LARGE SCALE PRODUCTION OF BIOCRUDE FROM MICROALGAE: EXPERIMENTAL MEASUREMENTS AND PROCESS SIMULATIONS TO ASSESS ITS ECONOMIC VIABILITY

Direttore della Scuola: Ch.mo Prof. Paolo Colombo

Coordinatore di indirizzo: Ch.mo Prof. Enrico Savio

Supervisore: Ch.mo Prof. Alberto Bertucco

Dottoranda: Elia Armandina Ramos Tercero

"We are like tenant farmers chopping down the fence around our house for fuel when we should be using Nature's inexhaustible sources of energy - sun, wind and tide. ... I'd put my money on the sun and solar energy. What a source of power! I hope we don't have to wait until oil and coal run out before we tackle that."

-Thomas Alva Edison

In conversation with Henry Ford and Harvey Firestone (1931)

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Abstract

This Ph.D. project has been addressed to evaluate the potential of microalgal technology for biofuel production. Different steps of the process, as well as technologies and concepts have been analyzed experimentally and by process simulations in order to assess the sustainability of the production of biofuel from microalgae.

An experimentation work on microalgae cultivation in untreated wastewaters is reported, including the selection of the optimal wastewater process stream, the nutrients removal efficiencies and the removal rates. Also, the effects of temperature, day/night irradiation and bacterial competition in steady-state biomass production are evaluated in order to integrate both technologies.

Downstream processing has been investigated with respect to anaerobic digestion and hydrothermal liquefaction (HTL) of microalgae biomass. The production of biogas is evaluated using whole and de-oiled microalgae, as a function of inoculum typology and biomass concentration, and the effect of the solvent used for oil extraction is tested. For HTL, the recovery and reuse of the water process is investigated, by recycling it into the HTL system, testing the effects of temperature and the number of recycles on the product yields.

An energy analysis of the entire process, considering several process routes and conditions is presented to verify and optimize its energy profits with respect to EROEI, and eventually a process which is energetically self-sufficient is proposed. Finally a techno-economic analysis of a large-scale plant of biocrude is reported, where data from experimental results and process simulations are used to calculate the oil selling price to achieve revenues from the production of biofuel from microalgae.

Riassunto

É ormai globalmente riconosciuto che l'energia da fonti fossili é in via di esaurimento. Attualmente circa l' 85% del fabbisogno energetico mondiale é prodotto da fonti fossili, ma i problemi di approvvigionamento ricadono sull'intera popolazione, dal momento che l'economia mondiale é molto sensibile alle variazioni del prezzo dei combustibili fossili, causando serie ripercussioni sui settori industriali e politici.

Da un punto di vista ambientale, i combustibili fossili generano una grande quantità di gas serra, con ripercussioni sugli ecosistemi e sulla qualità di vita (Crocker and Andrews, 2010). Lo sviluppo e lo sfruttamento di fonti di energia rinnovabile e sostenibile possono dare un importante contributo alla soluzione di questi problemi. Tra le diverse potenziali fonti di energia rinnovabile, i biocarburanti (biofuels) sembrano destinati a svolgere un ruolo importante nell'organizzazione globale di energia del futuro (Chen and Walker, 2011).

I biocarburanti provenienti da biomassa microalgale sono considerati come una delle alternative migliori e più a breve termine per produrre energia pulita (Amaro et al., 2012; Ghasemi et al., 2012; Mata et al., 2010). Le microalghe sono microorganismi capaci di convertire l'energia solare in energia chimica che può essere sfruttata come combustibili di diverse tipologie sia liquida sia gassosa. Le microalghe hanno numerosi vantaggi, tra cui:

- hanno una crescita rapida, possono raddoppiare la loro biomassa diverse volte al giorno quando sono nella fase esponenziale di crescita (Chisti, 2007);

- presentano una maggiore efficienza fotosintetica in confronto con altre piante oleose terrestri, questa caratteristica permette alle alghe di convertire l'energia luminosa solare in modo più efficiente.

- possono accumulare notevoli quantità di olio nelle loro cellule: la proporzione dei lipidi dipende dal metabolismo e delle condizioni di coltura;

- sono capaci di crescere in una ampia gamma di ambienti acquatici, tra cui i luoghi marginali non sfruttabili a fini agricoli;

- hanno mostrato un uso efficiente di nutrienti: per la crescita richiedono soltanto luce solare, una fonte di carbonio come CO_2 proveniente dai gas di combustione, e sostanze nutritive, in particolare azoto e fosforo che possono essere derivati da acque reflue;

- i lipidi possono essere estratti dalle microalghe e convertiti in biodiesel, mentre la biomassa residua dopo l'estrazione dell'olio (o anche l'intera biomassa prima dell'estrazione), può essere trasformata in etanolo, biogas, bioidrogeno, fornendo altri tipi di biocarburanti rinnovabili (Chisti, 2007).

- oltre ai biocarburanti, dalle microalghe si possono produrre prodotti chimici grezzi e raffinati (come antibiotici, pigmenti, steroidi), grazie alla loro struttura biochimica composta principalmente da proteine, lipidi e carboidrati.

La produzione di biocombustibili da microalghe é stata realizzata in impianti a scala pilota, ma la fattiblità della produzione di tali combustibili a livello industriale é ancora da verificare, così come la possibilità di sostituire in modo significativo i combustibili fossili.

Al giorno d'oggi alcune limitazioni della tecnologia di produzione di alghe sono ancora una questione aperta perché la produzione su larga scala é limitata dalla disponibilità di acqua, dalla fornitura di nutrienti e dalla necessità di ottimizzare il processo di estrazione d'olio e/o conversione della biomassa e dal bilancio energetico del processo. Tutti questi aspetti certamente influenzano il costo di produzione del bio-olio.

La fornitura di acqua dolce é insufficiente per sostenere una produzione costante di combustibili algali in qualsiasi scala. Anche se diverse specie di microalghe possono vivere in differenti tipi di acque (dolce, salmastra, acqua salina e acque reflue), l'acqua dolce é comunque necessaria per compensare le perdite per evaporazione. Anche se viene utilizzata acqua di mare, l'evaporazione dell'acqua cambia la salinità e di conseguenza la crescita e la produttività delle microalghe. Per ridurre al minimo il

consumo di acqua dolce é richiesto l'utilizzo di impianti di coltivazione chiusi e che includono il riciclo di acqua nel processo, riducendo al minimo la perdita (Batan et al., 2013). Inoltre, la disponibilità di nutrienti é un fattore critico. Secondo Chisti (2013) per la produzione di 82 milioni di tonnellate di biomassa algale, sono necessari circa 1,5 miliardi di tonnellate di anidride carbonica, 5,4 milioni di tonnellate di N (44% dell'uso attuale) e 1,1 milioni di tonnellate di P.

Il problema principale per l'azoto e il fosforo é che una richiesta così alta può diminuire la disponibilità di fertilizzanti nella coltivazione agricola. Un'altra fonte di carbonio che é stata studiata recentemente é il bicarbonato (Gardner et al., 2013; Gris et al., 2014): con specie di microalghe in grado di crescere in un mezzo alcalino, il bicarbonato potrebbe sostituire l'anidride carbonica, ma si tratta di una tecnologia in fase di sviluppo e la ricerca sperimentale per l'applicazione industriale é tuttora ad uno stadio preliminare.

Per quanto riguarda il problema dell'approvvigionamento di azoto e fosforo, per la produzione sostenibile di combustibili dalle microalghe é fondamentale il recupero di nutrienti all'interno del processo di produzione di biomassa, compresi quelli rimasti nella biomassa dopo l'estrazione dell'olio. Questi potrebbero essere recuperati attraverso digestione anaerobica (Chisti, 2013), una delle tecnologie più promettenti per il recupero di nutrienti ed energia (Frigon et al., 2013). La fattibilità di questa tecnologia é ancora da verificare poiché non sono disponibili molte informazioni sulla digestione della biomassa dopo l'estrazione dei lipidi. Le acque reflue sembrano essere molto promettenti come fonte di N e P per la crescita microalgale, unendo allo stesso tempo il trattamento biologico delle acque mediante la rimozione di questi componenti e quindi riducendo l'eutrofizzazione nell'ambiente acquatico. Questa tecnologia é stata studiata da molti ricercatori (Aslan and Kapdan, 2006; Pittman et al., 2011; Olguín, 2003), ma é importante valutare il suo impiego nella fornitura di sostanze nutritive in larga scala per la produzione di combustibili ed il contemporaneo trattamento delle acque (Lundquist et al., 2010). I valori presenti in letteratura riferiscono la diminuzione di sopra il 70% di N, P (Wang et al., 2010) con acque reflue sterili. Certamente é necessaro testare condizioni più realistiche, come la geometria del reattore e la configurazione del processo sulla base della concentrazione di nutrienti in acqua, la

competizione tra i batteri e microalghe, o diverse condizioni di coltivazione come la irradiazione solare o temperatura.

Inoltre, per rendere i combustibili microalgali un potenziale sostituto del petrolio é necessario ottimizzare il processo dal punto di vista energetico e aumentare l'efficienza riducendo i costi: i valori di *Energy Return on Energy Investments* (EROEI) del processo riportati in letteratura sono da 0,13 (Brentner et al., 2011) a 2.5 (Vasudevan et al., 2012), ma é richiesto un numero pari almeno ad 1, anche se 7 é il minimo per rendere il processo attraente (Chisti, 2008). Per questo motivo, si richiede una valutazione accurata delle condizioni operative del processo. Ad esempio, la bassa concentrazione di biomassa nella coltura dovuta alle elevate richiesta d'acqua per la crescita (al meno pari a 1%) rende la raccolta di biomassa e la essiccazione processi altamente energivori.

La valutazione energetica del processo deve includere l'analisi di diverse alternative per migliorare le rese di estrazione dell'olio e diversi metodi successivi per sfruttare l'energia dalla biomassa residua. La ricerca potrebbe includere la valutazione di altri possibili processi di conversione di biomassa come la liquefazione con acqua ad altra pressione (HTL), una tecnologia esente da solventi per il recupero dell'olio, la cui efficacia é stata dimostrata da diversi autori (Garcia Alba et al., 2012; López Barreiro et al., 2013a), ma in modo soltanto preliminare.

Dal punto di vista biologico, una serie di miglioramenti potrebbero cambiare il futuro della tecnologia delle microalghe, ad essempio l'aumento dell'efficienza fotosintetica, o la modifica genetica per aumentare l'accumulo di lipidi o di altri componenti (Chisti, 2013).

D'altra parte, dal punto di vista economico la sostenibilità dei processi di produzione di microalghe non é stato completamente chiarito. Valutazioni economiche diverse mostrano grandi variazioni tra loro, ed ogni singola differenza nel processo ha ripercussioni nei costi, dalla geometria del reattore alla trasformazione della biomassa e persino all'ubicazione dell'impianto, poiché questo dipo di processo richiede ingenti infrastrutture con relativo elevato costo d'investimento (Davis et al., 2011).

L'obiettivo della ricerca presentata in questa Tesi é di valutare il potenziale della tecnologia per la produzione di olio da microalghe, analizzando diverse alternative

e concetti sia in modo sperimentale che tramite simulazioni del processo.

Il **Capitolo 1** é una discussione introduttiva sulla situazione mondiale delle microalghe, recenti studi e gli ultimi risultati riportati su questa tecnologia. Dal punto di vista sperimentali, nei **Capitoli 2 e 3** di questa tesi si sono approfonditi, la coltivazione di microalghe in acque reflue e la capacità che presentano alcune specie di microalghe di crescere in acque reflue non trattate, verificata con la microalga *Chlorella protothecoides*. La crescita é stata valutata in acque provenienti da diversi step del di trattamento delle acque, per selezionare la stream ottimale per la crescita delle microalghe. Inoltre, é stata testata la efficienza nella rimozione di nutrienti e i tassi di rimozione in acque reflue reali. Nel **Capitolo 2** si riportano anche la produzione di biomassa in stato stazionario con alimentazione continua del effluente. Nel **Capitolo 3** sono stati studiati gli effetti della temperatura, l'irradiazione in ciclo giorno/notte e la competizione batterica sulla crescita di *C. protothecoides*, e la rimozione dei nutrienti con l'obiettivo d'integrare entrambe tecnologie in un approccio realistico. É stato infine proposto uno schema modificato dell'impianto di depurazione.

Il **Capitolo 4** presenta il lavoro sperimentale per la valutazione della capacità di produzione di biogas da microalghe e le loro velocità di degradazione nel processo di digestione anaerobica: sono state testate diverse condizioni come la tipologia dell'inoculo batterico e la concentrazione della biomassa algale all'inizio delle prove. Inoltre, questo capitolo riporta anche la ricerca nel recupero del contenuto energetico dalla biomassa residua dopo l'estrazione di olio, dimostrando che il metodo di estrazione dell'olio é un fattore importante. La produzione di biogas e sua corrispondente frazione di metano sono stati testati considerando l'effetto della miscela di solvente usato nella estrazione, e i risultati sono stati confrontati con quelli della biomassa microalgale prima della estrazione.

Il **Capitolo 5** é focalizzato sulla conversione di biomassa mediante il processo di liquefazione idrotermica (HTL) che viene svolto a temperature tra 200 °C e 375 °C (la pressione é quella necessaria per mantenere l'acqua in stato liquido), ed é caratterizzato da alte rese. Tuttavia, uno dei sottoprodotti é una fase acquosa con alto contenuto di componenti organici che deve essere trattata adeguatamente per evitare ulteriori costi. In questo capitolo si riporta il lavoro sperimentale svolto con l'obiettivo di recuperare

e riutilizzare l'acqua di processo mediante un riciclo nel sistema stesso. Inoltre si é misurato l'effetto della temperatura e del numero di ricicli nelle rese di produzione di olio, gas, residuo solido e la fase acquosa e la composizione dei prodotti.

Nel **Capitolo 6** si riporta l'analisi energetica del processo per la produzione di biocrudo: lo studio é stato svolto considerando diverse tipologie e condizioni di processo, i quali sono stati modellati e simulati dal simulatore di processo Aspen Plus^{TM}, col fine di verificare e ottimizzare i profitti energetici rispetto all'analisi del EROEI, e di proporre un processo energeticamente autosufficiente. Dei diversi processi studiati per ottenere energia della biomassa quello che utilizza la combustione di biomassa dopo estrazione dell'olio é risultato il più favorevole in termini energetici. In particolare, due casi di questo processo sono stati confrontati con un caso base, variando la provenienza dei requisiti energetici (calore ed elettricità), fornendoli sia da fonti esterne che dal processo steso.

In ultimo, nel **Capitolo 7** si riporta una valutazione tecnica di un impianto per la produzione di biodiesel da microalghe in cui si propone una nuova configurazione della sezione di crescita, un fotobioreattore ibrido, il Closed Pond Reactor (CPR). L'intero processo é stato simulato Aspen Plus^{TM} e ottimizzato per ottenere i migliori benefici in termini energetici. La progettazione e il dimensionamento delle attrezzature tecnologiche sono stati effettuati per ottenere una stima realistica dei costi, considerando sia CAPEX (costi di capitale) e OPEX (costi operativi). Nell'analisi economica si é valutata, la redditività del processo su scala industriale e sono stati calcolati i prezzi di vendita corrispondenti dell'olio e del biodiesel necessario per rendere la produzione economicamente sostenibile.

Foreword

This research project was developed at the Department of Industrial Engineering of the University of Padova (Italy), under the supervision of Prof. Alberto Bertucco. Part of the work reported in this Thesis (chapter 5) has been carried out at the Sustainable Process Technology group of the Twente University (The Netherlands) under the supervision of Prof. Wim Brilman. The fulfilment of the research project presented has involved the financial support of Mexican National Council of Science and Technology CONACyT, to whom the author grateful acknowledges. As a tangible result of the work completed during the Ph.D. school, a number of publications and presentations to conferences have been produced, as listed below.

Publications in Refereed Journals

1. **Ramos Tercero E. A.**, Sforza E., Bertucco A. (2013). Energy profitability analysis for microalgal biocrude production. Energy, vol. 60, p. 373–379, ISSN: 0360–5442, doi: 10.1016/j.energy.2013.08.003

2. **Ramos Tercero, E. A.**, Alibardi, L., Cossu, R., Bertucco, A. (2014). Anaerobic Digestion of Microalgal Residues to Enhance the Energetic Profit of Biocrude Production. In Chemical Engineering Transactions (Vol. 37, pp. 793–798). doi:10.3303/CET1437133.

3. **Ramos Tercero, E. A.**, Domenicali, G., Bertucco, A. (2014). Autotrophic production of biodiesel from microalgae: An updated process and economic analysis. Energy, 1–9. doi:10.1016/j.energy.2014.08.077.

4. **Ramos Tercero, E. A.**, Sforza, E., Morandini, M., Bertucco, A. (2014). Cultivation of *Chlorella protothecoides* with urban wastewater in continuous photobioreactor: biomass productivity and nutrient removal. Applied Biochemistry and Biotechnology, 172(3), 1470–1485. doi:10.1007/s12010–013–0629–9.

5. Sforza, E., **Ramos Tercero, E. A.**, Gris, B., Bettin, F., Milani, A., Bertucco, A. (2014). Integration of *Chlorella protothecoides* production in wastewater treatment plant: From lab measurements to process design. Algal Research. doi:10.1016/j.algal.2014.06.002.

Papers submitted for publication in Refereed Journals

1. **Ramos Tercero E. A.**, Bertucco A., Brilman W.D.F. Water process recycle in Hydrothermal Liquefaction of microalgae to enhance bio–oil yield. Energy and Fuels.

Papers or abstracts in Conference Proceedings

1. **Ramos Tercero E. A.**, Sforza E., Palma G., Giacometti G.M., Bertucco A. (2011). Evaluation of Energy Efficiency and Recovery in Microalgal Production Processes. International Algae Congress 2011, Berlin, Germany, 1–2 December 2011. Poster presentation.

2. **Ramos Tercero E. A.**, Sforza E., Palma G., Bertucco A. (2012). Energy Recovery Analysis for Microalgal Bio–oil Production. Young Algaeneers Symposium 2012, Wageningen, The Netherlands,14–16 June 2012. Poster presentation.

3. **Ramos Tercero E. A.**, Sforza E., Bertucco A. (2012). Autotrophic Growth of Microalgae in Wastewaters: an Experimental Investigation. Congress of GRICU 2012 – "Ingegneria Chimica: dalla nanoscala alla macroscala.", Montesilvano, Italy, 16–19 September 2012. Oral presentation.

4. **Ramos Tercero E. A.**, Bertucco A. (2012). Microalgae growth in wastewaters to improve the energy efficiency of bio-oil production process. II Simposio de becarios CONACyT, Strasburgo, France, 29–30 November 2012. Oral presentation.

5. **Ramos Tercero E. A.**, Sforza E., Morandini M., Bertucco A. (2013). Microalgal growth in urban wastewaters: A way to improve existing treatment performances. In: 3rd International Conference on Algal Biomass, Biofuels and Bioproducts, Toronto Canada, 16–19 June 2013. Oral presentation.

6. **Ramos Tercero E. A.**, Alibardi L., Cossu R., Bertucco A. (2014). Anaerobic Digestion of Microalgal Residues to Enhance the Energetic Profit of Biocrude Production.International Conference on Biomass, Florence Italy, 4–7 May 2014. Poster presentation.

7. **Ramos Tercero E. A.**, Alibardi L., Cossu R., Bertucco A. Biogas production from lipid-extracted microalgae testing different solvents for extraction. In: 5th International Symposium on Energy from Biomass and Waste, Venice, Italy, 17–20 November 2014. Oral presentation.

Introduction

Currently, about 85% of global energy demand is produced from fossil sources. It is well-known that fossil energy recently involves a pressure on resources depletion, a problem which affects the entire world population, since the world economy is highly sensitive to changes in the price of fossil fuels, causing serious repercussions on the industrial and political sectors. From an environmental point of view, fossil fuels generates big amount of GHG (greenhouse gas) emissions which affect the ecosystems and the quality of life (Crocker and Andrews, 2010).

The development and exploitation of renewable and sustainable energy sources can give a significant contribution to the solutions to these problems. Among the different potential sources of renewable energy, biofuels are expected to play an important part in the global energy organization of the future (Chen and Walker, 2011). In particular, biofuels from microalgae biomass are considered as one of the best and nearby alternatives for new clean energy (Amaro et al., 2012; Ghasemi et al., 2012; Mata et al., 2010). Microalgae are microorganisms capable to convert solar energy in chemical energy which can be exploit as fuel in diverse forms (e.g. liquid, gas, char). Microalgae have several advantages, the most important ones are:

- rapid growth, as they can double their biomass many times a day when in exponential phase of growth (Chisti, 2007);

- higher photosynthetic efficiency in comparison with other oleaginous terrestrial plants. This characteristic permits algae to convert solar light energy into chemical energy more efficiently;

- they accumulate considerable amount of oil in their cells, the proportion of lipids depending on the metabolic rate and cultivation conditions;

- they grow in a wide range of water environments, comprising those located in marginal places not exploitable for agriculture purposes;

- efficient use of nutrients, as they require only sunlight, a carbon source as CO_2 from flue gases, and nutrients, in particular nitrogen and phosphorus that could be derived from wastewater;

- the lipids from microalgae biomass can be extracted and converted to biodiesel, while the biomass resulting after oil extraction can be converted, by a wide number of conversion processes, into ethanol, biogas, biohydrogen, among other, providing other types of renewable biofuels (Chisti, 2007)

- besides biofuels, microalgae are reliable producers of fine chemicals (antibiotics, pigments, steroids among other), due to their biochemical structure composed principally by proteins, lipids and carbohydrates.

So far, the production of microalgae biofuels has been demonstrated in pilot scale facilities, but whether algal fuels can be produced in industrial and commercially way to replace fossil fuels is currently matter of debate. Nowadays, the development of large scale algae production technology is still limited by some open issues, such as the water availability, the supply of nutrients, the required enhancement of oil extraction and/or biomass conversion process and energetic profits of the process: all of these points certainly impacts the products prices. The water supply is insufficient to support any constant production of algal fuels in any scale anywhere. Even if different microalgae species can live in diverse kinds of water as fresh, brackish, saline water and wastewaters, freshwater is needed to compensate losses by evaporation, also when seawater is used, because evaporation changes the salinity and consequently the growth and productivity of microalgae. To minimize freshwater consumption, the use of closed cultivation facilities and water recycling in the process could be the solutions (Batan et al., 2013). Additionally, nutrients availability including carbon dioxide is questionable at industrial scale: according to Chisti (2013) to produce 82 million tons of algal biomass, it would be around 1.5 billion tons of CO_2, 5.4 million tons of nitrogen (44% of the world existing usage) and 1.1 million tons of phosphorous. Both the nitrogen and phosphorous supply would be in competition with crop agriculture, where they are largely used as fertilizer. Another source of carbon that recently has been investigated is bicarbonate (Gardner et al., 2013; Gris et al., 2014), with algae species able to grow in high alkaline medium. Bicarbonate solutions could substitute carbon

dioxide, but this technology is under development and more experimental investigation has to be performed for its implementation.

Therefore, for a sustainable production of microalgae fuels it is essential the recovery of nutrients within the algal process, including those remained in the biomass after oil extraction. This could be done by anaerobic digestion (Chisti, 2013), one of the most promising technologies for recovery nutrients and energy (Frigon et al., 2013), but more experimental assessments are required to evaluate the feasibility of this technology when applying it to biomass remaining after lipid extraction. Wastewater seems to be quite promising as source of N and P nutrients for microalgae growth, in a way that combines at the same time biological cleaning by removing these components from water. This technology has been investigated by a number of researchers, such as Aslan and Kapdan (2006), Olguín (2003) and Pittman et al. (2011). However, the use of wastewater as a nutrients source at the large scale, with both objectives, production of fuels and/or water treatment, has to be fully assesed. Lundquist et al. (2010) first proposed to couple the two process, and in the literature values of recovery above 70% of nitrogen, phosphorous and Chemical Oxygen Demand (COD) have been reported (Wang et al., 2010) with sterile wastewater. More realistic conditions certainly need to be tested, including the design of the reactor, the process configuration, the bacterial competition with microalgae and varying climatic conditions as sunlight irradiation or temperature.

In addition, in order to make microalgae a potential crude oil substitute, it is necessary to optimize the process from the energy standpoint, by increasing the energy efficiency and decreasing its losses. At the present, values of Energy Return on Energy Investment (EROEI) reported in literature span from 0.13 (Brentner et al., 2011) to 2.5 (Vasudevan et al., 2012), whereas higher values would be required, being 7 the minimum for energetic profitable process (Chisti, 2008). This point requires a thorough improvement of the process, where the low biomass concentration in the culture (in a high concentrated reactor the water content is more than 99%) makes the harvesting of biomass and drying highly energy consuming steps. These improvements must include the combination of different processes, to enhance the oil extraction yields and successive methods to exploit the energy from residual biomass. Among others,

the investigation of hydrothermal liquefaction for biomass conversion is attracting, as a solvent-free oil recovery technology whose functionality has been proved at lab level only (Garcia Alba et al., 2012; López Barreiro et al., 2013a).

From the biological point of view, ranges of improvement exist that could change the future of microalgae technology, as the increase of photosynthetic efficiency, or a modifcation of the metabolic pathway to enhance the lipid accumulation (or excretion) (Chisti, 2013).

In summary, from the economic point of view the sustainability of microalgae processes has not been totally clarified, as cost benefit analyses show large variations among themselves. Each single step difference in the process has repercussions on the costs, starting from the reactor geometry to end with the location of the plant (Davis et al., 2011).

For all the reasons above, the aim of this research project has been to evaluate the potential of microalgal technology for oil production, analyzing experimentally and by process simulations many steps of the process, technologies and concepts.

The topics addressed by this thesis are organized and subdivided in chapters as follows.

Chapter 1 is an introductory discussion on microalgae world situation, recent investigations and latest results reported for this technology.

Chapter 2 and 3 report the experimentation performed on microalgae cultivation in wastewaters, to verify the ability of microalgae to grow in untreated wastewaters aimed to the selection of the optimal wastewater process stream for microalgae growth. The nutrients removal efficiencies and removal rates in real wastewater are reported in **Chapter 2**, together with the steady-state biomass production with continuous feed of wastewater. In **Chapter 3** the effects of temperature, day/night irradiation and bacterial competition are studied, with respect to growth and nutrient removal of microalgae in order to integrate both technologies in a realistic approach, and a modified depuration scheme is proposed.

Chapter 4 presents the experimental work of the evaluation of biogas production capacity from microalgae and their degradability rates in the anaerobic digestion process, testing conditions such as inoculum typology and biomass concentration. Furthermore, the biogas/biomethane production is tested considering the effect of the

mixture of solvent used for oil extraction, and the results are compared with those from whole microalgal biomass.

Chapter 5 is focused on the biomass conversion by an alternative process, the hydrothermal liquefaction (HTL), reporting the experimental work to recover and reuse the water process, recycling it into the HTL system. The effect of the temperature and the number of recycles on the yields of bio-oil, gas, solid residue and aqueous phase are tested.

In **Chapter 6**, the energy analysis of the process to produce biocurde is investigated considering several process routes and conditions, in order to verify and optimize its energy profits with respect to EROEI, proposing a process which is energetically self-sufficient.

Chapter 7 describes the techno-economic evaluation of an industrial-scale plant for the production of biocrude from microalgae, using data from experimental results and process simulations carried out using Aspen Plus^{TM}, considering a hybrid photobioreactor. The oil selling price to achieve revenues was calculated. Finally in the conclusions, the summary of the thesis and the aspects that possibly need to be focused for ensuring future of this technology are discussed.

State of the art

1.1 World's energy situation and challenges

For long time, fossil fuels have been fundamental part of daily lives. It is widely recognized that energy systems currently in use, based on fossil fuels, involve not only pressure on resources which will depleted sooner or later, but also an increase in emissions of greenhouse gases (GHG). It is expected that this fact, combined with the exponential growth of emerging economies, will lead to an extraordinary increase in environmental impacts.

Developed countries have enjoyed power supplies in a plentiful and cheap way, by adopting an economy based on fossil energy, during the XX century, declared as "Petroleum Century". The use of oil allowed rapid economic expansion and enabled the modern ideal of personal automobile ownership (Crocker and Andrews, 2010).

Liquid fuels represent the most utilized source of energy at the present time, the dominance of fossil liquid fuels in the world market energy portfolio is projected to continue well beyond 2030 (Crocker and Andrews, 2010). So the crude oil demand is projected to grow, from 90 Million barrels per day (mb/d) in 2013 to 104 mb/d in 2040 (Agency, 2014) even though the developed world will see an overall decrease in demand for traditional petroleum feedstocks. In this respect, the transportation sector is expected to be the most affected.

1.1.1 The Peak Oil theory

The Peak oil theory represents the time at which the flowrate of a single oil field (or an entire region) reaches the absolute maximum value (Herold, 2012). Numerous oil reservoirs exploited at the moment were discovered decades ago. The combined

production of a number of fields also has a peaking profile which could represent the production from a particular country, region or even total global production. The most oil producing countries have already passed their peak in oil production, including the USA (1972), Iran (1974), Russia (1987), the UK (1999) and Norway (2001) (Dale et al., 2011). The problem of the whole peak existence argue is particularly relevant because the that world oil output is currently near to reach its highest level. It is recognized that about half of the resources available in the whole world have been produced, and at this point it cannot be denied that a depletion period and a forthcoming decline are arriving, due to the fossil nature of oil (Herold, 2012; Dale et al., 2011).

1.1.2   Biofuels synopsis

In recent years the world attention have led to substantial interest in developing renewable fuels, with the main objective to identify the best solution of energy that can achieve at least a partial replacement of traditional fossil fuels. In particular, biofuels derived from biomass and they may be either liquid, gases and solid.

Renewable fuels are classified in three categories or commonly named "generations" based on the raw materials used and the processing or production technology. First generation of liquid biofuels corresponds to those based on sugar and starch crops or vegetable oil as feedstock, such as corn, sugarcane, wheat, maize and vegetable oils. First generation biofuels were criticized for the direct competition with food supply, which causes the increasing on food prices and the effect on the poor, although in some areas they could be an additional source of income for poor farmers (Campbell et al., 2011; Mazzetto et al., 2013). Also the energy demand for their production was questioned.

Second generation biofuels comprises those derived from non–food crops such as *Jatropha curcas*, and are considered to have a minor impact on food markets and be more sustainable than first generation. For instance, *J. curcas* can grow easily in non-arable or wasteland and the equipment, and process already developed for biodiesel plants does not require major modification. Third generation of biofuels, like the second one, are made from non-food feedstock, differing from previous generation by its higher energy yields per hectare. The resulting fuel is also known as "green hydrocarbons" and is very similar to its petroleum equivalents (Fenton and Ó hUallacháin, 2012). The

research for more sustainable biofuels feedstock continues, and is recently focused on microalgae thanks to their numerous advantages, which are summarized below.

1.2   Microalgae overview

Microalgae as source of biofuels have gained considerable interest in recent years. The term "alga" refers to all the organisms having chlorophyll A and a mycelium with no difference into roots, leaves and stem (Williams and Laurens, 2010). Cyanobacteria are included in this definition, even though they are prokaryotic organisms. They are present all over the world, they are mainly distributed in waters, like rivers and oceans. Microalgae may have different types of cell organization: unicellular, colonial and filamentous (Richmond, 2004).

Microalgae, as all organisms with chlorophyll, perform the photosynthesis, according to which inorganic compounds and light energy are converted into organic matter. The energy is harvested by chlorophyll molecules, and the photosynthetic system is able to convert carbon dioxide and water to carbohydrates and oxygen. Microalgae can achieve energy efficiencies higher in comparison to terrestrial plants, as they can convert up to 5% of the incoming sunlight energy to biomass (Bertucco et al., 2014).

