the liquid was centrifuged at

7200 rpm for 3-4 min to eliminate the new solid precipitate produced when raising the liquid pH up to 7-7.5. The orthophosphate content in this final liquid was determined spectrophotometrically, and the extracted phosphorus was expressed in terms of the ratio between the P solubilized in the aqueous phase and the total amount of phosphorus in the raw digestate.

As a second attempt to solubilize the highest amount of P possible, the treatment was repeated, but in this case EDTA (ethylendiaminetetraacetic acid) was added after filtration of the solid particulate. EDTA is able to sequester metallic ions that would instead precipitate as insoluble phosphates. It was added in the form of $EDTANa_2Mg$, in a range of concentrations between 200 and 3000 mg/l EDTA. The mixture was left to react for 15 minutes, then treated with NaOH for neutralization, centrifuged and measured in its phosphate content.

2.7.2 Treatment with $NaHCO_3$

An alternative procedure for phosphorus solubilisation was to mix the raw digestate with different concentrations of $NaHCO_3$ (0.05-0.5 M). Then NaOH was added to reach a pH value of 8.5. This technique is similar to the "Olsen method", which is normally used for phosphorus extraction in soils. Bicarbonate ions (HCO_3^-) reduce the activity of metallic ions in solution such as calcium Ca^{2+} and aluminum Al^{3+}, thus increasing phosphate solubility. The mixture was left in a magnetically stirred beaker (≈500 rpm) for 1.5 h at room temperature. After the treatment with bicarbonate, the mixture was filtered with qualitative filtering disks by aspiration into a vacuum flask to eliminate the solid particulate. So, the phosphate content in the filtered liquid was measured spectrophotometrically (with the molybdate/ascorbic acid method).

Chapter 3
Experimental results

In this chapter the results of the experimental part of the thesis are reported. Experiments of anaerobic digestion of lipid extracted microalgae were carried out, both to characterize the biogas production and to obtain a liquid digestate which can be tested as a source of nutrients with the final aim to recycle the nutrients of the spent biomass. A control growth curve of *Chlorella vulgaris* is carried out in a modified BG11 medium, by substituting the N source with the aim to simulate the digestate composition. Dry weight is measured daily to estimate the mass-based maximum specific growth rate for *C. vulgaris*, a parameter needed for process simulation. Cultivations are performed in different media, obtained by supplying various sets of nutrients to the raw diluted digestate, and the growth results are compared. Several methods of phosphorus solubilisation are discussed, and the most effective is applied to treat the digestate prior to microalgal cultivation.

3.1 Anaerobic digestion

A first part of experimental work was carried out to assess the biogas production using algal biomass after lipid extraction. The chemical composition of algae and digestate was also characterized.

3.1.1 Lipid content in C. vulgaris

The lipid content in *C. vulgaris* had been previously evaluated by an external lab (Conycal S.L., Oviedo) through Soxhlet method, and it resulted to be 6.3 % by weight. As inhibition during anaerobic digestion is caused by chlorinated solvent residuals (Ramos Tercero *et al.*,2014), the mixture ethanol-hexane (2.5:1 v/v) was used, although this solvent is less efficient in lipid extraction. In fact, the average lipid content in whole microalgae (measured in our laboratory) resulted to be 4.18±0.62 % by weight.

3.1.2 Analysis of lipid-extracted biomass and anaerobic sludge and calculation of the amounts for BMP test

Both lipid extracted biomass and anaerobic sludge from Ca' Nordio plant were analyzed with respect to their total solids (TS) and volatile solids (VS) content. The results shown in Table 3.1 allowed the calculation of the amount of biomass inoculated in each bottle for biochemical methane potential (BMP) test.

Table 3.1 *Total solids (TS) and Volatile Solids (VS) content in anaerobic sludge and lipid extracted biomass.*

Anaerobic sludge		**Biomass**	
gTS/l	5.52 ± 0.040	***%TS on raw sample***	95.6 ± 0.1
gVS/l	11.3 ± 0.13	***%VS on raw sample***	89.0 ± 0.1
VS/TS (%)	48.6±0.5	***VS/TS (%)***	93.1 ± 0.1

A single AD bottle contains 250 ml of anaerobic sludge, or, if expressed in terms of mass of volatile solids:

$$5.519 \frac{\text{gVS}}{\text{l}} * 0.250\ \text{l} = 1.38\ \text{gVS sludge} \tag{3.1}$$

The optimum substrate/inoculum ratio is 0.5 (Kwietniewska & Tys, 2014). Thus, the amount of volatile solids of biomass to insert in every bottle is:

$$1.38\ gVS\ sludge * \frac{0.5\ gVSbiomass}{gVS\ sludge} = 0.69\ gVS\ biomass \tag{3.2}$$

The biomass resulted to have 89% VS on the raw sample (biomass and humidity); so, the amount of microalgal extracted powder to insert in each bottle is:

$$\frac{0.69\ gVS\ biomass}{0.89\ \frac{gVS\ biomass}{g\ biomass}} = 0.7752\ g\ biomass \tag{3.3}$$

3.1.3 Biochemical methane potential results

The results of BMP tests are summarized in the Tab. 3.2 and Fig.3.1.

Table 3.2 *BMP tests results.*

Biogas production after 40.7 days	347.3 ± 37.6	Nml/gSV
Methane production after 40.7 days (BMP)	150.2 ± 14.6	Nml/gSV
Final % CH_4 in biogas	43.3	%

It can be observed that a steep rise in biogas and methane cumulative production was present approximately in the first 10 days of anaerobic digestion. After this time, methane substantially reached a plateau, while a certain amount of biogas (mostly CO_2) was still produced. Considering $C_{0.2964}H_{0.4836}O_{0.1723}N_{0.0445}P_{0.0032}$ the empirical formula of the lipid spent biomass, as estimated from the elemental analysis of spent biomass by CHELAB S.r.l., the theoretical methane yield can be calculated:

$$CH_4\ yield\ = B_0 = \frac{1}{8}\left(\frac{4c+h-2o-3n}{12c+h+16o+14n}\right) * V_m = 444.2\ Nml\ \frac{CH_4}{gVS} \tag{3.4}$$

The biodegradability (BD_m) can be calculated from the ratio of the actual BMP on the theoretical methane potential (B_0):

$$BD_m = \frac{BMP}{B_0} * 100 = \frac{150.2}{444.2} * 100 = 34\% \quad (3.5)$$

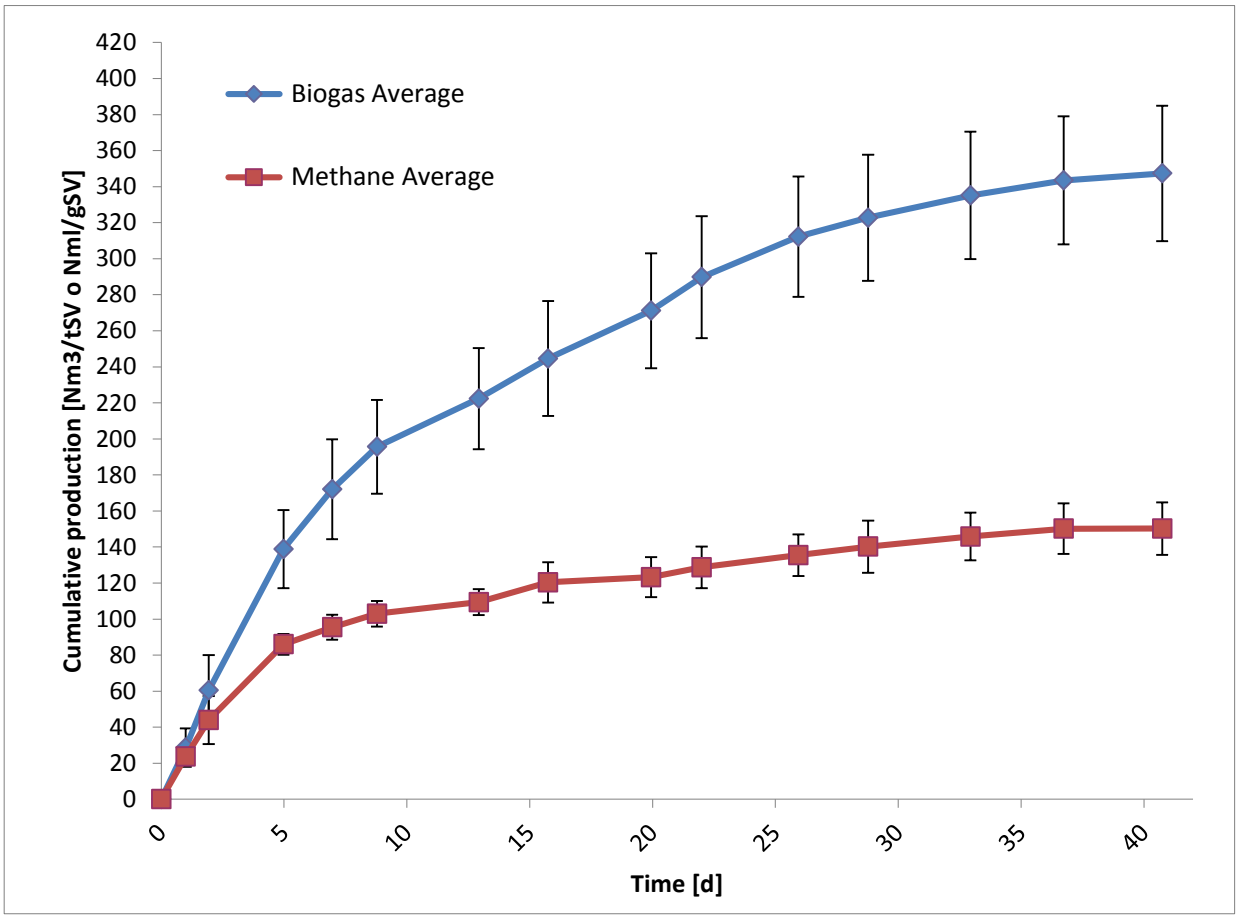

Figure 3.1 *Cumulative production curves for biogas and methane*

This value is lower than the ones reported in the literature (Zhao *et al.*, 2014), which range from 50 to 60% for lipid extracted *C. vulgaris*. This is due to the definition of biodegradability based on a ratio of methane production. If the experimental concentration of methane in the biogas is lower than expected for any reason, the ratio in Eq.3.5 will underestimate the biodegradability of the biomass. To verify this point, it is possible to calculate the theoretical volumetric ratio CH_4/CO_2 (R_G) based on the average carbon oxidation state *m* in the substrate, as explained in section §1.12.1:

$$m = \frac{-h + 2o + 3n}{c} = -0.0183 \quad (3.6)$$

$$R_G = \frac{4 - m}{4 + m} = 1.009 \quad (3.7)$$

The final experimental volumetric ratio CH_4/CO_2 in the biogas reaches a value of ≈0.76÷0.78, which is lower than 1.009. This demonstrates why methane fraction in the biogas is lower than expected, so biomass degradability is actually higher than 34%. To achieve a more realistic value, biodegradability should be defined as the ratio of the experimental and theoretical biogas yield, considering both methane and carbon dioxide:

$$BD_{biogas} = \frac{Experimental\ biogas\ production}{Theoretical\ biogas\ production} * 100 = \frac{EBP}{TBP} * 100 \quad (3.8)$$

The following calculation can be done for the denominator term the element composition of extracted biomass is known, and it contains 47.3% C on dry weight. 1 g of biomass (total solids) contains 0.473 g of C, which are equal to 0.04 mol of carbon. As both CH_4 and CO_2 contain one single atom of C, 0.04 mol of C correspond to 0.04 mol of biogas, based on the assumption that only two compounds are present. Ideally, every gas has a molar volume of 22.4 l/mol in normal conditions (0°C, 1 atm) and, considering a ratio VS/TS equal to 0.931, it results:

$$TBP = 0.04\ \frac{mol\ C}{gTS} * 0.931 \frac{gVS}{gTS} * 22.4 \frac{Nl}{mol} = 0.834\ Nl \frac{biogas}{gVS} \quad (3.9)\ ,$$

and therefore:

$$BD_{biogas} = \frac{347}{834} * 100 \approx 42\% \quad (3.10).$$

This value of biodegradability is higher than the previous one, but still lower than the ones from the literature. In order to enhance the degradation of the algal walls and improve biogas and methane yield, some pretreatments (ultrasound, microwaves) should be applied to the extracted biomass prior to perform the anaerobic digestion process.

3.2 Microalgal cultivation

The liquid fraction of the anaerobic digestate was then used to assess the microalgal growth on this substrate. The growth curves, measured in the digestate were compared to a control curve in standard growth media.

3.2.1 Control curves

In the anaerobic digestate, nitrogen is present in the almost exclusive form of ammonium. For this reason, the control curve was carried out by cultivating *Chlorella vulgaris* in a modified BG11 medium, to reproduce this N source of the digestate. Accordingly, N was supplied as ammonium chloride (NH_4Cl), instead of nitrate, which is the typical N form supplied in the culture media, but at the same concentration of BG11 (247 mg/l N). All other nutrients were

provided in the same amount and form as standard BG11. Culture conditions for control are listed in Table 3.3.

Table 3.3 *Culture conditions of the control*

Light intensity	*120 umol/(m^2s)*
Temperature	*27°C*
Volume	*100 mL (Drechsel bottle)*
CO_2	*5% v/v in air*

Growth curves were performed in triplicate, under axenic conditions and continuous irradiation. The pH of the culture was measured once a day and kept in the range 7.2-7.6 through NaOH addition. The control curve is shown in Fig. 3.2.

Growth rate constantachieved in these conditions was 2.040 ± 0.087 d^{-1}. The final biomass concentration reached by the culture was 2.147 ± 0.070 g/l. Absolute and relative nutrient consumptions and yields are summarized in Table 3.4. N_{fin}, N_{in}, P_{fin}, P_{in} represent the final and initial concentrations of nitrogen and phosphorus in the culture medium, expressed in mg/l. C_x represents the final biomass concentration, expressed in mg/l to make the nutrient-on-biomass yields ($Y_{N/x}$, $Y_{P/x}$) dimensionless. Y_N and Y_P represent the percent consumption of N and P respect to the initial amount. It is possible to observe that, in this case, all the phosphorus present in the medium was consumed.

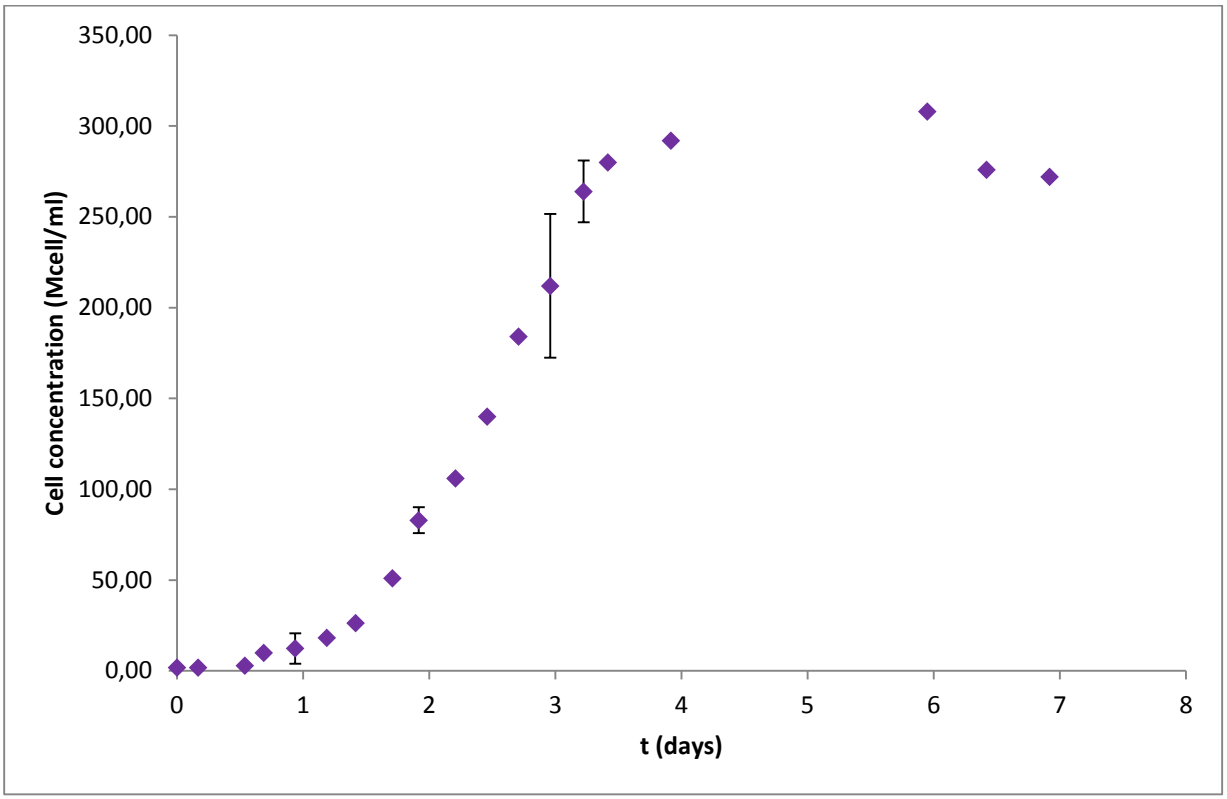

Figure 3.2 *Control growth curve of Chlorella vulgaris in modified BG11.*

Table 3.4 *Nutrient consumption in control cultivation.*

Medium	*Modified BG11 (control)*
$\boldsymbol{C_x}$ **(g/l)**	2.147 ± 0.070
$\boldsymbol{\Delta N\ (mg/l)\ =\ N_{fin} - N_{in}}$	126.1±29.1
$\boldsymbol{\Delta P\ (mg/l)\ =\ P_{fin} - P_{in}}$	4.95± 0.04
$\boldsymbol{Y_N = 100 * (N_{fin} - N_{in})/N_{in}}$	57%
$\boldsymbol{Y_P = 100 * (P_{fin} - P_{in})/P_{in}}$	100%
$\boldsymbol{Y_{N/x} = (N_{fin} - N_{in})/C_x}$	0.0619±0.0045
$\boldsymbol{Y_{P/x} = (P_{fin} - P_{in})/C_x}$	0.00231±0.00005

The sulphate (SO_4^{2-}) consumption was also analyzed throughout the whole growth process, in order to evaluate sulfur depletion (Fig. 3.3).

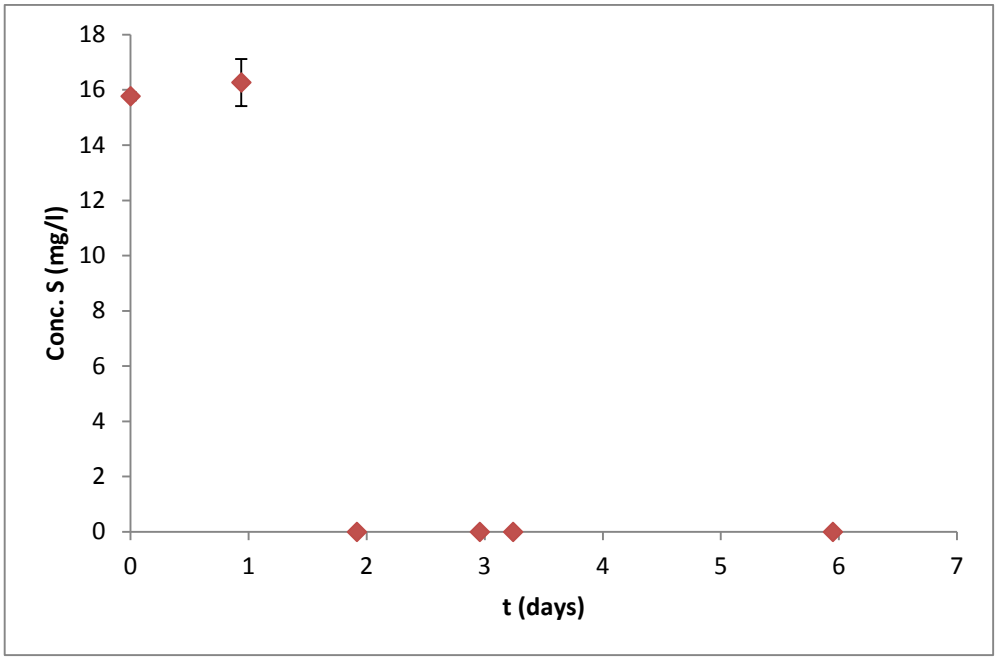

Figure 3.3 *Sulphur concentration during control culture growth.*

It can be noticed that S concentration remained constant in the first day of growth, then it was completely consumed after the second day. By considering that as an initial accumulation, sulphur is supposed to represent a limiting substrate for microalgal cultivation. To estimate the mass-based μ_{max}, the dry weight was measured for the control growth curve every day instead of cell concentration (Fig. 3.4). Mass-based growth rate μ_m was calculated as the slope

of the curve representing the natural logarithm of dry weight vs time. It resulted to be equal to 1.495 d^{-1}, which is lower than the cell-based one, 2.04 d^{-1}, but still comparable to the literature (Concas *et al.*, 2012). Thus, as no relevant nutrient/light limitation occurred in this process, a slightly higher value was assumed for the maximum specific growth rate μ_{max} (μ_{max} =1.55 d^{-1}), as estimated by Concas et al. (2012).

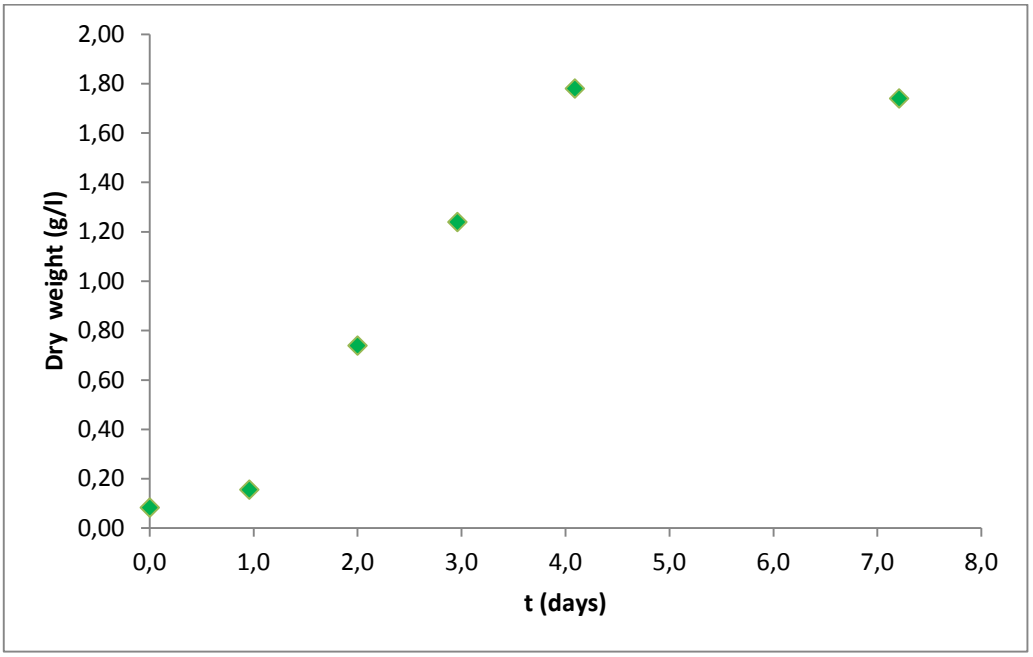

Figure 3.4 *Biomass dry weight during C. vulgaris cultivation.*

3.2.2 Growth curves in digestate with different nutrient additions

The effluent from anaerobic digestion was centrifuged in order to separate the liquid fraction from the solid one, to be recovered as a nutrient source in culture media. Nutrients content of liquid digestate was first analyzed by performing an element analysis, in order to investigate some possible lacks of macro- and micronutrients respect to BG11.

Table 3.5 shows that all micronutrients and ammonium are present in the diluted liquid digestate with a higher concentration respect to standard BG11, while P and S are almost absent and have to be added.

Ammonium-nitrogen concentration in the digestate resulted to be 524 mg/l $N - NH_4^+$: to perform growth experiments in digestate a dilution was then made so to obtain the same N concentration of the control (247 mg/l N as in BG11).