Nowadays, the identification of microalgae species is in progress: around 72,500 species are known, 44,000 have been named and published, but the "real" number could vary from 1 million specie to 350 million (Guiry, 2012).

Microalgae have different ways to assimilate the energy required for growth: most of them are photoautotrophic, but numerous species can also use organic substances (e.g. glucose) as carbon source to grow, in both the light and the dark (photoheterotrophic and heterotrophic growth respectively) (Chen et al., 2011). Furthermore, some species perform a mixotrophic metabolic mode, in which microorganisms have the capability of exploiting both organic (assimilated during the heterotrophic route, influenced by organic carbon availability) and inorganic carbon sources (fixed during photosynthesis, influenced by light intensity) (Hu et al., 2012).

Potentially, microalgae can provide fuels in several distinct forms: hydrogen via direct and indirect biophotolysis, biodiesel through transesterification of the lipid fraction, biogas via anaerobic digestion, bioethanol by fermentation of sugars, bio–oil via

thermochemical conversion as pyrolysis, hydrothermal liquefaction, combustible gas by gasification, solid fuels by hydrothermal carbonization or torrefaction and green diesel and gasoline through direct catalytic hydrothermal liquefaction (Bahadar and Bilal Khan, 2013).

1.2.1 Lipid accumulation

Biochemical composition of microalgae includes mainly carbohydrates, proteins, nucleic acids and lipids. The mass fraction corresponding to each component is strongly influenced by environmental conditions such as temperature, irradiation intensity, and nutrient availability. Also, a small portion of microalgae biomass corresponds to minerals containing elements such as Fe, K, Ca and Si.

With respect to the molecules energetically exploitable, the lipid fraction is of essential importance: microalgae accumulate fats as energy reserves, into the cell body in form of simple fatty acids and triglycerides, while membrane cells are mainly formed by phospholipids and glycolipids. The proportion of these two lipids depends on the metabolic rate and cultivation conditions (Torres et al., 2013).

Focusing on lipid production, when microalgae cells are in growing phase, membrane phospholipids and glycolipids generally dominate, but as the cells arrives to the stationary phase in cultivation curve, many species store triacylglycerols (TAGs) (Mus et al., 2013). Although average lipid contents ranges from 7% and 40%, dry weight (DW) some species may reach 70 wt% DW, and this characteristic is strongly specie-specific (Williams and Laurens, 2010; Chisti, 2007; Sforza et al., 2012a). Microalgae can be induced to accumulate considerable amount of lipids by changing environmental conditions causing a stress in cells. These condition are nitrogen and/or salt concentration in cultivation medium, light intensity, temperature, CO_2 concentration (Brennan and Owende, 2010). In particular nitrogen starvation ensures the best results to induce the lipid accumulation. However, as the growth is inhibited by low levels of nitrogen, recently a two-steps cultivation process have been proposed. The effects of the intensity of light provided to the system has been extensively studied. This variable affects the amount and composition of lipids accumulated: in general, low intensities mainly produce membrane lipids, whereas high intensities increase the TAGs (Sharma, 2010).

Numerous species, principally of eukaryotic microalgae are potential high–oil producers (National Academy of Sciences, 2012). Between them, the most renowned and investigated are *Tetraselmis, Dunaliella, Chlorococcum, Scenedesmus*, and *Chlorella*, and particularly *Neochloris oleoabundans* and *Botryococcus braunii* for their elevated oil accumulation; the species of *Nannochloropsis oculata* and *Nannochloropsis salina* from Eustigmatophyceae class; and the genera of *Isochrysis* and *Pavlova* from Haptophyta class (National Academy of Sciences, 2012).

In general, the aim is to maximize the amount of biomass with the best characteristics for the specific final product desired (e.g. high lipid content for biodiesel as final product) per unit area, time, or volume (Figure 1.1), involving also the maximization of the product output per unit of energy required, including nutrients, and other supplies. Besides lipid accumulation, many other criteria can be considered for algal strain selection, as fast assimilation of nutrients, fast growth, capability to survive in non–sterile environments, including variables that alter cost in the supply chain, and are important for evaluating the economic viability Chisti (2007).
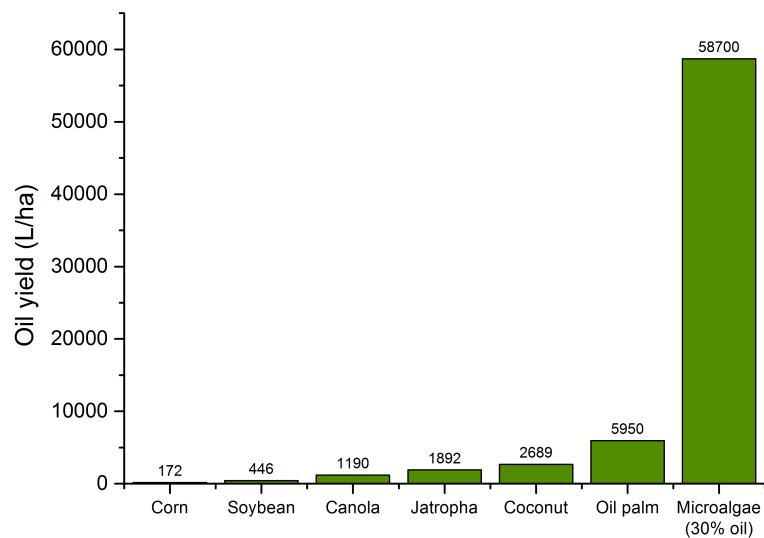
Figure 1.1: Oil yields from different feedstocks per unit of area from (Chisti, 2007)

1.2.2   Advantages of microalgae as source of fuels

The research about microalgae led to a overwhelming number of reports and scientific articles. In particular, many advantages of using microalgae are described and compared with other available feedstocks of previous biofuels generations (Beal et al., 2010b). Herein the most outstanding ones are reported:

- in comparison with all terrestrial plants, microalgae offer higher photosynthetic efficiency (corresponding to the percentage of solar energy stored in microalgal bodies as energetic compounds). Efficiency values have been reported from 3 to 8% and in some cases even 11% (Bertucco et al., 2014) compared to 0.5% of terrestrial plants. This characteristic is extremely important for biomass and lipid production, since it is directly proportional to areal productivity;

- the biological adaptation of these microorganisms makes them better suited to survive and reproduce in different environments. Microalgae can grow in a varied range of climates, in aquatic environments, including fresh, saline, brackish water or wastewater that can be sited in terrestrial locations comprising marginal lands like the desert, and also in areas not exploitable for agriculture;

- the accumulation of greenhouse gases CHG in the atmosphere (specifically CO_2) and the increasing production of wastewaters are a worldwide concern. These environmental problems can be decreased by microalgae, since they can fix nutrients from both, using CO_2 as carbon source and assimilating nitrogen and phosphorous polluting compounds present in wastewaters for their growth (Olguín, 2003).

Additionally, different microalgae species can live in a variety of environmental conditions. Thus, it is possible to find species best suited to local environments with extreme conditions of temperature of pH or specific growth characteristics. The same is not possible with other current biodiesel feedstocks (e.g. soybean, rapeseed, sunflower and palm oil) (Rawat et al., 2013). On the other hand, microalgae which can be grown in areas unsuitable for agricultural purposes (e.g. arid areas where the annual insolation is high or temperatures are not adequate) (Mata et al., 2010). In addition, microalgae

allow to obtain a biofuel which is free (or has very low amount) of sulfur (Ghasemi et al., 2012), so that pollutant emissions are lower than those associated with traditional fossil fuels. Furthermore, due to their biochemical composition (lipids, carbohydrates, and proteins) microalgal biomass has a wide range of exploitation possibilities.

1.3   General pathway for biofuels from microalgae

In the context of large scale microalgal cultivation, the process configuration is defined as the combination of economic viability, upstream processing and downstream processing (Rawat et al., 2013). In a scheme of a typical algal biofuel value chain, the microalgae species selection is the first variable to consider, which strongly depends on some geographic conditions (e.g. sunlight irradiation) and also on the design and operation of an optimal cultivation system. It follows the harvesting, dewatering step and following drying of biomass. Then there are two options: oil extraction to supply the biodiesel production or biomass exploitation for energy purposes.

1.3.1   Cultivation Systems and downstream processing

Factors which are determinant for the productivity of microalgae cultivation are: temperature, pH, nutrient medium supply and light distribution in the system. Photobioreactors are cropping systems aimed to facilitate the supply of nutrients and light to the microalgae cells and to adjust the environmental conditions to the microalgae species selected to achieve its growth. These cultivation systems can be designed as closed photobioreactors (PBR) or open ponds (OP). Although the term "photobioreactor" is typically used for closed systems, in a wider sense, it is also valid for open ponds.

PBR's are characterized by the accurate regulation of almost all important parameters for microalgal growth, and by the reduction of the risk of contamination and loss of CO_2. In addition, they allow optimal culture conditions, temperature control and flexible design. In the PBR's the direct exchange of gases between the culture broth and the atmosphere is usually limited, one of the main consequences being the accumulation of oxygen inside. Another disadvantage is the cost for the control of temperature, especially in hot climates (Richardson et al., 2012). Among all possible configurations, the more investigated for application to the commercial production

of high value chemicals from microalgae are tubular and flat panel PBR, both with some variances in inclination (horizontal, vertical, inclined), arrangement (spirals) and operation mode. The main advantage of these configurations is the high productivity and better control of culture conditions that they can reach, while high installation costs is the main disadvantage. They are now described separately

- *Tubular PBR's* They are suitable for outdoor cultivation because they can be operated at axenic conditions for long time (Michels et al., 2013). The cultivation temperature can be controlled, reaching faster growth rates and high productivity. Figure 1.2 shows a tubular reactor in which the entire microalgae culture passes through all tubes until the degassing zone. Some disadvantages of this geometry are: a) the mixing and mass transfer in tubular PBR are limited, causing high concentrations of O_2; b) photoinhibition problems are also common as depending on the mixing efficiency, the cells on the surface receive more light, while the central ones could be in the dark for longer time, depending also on the biomass concentration (Gebremariam and Zarmi, 2012).

Figure 1.2: Tubular photobioreactor. (Foto: IGV Biotech)

- *Flat panel PBR.* The main advantage of this reactor is the large illuminated surfaces (Figure 1.3), resulting in higher photosynthetic efficiencies. Moreover, it has been found that the dissolved O_2 concentrations are low. However,

problems are the difficulty of scaling, a difficult temperature control and possible hydrodynamic stress of certain species (Bahadar and Bilal Khan, 2013), that could change the characteristics of the biomass produced. Flat panel PBR'S are simple to sterilize and to operate in axenic conditions, compact and of low cost. The agitation system consists of injecting air from the bottom, which also contributes to the supply of CO_2, and the gas ventilation takes place at the top of the column. Nevertheless, so far there are not used for commercial applications.
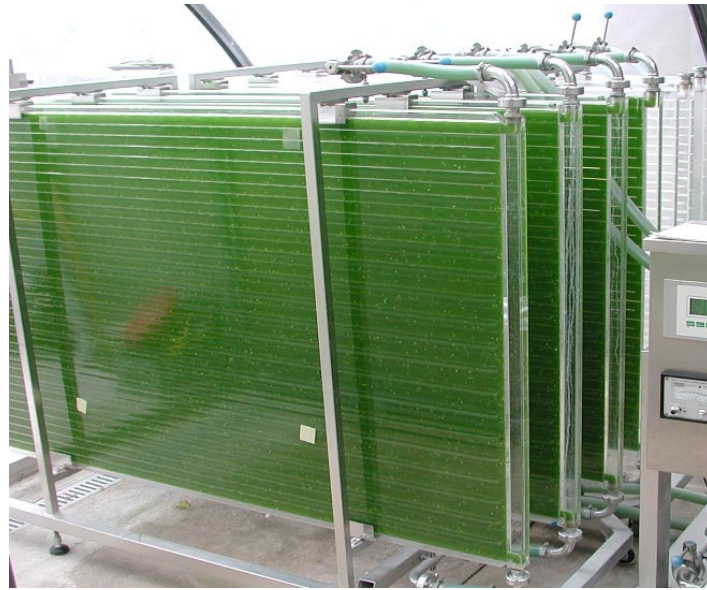
Figure 1.3: Fat panel photobioreactor. (Foto: IGV Biotech)

The **Open ponds** reactors (Figure 1.4) are mostly used in big scale, principally because of their simple construction, operation, durability and low installation cost (Slegers et al., 2013). However, the major limitations of open systems include difficult mixing, evaporation losses, and contamination of culture by environmental factors and other fast growing heterotrophic organisms. Therefore industrial scale productions are limited to species that grow in extreme conditions of pH, salinity (Rogers et al., 2013). OP's could be both natural systems (lakes, ponds) and artificial systems of which the raceway type is the most popular, even if other types exist like circular ponds. These last are stirred using a rotating blade travelling across the surface (similar to those for wastewater treatment) and are widely used in Japan, Taiwan and Indonesia for the production of microalgae. The raceway ponds commonly consists of a cavity into the soil to a depth of 15–20 cm (to ensure a correct light distribution), protected with

plastic liner to prevent percolation. The tank is divided into two channels and the circulation and mixing of the culture is performed by rotating paddles wheels, that in some cases also circulate through the pond.

Figure 1.4: Open pond reactor (Foto: Seambiotic Corporation)

1.3.2 Harvesting and dewatering

The steps of harvesting and dewatering are of paramount importance, since they affect the effectiveness of biomass processing. Microalgal biomass concentrations most commonly reported are around 0.1 and 0.5 g/l in OP's and 0.5 and 8.0 g/l in PBR'S (Ghasemi et al., 2012), so that the elimination of almost a liter of water from cultivation broth to produce a few grams of dry biomass is required, with a consequent energy duty for water pumping and treatment.

The most used harvesting methods are based on principles like particle size exclusion or separation by density, and include filtration, centrifugation, sedimentation, flotation, flocculation and bio–flocculation among others (Rawat et al., 2011). The density of the material to be separated can be modified by the addition of substances that cause particle aggregation (mostly metallic materials, such as Aluminium), but also by flocculats. In addition, the characteristics, disposition and effect of flocculants on the cultivation process must be studied, since the water after separation has to be recirculated to the photobioreactor.

Centrifugation and filtration are being considerably investigated, updates in these technologies are reported by Dassey and Theegala (2012); Rickman et al. (2012). They

can be used as single step or together with a preliminary separation. Centrifugation rapidly concentrates the biomass but involves high costs, while filtration could present some inefficiencies because the different sizes of algal cells tend to obstruct the filter. Vacuum filtration is effective with "large" algae (greater than 70 μm) when operated under required pressure combination with a filter aid. Both of these methods are able to concentrate from 5 wt% to 30 wt% the solids.

1.3.3 Processing routes

Biofuels are solid, liquid, or gaseous fuels derived from biomass. Liquid fuels can be used directly in the existing transportation network, and in engine turbine electrical power generators, while solid and gaseous fuels can be used for the production of electricity. Besides, from the biomass chemical products can be derived, and additionally power and chemicals can be derived from the complete exploitation of biomass components and residues in industrial, commercial, or urban applications, or agricultural or forestry, to obtain economic or energetic revenues. Biomass can be converted into useful forms of energy, by means of two main routes, the bio–chemical and the thermo–chemical ones (see Figure 1.5). Bio–chemical conversion includes: anaerobic digestion to produce biogas composed of methane and carbon dioxide or hydrogen (in dark fermentation), depending of conditions and bacteria medium, and fermentation for the production of ethanol. For thermo–chemical conversion more process options exists: combustion for the production of electrical or thermal energy, gasification, pyrolysis and hydrothermal gasification for gas fuel production, and hydrothermal liquefaction, from which liquid fuel is obtained (Toor et al., 2011). The selection of the process depends on the desired product and the biomass properties and characteristics.

1.3.3.1 Biodiesel from microalgal lipids

Biodiesel production from microalgae requires microalgal species with high lipid content and productivity. After the concentration and harvesting steps the drying of the algal cells is required to prepare them for lipid extraction. Drying is an energy demanding process, but thanks to it, the material can be mechanically treated to open up access for oil extraction (Xu et al., 2011). The oil extraction can be carried out with solvents,
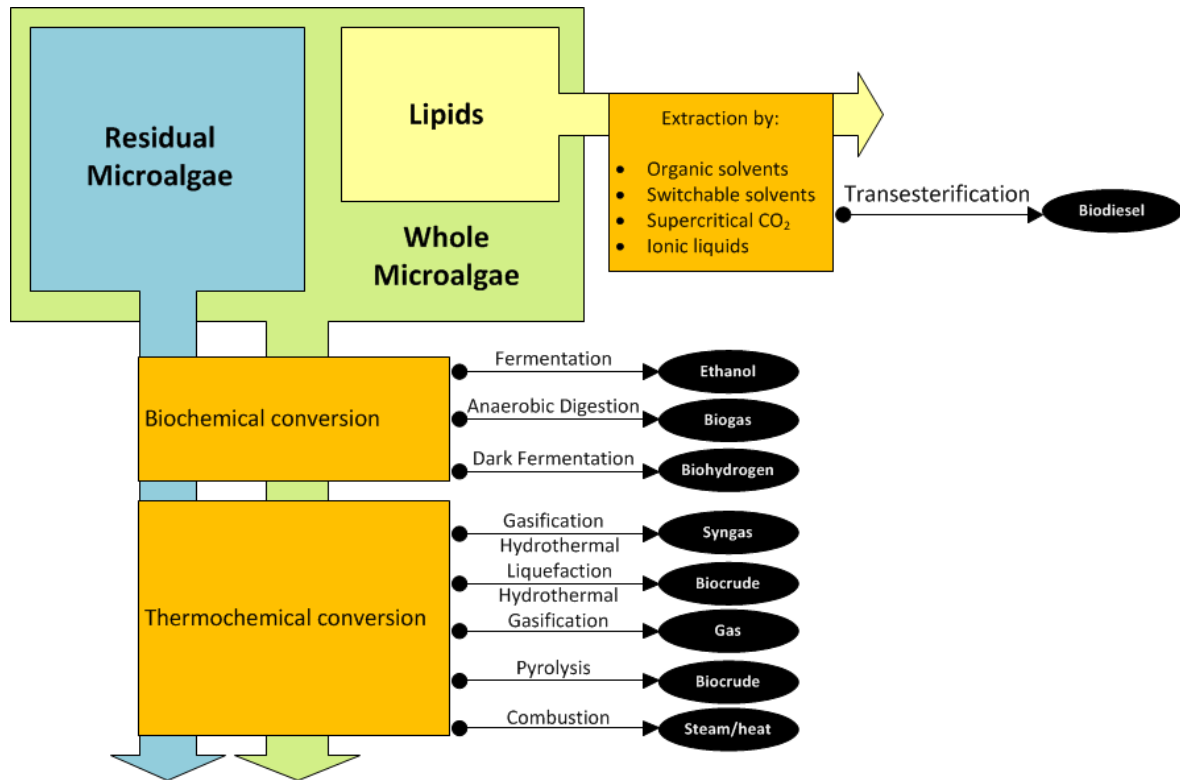
Figure 1.5: Biomass conversion routes

(organic or switchable) (Boyd et al., 2012), supercritical CO_2 (Hernández et al., 2014) and recently also by to ionic liquids (Choi et al., 2014). The desirable characteristics of a good solvent include high solvation properties, low toxicity, low cost, high availability. Examples of solvents (pure or mixed) include hexane, chloroform, methanol, ethanol, propanol, ethyl acetate, acetone. The solvent is separated from the algal oil, recovered, and reused. Solvent recovery is around 95 to 99.5% (Stephens et al., 2010). These step has some disadvantages that influence the efficiency and sustainability of the process, such as the large volume of solvents required and the sizing of the equipment for extraction and recovery and solvent vapors, which that could present a fire and explosion risk.

Switchable solvents are a new type of extracting media, as they exhibit two degrees of polarity: lipophylic in the non-ionic form and hydrophilic in the ionic one, a property that can be exploited in extractions to avoid the use and the disposal of solvents of different polarity. The extraction of hydrocarbons from dried and not dried *Botryococcus braunii* was shown by Samorì et al. (2010), but it is still an open issue and is under investigation. An alternative extraction method is the use of

Supercritical CO_2 ($SCCO_2$) which shows great potential due its relatively low critical pressure (7.38 MPa) and temperature (31.06 °C). It is high selective for non-polar lipids, so that, it does not solubilize phospholipids, which is important for biodiesel uses (Boyd et al., 2012), in addition, it can be easily separated from the extract and the residues, avoiding solvent traces. Nevertheless, $SCCO_2$ extraction has considerable economic and energetic costs due to the high pressures applied. On the other hand, ionic liquids are being investigated recently for extraction purposes. Ionic liquids are salts composed of reasonably large organic cations united with smaller anions, and they remain liquid at temperatures around 0–140 °C (Zhang, 2013). Their properties include good stability, high conductivity, low vapor pressure, non-flammability, and a wide miscibility range. Even if some authors reports reasonable good yields (Choi et al., 2014), further investigation are required.

1.3.3.2 Biochemical conversion processes

Anaerobic digestion (AD) is a process in which complex organic compounds are converted to carbon-dioxide and methane. The process is composed by 4 steps, the initial one is the hydrolysis and fermentation catalyzed by bacteria, followed by anaerobic oxidation of organic acids and alcohols to acetate (Batstone and Virdis, 2014), which is named acetogenesis, and the last step is methanogenesis mediated by bacteria. AD has been applied to either industrial slurries or domestic wastewaters. The current investigations in which AD is used to exploit the microalgal biomass energy, are addressed with different purposes, such as the species selection on the basis of methane yields (Mussgnug et al., 2010), or the test of marine and fresh water species (Frigon et al., 2013), even though and no significant difference was found between freshwater and marine microalgae. The methane production for tested strains varied between 227 and 410 mL CH_4/g TVS, and the best strains for the purpose were *Scenedesmus sp.*-AMDD, *Isochrysis sp.* and *Scenedesmus dimorphus*. Other microalgae with good performance were *Clamidomonas reinhardtii* and *Dunaliella salina* with around 390 and 330 ml CH_4/gTVS respectively, while the less favorable production was from *Scenedesmus obliquus* with 290 mLbiogas/gTVS (Mussgnug et al., 2010). In summary, the AD process is interesting since it permits the utilization of residues from

biodiesel production (Ehimen et al., 2011), but it may be that some previous steps (e.g. solvent extraction) change the efficiency and yields of the process.

1.3.3.3   Thermochemical conversion processes

Hydrothermal liquefaction (HTL) is a biomass conversion route to produce biocrude from wet microalgae biomass with high yields (López Barreiro et al., 2013a). HTL is carried out at temperatures from 200 °C to 370 °C and at a pressure corresponding to the water vapour pressure which maintains the water in the liquid state. In this process, besides the lipid fraction of microalgae, also proteins and carbohydrates are transformed, resulting in higher overall yields of biocrude (Valdez et al., 2014). Thus, the biofuel yields of low–lipid microalgae is increased and, additionally, these algae usually presents higher productivity than lipid rich ones. The conversion of microalgae components into biocrude is the highest for the lipid fraction, followed by proteins and in less amount by carbohydrates (Biller and Ross, 2011). The products of HTL are biocrude (or bio–oil), a gas phase mainly composed by CO_2, a solid residue and an aqueous phase, rich in soluble organics. The biocrude produced is energetically equivalent to conventional petroleum, is not suited for direct use in transportation engines, but it is a suitable renewable feedstock for co–refining in existing fossil refineries.

The Hydrothermal gasification process (also called supercritical water gasification, SCWG) is carried out in water at a supercritical state, above 374 °C and 22.1 MPa, wather is both a solvent and a reactant, and the product is a combustible gas. Most common temperatures tested for this process are from 400 to 700 °C and their corresponding supercritical pressure (López Barreiro et al., 2013a). Different microalgae species *Nannochloropsis sp.* or *Chlorella vulgaris* have been tested as well as some process conditions like temperatures and reaction times (Brown et al., 2010).

Pyrolysis is a the thermochemical conversion process of biomass, typically carried out at temperatures between 400 and 650 °C in the absence of O_2. This route produces a gas phase, a bio-char (solid phase) and a viscous fluid termed bio–oil, similar to that produced by HTL but with different properties. (e.g. higher water content, lower HHV) (Dickerson and Soria, 2013). Pyrolysis methods vary in residence time, temperature,

and heating rate, which affects the product yields. Pyrolysis of microalgae have been investigated, testing different species of microalgae (e.g. *Scenedesmus almeriensis*, *Nannochloropsis gaditana* and *Chlorella vulgaris*). As main products CO, CO_2 and H_2O, light hydrocarbons and H_2 were found (López-González et al., 2014).

In Gasification process, the main product is a gas mixture identified as syngas, consisting in CO, H_2,CO_2, N, and CH_4. The syngas is produced by the reaction of biomass with oxygen and steam. Nitrogen of the microalgae is reported to form ammonia during gasification. It can be recovered in the aqueous phase and then used as a source of nutrients for microalgae cultivation. A low microalgae concentration is required. Higher temperatures, low algae concentrations and longer residence times favor the algae gasification efficiency. The addition of catalysts to the capilaries resulted in higher yields of hydrogen and lower CO yields via enhanced water and gas shift activity (Singh and Olsen, 2011).

1.4 Wastewater treatment and microalgae cultivation: A win–win strategy

1.4.1 Wastewater treatment process

The actions for environmental protection, in relation to the problems connected to the discharge and treatment of wastewater from human activities, are started by recent laws with strict regulations consisting on:

1. controlling the correct and rational use of water resources

2. interventions of technological upgrading of sewage systems

3. implementation of purification treatments aimed to a higher removal of nutrients (nitrogen and phosphorus), organic and inorganic micropollutants and suspended solids

4. the reuse of treated wastewater

Besides the concerns associated with existing treatment technics, other concerns are associated to operational cost and high energy demand steps. The main pollutants in

urban waters are pathogens, nitrogen and phosphorous compounds, suspended solids, salts and oxygen demanding materials.

In order to remove these contaminants, the wastewater treatment process is divided into three main sections: primary, secondary treatment or biological section, tertiary treatments, and finally the corresponding sludge line.

Primary treatments consist of: a *screening section* to remove coarse materials from the stream that could damage process equipment and reduce effectiveness; *grit* usually consisting of a complex of sand, gravel or cinders with settling velocities superior than those of organic particles; *grease removal* to avoid problems and inhibition in aerobic biological treatment of anaerobic digestion and *primary clarifier* (Pittman et al., 2011). Secondary treatments are based on biological processes when microorganisms use carbon, nitrogen and phosphorus contained in the wastewater as nutrients, reducing they concentration in the wastewater flows. Traditionally these microorganisms are a complex of bacteria, but recently the research has pointed to use microalgae instead. The second treatments comprises *oxidation* step carried out in an aerated tank where activated sludges degrade the organic matter in activated sludge suspension and pre–treated water, both products are separated and the treated water passes to the next step of *nitrification–denitrification*, which is also performed by microorganisms that naturally transform ammonia to nitrate in aerobic conditions and nitrate to nitrogen gas in anoxic conditions (Olguín, 2012). Nitrification–denitrification step is followed by a *second sedimentation.*

The tertiary treatment include *filtration* and *disinfection*, in order to remove suspended, colloidal and dissolved components as calcium, potassium, sulphates, nitrate and phosphate, and some complex synthetic organic compounds Metcalf and Eddy (2004). Also, the sludge produced requires treatment for a final disposal: the sludge line is composed of *thickening*, *stabilization* and *dehydration*, stabilization is aimed to reduce the bacterial load by two possible routes, chemical (with doses of lime and chlorine) and biological, by composting or anaerobic digestion (Gray, 2010).

1.4.2 The coupled process

The integration between the microalgae biomass production and wastewater treatment processes could become a win–win strategy, in which both of them,

suitably combined, enable to achieve environmental and economical advantages. These synergies are refered to nutrients sources for microalgae cultivation, water demand, greenhouse gasses emissions and energy requirements.

On the other hand, in the production process of microalgae, significant constrains still need to be overcome before microalgae–based biofuel production becomes cost–effective and can impact the world's supply of transport fuel (Lam and Lee, 2012). In order to fulfill the economic limitations of microalgae production, a multipurpose approach could be applied for large scale cultivation. Numerous studies have recently focused to reduce nitrogen and phosphorus pollution from wastewater: Singh and Thomas (2012); Prathima Devi et al. (2012); Boelee et al. (2011); Sahu et al. (2013a). Accordingly, wastewater could be exploited to cultivate microalgae at a lower cost with the additional benefit of eliminating pollutants from the environment (Pittman et al., 2011). This dual purpose system is gaining popularity and is an attractive alternative to microalgae–based systems aimed solely at biodiesel production, thanks to its advantages of reducing energy, fertilizer and freshwater costs (Olguín, 2012). In fact, the use of microalgal biomass for nutrients removal could improve the current wastewater treatment technology since the biological nutrient removal through nitrification–denitrification process suffers from high energy demand (Metcalf and Eddy, 2004). Using microalgae in these plants could improve their performances, reducing the total energy demand and produce a high energy content feedstock, that could be exploited by applying a biorefinery approach.

1.4.3 NASA OMEGA System

Recently a novel project has been developed at the United States National Aeronautics and Space Administration (NASA) named OMEGA, which was born as a way to control agricultural wastewater from cities near to the coast (NASA, 2011). The OMEGA system (Figure 1.6) consists of an closed floating PBR built of a plastic material with special properties, economicity, transparency and flexibility. The inlet to the reactor are municipal wastewater and freshwater microalgae species. The OMEGA reactor float on the water surface and uses sunlight for microalgae growth. Also CO_2 is added, while the temperature is controlled by the sea water around of the reactor, and the mixing required is provided by the waves. The OMEGA reactor besides to exploit

solar, wind and wave energy, uses the chemical energy from wastewater and seawater for osmosis.
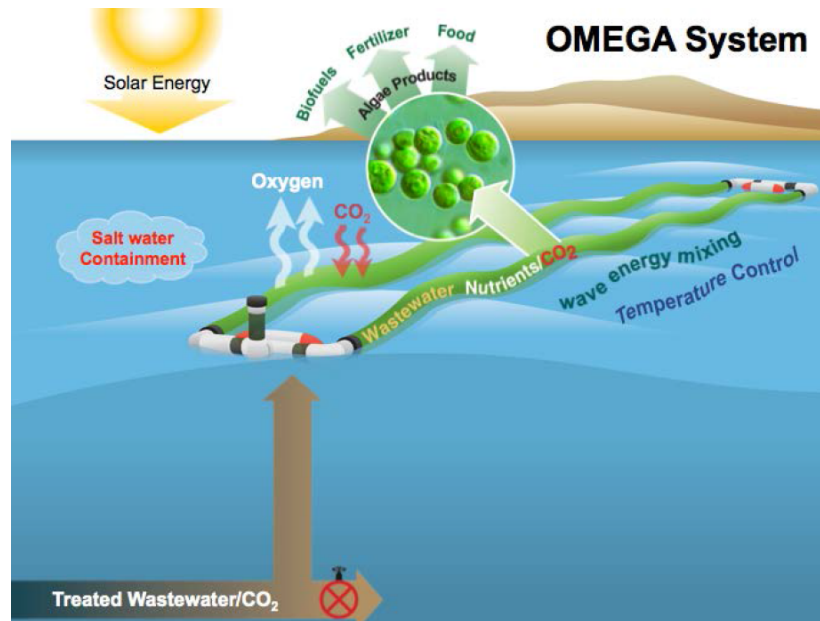
Figure 1.6: OMEGA system NASA (2011)

1.5 Updated process pathway from microalgae to biofuels (NREL report 2014)

The National Renewable Energy Laboratory (NREL) published a report to explore the design process and economics of many bio—fuels production pathways, aimed to determine an "absolute plant gate price" for biofuels, this plant gate price is being referred to as the "minimum fuel selling price" or MFSP, which can be used to assess the cost-competitiveness and market penetration potential. In this section a summary of this interesting and very recent work is presented.