Table 3.5 *Comparison of nutrients between BG11 and raw liquid digestate*

Element	***Raw liquid digestate***	***BG11***	***Δ(BG11-rld)***
N-NH_4^+ (g/l)	524	247	-277
P- PO_4^{3-}(g/l)	0.88	5.4	4.52
S (g/l)	0	0.009798	0.009798
Ca (g/l)	0.177	0.0098	-0.1672
Co(g/l)	<0.001	0.000012	-
Fe (g/l)	0.0061	0.001279	-0.004821
Mg (g/l)	0.051	0.0131	-0.0379
Mn (g/l)	<0.0025	0.0005	-
Mo (g/l)	<0.001	$1.7 \cdot 10^{-7}$	-
K (g/l)	0.083	0.0152	-0.0678
Cu (g/l)	<0.001	0.000002	-
Zn (g/l)	<0.001	0.000011	-

Three cases can be distinguished, in which three different cultivation media were used:

1) no additional nutrient was supplied to the cultivation medium;

2) phosphate was added to reach BG11 P concentration (5.4 mg/l), considering that phosphorus content in the aqueous phase of the digestate was found to be 0.88 mg/l;

3) also sulphate was added to favor the growth process. Digestate contained approximately no S as it mostly ended up in gaseous H_2S. Thus, it was entirely added to match S concentration of BG11.

Growth curves in all media were performed at exactly the same conditions as control.
The growth curve measured in the raw diluted digestate, with no nutrient addition, is shown in Fig. 3.5. It can be noticed that *C. vulgaris* grew scarcely (reaching a cell concentration of $13x10^6$ cells/ml) because of substantial lack of nutrients (P and S in particular). Also the growth rate was far lower than in control cultivation (μ=1.03 d^{-1}). Fig. 3.6 shows the cell concentration vs time in the case where only phosphorus was added. It can be observed that phosphate addition improved cellular growth, but cells multiplication stopped at about 70 million cells/ml. In this case, growth rate resulted to be 1.98 d^{-1}, which is similar to the control. Considering a possible sulphate limitation, a growth curve with S addition was eventually carried out. In this case, microalgal growth was comparable to the control, as all nutrients were provided in the correct amount, reaching a final concentration of ≈ $340x10^6$ cells/ml, as shown in Fig. 3.7. Also cells grew at a comparable rate as control (μ=2.08 d^{-1}). This substantial similarity between the two cases and control confirmed that all micronutrients

were present in a sufficient amount to guarantee algal growth in the aqueous phase of the digestate, which only lacked in P and S macronutrients.

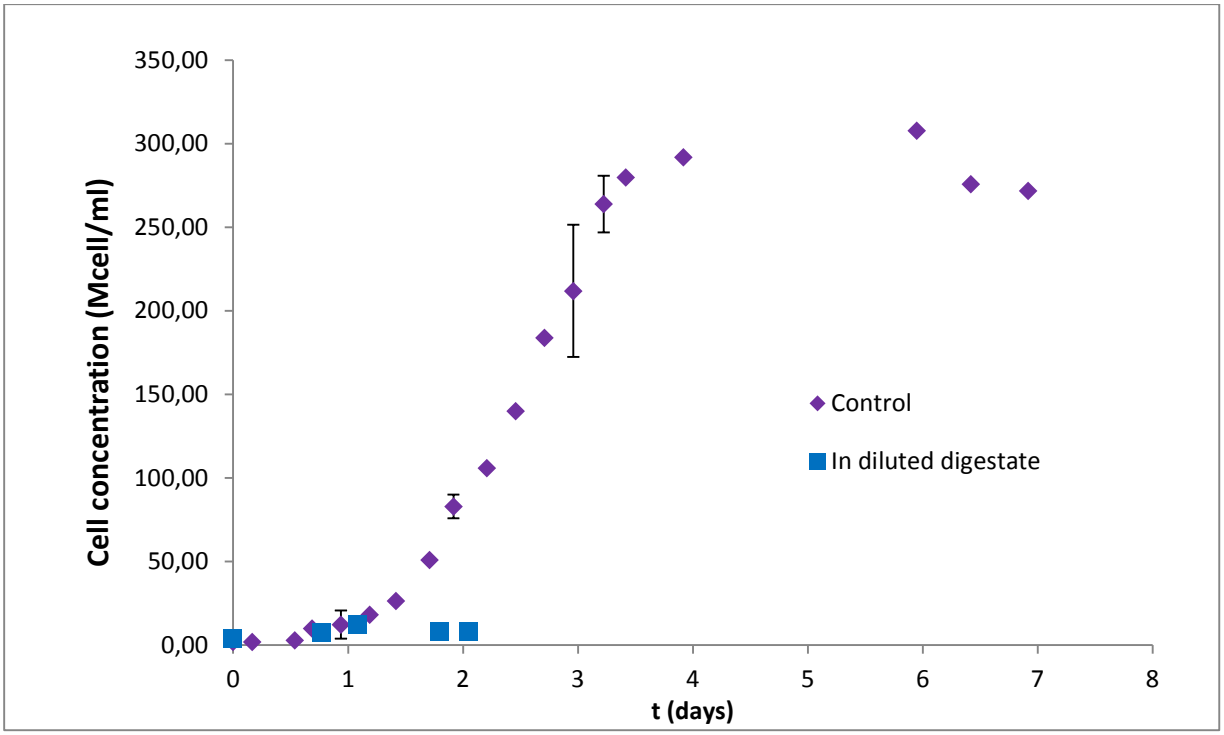

Figure 3.5 *Growth curve in raw untreated digestate with no further nutrient added, compared with control curve.*

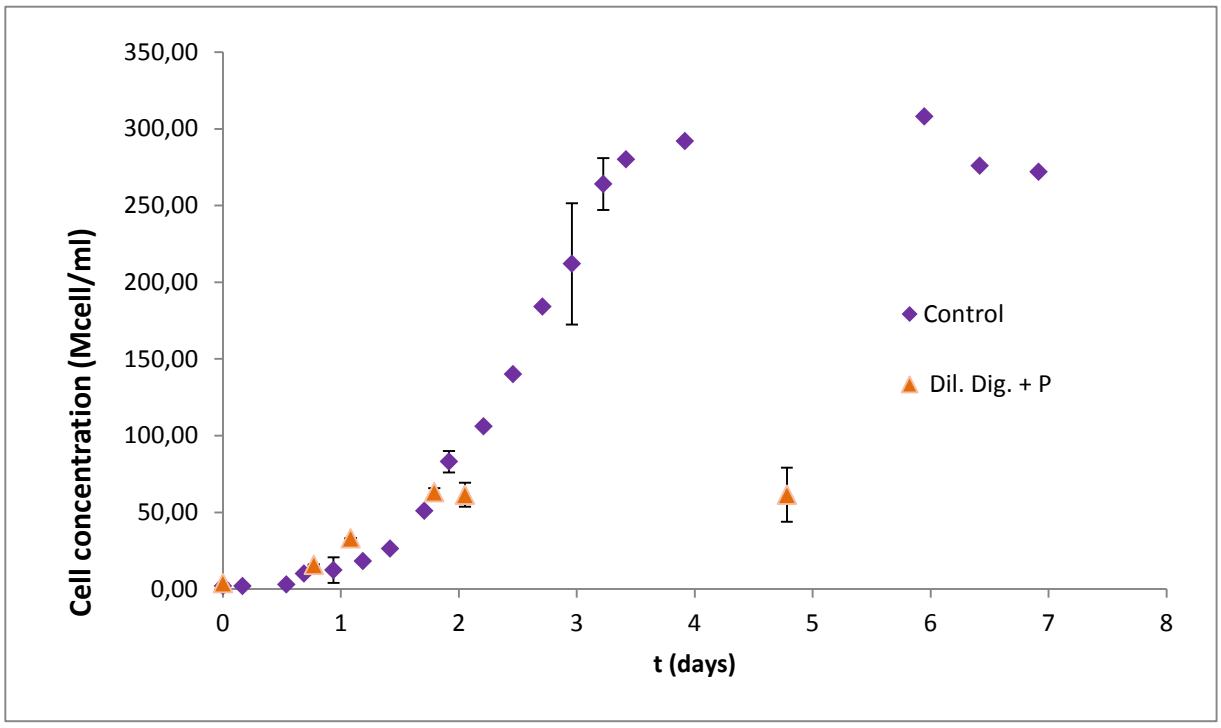

Figure 3.6 *Growth curve in raw untreated digestate with the addition of the correct amount of phosphorus, compared with control curve.*

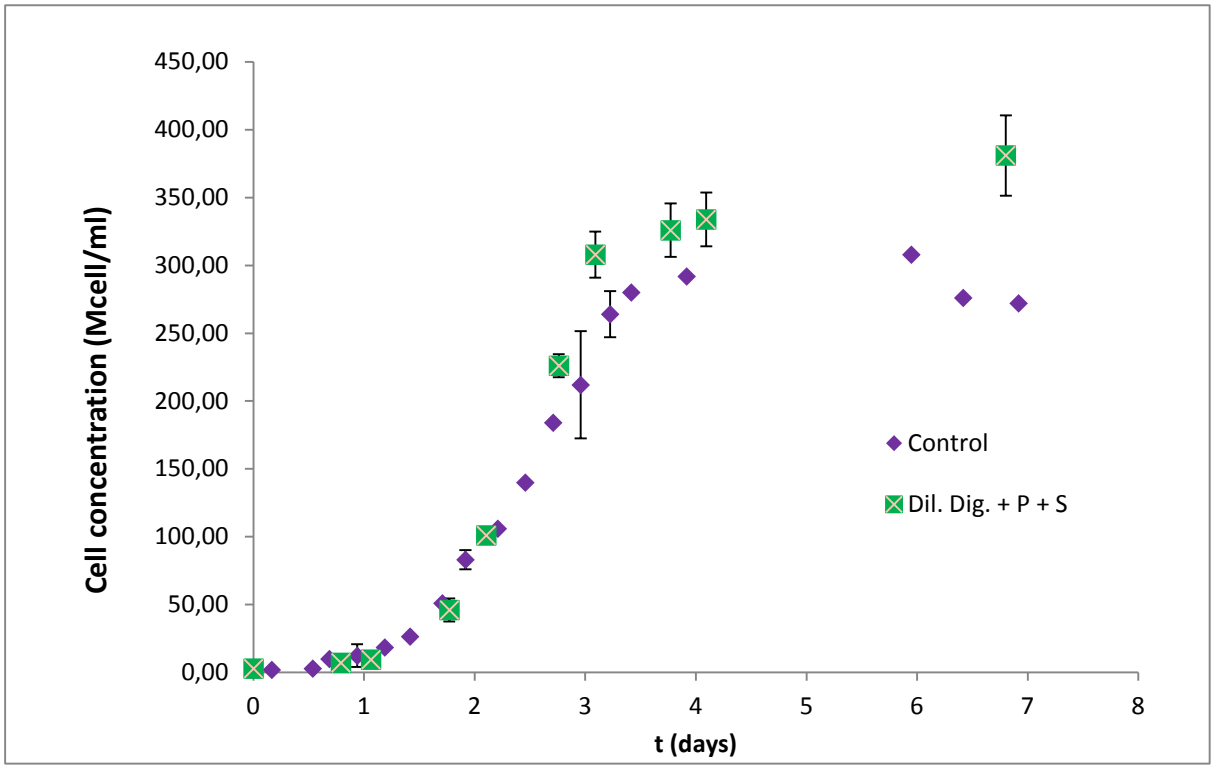

Figure 3.7 *Growth curve in raw untreated digestate with the addition of the correct amount of phosphorus and sulphur, compared with control curve*

Table 3.6 summarizes growth rates in all considered culture media. It can be observed that in the case of diluted digestate with no addition, growth rate was significantly lower than the one in modified BG11, while in the other cases it was comparable to the control.
Thus, growth rate is probably mostly influenced by the amount of phosphorus available in the culture medium.

Table 3.6 *Growth rates in several media*

Medium	Growth rate (d^{-1})
Modified BG 11 (control)	2.0401
Untreated diluted digestate	1.0271
Untreated diluted digestate + P	1.9806
Untreated diluted digestate + P + S	2.0758

Final biomass concentrations, nutrient consumptions and yields in all the media are reported in Table 3.7.

Table 3.7 *Final biomass concentrations, nutrient consumptions and yields in digestates with different levels of nutrient addition.*

Case		***1***	***2***	***3***
Medium	*Control*	*Raw diluted digestate*	*Raw diluted digestate +P*	*Raw diluted digestate +P+S*
C_x **(g/l)**	2.15 ± 0.07	0.09±0.01	0.88± 0.28	1.99 ± 0.16
$\Delta N\ (mg/l) = N_{fin} - N_{in}$	126.1±29.1	6.07 ±2.43	46.89±13.76	105.73±4.45
$\Delta P\ (mg/l) = P_{fin} - P_{in}$	4.95± 0.04	0.184±0.043	2.62±0.30	3.90 ± 0.18
$Y_N = 100 * (N_{fin} - N_{in})/N_{in}$	57%	3%	22%	49%
$Y_P = 100 * (P_{fin} - P_{in})/P_{in}$	100%	28%	43%	88%
$Y_{N/x} = (N_{fin} - N_{in})/C_x$	0.0619±0.0045	0.0662±0.0166	0.0535±0.0016	0.0532±0.0019
$Y_{P/x} = (P_{fin} - P_{in})/C_x$	0.0023±0.0001	0.0020±0.0002	0.0032±0.0014	0.0022±0.00009

It is noteworthy that:

- in case 1, almost no biomass is produced, N and P consumption is drastically lower than in the control, due mostly to P and S limitation;
- in case 2, biomass production is more abundant than in case 2; N and P consumption is lower than in control but significantly higher than in case 1, as only S is limiting;
- in case 3, biomass production and nutrient consumption are higher than both previous cases (slightly lower than control but comparable).

However, nutrient consumption is directly linked to biomass production, thus their ratios (nutrient/ biomass yields) are comparable in all cases. In particular, phosphorus yield was the highest in case 2, where P was supplied abundantly while S was not, maybe because of an hypothetical *luxury uptake* by the microalgae.

3.3 Phosphorus solubilisation

The utilization of digestate as nutrient source for microalgal growth gave rise to some unsolved issues, one of them is the substantial lack of fundamental macronutrients such as phosphorus and sulphur.

Sulphur is mostly lost as H_2S in the biogas during the anaerobic digestion operation, so little can be done to recover it in terms of chemical treatment of the digestate. In literature, some other solutions are reported to oxidize sulfide to sulphate during AD operation. For example, microaeration can be performed in the anaerobic digester, or the AD unit can be integrated

with an external bioreactor with a culture of sulfur-oxidizing bacteria (SOB). SOB can be present in an alkaline suspension or immobilized on various carriers to act as a biofilter (Pokorna & Zabranska, 2015).

Concerning phosphorus, most of it is precipitate in the solid phase of the digestate due to the formation of insoluble phosphate salts, such as $Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$, $Fe_3(PO_4)_2$. Thus, some attempts to solubilize it in the aqueous phase and make it available as a nutrient in a cultivation medium have been made: digestate acidification, EDTA addition and bicarbonate treatment.

The results of these experiments are expressed in % of solubilized phosphorus with respect to the P in the orthophosphate form, which was present in the digestion bottles. P concentration in BMP bottles can be calculated, as the P content in both sludge and extracted biomass are known. Anaerobic Ca'Nordio sludge is characterized by a concentration of 16.98 mgP/gSV, while the lipid-spent biomass has a P content of 13.3 mgP/g(dry biomass), as the dry biomass is 95.6% of the total weight. It can be thus calculated that 1 L of mixture sludge+biomass contains approximately 133.2 mg of P.

3.3.1 Acidification of the digestate

The raw digestate was mixed with a given amount of sulfuric acid (H_2SO_4) and left in a magnetically stirred beaker (≈500 rpm) for 120±10 min at room temperature. As a first attempt, the acid was gradually added at the beginning in order to reach fixed pH values (2,4,5); H_2SO_4 was chosen as it is one of the least expensive mineral acids, and also because it could be exploited as an added sulfate source for the culture medium. After the acid treatment, the mixture was filtered with filtering paper disks to eliminate the solid particulate. The filtered liquid was treated with NaOH to neutralize the acidic effect and take the pH up to 7.25, in order to make the treated digestate utilizable as a cultivation medium, independently of the dilution to apply prior to cultivation. It must be remembered that *Chlorella vulgaris* can only thrive and produce the desired lipids in a pH range from 7 to 9 (Xia & Murphy, 2016). After alkaline treatment, the liquid was centrifuged at 7200 rpm for 3-4 min to eliminate the new solid precipitate that appeared while changing the liquid pH up to 7-7.5. This centrifuged phase was then analyzed for its phosphate content in order to evaluate its suitability as a nutrient source for algal cultivation. The results are shown in Fig. 3.8.

It can be clearly seen that after the simple acidification/filtration step, the lower pH was reached through H_2SO_4 addition, the more phosphorus was extracted from the solid into the liquid phase. Instead, after neutralization with soda, most of the soluble phosphate precipitated again as metallic (Cu, Fe, Mg, Ca) salts. In fact, the solution assumed a dark blue/green color, these salts were then removed through centrifugation, and so was done for most of the phosphorus.

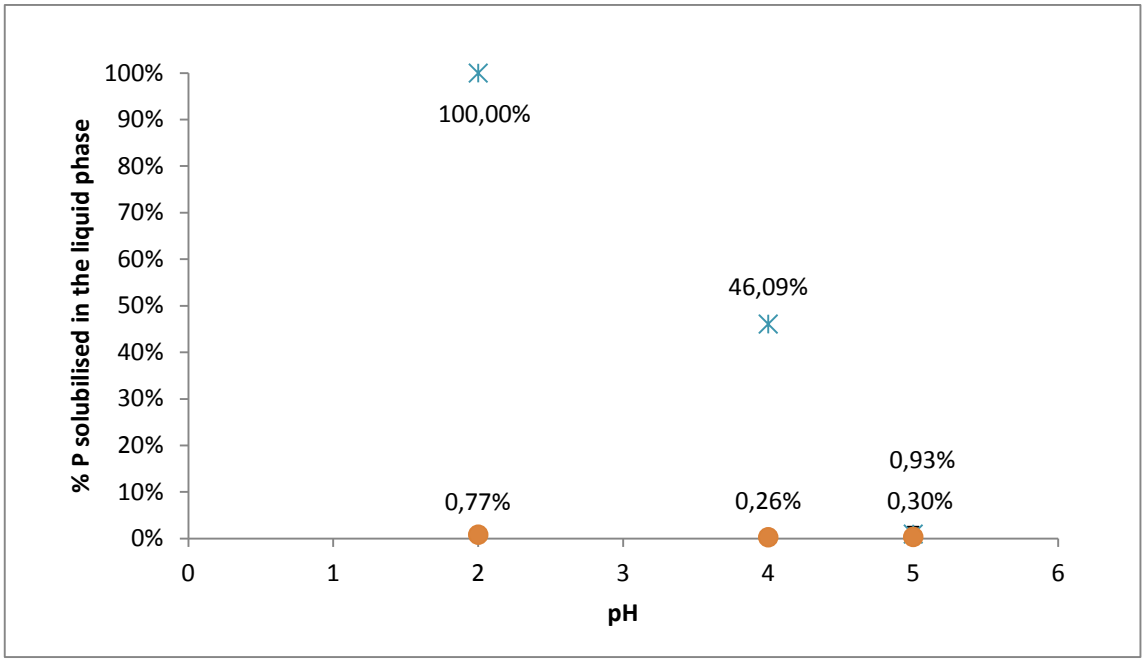

Figure 3.8 *Phosphorus percentage in the liquid phase after several treatments. The blue stars represent the acidified and filtered digestate at a specific pH, while the orange dots represent the liquid digestate after neutralization from that particular pH to a value of 7.25 and subsequent centrifugation.*

Re-precipitation was more evident while raising pH value with soda. In this second experiment, whose results are reported in Fig.3.9, the acidification pH was fixed at a value of 2, while the pH of the neutralized/centrifuged digestate was set at three different values (7,7.25,7.5).

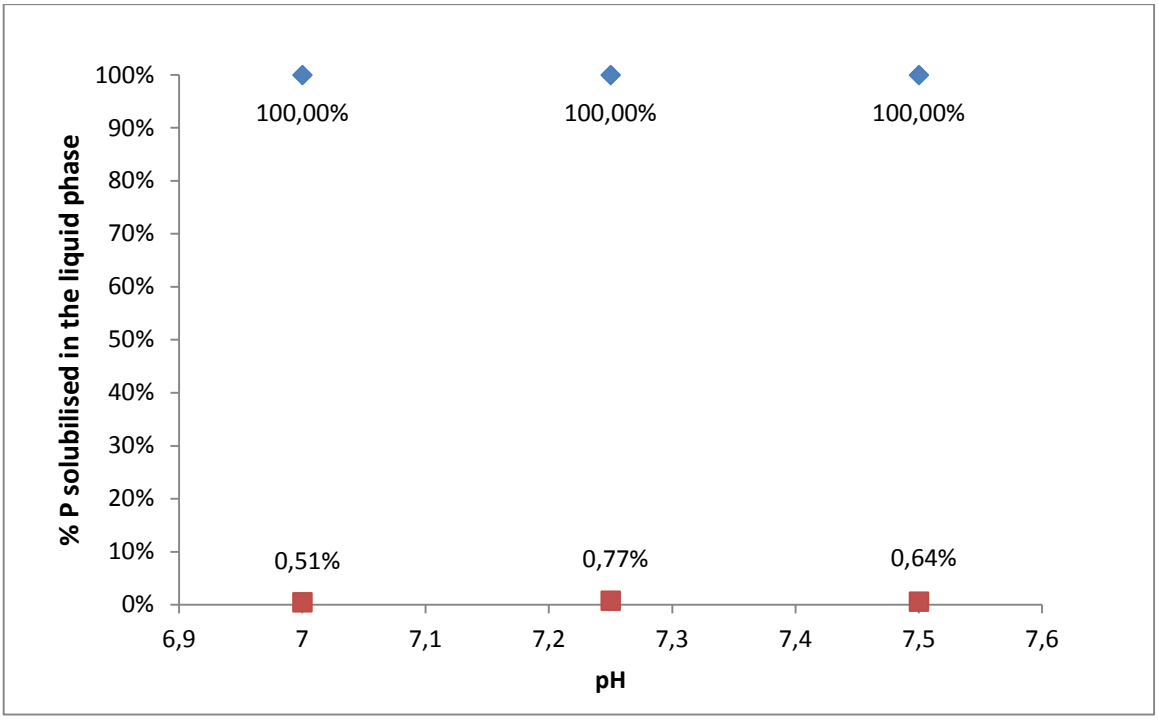

Figure 3.9. *Phosphorus percentage in the aqueous phase after several treatments. The blue dotsrepresent acidified and filtered digestate at pH 2, while the orange squares represent liquid digestate after neutralization from pH=2 to a value of 7,7.25,7.5, respectively and subsequent centrifugation.*

No matter the conditions, at most only 1% of the P in the digestate was available in the final liquid, a quantity comparable to the case of untreated centrifuged digestate, and still insufficient as P source for microalgal cultivation. So, the acidification/centrifugation method proved to be unfeasible for chemical phosphorus recovery from the liquid digestate fraction.

3.3.2 Acidification and EDTA addition

In order to overcome re-precipitation problems during neutralization, EDTA (ethylendiaminetetraacetic acid) was added after filtration of the solid particulate. EDTA is able to capture metallic ions that would instead precipitate as insoluble phosphates:

$$M^{n+}_{(aq)} + [EDTA]^{4-}_{(aq)} \leftrightarrow [EDTA - M]^{4-n}_{(aq)} \tag{3.11}$$

This sequestration leaves the phosphate ion PO_4^{3-} free to be dissolved in the aqueous phase and available as a nutrient source for microalgae cultivation.

The mixture was left to react for ≈15 minutes, then NaOH was added for neutralization, centrifugation was performed and the phosphate content was measured.

From the diagram in Fig. 3.11, it is clear that the more EDTA is added, the more phosphorus is dissolved in the liquid phase. However, to reach significant concentrations of P, more than 3 g/l of expensive EDTA must be added to the digestate, besides all other compounds (H_2SO_4, NaOH). This could hinder the economical sustainability of phosphorus recovery from the digestate by EDTA addition. Therefore, other solutions should be investigated.