The report of Davis et al. (2014a) develops a Techno–economic analysis (TEA) for an alternative approach to evaluate and achieve a MFSP, i.e. a process aimed to the fractionation of algal biomass and to the selective conversion of the major biomass constituents to fuel products, specially carbohydrates to ethanol and lipids to renewable diesel blendstock. The fractionation process considered at the NREL report is based on the conversion of each particular component of the biomass. The economics of this conceptual process uses the best available equipment and raw material costs and an

"$n^{th}-plant$" project cost structure and financing. The prospective or the plant designed reports a MFSP of \$4.35/GGE in 2022 (\$4.57/gal diesel and \$2.95/gal ethanol in 2011 dollars).
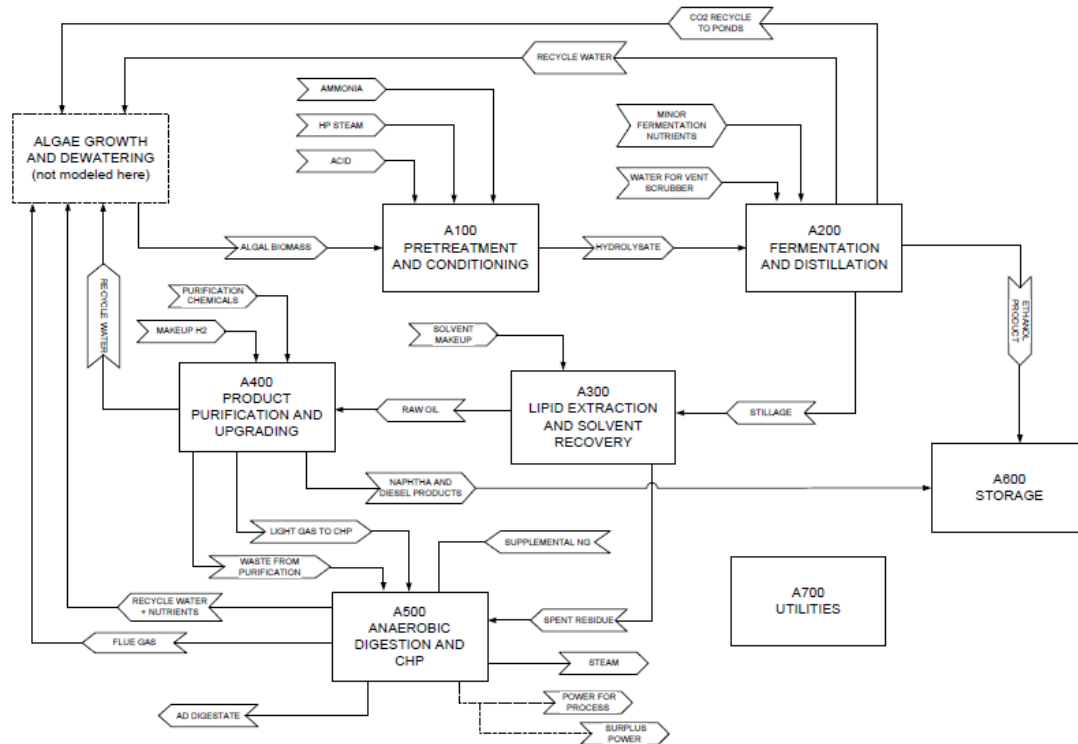
Figure 1.7: General process flowsheet diagram reported in Davis et al. (2014a)

1.5.1   Process overview

The process described in the NREL report is divided into seven sections (see Figure 1.7), the feedstock being algal biomass delivered after upstream dewatering to 20 wt% solids. These are pretreated with a dilute stream of acid, followed by fermentation of the resulting sugars to ethanol, afterwards by distillation and solvent extraction of the stillage to recover the fatty acids. The proposed process also includes the hydrotreating of lipid products, anaerobic digestion and combined heat and power, storage products, and public facilities.

The process was modeled thermodynamically for each unit operation by Aspen, using the material and energy balance data to determine the number and size of equipment items. The cost estimates along with the plant operating expenses are also calculated using Aspen model, and the simulation data are used in a discounted cash flow rate of

return (DCFROR) analysis to determine a MFSP for the refined diesel and ethanol, combined together based on energy content of each stream. This MFSP, reported in \$/GGE is required to obtain a net present value (NPV) of zero for a 10% internal rate of return (IRR) after taxes, after 7 years of recovery period (not including the gas turbine of the power plant which has 20 years of recovery period). The MFSP resulted is valid for the process conditions. The key assumption of the $n^{th} - Plant$ is that the analysis does not describe a single plant, it assumes that numerous plants using the same technology have already been built and are in operation.

The analysis considers the feedstock as dewatered algal biomass feedstock with 20 wt% solids, not including upstream biomass production and dewatering. It assumes a cost of \$430/ton of biomass on ash-free dry weight (AFDW) basis previously reported in Biddy et al. (2013), this cost is divided in a cost for algal biomass cultivation of \$340/ton and dewatering \$87/ton. An annual feed rate is assumed as 1,215 metric ton/day AFDW of algal biomass, implying an annual average productivity of 30 g/m^2/day in an open pond system.

Biomass is divided on the basis of the target composition, and cultivation time is set in order to reach this compositions, e.g. high protein biomass "HPSD" is obtained by harvesting prior to nutrient depletion, the the cultivation time is around 3 to 5 days for high carbohydrate biomass "HCSD" and 6 to 9 days for high lipid biomass "HLSD", using fresh water media. The design and construction of the plant is programed for 36 months, the start–up time is 0.5 year, the facility on-stream time is 90% (330 days/year or 7,920 hours/year).

1.5.2 Process Design and Cost Estimation Details

For **Pretreatment of the biomass** the average fed biomass is assumed as 1,215 metric ton/day (annual), but the material diverted to be dried in the summer is 35% of total summertime feed rate which corresponds to 2,229 ton/day AFDW, thus 780 ton/day of dry biomass (AFDW basis) is diverted away to drying and 1,449 ton/day is sent on to the pretreatment fractionation step.

The dryer is rotary drum type, and uses natural gas as fuel. The purchase cost for each of the 12 dryer is \$905,000 (2013–dollars) with flow capacity up to 2,520 kg/hr of moisture–free biomass. The pretreatment area including dryers, acid pretreatment, and

conditioning contributes with \$0.50/GGE to the MFSP. About 56% of this contribution is attributed to capital cost, of which acid pretreatment equipment impacts for the large preponderance of total capital expenses. The total installation cost for this section is \$65,564,413 (2013–dollars).

In the **Fermentation and Distillation** process step, the soluble sugars are fermented to ethanol by *Saccharomyces cerevisiae*. Fermentation is carried out in batch bioreactors for 1.5 days, then, the fermentation broth is sent to ethanol purification consisting of beer and rectification distillation columns and vapor phase molecular sieve adsorption, which concentrates the ethanol product up to 99.5%. The vapor above from the beer column and fermentation is composed by CO_2, ethanol, and other components in less amount, a vapor which is sent to a scrubbing column in order to recover the volatilized ethanol. The stillage from the beer column, containing water and all remaining components of algae, is sent to lipid extraction step. The design of this processes assumes a feed rate of 1,449 ton/day in the summer case, so that the production of ethanol is around 3,263 gal/h in the summer design case. The total installation cost for this section is \$65,564,413 (2013–dollars).

The **Lipid Extraction and Solvent Recovery** area points to the extraction of the lipid fraction of the outlet stream from the fermentation and distillation section. These lipids are subsequently cleaned and upgraded to biodiesel. The stillage product from the beer column passes to a liquid-liquid countercurrent multi–stage extraction column, which uses hexane as an extraction solvent. The extracted oil phase together with solvent, fatty acid, polar lipid impurities, and a small amount of water, are sent to the stripping column to recover the solvent, reaching a purity of around 99.7% in total lipids stream. The aqueous product is sent to anaerobic digestion section. The extraction columns were design for a feed rate of 5,894 kg/h of total lipid. Under the maximum summer/spring scenario based on the capacity design, 16 equivalent columns operate in parallel. Each column is designed with a diameter of 6 ft for the agitated zone, and 9 ft diameter as the expanded ends, and an overall height of 60 ft. Each column requires 40 hp of power. The resulting purchase cost is \$1,980,000 per column, corresponding to the 30% of the total equipment installed costs. The solvent stripping cost and associated reboilers were quoted as \$714,000 and \$150,000 for the distillation

column and reboiler respectively, based on purchase costs per unit in 2009–dollars. The total installation cost for this section is \$19,560,332 (2013–dollars).

The **Product Purification and Upgrading** section initially removes impurities of the lipid product which would be problematic for the catalytic upgrading step. Lipid purification consists of a series of steps to remove gums, metals, and other impurities with the use of phosphoric acid, wash water, silica, and clay. The bottoms product from the solvent recovery stripping column contains 98% neutral lipid, 1.7% polar lipid impurity components, 0.3% hexane, and trace amounts of water. The bleaching, demetallization, and degumming cost estimates were furnished by Harris Group, the installed cost is \$7,000,000 based on the summer/spring design capacity. A power demand of 0.01 KWh/kg oil feed rate was assumed. Cost estimate includes costs for reactors, makeup and recycle gas compressors, fired heater, separation vessels, and distillation. The installation cost for this section based on the design summer/spring capacity is \$48,296,790.

Anaerobic Digestion/CHP. The aqueous product exiting from the lipid extraction column is united to the waste stream from the lipid purification step, then cooled and sent to the anaerobic digestion reactor. This step is used to provide a stabilization of wastewater treatment sludge, but also to integrate the process with algae cultivation. Anaerobic digestion is used to recover nutrients as nitrogen and phosphorus, but also other minor nutrients, to be recycled to the cultivation step, as well as to recover the residual carbon content in biogas for heat and power uses. The digestion is carry out at 35°C with 20–day hydraulic retention time, volatile solids loading factor of around 3.3 g/L day. The resulting total volume required for the digestion reactor was 121,500 m^3, requiring two digester units each sized at 16.1 MM gal which ensure, methane yield 0.3 L CH$_4$/g TS. The total slurry feed rate to the anaerobic digestion in the summer/spring design case is 243,500 kg/h (of volatile solids), the effluent is recycled to the algal cultivation, thus reducing the amount of makeup nutrients required for biomass growth. The cost of this section assumes that the anaerobic digestion units has a total purchase cost of \$25.8MM (2012 dollars), and includes also a digestate centrifuge, rated as 5% of the cost of the anaerobic digestion system, and a gas turbine. The total installation cost for this section is \$21,558,209.

Cultivation of *Chlorella protothecoides* in urban wastewater: biomass productivity and nutrient removal

The capability to grow microalgae in non-sterilized wastewater is essential for an application of this technology in an actual industrial process. Batch experiments were carried out with the species in non-sterilized urban wastewater from local treatment plants, to measure both the algal growth and the nutrient consumption. *Chlorella protothecoides* showed a high specific growth rate (about 1 d^{-1}), and no effects of bacterial contamination were observed. Then, this microalga was grown in a continuous photobioreactor with CO_2–air aeration in order to verify the feasibility of an integrated process for the removal of nutrient from real wastewaters. Different residence times were tested, and biomass productivity and nutrients removal were measured. A maximum of microalgae productivity was found at around 0.8 d of residence time, in agreement with theoretical expectation in the case of light-limited cultures. In addition, N-NH_4 and P-PO_4 removal rates were determined in order to model the kinetic of nutrients uptake. Results from batch and continuous experiments were used to propose an integrated process scheme of wastewater treatment at industrial scale including a section with *C. protothecoides.*

2.1 Introduction

In the recent decades, problems of resources scarcity, energy demand and pollution are emerging dramatically, due to population increase and raise of life quality in new region of the world. To date, more than 80% of the world's energy use still originates from combusting fossil fuels (Brennan and Owende, 2010). The potential of microalgae as an alternative for energy source is subject to intense academic and industrial research (Acién Fernández et al., 2012; Demirbas and Fatih Demirbas, 2011).

However, significant obstacles Lam and Lee (2012) still need to be overcome before microalgae–based biofuel production becomes cost–effective and can impact the world's supply of transport fuel. In order to fulfill the economic constraints of microalgae production, a multipurpose approach must be applied for large scale cultivation. Numerous studies have recently focused to reduce nitrogen and phosphorus pollution from wastewater (Singh and Thomas, 2012; Prathima Devi et al., 2012; Boelee et al., 2011; Sahu et al., 2013a). In particular, these nutrients contained in wastewaters could be exploited to cultivate microalgae at a lower cost with the additional benefit of eliminating pollutants from the environment (Pittman et al., 2011). The integration of microalgal biomass production and wastewater treatment could become a double purpose strategy, in which both of these processes, suitably combined, enable to achieve environment and economic advantages, according to Lam and Lee (Lam and Lee, 2012). This dual purpose system is gaining popularity and is an attractive alternative to microalgae–based systems aimed solely at biodiesel production, thanks to its advantages of reducing energy, fertilizer and freshwater costs (Olguín, 2012). In fact, the use of microalgal biomass for nutrients removal could improve the current wastewater treatment technology. The biological nutrient removal through nitrification-denitrification process suffers from high energy demand (Metcalf and Eddy, 2004). Using microalgae in these plants could improve their performances, reducing the total energy demand of the plant and producing a high energy content feedstock, that could be exploited by applying a biorefinery approach. Even if the capability of microalgae to uptake nutrients from wastewater is well known, and novel technologies on this topic are under investigation (as microalgal immobilization for instance, De-Bashan and Bashan (2010)), the feasibility of this process at the

large scale is far to be demonstrated and the current literature is generally lacking experimental results concerning real and untreated wastewaters under continuous operation conditions. Recent papers (McGinn et al., 2012; Ruiz et al., 2013) report about continuous experiments with *Scenedesmus obliquus* exploiting nutrients from wastewaters, and confirm a rapidly growing interest in evaluating an effective algal production of this type, that can be applied at the large scale. Furthermore, the concentration and quality of nutrients in domestic wastewater is highly variable, depending on weather, season and typology of the plant, and an algae production at large scale based on wastewaters must be improved by manipulating operating conditions (Park et al., 2011). Thus, a general comprehension of a depuration algae-based process is still challenging.

This chapter is aimed to verify algal growth capabilities using real and untreated wastewater, and to assess key design criteria and the technical feasibility of biofixation using microalgae. From a chemical and process engineering standpoint, operation in continuous photobioreactor is needed. In addition, the nitrogen and phosphorous consumption has to be measured in order to determine the kinetics of the process. Accordingly, it will be demonstrated the possibility of growing microalgae continuously in reactors working at steady state and fed by untreated wastewater, which are simultaneously depurated from nutrients. Based on experimental results, some preliminary considerations about the assessment of an algal based wastewater treatment process at industrial scale will be done.

2.2 Materials and methods

Chlorella protothecoides 33.80 (from SAG Goettingen, Germany), was maintained in liquid BG11 medium (Rippka et al., 1979) for the inoculum. Microalgae were cultured in different wastewaters sources, sampled from two different treatment plants in the northern part of Italy: Montecchio Maggiore, Vicenza (VI) which treats mixed domestic and industrial wastewater, and Camposanpiero, Padova (PD) which treats domestic wastewater only. Types of waters and denominations are reported in Figure 2.1. The typical nutrient content of these wastewaters is reported in Table 2.1. In order to remove the particulate, waters have been subjected to a first filtration treatment by

Table 2.1: Types of wastewaters and typical nutrients concentration.

Source	Wastewater typology	ID	N-NO$_3$	N-NH$_4$	P	N-NO$_2$	COD
Montecchio	After primary treatment	VI-I	9.31	44.46	8.00	2.21	310
	Process outstream	VI-II	34.67	1.66	1.02	2.24	ND
	After primary treatment	PD-I	18.83	63.83	9.44	3.34	272
Camposanpiero	Process outstream	PD-II	24.67	0.89	1.64	3.01	34
	Centrifuge of sludge	PD-III	158	139.63	7.9	<0.05	2010
	Centrifuge after anaerobic digester	PD-IV	64.94	1144.05	53.7	95.08	3870

paper filter of 10 μm of particles retention. No sterilization treatment was carried out, excluding some experiments, as indicated in the results section. In these cases, the sterilization was performed by autoclave, at 121 °C for 20 min.

2.3 Experimental set–up

The experiments were carried out in both batch and continuous processes. Batch experiments were performed in glass bottles of 250 mL, continuously mixed by stirring magnet and bubbling air enriched with 5% v/v of CO_2. The total flow rate was 1 L h^{-1}. The preinoculum were also grown in this culture systems. The temperature was controlled at 23°C in an incubator (Frigomeccanica Andreaus, Padova), and artificial light (white neon lamps OSRAM) was provided continuously at intensity of 100 μE m^{-2} s^{-1} of PAR (Photosynthetic Active Radiation) measured by a photoradiometer (Model LI–189, LI–COR, USA). Each experiment started with an initial microalgae inoculation of $OD_{750} = 0.5$, corresponding to a cell concentration of about 20x10^6 cells/mL. Continuous flow experiments were carried out in a flat plate vertical reactor of 250 mL of volume (see Figure 2.2). By tracer experiments it was shown that such a reactor behaves like a perfectly mixed one (CSTR, or chemostat). *C. protothecoides* was inoculated at the beginning into the reactor with non–sterilized wastewater medium. Once reached a significant concentration (the order of 10^8 cells/mL), the operational mode was switched from batch to continuous, feeding non-sterilized wastewater by a peristaltic pump (Sci–Q 400, Watson Marlow,USA). The desired value of the liquid level in the reactor was controlled by an overflow pipe, and the outlet flow was collected
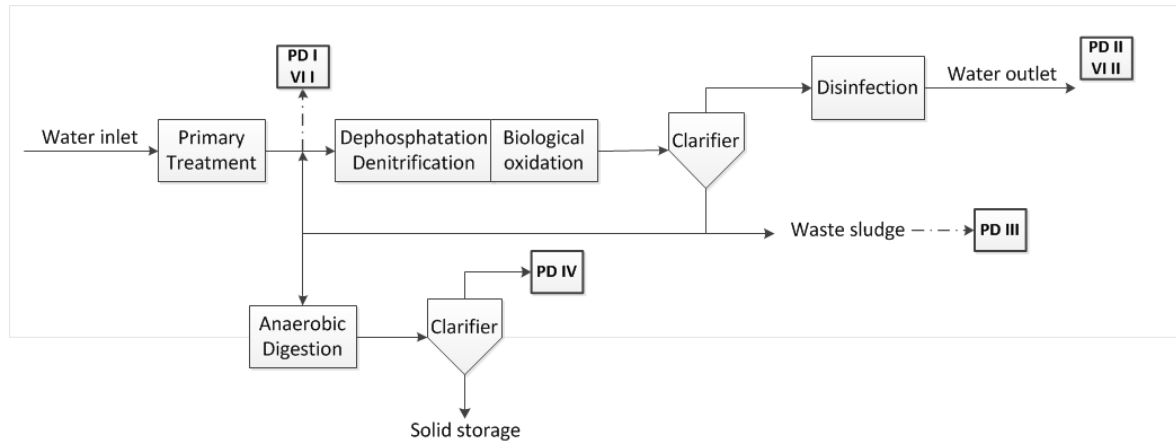
Figure 2.1: Simplified scheme of urban wastewater treatment plant and waters used in experiments: PD I and VI I (water after primary treatment), PD II and VI II (water after final process), PD III (water after sludge centrifugation) and PD IV (water after centrifugation of anaerobic digested).

in a bottle. The residence time (τ) was directly controlled by the peristaltic pump. The conditions of mixing, temperature and lighting were the same as those of the batch systems. Microalgae growth was tested as a function of residence time. Furthermore two different wastewater were tested at the same τ=1.26 d in order to investigate the feed quality effects.

Due to the nutrient content of sampled wastewater, which was not constant with time, all batch experiments were carried out with water from a single sampling from the plant, and nutrients were measured at time 0 of each growth curve, in order to verify the effective consumption by microalgae. In a second sampling, wastewater were collected for feeding the continuous experiments, in order to analyze the effect of τ as the main operating variable.

2.4   Analytical methods

The growth was monitored daily by spectrophotometric analysis of the optical density (measured at 750 nm, by double beam spectrophotometer UV–Visible UV 500 from Spectronic Unicam, UK) correlated to cell concentration, measured with a Bürker Counting Chamber (HBG, Germany). Specific growth rates in batch experiments were measured by linear regression of 6–10 experimental points of logarithmic phase of growth, from two independent biological replicates. At the end of the growth curve the final concentration of biomass of each experiment was measured as dry weight
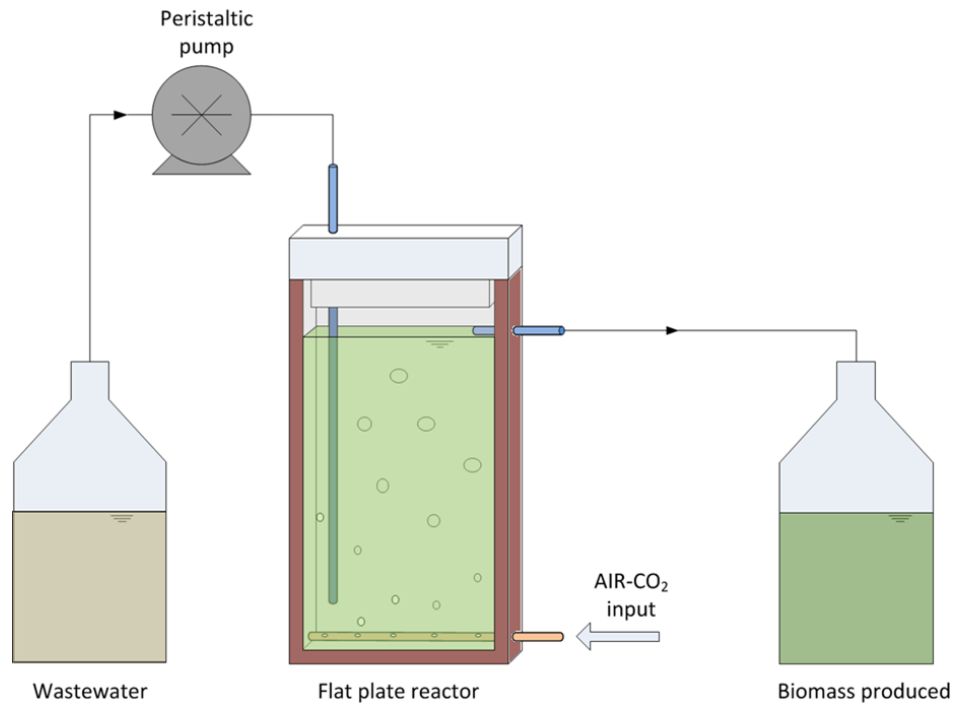
Figure 2.2: Scheme of lab-scale reactor of continuous flow experiment. Not in scale.

(DW) in terms of g/L. DW was measured gravimetrically in cells previously harvested with a 0.22 μm filter, the filters were dried for 4 hr at 100 °C in a laboratory oven. In continuous experiments, biomass concentration in terms of cell/mL and DW were determined daily in duplicate, and the steady state concentration was averaged on 5 to 10 experimental points. The nutrients analyzed were nitrate ($N-NO_3$), ammonium ($N-NH_4$) and phosphate ($P-PO_4$), assessed daily using standard methods in water and wastewater (APHA-AWWA-WEF, 1992). A sample of culture was filtered in order to measure only dissolved nutrients (0.2 μm), and nitrate, ammonium, phosphate and nitrites were measured by test kits provided by St. Carlo Erba Reagenti (Italy). The data were found consistent with those provided by the measurements in treatment plants. Nitrate analysis kit (code 0800.05482, Carlo Erba reagenti) is based on reduction of nitrates to nitrites that react with sulfanilic acid producing diazonium ion. By the reaction with gentisic acid, a dying molecule is produced and detected at 445 nm Spectronic Unicam UV–500 UV–visible spectrometer. Ammonium (code 0800.05405. Carlo Erba reagenti) is measured indirectly by the absorbance (at 420 nm) of an indophenolic complex produced by reaction of ammonia with phenolic derivatives. Phosphorus (code 0800.05455, Carlo Erba reagenti) is measured by the

formation of a dyed complex (690 nm) between orthophosphate ion and molybdenum under reducing environment. Nitrites (code 0800.05485, Carlo Erba reagent) is based in the reaction of nitrite ion with sulfanilic acid, then the diazonium salt reacts with a-naphthylamine generating a nitrogenized dye which absorbs at 520 nm. Chemical oxygen demand (COD) was measured by an analytical kit provided by Sigma–Aldrich, USA (AQUANAL®) and is based on oxidation of organic compounds by potassium dichromate in sulfuric acid solution

2.5 Results and discussion

2.5.1 Batch experiments: algal growth in different wastewaters

C. protothecoides, a common species used to produce biodiesel, was chosen because of its capability to grow with remarkable performances in untreated wastewaters (see table 2.2 for specific growth rates). First, the capability of *C. protothecoides* of growing in the different wastewaters was tested. The experiments were carried out in vertical bubbling reactors illuminated with artificial continuous light (100 μE m^{-2} s^{-1}) and mixed by aeration with 5% v/v of CO_2 in air. In order to verify the effect of competition for nutrients between bacterial population and microalgae, the effect of sterilization was observed in the experiments with waters derived from the out stream of primary treatment of VI plant. The sterilization by autoclave did not influence the growth kinetic constant (values of 1.04 d^{-1} and 1.01 d^{-1} for sterilized and non–sterilized water were found respectively), showing that the endogenous bacterial contamination did not affect the algal growth rate. As can be seen in Figures 2 and 3A, with the corresponding wastewater from PD, demonstrating that, although the inlet flow of wastewater in a treatment plant is wide variable, *C. protothecoides* is able to grow in different waters with similar performances, and confirming its strong resistance to chemical and to competition with native microflora. We also tested the capability of *C. protothecoides* of growing in final treated water, in order to assess the possibility to decrease the concentration con N and P in water outlet. As reported in Figure 2.3B, microalgae can grow in these waters (derived both from VI and PD), but with a lower biomass concentration due to the low content of nutrients available. Concerning the nutrient uptake (data shown in table 2.2), in all cases *C. protothecoides* consumed
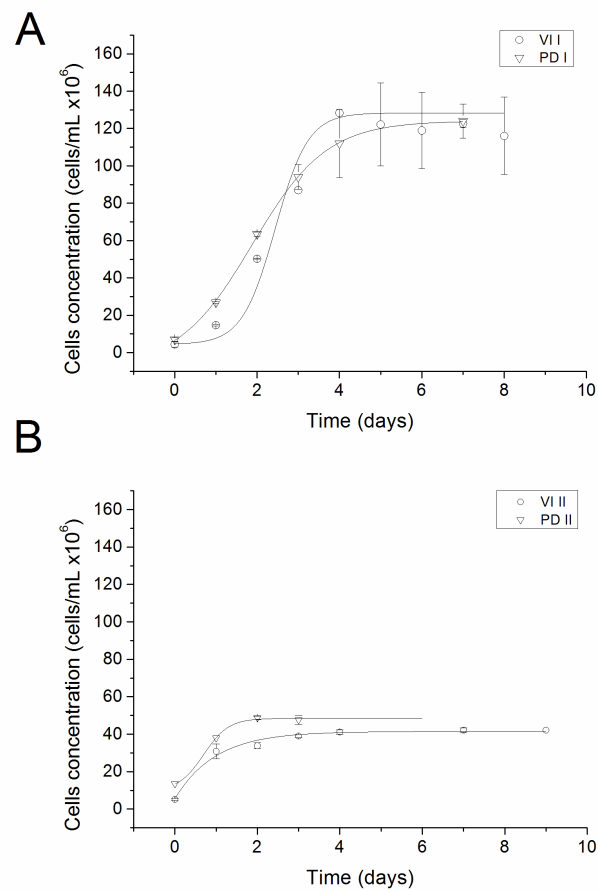
Figure 2.3: Growth curves of C. protothecoides in urban wastewaters from Montecchio (circle) and Camposanpiero (triangle) treatment plants, in wastewaters from the primary treatment (A) and from the outstream of the process (B). Solid lines are eye guides.

high percentages of N and P contained in the waters. In particular, N-NO$_3$ and N-NO$_2$, present in lower concentration, were totally consumed in the early stage of the growth curve. Thus, these nutrients were not considered in the discussion of further experiments because of their fast kinetic of consumption. The N-NH$_4$ was efficiently removed from primary wastewaters (VI–I and PD–I), while in final waters (VI–II and PD–II) the ammonium consumption was lower, due to the scarcity of P in this type of water, that was limiting for algal growth.

The nutrient consumption by microalgae is mainly related to the N:P ratio. On the other hand, the composition of wastewater is widely variable. The ideal range of N:P mass ratio for microalgal growth is 6 to 13 (Olguín, 2012; Arbib et al., 2013), confirming that the water used in this study are suitable for algal bioremediation. As reviewed

by Olguín (2012), the COD removal strongly depends on various factors, such as the characteristic of the specific type of wastewater utilized and the microalgal species. On the other hand, Hu and co-workers (Hu et al., 2012) suggested that the CO_2 bubbling could affect the COD uptake, by shifting the metabolism of *A. protothecoides* (a taxonomic synonymous of *C. protothecoides*) to autotrophic metabolism only. In fact, they observed that the COD reduction rate is inversely related to the CO_2 concentration. On the other hand, they obtained a partial COD reduction, while in this work the organic matter concentration remained constant. This could be explained by the preparation of preinoculum, that was grown at concentration of CO_2 higher than the atmospheric one, probably leading to an adaptation of metabolism to environmental conditions, that could affect the capability of *C. protothecoides* to use organic carbon. In fact, the mixotrophic capability of *C. protothecoides* is strictly related to the limiting concentration of CO_2 and the absence of light, as previously demonstrated (Sforza et al., 2012b). Anyway, CO_2 is the main nutrient needed to obtain a sufficient productivity of algal biomass, that could make the algal remediation process feasible at large scale. Thus, our results suggest that *C. protothecoides* was able to efficiently remove N and P, achieving a good biomass production, but a two steps depuration process is required, and an activate sludge reactor is needed, in order to achieve a COD reduction.

Table 2.2: Growth kinetic and nutrients consumption in batch experiments with waters from primary treatment and final treatment.