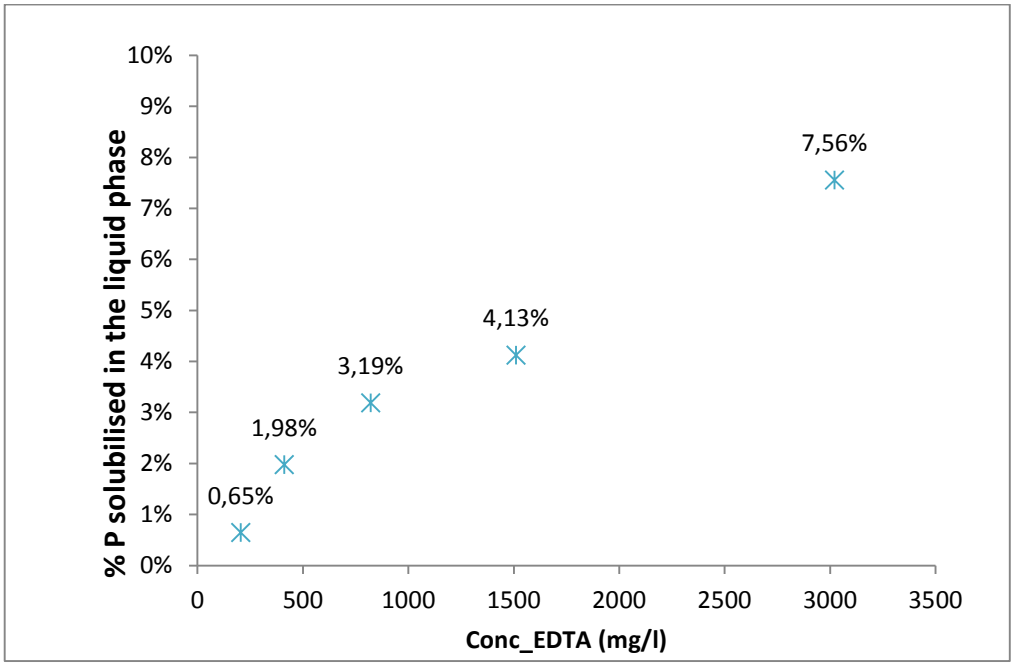

Figure 3.10 *Phosphorus percentage in the liquid phase after acid treatment and EDTA addition, at various EDTA concentrations.*

3.3.3 Treatment with sodium bicarbonate

A procedure called "Olsen method" (Horta & Torrent, 2007), normally used to extract and determine phosphorus in soils, was tested to extract phosphorus from the solid part of the digestate into the liquid one. This method involves the use of a cheap and non-toxic substance such as sodium bicarbonate ($NaHCO_3$). The presence in solution of bicarbonate ions (HCO_3^-) reduces the activity of metallic ions such as calcium Ca^{2+} and aluminum Al^{3+}, thus increasing phosphate solubility. Accordingly, the raw digestate was mixed with different amounts of $NaHCO_3$ and then NaOH was added to reach a mixture pH equal to 8.5, as required by the Olsen method. The reacting mixture was left in a magnetically stirred beaker (≈500 rpm) for 90±10 min at room temperature. After the treatment with bicarbonate, the mixture was filtered with qualitative filtering disks to eliminate the solid particulate. The filtered liquid was then measured spectrophotometrically (with the molybdate/ascorbic acid method) to find out its effectiveness in solubilizing phosphorus.The results are shown in Fig. 3.11.

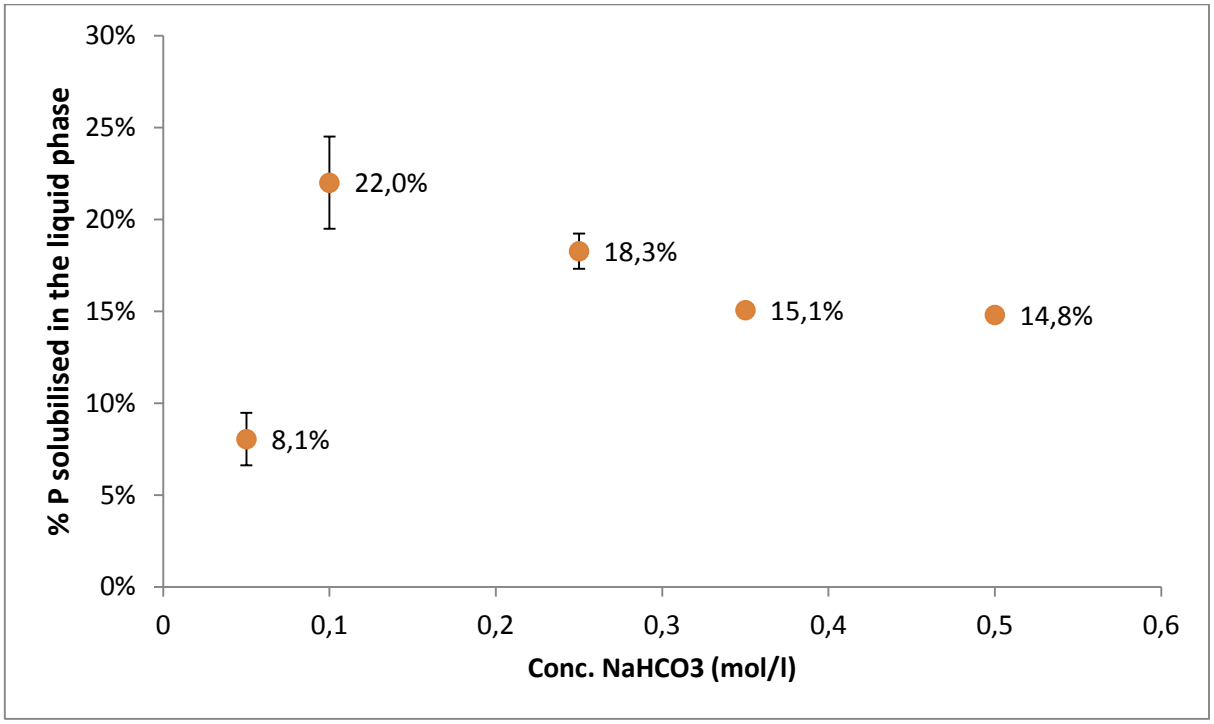

Figure 3.11 *Phosphorus percentage extracted in the liquid phase after treatment with sodium bicarbonate, at different bicarbonate concentrations.*

The percent of solubilized phosphorus in the liquid digestate showed a maximum at a concentration of bicarbonate of 0.1 mol/l. As almost 30 mg/l P (corresponding to ≈22% of the total amount) were extracted using sodium bicarbonate, this method seems promising for the purpose of phosphorus recovery. This quantity is significantly higher than in the case of

untreated digestate, and would be sufficient to sustain a microalgal cultivation in terms of phosphorus requirements.

3.4 Medium setup for algal cultivation in treated digestate

200 ml of raw digestate were treated with $NaHCO_3$ 0.1 M at pH=8.5, then filtered to eliminate the solid fraction. The final liquid was analyzed with respect to ammonium and phosphate contents to evaluate the dilution to apply prior to using this treated digestate as a cultivation medium. Its content in ammonium-nitrogen resulted to be 534±14 mg/l N, while the concentration of phosphorus was 8.78±0.18 mg/l P. This lower phosphorus content than in experiments with 10 ml of material was probably caused by mixing problems and/or inhomogeneity of the solid particulate in the digestate. As the ammonium-nitrogen content was little more than double than the one in modified BG11, it was decided to apply a 1:2 dilution in deionized water.

3.5 Cultivation in treated digestate

C. vulgaris was cultivated in the diluted liquid phase of the treated digestate, at the same conditions as all previous cultures. Sulphur was added in the same amount as standard BG11, while no additional phosphorus nor micronutrients were supplied. The growth curve of this case, compared to control, is shown in Fig. 3.12.

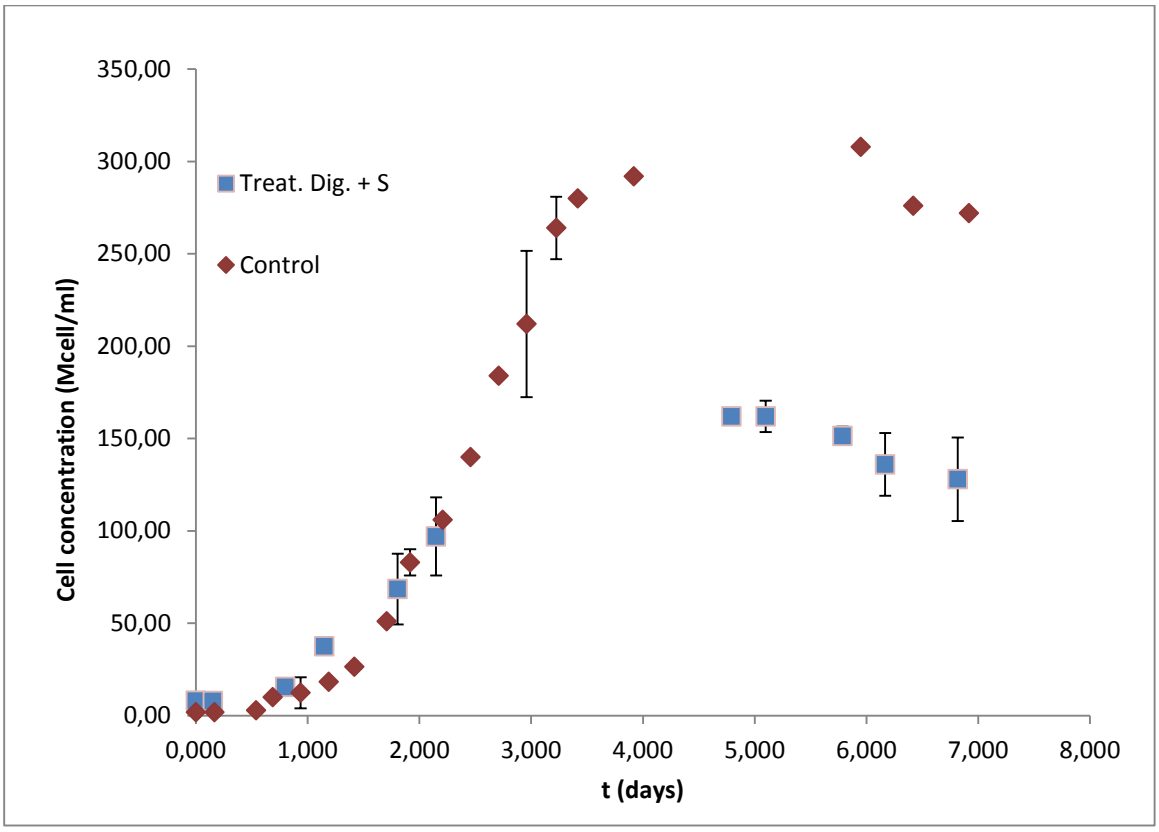

Figure 3.12 *Growth curve of C. vulgaris in treated diluted digestate compared to control.*

It can be observed the growth behavior of microalgae in treated digestate followed the one of the control up to a certain point (≈160 Mcell/ml), then the cellular concentration reached a plateau instead of rising any further. Growth rate was equal to 1.45±0.27 d^{-1}, a value which is lower than in control but still acceptable.

Table 3.8 reports the final biomass concentration and nutrient consumptions/yields in the treated digestate in comparison to control in modified BG11. As expected, the final concentration that the biomass reached in the digestate was lower, so were the absolute N and P consumption. In both cases all the phosphorus available was effectively consumed. The relative yields (nutrient consumed/biomass produced) resulted to be comparable as well.

Table 3.8 *Final biomass concentrations, nutrient consumptions and yields in treated digestate (in which S was added) compared to control.*

Medium	*Control*	*Diluted treated digestate+S*
$\boldsymbol{C_x}$ **(g/l)**	2.15 ± 0.07	1.47±0.01
$\boldsymbol{\Delta N\ (mg/l)\ =\ N_{fin} - N_{in}}$	126.1±29.1	108.3 ±5.9
$\boldsymbol{\Delta P\ (mg/l)\ =\ P_{fin} - P_{in}}$	4.95± 0.04	3.0±0.1
$\boldsymbol{Y_N = 100 * (N_{fin} - N_{in})/N_{in}}$	57%	47%
$\boldsymbol{Y_P = 100 * (P_{fin} - P_{in})/P_{in}}$	100%	100%
$\boldsymbol{Y_{N/x} = (N_{fin} - N_{in})/C_x}$	0.0619±0.0045	0.0737±0.0033
$\boldsymbol{Y_{P/x} = (P_{fin} - P_{in})/C_x}$	0.0023±0.0001	0.00204±0.00002

Chapter 4
Process simulation

In this chapter the process simulation of the production and anaerobic digestion of microalgal species *Chlorella vulgaris* is discussed. The whole setup of the simulation is described in all its steps, including models, stoichiometry, kinetics, flowsheet building and calculations. First, a case with the photobioreactor (PBR) only, modeled as a plug flow reactor (PFR), is studied, to compare the results with experimental data in batch cultures, as well as to analyze the effect of substrate limitations. Then, a base case with the whole flowsheet is described in terms of N, P and water balances. Energy balances are applied to give an estimate of the areal requirements and of the geometric features of the PBR. A sensitivity analysis is performed to evaluate the effect of several variables on nutrients loss and external makeup requirements. Eventually, a brief analysis is performed on the absorption of CO_2 in water to verify possible effects on it due to and the presence of the other nutrients in the liquid stream.

4.1 Simulation model and chemical equilibrium

The simulation was carried out using Aspen Plus process simulator (V.8.2). The components which were considered are listed in Table 4.1.

Table 4.1 *List of components included in the Aspen Plus simulation.*

1	O2	8	H3O+	15	AMMON-01 (NH_4Cl)
2	N2	9	AMMONIA (NH_3)	16	DIPOT-01 (K_2HPO_4)
3	CO2	10	NH2COO-	17	K+
4	H2O	11	H2PO4-	18	HCL
5	NH4+	12	HCO3-	19	CL-
6	OH-	13	CO3--	20	H3PO4
7	PO4---	14	HPO4--	21	CH4

The Electrolyte-NRTL (Elec-NRTL) model was adopted for equilibrium calculations, as it is capable of dealing with the ionic species included in the algal growth. Through the *Elec-Wizard*, the reactions (listed in Table 4.2) of equilibrium and salt dissociation were included in GLOBAL chemistry. The formation of solid carbon and salt species was neglected, thus avoiding the inclusion of a SOLID substream, which would complicate the simulation without relevant improvements in terms of comparability with experimental results.

Table 4.2 *Equilibrium and dissociation reactions in GLOBAL chemistry*

Reaction	Type	Stoichiometry
1	Equilibrium	$H_2O + HPO_4^{2-} \leftrightarrow H_3O^+ + PO_4^{3-}$
2		$H_2O + H_2PO_4^- \leftrightarrow H_3O^+ + HPO_4^{2-}$
3		$H_3PO_4 + H_2O \leftrightarrow H_3O^+ + H_2PO_4^-$
4		$HCl + H_2O \leftrightarrow Cl^- + H_3O^+$
5		$NH_3 + HCO_3^- \leftrightarrow H_2O + NH_2COO^-$
6		$NH_3 + H_2O \leftrightarrow OH^- + NH_4^+$
7		$H_2O + HCO_3^- \leftrightarrow CO_3^{--} + H_3O^+$
8		$2\,H_2O + CO_2 \leftrightarrow HCO_3^- + H_3O^+$
9		$2\,H_2O \leftrightarrow OH^- + H_3O^+$
AMMON-01	Dissociation	$NH_4Cl \rightarrow Cl^- + NH_4^+$
DIPOT-01		$K_2HPO_4 \rightarrow HPO_4^{2-} + 2\,K^+$

4.2 Algal growth reaction stoichiometry and kinetic model for PBR

The reaction stoichiometry of the microalgal growth was calculated from the raw formula, which was obtained by a supposed composition of the *Chlorella vulgaris* biomass. C, H, N weight fractions were retrieved from the elemental analysis of the whole algae performed by Conycal S.r.l; P content was determined by the element analysis by Chelab S.r.l. and confirmed by literature data (Concas *et al.*, 2012; Alcàntara *et al.*, 2013); the remaining fraction was assumed to be composed of oxygen. The resulting composition (in both molar and mass terms) is shown in Table 4.3.

Table 4.3 *Elemental analysis of C. vulgaris biomass in terms of molar and mass fractions*

Element	*Molar fraction*	*Mass fraction*
C	*0,2848*	*0,4645*
H	*0,4984*	*0,0677*
N	*0,0386*	*0,0734*
O	*0,1749*	*0,3803*
P	*0,0033*	*0,0139*

The stoichiometry of the reaction producing algal biomass is thus:

$$0.22\ H_2O + 0.0386\ NH_4^+ + 0.2848\ CO_2 + 0.00165\ H_2PO_4^- + 0.00165\ HPO_4^{2-} \rightarrow \rightarrow\ C_{0.2848}H_{0.4984}O_{0.1742}N_{0.0386}P_{0.0033} + 0.297125\ O_2 + 0.03365\ H_3O^+ \quad (4.1)$$

The process simulation was carried out with the help of a FORTRAN subroutine, in order to apply User-defined kinetics in the PBR, which was simulated as a PFR (RPlug in Aspen Plus). Aspen Plus V8.2 is based on the Intel Fortran compiler XE 2013 and Microsoft Visual

Studio 2013. The reaction rate used in the simulation is based on Monod kinetics with multiple limiting substrates ($CO_2, NH_4^+, H_2PO_4^- + HPO_4^{2-}$):

$$R = \mu_{max} * C_X * \frac{C_{CO_2}}{K_{CO_2}+C_{CO_2}} * \frac{C_{NH_4^+}}{K_{NH_4^+}+C_{NH_4^+}} * \frac{C_{\left(HPO_4^{2-}+H_2PO_4^-\right)}}{K_{\left(HPO_4^{2-}+H_2PO_4^-\right)}+C_{\left(HPO_4^{2-}+H_2PO_4^-\right)}} \quad \left[\frac{kg}{m^3 s}\right] \quad (4.2)$$

where:

μ_{max} $[d^{-1}]$ = maximum specific growth rate, assumed using experimental data;

K_i $\left[\frac{kg_i}{m^3}\right]$ = half saturation constant for substrate *i* (listed in Table 4.4);

C_i, C_X $\left[\frac{kg}{m^3}\right]$ = substrates and biomass concentration.

Table 4.4 *Values of K_i for considered substrates.*

Substrate	CO_2	NH_4^+	$H_2PO_4^- + HPO_4^{2-}$
$\mathbf{K_i}$ $\mathbf{[kg_i/m^3]}$	4.752E-03	24.84E-03	4.943E-03

The half saturation constants for nutrients were retrieved from the literature data for *C. vulgaris* (Concas *et al.*, 2012), while parameter μ_{max} (maximum specific growth rate) has to be determined experimentally. Note that μ_{max} based on mass and μ_{max} based on cells can be different, since the average cell size may vary considerably during the growth process. During exponential phase, nonetheless, in case of non-inhibiting and non-limiting light, the two values are comparable (Gris, 2012). Furthermore, $\mu_{max,mass}$ should be lower or at most equal to $\mu_{max,cells}$, as cells are not supposed to increase their volume during exponential growth. The value 1.55 d^{-1} was selected, as estimated in section §3.2.1).

4.3 Fortran subroutine

The Fortran subroutine source code is reported textually in Appendix 1, as well as the procedure to compile the program to obtain an object linkable to the simulator. The calculations performed in this subroutine are now described. First, all Aspen COMMON blocks are included, to allow the interaction between the user and Aspen Plus units and properties, and all variables are declared. The program receives from the simulator the total mole flowrate *M* and the vapor fraction in the inlet stream v_{frac}, then it calculates the total molar flowrate in the liquid phase with the equation:

$$MM = M * \left(1 - v_{frac}\right) \quad (4.3)$$

The liquid volumetric flowrate in the inlet V_{in} is calculated by multiplying MM by the molar volume of the liquid mixture, *stwork_vl*. Then the residence time $\tau_{PFR}$is calculated in this way:

$$\tau_{PFR} = vliq * \frac{xlen}{V_{in}} \quad (4.4)$$

where *vliq*, for a PFR, is the cross-sectional area occupied by the liquid phase in the reactor and *xlen* is its length; thus, the product of these two variables represents the volume of the reacting liquid mixture in the PFR. The vapor and liquid fractions in the reactor are calculated as:

$$\%LIQ = 100 * \frac{vliq}{vliq+vvap}, \quad \%VAP = 100 * \frac{vvap}{vliq+vvap} \quad (4.5)$$

where *vvap* is the reactor cross-sectional area occupied by the vapor phase. The variables *vvap*, *vliq* and *xlen* are retrieved from the simulation as common blocks variables, and so is the ALGA mass flowrate. The mass flowrates of the other components involved in the reaction are then calculated:

$$M_i = MM * PM_i * X_i \quad (4.6)$$

where all the values of PM_i (the molecular weight of each species) are directly provided by the user, while the molar fractions in the liquid feed X_i are called from the simulation. Then, ALGA and nutrient weight concentrations are calculated:

$$C_i = M_i/V_{in} \quad (4.7)$$

In the particular case of orthophosphates, they are considered as one single species in the kinetics:

$$C_{(H_2PO_4^- + HPO_4^{2-})} = (M_{H_2PO_4^-} + M_{HPO_4^{2-}})/V_{in} \quad (4.8)$$

The mass concentrations are used to calculate the reaction rate *R*, according to eq. 4.2. Note that, in the Monod kinetics, the factors

$$limit_i = \frac{C_i}{C_i+K_i} \quad (4.9)$$

can be defined as "limiting factors". These terms, whose values are between 0÷1, allow to understand the limiting nutrient(s); the closer they are to 0, the more they reduce the algal growth rate, as a result of the lack of one or more substrates. The rates of production or consumption of the compounds participating the reaction are calculated and supplied to the simulator. In an Rplug unit, the rate per unit volume (*R*) is multiplied by the cross-sectional area covered by the reacting phase. The definition of these rates are in terms of mass for the ALGA, which is a non-conventional component, and in molar terms for all the other species (conventional components):

- production/consumption rates for conventional components: $\left(\frac{kmol}{m^3 s}\right) * (m^2) = \frac{kmol}{ms}$

- production/consumption rates for non-conventional components: $\left(\frac{kg}{m^3 s}\right) * (m^2) = \frac{kg}{ms}$

Thus, in the case of ALGA, the production rate is given by:

$$r_{ALGA} = R * vliq \tag{4.10}$$

while for all the other components, the production or consumption rate are calculated as:

$$r_i = \frac{R}{PM_{ALGA}} * vliq * v_i \tag{4.11}$$

where $PM_{ALGA}$is the molecular weight of the biomass obtained from its molar composition:

$$PM_{ALGA} = 12c + h + 14n + 16o + 31p \tag{4.12},$$

where *c,h,n,o,p* are the molar fractions in the biomass of C,H,N,O,P respectively.

The pH of the reactor outlet is calculated as well:

$$pH = -Log \frac{C_{H_3O^+}}{PM_{H_3O^+}} \tag{4.13}$$

A text file with all the relevant variables concerning the PFR is created at each iteration; it can be useful to understand in what direction the iterating steps are moving to the results of simulation convergence.

4.4 Nutrient calculations

The CO_2 flowrate to be fed to the culture was calculated by scaling up experimental data. For a culture of 300 ml with a residence time of 1 day, a flowrate of 0.5 l/h of air with a 5% v/v CO_2 is normally adopted on a lab scale. In this simulation, a cultivation flowrate of 10000 l/h was considered, thus:

$$0.5 \frac{l}{h} air : 300 \frac{ml}{day} = x : 10000 \frac{l}{h} \tag{4.14}$$

$$x = \frac{2.4 * 10^8 * 0.5}{300} \frac{l}{h} air = 400000 \frac{l}{h} air\ with\ 5\%\ v/v\ CO_2 \tag{4.15}$$

The equivalent molar flowrate is ≈17.0 kmol/h (as calculated by Aspen Plus), and the composition specification is given in terms of molar fractions (0.21 for O_2, 0.74 for N_2 and 0.05 for CO_2).