Water	Growth rate (d-1)	Nutrient consumption (% of initial)			Final concentration (mg/L)		
		N-NO$_3$	**N-NH$_4$**	**P-PO$_4$**	**N-NO$_3$**	**N-NH$_4$**	**P-PO$_4$**
VI I (sterilized)	1.04	>95	92	71	<5	1.76	0.52
VI I	1.01	>96.3	97	70	<5	1.14	0.79
VI II	0.97	>95	33	50	<5	1.01	0.33
PD I	1.02	>90	94	62	<5	1.88	0.53
PD II	1.03	>90	4	68	<5	0.63	0.17

Finally, the microalgal growth in other waters derived from different step of the depuration process was tested. PD–III is the water from centrifugation of sludge and PD–IV is from centrifugation after the anaerobic digester. As shown in Figure 2.4A, *C. protothecoides* is able to grow in water from sludge centrifugation only after

sterilization, suggesting that the high concentration of bacteria in this water strongly competed with algal growth. Concerning waters derived from the anaerobic digester, we found that microalgae growth is strongly inhibited even if water was sterilized or diluted (Figure 2.4B). The effect is probably due to both the high concentration and the quality of the organic matter contained in this water.
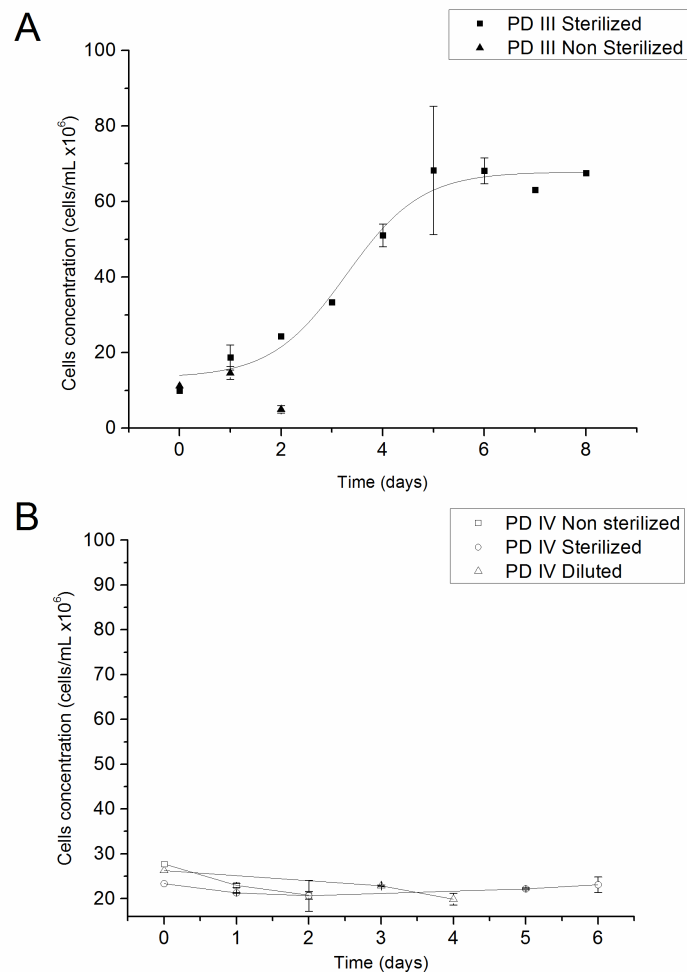
Figure 2.4: Growth curves of C. protothecoides in wastewaters after centrifugation of sludge (A) and after centrifugation of solids from anaerobic digestion (B). Solid lines are eye guides.

In summary, from the comparison of the different wastewaters tested, the best growth rate and final biomass concentration were obtained when using the wastewater sampled after the primary treatment, that showed a sufficient content of nutrients and an irrelevant bacterial contamination if compared with algal growth. This water was then used for further experiments in the continuous reactor.

2.5.2 Continuous flow experiments: biomass productivity

C. protothecoides was grown in a continuous flow reactor fed by non–sterilized wastewaters collected after the primary treatment, from both Vicenza and Padova, in order to evaluate biomass productivity and nutrients uptake as a function of the residence time at steady–state operating conditions. A first run was carried out using VI–I water in order to demonstrate that a steady state can be achieved, even if the untreated wastewater has an endogenous bacterial contamination. As can be seen from Figure 2.5, after 5 days of batch operation the mode was switched to continuous one. The inlet flow rate was 198 mL d^{-1} resulting in a residence time of 1.26 d. The outlet biomass concentration became stationary after further 5 days, with a steady-state concentration of about 0.47 g L^{-1}. The pH value remained constant (at about 7.5) during the experiment as well as the bacterial concentration, showing that bacteria did not compete with algal growth in a continuous system. In fact, the reactor is designed to optimize the growth conditions of microalgae, and the native microflora of the waste stream, mainly heterotrophic, is washed out, confirming what was suggested also by McGinn et al. (McGinn et al., 2012).
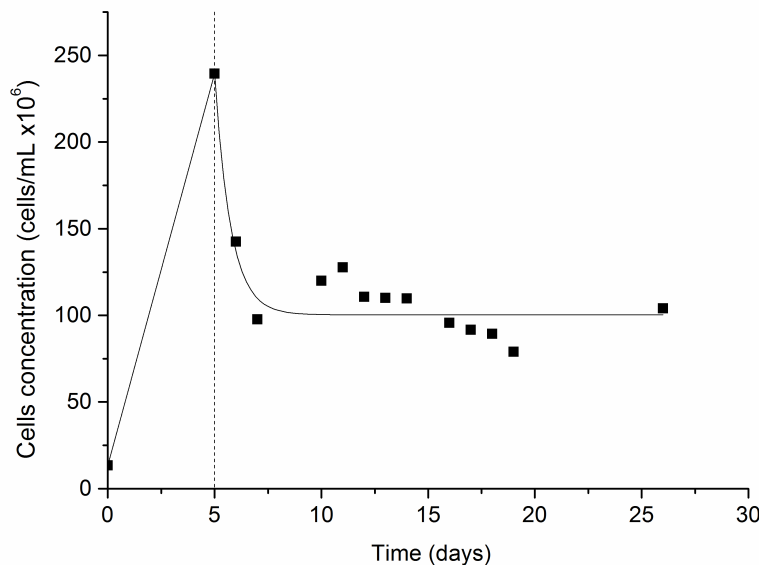
Figure 2.5: Continuous experiment of C. protothecoides growth with wastewaters after primary treatment (non-sterilized) from Montecchio.

Similar results were obtained with PD–I water: in this case five residence times

(0.65, 0.80, 1.01, 1.26 and 1.97 d) were tested, and the values are reported in Figure 2.6 in terms of cell concentrations. It was confirmed that pH values remained constant throughout all the experiments.
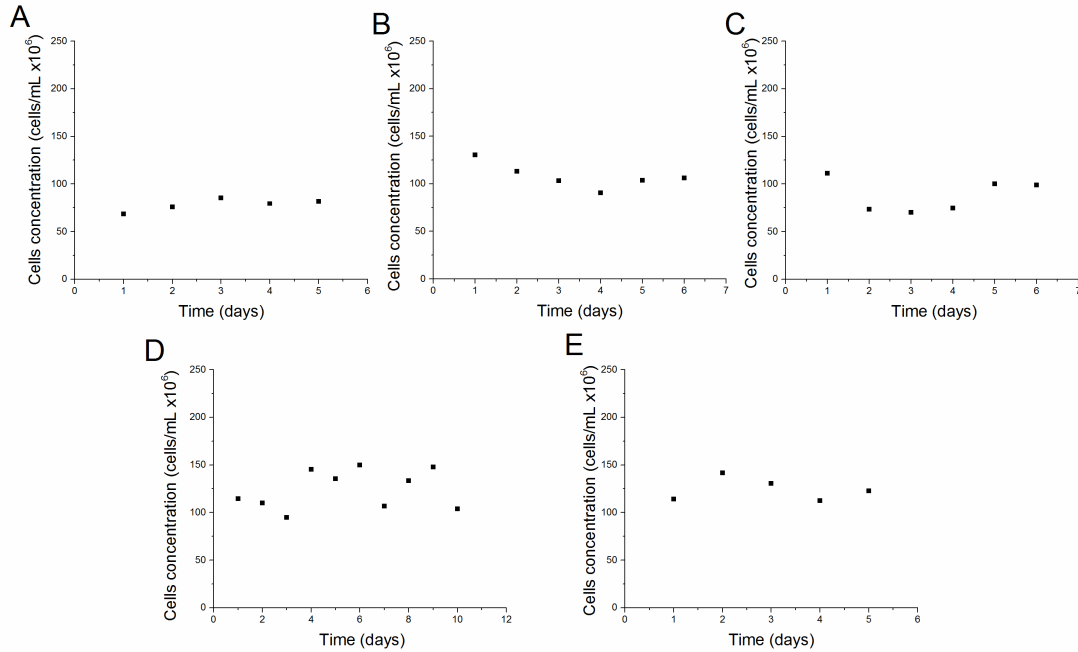
Figure 2.6: Continuous experiment of C. protothecoides growth with wastewaters after primary treatment (non sterilized) from Camposanpiero at five different residence times (0.64, 0.8, 1.01, 1.26, 1.97 d).

A. protothecoides was previously grown only in batch and semi-continuous system by Zhou et al. (2012), with autoclaved waters. In this work it was demonstrated that *C. prothotecoides* is a strong strain that can be cultivated in continuous systems, even if in the reactor is fed by unsterilized wastewater, confirming a quite interesting resistance to competition with native microflora. Other species such as *Scenedesmus obliquus* were cultivated in continuous system fed by sterilized wastewaters (McGinn et al., 2012) and by secondary waters with low nutrient concentration (Ruiz et al., 2013).

In Figure 2.7 results of biomass productivity as a function of residence time are reported. It is shown that the outlet biomass concentration at steady state increased with the residence time up to a value of 0.80 g L^{-1} at $\tau = 1.97$ d. In the same figure productivity values are also displayed.

Productivity is defined in terms of kg of biomass produced per day and per litre of reactor, i.e. the production rate per unit reactor volume r_{Xu}. According to the
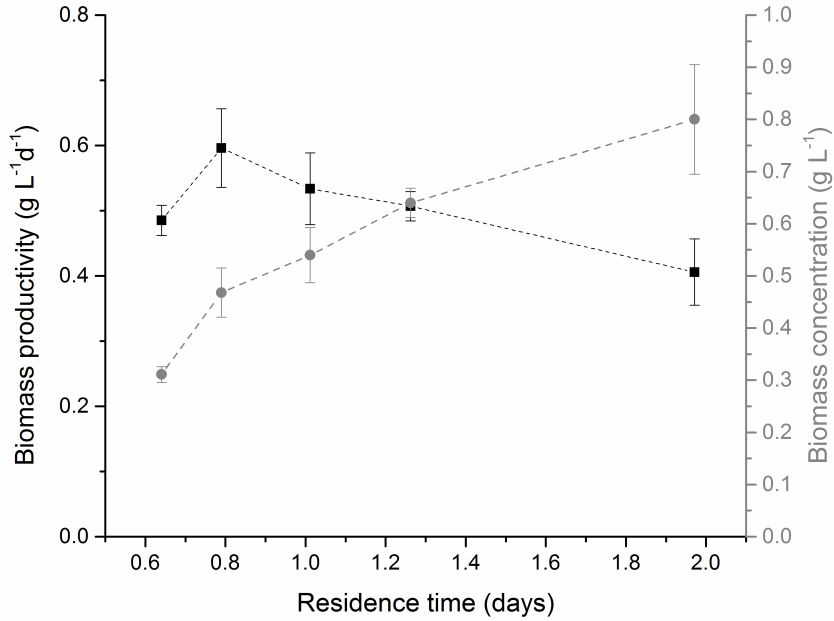
Figure 2.7: Biomass concentration (circle) and productivity (square) at different residence times in continuous reactor.

steady–state material balance (Equation 2.1) for a biological CSTR:

$$-c_{Xu} + r_{Xu} \cdot \tau = 0 \qquad (2.1)$$

productivity can be calculated as the ratio of outlet concentration c_{Xu} and the residence time. From Figure 2.7 it can be seen that the values are indeed satisfactory (according to the literature, 1.5 g $L^{-1}d^{-1}$ area top reference value), considering that no extra nutrients are fed to the reactor. In addition, a maximum of productivity is evidenced experimentally, corresponding to $\tau = 0.80$ d. Over this value of residence time, the biomass productivity decreased. A similar trend is reported also in Ruiz et al. (2013), which applied the Verhulst logistic kinetic model to estimate the maximum productivity. However, they found a discrepancy between the model and the experimental data, in particular for residence times lower than 0.9 d. In our case, if we apply their equation for the residence time which maximizes productivity.

$$\tau_{optimum} = \frac{2}{\mu} \qquad (2.2)$$

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and consider a specific growth rate of about 1 d^{-1} for *C. protothecoides*, it again the maximum productivity would be expected at $\tau=2$ d. As our maximum was measured at about $\tau=0.8$ d, again the theoretical prediction developed by Ruiz et al. (2013) does not match with experimental results. Work is in progress to improve the Verhulst model.

The maximum in biomass productivity can be explained by considering that, even if microalgae concentration increases with residence time, its rate of increase decreases owing to self–shading effects occurring when it gets higher. In this condition part of the reactor is in the dark, so that the overall light exploitation gets lower, and the productivity decreases accordingly. This light limitation hypothesis is confirmed by the comparison of the biomass–light yield values of steady–state concentration obtained at residence times higher than 0.8 d. By assuming a biomass energy content of about 20 MJ/kg (average of data reported in literature, i.e. Li et al. (2012b); Phukan et al. (2011); Sturm and Lamer (2011)) and considering the energy of the light impinging the panel, is it possible to calculate the energy conversion efficiency related to the PAR. In the case of PD–I water the fraction of PAR converted into microalgae increased when decreasing the residence time (5.18, 6.47, 6.81, 7.59 % at residence times of 1.97, 1.26, 1.01 and 0.80 d respectively). The biomass–light yield value obtained at 0.64 d of residence time corresponds to 6.19% of PAR, which is lower because it is close to washout condition, where biomass is removed from the reactor at higher rate, leading to an inefficient exploitation of light, even if no shading effects occur. The light limitation hypothesis could be applied also in the case of Ruiz et al. (2013), that cultivated microalgae in a reactor of 44 mm of depth. However, the nutrient limitation could also play a role in the kinetics of growth. This point can be discussed by considering the measurements of nutrient removal in the continuous reactor, reported in Figure 2.8. Here, it can be seen that the nitrogen consumption at $\tau = 0.64$ d is lower than in the others cases. An increment is observed while increasing residence time, where microalgae were able to consume about the 93% of phosphorus and about 72% of nitrogen. These results suggest that the growth-limiting nutrient is phosphorous and that it starts to become limiting due to the increasing residence time. Such finding is confirmed if compared to the results obtained with VI–I water, where an opposite trend
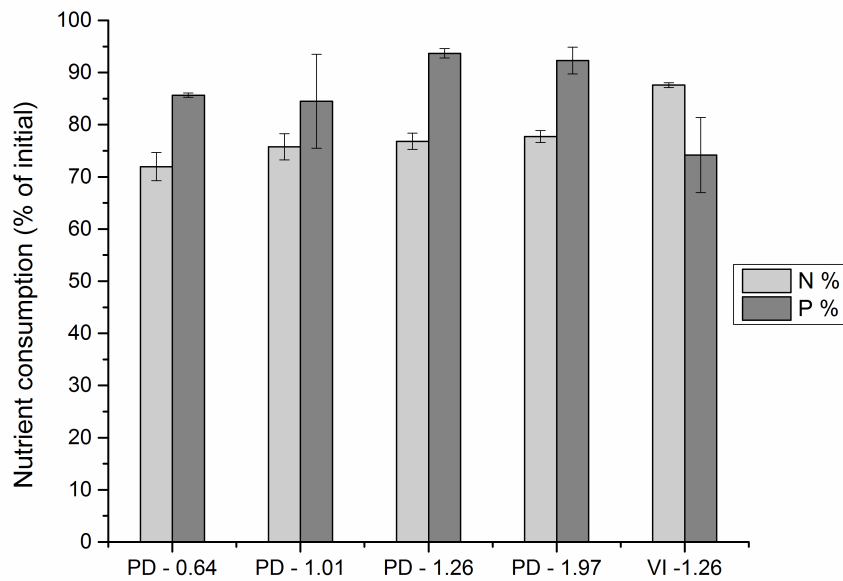
Figure 2.8: Nutrient consumption in continuos reactor.

was observed. In fact, in this case a higher percentage of N was removed, while only a 73% of P was consumed, suggesting that nitrogen could become limiting here. The different behavior is due to the different N/P ratio of the two waters (14.28 and 17.91 for VI and PD respectively), confirming that a higher consumption of N is possible only if P is not limiting.

2.5.3 Nutrients removal rates

Batch experiments were carried out to investigate the effect of the initial P-PO$_4$ and N-NH$_4$ concentration on nutrient uptake by microalgae and to measure the related kinetics of nutrient consumption. Real wastewater from PD plant was used, and the initial concentration was modified by adding artificial nutrients, or diluting the starting wastewater, specifically for each experimental measurement, to cover the concentration ranges of interest. The microalgal growth and nutrient concentration were monitored during each run. Figure 2.9A reports the first three points of concentrations profiles for P-PO$_4$. These data were used to evaluate the initial reaction rates of nutrient removal by the slope of a linear interpolation, and are summarized in Table 2.3 as a function of the initial concentration. In the same table also the specific rate of phosphorous

removal (r_P/C_X, being C_X the microalgae concentration) is reported: it increases from 0.04 to 0.24 mg P-PO$_4$/mg biomass, while increasing P-PO$_4$ initial concentration from 1.73 to 11.25 mg L^{-1}. In a similar way, Figure 2.9B reports the first three points of N-NH$_4$ concentrations profiles, together with a linear interpolation. The initial reaction rates are summarized in Table 2.4 as a function of the initial concentration, together with the specific rates of nitrogen removal (r_N/C_X).

Table 2.3: Kinetic parameters of phosphorous uptake

Initial P-PO$_4$ concentration (mg/L)	Removal rates (mg/L /d)	r$_i$/C$_X$
1.73	5.13	0.04
6.23	20.17	0.16
11.25	32.43	0.24

Table 2.4: Kinetic parameters of nitrogen uptake

Initial N-NH$_4$ concentration (mg/L)	Removal rates (mg/L /d)	r$_i$/C$_X$
26.13	31.42	0.34
45.40	32.20	0.50
76.72	38.60	0.51

The kinetics of removal of nutrient i was assumed to obey the Monod kinetics for both P and N (Equation 2.3):

$$\frac{r_i}{C_X} = \mu_{max,i} \frac{C_i}{C_i + K_i} \tag{2.3}$$

where $\mu_{max,i}$ is the reaction rate constant and K_i is the half saturation constant of nutrient i. By correlating the data measured to these parameters, it was obtained:

$$\mu_{max,P} = 0.849 \left[\frac{mgP - PO_4^-}{mg_{biomass}\, d} \right] \tag{2.4}$$
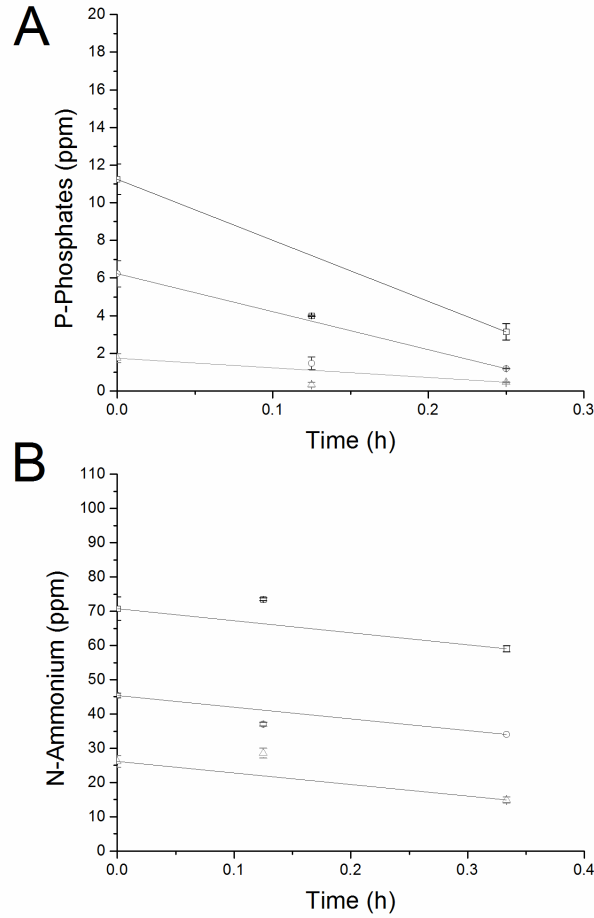
Figure 2.9: First three point of consumption of P(A) and N (B) at different initial concentrations.

$$\mu_{max,N} = 0.688 \left[\frac{mgN - NH_4}{mg_{biomass} \, d} \right] \tag{2.5}$$

Aslan and Kapdan (Aslan and Kapdan, 2006) investigated the nutrients kinetics uptake in artificial wastewater by *Chlorella vulgaris*. They found a specific reaction rate constant $\mu_{max}/C_P = 0.5$ mg P-PO$_4$/mg (chl a) and a half saturation constant K_P =10 mg L^{-1}. Their specific reaction rate is higher than the one we measured, even accounting for the different units (we refer to total biomass, while Aslan and Kapdan to the chl a, a part of the biomass). About the N-NH$_4$ removal rates, the same authors reported values of $\mu_{max}/C_N = 1.5$ mg N-NH$_4$/mg (chl a) and K_N =31.5 mg L^{-1}, which give higher specific reaction rates than in our case, as well. However, the pigment content of microalgae is variable depending on environmental condition and growth

phase. Thus, measuring the uptake of nutrient normalized on biomass concentration would give more accurate results. The results about nutrient removal rates confirmed that, depending on the nutrient concentration in the wastewater, P is the limiting nutrient affecting the microalgal growth for the wastewaters considered.

$$K_P = 28.2 \left[\frac{mgP - PO_4^-}{l} \right] \tag{2.6}$$

$$K_N = 23.4 \left[\frac{mgN - NH_4}{l} \right] \tag{2.7}$$

2.6 From experimental data to depuration process design

Based on the experimental data discussed above, an improved scheme of biological wastewater treatment including a microalgae step is proposed (see Figure 2.10). Here a tank reactor where microalgae treat the wastewater, was added after the primary treatment and before the aeration tank. In this step microalgae use nutrients to grow, achieving the double goals of depurating water from N and P, and producing a valuable biomass that could be separated and exploited for energy or high-value material production purposes. The water stream out of this tank is delivered to an activated sludge reactor, where organic matter is degraded by bacteria, as in the traditional process. It is important to consider that a certain amount of phosphorus and nitrogen has to be fed to the activated sludge reactor, to permit the removal of COD. In fact, bacteria need a ratio of COD:N:P =100:5:1 (Spanjers and Vanrolleghem, 1995) in order to efficiently remove organic matter. Thus, by considering the kinetic of nutrients removal, it is possible to design the microalgal reactor that can provide a higher biomass productivity and an appropriate nutrient consumption. We point out that the effectiveness of this system is strongly affected by the ratio of nutrients in the incoming water, and the values of the system parameters have to be measured as shown above. A deeper analysis should be done in order to assess the feasibility of this process, related in particular to the economic costs associated with the improved process.
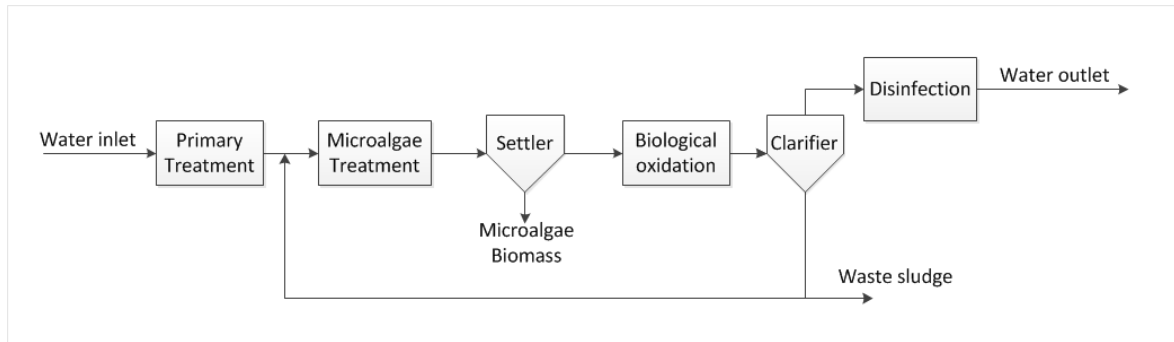
Figure 2.10: Simplified scheme of urban wastewater treatment plant integrated with biomass production step.

2.7 Final remarks

In this chapter, the problem of culturing *C. protothecoides* in raw urban wastewaters has been investigated in view of possible industrial applications. It was shown that using untreated wastewater as culture medium without additional nutrient supply is suitable to attain a good performance of a process whose aim are simultaneously microalgal production, and efficient removal of N and P compounds. In addition to a remarkable growth rate of the species, an efficient nutrients removal was also measured, proving that, since N and P contained in real wastewaters were exploited by microalgae as nutrients. P and N degradation kinetics were determined in real and untreated water, and the kinetic constants were correlated to these data. In order to assess the applicability of this idea at the industrial scale, *C. protothecoides* was cultivated in a continuous flow photobioreactor, fed with non-sterilized wastewater. A steady-state operation was obtained with a considerable biomass concentration. The continuous reactor was operated at different residence times, in order to evaluate biomass productivity and nutrients uptake. Whereas the outlet biomass concentration was increasing with residence time, a maximum in productivity was found at 0.8 d of residence time, due to light limitation phenomena on cell growth. An efficient nutrient removal was obtained in the continuous PBR. As well was also shown that the competition with endogenous bacterial contamination did not affect the algae growth in the continuous reactor. Eventually a possible process for the integration of wastewater treatment at industrial scale including a section with *C. protothecoides* was proposed.

Symbols

PD	Padova treatment plant
VI	Vicenza treatment plant
PD-I	Padova treatment plant, after primary sedimentation
VI-I	Vicenza treatment plant, after primary sedimentation
PD-II	Padova treatment plant, process out stream
VI-II	Vicenza treatment plant, process out stream
PD-III	Out stream of centrifuge of sludge, Padova
PD-IV	Centrifuge post anaerobic digester, Padova
τ	Residence time [d]
r_{Xu}	Biomass reaction rate [mg L^{-1} d^{-1}]
c_{Xu}	Outlet biomass concentration [mg L^{-1}]
r_p	Phosphorous reaction rate [mg L^{-1} d^{-1}]
C_P	Phosphorous concentration [mg L^{-1}]
X_i	Biomass concentration [mg L^{-1}]
μ	Reaction rate constant [mg substrate mg^{-1} biomass d^{-1}]
$\mu_{max,i}$	Reaction rate constant, maximum [mg substrate i mg^{-1} biomass d^{-1}]
K_i	Half saturation constant [mg substrate i L^{-1}]
r_N	Nitrogen reaction rate [mg L^{-1} d^{-1}]
C_N	Nitrogen concentration [mg L^{-1}]
k	Reaction rate constant [d^{-1}]

Integration of *Chlorella protothecoides* production in wastewater treatment plant: from lab measurements to process design

The exploitation of microalgae in a wastewater treatment process is currently an open issue, and its actual applicability is still under investigation. In this chapter the effects of temperature, day/night irradiation and bacterial competition were studied on growth and nutrient removal of *C. protothecoides* cultivated in real and unsterilized primary urban wastewater. *C. protothecoides* showed a linear dependence of growth rate on temperature under continuous irradiation with a maximum at 30 °C. Continuous flow experiment under day-night irradiation condition showed that a cyclic steady state was achieved, with significant differences in biomass concentration and nutrient removal after dark and light periods. The presence of a native microflora in wastewater did not affect the microalgal growth, both in batch and continuous flow experiments. N and P were efficiently removed in all condition tested, while the COD was not consumed by microalgal biomass. Thus, in view of a large scale application, a two steps depuration process would be required, where the photobioreactor will remove nutrients as N and P and an activated sludge reactor downstream will reduce the organic matter. The experimental data obtained were used to design a possible process of this type.

3.1 Introduction

It is worldwide recognized that the demand of liquid fuels is expected to grow quite fast, leading to a tremendous development of biofuel production technologies. Among these, in the last decade the biodiesel derived from the cultivation of microalgal biomass has been proposed as an effective method to produce high quality, sustainable and renewable biofuel (Acién Fernández et al., 2012). However, industrial processes based on the use of fresh water, synthetic CO_2 and chemical fertilizers are not economically sustainable (Lam and Lee, 2012). In fact, many LCA studies have shown that the whole process has a high energy demand that lead to a negative overall energy balance (Lardon et al., 2009; Brentner et al., 2011), and many authors have highlighted that the environmental and economic benefits of microalgae utilization are not always clear and effective when passing from laboratory to large scale (Lardon et al., 2009; Davis et al., 2011; Lundquist et al., 2010). For these reasons, nowadays, there is no commercial plant producing and processing microalgae biomass into biofuels yet (Lam and Lee, 2012). One of the major issues related to microalgae biomass production concerns the nutrients availability (Jorquera et al., 2010; Sander and Murthy, 2010), as the cultivation of microalgae at industrial scale for biofuels production requires a large amount of nutrients, typically nitrogen and phosphorous. The idea of exploiting nutrients from wastewaters to grow microalgae goes back to the 50's, when early studies were carried out by Oswald's group (Oswald et al., 1953), but it is only in the last decade that researches have focused on this field (Boelee et al., 2011; Singh and Thomas, 2012; Sahu et al., 2013b; Patel et al., 2012), due to the need to find more sustainable solutions for fuel production within a short time. In addition, coupling the microalgal production with water treatment can improve its sustainability, as the nitrification–denitrification processes usually implemented for nutrient reduction are actually high energy-intensive and require huge capital costs (Metcalf and Eddy, 2004). A carefully engineered approach could improve the overall process yield, reducing the total energy demand and, at the same time, producing a biomass feedstock with high energy content. Some key issues for industrial applicability remain still unsolved, such as environmental fluctuations of temperature, light availability and possible competition with native microflora present in wastewaters. Along the year, wastewaters in treatment plants are

characterized by significant temperature changes, depending on geographical position and climate that affect the sludge growth kinetic. Wastewater temperature is usually higher than that of the water supply thanks to the addition of solid waste and warmer water from household appliances, so that their temperatures usually range from 10°C to 30°C (Metcalf and Eddy, 2004) at mid-latitudes.

In addition, in an actual photobioreactor operated outdoor at mid–latitudes, the light conditions are widely variable, so that a number of issues must be thoroughly addressed, i.e. reactor orientation, light variation during the day, reflection of light, light gradient in the reactor (Slegers et al., 2011). Obviously, when using wastewater as the feed for microalgae cultivation, the wastewater cannot be previously sterilized due to the enormous volumes to be processed. In these conditions, many species (including bacteria) would necessarily coexist in the culture together with microalgae. A classical wastewater treatment biological reactor is characterized by the presence of thousands species belonging to almost all of biological kingdoms (Metcalf and Eddy, 2004). Due to this huge genomic and phenotypic variability, the study of this ecosystem (the so–called "activated sludge") is extremely complex (Forster et al., 2002), even if improving knowledge of the ecosystem composition could lead to a better understanding of the biochemical reactions occurring within the reactor, thus allowing the enhancement of its performance (Nielsen et al., 2012). To date, some microalgal bacteria consortia have been tested by many authors with the aim of nutrient removal from water (Boelee et al., 2011; Singh and Thomas, 2012; Sahu et al., 2013b; Patel et al., 2012). The feasibility of this process at industrial scale in continuous systems is not clear, in particular concerning a long term competition between bacteria and photosynthetic organisms.

The objective of this chapter is to assess the exploitation of wastewater as a nutrient source for microalgal growth, in order to better understand this process, and to give a contribution towards its industrialization and large-scale application. The influence on growth of several parameters, as the effect of temperature and real irradiation, COD consumption and competition with native microflora were considered. To this aim, *C. protothecoides* was cultivated in real non–sterilized wastewaters, taking into account the effect on growth kinetics of actual temperature range and alternation of day–night cycle. Nutrients removal was measured: in particular we focused on nitrogen,

phosphorus and chemical oxygen demand (COD) concentration, in view of developing an integrated system where bacteria and microalgae cooperate in the water treatment process. Bacteria contamination in different conditions tested was also considered as a rough measure of a possible coexistence in a continuous bioreactor. Experiments under day–night irradiation were carried out both in batch and in continuous, in order to verify the biomass productivity. Starting from experimental results, the model parameters of nutrient kinetic uptake were correlated to the measured data. Eventually, a possible integrated process was proposed and nutrient, biomass and energy balances were applied to a preliminary process design scheme.