Nitrogen was provided in two forms, ammonium chloride (NH_4Cl) and ammonia (NH_3), in order to have the possibility to arbitrarily modify the pH in the reactor, which depends on their ratio. The pH in the PBR is a relevant parameter as it influences the distribution of the two species of orthophosphates that are assimilated to produce microalgal biomass. The ratio of the two N forms can be modified by changing a fictitious parameter, the "pH" of the

nutrient makeup, as will be explained in the following. As ammonium chloride dissociates completely, the following equilibrium between ammonium ion and ammonia is then established:

$$NH_4^+ + H_2O \leftrightarrow NH_3 + H_3O^+ \tag{4.16}$$

The pK_a of this equilibrium is a function of temperature:

$$pK_a = -Log\left(\frac{[NH_3][H_3O^+]}{[NH_4]}\right) = 0{,}0901821 + \frac{2729{,}92}{T\ (K)} \tag{4.17}$$

The pH of the makeup is set arbitrarily to modify the pH in the reactor ($pH = -Log[H_3O^+]$). The molar fraction of ammonia, defined as:

$$F = \frac{[NH_3]}{[NH_4^+] + [NH_3]}, \tag{4.18}$$

needs to be calculated. To do so, the following equations are used.

$$pK_a - pH = Log[H_3O^+] - Log\left(\frac{[NH_3][H_3O^+]}{[NH_4^+]}\right) = Log\left(\frac{[NH_4^+]}{[NH_3]}\right) \tag{4.19}$$

$$10^{(pK_a - pH)} = \frac{[NH_4^+]}{[NH_3]} \quad ; \quad 1 + 10^{(pK_a - pH)} = \frac{[NH_4^+] + [NH_3]}{[NH_3]} = \frac{1}{F}$$

$$F = \frac{1}{1 + 10^{(pK_a - pH)}} \tag{4.20}$$

If N_{tot} is the total nitrogen mass flow rate (kg/h) to be distributed into the two forms, the mass flow rate of ammonia (kg/h) to be fed in the reactor is:

$$N_{\ NH_3} = N_{tot}\left(\frac{PM_{NH_3}}{PM_N}\right) * F \tag{4.21}$$

The remaining nitrogen flowrate is provided as NH_4Cl:

$$N_{NH_4Cl} = N_{tot}\left(\frac{PM_{NH_4Cl}}{PM_N}\right) * (1 - F) \tag{4.22}$$

The phosphate makeup is supplied through one component only, dipotassium phosphate (K_2HPO_4), which completely dissociates and splits into various forms of orthophosphate ions, according to the pH of the solution. Within a pH=5÷9, the two main ions are $H_2PO_4^-$ and HPO_4^{2-}, so that in a situation of pH≈7, their concentrations are comparable, in fact in the reaction stoichiometry they are set to be consumed equally. If the goal is to achieve a fixed concentration of a particular species at the reactor inlet, deciding the salt makeup is not trivial

because nutrients are fed to the process in a different form from what is really consumed. The flowrates of salts and ammonia can be defined, but the ion distribution derives from equilibrium calculations made by the simulation model itself, which takes into account all the equilibria at the same time in the specified chemistry. Thus, no design specification can be set on nutrient makeup.

4.5 Simplified flowsheet with photobioreactor

First, a simplified flowsheet with the PBR alone was developed to study algal growth, nutrient requirements, limitations and profiles along the reactor, as well as to make a comparison with experimental data.

The PBR model of Aspen Plus better represents the progression of the reaction and the contemporary adjustment of ionic species concentration due to the shifting of chemical equilibria. CO_2 is fed as gaseous phase (5% v/v in air), and it dissolves in the reactor liquid feed. This is composed of water and the two macronutrients (N and P). The reaction products are flashed to eliminate the residual gases, i.e. unreacted CO_2, produced O_2, which increases the one in the air, ammonia and inert N_2. As a base case, two fixed concentrations of the main macronutrients in the reaction medium were selected to ensure large excess and avoid limitations.

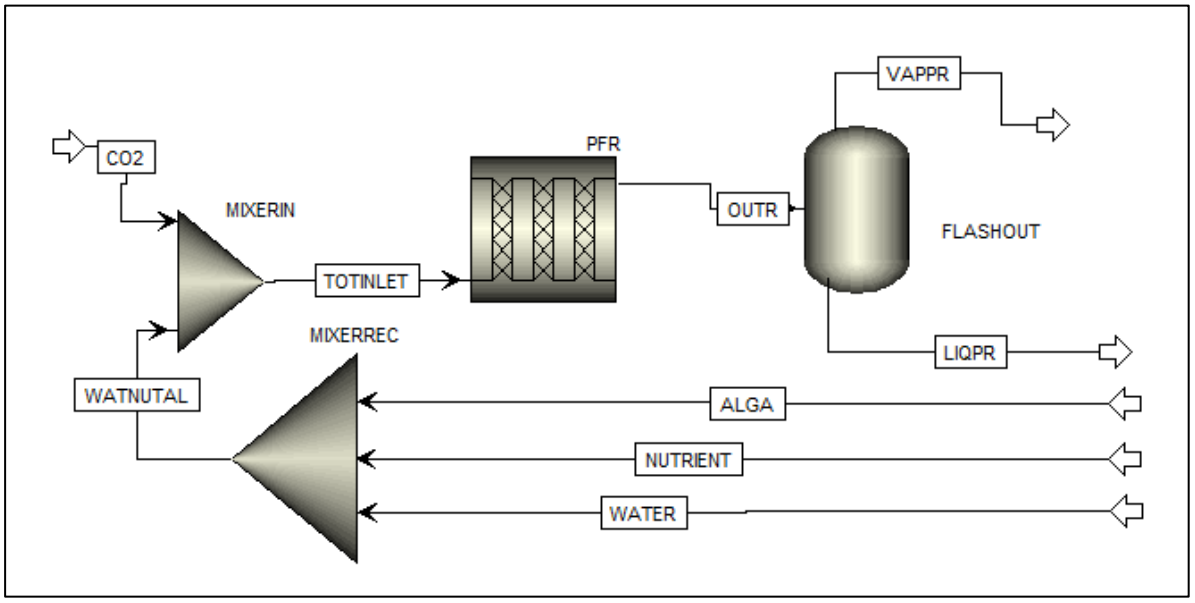

Figure 4.1 *Simplified flowsheet of the microalgae production section.*

In Table 4.5, the nutrient supplies needed to obtain concentration values similar to pH obtained in laboratory scale cultures (≈7.3) are reported.

Table 1.5 *Macronutrients concentration in the reaction medium and nutrient supply flowrates.*

Macronutrient	**Concentration**	***Nutrient supply***
Nitrogen	494 mg/l (as BG11 medium 2X)	2.5 kg/h NH_3 11 kg/h NH_4Cl
Phosphorus	89 mg/l(as in medium with P excess used in lab scale continuous cultivation)	5 kg/h K_2HPO_4

The flow rate of algal biomass in the reactor inlet stream is 2 kg/h, to produce a reasonable inlet concentration value of 0.2 g/l. Water flowrate in the process is 10000 kg/h. The temperature is set to 28°C (301.15 K). The PFR is 3760 m long, with a diameter of 1.9 m, a geometry which leads to a residence time τ_{PFR} equal to 1 day.

Tables 4.6 and 4.7 show the results of the simulation of this simplified case. The complete stream tables are reported in Appendix 2. The mass flowrates of N in all the streams were calculated by multiplying the sum of the molar flowrates of the N containing species (NH_3, NH_4^+, $NH2COO^-$) by the atomic weight of N. The same was done for P, considering the orthophosphate species ($H_2PO_4^-$, HPO_4^{2-} , PO_4^{3-} , H_3PO_4).

Table 4.6 *Stream results, in terms of mass flowrates of nutrients, water and biomass, in a case study in the simulation with the reactor only.*

MASS FLOWRATE kg/h	**ALGA**	**CO2**	**LIQPR**	**NUTRIENT**	**OUTR**	**TOTINLET**	**VAPPR**	**WATER**	**WATNUTAL**
N	0	0	4.492	4.934	4.504	4.934	0.0115	0	4.934
P	0	0	0.808	0.890	0.808	0.890	0	0	0.890
WATER	0	0	9983	0.00	9995	9997	11.9	10000	10000
ALGA	2	0	7.86	0	7.86	2.00	0.00	0.00	2.00
CO2	0.00	37.41	0.38	0.00	22.04	30.82	21.66	0.00	0.00

It is noteworthy that the geometric volume of the reactor is much larger than the volume of the reacting (liquid) mixture, as almost all the mixture (≈98%) entering the PFR is in gaseous phase in volumetric terms. With a residence time of 1 day, 5.86 kg/h of biomass are produced, and the ALGA concentration rises from a value of ≈0.200 g/l to ≈0.786 g/l.

Table 4.7 *Additional results for the reactor in a case study in the simplified simulation.*

Inlet biomass concentration C_{xin} (g/l)	0.200
Outlet biomass concentration C_{xout} (g/l)	0.786
Max specific growth rate μ_{max} (d^{-1})	1.55
Reaction rate R [kg/(sm^3)]	1.01
Rate reduction factors due to limitation	
limitC	0.889
limitN	0.958
limitP	0.981
Reactor liquid phase holdup (m^3)	238
Reactor total volume (m^3)	10660
pH reactor INLET	7.24
pH reactor OUTLET	7.31

This simulation result is validated by the dry weight measurement in the experimental batch curves, which were exploited to estimate the parameter μ_{max} (section §3.2.1). It must be remembered that, concerning the species concentrations, the behavior in a plug flow reactor in a space coordinate is the same as for a batch reactor along with in time. In batch cultures, after one day of exponential growth (this phase also characterizes continuous cultures), the biomass concentration increased from 0.16 g/l to 0.74 g/l; these values are similar to the initial and final ones in the PFR with a τ=1 day, validating our estimate of the parameters in the Monod kinetic model from experimental values. Nutrient consumption is fixed by stoichiometry, and, because of their definition, the yields of consumed nutrients on biomass produced are numerically equal to the weight fraction of that substrate (N,P) in the biomass (0.0734 kg N/kg ALGA and 0.0139 kgP/kg ALGA). These yields are comparable to the experimental values and to literature data (Crofcheck *et al.*, 2012; Sforza *et al.*, 2014), but they are higher than the ones obtained during our experimental cultures, especially in the case of phosphorus. It means that the whole algal biomass used for lipid extraction (the one provided by NEOALGAE™) is actually richer in N and P than the one grown in batch cultivations.

The pH at the reactor inlet was set to reproduce a realistic experimental value. The pH in the reactor is almost constant, as it slightly rises from7.24 to 7.31). This result can be explained by the conversion of bicarbonate to CO_2 which also produces OH^- ions and increases the pH along the reactor. The CO_2 in gas feed is absorbed in the liquid medium, and is then converted into the algal biomass; as soon as it is consumed, because of the thermodynamical equilibrium between phases, it is reabsorbed again from air. The profile in Fig.4.2 shows how the CO_2 content is reduced in both the liquid and vapor phases.

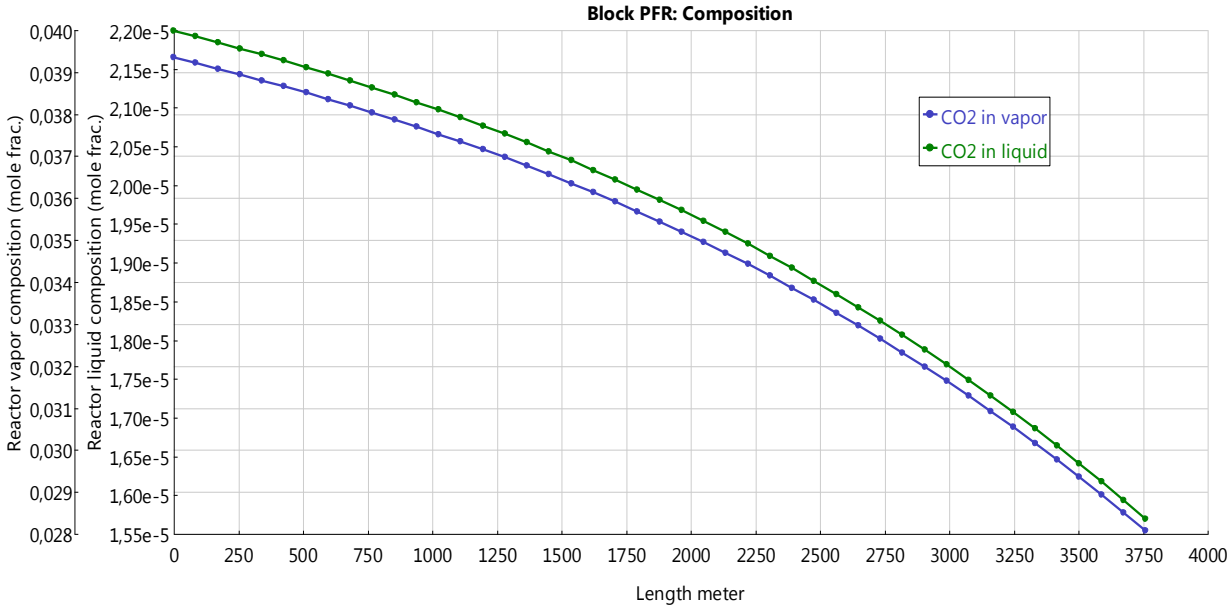

Figure 4.2 *Molar fraction profiles of CO_2 in the PFR, in both liquid and vapor phases.*

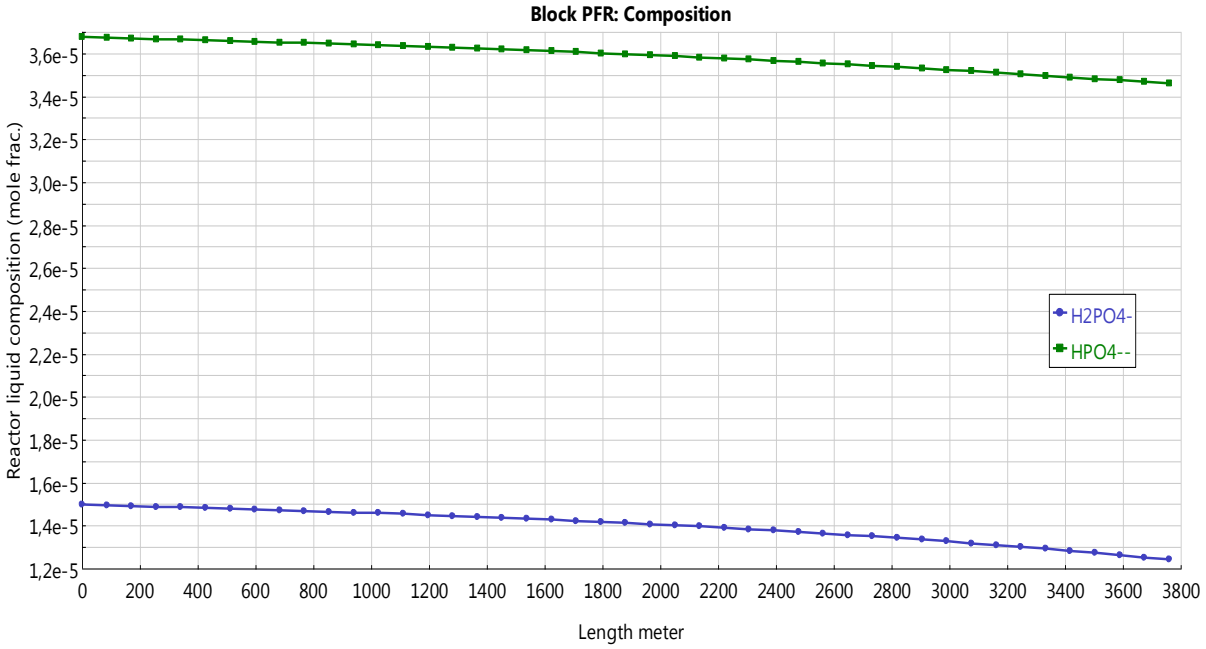

Figure 4.3 *Orthophosphate ions molar fraction profiles in the liquid phase of the PFR, in non-limiting conditions (494 mg/l N, 89 mg/l P).*

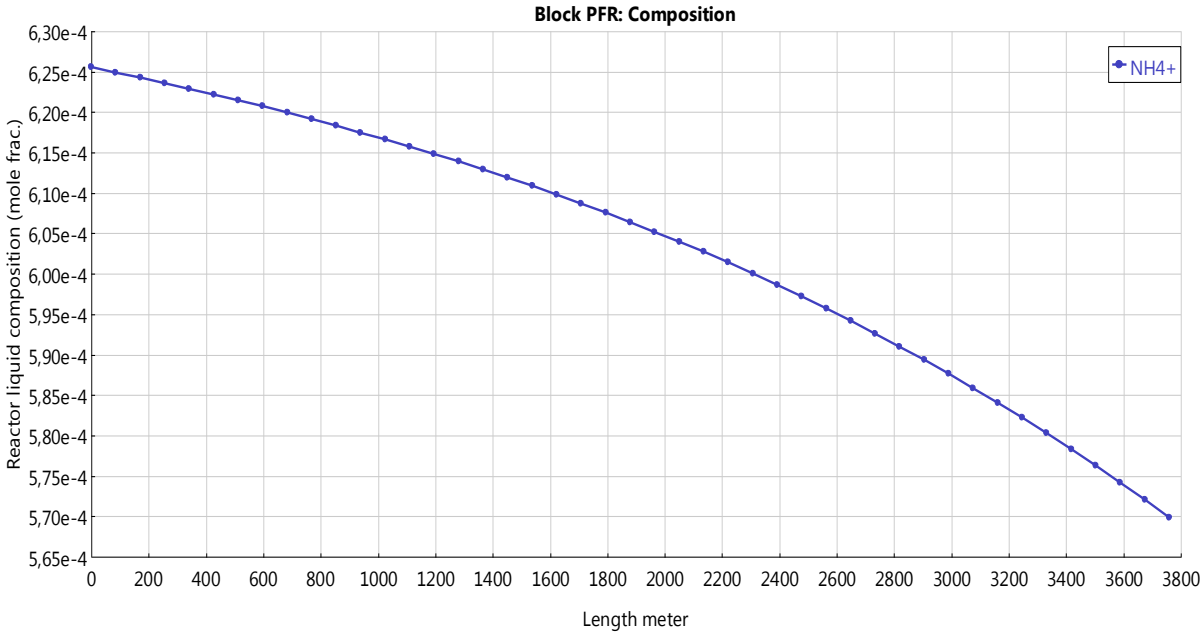

Figure 4.4 *Ammonium ion molar fraction profile in the liquid phase of the PFR in non-limiting conditions (494 mg/l N, 89 mg/l P).*

Fig.4.3 and Fig.4.4 show the other nutrients molar fraction profiles in the liquid media along the reactor length, in which their consumption is evident, even though they do not reach values close to zero, as in this case all nutrients are supplied in excess. In case of limiting N and P (4.94 mg/l N, 0.89 mg/l P in the reactor inlet), a change in the profile is noticed, showing how nutrients are being almost completely depleted (Fig. 4.5-4.6)

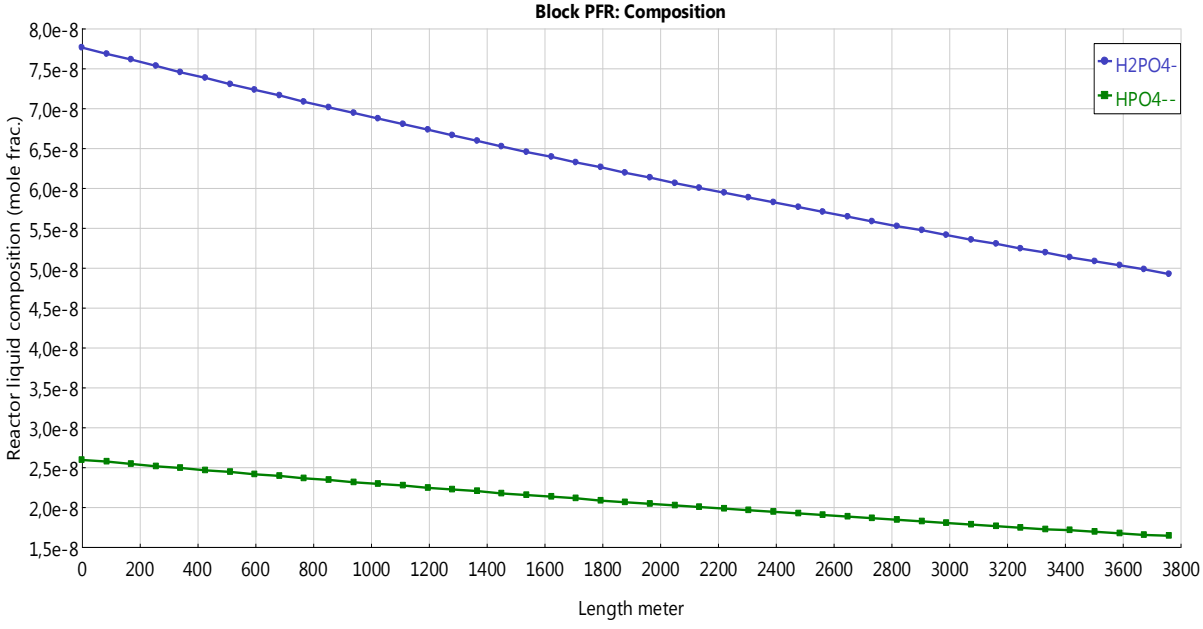

Figure 4.5 *Orthophosphate ions molar fraction profiles in the liquid phase of the PFR, in limiting conditions (4.94 mg/l N, 0.89 mg/l P)*

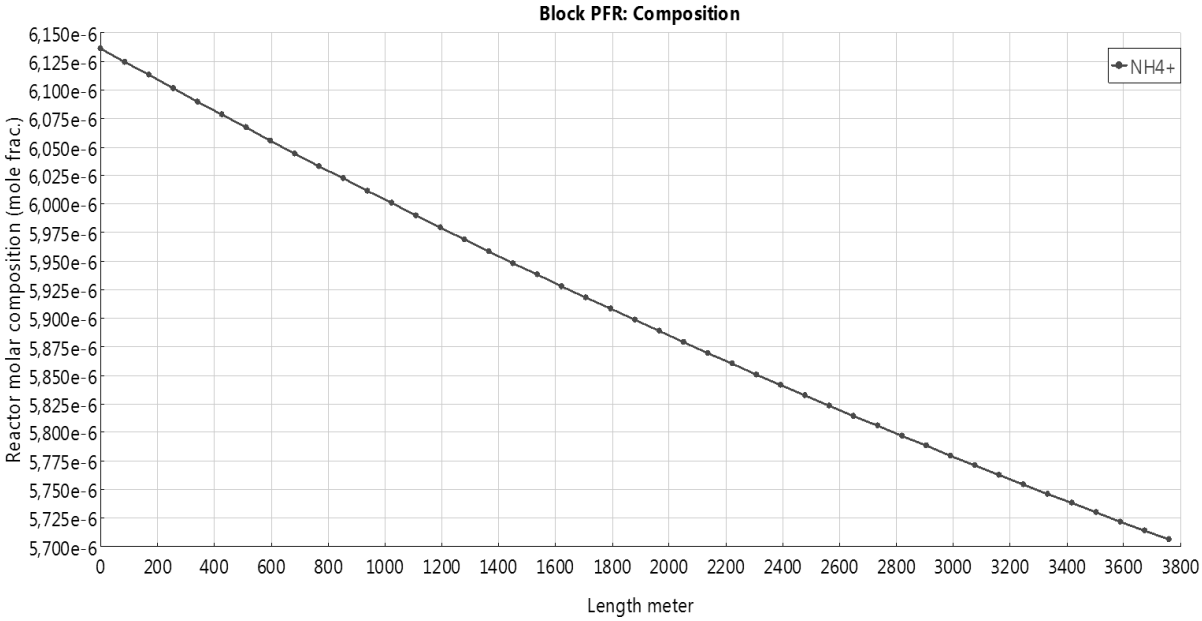

Figure 4.6 *Ammonium ion molar fraction profiles in the liquid phase of the PFR, in limiting conditions (4.94 mg/l N, 0.89 mg/l P).*

A sensitivity analysis is performed on the nutrient feed concentrations of N and P to better understand the relevant effect that the limitation of N and P has on biomass productivity. Obviously, the scarcer and more limiting is one of the two substrates in the reactor inlet, the lower is the overall biomass productivity; the limiting factors values for that nutrient corresponding to each point are reported (Fig. 4.7-4.8).

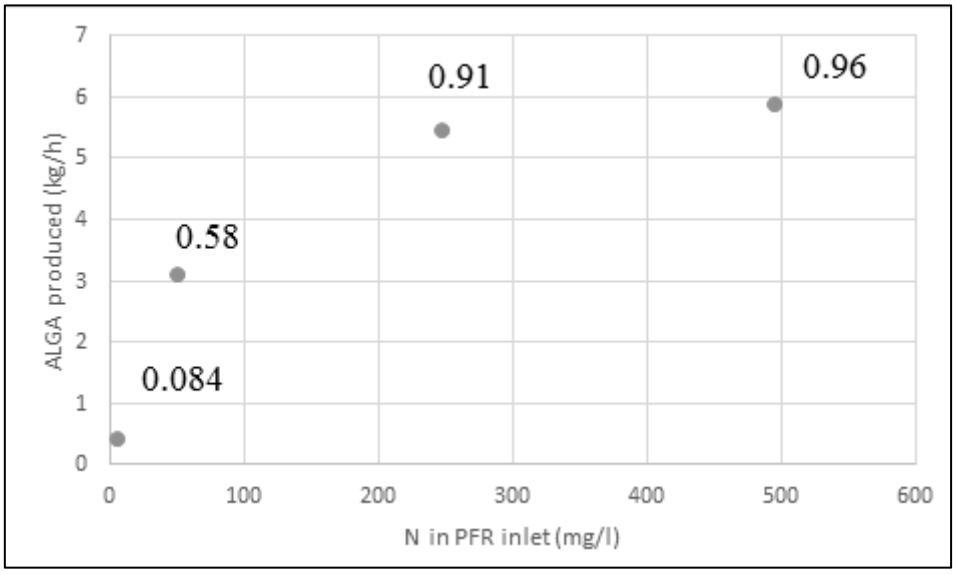

Figure 4.7 *Biomass productivity at different nitrogen concentrations in the reactor inlet medium (at a fixed and non-limiting concentration of P equal to 89 mg/l).The limiting factors for N are reported for each point.*

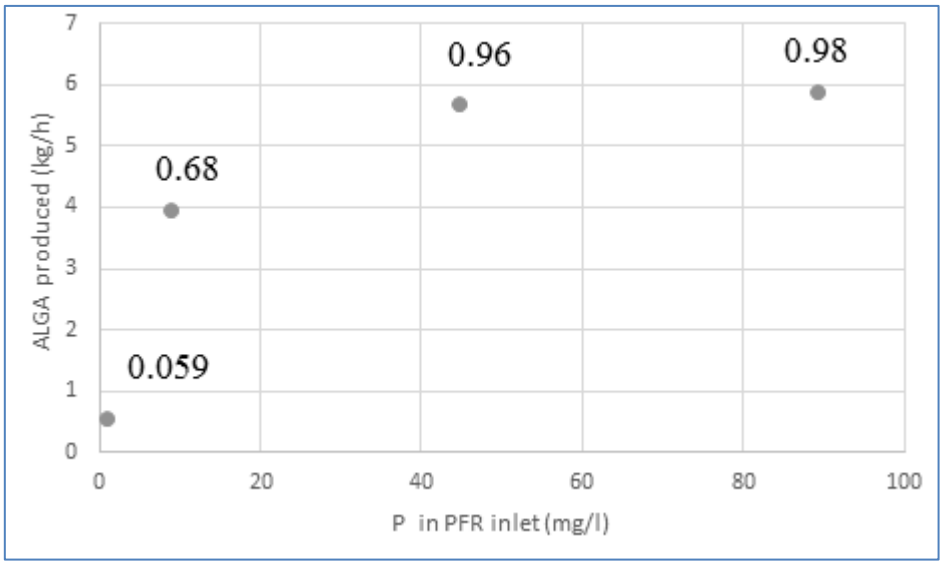

Figure 4.8 *Biomass productivity at different phosphorus concentrations in the reactor inlet medium (at a fixed and non-limiting concentration of N equal to 494 mg/l). The limiting factors for Pare reported for each point.*

4.6 Complete flowsheet

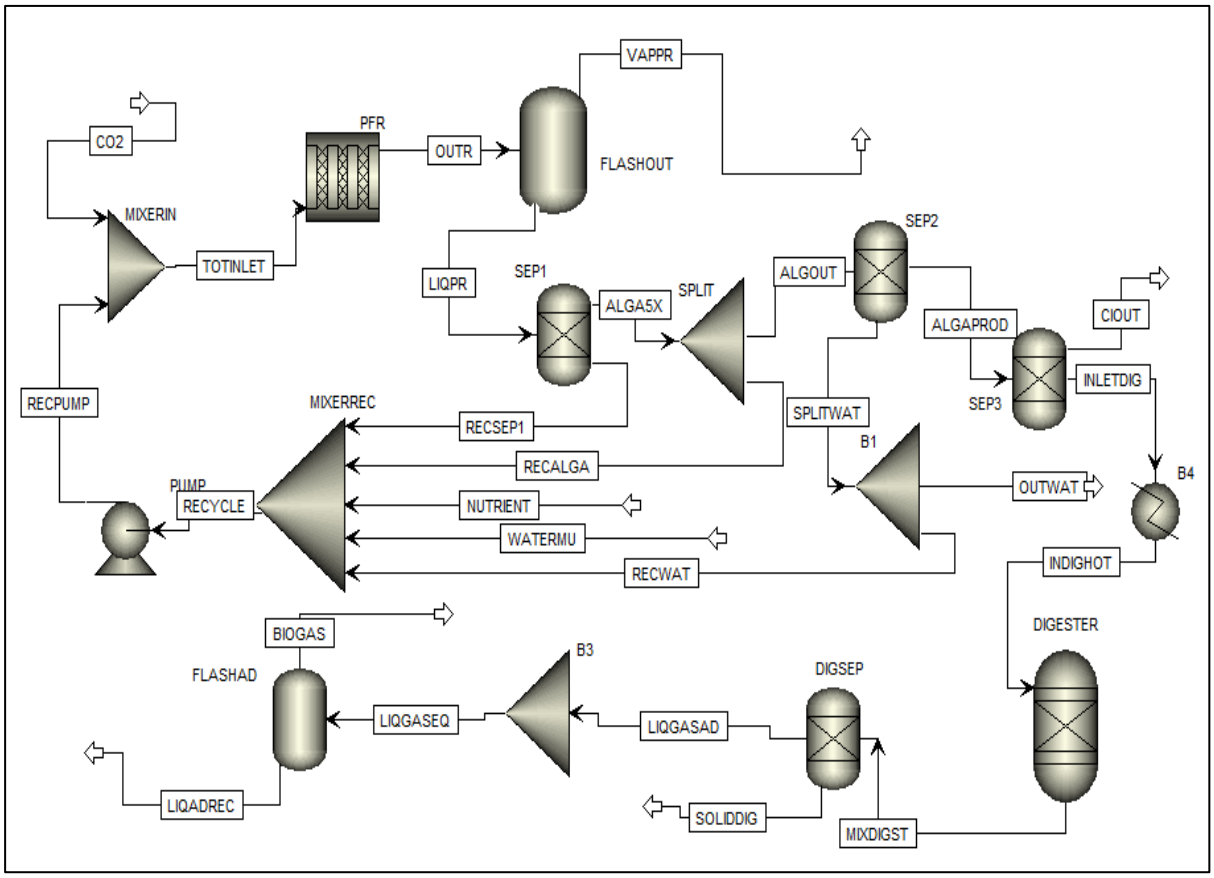

Figure 4.9 *Complete flowsheet of the production and anaerobic digestion of microalgal biomass.*

Fig. 4.9 shows the complete flowsheet of both the sections of production and anaerobic digestion of the microalgal biomass, with water and nutrient recycling streams. The reactor conditions and geometry are unvaried respect to the previous case. The reactor feed is composed of water, N in the form of ammonia and ammonium, P in the form of orthophosphate ions, CO_2 and microalgal biomass also in this case. The most evident difference consists in the fact that all the biomass and most of the water (containing the greatest part of nutrients) in the reactor inlet stream, come from recycle streams, with a little water and nutrients makeup to compensate for losses in the outlet streams. In this simulation two design specifications (DS) are applied, which are listed in Table 4.8.

Table 4.8 *Design specifications for the simulation.*

DS	Target	Varying
1	RECYCLE flowrate= 10000 kg/h	Water makeup WATERMU
2	ALGA flowrate in reactor inlet TOTINLET= 2 kg/h	Split fraction in SPLIT

DS1 was set on the recycle stream and not on the PFR inlet, because it is necessary to consider only the liquid part of the reactor feed; a little amount of air rich in CO_2 solubilizes in it but is negligible in its mass fraction in TOTINLET, whose flowrate is close to 10000 kg/h anyway). Both design specifications act as a single one which fixes the biomass concentration in the reactor inlet equal to 0.2 g/l.
Downstream to the reactor, the following units are considered:

- the flash FLASHOUT (with no duty or T/P change applied) eliminates the gaseous phase in the reactor outlet;
- the liquid (and solid) part coming from FLASHOUT is sent to a separator (SEP1), acting as a first stage of separation by gravity (sedimentation). Here it is aimed that the biomass is concentrated fivefold (5X), so 80% of the mixture water+nutrients is recycled back to the PFR feed in the stream RECSEP1;
- the concentrated biomass is sent to a split (SPLIT), where part of this stream is sent back to the reactor to provide the desired initial microalgae concentration in the PFR inlet, as well as to recycle another share of water and nutrients;
- the remaining part (ALGOUT) is sent to a separator (SEP2), acting as a centrifuge. Here the biomass reaches a fraction of 20% of dry weight, a reasonable value in centrifugation operations, and for digesters feed (“wet” anaerobic digestion). This product (ALGAPROD) is sent to the AD section. To define the fraction *SEP* of water and all

dissolved substances to send to the stream ALGAPROD, a calculator (DWINAD) was implemented, which performed the following calculation:

$$SEP = \left(\frac{1-F_{DW}}{F_{DW}}\right) * \frac{M_{ALGA,ALGOUT}}{M_{H2O,ALGOUT}} \quad (4.23)$$

where $F_{DW}$is the fixed fraction of dry weight of the biomass in the digester inlet (equal to 0.2 in this case), $M_{ALGA,ALGOUT}$ and $M_{H2O,ALGOUT}$ are the ALGA and water flowrate in the stream ALGOUT, respectively;

- the nutrient-rich water extracted from the centrifuge is sent to another SPLIT (B1), where almost all is recovered and sent back to the reactor, while a little fraction of it is wanted as a purge to account for losses in the real process operation;

- the stream entering the anaerobic digestion section (ALGAPROD) is first directed through a separator (SEP3), which eliminates all the ions present in the stream, letting water and alga only into the digester. This is a fictitious unit that works to simplify the simulation in terms of convergence, and acts as a purge which avoids the accumulation of counter ions (such as Cl^- and K^+). Furthermore, it allows an easier definition of the yields in the digester and a better understanding of nutrient losses and recovery possibilities. The simulation model for chemical equilibrium is simplified to study the behavior of the 3 macronutrients (C, N, P); it does not account for all the other ions which are normally present in the digestate and in the culture medium (Mg^{2+}, Ca^{2+}, etc..).

- the stream INLETDIG is thus composed of water and biomass only, and is heated up to 35°C to simulate the temperature conditions of a mesophilic digester;

- the anaerobic digestion unit is simulated with a RYield reactor, which allows to set the yields of the products of the process. These yields have been automatically implemented in the unit through a calculator (YIELDSAD). To obtain these values, a fictitious stoichiometry, obtained from atomic balances, was adopted. For a general algal biomass with known molar composition, the reaction is:

$$C_cH_hN_nO_oP_p + \left(-\frac{h}{4} + \frac{11p}{4} - \frac{o}{2} + c - \frac{3n}{4}\right) H_2O \rightarrow$$
$$\rightarrow \left(\frac{h}{8} + \frac{5p}{8} - \frac{o}{4} + \frac{c}{2} - \frac{3n}{8}\right) CH_4 + \left(-\frac{h}{8} - \frac{5p}{8} + \frac{o}{4} + \frac{c}{2} + \frac{3n}{8}\right) CO_2 + nNH_3 + pH_3PO_4 \quad (4.24)$$

where, as said before, *c*, *h*, *n*, *o*, *p* are the molar fractions in the alga of the elements C, H, N, O, P, respectively. After replacing these values for the specific algal biomass composition, the following stoichiometry can be obtained:

$$C_{0.2848}H_{0.4984}O_{0.1749}N_{0.0386}P_{0.0033} + 0.11125H_2O \rightarrow$$

$$\rightarrow 0.1487375\ CH_4 + 0.1360625\ CO_2 + 0.0386NH_3 + 0.0033H_3PO_4 \quad (4.25)$$

The yields in a RYield unit are defined as the mass ratio between the product and the non-inert feed (both water and ALGA); in the case of methane, carbon dioxide, ammonia and phosphoric acid, they were calculated using the following equation:

$$Y_{i,dig} = F_{DW} * BD * \nu_i * \frac{PM_i}{PM_{ALGA}}, \quad (4.26)$$

where F_{DW} is the dry content of algae in the feed, BD is the fixed degree of biodegradability of the microalgal biomass, ν_i is the stoichiometric coefficient of the product *i*, PM_i is its molecular weight. A fraction of algal biomass remains undigested and maintains its original composition, due to partial degradability of cell wall ; in fact, some chemical, thermal or physical pretreatments of the biomass are required prior to the digestion process to enhance the process itself. The yield of the unreacted biomass is:

$$Y_{ALGA} = F_{DW} * (1 - BD) \quad . \quad (4.27)$$

Because of their definition, with respect to the mass balances, the sum of the yields is equal to 1; thus, water yield is calculated subtracting all the other calculated yields from the unity;

- the effluent from the AD unit (MIXDIGST) is sent to a liquid/solid separator (DIGSEP); the algal biomass is sent to the solid product (SOLIDDIG), as well as part of the phosphorus, to consider its loss by precipitation. All other compounds are sent into the aqueous product (LIQGASAD), which is actually a 2-phase mixture (gas/liquid).

- The mixer B3 is just a fictitious unit to restore the Elec_NRTL model for equilibrium calculation; in fact, to avoid charge imbalances, NRTL was adopted as a thermodynamic model in both DIGESTER and DIGSEP blocks. No ionic equilibrium was calculated in the section between the DIGESTER and B3, in which the only species that are present are the digestion products as defined in the RYield unit.

- The stream LIQGASEQ, in which all ionic equilibria are restored, is flashed to separate the BIOGAS from the aqueous effluent. This liquid (LIQADREC) leaves the process as it is considered as a nutrient-rich medium to be stored in a tank and exploited when necessary, together with fresh nutrients supply, accomplishing the purpose of nutrient recycling.

4.6.1 Convergence of the simulation

To facilitate the convergence, which can be hard because of the multiple recycle streams, the following steps are suggested:

- run the simulation with all the recycle streams (except RECALGA) open;
- close RECSEP1;
- run the program again;
- close RECWAT too.

After closing the recycle streams, charge balance warnings may arise in the simulation. They could be due to an inhomogeneous distribution of phosphorus into the two predominant forms of orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}), as stoichiometry establishes that they are consumed at the same rate to produce biomass. Thus, if one of the 2 ions concentration is decreased to zero (while all other electrolytic reactants keep on diminishing as a consequence of the reaction), a charge imbalance will emerge in the PFR. Since this warning may occur even if both phosphate ions are far more than sufficient to carry out the reaction. it may be due to some tolerance setup in the convergence or integration methods of the simulator units. These problems are common when large recycle streams are involved in the simulation. However, if the difference between anion and cation fluxes in all involved streams were more than 3 orders of magnitude lower than their absolute values (relative error < 10^{-3}), and also all the results were reasonable in terms of biomass production and nutrient consumption, it was decided to neglect these warnings. It was seen that the relative error between cations and anions is higher when more recycle streams are closed, in limiting substrates conditions and if pH is too low (<5) or too high (>9).

Apart from closing the recycle streams one by one, according to what is suggested above, another possibility is to keep the pH in the range 5-9 (to achieve a homogeneous phosphorus speciation) every time a recycle stream is closed, by modifying the ratio ammonia/ammonium chloride as explained before.

The pH value towards which the simulation is converging can be checked at each iteration in the text file created by the Fortran subroutine. Thus, if pH is consistently leaning towards excessively low or high values in the reactor, the amount of NH_3 and NH_4Cl can be immediately recalculated and reset in the NUTRIENT stream input to move the PFR pH into the desired range.

If a reactor whose pH is expected to be out of this interval has to be studied, it is suggested to achieve a converged simulation with a pH within the range 5-9 and then move it slowly towards the chosen value without reinitializing the run.

As a further suggestion, it can be noticed that the fictitious pH of the makeup is always higher than the one that is desired in the reactor, also considering the acid effect that CO_2 has on the RECYCLE stream prior to entering the reactor.

4.7 Degrees of freedom

The degrees of freedom of the process considered are:

- the residence time in the reactor τ_{PFR};
- the split ratio (S_{B1}) in the B1 split into the stream RECWAT (i.e. the recycled fraction of that stream containing water and nutrients);
- the P flowrate in the NUTRIENT makeup P_{mu} (supplied as K_2HPO_4). From P_{mu}, the N flowrate N_{mu} (supplied as NH_3 and NH_4Cl in a ratio leading to a reasonable pH in the PFR) is calculated stoichiometrically from the N/P ratio in the biomass composition;
- the biodegradability of the algal biomass in the anaerobic digester (BD);
- the fraction of P in the digestion effluent that is dissolved in the liquid phase ($F_{P,liq}$).

4.8 Base case

4.8.1 N, P, water balances

It is possible to analyze a case study as a base case for the simulation. The degrees of freedom values are summarized in Table 4.9. Complete stream tables are reported in Appendix 3. N, P and water balances are shown in Fig. 4.10, 4.11 and 4.12.

Table 4.9 *Chosen values for the degrees of freedom in the base case simulation.*

Degree of freedom	Chosen value	Motivation
P_{mu} (kg/h)	0.1	to avoid limitation
BD	0.54	literature data
$F_{P,liq}$	0.2	experimental results
τ_{PFR} (days)	1	experimental setup
S_{B1}	0.97	centrifuge operation

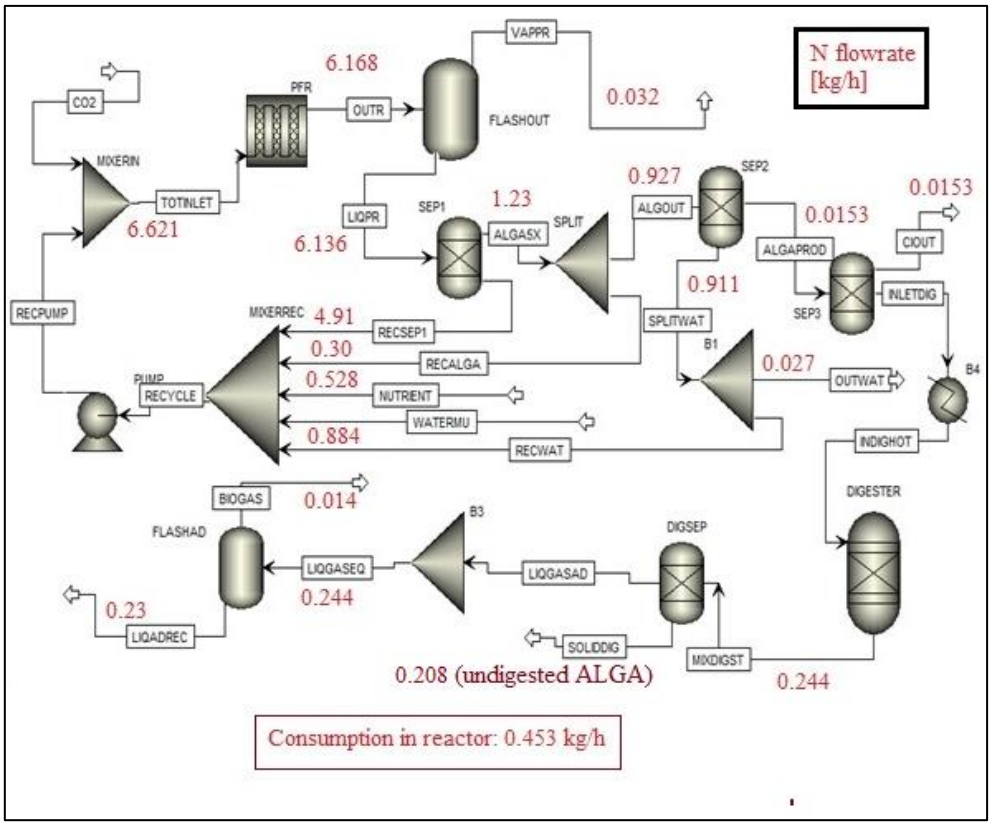

Figure 4.10 *N balances, in terms of mass flow rates (kg/h of N), in the final flowsheet.*

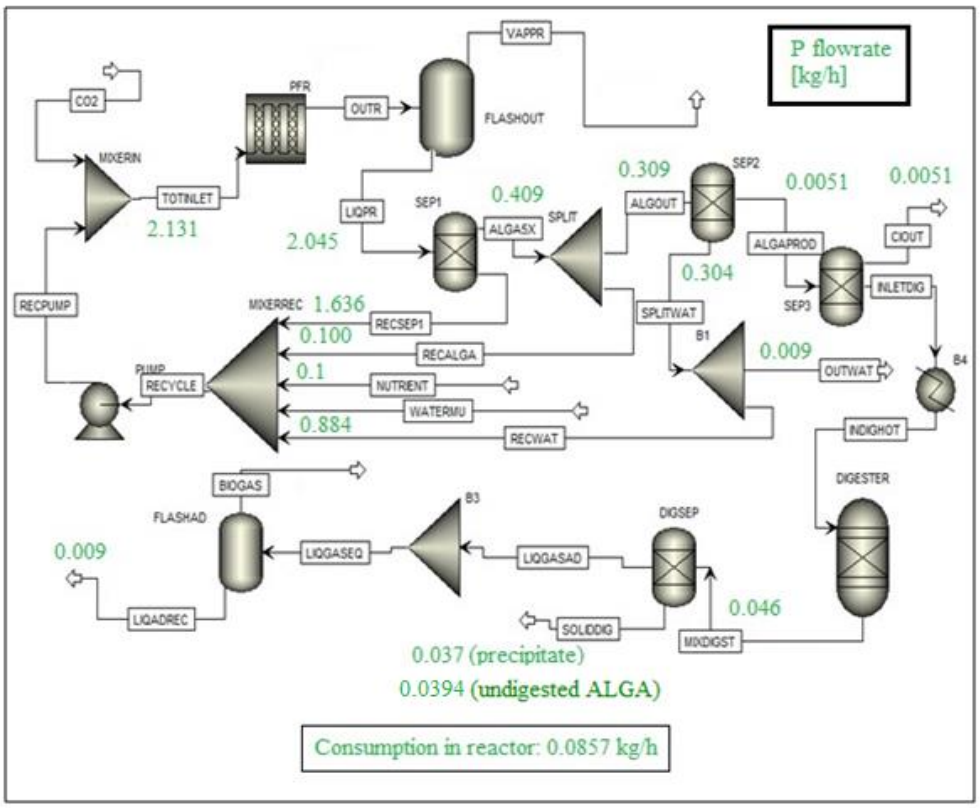

Figure 4.11 *P balances, in terms of mass flow rates (kg/h of P), in the final flowsheet.*

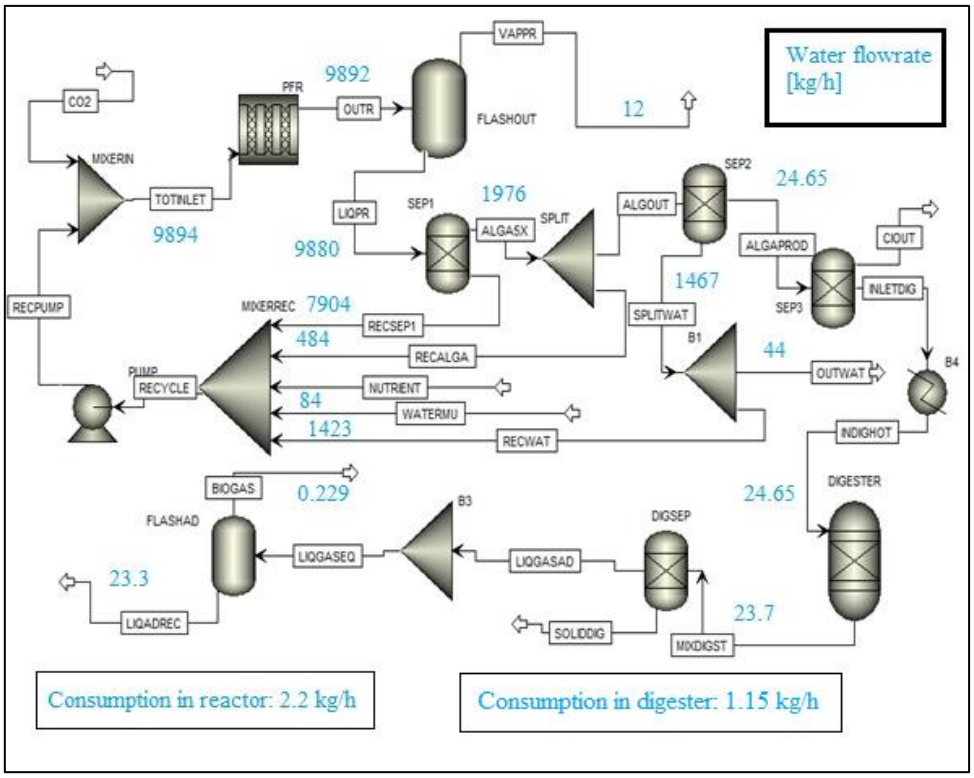

Figure 4.12 *Water balances, in terms of mass flow rates (kg/h of water), in the final flowsheet.*

Table 4.10 shows some additional data related to the PFR (an output from FORTRAN subroutine).