3.2 Materials and methods

3.2.1 Microalgae strains and growth experiments

C. protothecoides 33.80 (from SAG Goettingen, Germany), was maintained in liquid BG11 medium (Rippka et al., 1979) for the pre–inoculum. Microalgae were cultured in wastewaters sampled from a treatment plant (ETRA S.p.A.) in Camposanpiero, Padova (PD), Italy, which treats domestic wastewater. The water was sampled after the primary treatment. No sterilization treatment was carried out (excluding one experiment): in this case the sterilization of wastewater was performed by autoclave at 121°C for 20 min). Water was maintained in refrigerator before each experiment. Experiments of microalgal growth were carried out both in batch and continuous systems. Due to the nutrient content of sampled wastewater, which was not constant with time, all batch experiments were carried out with water from a single sampling from the plant, and nutrients were measured at time 0 of each growth curve, in order to verify the effective consumption by microalgae. Initial nutrient concentrations are reported in Table 3.1. Each batch experiment was carried out in at least two replicates, and started with an initial microalgae inoculation of $OD_{750} = 0.5$, corresponding to a cell concentration of about $20x10^6$ cells mL^{-1}.

As reported in Figure 3.1A (data provided from the treatment plant where waters were sampled), inlet wastewaters in a treatment process are exposed to seasonal temperature fluctuations. Thus, a number of experiments were performed at 10, 15, 23 and 30°C under continuous irradiation of 100 μmol photons m^{-2} s^{-1}. Other batch

Table 3.1: Initial and final concentration of nutrients in batch experiments under continuous irradiation at different temperatures.

Temperature	Total N				Total P			
	Initial		Final		Initial		Final	
($°$C)	(mg L^{-1})		(mg L^{-1})		(mg L^{-1})		(mg L^{-1})	
	Mean	St.Dev	Mean	St.Dev	Mean	St.Dev	Mean	St.Dev
10	38.71	1.02	10.97	8.52	2.53	0.06	0.006	0.003
15	31.51	2.62	1.01	0.09	2.61	0.07	0.01	0.04
23	28.74	0.26	1.87	0.86	2.34	0.02	0.01	0.03
30	38.48	1.22	4.99	0.12	2.47	0.02	0.01	0.007

experiments were carried out to verify the effect of bubbling of CO_2 and air-only on COD consumption and bacterial competition, at 23 $°$C and 100 μmol photons m^{-2} s^{-1} of irradiation.

A continuous flow experiment was carried out in order to test *C. protothecoides* behavior in more realistic conditions at 23$°$C and day-night irradiation (sunlight cycle of October in Padova, Italy). This continuous experiment was performed with water collected during a second sampling at the water treatment plant, and the initial nutrient content was specifically measured. In order to remove the suspended solids and avoid tubes clogging in the continuous flow experiments, waters have been subjected to a first filtration treatment by paper filter of 100 μm of particles retention. This resulted in a lower COD concentration of water used in continuous experiment, with respect to that of batch experiments.

3.2.2 Experimental apparatus

Pre–inoculum and batch experiments were performed in glass bottles of 250 mL, continuously mixed by magnetic stirring and bubbling air enriched with 5% v/v of CO_2, which also provided a non-limiting CO_2 supply. The total gas flow rate was 1 L h^{-1} for each bottle. The temperature was controlled by an incubator (Frigomeccanica Andreaus, Padova), and artificial light (white neon lamps OSRAM) was provided continuously at intensity of 100 μmol m^{-2} s^{-1} of PAR (Photosynthetic Active Radiation) photons measured by a photoradiometer (Model LI–189, LI–COR, USA). Continuous flow experiment was carried out in a flat plate vertical reactor of 250
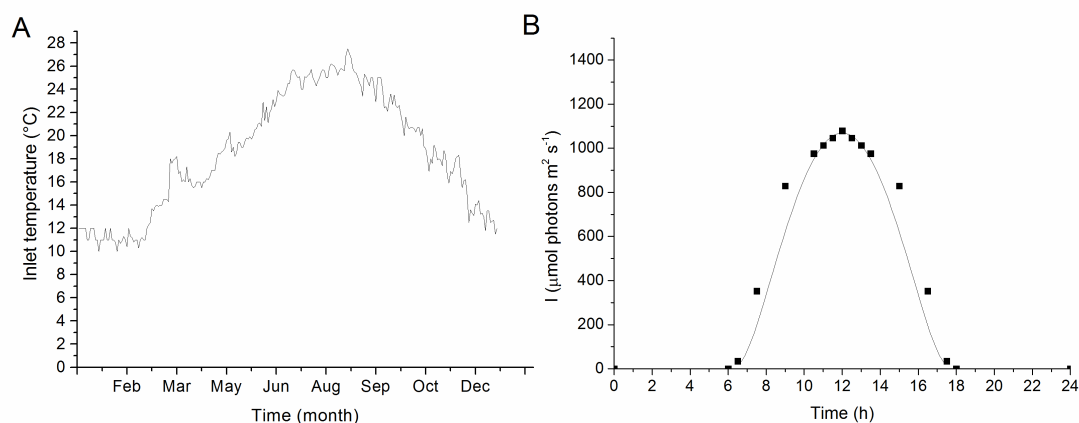
Figure 3.1: A reports annual trend of inlet wastewater temperatures at Camposampiero plant (data from ETRA s.p.a). In B irradiation profile (lines) from (PVGIS, Solar Irradiation Data, 2013) database and measures of light impinging experimental PBR (black square) of October month are reported.

mL of volume, reasonably assumed as a perfectly mixed reactor (CSTR, or chemostat) as confirmed by tracer experiments (Bertucco et al., 2014). Alternated day–night cycles were generated with a LED lamp (Photon System Instruments, SN–SL 3500-22). The light intensity as a function of time was simulated so that to provide the PBR with the same PAR amount of energy received under natural conditions at the selected latitude. PVGIS, Solar Irradiation Data (2013) is an online available database of typical day evolution of irradiation on a given surface for any earth location and time of year. This software was used as the source of irradiation data for the location of Padova, Italy (Figure 3.1B). An incident angle of 35° was applied, as the default setting of the database, in order to exploit the maximum solar energy. The light intensities were measured both at the front and at the back side of the reactor by the photoradiometer, in order to verify the actual light absorbed by the panel.

C. protothecoides was inoculated at the beginning into the reactor with non-sterilized fresh wastewater medium. To prevent the occurring of washout, the reactor operation was started in batch mode. Once reached a significant concentration (the order of 10^8 cells mL^{-1}), the operational mode was switched from batch to continuous, feeding non–sterilized wastewater by a peristaltic pump (Sci–Q 120S , Watson Marlow, USA). Inlet wastewater storage tank was continuously mixed with a magnetic stirrer, and maintained at the same temperature of the reactor. In order to avoid any increase of

contamination, the water in the feed tank was replaced every day. The liquid level in the reactor was controlled with an overflow tube placed close to the top, and the outlet flow was collected in a bottle. So, the residence time in the reactor (τ) was directly controlled by the peristaltic pump. A flow rate of 130 mL d^{-1} was set, thus leading to a residence time (τ) of 1.9 days. Steady-state operation was reached after 5 days and maintained for further 20 days. The total duration of the continuous experiment was 30 days.

3.2.3 Analytical methods

The biomass concentration was monitored daily by spectrophotometric analysis of the optical density (OD) (measured at 750 nm, by double beam spectrophotometer UV–Visible UV 500 from Spectronic Unicam, UK) correlated to cell concentration, measured with a Bürker Counting Chamber (HBG, Germany), for both batch and continuous experiments. Specific growth rates in batch experiments were measured by linear regression of six to ten experimental points of logarithmic phase of growth, from two or three independent biological replicates. The concentration of biomass was also measured as dry weight (DW) in terms of g L^{-1}. DW was measured gravimetrically in cells previously harvested with a 0.22 μm filter, and then dried for 4 h at 80 °C in a laboratory oven. In continuous experiments, biomass concentration in terms of cell mL^{-1} and DW were monitored twice a day: samples were collected at the end of the light phase and of the dark phase of the day/night cycle, and the steady state concentration was averaged on five to ten experimental points. The nutrients analyzed were ammonium (N–NH$_4$) and phosphate (P–PO$_4$), assessed daily using standard methods in water and wastewater (APHA-AWWA-WEF, 1992). N–NO$_3$ concentration (test kits provided by Carlo Erba Reagenti, Italy, code 0800.05482) was measured at the beginning of the experiments (data not shown), but it was found at very low concentration and it was consequently not considered, as also reported in the previous chapter. Samples of culture were filtered in order to measure only dissolved nutrients (0.2 μm), and ammonium was measured by test kits provided by Carlo Erba Reagenti (Italy) (code 0800.05405). The data were found consistent with those provided by the measurements in treatment plants. Ammonium is indirectly measured by the absorbance (at 420 nm) of an indophenolic complex produced by

reaction of ammonia with phenolic derivatives. Orthophosphates were measured by a modified analytical method described in APHA–AWWA–WEF, 1992. The mixed reagent was prepared immediately before the analysis (since it become unstable in 3–4 hours), and is composed by sulphuric acid (2.5 N), potassium antimonyl tartrate (0.034 g L^{-1}), ammonium molybdate (1.5 g L^{-1}), ascorbic acid (0.27 g L^{-1}). 250 μL of this mixture were used for 2.5 mL of water sample and incubated for 5 min. The absorbance of the sample due to the colorimetric reaction was measured spectrophotometrically at 705 nm. COD is the method to measure the amount of organic matter, expressed in mg L^{-1} of oxygen required to oxidize the organic matter by using a strong oxidant. COD was measured by an analytical kit provided by Sigma–Aldrich, USA (AQUANAL$^{\circledR}$) based on the oxidation of organic compounds by potassium dichromate in sulfuric acid solution. In order to determine the effect of indigenous bacterial population in wastewaters, the growth of *C. protothecoides* in wastewater medium sterilized by autoclave was measured, and compared to those in non-sterilized one. These experiments were carried out in in vertical batch bubbling reactors of 250 mL of volume, illuminated with artificial continuous light (100 μmol m^{-2} s^{-1}) and mixed by aeration enriched with CO_2 (5% v/v). Native microflora contamination was controlled by a non–specific plate counting method, as described in Görs et al. (2009), by counting the CFU (Colony Forming Units) of a sample of given volume in LB medium. Petri plates were incubated at 37°C and the count was performed after 24 hours.

3.2.4 Statistical Analysis

T–student tests were applied to ascertain significant differences in biomass concentration in terms of g L^{-1}, cellular weights at different temperatures, nutrient concentration and cellular concentration in the continuous experiment between samples of the light and the dark phase. The level of statistical significance was assumed for P≺0.05.

3.2.5 Mass and energy balances

Microalgal reactor design was based on biomass, phosphorous and nitrogen mass balances. In particular the reactor was assumed as a perfectly mixed reactor (CSTR), working at steady state. Thus, by considering an accumulation term and an inlet

biomass concentration equal to zero, the biomass balance can be expressed in Equation 3.1:

$$0 = -C_{xo} + r_x \cdot \tau \tag{3.1}$$

where τ is the residence time and r_x the growth rate. The subscript "o" refers to outlet conditions. The residence time is calculated by:

$$\tau = \frac{V_r}{Q} \tag{3.2}$$

To model the biomass growth rate as a function of temperature, the Arrhenius equation was assumed, written in as:

$$\mu_{max} = A \cdot e^{\frac{-E_A}{RT}} \tag{3.3}$$

where the parameters A and $-E_A/RT$ were fitted to experimental measurements. Concerning nutrients, the mass balance of component "i" is expressed in Equation 3.4:

$$0 = -C_{ie} + C_{io} + r_i \cdot \tau \tag{3.4}$$

Where subscript "e" means inlet conditions. The nutrient consumption rate r_i is assumed to obey the Monod kinetic (Equation 3.5):

$$r_i = -\frac{\mu_{i,max} \cdot C_{io}}{k_i + C_{io}} \cdot C_{xo} \tag{3.5}$$

In summary, the nutrient balance around the microalgal reactor is represented by:

$$C_{ie} - C_{io} - \frac{\mu_{i,max} \cdot C_{io}}{k_i + C_{io}} \cdot C_{xo} \cdot \tau = 0 \tag{3.6}$$

The kinetic parameters $\mu_{i,max}$ and k_i for N and P in real wastewater were experimentally measured as described in chapter 2. In order to verify the Monod–based model for nutrient consumption, the N and P balances (Equations 3.6) were used to compare experimental data from Chapter 2., by substituting the inlet nutrient concentrations C_{Ne} and C_{Pe}, residence time τ and biomass concentration C_{xo}, measured

in each continuous experiment, and solving them with respect to C_{No} and C_{Po} in order to calculate the ΔC_P and ΔC_N accordingly.

The biomass production rate r_x can be linked to the nutrient specific production rate through the Biomass/Nutrient yield $Y_{x/i}$:

$$-\frac{dC_x}{dC_i} = Y_{x/i} \tag{3.7}$$

Therefore,

$$r_x = Y_{x/i} \cdot r_i \tag{3.8}$$

The N and P yields were calculated from the ratio of biomass concentration on actual nutrient consumption of continuous experiments at constant and seasonal illumination carried out in chapter 2, in this chapter, and in other continuous experiment data published by other Authors (Reis et al., 1996; Ruiz et al., 2013). Yields values were found to depend on light intensity. Richmond (2004) reported a general effect of illumination on nutrient uptake, while Powell et al. (2008) focused in particular on phosphorus content in the biomass which, in continuous cultures, was found affected mainly by light intensity and temperature. In our experiments, by considering not only the light provided to the reactor, but also the actual light absorbed (calculated from the irradiance measured at the front and back side of the reactor). E_{PAR} is calculated in Equation 3.9:

$$E_{PAR} = \frac{E_x}{E_{abs}} = \frac{C_{xo} \cdot Q \cdot LHV}{(E_i - E_o) \cdot S_{PBR}} \tag{3.9}$$

as ratio of the energy stored by biomass E_x, and the energy absorbed E_{abs}. E_{abs} is given by the difference between the incident energy E_i and the back irradiance E_o, referred to the exposed surface of the reactor, S_{PBR}. The energy stored in biomass E_x was evaluated based on the low heating value LHV (KJ g^{-1}) of the biomass produced. By applying the same approach on literature data, similar trends were found with data from Reis et al. (1996) for nitrogen uptake of *Phaeodactylum tricornutum* and those reported by Ruiz et al. (2013) for P uptake in *S. obliquus*. In addition, we compared our results of *C. protothecoides*, with data of nutrient yields for other species, which were

collected during experiments in the same reactor. All of these data appear consistent. Based on the experimental correlation, nutrient yields were evaluated, corresponding to the light efficiency value assumed for calculation (see Table 3.3 later). To design the photobioreactor for nutrient removal, the biomass production rate r_x in the biomass balance Equation 3.1 was related to nutrient balances by Equation 3.8. Thus, by considering the resulting equation and the mass balance on nutrient (Equation 3.6), a system of two equations is obtained which can be solved to calculate τ and C_{xo}. Then, the volumetric productivity of biomass can be calculated as:

$$P_x = \frac{C_{xo}}{\tau} \tag{3.10}$$

Concerning the energy balance, by considering that biomass productivity is directly linked to the specific energy irradiation available, the biomass production depends on the incident solar energy. In fact, in order to produce 1 kg of biomass, 20 MJ of energy from solar irradiation are necessary, according to the LHV of microalgae. Data of solar irradiation were obtained from PVGIS, and used to calculate a maximum areal productivity P_m, by considering a photosynthetic efficiency η of 7% (Chisti, 2013), which corresponds to E_{PAR} of 16.3%. To design a flat panel photobioreactor, by assuming this theoretical areal productivity, and the volumetric productivity required 3.9, the height of the reactor can be calculated as:

$$H = \frac{P_m}{P_x} \tag{3.11}$$

Finally, on the base of the volumetric flowrate of wastewater in real treatment plants (Q_{24}), the surface needed by the photobioreactor results:

$$S = \frac{Q_{24}}{H} \tag{3.12}$$

3.3   Results and discussion

3.3.1   Effect of temperature on *C. protothecoides* growth

As reported, inlet wastewaters in a treatment process are exposed to seasonal temperature fluctuations (Figure 3.1A). Accordingly, *C. protothecoides* was cultivated

at 10, 15, 23 and 30°C under continuous irradiation in order to evaluate the effect of temperature on growth. Results are reported in Figure 3.2A. The specific growth rate calculated on cell number basis was found to be linearly correlated to temperature (R^2 = 0.997), even if the temperature affected the biomass concentration obtained in term of g L^{-1}. In fact, the cellular mass tends to slightly increase with decreasing temperature, showing a significant difference between cellular weights measured at low temperatures (10 and 15°CC) and those measured at higher temperatures (23 and 30°C). This also affected the calculation of specific growth rates, which resulted different if calculated on cell number or on dry weight basis (Figure 3.2A, with a R^2 = 0.979).
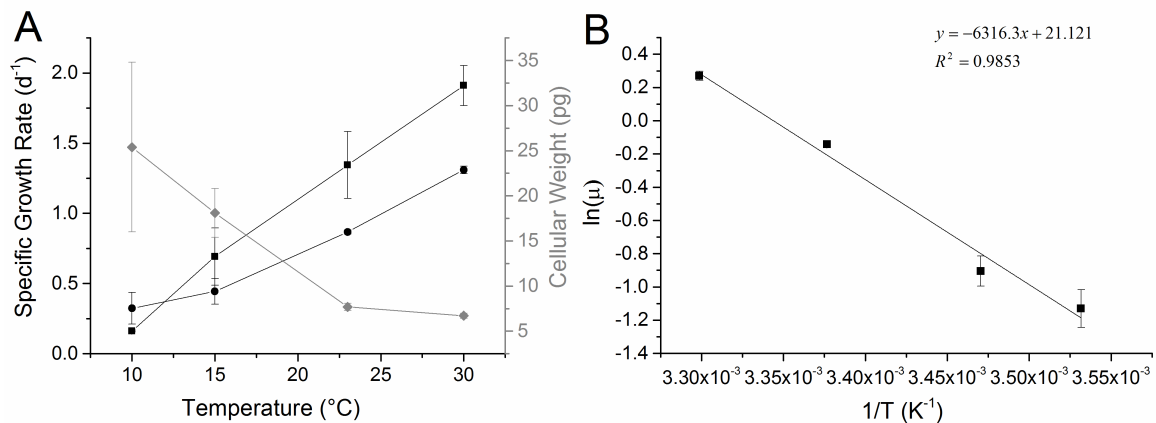
Figure 3.2: A *C. protothecoides* growth rate calculated on number of cell (black squares) and on dry weight basis (black circles) and final dry weight per cell at stationary phase (grey) at different temperatures under continuous irradiation. **B** reports the Arrhenius plot from measured data. The linearization was used to evaluate the kinetic parameters A and E$_a$/R.

The response to temperature changes is strongly dependent on the species considered (Li et al., 2013b; Xu and Hu, 2013). In particular Xu and Hu (2013) showed an extensive adaptability of *Chlorella sp.* to changing temperature (2°C - 42°C). Aleya et al. (2011) obtained a similar trend of specific growth rate testing another species of *Chlorella* at different temperatures (from 10 to 35°C). *C. protothecoides* showed a remarkable capability of nutrients consumption: ammonia and phosphorus were efficiently removed from the wastewater as reported in in Table 3.1. In order to evaluate the effect of temperature on biomass growth in an actual process, the specific growth rate data calculated on the DW basis were used to estimate the parameters of the Arrhenius plot (Equation 3.3). The values of parameters for *C. protothecoides* calculated from the linearization (Figure 3.2B) were $A = 1.49 \times 10^9 \ d^{-1}$ and $E_a/R =$

6316.3 K. The value E_a was then calculated as 52.48 kJ mol^{-1}, that is consistent to those reported for *C. reinhartdii* by Le Borgne and Pruvost (2013), i.e. E_a =62.69 kJ mol^{-1} and A=$1.34 \times 10^{10} d^{-1}$.

3.3.2 Continuous production of biomass under day–night cycle

A continuous flow experiment was carried out in order to test *C. protothecoides* behavior in more realistic conditions at 23°C and day–night irradiation (sunlight cycle of October in Padova, Italy). The reactor operated first in batch mode until a concentration of 110×10^6 cells mL^{-1} was reached. A continuous operation mode was then applied and a steady state concentration was eventually reached. Under steady-state condition, all the variable values remained constant (Figure 3.3): the cell concentration was stable at about 108.6 ± 8.75 millions of cell mL^{-1} and, even if a difference between light phase and dark phase was observed, the mean value of dark phase was not significantly different from the one of light phase for $P \prec 0.05$ (Figure 3.3A). This could be due to the error in measuring the cell concentration by optical microscope, affected by the high dilution needed to count the cells in the Burker chamber. However, the data obtained define a clear difference (statistically significant) in the biomass concentration, in terms of g L^{-1}, between the dark and light phases (0.64 and 0.79 g L^{-1} respectively): at the end of the dark phase the biomass concentration decreased by 25% with respect to the end of the light phase, despite the cell concentration seems to remain constant (Figure 3.3B). Alternatively, these data could reveal a biomass loss (cell density) of about 25% during the dark phase, calculated referring to the average cell concentration between dark and light phases. Ogbonna and Tanaka (1996) obtained comparable results by cultivating *Chlorella pyrenoidosa* under different light–dark cycles, highlighting a biomass loss after dark phase of about 25%, similar to those of the reported in this thesis. In addition, the death kinetic of algal cell is slow, as reported also in Dehning and Tilzer (1989), who observed the capability of *Scenedesmus acuminatus* of surviving under dark for at least 10 days, without any significant reduction in cell number. Thus, the reduction of cell biomass in day/night experiments compared to continuous light experiments could be due to an intracellular biomass loss due to dark respiration (Lee and Lee, 2001), with a lower growth kinetics that determine a partial washout in the continuous

system. However, even if in these conditions, a good biomass productivity was obtained corresponding to 0.34 and 0.42 g L^{-1} d^{-1} for the dark and the light phases, respectively.

Concerning the nutrient removal (see Figure 3.3C and 3.3D), also N–NH$_3$ and P–PO$_4$ outlet concentrations values were found stable. Analysis on the wastewater fed to the reactor show inlet values of 24.27 mg L^{-1} for N-NH$_3$ and 1.62 mg L^{-1} for P–PO$_4$, leading to outlet values of 0.760 ± 0.35 mg L^{-1} and 0.639 ± 0.38 mg L^{-1} at the end of the dark phase and 0.399 ± 0.07 mg L^{-1} and 0.201 ± 0.06 mg L^{-1} at the end of the light phase, respectively. Thus, data revealed that during the light phase there is a higher consumption of nutrients (statistically significant), for both N–NH$_3$ and P–PO$_4$, which is compatible with the higher biomass concentration measured during the light phase. The consumption of nutrients during night phase could be explained by a maintenance metabolism. For instance, Ogbonna and Tanaka (1996) observed an increase of protein content in *Chlorella sp.* during night, while Needoba and Harrison (2004) demonstrated an accumulation of an intracellular N pool at night in diatoms, which is then consumed during the day.

3.3.3 Bacteria competition

In order to determine whether the indigenous bacterial population in wastewaters may affect the microalgal growth, the growth of *C. protothecoides* in wastewater medium sterilized by autoclave was measured, and compared to those in non–sterilized (Figure 3.4A). No differences in algal growth were detected, suggesting that the presence of native microflora in the wastewater did not influence the algal growth. In addition, during the growth curve, samples of cultures were plated in LB petri dishes, and a CFU count was performed, in order to have a rough measure of the evolution of aerobic bacterial community over time. As a control, the CFU measurement was also carried out in the case of sterilized water, and an appearance of colonies was not observed (data not reported). In non–sterilized waters the number of CFU increased during time, as reported in Figure 3.4B. However, the growth rate of native microflora is quite slow, suggesting that the experimental conditions favored the microalgal growth instead of the bacterial one. This is probably due to the addition of CO$_2$ to the air bubbling, that might inhibit bacterial growth. To verify this hypothesis, a growth curve in air bubbling was carried out, without the addition of CO$_2$. The microalgal growth
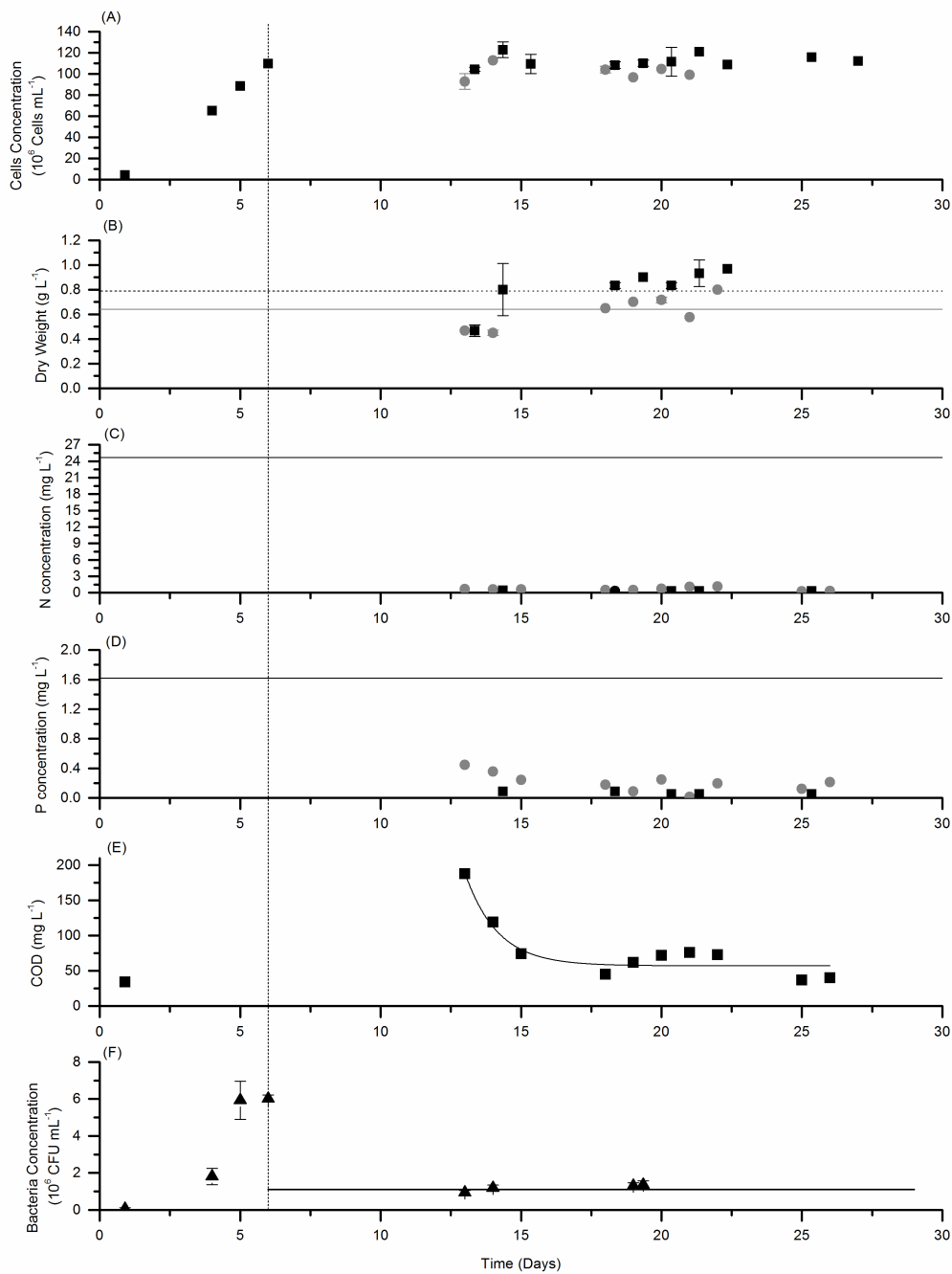
Figure 3.3: Results of continuous experiment under day-night irradiation. The vertical dotted line outlines the transition from batch mode to continuous one, the data after dark and light phase are reported in grey and black respective. **A** reports the cell concentrations and **B** the dry weight. The dotted line refers to the mean value of samples of light phase, while the solid one the samples of dark phase. In **C** the nitrogen and in **D** the phosphorus concentration are reported. The solid lines indicate the corresponding inlet concentration of nutrients. In **E** the trend of COD (solid line is an eye guide only) and in **F** the CFU mL^{-1} concentration during time are reported.

rate was found lower (Figure 3.4A), due to the limiting CO_2 concentration, while the CFU concentration was found higher than that observed in air–CO_2 bubbling (Figure 3.4B), suggesting an enhanced bacterial growth rate in this conditions.

Thus, by changing the growth conditions, the bacterial contamination could be limited with the aim to stimulate microalgal growth and productivity. In order to assess the possible effect of a coexistence of bacteria and algae in a continuous process, the CFU numbers were also monitored in the continuous experiment reported (see Figure 3.3E and 3.3F for CFU data). During the first batch phase, the CFU increased with time, but when the peristaltic pump was turned on, the bacterial concentration started to decrease, settling down to very low concentration. This results suggested that, in a highly controlled system, by setting the culture conditions, it is possible to stimulate the algal growth at the expense of bacterial population, that tend to be washed out from the reactor. This was also qualitatively observed by McGinn et al. (2012), that found a lower concentration of invading organisms in a continuous system.

3.3.4 COD removal in wastewaters by microalgae

The nutrient removal kinetics was investigated, in particular for the COD. In all batch experiments we observed, as a similar trend, that the COD remained constant during the exponential phase, but an increase was observed in the stationary phase, probably due to the accumulation of degradation matter in suspension due to the cells death (Figure 3.4C). The COD trend is quite different for the culture grown under air bubbling (triangles) where the stationary phase occurred at the second day of growth, and the accumulation of degradation materials started earlier.

In the literature it is stated that some microalgal species can perform COD consumption, but it seems that COD is removed only when its concentration is quite high (more than 2000 ppm according to Li et al. (2011)). On the opposite, COD concentration in primary treated wastewater is typically in the range between 200 and 300 ppm, conditions at which other authors reported the inefficiency of microalgae in organic matter removal (Ruiz et al., 2013; Li et al., 2011; Wang et al., 2010). These data suggest that microalgae cannot degrade COD when its concentration is lower than a not yet determined threshold value. On the other hand, the CO_2 bubbling could affect the COD uptake, by shifting the metabolism of *C. protothecoides* to autotrophic
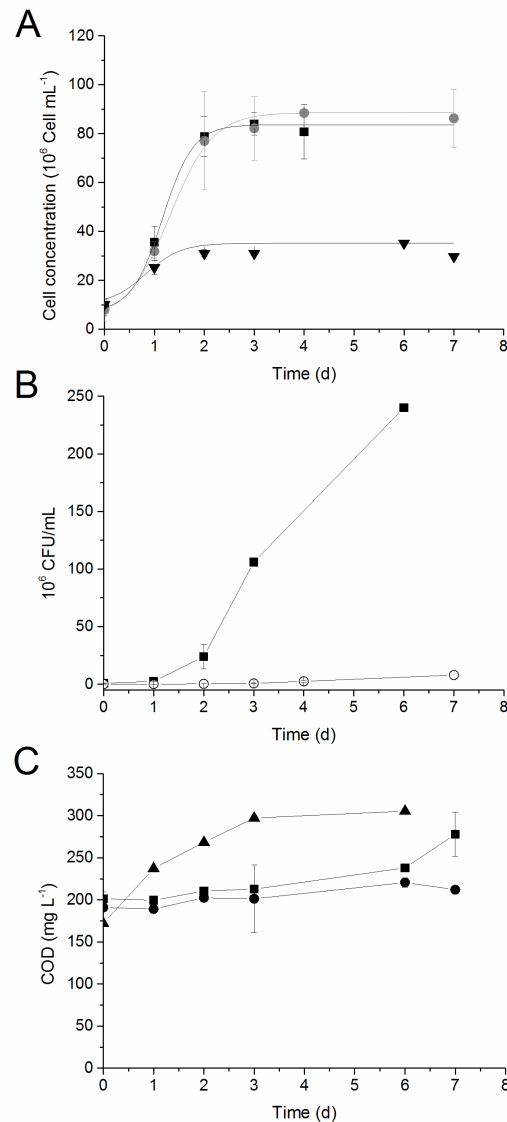
Figure 3.4: In **A** the growth curve of *C. protothecoides* in non–sterilized (grey circles) and sterilized (black squares) water under air–CO_2 bubbling are reported. Black triangles refers to algal growth in non–sterilized condition under only air bubbling. In **B** the bacterial CFU mL^{-1} count in non–sterilized media under CO_2-air (open circles) or only air bubbling (black squares) are reported. In **C** the COD measurements for each experiments are reported (triangles for air bubbling, circles for non-sterilized and squares for sterilized water).

metabolism only (Hu et al., 2012). In this case, even if the absence of light during the night should induce mixotrophic capability, the continuous CO_2 bubbling stimulates only the autotrophic metabolism, and probably inhibits the respiration in the dark, as observed also by Sforza et al. (2012b). Concerning the COD measurements carried out in the continuous reactor (see Figure 3.3E), compared to the increased content of COD during the batch phase, the values decreased at steady state condition, but

the mean value is similar to the initial concentration in the wastewater. The high values measured in the first days are probably due to the accumulation of death cell components during the batch phase, that are subsequently eluted when the reactor is switched to continuous mode. Anyway, in this operating conditions it resulted that the consortium *C. protothecoides*–native microflora cannot assimilate the organic matter, according to both the batch and the continuous experiments, reported also in the previous chapter.