Table 4.10 *Additional data related to the PFR in the base case.*

Inlet biomass concentration C_{xin} (g/l)	0.2
Outlet biomass concentration C_{xout} (g/l)	0.82
Max specific growth rate μ_{max} (d^{-1})	1.55
ALGA production (kg/h)	6.163
Reaction rate R [kg/(sm^3)]	1.10
Rate reduction factors due to limitation:	
limitC	0.906
limitN	0.967
limitP	0.992
Reactor liquid phase holdup (m^3)	238
Reactor total volume (m^3)	10660
pH reactor INLET	7.51
pH reactor OUTLET	7.61

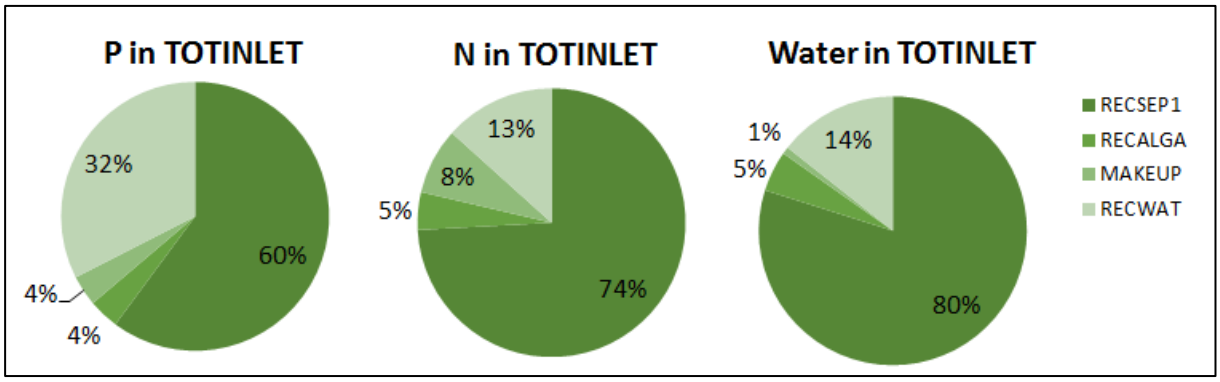

Figure 4.13 *P, N and water contributions in recycle and makeup streams to the reactor inlet flowrate of the same compound.*

Fig. 4.13 shows the share of the makeup and several recycle streams to the P, N and water flowrates in the PFR inlet. It can be observed that in all three cases the highest contribution is given by the nutrient-rich water recycle from the sedimentation unit (RECSEP1), followed by the one recovered from the centrifuge (RECWAT). Much smaller fractions of N, P and water are provided to the reactor by the recycle stream containing the biomass (RECALGA) and by the nutrient/water makeup. It is noteworthy that the liquid coming out of AD can be stored separately to be used as a nutrient source. Furthermore, the ammonia that is lost in the gaseous streams (BIOGAS and VAPR) can be easily recovered, through absorption in water, and reused as a nitrogen source for algal cultivations. Note that the stream CIOUT comes from a fictitious unit (SEP3), which is only functional to the simulation, but does not exist in the actual plant. Therefore, the N and P in CIOUT are not actually lost and can be assumed as completely recycled. These three aspects reduce the actual amount of fresh makeup to be supplied to the process, with the shares depicted in Fig. 4.14.

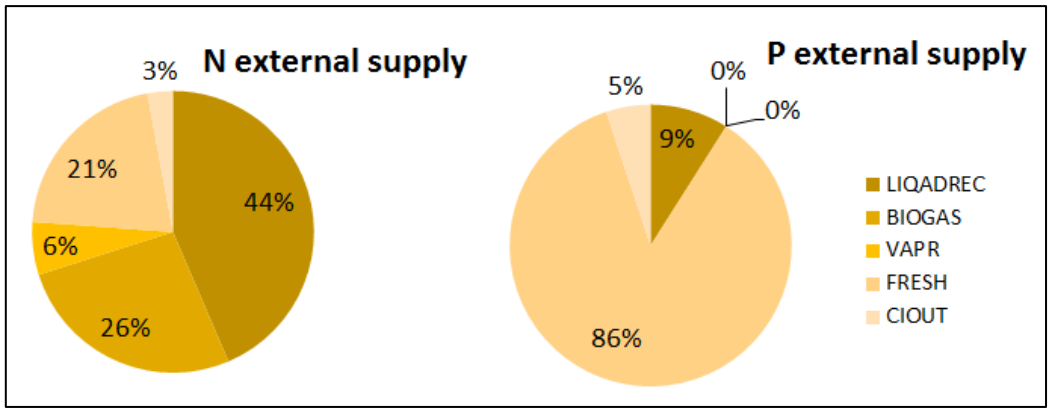

Figure 4.14 *Distribution of N and P in the external nutrient supplies, including the liquid recycled from AD, the ammonia absorbed from BIOGAS and VAPR, the actual fresh nutrient makeup and fictitious stream CIOUT.*

N is mostly provided by the recycle stream from AD (LIQADREC) and from the biogas, while, unfortunately, in this case, most P has to be provided as fresh input (as a phosphate salt).

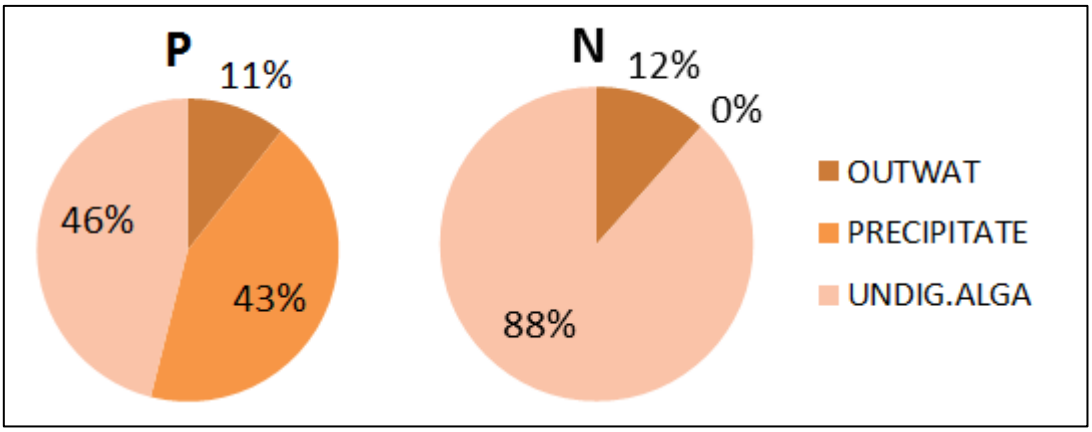

Figure 4.15 *Distribution of P and N in the stream representing nutrient losses.*

Fig. 4.15 shows the distribution of P and N losses in the outlet streams that cannot be recovered, corresponding to the nutrient-rich water lost in the centrifuge (OUTWAT), the solid precipitate in the digestate and the nutrients assimilated into the algal biomass which is not degraded during the anaerobic digestion process. Phosphorus is mainly wasted in the precipitate and in undigested alga, while nitrogen is mainly lost in the latter form, as it is reasonably assumed to be completely soluble in the liquid fraction of the digestate.

4.8.2 Areal requirement and geometric characteristics of the reactor

The energy balance for the PBR was also considered, by assuming an annual solar irradiation at middle latitudes of about 4500 MJ/(m^2yr) and a photosynthetic efficiency of 0.07 (Sforza *et al.*, 2014); the maximum areal productivity obtainable with the available solar energy in Padua resulted:

$$43\,\frac{g}{day*m^2} = 1.792 * 10^{-3}\,\frac{kg}{h*m^2} = P_{m,PD} \quad . \tag{4.28}$$

In the base case, to assure a biomass production $ALGA_{prod} = 6.163 kg/h$, the areal requirement is:

$$A_{sur} = \frac{6.163\frac{kg}{h}}{1.792*10^{-3}\frac{kg}{h*m^2}} = 3440\ m^2 = 0.344\ ha \quad . \tag{4.29}$$

The reactor length is considered as the one of the PFR (L=3760 m), to keep all the profiles along its space coordinate valid; thus, the width *W* of an hypothetical open pond having the shape of a channel with rectangular section is :

$$W = \frac{A_{sur}}{L} = \frac{3440}{3760} m = 0.915\ m \quad . \tag{4.30}$$

Considering the volumetric productivity of the biomass as the ratio between the difference of its concentrations at the outlet and the inlet of the reactor and the residence time

$$P_x = \frac{C_{xout} - C_{xin}}{\tau_{PFR}} = \frac{0.62 \frac{kg}{m^3}}{24\ h} = 0.0258 \frac{kg}{m^3 h} \quad , \qquad (4.31)$$

the height of the liquid level in the pond can be calculated as:

$$H = \frac{P_{m,PD}}{P_x} = \frac{\left(1.792 * 10^{-3} \frac{kg}{hm^2}\right)}{0.0258\ \frac{kg}{hm^3}} = 0.070\ m = 7\ cm \qquad (4.32)$$

The velocity of the liquid culture in the pond can be calculated by dividing the volumetric flowrate (≈10 m^3/h) by the cross-sectional area of the reactor:

$$v_{pond} = \frac{V_{in}}{HW} = \frac{10 \frac{m^3}{h}}{0.07m * 0.915m} \approx 156 \frac{m}{h} = 0.043 \frac{m}{s} = 4.3\ cm/s \qquad (4.33)$$

The geometric characteristics of the pond are shown in Fig. 4.16.

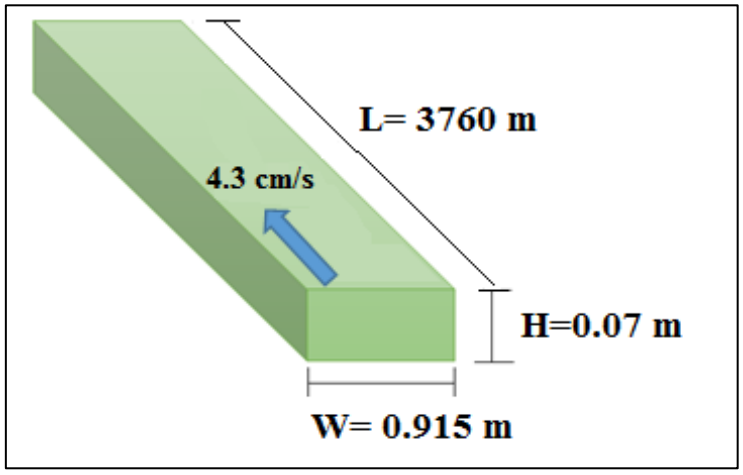

Figure 4.16 *Geometric characteristics of the pond and liquid velocity to ensure the algal productivity of the base case, as a result of energy balances.*

4.9 Sensitivity analysis

A sensitivity analysis was performed to understand the effect of the degrees of freedom on nutrient loss and on the possibility to reduce the fresh makeup of N and P needed.
The main results and indices are reported below, while in Appendix 4 all the following variables (retrieved from the simulation or calculated) are listed for each case considered:

- nutrient makeup flowrates
- limiting factors for C,N,P;
- algal production $ALGA_{prod}$ (kg/h);

- absolute flowrates of N and P (kg/h) in streams VAPR, BIOGAS, OUTWAT, SOLIDDIG (as precipitate, for P only);

- absolute losses of N (N_{UA}) and P (P_{UA}), in kg/h, in the stream SOLIDDIG due to the undigested alga, calculated as:

$$N_{UA} = ALGA_{prod} * (1 - BD) * w_{N,ALGA} \tag{4.34}$$

$$P_{UA} = ALGA_{prod} * (1 - BD) * w_{P,ALGA} \tag{4.35}$$

  where $w_{N,ALGA}$ and $w_{P,ALGA}$are the mass fractions in the algal biomass, as listed in Table 4.2;

- percent reductions $N_{red,AD}$ and $P_{red,AD}$ in the nutrients makeups needed, thanks to the addition of an anaerobic digestion section to the plant:

$$N_{red,AD} = \frac{N_{ext,AD} - N_{ext,noAD}}{N_{ext,noAD}} * 100 \qquad P_{red,AD} = \frac{P_{ext,AD} - P_{ext,noAD}}{P_{ext,noAD}} * 100 \tag{4.36}$$

  The external supplies of N and P may include the AD section in the flowsheet ($N_{ext,AD}$, $P_{ext,AD}$) or exclude it ($N_{ext,noAD}$, $P_{ext,noAD}$). They are calculated by subtracting the flowrates of the nutrients in the streams that can be effectively recovered from the fresh NUTRIENT makeup, according to:

$$N_{ext,AD} = N_{NUTRIENT} - N_{VAPR} - N_{CIOUT} - N_{LIQADREC} - N_{BIOGAS} \tag{4.37}$$

$$N_{ext,noAD} = N_{NUTRIENT} - N_{VAPR} - N_{CIOUT} \tag{4.38}$$

$$P_{ext,AD} = P_{NUTRIENT} - P_{CIOUT} - P_{LIQADREC} \tag{4.39}$$

$$P_{ext,noAD} = P_{NUTRIENT} - P_{CIOUT} \quad . \tag{4.40}$$

  These variables are useful to understand the actual reduction in the fresh nutrient makeup, if anaerobic digestion were adopted as a nutrient recycling technique. Thus, they are reported everywhere in the sensitivity analysis;

- the total specific losses, in g/(kg ALGA produced) of N ($Nwast$) and P ($Pwast$), calculated by dividing the absolute losses by $ALGA_{prod}$. They include the N and P flowrates in OUTWAT and SOLIDDIG (this solid stream contains two contributions to the loss, one due to precipitation and one due to the nutrient content of the undigested algal biomass);

- the percent of the wasted N and P ($\%loss_{N/P}$) with respect to the whole amounts of N and P which are necessary to produce 1 kg/h of ALGA:

$$\%loss_N = \frac{100*(Nwast)}{(Nwast)+(Nassim)}; \quad \%loss_P = \frac{100*(Pwast)}{(Pwast)+(Passim)} \tag{4.41}$$

where $Nassim$ and $Passim$ are the N and P actually assimilated by the algal biomass. The goal in this case is to understand how much of the supplied nutrient is effectively stored in the microalgae and how much is wasted due to inefficiencies in nutrient recycling. These variables are reported in the tables summarizing the results of the sensitivity study.

4.9.1 Sensitivity on P flowrate

A first sensitivity analysis was performed varying the phosphorus makeup (N is calculated accordingly, in a stoichiometric ratio with P). The results are shown in Table 4.11. It can be noticed that the higher is P_{mu}, the more nutrients are lost with respect to what is stored in the algal biomass, and the lower is the reduction in the makeup due to anaerobic digestion. Though rising P_{mu}may seem unfavorable from a nutrient point of view, it is suggested to avoid nutrient limitation in the reactor, because this would decrease the performances in terms of biomass production. This would require a greater reactor volume and thus a higher land requirement to obtain the same algal productivity. Indeed, this operational reason led to the choice of 0.1 as P_{mu} for the base case simulation.

Table 4.11 *Results of the sensitivity analysis at different P makeup flowrates (BD=0.54, $F_{P,liq}$=0.2,τ_{PFR}=1d, S_{B1}=0.97.*

P_{mu} (kg/h)	N_{mu} (kg/h)	*limit N*	*limit P*	*limit C*	ALGA prod (kg/h)	% N makeup reduction thanks to AD	% P makeup reduction thanks to AD	%lossN (on total N needed for 1 kg of alga)	% lossP (on total P needed for 1 kg of alga)
0.1	0.528	0.97	1	0.9	6.19	-50.7	-9.91	34.6	50.0
0.08	0.423	0.9	0.98	0.91	5.36	-53.0	-10.40	29.5	45.0
0.05	0.264	0.71	0.9	0.92	3.54	-53.7	-10.78	21.1	34.2
0.01	0.053	0.23	0.69	0.92	0.713	-54.0	-10.85	5.08	9.42

4.9.2 Sensitivity on biodegradability in the anaerobic digester

From Table 4.12, it can be observed that the higher is the biodegradability of the microalgae in the anaerobic digester, the greater is the reduction of nutrient makeup due to presence of the AD section. Furthermore, with an increased biodegradability, less nitrogen is wasted with respect to the one assimilated by the algal biomass. The reduction effect is much less evident on phosphorus. This is due to the fact that where more biomass is degraded, more phosphorus is surely given off by the algae, but at the same time more P precipitates as insoluble phosphate salts. The effects change significantly if the sensitivity analysis on biodegradability is performed at a higher fraction of solubilized P ($F_{P,liq}$): in fact, in this case (Table 4.13), a

higher biodegradability corresponds to a remarkably lower loss of P to produce 1 kg of algal biomass.

Table 4.12 *Results of a first sensitivity analysis at different biodegradability values in the AD unit ($F_{P,liq}$=0.2,τ_{PFR}=1d, S_{B1}=0.97, P_{mu}=0.1 kg/h).*

BD	**% N makeup reduction thanks to AD**	**% P makeup reduction thanks to AD**	**%lossN (on total N needed for 1 kg of alga)**	**% lossP (on total P needed for 1 kg of alga)**
0.300	-28.4	-5.53	43.2	51.1
0.540	-51.0	-9.87	34.2	50.0
0.700	-66.1	-12.8	26.5	49.2
0.850	-80.2	-15.5	17.4	48.4

Table 4.13 *Results of a second sensitivity analysis at different biodegradability values in the AD unit ($F_{P,liq}$=0.6,τ_{PFR}=1d, S_{B1}=0.97, P_{mu}=0.1 kg/h).*

BD	**% N makeup reduction thanks to AD**	**% P makeup reduction thanks to AD**	**%lossN (on total N needed for 1 kg of alga)**	**% lossP (on total P needed for 1 kg of alga)**
0.300	-28.4	-16.4	43.2	48.1
0.540	-51.0	-29.4	34.2	43.9
0.700	-66.1	-38.1	26.5	40.7
0.850	-80.2	-46.2	17.4	37.4

Besides, more P can obviously be recovered and used to reduce the fresh amount of makeup. This analysis shows that between the two variables $F_{P,liq}$ and *BD*, the former is more relevant if the goal is to improve the possibility to recycle the expensive P thanks to anaerobic digestion. So, the priority is to find a particular treatment that enhances phosphorus dissolution in the liquid fraction of the digestate. After solving this problem, it will also be highly useful to increase algal biodegradability (through several pretreatments of the biomass prior to digestion) to release more nutrient from the lipid-spent biomass and make it available for new algal cultivations.

4.9.3 Sensitivity on P recovery in the liquid fraction

In this case, Table 4.14 confirms that, if a treatment of the digestate were capable to increase the amount of phosphorus available in the aqueous phase of the digestate, less makeup would be necessary and less P would be wasted to produce 1 kg of ALGA (with respect to the assimilated one). It can easily be seen that no effect is found with respect to nitrogen.

Table 4.14 *Results of the sensitivity analysis at different P recoveries in the liquid phase of the digestion effluent (τ_{PFR}=1d, S_{B1}=0.97, P_{mu}=0.1 kg/h, BD=0.54)*

$F_{P.liq}$	% N makeup reduction thanks to AD	% P makeup reduction thanks to AD	%lossN (on total N needed for 1 kg of alga)	% lossP (on total P needed for 1 kg of alga)
0.2	-51.0	-9.87	34.2	50.0
0.4	-51.0	-19.6	34.2	47.1
0.6	-51.0	-29.4	34.2	43.9
0.8	-51.0	-39.2	34.2	40.3

4.9.4 Sensitivity on residence time in the PBR

As reported in Table 4.15, a higher residence time leads to a greater biomass production, but the effect is a lot more evident when increasing it from 0.5 d to 1 d than when increasing it from 1 d to 2 d. The same asymptotic behavior can be noticed in the reduction in N and P makeup quantities and in the N loss with respect to the N stored in the biomass. Instead, a minimum is shown in P loss percent (at 1 d), which decreases significantly from 0.5 to 1 d of residence time. Also due to the possible operational and building costs, it seems useless to increase τ_{PFR} above an optimal value (in this case 1 d), in terms of the losses and the recovery possibilities of the two macronutrients.

Table 4.15 *Results of the sensitivity analysis at different P recoveries in the liquid phase of the digestion*

τ_{PFR} (d)	ALGA prod (kg/h)	% N makeup reduction thanks to AD	% P makeup reduction thanks to AD	%lossN (on total N needed for 1 kg of alga)	% lossP (on total P needed for 1 kg of alga)
0.5	2.09	-36.3	-14.5	27.0	48.6
1	6.16	-51.0	-9.87	34.2	50.0
1.5	7.13	-52.6	-6.33	34.9	50.9
2	7.17	-52.7	-6.25	34.9	51.0

4.9.5 Effect of increasing the recycle in RECWAT

Table 4.16 shows that increasing the recycle form RECWAT (which is the nutrient-rich stream coming from the centrifugation unit) has a positive effect in terms of algal production and nutrient saving, as less N and P are lost in the stream OUTWAT.
However, it seems unlikely to be able to recover practically all the water from the centrifuge, as some purge streams must always be present in a process.

Table 4.16 *Effect of increasing the recycled fraction of nutrient-rich water from the centrifuge ($F_{P,liq}$=0.2,τ_{PFR}=1d, S_{B1}=0.97, P_{mu}=0.1 kg/h).*

S_{B1}	ALGA prod (kg/h)	% N makeup reduction thanks to AD	% P makeup reduction thanks to AD	%lossN (on total N needed for 1 kg of alga)	% lossP (on total P needed for 1 kg of alga)
0.97	6.16	-51.0	-9.87	34.2	50.0
0.99	6.24	-53.1	-13.3	32.9	49.0

4.10 CO_2 absorption in the culture medium

A last point addressed was the possibility of avoiding a gas feed in the PFR, by supplying the carbon source in the form of carbonates rather than of CO_2. Therefore, a simple simulation flowsheet, based on an absorption tower (Fig. 4.17), was developed to accomplish:

1. understanding if the electrolytes coming from the nutrient makeup influence negatively CO_2 absorption in the culture medium;
2. evaluating the loss of ammonia by volatilization in the product as a result of a stripping performed by the gaseous stream.

The thermodynamic model for the equilibrium calculation was ElecNRTL, as before. The flowsheet is composed of a simple RadFrac unit, characterized by 8 ideal stages, with no reboiler nor condenser, working at T=28°C and P=1 atm. The stream FLUEGAS entering the column at the bottom contains CO_2 and N_2 at a fixed composition. A certain amount of CO_2 and N_2 is dissolved into the liquid stream H2O, which is fed to the top of the absorber. The CO_2-enriched liquid stream (WATERCO2) exits the column at the bottom, while the gaseous stream which is poorer in CO_2 (FLUEOUT), is sent out from the top. Three cases are defined, varying the flowrate and the composition of the gaseous stream, with or without nutrients in the liquid feed in each case:

1. total mass flowrate of 400000 l/h, 10 % v/v of CO_2 (as in the previous simulation);
2. total mass flowrate of 400000 l/h, 100 % v/v of CO_2;
3. total mass flowrate of 40000 l/h, 100 % v/v of CO_2.

To represent a similar situation as in the reactor in the main simulation flowsheet, water feed flowrate was set to a value of 10000 kg/h, while nutrient flowrates, when present, were fixed at 2.5, 11 and 5 kg/h of NH_3, NH_4Cl, and K_2HPO_4, respectively, to reproduce the base case conditions of 494 mg/l N, 89 mg/l P.

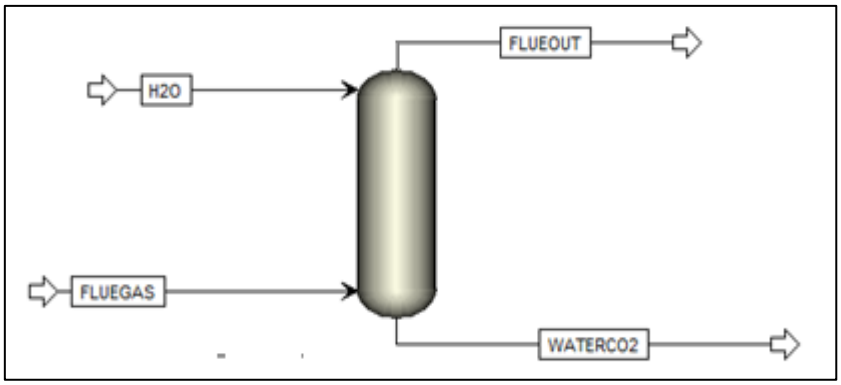

Figure 4.17 *Scheme of the CO_2 absorption tower.*

The loss of ammonia by volatilization is expressed as the % of N in the stream FLUEOUT with respect to N in the inlet stream H2O. The results are summarized in Table 4.17.

Table 4.17 *Results of the analysis performed on CO2 absorption in water and ammonia loss, in different conditions of gas flowrate/composition, with or without nutrients in the liquid stream.*

Case	**Gas feed**	**Nutrients in the liquid feed**	**Dissolved CO_2 (kg/h)**	**N-NH_3 loss (%)**
1	400000 l/h 10% v/v CO2	No	1.35	-
		Yes	1.33	0.105
2	400000 l/h pure CO2	No	13.4	-
		Yes	13.2	0.01
3	40000 l/h pure CO2	No	13.2	-
		Yes	13.0	0.001

These concluding remarks are proposed:

- nutrients do not interfere in a sensible way with CO_2 absorption;
- ammonia loss due to air stripping is irrelevant (even lower in the case of pure CO_2);
- the amount of CO_2 that is dissolved in the liquid stream from the pure gas is remarkably higher (approximately tenfold) than the one in the case of CO_2 10% v/v, independently of the total flow rate of the stream with CO_2 100% v/v, which could therefore be minimized.

The results confirm that it would be more convenient, in terms of mass transfer and absorbed amounts of CO_2, to feed pure carbon dioxide to the culture; it would be much more expensive in terms of the operating costs of the photobioreactor, but it would allow to reduce its volume of orders of magnitude, with all the advantages in terms of building costs and land

requirements.

According to the base case, where approximately 6 kg/h of algal biomass are produced, the flowrate of CO_2 that is fixed by the microalgae (i.e. consumed in the reactor) is:

$$ALGA_{prod} * w_{c,alga} * \frac{PM_{CO_2}}{PM_C} = \frac{6\ kg}{h} * 0.4645 * \frac{44}{12} = 10.2\ kg/h$$

If all the needed flowrate of carbon dioxide had to be dissolved and consumed in one single step, the dissolved amount from a flowrate of 400000 l/h at 10%v/v would not be sufficient. However, as the reaction goes on along the PFR, thanks to the thermodynamic equilibrium, some CO_2 is solubilized from the gas to the liquid phase to compensate for the one that is consumed in the aqueous medium. In this way, 10.2 kg/h are consumed in total (summing up the mass balance of CO_2 in both liquid and vapor phase) in the PBR. On the other hand, when more than 10.2 kg/h of CO_2 are absorbed, it is theoretically possible to run the PFR without a gas phase inlet.

To complete this analysis, an example of the profile of the mass fraction of CO_2 dissolved in water along the absorption column is shown in Fig. 4.18. The mass fraction increases from stage 1 (top) to stage 8 (bottom), but the profile is almost horizontal in most of the column ideal stages, showing that their number is higher than the one actually needed to accomplish the absorption process.

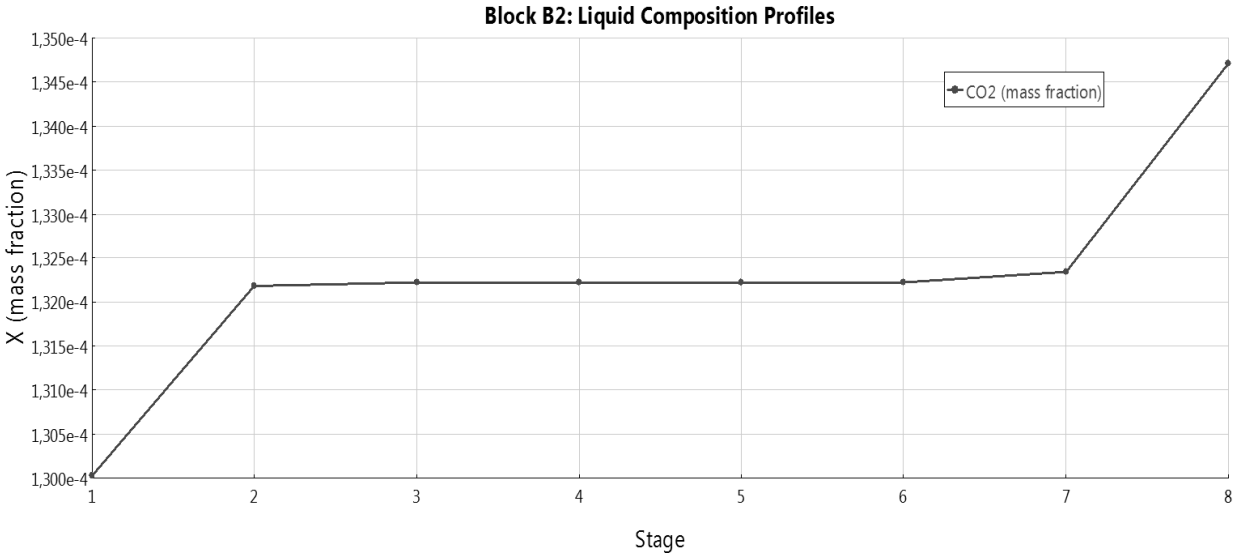

Figure 4.18 *Profile of the CO_2 mass fraction (case 1 with nutrients) in the liquid along the absorbing column ideal stages (1=top, 8=bottom).*

Conclusions

In order to make third generation biofuels sustainable and more competitive with fossil fuels, a study was performed on nutrient recycling from the biomass after lipid extraction. This residual fraction retains almost all the nitrogen and the phosphorus assimilated by microalgae. In this case, anaerobic digestion was studied as a technology to specifically recover these elements. An extraction with solvent was performed to evaluate the lipid content of microalgal species *Chlorella vulgaris*. The residual biomass was used as substrate in a lab scale anaerobic digestion, to evaluate the biochemical methane potential (BMP) and to recycle the effluent as nutrient source for the cultivation. The digestate was centrifuged to separate the solid and the liquid fraction. The latter was then analyzed to point out deficiencies in macro- and micronutrients with respect to the synthetic medium (control). Phosphorus and sulphur were found to be the limiting components: P ended up almost completely in the solid precipitate as insoluble phosphate salts; S was mainly lost in the biogas as H_2S. Instead, ammonium-nitrogen and micronutrients resulted to be in a sufficient concentration to sustain algal cutltures. Batch growth curves were carried out using the liquid digestate as main nutrient source, but the results showed that the addition of both $H_2PO_4^{2-}$ and SO_4^{2-} was necessary to assure a final biomass concentration comparable to the control. Several methods were tested to increase phosphorus dissolution from the solid and availability in the liquid fraction of the digestate. In terms of P extraction and hypothetical costs on an industrial scale, the most efficient one proved to be the treatment of the mixture with sodium bicarbonate. The pretreated digestate was filtered and used as nutrient source for an algal cultivation, with SO_4^{2-} as the only external supply. The final biomass concentration and the growth rates showed a sensible improvement with respect to untreated digestate with no addition, though still being lower than control. Some micronutrient lacks due to interaction with sodium bicarbonate and precipitation will have to be investigated in this case. Furthermore, sulphur loss in the biogas digester should be avoided, in order to be able to recycle this nutrient too to the cultivation. Some design solutions in the digester could be adopted, such as biofilters, sulphur oxidizing bacteria and microaeration. However, the priority in the future is to improve the recovery of P in the liquid fraction of the digestate. This could be achieved through combined chemical treatments of the digestate or design changes in the digester, such as a two-stage configuration or anaerobic membrane bioreactors (AMBR). A second aim was to develop a model for the simulation of the industrial scale process of microalgal cultivation with enhanced nutrient recovery.

As far as the simulation of the sole reaction process is considered, the results are validated by experimental data in terms of nutrient consumption, pH and biomass productivity. This confirms the reasonability of the choice of model and parameters. The complete flowsheet,

instead, allowed to understand the contribution of the anaerobic digestion to the possibility of reducing the external supply. However, first of all, it is necessary to recycle as much water as possible from the separation units (sedimenter and centrifuge), not only to reduce the water footprint of the process, but also to recover to the cultivation section all the nutrients that it contains. A base case showed that, despite the improvements on phosphorus solubilisation into the liquid fraction of the digestate, the anaerobic digestion does not greatly affect the makeup requirements of phosphate fertilizers. Instead, it greatly reduces the need of external nitrogen supply (almost 80% less). This difference is due to the fact that, while ammonium nitrogen is almost completely soluble in the aqueous phase, most of the P is lost in the precipitate. To increase the solubility of phosphorus into the liquid fraction of the AD effluent is the absolute priority. Until this goal is reached, enhancing the biodegradability of the alga would be quite useless in terms of P recycling. A combination of improving algal biodegradability and P recovery in the liquid digestate could cut up to half of the external phosphate fertilizer needs. The sensitivity analysis showed that increasing the residence time of the reactor to a value greater than 1 d is not worthwhile, as it contributes only to increase volumes and land requirements, without a substantial advantage. The simulation of the absorption of CO_2 in nutrient-rich water showed that no major negative interference between CO_2 and other nutrients availability in the medium is present. In fact, CO_2 is solubilized in a sufficient amount to sustain algal growth, and the stripping of ammonia by the gaseous stream is irrelevant. So, it will be possible to perform the cultivation by feeding carbon in a liquid medium, rather than as gaseous CO_2.

Appendix A

Fortran subroutine and compiling procedure

```
C     User Kinetics Subroutine for RPLUG
C
      SUBROUTINE pfrnew   (SOUT,   NSUBS,  IDXSUB,   ITYPE,  NINT,
     2                     INT,    NREAL,  REAL,     IDS,    NPO,
     3                     NBOPST, NIWORK, IWORK,    NWORK,  WORK,
     4                     NC,     NR,     STOIC,    RATES,  FLUXM,
     5                     FLUXS,  XCURR,  NTCAT,    RATCAT, NTSSAT,
     6                     RATSSA, KCALL,  KFAIL,    KFLASH, NCOMP,
     7                     IDX,    Y,      X,        X1,     X2,
     8                     NRALL,  RATALL, NUSERV,   USERV,  NINTR,
     9                     INTR,   NREALR, REALR,    NIWR,   IWR,
     *                     NWR,    WR,     NRL,      RATEL,  NRV,
     1                     RATEV)
C
      IMPLICIT NONE
C
C     DECLARE VARIABLES USED IN DIMENSIONING
C
      INTEGER NSUBS, NINT,  NPO,   NIWORK,NWORK,
     +        NC,    NR,    NTCAT, NTSSAT,NCOMP,
     +        NRALL, NUSERV,NINTR, NREALR,NIWR,
     +        NWR
C
C.....RPLUG...
#      include "rplg_rplugi.cmn"
#      include "rplg_rplugr.cmn"

c      DECLARE PFR VARIABLES

      EQUIVALENCE (XLEN, RPLUGR_UXLONG)
      EQUIVALENCE (DIAM, RPLUGR_UDIAM)
C
C.....REACTOR PROPERTIES...
#     include "rxn_rprops.cmn"
      EQUIVALENCE (TEMP, RPROPS_UTEMP)
      EQUIVALENCE (PRES, RPROPS_UPRES)
      EQUIVALENCE (VFRAC, RPROPS_UVFRAC)
      EQUIVALENCE (BETA, RPROPS_UBETA)
      EQUIVALENCE (VVAP, RPROPS_UVVAP)
      EQUIVALENCE (VLIQ, RPROPS_UVLIQ)
      EQUIVALENCE (VLIQS, RPROPS_UVLIQS)

#     include "shs_stwork.cmn"
C
C     INITIALIZE RATES
C
C     DECLARE ARGUMENTS
C
      INTEGER IDXSUB(NSUBS),ITYPE(NSUBS), INT(NINT),
     +        IDS(2),NBOPST(6,NPO),IWORK(NIWORK),
```

```
     +        IDX(NCOMP),   INTR(NINTR),  IWR(NIWR),
     +        NREAL, KCALL, KFAIL, KFLASH,NRL,
     +        NRV,   I
      REAL*8 SOUT(1),      WORK(NWORK),
     +       STOIC(NC,NSUBS,NR),  RATES(1),
     +       FLUXM(1),     FLUXS(1),     RATCAT(NTCAT),
     +       RATSSA(NTSSAT),      Y(NCOMP),
     +       X(NCOMP),     X1(NCOMP),    X2(NCOMP)
      REAL*8 RATALL(NRALL),USERV(NUSERV),
     +       REALR(NREALR),WR(NWR),      RATEL(1),
     +       RATEV(1),     XCURR, vol, k, d, PM(7)
C
C     DECLARE LOCAL VARIABLES
C
      INTEGER IMISS
      REAL*8 REAL(NREAL),  RMISS, XLEN,  DIAM,  TEMP,
     +       PRES,  VFRAC, BETA,  VVAP,  VLIQ,
     +       VLIQS,M(9), C(6), V, tau(1), R, MM(2),
     +        deno, CO2,fosf, NH4, pOH,pH

C
C     BEGIN EXECUTABLE CODE
C
      k = 0.0000179  ! s-1, ovvero 1.55 day-1

! restituzione della portata molare totale kmol/s
       M(2) = SOUT(NCOMP_NCC+1)

! portata molare totale in fase liquida  kmol/s
       MM(1) = M(2)*(1-vfrac)

! restituzione del PM della miscela (kg/kmol)
       PM(1) = SOUT(NCOMP_NCC+9)
       PM(2) = 44                                ! PM CO2
      PM(3) = 18                  ! PM NH4+
      PM(4) = 17                  ! PM OH-
      PM(5) = 96.97               ! PM H2PO4-
      PM(6) = 19                  ! PM H3O+
      PM(7) = 95.97               ! PM HPO4--

! portata volumetrica calcolata m3/s

       V = MM(1)*(stwork_vl)    !stwork_vl=volume molare miscela

! calcolo del tempo di permanenza
       tau(1) = VLIQ*XLEN/V        !vliq=CROSS-SECTIONAL AREA (m2), V=portata
volumetrica

! portata ponderale di alghe kg/s
       M(1) = SOUT(ncomp_ncc+9+1)

! calcolo della portata ponderale di CO2, NH4+,OH- e PO4--- kg/s in fase liquida
       M(3) = MM(1)*X(3)*PM(2)           !CO2
      M(4) = MM(1)*X(5)*PM(3)    !NH4+
      M(5) = MM(1)*X(6)*PM(4)    ! OH-   !viene la stessa cosa nei due modi
      M(6) = MM(1)*X(11)*PM(5)   !H2PO4-
      M(7) = MM(1)*X(8)*PM(6)    !H3O+
      M(8) = MM(1)*X(14)*PM(7)   !HPO4--

! calcolo della concentrazione di alghe e di nutrienti   kg/m3
       C(1) = M(1)/V   ! conc alghe
```

```
       C(2) = M(3)/v  ! conc CO2
      C(3) = M(4)/v   ! conc NH4+
      C(4) = M(5)/v  ! conc OH-
      C(5) = (M(6)+M(8))/v  ! conc H2PO4- + HPO4--
      C(6) = M(7)/v  ! conc H3O+


! calcolo della velocità di reazione [kg/m3 s]

       deno=(4.752e-3+C(2))*(4.943e-3+C(5))*(24.84e-3+C(3))
       CO2=C(2)/(4.752e-3+C(2))
       fosf=C(5)/(4.943e-3+C(5))
       NH4=C(3)/(24.84e-3+C(3))
       R=k*C(1)*C(2)*C(3)*C(5)/deno
       pOH = - log10(C(4)/15)
       pH = - log10(C(6)/19)

        RATES(Ncomp_ncc+1) = R*(vliq)                          ! biomassa
! i rates dell'alga sono in kg/s. i rates di quelli convenzionali sono in kmol/s. MAH.
        !quindi moltiplica per nui e dividi per PMalga
       RATEs(4) =  -0.029903493*R*vliq       !acqua
       RATEs(5) = -0.005246703*R*(vliq)     ! ammonio
       RATEs(3) = -0.038711431*R*(vliq)        ! CO2
       RATEs(1) =  0.040386706*R*(vliq)        ! ossigeno
       RATES(11)= -0.000224276*R*(VLIQ)    ! diidrogenofosfato
       rates(8) = 0.004573875*R*vliq         !H3O+
        rates(14)= -0.000224276*R*(VLIQ)    ! MONOidrogenofosfato


c  WRITE RESULTS IN A TXT FILE-----------------------------------------------

      open(1,FILE='PFRRESULTS.dat')

       write (1, *) tau(1)/3600, "tempo in h"
       write (1, *) c(2), "concentrazione co2 in g/l"
       write (1, *) c(1), "concentrazione bio in g/l"
       write (1, *) V, "portata volumetrica in m3/s"
       write (1, *) PM(1), "peso molecolare miscela"
      write (1, *) Rates(1), "velocità produzione O2"
      write (1, *) Rates(3), "velocità consumo CO2"
      write (1, *) Rates(5), "velocità consumo ammonio"
      write (1, *) R, "velocità di reazione R"
      write (1, *) c, "concentr kg/m3__ alghe CO2 NH4+ OH- H2PO4- H30+ "
      write (1, *) M, "portate ponderali kg/s"
      write (1, *) X, "frazioni molari "
      write (1, *) deno, "denominatore totale"
      write (1, *) CO2, "correzione veloc per CO2 limitante"
      write (1, *) NH4, "correzione veloc per NH4 limitante"
      write (1, *) fosf, "correzione veloc per fosfati limitanti"
      write (1,*) 100*vliq/(vliq+vvap), "% fase liquida reattore"
      write (1,*) 100*vvap/(vliq+vvap), "% fase gassosa reattore"
      write (1,*) MM(1), "portata molare totale in fase liquida"
      write(1,*) k*3600*24, "veloc di reaz max specifica, day-1"
      write (1,*) pOH, "pOH reattore"
      write (1,*) pH, "pH reattore"

       close(1,STATUS='keep')

      RETURN
      END
```

To compile the program (called “pfrnew), the Fortran source pfrnew.f , written in Visual Studio 2013, must be compiled by the Fortran compiler using the `aspcomp` procedure. For example, in an Aspen Plus Simulation Engine window (available in Start | Programs | AspenTech | Process Modeling <version> | Aspen Plus), type:

```
aspcomp pfrnew.f
```

In order to be able to compile the program, the window must be referring to the same position (directory) in which the `pfrnew.f` file is present. To do so, if the directory “`nameofthedirectory`”, which contains the Fortran file `pfrnew.f`, is placed into the local disk (C:), the command is:

```
cd C:\nameofthedirectory
```

The simplest method of supplying Fortran user models to Aspen Plus is by putting the user model’s object module files (which appears as the result of the aspcomp command) in the same run directory of the Aspen Plus file. In this case, the object module file would be called “`pfrnew.obj`”. During the run, the Aspen Plus simulator, in which PFRNEW was set as the subroutine for the reaction kinetics, calls the file `pfrnew.obj` and executes all the statements that are present.

Appendix B

Stream tables for the base case in the simplified flowsheet

	ALGA	CO2	LIQPR	NUTRIENT	OUTR	TOTINLET	VAPPR	WATER	WATNUTAL
Substream: MIXED									
Mass Flow kg/hr									
O2	0	114,2357	0,0837907	0	121,8049	114,2357	121,7211	0	0
N2	0	352,4096	0,1246512	0	352,4096	352,4096	352,2849	0	0
CO2	0	37,40833	0,3831579	0	22,04069	30,81657	21,65753	0	0
H2O	0	0	9983,207	1,00E-08	9995,124	9997,305	11,91712	10000	9999,997
NH4+	0	0	5,703927	3,710154	5,703927	6,2658	0	0	3,709256
OH-	0	0	6,51E-05	4,73E-16	6,51E-05	5,52E-05	0	1,91E-05	3,05E-03
PO4---	0	0	6,46E-05	8,33E-03	6,46E-05	6,06E-05	0	0	4,10E-03
H3O+	0	0	9,34E-06	7,66E-21	9,34E-06	1,09E-05	0	2,13E-05	1,87E-07
AMMONIA	0	0	0,0768319	2,499238	0,0908168	0,0833177	0,0139849	0	2,500086
NH2COO-	0	0	9,32E-03	0	9,32E-03	0,0105884	0	0	0
H2PO4-	0	0	0,6680732	4,16E-03	0,6680732	0,8086411	0	0	0,0220753
HCO3-	0	0	7,446208	0	7,446208	9,111267	0	0	0
CO3--	0	0	0,0157995	0	0,0157995	0,0168783	0	0	0
HPO4--	0	0	1,841912	2,742739	1,841912	1,954966	0	0	2,729284
K+	0	0	2,244728	2,244728	2,244728	2,244728	0	0	2,244728
HCL	0	0	2,63E-12	1,80E-13	2,96E-12	3,31E-12	3,32E-13	0	5,66E-14
CL-	0	0	7,290652	7,290652	7,290652	7,290652	0	0	7,290652
H3PO4	0	0	2,30E-06	8,33E-12	2,30E-06	3,19E-06	1,53E-21	0	1,63E-09
Total Flow kmol/hr	0	17	554,9003	0,6441974	572,4342	572,5788	17,53391	555,0844	555,7285
Total Flow kg/hr	0	504,0536	10009,1	18,5	10516,69	10522,55	507,5947	10000	10018,5
Total Flow l/hr	0	399838,14	10035,588	3556,053	442983,78	441155,22	432948,24	10036,62	10049,904
Temperature K		301,15	301,15	301,15	301,15	300,8291	301,15	301,15	301,2035
Pressure atm	1	1,05	1	1	1	1	1	1	1
Substream: $TOTAL									
Total Flow kg/hr	2	504,0536	10016,95	18,5	10524,55	10524,55	507,5947	10000	10020,5
Substream: NC									
Mass Flow kg/hr									
ALGA	2	0	7,857145	0	7,857145	2,0001	0	1,00E-04	2,0001