3.3.5 Design of an integrated process of water treatment and algal production

Based on experimental measurements reported in previous sections, a combined process with microalgae and conventional activated sludge can be proposed. According to our experimental results, it was assumed that the COD concentration is not modified in the microalgal reactor. Even if working in the absence of CO_2 could stimulate a mixotrophic algal growth, the productivity of algal biomass would be decreased , as the CO_2 is the main nutrient needed to obtain a sufficient production of algal biomass (refer to 3.4C for algal growth curve under air bubbling). On the other hand, *C. protothecoides* was able to efficiently remove N and P, achieving a good biomass production. Therefore, a two steps depuration process is required, where an activate sludge reactor is also needed, in order to achieve a COD reduction. Thus, in the process currently proposed, the microalgae reactor removes nutrients, while activated sludges reduce the organic matter of the wastewater. By considering that the native microflora of waters after primary treatment did not affect the algal growth, while the bacteria population of activated sludge resulted competitive with microalgae, it is convenient to place the photobioreactor before the activated sludge reactor. We refer to Figure 3.5 for the block flow diagram of this process. All calculations were performed in order to achieve an outlet pollutants concentration under the limits imposed by territorial law (Total P $\leq$ 1 mg L^{-1}, Total N $\leq$ 15 mg L^{-1}, COD $\leq$ 125 mg L^{-1}, for active population of 10 000 to 100 000 habitants) (Norme, 2006). Nutrient and COD loadings vary significantly along the day, so an equalization basin is needed before the algal treatment to normalize nutrients concentration during day, and permit an
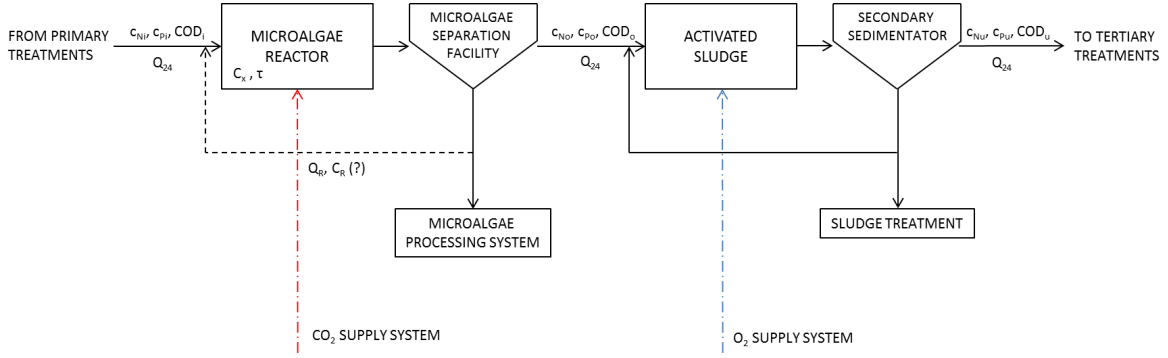
Figure 3.5: Process scheme proposal: the microalgae reactor followed by conventional activated sludge process.

easier operation of the entire wastewater treatment process. The microalgal reactor need to be equipped with a separation facility (centrifuge, decanter or sedimentation tank) for the biomass collection, and could include also a recirculation line if the requested biomass concentration is not autonomously developed. The water is then delivered to the activated sludge reactor where the organic matter is degraded. To design the microalgal section it must be taken into account that the activated sludge process requires a certain amount of nutrients for bacterial biomass production, so the nutrient consumptions must be limited to avoid the failure of the treatment step downstream. Activated sludge bacteria consortium is characterized by an average elemental composition corresponding to BOD:N:P=100:5:1 (Metcalf and Eddy, 2004), where BOD refers to biochemical oxygen demand. By considering the nutrient content of wastewater used in this chapter (with a COD, N and P average concentrations of about 270, 40, 4.5 mg L^{-1}, respectively), and by assuming a BOD/COD ratio of 0.5, the nutrient concentrations exiting the microalgal reactor $C_{N_{o,min}}$ and $C_{P_{o,min}}$ (where the "min" subscript refers to the minimum concentration required for bacterial growth) should be 6.8 and 1.36 mg L^{-1}, respectively.

Thus, the reactor design was based on nutrient balances expressed by Equations 3.6 and 3.8. In order to verify the applicability of the model assumed, the N and P kinetic parameters were used to compare experimental data from Chapter 2 (see Table 3.2) to values calculated by applying Equation 3.6 (based on N and P) at the same inlet conditions (inlet nitrogen and phosphorus concentrations) and running parameters (residence time and measured biomass concentration). Results of these calculations are reported in Figure 3.6. The adopted model seems to well reproduce the real conditions
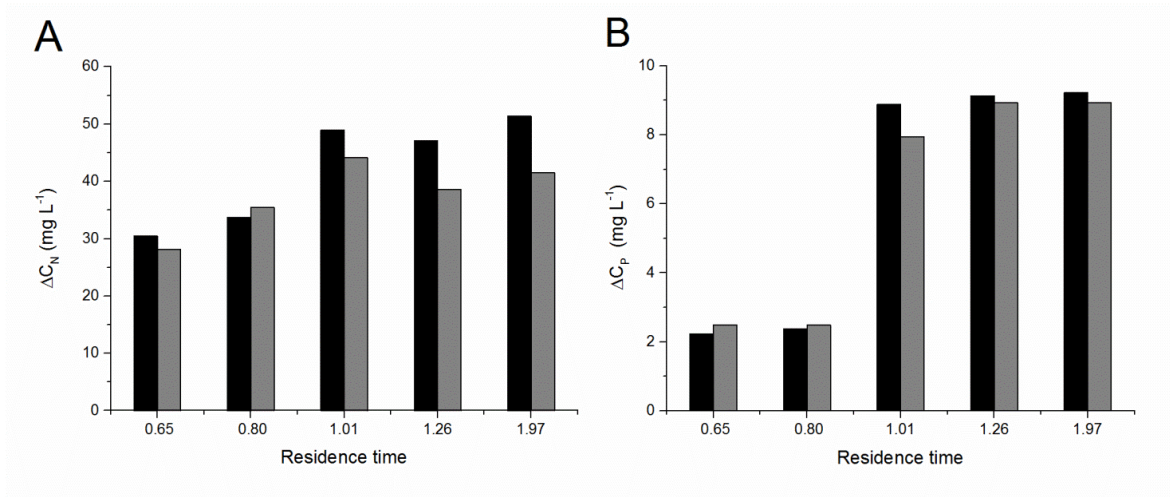
Figure 3.6: Process scheme proposal: the microalgae reactor followed by conventional activated sludge process.

with acceptable errors, in particular for phosphorus, that is the limiting nutrients in this type of waters.

Table 3.2: Measured nutrient consumption and biomass concentration of continuous experiments at different residence times.

			Residence time				
Parameter	Symbol	Unit	0.65	0.8	1.01	1.26	1.97
Biomass conc.	C_{x0}	mg L^{-1}	307	468	510	640	800
Inlet N	C_{Ne}	mgN L^{-1}	37.4	37.43	52.56	49.35	52.56
Inlet P	C_{Pe}	mgP L^{-1}	2.59	2.59	9.43	9.53	9.43
N consumption	ΔC_{Ne}	mgN L^{-1}	28.1	35.43	44.12	38.56	41.52
P consumption	ΔC_{Pe}	mgP L^{-1}	2.39	2.49	7.95	8.93	8.93

Microalgal reactor design could be based on phosphorus or nitrogen balances, depending on whichever of these balance is more precautionary for the activated sludge process downstream, coupled with microalgae biomass balance. In this chapter, the reactor design was based on P balance, which resulted to be the limiting nutrient in the wastewater considered. With respect to this, the amount of P that has to be consumed is 3.14 gP m^{-3}(see Table 3.3). The calculation takes into account that a certain amount of P ($C_{P_{o,min}}$) is necessary for the activated sludge reactor downstream. A CSTR working at steady–state conditions was assumed. Thus, by applying the P balance Equation 3.6 and the biomass balance Equation 3.2 on values reported in

Table 3.3, τ and C_x were calculated as 0.74 d and 108 mg L^{-1} respectively. Biomass productivity associated to these values of residence time and biomass concentration resulted about 146 mg L^{-1} d^{-1}. The corresponding nitrogen outlet concentration from the microalgal reactor can be calculated by Equation 3.6 on N basis. With these residence time and biomass concentration we obtained 17.22 mg L^{-1}, that is higher than $C_{N_{o,min}}$ required, and will reach a value lower than that imposed by territorial laws after the activated sludge reactor.

Table 3.3: Process parameters used in the design proposed. Kinetic parameters of N and P consumption rate and biomass yields

Data	Symbol	Value	Unit
Total COD concentration inlet	COD	270	mg L^{-1}
Total N concentration inlet	C_{Ni}	40	mgN L^{-1}
Total P concentration inlet	C_{Pi}	4.5	mgP L^{-1}
Total N concentration outlet	$C_{No,min}$	6.8	mgN L^{-1}
Total P concentration outlet	$C_{Po,min}$	1.36	mgP L^{-1}
Carbon–biomass yield	Y_C	0.527	mgC mgBiomass^{-1}
Biomass–Nitrogen yield	$Y_{x/N}$	8.61	mgBiomass mgN^{-1}
Biomass–Phosphorous yield	$Y_{x/P}$	34.48	mgBiomass mgP^{-1}
Nitrogen max consumption rate	$\mu_{N,max}$	0.668	mgN mgBiomass^{-1} d^{-1}
Phosphorous max consumption rate	$\mu_{P,max}$	0.849	mgP mgBiomass^{-1}d^{-1}
Nitrogen half–saturation constant	k_N	23.4	mgN L^{-1}
Phosphorous half–saturation constant	k_P	28.2	mgP L^{-1}

The energy balance was also considered, by assuming an annual solar irradiation at middle latitudes of about 4500 MJ m^{-2} y^{-1} (data from (PVGIS, Solar Irradiation Data, 2013) for north Italy), and a photosynthetic efficiency of 0.07: the maximum productivity obtainable with the available solar energy in Padova resulted about 43 g m^{-2} d^{-1}. As a consequence, an horizontal flat panel reactor seemed the better configuration in order to maintain the plant costs low, and to efficiently capture the light available. By applying Equation 3.11, the height of the PBR can be calculated as about 30 cm. Starting from these results, a set of surface areas for the microalgal reactor can be proposed, based on different wastewater treatment plant dimensions, considering a daily water consumption per equivalent inhabitant (E.I.) of 0.3 m^3 d^{-1}

E.I.$^{-1}$ and an inflow sewer coefficient of 0.8 (i.e. the amount of water consumed that flows into sewer). Results of calculations are summarized in Tables 3.4 and 3.5.

Table 3.4: Design results for a pre-microalgal reactor calculated for a base case, and in winter and summer seasons.

Variable	Base case	Winter	Summer
C_{Po} [mg L^{-1}]	1.36	1.36	1.36
C_{No} [mg L^{-1}]	17.23	17.25	17.81
τ [d]	0.74	1.69	0.56
C_x [mg L^{-1}]	108.27	108.27	108.27
P_x [mg L^{-1}d^{-1}]	145.71	64.06	192.36
E[MJ m^{-2} d^{-1}]	12.44	5.8	23.9
η	0.07	0.07	0.07
Operative days [d]	365	90	90
$P_{m,PD}$[g m^{-2} d^{-1}]	43.54	20.31	83.73
H [m]	0.298	0.317	0.435

In order to consider the temperature fluctuation in different seasons, we applied the same calculation methods to winter and summer temperature, on which the kinetic parameters for N and P consumption depend, and considering the solar irradiation at the same latitude in the two seasons. Results are reported in Tables 3.4 and 3.5. Of course, the surface needed in winter is higher than in summer, due to the slower kinetic of nutrient uptake and the lower energy availability. From a technical point of view, there are two possibilities to overcome this issue: using an averaged value of area and verify the different nutrients outlet concentration, or using a modular reactor that can be partially operated during the summer season. Concerning the first hypothesis, an averaged surface of 2.35 ha with a resulting τ and C_{xo} of 1.23 d and 66.3 mg L^{-1} respectively could lead to a too high outlet concentration of P and N during winter season (C_{No} of 30.3 and C_{Po} of 2.58 mg L^{-1}), over the limit imposed by the law. Thus, the only acceptable solution is a partitioned photobioreactor, where the surface can be managed as a function of temperature and energy available along the year. In any case, a quite large surface for nutrient removal is required, and this should be carefully considered in applying such integrated microalgal–depuration process. Finally we point out that these are only preliminary results, because the effect of actual fluctuating

Table 3.5: Microalgal reactor area needed to assure a requested productivity depending on different wastewater flowrates in base case and winter and summer seasons.

Population served	Q^{24}	Reactor surface for base case	Reactor surface for winter	Reactor surface for summer
E.I.	$m^3 \ d^{-1}$	ha	ha	ha
30000	7200	1.8	3.8	0.9
60000	14400	3.6	7.7	1.9
100000	24000	5.9	12.8	3.1
150000	36000	8.9	19.2	4.7

radiation of seasons is not considered in this model. A more complex modelling of growth rate would be required as a function of fluctuating illumination, which is still under investigation.

3.4 Final remarks

In this chapter a possible exploitation of microalgae in a wastewater treatment process was assessed from both the experimental and the process design points of view. *C. protothecoides* was cultivated in real and unsterilized primary urban wastewater and the effect of temperature was investigated. The effect of day/night irradiation in a continuous system was also verified, and a difference was found on biomass and nutrient concentrations between the dark and the light period. The possible competition with native microflora was considered, but it did not affect the algal growth, in particular under continuous cultivation, where the bacterial growth is slow, due to the presence of bubbling CO_2, and bacterial washout condition occurred. Concerning the nutrient consumption, N and P were efficiently removed in all the conditions tested, while the COD was not consumed by microalgal biomass. Based on experimental results, a two step depuration process was proposed, where a first photobioreactor removes nutrients as N and P, and a subsequent activated sludge reactor reduces the organic matter. The kinetic parameters of nutrient consumption were checked on experimental measures and the biomass and energy balances were applied to calculate a possible productivity and to design the process. It was found that cold and warm seasons strongly influence design results and that the area required for the photobioreactor is indeed quite large.

Symbols

C_{xo}	inner and outlet microalgal biomass concentration [mg L^{-1}]
r_x	biomass production rate [d^{-1}]
τ	reactor residence time [d]
V_R	reactor volume [m^3]
Q	volumetric flow rate [m^3 d^{-1}]
μ_{max}	maximum specific growth rate of biomass [d^{-1}]
A	pre-exponential factor of Arrhenius equation [d^{-1}]
T	temperature [K]
E_A	activation energy [J mol^{-1}]
R	gas constant [J mol^{-1} K^{-1}]
r_i	specific consumption rate of the nutrient i [mg mgBiomass^{-1} d^{-1}]
C_{ie}	inlet concentration of component i [mg L^{-1}]
C_{io}	outlet concentration of component i [mg L^{-1}]
$\mu_{i,max}$	maximum specific consumption rate of component i for *C. protothecoides* [mg mgBiomass^{-1} d^{-1}]
k_i	half-saturation constant of component i for *Chlorella protothecoides* [mg L^{-1}]
$Y_{x/i}$	biomass/nutrient yield
P_x	volumetric biomass productivity [mg L^{-1} d^{-1}]
η	photosynthetic efficiency
$P_{m,PD}$	maximum theoretical areal productivity [g m^{-2} d^{-1}]
E_{PAR}	light use efficiency [%]
E_x	power stored in biomass [KJ d^{-1}]
E_{abs}	energy absorbed by the reactor [KJ d^{-1}]
E_o	back irradiance of the reactor [KJ m^{-2} d^{-1}]
E_i	incident irradiance on the reactor [KJ m^{-2}d^{-1}]
S_{PBR}	surface of lab reactor[m^2]
LHV	low heat value of microalgal biomass [KJ g^{-1}]
H	height (and depth) of the reactor [m]
Q_{24}	water flow rate in wastewater treatment plant [m^3 d^{-1}]
S	reactor surface [m^2]

Anaerobic digestion of whole microalgae and microalgae residues

In order to reduce the energetic costs and to make microalgae cultivation more attractive, the possibility of exploiting the energetic content of microalgal biomass residues after oil extraction by anaerobic digestion was studied. Two microalgal species, *Scenedesmus obliquus* and *Chlorella protothecoides* were tested for biogas production, before oil extraction. Biochemical Methane Potential (BMP) tests were carried out to evaluate biogas production capacity from microalgae and degradability rates. Two different kinds of inocula were used to compare the specific hydrolytic capacities and to assess the most suitable one to maximize the biogas conversion of microalgae. As the biocrude production process from microalgae causes a large amount of biomass residues which can be energetically exploited through anaerobic digestion, experimental runs of biogas production were carried out and methane yields were measured, using *Scenedesmus obliquus* biomass prior to lipid extraction (FSO) and after oil removal, using different mixtures of solvents as methanol:chloroform (SOMC), hexane:isopropanol (SOHI) and acetone:dichloromethane (SOAD), in order to find out the most suitable combination in energy terms to produce biocrude and/or biogas.

4.1 Introduction

In view of future exploitation of microalgae for biofuels production, the energetic profitability must be maximized by means of bioprocesses and technological chains that

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⁰Part of this chapter has been published in Chemical Engineering Transactions

increase the energy return on energy investment (EROEI) of the process. To this aim the reuse of biomass residues obtained after oil extraction plays an important role on increasing EROEI for biodiesel production from microalgae. The energetic utilization of residues after biodiesel production provides benefits also from an economic point of view. The production of biocrude is in fact not sustainable yet (Campbell et al., 2011) as the costs of biocrude and biodiesel are still high and not competitive with commercial products (Davis et al., 2011). Methane production by means of anaerobic digestion (AD) from biomass residues could be a practical and competitive alternative to enhance energy return and to reduce costs of biodiesel production from microalgae. Current investigations in which AD is used for microalgal biomass to produce biogas is carried out with different purposes, e.i. to integrate AD in biorefinery facility (Mussgnug et al., 2010). Methane potential productions from microalgae are species–specific and vary from about 200 to 400 mL CH_4/g VS (VS = Volatile solids) Frigon et al. (2013). Different digestion conditions in terms of temperature, biomass concentration, carbon to nitrogen ratio and retention time for biogas production from *Chlorella* residues after biodiesel production via transesterification, were evaluated by Ehimen et al. (2011). Methane potential production can be also influenced by microalgae growth conditions, i.e. autotrophic growth, using wastewater as resource of nutrients (Alcántara et al., 2013), or mixotrophic growth (Singh et al., 2011). The effect of substrate/inoculum ratio was also investigated (Alzate et al., 2012).

In this chapter the Biomethane Potential (BMP) of *S. obliquus* and *C. prototbecoides* was investigated. Two kinds of inocula and different substrate concentration into the reactors were tested in order to determine the applicability of anaerobic digestion of microalgae and to compare their specific hydrolytic capacities. Hydrolysis in fact represents the limiting factor in anaerobic digestion of complex organics (Vavilin et al., 2008; Trzcinski and Stuckey, 2012) and a rapid and efficient hydrolysis can improve the overall conversion of microalgae into biogas. Also the BMP of *S. obliquus* residues after lipid extraction was tested, evaluating the effect of different solvents for extraction process, on biogas, methane concentration and oil yields. Kinetic constants and production rates of biogas and methane were determined. Furthermore, *S. obliquus* biomass after lipid extraction was tested as a substrate for anaerobic

digestion: the biogas production and methane yields were determined considering different solvents mixtures, methanol:choloform (SOMC), hexane:isopropanol (SOHI) and acetone:dicholomethane (SOAD).

4.2 Materials and methods

4.2.1 Microalgae species

Two species of microalgae were utilized in the experiments, *S. obliquus* 276–7 and *C. protothecoides* 33.80 were obtained from SAG–Goettingen and cultured in freshwater media (BG11) Rippka et al. (1979). Maintenance and propagation of cultures were performed using the same medium added with 10 g L^{-1} of Plant Agar (Duchefa Biochemie). Temperature was controlled at $23 \pm 1°C$ in a growth chamber. *S. obliquus* was grown in a batch flat panel reactor of 50 L, bubbled with air enriched with CO_2 to maintain the pH between 6 to 7 and provide a non–limiting concentration of carbon source the reactor was provided with a light irradiance of 200 μE m^{-2} s^{-1}, measured by a photoradiometer (Model LI–189, LI–COR, USA). While *C. protothecoides* was grown in continuous flat panel reactor of 2 L, under CO_2–air bubbling (5%v/v), and irradiated by fluorescent tubes at the intensity of 237 μE m^{-2} s^{-1}. The biomass was centrifuged, dried and pulverized for the experiments.

4.2.2 Anaerobic inoculum

Biogas production experiments were carried out using two different types of inocula. The first inoculum (hereafter named G) was an anaerobic granular sludge, collected from a real scale Upflow Anaerobic Sludge Blanket (UASB) digester of a brewery factory located in Padova, Italy. Inoculum G was composed by a Total Solids (TS) concentration of 11 % and a Volatile Solids (VS) concentration of 70 % referred to dry weight. The second inoculum (hereafter named CN) was an anaerobic sludge collected from an anaerobic digester of sewage sludge from a municipal wastewater treatment plant located in Padova, Italy. Inoculum CN was sampled twice. The first sample (CN–A) was utilized in the experiments reported in section 4.3.3 characterized by a TS concentration of 50g/L and a VS concentration of 55 % referred to dry weight; the second sample (CN–B) was utilized in the experiments reported in section 4.3.3, its TS and VS concentrations were 20 g/L and 60 % respectively.

4.2.3 Experimental set up

All BMP test were carried out in batch conditions, in reactors of 0.5 L hermetically closed by means of silicon plug, enabling sampling of the gas produced during fermentation. The working volume of each reactor was 0.25 L. After setting up, the reactors were flushed with N_2 gas for 3 min, in order to achieve the anaerobic conditions, then they were incubated without stirring in a thermostatic chamber at $35 \pm 2°C$. Blank tests were prepared to measure the biogas produced by inoculum. Biogas volume was measured using the displacement principle accordingly, the biogas produced is cumulated in the reactor rising its pressure, the gas is transferred to a vessel containing saline solution whose volume is moved, which in turn corresponds to the volume of gas produced, measured with a graduated cylinder.

In this respect, it was used an acidified (pH$\prec$3) and saline (NaCl 25 %) solution in order to avoid the dissolution of methane and carbon dioxide into the liquid. Conversely, the composition of biogas in terms of carbon dioxide and methane was measured using a portable gas analyzer (LFG 20, Eco–Control). The incubation time was approximately 60 days. Batch tests were carried out in triplicates for each sample and three control tests (without microalgae) for each condition as well.

4.2.4 Analytical procedure

Chemical and physical characterization parameters were measured in accordance to standard methods (APHA, AWWA, 1999). Data on biogas and methane productions are reported in terms of 1 atm of pressure and 0 °C of temperature.

Methane and carbon dioxide volumes produced in the time interval between two subsequent measurements were calculated using a model accounting for the gas concentration at time *t* and time *t-1*, together with the total volume of biogas produced at time *t*, the concentration of the specific gas (methane or carbon dioxide) at times *t* and *t-1*, and the volume of the reactors' head space (Ginkel et al., 2005). The following equation was applied:

$$V_{C,t} = C_{C,t} \cdot V_{G,t} + V_H \left(C_{C,t} - C_{C,t-1} \right) \tag{4.1}$$

where: $V_{C,t}$ is the volume of generic biogas (CH_4 or CO_2) produced in the interval between t(d) and $t - 1$(d); $C_{C,t}$ and $C_{C,t-1}$ represent the gas (CH_4 or CO_2) concentrations measured at times t(d) and $t - 1$(d); $V_{G,t}$ is the volume of biogas produced between time t(d) and $t - 1$(d); V_H is the volume of the reactors' headspace. To compare results obtained from the batch tests, data were interpolated using a Gompertz equation (Favaro et al., 2013). The Gompertz equation used is as follows:

$$B(t) = B_0 \cdot exp \left\{ -exp \left[k \cdot e \frac{(\lambda - t)}{B_0} \right] + 1 \right\} \tag{4.2}$$

where: $B(t)$ is the cumulative biogas or methane production at time t (d) (mL/g VS); B_0 is the maximum biogas or methane production (mL/g VS); k is the biogas/methane production rate (mL/d g VS); λ is the latency phase (d); e is Euler's number. The quantities B_0, λ, and k constants have been obtained based on experimental data the for biogas and for methane, with a non–linear regression. Total lipids of dried *S. obliquus* biomass were extracted according to Bligh & Dyer method in a Soxhlet apparatus for an overnight period, using three solvent mixtures, chloroform:methanol 1:2, hexane:isopropanol 3:2 and acetone:dichloromethane 1:1. The lipid content was measured gravimetrically after solvent removal using rotary evaporator. The residual biomass was subjected to a thermal treatment at 90 °C for 2 h in order to ensure the total elimination of solvent.

4.3 Results and discussion

4.3.1 BMP tests, effect of inocula and S/I ratio

BMP of *S. obliquus* was investigated with two Substrate/Inoculum (also identified as Food/Microorganism) ratios (S/I ratio as g VS substrate/g VS inoculum). The S/I ratios applied were 0.5 and 0.1, using both the inocula reported in section 4.2.2. The best yield was obtained with inoculum CN and 0.5 of S/I ratio, resulting in a biogas production of 420 mL gVS^{-1} composed by 55 % of methane. The suitability of fresh *S. obliquus* biomass for the production of biogas is show in Figure 4.1, where production as a function of time is shown, comparing the performance of both inocula, with S/I ratio of 0.5 (Figure 4.1A) and 0.1 (Figure 4.1B). It can be easily noted that maximum biogas production is achieved with the flocculent sludge (CN) in a shorter
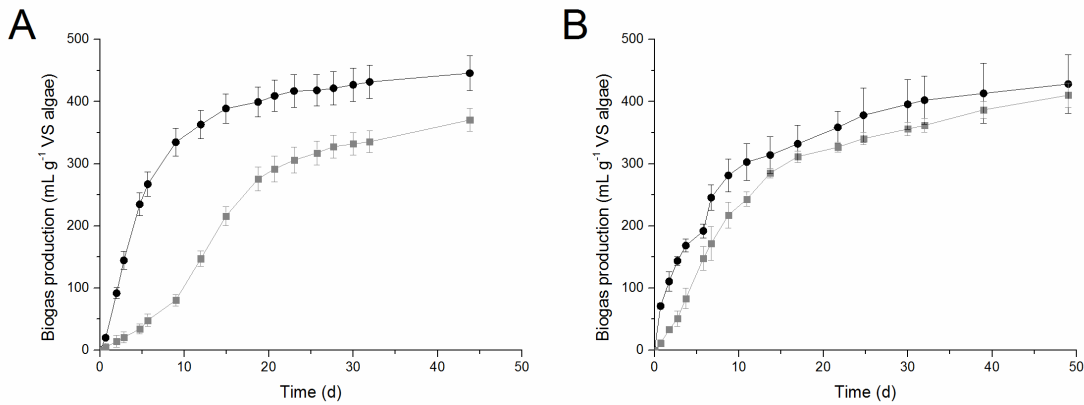
Figure 4.1: *S. obliquus* biogas production curves in mL/gVS of microalgae biomass, over time. A) S/I ratio of 0.5, B) S/I ratio of 0.1. Black circles represent inoculum CN, grey squares inoculum G.

period. With both organic loads, methane production started almost immediately. On the other hand, by using the granular sludge (G), an initial adaptation time of about a week was observed at higher S/I ratio. With the lower S/I value this lag phase is almost undetectable. This is in agreement with studies on hydrolysis rates indicating that, at low S/I ratios, the higher amount of bacteria leads to a higher enzyme availability and a faster substrate hydrolysis and consumption (Vavilin et al., 2008; Trzcinski and Stuckey, 2012). In addition, it is possible to observe that the slope of the curve, i.e. the production rate, varied considerably between granular sludge and flocculent sludge when the S/I is higher, whereas if the S/I ratio is lower with both sludges the difference is minimum.

The same behavior is observed in the cumulate production of biogas. The difference in production is more marked when the ratio is higher, although in all cases the net methane production is good. Biogas production and composition of CO_2 and CH_4 were also investigated with the specie *C. protothecoides* in order to compare it performance and to investigate if production could be influenced by the microalgae species. In this case, a S/I ratio of 0.5 was used, using both inocula described previously. Results are shown in Figure 4.2. It can be observed that, with both the inocula, the trend of biogas production was very similar to that obtained with the species *S. obliquus* at the same S/I ratio, highlighting a lag phase with G inoculum and immediate production with CN sludge. Although the final production of biogas was relatively lower for *C. protothecoides* with both inocula, a major difference was observed with CN, probably
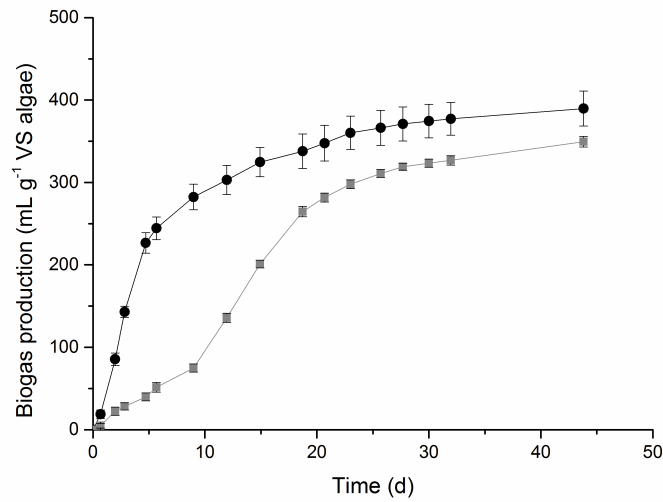
Figure 4.2: *C. protothecoides* biogas production curves in mL/gVS of microalgae biomass, over time. Testing the different inocula, CN is represented by black circles, and G by grey squares, both experiments with S/I ratio of 0.5.

due to the difference in biochemical composition of microalgae species, causing the variance in BMP (Sialve et al., 2009). Values of biogas production and composition are reported in Table 4.1.