Appendix C

Stream tables for the base case in the complete flowsheet

	ALGA5X	ALGAPROD	ALGOUT	BIOGAS	CIOUT	CO2	INDIGHOT	INLETDIG
phase	LIQUID	LIQUID	LIQUID	VAPOR	MIXED	VAPOR	LIQUID	LIQUID
Substream: MIXED								
Mass Flow kg/hr								
O2	0,016123	2,01E-04	1,22E-02	0	2,01E-04	114,282	0	0
N2	0,0239	2,98E-04	1,80E-02	0	2,98E-04	352,5522	0	0
CO2	0,090832	1,13E-03	0,06858	2,111799	2,28E-02	37,42347	0	0
H2O	1975,952	24,65488	1491,901	0,229233	1,75E-02	0	24,65488	24,65488
NH4+	1,534098	1,91E-02	1,16E+00	0	1,04E-02	0	0	0
OH-	3,42E-05	4,26E-07	2,58E-05	0	2,69E-11	0	6,03E-08	4,70E-08
PO4---	1,67E-04	2,08E-06	1,26E-04	0	8,54E-05	0	0	0
H3O+	9,35E-07	1,17E-08	7,06E-07	0	9,57E-14	0	6,74E-08	5,26E-08
AMMONIA	3,97E-02	4,96E-04	3,00E-02	1,69E-02	5,53E-04	0	0	0
NH2COO-	1,66E-02	2,07E-04	1,25E-02	0	2,91E-02	0	0	0
H2PO4-	1,26E-01	1,57E-03	9,52E-02	0	3,21E-04	0	0	0
HCO3-	4,816311	6,01E-02	3,636452	0	1,18E-03	0	0	0
CO3--	3,15E-02	3,93E-04	2,38E-02	0	1,42E-07	0	0	0
HPO4--	1,141362	1,42E-02	8,62E-01	0	1,54E-02	0	0	0
AMMON-01	0	0	0	0	0	0	0	0
DIPOT-01	0	0	0	0	0	0	0	0
K+	7,25072	9,05E-02	5,474501	0	9,05E-02	0	0	0
HCL	7,86E-13	9,81E-15	5,94E-13	0	4,20E-15	0	0	0
CL-	5,854686	7,31E-02	4,420455	0	7,31E-02	0	0	0
H3PO4	1,58E-07	1,97E-09	1,19E-07	2,66E-24	9,95E-12	0	0	0
CH4	0	0,00E+00	0,00E+00	0,939982	0,00E+00	0	0	0
Total Flow kmol/hr	110,2164	1,375221	83,2165	0,120296	7,16E-03	17,00688	1,368554	1,368554
Total Flow kg/hr	1996,895	24,91618	1507,713	3,297948	2,61E-01	504,2577	24,65488	24,65488
Total Flow l/hr	1992,306	24,8589	1504,248	3154,66	14,04	400000	24,8004	24,7452
Temperature K	301,15	301,15	301,15	320,6045	301,15	301,15	308,15	301,15
Pressure atm	1	1	1	1	1	1,05	1	1
Vapor Frac	0	0	0	1	7,90E-02	1	0	0
Liquid Frac	1	1	1	0	0,920987	0	1	1
Substream: $TOTAL								
Total Flow kg/hr	2005,058	31,0799	1513,876	3,297948	2,61E-01	504,2577	30,8186	30,8186
Substream: NC								
Mass Flow kg/hr								
ALGA	8,163558	6,16372	6,16372	0	0	0	6,16372	6,16372

	LIQADREC	LIQGASAD	LIQGASEQ	LIQPR	MIXDIGST	NUTRIENT	OUTR	OUTWAT
phase	LIQUID	MIXED	MIXED	LIQUID	MIXED	MIXED	MIXED	LIQUID
Substream: MIXED								
Mass Flow kg/hr								
O2	0	0	0	0,080615	0	0	122,3274	3,59E-04
N2	0	0	0	0,119502	0	0	352,6709	5,32E-04
CO2	8,23E-03	2,712581	2,120029	0,454158	2,712581	0	27,25175	2,02E-03
H2O	23,29417	23,74857	23,5234	9879,762	23,74857	1,00E-08	9891,742	44,01737
NH4+	0,258092	0	0,258092	7,67049	0	1,05E-01	7,67049	3,42E-02
OH-	2,24E-06	0	2,24E-06	1,71E-04	0	4,69E-16	1,71E-04	7,61E-07
PO4---	1,72E-05	0	1,72E-05	8,35E-04	0	1,03E-02	8,35E-04	3,72E-06
H3O+	1,14E-08	0	1,14E-08	4,68E-06	0	1,02E-20	4,68E-06	2,08E-08
AMMONIA	2,03E-02	0,297323	3,72E-02	1,99E-01	0,297323	0,542098	2,38E-01	8,85E-04
NH2COO-	5,80E-02	0	5,80E-02	8,29E-02	0	0	8,29E-02	3,69E-04
H2PO4-	1,53E-03	0	1,53E-03	0,630557	0	2,39E-04	0,630556	2,81E-03
HCO3-	0,746672	0	0,746672	24,08155	0	0	24,08155	0,107291
CO3--	1,57E-02	0	1,57E-02	0,157287	0	0	0,157287	7,01E-04
HPO4--	2,72E-02	0	2,72E-02	5,706808	0	0,298679	5,706809	2,54E-02
AMMON-01	0	0	0	0	0	0	0	0
DIPOT-01	0	0	0	0	0	0	0	0
K+	0	0	0	36,2536	0	0,251993	36,2536	0,161521
HCL	0	0	0	3,93E-12	0	8,07E-15	4,45E-12	1,75E-14
CL-	0	0	0	29,27343	0	2,03E-01	29,27343	1,30E-01
H3PO4	1,33E-09	2,93E-02	1,33E-09	7,89E-07	0,146532	3,18E-13	7,89E-07	3,52E-09
CH4	0,138299	1,078281	1,078281	0	1,078281	0	0	0,00E+00
Total Flow kmol/hr	1,331092	1,464852	1,451388	551,0818	1,466048	5,31E-02	568,7634	2,455238
Total Flow kg/hr	24,56811	27,86606	27,86606	9984,473	27,98329	1,4122	10498,09	44,48389
Total Flow l/hr	25,17126	3622,788	3179,765	9961,53	3622,783	779,2194	446548,2	44,3817
Temperature K	320,6045	308,15	320,6026	301,15	308,15	301,15	301,15	301,15
Pressure atm	1	1	1	1	1	1	1	1
Vapor Frac	0	0,09715	0,082882	0	0,097067	0,597985	0,031088	0
Liquid Frac	1	0,90285	0,917118	1	0,902933	0,402016	0,968912	1
Substream: $TOTAL								
Total Flow kg/hr	24,56811	27,86606	27,86606	9992,637	30,8186	1,4122	10506,25	44,48389
Substream: NC								
Mass Flow kg/hr								
ALGA	0	0	0	8,163558	2,835311	0	8,163558	0

	RECALGA	RECSEP1	RECWAT	RECYCLE	SOLIDDIG	SPLITWAT	TOTINLET	VAPPR	WATERMU
phase	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	MIXED	VAPOR	LIQUID
Substream: MIXED									
Mass Flow kg/hr									
O2	3,95E-03	0,064492	1,16E-02	0,080055	0	1,20E-02	114,362	122,2468	0
N2	5,85E-03	0,095602	1,72E-02	0,118672	0	1,77E-02	352,6709	352,5514	0
CO2	0,022251	0,363326	0,065424	0,190612	0	0,067447	36,36188	26,79759	0
H2O	484,0518	7903,81	1423,228	9894,534	0	1467,246	9893,983	11,98019	83,51492
NH4+	0,37581	6,136392	1,10E+00	7,954433	0	1,14E+00	8,301606	0	0
OH-	8,37E-06	1,37E-04	2,46E-05	4,06E-04	0	2,54E-05	1,31E-04	0,00E+00	1,59E-07
PO4---	4,09E-05	6,68E-04	1,20E-04	2,20E-03	0	1,24E-04	6,77E-04	0,00E+00	0
H3O+	2,29E-07	3,74E-06	6,74E-07	1,99E-06	0	6,95E-07	5,91E-06	0,00E+00	1,78E-07
AMMONIA	9,74E-03	1,59E-01	2,86E-02	4,87E-01	0	2,95E-02	1,95E-01	3,91E-02	0
NH2COO-	4,06E-03	6,63E-02	1,19E-02	2,01E-01	0	1,23E-02	7,50E-02	0,00E+00	0
H2PO4-	3,09E-02	0,504445	9,08E-02	0,293501	0	9,36E-02	0,801133	0	0
HCO3-	1,179858	19,26524	3,469066	23,93585	0	3,576357	26,04075	0	0
CO3--	7,71E-03	0,12583	2,27E-02	0,371034	0	2,34E-02	0,134612	0	0
HPO4--	0,279601	4,565447	8,22E-01	6,304343	0	8,48E-01	5,803521	0	0
AMMON-01	0	0	0	0	0	0	0	0	0
DIPOT-01	0	0	0	0	0	0	0	0	0
K+	1,776219	29,00288	5,222509	36,2536	0	5,38403	36,2536	0	0
HCL	1,93E-13	3,14E-12	5,66E-13	1,67E-12	0	5,84E-13	5,26E-12	5,18E-13	0
CL-	1,434231	23,41874	4,216981	29,27343	0	4,347403	29,27343	0	0
H3PO4	3,87E-08	6,31E-07	1,14E-07	1,55E-07	0,117225	1,17E-07	1,24E-06	5,49E-22	0
CH4	0,00E+00	0	0,00E+00	0	0	0,00E+00	0	0	0
Total Flow kmol/hr	26,99985	440,8654	79,38604	551,9342	1,20E-03	81,84128	568,9127	17,68161	4,635783
Total Flow kg/hr	489,1821	7987,578	1438,312	10000	0,117225	1482,796	10504,26	513,615	83,51492
Total Flow l/min	488,0579	7969,224	1435,007	9976,098	0,0942	1479,389	443923,9	436586,6	83,82078
Temperature K	301,15	301,15	301,15	301,1824	308,15	301,15	300,5572	301,15	301,15
Pressure atm	1	1	1	1	1	1	1	1	1
Vapor Frac	0	0	0	0	0	0	0,030955	1	0
Liquid Frac	1	1	1	1	1	1	0,969045	0	1
Substream: $TOTAL									
Total Flow kg/hr	491,1819	7987,578	1438,312	10002	2,952536	1482,796	10506,26	513,615	83,51502
Substream: NC									
Mass Flow kg/hr									
ALGA	1,999838	0	0	1,999955	2,835311	0	1,999955	0	1,00E-04

Appendix D

Complete results of the sensitivity analysis

D.1 Sensitivity on P flowrate in the makeup

BD	0.54
$F_{P.liq}$	0.2
τ_{PFR} (d)	1
S_{B1}	0.97

P_{mu} (kg/h)	N_{mu} (kg/h)	*limit N*	*limit P*	*limit C*	ALGA prod (kg/h)	Loss N in VAPR (kg/h)	Loss N in BIOGAS (kg/h)	N_{UA} (kg/h)	Loss N in OUTWAT (kg/h)	TOT N loss in sol./liq. (kg/h)
0.1	0.528	0.97	1	0.9	6.19	0.0253	0.0140	0.209	0.0302	0.239
0.08	0.423	0.9	0.98	0.91	5.36	0.0161	0.0121	0.181	0.0078	0.189
0.05	0.264	0.71	0.9	0.92	3.54	0.0013	0.0080	0.120	0.0019	0.121
0.01	0.053	0.23	0.69	0.92	0.713	0.0002	0.0016	0.0241	0.0001	0.024

P_{mu} (kg/h)	Loss P in OUTWAT (kg/h)	P_{UA} (kg/h)	Loss P in SOLIDDIG (PRECIP) (kg/h)	Total P loss (kg/h)	% N makeup reduction thanks to AD	% P makeup reduction thanks to AD
0.1	0.0088	0.0396	0.0373	0.0857	-50.7	-9.91
0.08	0.0035	0.0343	0.0323	0.0701	-53.0	-10.40
0.05	0.0006	0.0226	0.0213	0.0445	-53.7	-10.78
0.01	0.0001	0.0046	0.0043	0.0089	-54.0	-10.85

P_{mu} (kg/h)	Specific loss N in VAPR (g/kg alga prod)	Specific loss N in BIOGAS (g/kg alga prod)	Specific N_{UA} (g/kg alga prod)	Specific loss N in OUTWAT (g/kg alga prod)	Total N loss in sol./liq (g/kg alga prod)	%lossN (on total N needed for 1 kg of alga)
0.1	4.11	2.27	33.9	4.90	38.9	34.6
0.08	2.61	1.97	29.4	1.27	30.7	29.5
0.05	0.218	1.30	19.4	0.309	19.7	21.1
0.01	0.0332	0.262	3.91	0.0153	3.93	5.08

P_{mu} (kg/h)	Specific loss P in OUTWAT (g/kg alga prod)	Specific P_{UA} (g/kg alga prod)	Specific loss P in SOLIDDIG (PRECIP) (g/kg alga prod)	Total P loss (g/kg alga prod)	% lossP (on total P needed for 1 kg of alga)
0.1	1.43	6.43	6.02	13.9	50.0
0.08	0.575	5.57	6.02	11.4	45.0
0.05	0.0917	3.67	6.02	7.21	34.2
0.01	0.0089	0.741	6.02	1.45	9.42

D.2 Sensitivity on biodegradability in the anaerobic digester

CASE 1	
P_{mu} **(kg/h)**	0.1
$F_{P.liq}$	0.2
τ_{PFR} **(d)**	1
S_{B1}	0.97

BD	**ALGA prod (kg/h)**	**Loss N in VAPR (kg/h)**	**Loss N in BIOGAS (kg/h)**	N_{UA} **(kg/h)**	**Loss N in OUTWAT (kg/h)**	**TOT N loss in sol./liq. (kg/h)**
0.300	6.16	0.0321	0.0022	0.317	0.0273	0.344
0.540	6.16	0.0321	0.0139	0.208	0.0273	0.236
0.700	6.16	0.0321	0.0290	0.136	0.0273	0.163
0.850	6.16	0.0321	0.0482	0.0679	0.0273	0.095

BD	**Loss P in OUTWAT (kg/h)**	P_{UA} **(kg/h)**	**Loss P in SOLIDDIG (PRECIP) (kg/h)**	**Total P loss (kg/h)**	**% N makeup reduction thanks to AD**	**% P makeup reduction thanks to AD**
0.300	0.0091	0.0600	0.0206	0.0897	-28.4	-5.53
0.540	0.0091	0.0394	0.0371	0.0856	-51.0	-9.87
0.700	0.0091	0.0257	0.0481	0.0829	-66.1	-12.8
0.850	0.0091	0.0129	0.0584	0.0803	-80.2	-15.5

BD	**Specific loss N in VAPR (g/kg alga prod)**	**Specific loss N in BIOGAS (g/kg alga prod)**	**Specific** N_{UA} **(g/kg alga prod)**	**Specific loss N in OUTWAT (g/kg alga prod)**	**Total specific N loss in sol./liq (g/kg alga prod)**	**%lossN (on total N needed for 1 kg of alga)**
0.300	5.21	2.26	51.4	4.44	55.9	43.2
0.540	5.21	2.19	33.8	4.44	38.2	34.2
0.700	5.21	2.12	22.0	4.44	26.5	26.5
0.850	5.21	2.06	11.0	4.44	15.5	17.4

BD	**Specific loss P in OUTWAT (g/kg alga prod)**	**Specific** P_{UA} **(g/kg alga prod)**	**Specific loss P in SOLIDDIG (PRECIP) (g/kg alga prod)**	**Total P loss (g/kg alga prod)**	**% lossP (on total P needed for 1 kg of alga)**
0.300	1.48	9.73	3.34	14.6	51.1
0.540	1.48	6.40	6.02	13.9	50.0
0.700	1.48	4.17	7.80	13.4	49.2
0.850	1.48	2.09	9.47	13.0	48.4

CASE 2	
P_{mu} **(kg/h)**	0.1
$F_{P.liq}$	0.6
τ_{PFR} **(d)**	1
S_{B1}	0.97

BD	**ALGA prod (kg/h)**	**Loss N in VAPR (kg/h)**	**Loss N in BIOGAS (kg/h)**	N_{UA} **(kg/h)**	**Loss N in OUTWAT (kg/h)**	**TOT N loss in sol./liq. (kg/h)**
0.300	6.16	0.0321	0.0021	0.317	0.0273	0.344
0.540	6.16	0.0321	0.0131	0.208	0.0273	0.236
0.700	6.16	0.0321	0.0273	0.136	0.0273	0.163
0.850	6.16	0.0321	0.0457	0.0679	0.0273	0.095

BD	**Loss P in OUTWAT (kg/h)**	P_{UA} **(kg/h)**	**Loss P in SOLIDDIG (PRECIP) (kg/h)**	**Total P loss (kg/h)**	**% N makeup reduction thanks to AD**	**% P makeup reduction thanks to AD**
0.300	0.009	0.060	0.010	0.0794	-28.4	-16.4
0.540	0.009	0.039	0.019	0.0671	-51.0	-29.4
0.700	0.009	0.026	0.024	0.0589	-66.1	-38.1
0.850	0.009	0.013	0.029	0.0512	-80.2	-46.2

BD	**Specific loss N in VAPR (g/kg alga prod)**	**Specific loss N in BIOGAS (g/kg alga prod)**	**Specific** N_{UA} **(g/kg alga prod)**	**Specific loss N in OUTWAT (g/kg alga prod)**	**Total specific N loss in sol./liq (g/kg alga prod)**	**%lossN (on total N needed for 1 kg of alga)**
0.300	5.21	0.335	51.4	4.44	55.9	43.2
0.540	5.21	2.12	33.8	4.44	38.2	34.2
0.700	5.21	4.44	22.0	4.44	26.5	26.5
0.850	5.21	7.41	11.0	4.44	15.5	17.4

BD	**Specific loss P in OUTWAT (g/kg alga prod)**	**Specific** P_{UA} **(g/kg alga prod)**	**Specific loss P in SOLIDDIG (PRECIP) (g/kg alga prod)**	**Total P loss (g/kg alga prod)**	**% lossP (on total P needed for 1 kg of alga)**
0.300	1.48	9.73	1.67	12.9	48.1
0.540	1.48	6.40	3.01	10.9	43.9
0.700	1.48	4.17	3.90	9.5	40.7
0.850	1.48	2.09	4.74	8.3	37.4

D.3 Sensitivity on P recovery in the liquid fraction of the digestate

P_{mu} (kg/h)	0.1
BD	0.54
τ_{PFR} (d)	1
S_{B1}	0.97

$F_{P.liq}$	ALGA prod (kg/h)	Loss N in VAPR (kg/h)	Loss N in BIOGAS (kg/h)	N_{UA} (kg/h)	Loss N in OUTWAT (kg/h)	TOT N loss in sol./liq. (kg/h)
0.2	6.16	0.0321	0.0139	0.208	0.0273	0.236
0.4	6.16	0.0321	0.0135	0.208	0.0273	0.236
0.6	6.16	0.0321	0.0131	0.208	0.0273	0.236
0.8	6.16	0.0321	0.0127	0.208	0.0273	0.236

$F_{P.liq}$	Loss P in OUTWAT (kg/h)	P_{UA} (kg/h)	Loss P in SOLIDDIG (PRECIP) (kg/h)	Total P loss (kg/h)	% N makeup reduction thanks to AD	% P makeup reduction thanks to AD
0.2	0.0091	0.0394	0.0371	0.0856	-51.0	-9.87
0.4	0.0091	0.0394	0.0278	0.0763	-51.0	-19.6
0.6	0.0091	0.0394	0.0185	0.0671	-51.0	-29.4
0.8	0.0091	0.0394	0.0093	0.0578	-51.0	-39.2

$F_{P.liq}$	Specific loss N in VAPR (g/kg alga prod)	Specific loss N in BIOGAS (g/kg alga prod)	Specific N_{UA} (g/kg alga prod)	Specific loss N in OUTWAT (g/kg alga prod)	Total specific N loss in sol./liq (g/kg alga prod)	%lossN (on total N needed for 1 kg of alga)
0.2	5.21	2.26	33.8	4.44	38.2	34.2
0.4	5.21	2.19	33.8	4.44	38.2	34.2
0.6	5.21	2.12	33.8	4.44	38.2	34.2
0.8	5.21	2.06	33.8	4.44	38.2	34.2

$F_{P.liq}$	Specific loss P in OUTWAT (g/kg alga prod)	Specific P_{UA} (g/kg alga prod)	Specific loss P in SOLIDDIG (PRECIP) (g/kg alga prod)	Total P loss (g/kg alga prod)	% lossP (on total P needed for 1 kg of alga)
0.2	1.48	6.40	6.02	13.9	50.0
0.4	1.48	6.40	4.51	12.4	47.1
0.6	1.48	6.40	3.01	10.9	43.9
0.8	1.48	6.40	1.50	9.4	40.3

D.4 Sensitivity on residence time in the PBR

P_{mu} (kg/h)	0.1
BD	0.54
$F_{P.liq}$	0.2
S_{B1}	0.97

τ_{PFR} (d)	ALGA prod (kg/h)	Loss N in VAPR (kg/h)	Loss N in BIOGAS (kg/h)	N_{UA} (kg/h)	Loss N in OUTWAT (kg/h)	TOT N loss in sol./liq. (kg/h)
0.5	2.09	0.250	0.0047	0.0705	0.0971	0.168
1	6.16	0.0321	0.0139	0.208	0.0273	0.236
1.5	7.13	0.0007	0.0161	0.241	0.0018	0.243
2	7.17	0.0002	0.0162	0.242	0.0004	0.243

τ_{PFR} (d)	Loss P in OUTWAT (kg/h)	P_{UA} (kg/h)	Loss P in SOLIDDIG (PRECIP) (kg/h)	Total P loss (kg/h)	% N makeup reduction thanks to AD	% P makeup reduction thanks to AD
0.5	0.0551	0.0133	0.0125	0.0810	-36.3	-14.5
1	0.0091	0.0394	0.0371	0.0856	-51.0	-9.87
1.5	0.0005	0.0456	0.0429	0.0890	-52.6	-6.33
2	0.0002	0.0459	0.0431	0.0892	-52.7	-6.25

τ_{PFR} (d)	Specific loss N in VAPR (g/kg alga prod)	Specific loss N in BIOGAS (g/kg alga prod)	Specific N_{UA} (g/kg alga prod)	Specific loss N in OUTWAT (g/kg alga prod)	Total specific N loss in sol./liq (g/kg alga prod)	%lossN (on total N needed for 1 kg of alga)
0.5	11.4	15.8	21.0	22.2	27.2	27.0
1	33.8	4.43	20.0	21.4	38.2	34.2
1.5	39.1	0.286	18.3	20.0	39.4	34.9
2	39.3	0.0704	18.15	19.8	39.4	34.9

τ_{PFR} (d)	Specific loss P in OUTWAT (g/kg alga prod)	Specific P_{UA} (g/kg alga prod)	Specific loss P in SOLIDDIG (PRECIP) (g/kg alga prod)	Total P loss (g/kg alga prod)	% lossP (on total P needed for 1 kg of alga)
0.5	8.94	2.16	2.03	13.1	48.6
1	1.48	6.40	6.01	13.9	50.0
1.5	0.073	7.40	6.96	14.4	50.9
2	0.0399	7.43	7.00	14.5	51.0

D.5 Effect of increasing water recycle from the centrifuge

P_{mu} (kg/h)	0.1
BD	0.54
$F_{P.liq}$	0.2
τ_{PFR} (d)	1

S_{B1}	ALGA prod (kg/h)	Loss N in VAPR (kg/h)	Loss N in BIOGAS (kg/h)	N_{UA} (kg/h)	Loss N in OUTWAT (kg/h)	TOT N loss in sol./liq. (kg/h)
0.97	6.16	0.0321	0.0139	0.208	0.0273	0.236
0.99	6.24	0.0403	0.0141	0.211	0.0107	0.221

S_{B1}	Loss P in OUTWAT (kg/h)	P_{UA} (kg/h)	Loss P in SOLIDDIG (PRECIP) (kg/h)	Total P loss (kg/h)	% N makeup reduction thanks to AD	% P makeup reduction thanks to AD
0.97	0.0091	0.0394	0.0371	0.0856	-51.0	-9.87
0.99	0.0049	0.0399	0.0375	0.0823	-53.1	-13.3

S_{B1}	Specific loss N in VAPR (g/kg alga prod)	Specific loss N in BIOGAS (g/kg alga prod)	Specific N_{UA} (g/kg alga prod)	Specific loss N in OUTWAT (g/kg alga prod)	Total specific N loss in sol./liq (g/kg alga prod)	%lossN (on total N needed for 1 kg of alga)
0.97	5.21	2.26	7.47	33.8	38.2	34.2
0.99	6.53	2.29	8.82	34.2	35.9	32.9

S_{B1}	Specific loss P in OUTWAT (g/kg alga prod)	Specific P_{UA} (g/kg alga prod)	Specific loss P in SOLIDDIG (PRECIP) (g/kg alga prod)	Total P loss (g/kg alga prod)	% lossP (on total P needed for 1 kg of alga)
0.97	8.94	2.16	2.04	13.9	50.0
0.99	1.48	6.40	6.02	13.3	49.0

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