Table 4.1: Biogas and methane production from fresh microalgae (n=3; ±SD)

Species	Inoculum	S/I (gVS/gVS)	Biogas (mL/g VS)	Methane (mL/g VS)	% Methane
S. obliquus	CN	0.1	395 ± 40	215 ± 4.3	55
		0.5	420 ± 26	230 ± 3.8	55
	G	0.1	350 ± 10	190 ± 7.2	55
		0.5	331 ± 17	176 ± 8.4	53
C. protothecoides	CN	0.5	371 ± 20	206 ± 8.9	56
	G	0.5	319 ± 4.9	166 ± 4.9	52

4.3.2 Production rates and kinetic parameters

Results of kinetic analysis of biogas and methane productions are reported in Table 4.2. Biogas production rate with inoculum CN resulted higher than those from tests with inoculum G, even in the cases with lower S/I ratio. This can be explained by the fact that inoculum CN is a flocculent type of anaerobic biomass. Therefore the

distribution of inoculum in the reactor is more homogenous in this case, allowing a higher contact between bacteria and microalgae. Inoculum G is characterized by fast settleability and bacteria are grouped in complete communities only in the granule. The contact between the substrate and the inoculum is limited and distribution of organics to be degrades is mainly guided by diffusion effects without constant mixing of the reactors. This effect influenced mainly the first phases of the anaerobic degradation, the hydrolysis of organics, resulting in lower biogas production rates in the first 10 days of degradation. After this initial phase, biogas and methane productions reached results comparable with CN inoculum (at day 30 of the curve).

Table 4.2: Kinetic constants and production rates of biogas and methane obtained from data interpolation

Species	Inoculum	S/I	Biogas				Methane			
			B_0 (mL/g VS)	k (mL/d g VS)	λ (d)	R^2	B_0 (mL/g VS)	k (mL/d g VS)	λ (d)	R^2
S. obliquus	CN	0.1	394.6	34.93	0	0.988	213.88	20.88	0	0.986
		0.5	415.07	48.92	0.18	0.997	228.89	29.61	0.31	0.998
	G	0.1	386.64	23.5	0.27	0.994	208.79	13.14	-0.08	0.995
		0.5	362.79	19.58	4.08	0.998	188.01	12.05	5.15	0.998
C. protothecoides	CN	0.5	360.6	47.12	0.19	0.994	198.79	28.49	0.29	0.996
	G	0.5	354.07	17.43	3.11	0.997	178.8	12.5	6.27	0.998

As can be observed in Table 4.2, the production rates of biogas and methane (k) are proportionally correlated in all the cases investigated, the higher the biogas production rate the faster the methane production. Higher speeds were observed with *S. obliquus* with CN and S/I of 0.5, being 48.92 and 29.61 (mL/d g VS) in biogas production and methane respectively, followed by 47.12 and 28.49 (mL/d g VS) achieved with *C. protothecoides* with CN and S/I of 0.5. In these cases little influence was observed with respect of the microalgae species. The same behavior can be noticed for the biogas production curves, having rates very similar in both species with inoculum G, 17.45 (mL/d g VS) with *C. protothecoides* and 19.58 (mL/d g VS) with *S. obliquus*, while methane around 12 (mL/d g VS) for both. In this case the rate was less than a half then comparing G with CN. The results indicate that inoculum CN is characterized by faster hydrolytic capacity for the specific type of substrate used. This fact could explain the lag phase with inoculum G of about 3 to 4 days for biogas and 5 to 6 days for methane. When substrate concentration increased in the reactors, without constant mixing, the

methanogenic bacteria only degrade the substrate that is in direct contact with them. On the contrary, this behavior is not observed when the S/I ratio was lower because a higher concentration of microorganisms was present and a higher contact area with the substrate was possible.

4.3.3 Biogas from de-oiled biomass: preliminary results

Previous work in energetic analysis of biocrude production indicates that the process can be energetically self–sufficient as long as the energy in the residual biomass is exploited (Chapter 6). For this reason the BMP of de–oiled biomass was experimented. The mixture chloroform/methanol is considered as an excellent solvent for extracting lipids from biomass of microalgae (Lam and Lee, 2012). However BMP tests showed evidence of strong inhibition of methanogenic activity during tests within microalgal residues after oil extraction with this mixture. Test was carried out using de–oiled *S. obliquus* as substrate, under S/I ratio of 0.5 and 0.3 with CN inoculum. As reported in Figure 4.3, methane was not detectable in biogas and only CO_2 was produced. From these results it can be hypothesized that the chloroform/methanol mixture, even if particularly volatile and probably largely removed from the biomass before digestion, can still produce inhibitory effects for methanogenic bacteria even at a lower concentration of substrate with the same concentration of bacteria (S/I 0.3). The same behavior was observed by Zhao et al. (2012). Biogas analysis showed also concentration of H_2S, higher than 1,000 ppm (data not shown). Apparently the methanogenic inhibition allowed sulfur–reducing bacteria to predominate the final digestion phases thanks to their lower sensibility to inhibition or unfordable digestion conditions if compared to methanogens (Deublein and Steinhauser, 2008).

4.3.4 BMP of residual microalgae, effect of solvents in biogas yields

In order to determine the ultimate biogas and methane yield from the microalgae used, as well as to evaluate their biodegradability, an array of batch tests were conducted. To evaluate the lipid removal yields and observe the effect of solvents in the digestion, from *S. obliquus*, testing three mixtures of solvents, chloroform:methanol 1:2 (SOMC), hexane:isopropanol 3:2 (SOHI) and acetone:dichloromethane 1:1 (SOAD), lipids were extracted by Soxhlet method until the solvent condensed on the top was
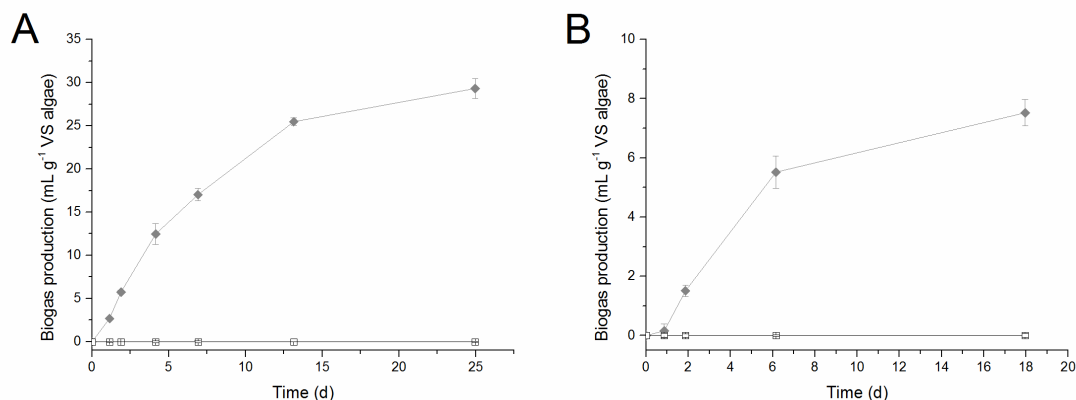
Figure 4.3: Cumulative CO_2 (grey rhombus) and CH_4 (squares) production in mL/g VS of de–oiled microalgae biomass, over time, A) S/I = 0.5, B) S/I = 0.3.

transparent. The biomass recovered after extraction was treated at 70°C for 2 hr in order to eliminate solvent residues and the lipid mass was measured gravimetrically after solvent removal using rotary evaporator. These experiments were conducted with inoculum CN-B at the experimental conditions explained in section 4.2.3.

Table 4.3: Lipid extraction yields, product yields and function of solvent mixture

Solvent	Methanol/ chloroform	Acetone/ dichloromethane	Hexane/ isopropanol	None
Lipids extracted	34%	8.90%	8.30%	0%
Biogas Prod. (mL/gVSalga)	150	274	287	330
Methane Prod. (% v/v)	61	58	74	57
(mL/gVSalga)	91	158	212	188

In Table 4.3 the yields of lipids extracted with each solvent mixture and the methane production with their respective biomass residues are reported, it is observed a biogas production ranged from 150 to 330 mL/gVSalga. Notably, the production can be correlated with the amount of lipids extracted, showing that with whole biomass the biogas yields is higher, and it decreases when the remaining lipid concentration in microalgae substrate decreases, with important exceptions. For example, the concentration of methane in biogas and the time required to produce the gas change,

as they are influenced by the solvent used and its efficiency for lipid extraction. Chloroform/methanol, regarded as the best solvent for lipid extraction (Table 4.3) from microalgae biomass, is widely used in Soxhlet and Bligh & Dyer methods, however BMP tests showed an extensive inhibition within this solvent extracted residues (Figure 4.4). Nevertheless, after more than 40 days a biogas productions is observed, probably by inoculum adaptation.

The Acetone/Dicholormethane mixture also presents an inhibitory effect, but the adaptation period is shorter than SOMC, around 20 days. This suggests that small quantities of chloromethanes compounds even after drying can be not removed from the biomass, and produce a significant inhibitory effect during digestion. Chloroform and dichlorometane are retained within the biomass, and the time of bacteria adaptation depends on the Clorine composition in the component, in other words, chloroform has 3 atoms of Cl in its molecular structure and presents an adaptation period longer than that one with dicholormethane which has 2 Clorine atoms in its molecule. Chlorine severely inhibited both acid fermentation and methanogenic reactions at very low concentration (Zhao et al., 2012; Hu and Sommerfeld, 2008), while it is not affecting hydrolysis process. As can be observed in Figure 4.4 and Table
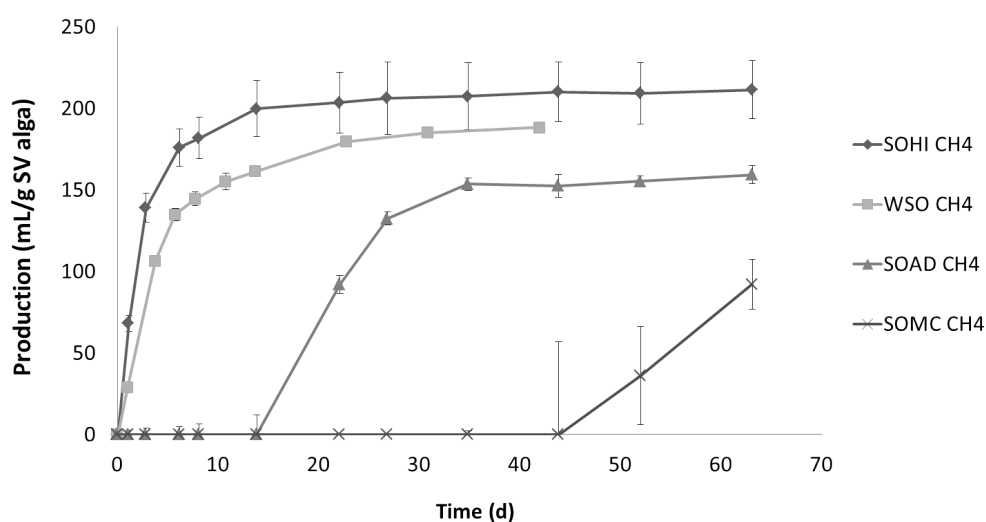
Figure 4.4: Cumulative CH_4 production in mL/g VS of de-oiled *S. obliquus* biomass over time, extracted with different solvents hexane:isopropanol 3:2 (SOHI), acetone:dichloromethane 1:1 (SOAD) and chloroform:methanol 1:2 (SOMC) and whole *S. obliquus* (WSO).

4.3, when the mixture hexane/isopropanol is used, the digestion of the biomass residues presents the second highest production of biogas, but the higher methane composition of around 74% corresponding to 212 mL/gVSalga. This production is inclusively higher than that obtained with the whole biomass (188 mL/gVSalga), a fact which could be explained because the lipid extraction process disrupts the cell wall of microalgae, hydrolyzing organic molecules and increasing their solubilization, consequently probably also increasing the bioavailability of the intracellular organic components of the microalgae to the anaerobic bacteria. For these reasons, the extraction process with hexane/isopropanol can be considered as pretreatment process that enhances the biodegradability of microalgal biomass (Ramos-Suárez and Carreras, 2014).

4.4   Final remarks

In this chapter, anaerobic digestion of microalgal biomass was investigated, resulting in a feasible operation with degradation kinetics comparable to those of conventional biomass used for biogas production. Reuse of biomass residues after oil extraction could improve the energy profits of the process, but it is important to highlight the essential role of the extraction method, in particular of the solvents. The mixture chloroform:methanol should be further investigated to propose an alternative method to totally eliminate the solvent content in the biomass. In addition different solvent mixtures for extraction must be investigated, if the aim is to produce biomethane with de-oiled biomass. Anaerobic digestion can play a central role in the biorefinery concept for microalgal energy exploitation, as it is a well establish technology and fresh microalgae represent suitable substrates for biogas production. In microalgae production process, combination of bioprocesses with chemical processes anyway must be assessed from a global point of view in order to define the optimal process schemes that allow the highest energy conversion rates maintaining the best process condition for the biological degradation.

Process water recycle in Hydrothermal Liquefaction of microalgae to enhance bio-oil yield

In this chapter the effect of recycling the process water of Hydrothermal Liquefaction (HTL), to the HTL reactor was investigated, aiming to develop a solvent–free process. When recycling twice the aqueous phase at 220°C, 240°C and 265°C, a significant increase in oil production with recycle number is observed at all temperatures. An extended series of recycle experiments was performed at 240°C, showing that the oil yield increased up to stationary level, after 6 recycles. To investigate the role of accumulated compounds as acetic acid, additional experiments were carried out with dilution and spiking of recycled aqueous phase. No relationship between the oil yields obtained and the acetic acid concentration was found. Aqueous phase recycling not only increases oil yield but also enables to reduce operating temperature (and hence costs) and is therefore an essential element in a solvent-free process for biocrude production from microalgae.

5.1 Introduction

Microalgae seem to be a feasible option as source of high value and environmental friendly fuels. Microalgae present well–known advantages as CO_2 fixation, wastewater depuration capacity and growth rates higher than other terrestrial plants (Brennan

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and Owende, 2010). However, algae processes for biofuels production currently cannot compete with respect to traditional fossil fuels for economic reasons (Sun et al., 2011). To improve this situation many efforts were directed to increase lipid yields. However, on the other hand, the use of high–lipid microalgae species does not automatically result in high oil yields as these species usually do not show high growth rates. Another pending glitch is the water elimination after the microalgae cultivation process, as this is generally required for an efficient lipid extraction, but this step is also energetically expensive. These complications could be in some way avoided by selecting a suitable thermochemical biomass conversion process (instead of lipid oil extraction) and by optimizing its operation conditions besides the recovery of the oil part of the biomass with higher yields. The principal advantage of biomass conversion process is, the thermochemical transformation of the no–oil portion in a biocrude.

Hydrothermal liquefaction (HTL) is a biomass conversion process that in recent years has generated strong interest. Through this process, biocrude from wet microalgae biomass can be produced with high yields (López Barreiro et al., 2013a). HTL is carried out at temperatures from 200 °C to 370 °C and at pressure corresponding to water vapour pressure which maintains the water in the liquid state. In this process, besides the lipid fraction of microalgae, also proteins and carbohydrates are transformed, resulting in higher overall yields of biocrude (Valdez et al., 2014). Thus, the biofuel yields of low–lipid microalgae is increased and, additionally, these algae usually presents higher productivity.

The conversion of microalgae components into biocrude is the highest for the lipids fraction, followed by proteins and in less amount by carbohydrates (Biller and Ross, 2011). The products of HTL are biocrude (or bio–oil), a gas phase composed in major part by CO_2, a solid residue and an aqueous phase, rich in soluble organics. So far, temperatures covering the whole HTL range, reaction times varying from 5 to 120 min and screening of different microalgae species have been investigated to determine their suitability and the optimum conditions for HTL (Garcia Alba et al., 2012). Marine species like *Phaeodactylum tricornutum*, *Tetraselmis suecica*, *Nannochloropsis gaditana*, *Porphyridium purpureum* and *Dunaliella tertiolecta* and fresh water species like *Scenedesmus obliquus*, *Scenedesmus almeriensis*, and *Chlorella vulgaris* have been

reported by López Barreiro et al. (2013b).

The use of catalysts, heterogeneous ones like Pd/C, Pt/C, Ru/C, Ni/SiO$_2$-Al$_2$O$_3$, CoMo/γ-Al$_2$O$_3$ and zeolite,(Duan and Savage, 2011) homogeneous catalysts like alkali salts (K$_2$CO$_3$ or KOH (Singh et al., 2014), Na$_2$CO$_3$ (Ross et al., 2010)) and organic acids like acetic acid of formic acid (Ross et al., 2010) have been studied in HTL process to evaluate their effect on oil yields, which were improved with all heterogeneous catalyst tested (Duan and Savage, 2011). Also, using K$_2$CO$_3$ and KOH, the oil yields were higher in comparison with both no catalyst (Singh et al., 2014) and organic acids, but with organic acids (acetic acid) the high heating value (HHV) of oil was higher (Ross et al., 2010).

More recently, aqueous phase, i.e. process water, from HTL has received more attention for two main reasons: its high content of C, N and P (Sudasinghe et al., 2014; Gai et al., 2014) is suitable to be used in the cultivation step and, since it is contaminated and expensive to dispose, it needs additional treatment (Zhu et al., 2013). Components of aqueous phase from HTL of *Chlorella pyrenoidosa* were determined at different operation conditions, Gai et al. (2014) reports that the solid ratio in HTL reactor is the predominant parameter affecting the concentration of nutrients in the co–product, observing the increment in organic acids concentration at optimized conditions for energy recovery.

When using this water for microalgae cultivation and microbial cultivation, (Nelson et al., 2013; Orfield et al., 2014) i.e. for nutrient recycling, high dilution rates are required. Garcia Alba et al. (2013) found that microalgae growth rates and steady state concentration are reduced considerably, when algae are cultivated in the HTL aqueous phase (diluted with water) only, while with addition of micronutrients the growth rates and concentration are similar to those with standard growth medium. Another recycling study was reported by Biller et al. (2012) where dilution rates ran from 200X to 600X to achieve microalgae growth. However *C. vulgaris* was able to grow in HTL aqueous phase diluted 50X, under sterile conditions (Du et al., 2012). Other authors propose catalytic hydrothermal gasification (CHG) of the aqueous phase (Davis et al., 2014b; Elliott et al., 2013) to exploit the significant amount of carbon from the feedstock remained in this phase, resulting in considerable fraction of CO$_2$ and ammonia

(Davis et al., 2014b). (Orfield et al., 2014) report a Life Cycle Assessment (LCA) study comparing two pathways for reuse of aqueous phase, CHG and *Escherichia coli* cultivation, being this last added to HTL of microalgae, they highlight the importance of the aqueous product reuse that could reduce oil prices (if microbial biomass increase considerable) and enhance the Energy Return on Energy Investment (EROEI) values using CHG.

Few studies have been carried out aimed to recycle the aqueous phase into the HTL process itself. Li et al. (2013a) improved hydrochar production via HTL of *Salix psammophila*, a desert shrub, by reusing the effluent. While Zhu et al. (2015) enhance oil yields (from 34.9 to 38.4 wt%) and HHV of catalyzed HTL of barley straw, recirculating the aqueous product three times, however solid residues showed also an increment, in fact higher than biocrude. Since HTL applied to microalgae seems to be a versatile and promising process, various studies around the world are engaged in this problem both at laboratory level and in pilot plants. True commercial operation has not been reported so far, mainly because the industrial production of algal biofuels via HTL has still open challenges, including scale up, oil upgrading, catalysts selection and recovery and other issues such, the accumulation of certain compounds which may affect the growth of algae (Tian et al., 2014), and the use of organic solvents to recover the biocrude which affects oil yields and composition, Valdez et al. (2011) increase biocrude yields but decreases its quality, extracting also some water–soluble organics (Xu and Savage, 2014).

In this chapter aqueous phase recycle from HTL of a low–lipid microalgae, *C. vulgaris*, to the HTL process has been investigated, due to its elevated concentration of high organic compounds (from 15 to 43% of carbon from microalgae biomass remain in the aqueous phase (Li et al., 2013a)). The potential to add this step to the HTL process to enhance oil productivity at different temperatures as well using a "continuous" recycle has been evaluated. In order to understand the oil yield change, experiments with addition of acetic acid (earlier identified as catalyst for HTL process) (Ross et al., 2010) and diluted aqueous phase are also presented. The compositions of all products were measured, with special attention to aqueous co–product and biocrude along the recycles and molecular weight spectra, elemental composition and several light and

mid-boiling compounds in biocrude are reported. To our knowledge, this potential step in microalgae process has not been presented in published research up today.

5.2 Materials and Methods

5.2.1 Microalgae feedstock

The microalgae species *C. vulgaris* used in this study has been purchased from a commercial source, in powder form. The proximate and ultimate analyses for *C. vulgaris* are reported in Table 5.1, together with their biochemical composition provided by the supplier.

For proximate analysis, *C. vulgaris* was dried at 105 °C for 24 h to quantify the moisture. After moisture elimination, ash content was determined treating the residue at 550 °C for 5 h. The C, H, and N fractions of the dry ash free (d.a.f.) algae were measured in duplicate by the elemental analyzer Thermo Scientific Flash 2000, CHN–S, while Oxygen was obtained by difference, and the HHV was calculated according to Boie's formula (see section 5.2.4).

Table 5.1: Proximate, ultimate and biochemical composition of *C.vulgaris* used in this experiments

Microalgae specie	*Chorella vulgaris*	
	C (wt%)	46.7
Elemental Composition	H (wt%)	6.8
	N (wt%)	7.4
	O* (wt%)	39.0
Lipids (wt%)	6.3	
Carbohydrates (wt%)	33.5	
Proteins (wt%)	49.5	
Ash (wt%)	7.2	

Biochemical composition (lipids, carbohydrates and proteins) was provided by the supplier. *calculated by difference.

5.2.2 Experimental Setup and products recovery

The experiments were carried out in a stainless steel autoclave of 45 mL, operated in batch mode and mechanically stirred. The temperature inside the reactor was

reached by means of a fluidized sand bath in which the autoclave is submerged. The temperature inside the autoclave is measured by a thermocouple located in the bottom plug. The pressure is measured through a gas connection of the top plug to a pressure transmitter. All pressure and temperature data were monitored for the entire duration of the experiments from outside the reaction room for safety reasons. The reactor was charged with 1.85 g (d.a.f.) of dry microalgae and 15 mL of distillated water for the first run, resulting in a concentration of 11 wt%. In subsequent runs the aqueous phase was recycled, adding new (dry) microalgae biomass, around 1.85 g (d.a.f.) in each experiment. After each reactor loading the autoclave was tightly closed, assembling the mechanical mixer device in the lid of the top. Subsequently, a leakage test was conducted, flushing three times Nitrogen at 60 bar, to sweep off the air present inside the reactor and to achieve a start reaction atmosphere composed by only Nitrogen, at around 5.5 bar of pressure. The reaction time was 30 min, excluding the heating time (approx. 7 to 10 min). After this 30 min. of holding time, the autoclave was fast quenched in a water bath until room temperature, for products recovery. Three holding temperatures (220 °C, 240 °C and 265 °C) were applied to evaluate the temperature effect on yields obtained. The experiment with multiple step ("continuous") recycle of the water phase was carried out at 240 °C. After the quenching and before disassembly the autoclave, the gas produced was collected and measured by a liquid displacement system, using a saline solution in order to avoid the dissolution of methane and carbon dioxide into the liquid. Gas was sampled using a 50 mL syringe for composition analysis. To reduce the use of non–environmental friendly solvents to recover the products and to obtain an aqueous phase free of solvent, the remained content of the reactor was filtered through filter, 6μm pore size, to separate the oily solids and to recover the aqueous phase for its recycle. The procedure for products collection is shown in Figure 5.1.

The mixture containing both the oil produced and the solids, see section 5.3.1. After its recovery, the mixture was vacuum filtered by system made up of a Buchner funnel, a side arm flask, and a pump, using a glass microfiber filter Whatman GF/B, 1 μm pore size. The residue still present in the vessel was washed with dichloromethane (DCM), this way the solvent helped to wash the filter and ensure that the filter cake did
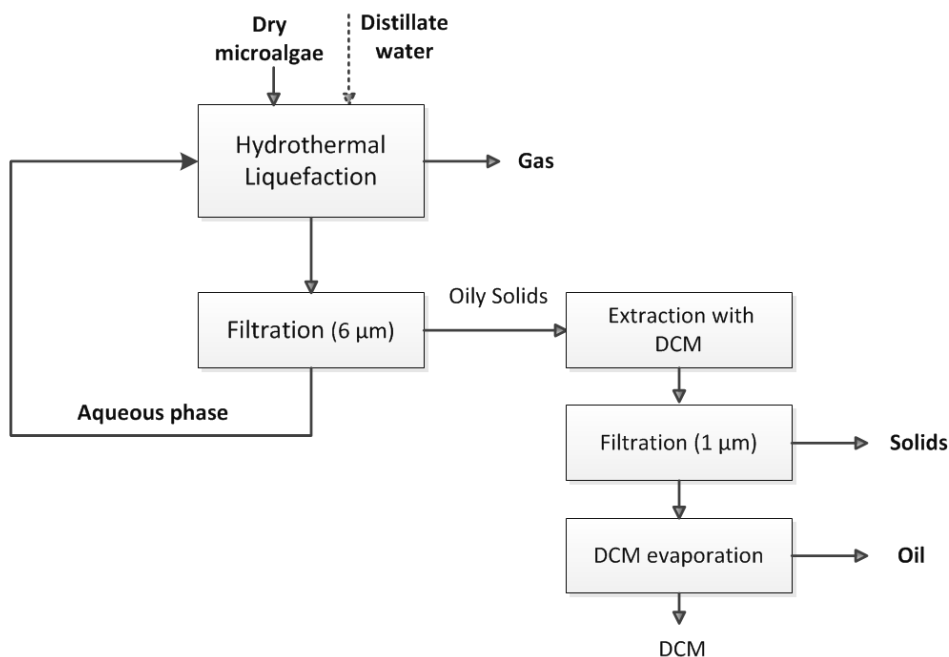
Figure 5.1: Procedure scheme for product collection. Distillate water was added only in the first run, for the successive rounds aqueous phase was used instead.

not contain oil, but only solid residues. To remove the solvent, the solids were dried at 105 °C for 24 h and quantified. The water soluble organics of the final aqueous phase were quantified on a 2 mL samples which were heated in the oven at 100 °C for 24 h so, a mixture of organics and ash soluble was obtained. Then, the mixture was treated at 550 °C for 5 h to quantify the ash which was subtracted from the previous organics weight, to calculate the organics d.a.f. In order to achieve a maximal product recovery and to close mass balances, the reactor was rinsed with DCM after recover the products from the last run, the resulting mixture was vacuum filtered (glass microfiber filter, Whatman GF/B, 1 μm pore size), and DCM was evaporated, the oil and the solids were dried at 105 °C for 24 h.

5.2.3 Analytical procedures

The gas samples were analyzed by a gas chromatograph, (Varian Micro GC CP–4900 compound with analytical columns: Molsieve 5A (10 m) and PPQ (10 m), using helium as carrier gas. For the identification of several light and mid–boiling compounds, and particularly for the quantification of acetic acid in water phase, were used a Gas Chromatography–Mass Spectrometry (GC–MS) analyzer (GC 7890A MS 5975C – Agilent Technologies) equipped with a capillary column (Agilent HP–% MS,

HP19091S– 433). Samples were dissolved in acetone (50 mg aqueous phase/gr acetone) and filtered (Whatman 0.2 μm filter). To identify the elemental composition of oil and solid residue, in terms of nitrogen, carbon and hydrogen (in wt%) the elemental analyzer (EA, Thermo Scientific Flash 2000) was utilized. Oxygen was calculated by difference. All the samples were analyzed in duplicate and the average values were taken. In addition, the molecular weight distribution of the oils was obtained by Gel Permeation Chromatography (GPC) using an Agilent 1200 series HPLC system with 3 GPC PLgel 3 μ m MIXED–E columns connected in series. The column temperature was 40 °C, with a flow of 1 ml/min and tetrahydrofuran (THF) was used as solvent.

5.2.4 Definitions and calculations

The product yields, which include gas, oil, solids and water–soluble organics were determined by Equation 5.1, based on the total mass of each product "i" over the mass of microalgae (d.a.f.) loaded into the reactor.

$$Y_i(wt\%, daf) = \frac{m_i}{m_{algae}} \cdot 100 \tag{5.1}$$

The higher heating value of microalgae feedstock, biocrude and residual solids was determined according to Boie's formula, Equation 5.2, based on the experimental content of C, H, N and O in wt%, wherein the latter is calculated by difference.

$$HHV_{Boie}(MJkg^{-1}) = 0.3516 \cdot C + 1.16225 \cdot H - 0.1109 \cdot O + 0.0628 \cdot N \tag{5.2}$$

The total energy converted in the HTL process from algae to bio-oil was calculated by Equation 5.3), expressed in %, based on the HHV of oil produced and microalgae d.a.f., as well as their masses.

$$Energy\ recovery(\%) = \frac{HHV_{oil} \cdot m_{oil}}{HHV_{algae} \cdot m_{algae(daf)}} \tag{5.3}$$

It should be noticed that the external energy input, required to achieve the temperature in the reaction system is not taken into account.

5.3 Results and discussion

5.3.1 Water phase recycle at different temperatures

All HTL experiments were carried out in batch mode at 220 °C, 240 °C and 265 °C, with a residence time of 30 min. In these tests, the aqueous phase produced was recirculated twice, making it a total of three runs per experiment. In each recycle run, new dry algae material was fed to the reactor and all oil/solid material from the previous run was removed. In this recovery no additional organic solvent was used for extraction, or to induce phase separation, so as to improve the sustainability of the process. Upon visual inspection of the products, the solids have a 'wet' appearance at temperature of 220 °C (Figure 5.2A) as well in the first run at 240 °C (Figure 5.2B). With increasing number of recycles, the mass of solids increased and its aspect changed from a true solid to a slurry–type texture, and was finally converted to a free–flowing fluid in the last run at 265 °C (Figure 5.2C). This trend can be completely observed in Figure 5.2.
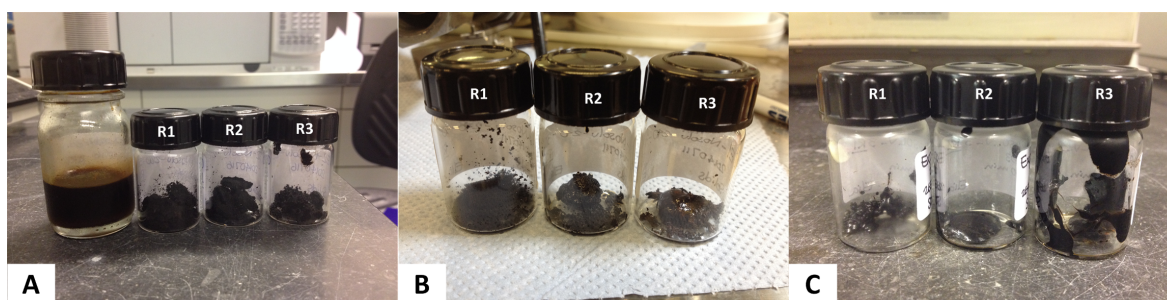

Figure 5.2: Oily solids after 6μm filtration from recycles (R1 = run1, R2 = run2 and R3 = run3) at different temperatures. A) 220°C (the first vessel correspond to water phase after 3th run), B) 240°C and C)265°C.

The microalgae species *C. vulgaris*, used in this research, has a lower oil content: in our case only 6.3 % of oil could be extracted by the soxhlet method. (Bligh and Dyer, 1959). Although according to literature data values up to 25 % of oil in this species have been reported, it is well known that the amount of lipids varies depending on e.g. the culture conditions and strain. Despite its low lipid productivity, *C. vulgaris* has great advantages such as a fast growth rate and the ability to grow in non–axenic conditions, such as wastewaters. (Cabanelas et al., 2013). As can be observed in Figure 5.3, at 220 °C the oil yield increases from 13.3 % in the first run to 21.2 % in the third

run (after 2 water phase recycles), the solid residue trend is not clear. The results for the solids yield are in line with those obtained by Garcia Alba et al. (2012). The low content of oil in this microalgae is not a constraint for the production of biocrude but definitely influences the biocrude yields (Li et al., 2014). The low lipid content makes the overall biocrude yield somewhat lower than in studies at comparable conditions with other algae (Valdez et al., 2014; Garcia Alba et al., 2012). The behavior of product yields as a function of temperature is displayed in Figure 5.3. The oil yield increases while the solid residue decreases with increasing temperature. Our results match with this tendency, that has been fairly reported in literature. At 240 °C of reaction temperature (Figure 5.3) the starting yield for oil is 14.0 wt% in the first run, leading to increase to more than double (31.5 wt%) after 2 recycles. The same trend is observed at a temperature of 265 °C where the minimum conversion of oil is 18 wt% reaching to 35.6 wt% after the second recycle. On the other hand, the solid residue yields does not seem to be affected by the aqueous phase recycling, but merely depending upon process temperature. The gas yields show an incremental increase, especially after the first recycle, at all the three reaction temperature tested (Figure 5.3), probably due to decomposition of recycled aqueous phase organics. The water–soluble organics were measured at the end of the third run, in basis of the total mass of algae fed (around 5.5 – 5.6 g d.a.f), and values in mass and wt% are reported in Table 2, where a decrease in organics with increasing temperature can be observed. The yield of each of the products shows a typical dependence on the temperature, indicating the main reaction mechanisms (hydrolysis, depolymerisation or repolymerisation) for the different temperature ranges (Yuan et al., 2009). In our aqueous phase recycling experiments the gas yield and solid yield are essentially independent of the aqueous phase composition used. This suggests that the increase in the observed oil yield is merely due to the saturation of the aqueous phase with organics.

The elemental composition of oils and their HHV are reported in Table 5.2, where it can be clearly seen that the amount of N increases with temperature and process water recycles, while the C-content decreases a bit as temperature raises and for each subsequent recycle. This affects also the HHV which declines approximately 1 MJ/kg from run 1 to run 3 at all the three temperatures tested. This might be
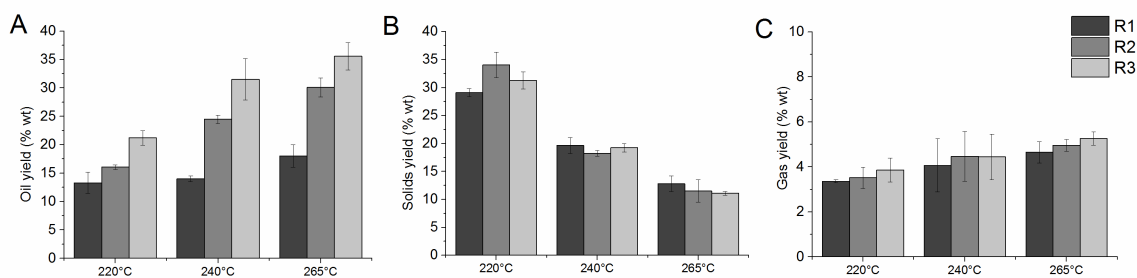
Figure 5.3: HTL Product yields along runs (R1 = run1, R2 = run2 and R3 = run3) in terms of A) oil, B) solids residue and C) gas at different temperatures 220°C, 240°C and 265°C.

explained by the increased content of nitrogenous compounds, originating from the proteins presence in the aqueous phase. It is likely that large protein molecules have been broken in previous and current runs and repolymerize in the following runs with the new biomass fed, which in turn is also broken down. The breakdown of protein and repolymerization mechanisms for the fragments formed strongly depend on the temperature and residence time (Singh et al., 2014; Brand et al., 2014).

Table 5.2: Comparison of biocrude composition, heating values and total water soluble organics at different temperatures.

	N (wt%)	C (wt%)	H (wt%)	O* (wt%)	HHV (MJ/kg)	molar O/C	molar H/C	Energy recovery (%)	Water-soluble Organics
					220°C				
R1	3.4	69.5	9.5	17.5	33.8	0.19	1.64	20	
R2	4.3	69.6	9.5	16.6	33.9	0.18	1.63	26	
R3	5.7	68.6	9.0	16.7	33.1	0.18	1.58	33	1.8 g (33%)
					240 °C				
R1	4.7	71.5	9.3	14.6	34.6	0.15	1.55	24	
R2	6.0	69.8	8.8	15.4	33.5	0.17	1.52	40	
R3	6.9	69.5	8.7	14.9	33.3	0.16	1.50	50	1.6 g (29%)
					265 °C				
R1	5.9	72.2	8.6	13.2	34.0	0.14	1.44	31	
R2	6.9	70.6	8.5	14.0	33.4	0.15	1.44	48	
R3	7.4	69.8	8.6	14.2	33.1	0.15	1.48	58	1.2 g (21%)

* calculated by difference. Water-soluble organics measured after the 3th run, the percentages are
  in basis of the total algae fed.

5.3.2   Continuous recycle of water phase

In order to estimate a maximum productivity of oil, a HTL test with continuous (i.e. repeated several times) recycle of the water phase was performed, at 240 °C of reaction temperature and a residence time of 30 min. This temperature was selected because it is a good compromise between the average production of oil and effortless filtration. It was found that the yield of bio–oil increased continuously with the number of water recycles. In Figure 5.4B the ongoing change in viscosity of the product can be visually observed, this can be seen in Figure 5.4 where the oil yield spars from 14.3 wt% in the first run and reaches maximum of 42.2 wt% of oil after 6 recycles. The water–soluble organics reach 2.6 g after 7 runs, correspondent to 20.1 % of the total mass of algae fed. The pH of the aqueous phase after the first run was 6 and continuously decreased to 4 in the last run.

The molecular weight distribution of the oils was obtained by Gel Permeation Chromatography (more information in Appendix A), can be seen the decreasing in molecular weight of the oil with the recycle number. Specifically it decreases at the peak of 1000 g/mol and increases in the range of 200 g/mol to 300 g/mol. The same trend can be seen also in the oils produced at 200 °C and 265 °C. From the recycling experiments it has been shown that a stable and high yield in oil production can be reached. However, the increasing nitrogen content in the oil composition and (slightly) reduced HHV values (Figure 5.5), indicate a decrease of the biocrude quality with recycle number. This nitrogen content could negatively affect its potential application as feedstock for transportation fuel applications. As observed in previous experiments (section 3.2), the gas production, increases gradually, and its composition with special emphasis on CO and CO_2 provides an idea of the chemical pathway involved. CO in the first run has a value of $2.23x10^{-3}$ $g_{CO}/g_{algae(d.a.f.)}$ and progressively decreases down to $4.08x10^{-4}$ $g_{CO}/g_{algae(d.a.f.)}$ in run 7, whereas CO_2 has a slight increase from $3.39x10^{-2}$ $g_{CO_2}/g_{algae(d.a.f.)}$ up to $4.5x10^{-2}$ $g_{CO_2}/g_{algae(d.a.f.)}$. A general view of the reaction pathway based on our results and according to Peterson et al. (2008) suggests that both decarbonylation and decarboxylation reactions occur, whereas decarboxylation is clearly dominating. With aqueous phase recycling, decreasing pH and increasing organic acid content, the amount of CO further decreases significant and the CO_2
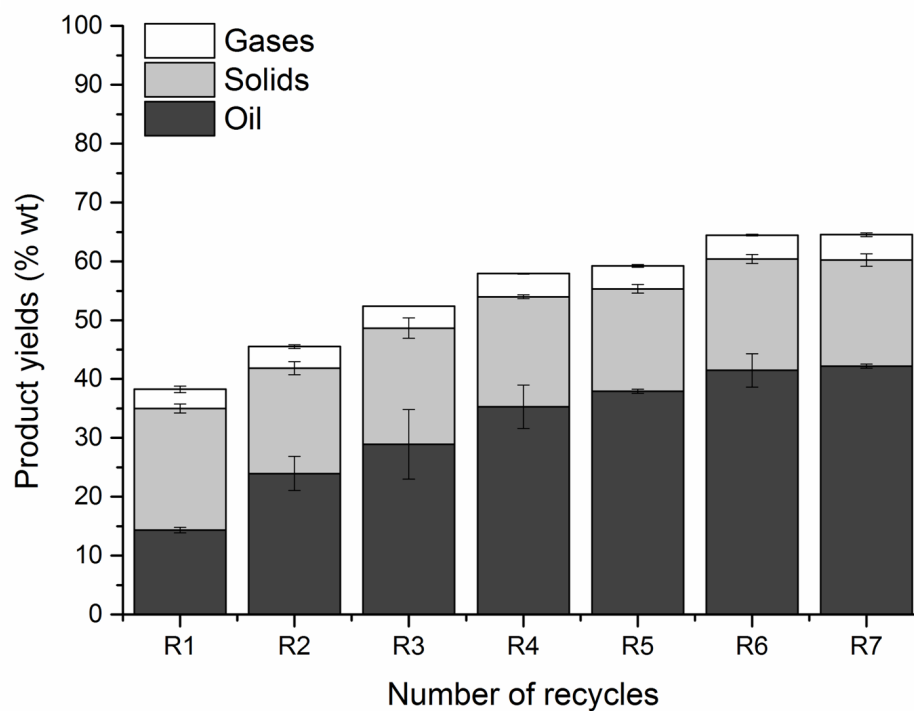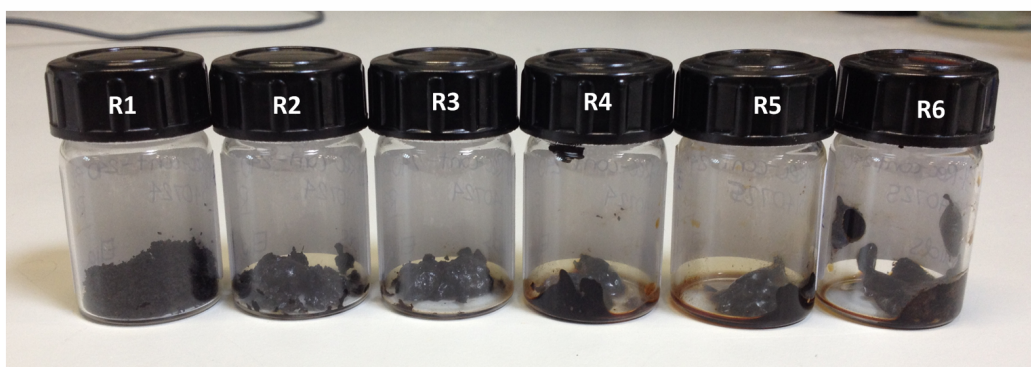
Figure 5.4: A) Gas, oil and solid residue yields and B) Oily solids along aqueous phase recycles at 240°C with 30 min reaction time.

content increases, even more than the reduction in CO. The chemical pathways and interactions between the major components of biomass is far from being completely understood. The compounds in oil and water phase are numerous and identified and characterized to a limited extent only (Torri et al., 2012). The complexity even increases with the recycling of the components in the aqueous phase from a previous HTL experiment. Identification of all compounds and reaction pathways is far beyond the scope of this paper.
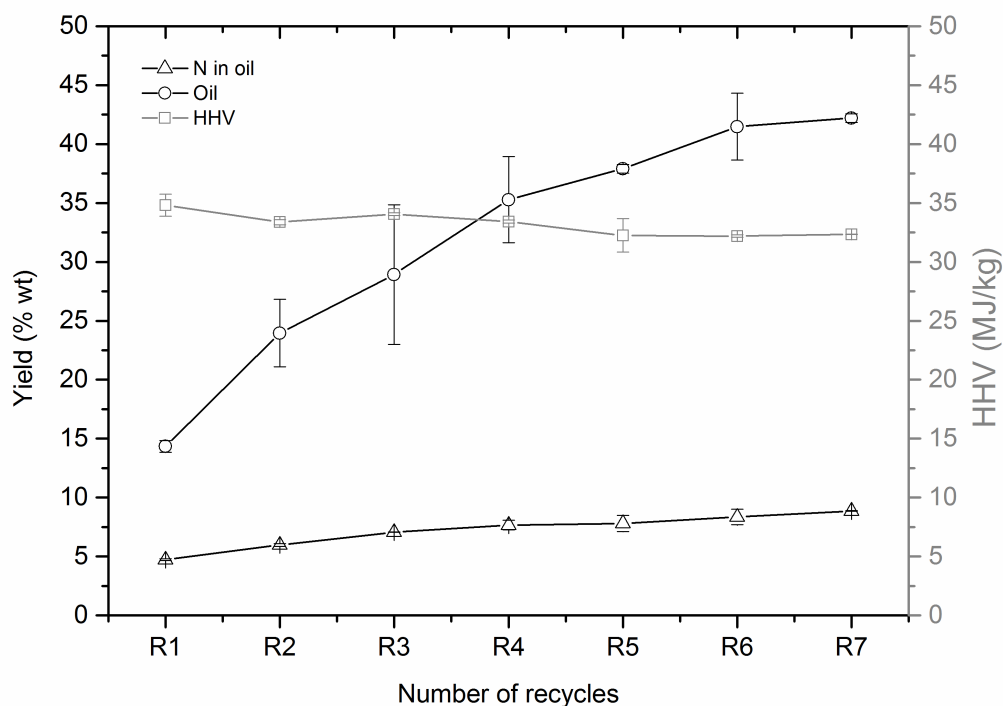
Figure 5.5: *C. vulgaris* oil yields, Nitrogen content and HHV (MJ/kg) of oils at along aqueous phase recycles at 240 °C with 30 min residence time.

5.3.3 Aqueous phase composition

The molecular identification, of the more dominating organic compounds in water provides some information on the mechanisms of the reactions occurring. Aqueous phases composition was analyzed, and light and mid–boiling compounds were detected by GC–MS analysis. The results of HTL process water resulting from experiments at different temperatures were analyzed after the third run, while for the "continuous" experiment the water phase was analyzed all along the test. The components detected in major quantity in the HTL aqueous phase at 220 °C were dl–Alanyl–l–leucine O–Methyl S–2–diisopropylaminoethyl, ethylphosphonothiolate, Phenol and 3,5–dimethoxy– and acetic acid, the latter one being the main component with 34 %. At 265 °C more components were identified, 3–Penten–2–one, 4–methyl– and 2–Pentanone, 4–hydroxy–4–methyl– were prominent followed by acetic acid and 2,2,6,6-Tetramethyl–4–piperidone among other heterocyclic compounds like pyrazine. Along water recycles the composition of aqueous phase was changing. It is important to

highlight the high fraction of acetic acid in water phase. The acetic acid concentration in each recycle was observed, ranging from 2.4 g/L after the first run, to concentrations about 8 g/L in run 7. Aqueous phase components reported in this chapter were obtained using microalgae species *C. vulgaris*, with very low content of oil (6.3%) and high protein content of around 50 wt%. As proteins are the main source of nitrogen compounds, it is important to highlight that results of this study in terms of oil yield and composition of oil and aqueous phase are influenced by the gross biochemical composition of the microalgae used both, with and without recycling of the process water.

5.3.4   About the influence of Acetic acid?

As mentioned above, acetic acid is one of the main components of HTL process water. Its catalytic effect to enhance the bio–oil yield has been investigated by Ross et al. (2010) for HTL operation at 300 °C and 350 °C for two microalgae species with different oil content. Comparing the yields using organic acids and alkali catalysts, a slightly increase is reported when using acetic acid. As the role of acetic acid as catalyst may depend on the reaction conditions applied, a further study on its effect in case of process water recycling seems relevant and results of these experiments are reported in this section. The results of the GC–MS analysis used to identify the light and mid–boiling compounds of the aqueous phase, report accumulation of acetic acid in the continuous recycle experiments (section 5.3.2). To identify whether the increasing acetic acid content influenced the liquefaction process, an experiment was performed using a solution of acetic acid as catalyst. The acid concentration applied was 6 g/L and the tests were carried out at 240 °C with a residence time of 30 min, using the same method for separation and analysis of products as in previous experiments. For further qualitative comparison, tests at the same conditions but without additional acetic acid were performed. The results show a minimal change in the yields of the products; a slight increase of only 0.62% in oil yield was detected when acetic acid is used, a value which is with the experimental error. Furthermore, also the measured chemical properties of oil were not affected by the use of acetic acid, neither significant changes in elemental composition were identified, values of 5.2 (wt%) N, 70.33 (wt%) C, 8.89 (wt%) H and 15.58 (wt%) of O using acetic acid are reported, compared

with 5.18 (wt%) N, 70.77 (wt%) C, 9.11 (wt%) H and 14.94 (wt%) of O, from the equivalent experiment without acetic acid. The molecular weight distribution of the oils recovered is a further point to highlight, since no significant changes were detected when acetic acid is used and when is not, figure 5.6 shows the diagrams of molecular weight distribution of oils obtained in both reactions, the peak of molecular weight below (100 g/mol) possible corresponds to the degradation products of the GPC–eluent used (THF).
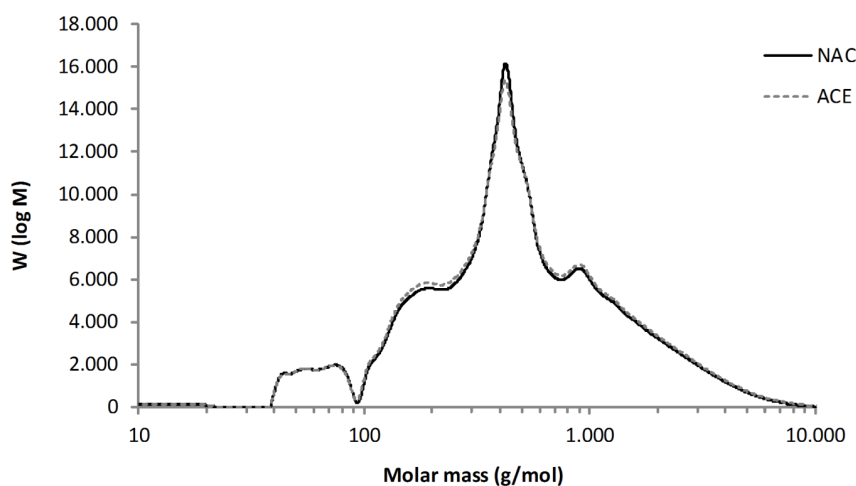

Figure 5.6: Molecular weight distribution by GPC analysis of oil obtained at 240°C with 30 min reaction time. ACE = Acetic acid initial concentration 6.0 g/L, NAC without acetic acid.

On the other hand, although the change in elemental composition is minimum, it causes a variation in HHV values, from 33.6 MJ/kg when acetic acid is present, to 34.1 MJ/kg when it was not used. A similar behavior was also observed by Ross et al. (2010) besides an increasing in oil yield using organic acids in comparison with alkali catalyst. Li et al. (2013a) also reports an improvement in oil yield using *Salix psammophila*, containing more than 25 wt% of lignin. The role of acetic acid in Changjun experiments was identified as acid catalysis of the hydrolysis reactions of lignin. In this chapter, on the contrary, *C. vulgaris* does not contain (significant amounts of) lignin. Based on our results, the oil yields do not seem to be correlated to acetic acid content with water recycling, as can be observed in figure 5.7. In this figure the oil yield and the initial concentration of acetic acid in the water phase are represented. All the points refer to experiments at 240 °C and 30 min of residence time. The black dots were

obtained from the "continuous" recycling experiments, where a cumulative production of acetic acid with water recycling has been reported (and the increasing in oil yields); the squares represents the results of the experiments with added acetic acid and its equivalent without acetic acid and, finally, the triangles refer to experiments in which water phase from another run is diluted with demiwater (for details, see section 5.3.5) to start concentrations of respectively 2 and 4 g/l.
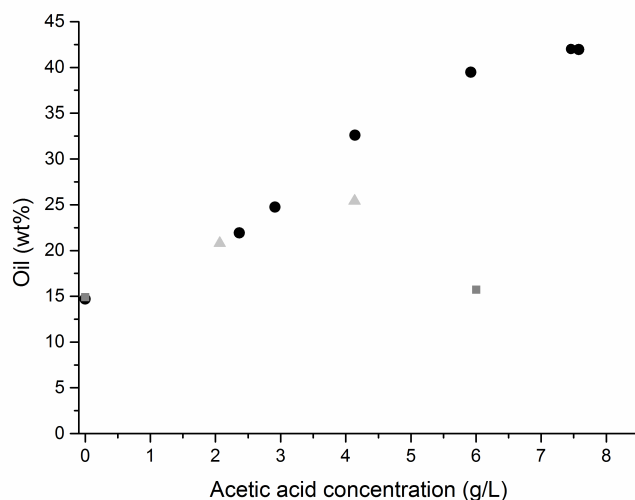

Figure 5.7: Oil yields as function of initial acetic acid concentration in reaction medium. Gray squares represents the results of the acetic acid experiment, triangles are from dilution of water phase experiments and circles are the continuous recycle experiment results. All the results were obtained at 240°C with 30 min reaction time.

Pointing attention to the water phase composition, it is still possible that the organic components based on their concentration change the solvation properties of the aqueous medium, although it is well known that the properties of water changes with the temperature (e.g. when approaches to its critical temperature of 374 °C the behavior changes from non–polar to polar), probably the aqueous phase could increasingly accept less organics, altering the mechanism to the formation of oils. Besides, the organic molecules present in aqueous phase have molecular structures with short hydrocarbon chains and therefore low molecular weight, this would explain the increasing content in compounds of low molecular weight in the oil as number of recycles are performed (see Appendix A).

5.3.5 Water phase dilution experiments

In order to verify how the concentration of organic components in process water affects the properties of this co–product, and to identify the direct influence on the oil yields, two HTL experiments were conducted at 240 °C with residence time of 30 min and a biomass concentration of 11 wt%, as in previous tests, using the same protocol for product recovery and analysis. In these experiments aqueous phase from run 7 from the continuous recycle experiment was used; in the first run it was diluted 1:2, while in the second 1:4, using distillate water in both cases. Table 5.3 reports the yields and elemental composition of oils from both experiments. The highest oil yield was obtained in the test D–1:2, where the initial concentration of organics was 0.10 g_{org}/g_{ap} (for D–1:4 it was 0.05 g_{org}/g_{ap}, the net weight percent of organics in aqueous phase were 26.5 and 33.1, for D–1:2 and D–1:4 respectively). The values of Table 5.3 are in agreement with the "continuous" experiments, in which the yield of soluble organics steadily decrease (on algae basis) as function of recycles. It was mentioned that there is accumulation of small organic compounds upon recycling and some of the major were identified. This accumulation will however level off, till a kind of steady state is reached. This implies that the water phase gets "saturated" with the smaller organic compounds and hence more oil will be recovered in the organic phase when starting with an aqueous phase that already contains a significant amount of organics. However, when the aqueous phase is more concentrated in organics, these compounds may also show more tendency to polymerize to higher molecular weight compounds reporting directly to the oil phase. This hypothesis could be supported by the N in oil, which is proportional to the oil yields. No significant change was detected in HHV.

Table 5.3: Comparison of oil yield, composition, heating values and energy recovery at different dilution of organic matter in initial aqueous phase.

Dilution	Oil yield (wt%)	N (wt%)	C (wt%)	H (wt%)	O (wt%)	HHV (MJ/kg)	Energy recovery %
1:4	20.8	7.0	69.5	8.6	15.0	33.1	34
1:2	25.4	7.3	69.0	8.7	15.0	33.2	41

5.3.6 Energy recovery ratios

The energy recovery has been evaluated as the energy obtained as biocrude divided by the heating value of the microalgae biomass fed to the system. This parameter determines the maximum efficiency of the reaction, but does not include energy consumption during operation. The maximum energy recovery was 68%, obtained in the 7th run at 240 °C, and this value corresponds to the highest oil yield.

Table 5.4: *C. vulgaris* HTL biocrude O/C and H/C ratios and energy recovery along aqueous phase recycles at different temperatures with 30 min residence time.

	N (wt%)	C (wt%)	H (wt%)	O (wt%)	HHV (MJ/kg)	Molar O/C	Molar H/C	Energy recovery (%)
R1	4.7	71.7	9.4	14.2	34.8	0.15	1.57	25
R2	6.0	69.5	8.8	15.6	33.4	0.17	1.53	40
R3	7.1	70.5	8.9	13.5	34.1	0.14	1.51	49
R4	7.7	69.5	8.7	14.2	33.4	0.15	1.50	59
R5	7.8	67.5	8.4	16.2	32.3	0.18	1.50	61
R6	8.4	67.5	8.5	15.6	32.2	0.17	1.52	67
R7	8.9	67.3	8.4	15.4	32.3	0.17	1.50	68

Next to the energy recovery into oil, Table 5.4 and Table 5.2 also reports the molar O/C and H/C ratios of the oil, in both series of experiments; at three different temperatures and with increasing water recycle number. It can be observed that the O/C and H/C ratios are close to that of crude oil. In addition a reduction in O/C and H/C ratios of oil with increasing temperature was observed, as already been reported by other authors (Garcia Alba et al., 2012), and with increasing aqueous phase recycling. The reduction of these ratios is due to the increasing content of aromatic compounds. For the 265 °C oil, these ratios do not seem to follow this trend any longer, while N still increases significantly and C slightly decreases. This is probably related to changes in nature and composition of the organic compounds in the oils.

5.4 Final remarks

In this chapter, process water from hydrothermal liquefaction of the low–lipid microalgae *C. vulgaris* was recycled into the HTL reactor. In the procedure followed

the solid and oil product phases were collected together, without using any additional solvent. A strong increase in oil yields was observed upon aqueous phase recycling, for all the temperatures tested. Continued recirculation resulted in a stationary phase for the yield, which was reached after approximate six recycles. The maximum oil productivity for above algae was 42.2 wt% at the 7th recycle at 240°C and 30 minutes process time for each run. This yield corresponded also to the highest energy recovery ratio for the oil, equal to 68 % of the HHV of the algae fed to the system. The increase in biocrude yield upon water recycling is probably mainly due to saturation of the aqueous phase with organics and to some extent to the repolymerization of nitrogen–rich organic compounds present in the aqueous phase, supported by the observed corresponding increase in N content in the oil, and a slightly lower HHV value of around 33 MJ/kg. The possible influence of acetic acid on oil yield was investigated in this chapter. However it was not possible to confirm any relationship between the oil yields obtained and the acetic acid concentration. Aqueous phase recycling is therefore proposed as novel step in biocrude production from microalgae by HTL, since the presence of soluble–organic compounds in the water phase leads to increased biocrude production at lower temperatures and reduces energetic costs by ensuring high oil yields at relatively low temperatures, also to avoiding the use of solvents for oil recovery.

Energy profitability analysis for microalgal biocrude production

This chapter presents the results of a thorough Energy Return on Energy Investment (EROEI) analysis for biocrude production from microalgae processes. We have investigated different alternatives to maximize the energy recoveries of each process considered, which has been modeled and simulated by Aspen Plus^{TM}. The estimates for feeds and recirculation of microalgae and nutrients, working conditions, systemization and equipment requirements were obtained using both literature and own experimental data. The Pinch Technology Analysis was applied to optimize the process operating conditions. It was found that the process which uses the combustion of biomass after biocrude extraction is the most favorable one in energy terms. In particular, 2 cases of this type were addressed and compared to a *Base case* where all energy requirements (heat and electrical) are provided from external sources: *Case 1*, when electricity is supplied externally whereas the biomass and part of the biocrude produced are burned to meet the requirements of thermal energy, and *Case 2*, where the energy recovered from both, biomass and part of biocrude are used to fulfill all heat and electricity duties. These cases were analyzed also when the nitrogen needed for microalgae growth is obtained from wastewaters. Favorable EROEI figures could be calculated.

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[0]Part of this chapter has been published in Energy

6.1   Introduction

The increasing demand of fossil fuels and the forecast of depletion of oil reservoirs within few decades is going to lead to a steady increase of oil prices in the near future, which is expected to heavily affect all the economic activities, in particular the transportation sector. Fossil fuels contribute with a share of 88% to the total global consumption of primary energy, with oil 35% , coal 29% and natural gas 24% as the major sources (Brennan and Owende, 2010). This problem is common to the entire world population, with special regard to industrialized countries. The world economy is highly sensitive to changes in the price of fossil fuels, with serious repercussions on the industrial, commercial, alimentary, social, political attitudes.

From an environmental point of view, in the European Union (EU) more than 60% of greenhouse gas (GHG) emissions are originated from energy sector, in addition, 20% is caused by the transport sector. Owing to the development of new growing economies, such as China and India (Mata et al., 2010), there will be an ever more significant contribution to global warming and GHGs, with negative impacts on human life, e.g., the accumulation of CO_2 both in the atmosphere and in the oceans, turning the water pH gradually to more acidic and affecting negatively the marine ecosystem biodiversity (Bates and Peters, 2007), and the melting of glaciers, leading to the reduction of salinity in sea waters and to the increase of the sea level rising the risk of flooding in coastal areas. For these and other reasons, reducing the GHG emissions and concentrations in the environment is a urgent need and a crucial issue to be addressed properly in the shortest time.

Exploitation of renewable and sustainable energy sources can give a significant contribution to the solutions to these problems, however it is essential to check that it is performed in a sustainable way. Among other alternatives, oil from microalgal biomass seems to have a great potential to replace fossil fuels and crude oil. In principle, it presents a number of advantages, such as the use of atmospheric CO_2 to produce biomass, the possibility of achieving massive production (the oil content in microalgae can be higher than 80% in dry weight, DW), the exploitation of biocrude as a feedstock for bulk chemicals with high market value, and a yield of more than 100 metric tons of biomass (DW) per hectare per year (the maximum theoretical value is

around 280 t, (Bilanovic et al., 2012)), in addition, microalgae do not compete directly with food crops for land. The possibility of sustainable fuel production from microalgae has generated tremendous interest in recent years (Warner, 2012) and a major point under discussion is about the feasibility of utilizing microalgal biomass to contribute or supply total or large part of the world demand of energy. Industrial processes have to be envisaged, designed, analyzed and proved to be sustainable on three sides at least: energetically, environmentally and economically. As a first step, the aim of this paper is to address the energy sustainability issues of a general process suitable to produce microalgae at the large scale, to propose a number of process improvements, and to give a contribution to assess the industrial microalgae production in profitability terms.

When the sustainability of a novel source or pathway to obtain biofuels has to be evaluated, an indicator widely used to check the energetic profitability of any process in which the goal is the production of energy is the Energy Return on Energy Investment (EROEI). EROEI indicates how favorably the primary energy input to the process is used to obtain the energy-carrier product, and provides information of the advantages that can be achieved by selecting one process (rather than others) to exploit the primary energy available in the feed. In this way, the EROEI analysis favors those systems that produce the best energy payback (Raugei et al., 2012). It is noted that such a methodology does not take into account other environmentally relevant factors like deforestation, land use, contamination and others (Font de Mora et al., 2012), as it is focused onto the first and crucial factor, i.e. energy.

EROEI can be calculated for renewable as well as non-renewable primary energy inputs. If a process option brings an EROEI greater than 1 (possibly much greater than 1), a good payback will be provided by the primary energy investment (Bardi, 2005). This indicator can be calculated for any fuel production processes, thus allowing their comparison based on the ratio between energy obtained and energy required, and a consequent selection of the best performing way for exploiting different primary energy sources. Examples retrieved from the literature refer, among others, to processes producing vegetable oils (Grau et al., 2013), photovoltaic energy (Raugei et al., 2012), and microalgal oil. Values of EROEI reported in this last case span from 0.13 (Brentner et al., 2011), who investigated the entire process, including the biodiesel production

section, to 2.5 (Vasudevan et al., 2012), whereas those related to petroleum production by both conventional extraction and enhanced oil recovery span between 18 (Gagnon et al., 2009) and 40 (Dale et al., 2011). A relation between oil EROEI values and oil price is studied and modeled by Heun and de Wit (2012): at the present time, oil price increases are observed when EROEI is below 10.

The different values calculated for microalgae-based processes depend on a number of variables including the process design, the bioreactor type (open or close photobioreactors), the productivity of the species in different conditions, the raw materials, the downstream methods and equipment, and the energy integration system. Energy, as a physical quantity, is not affected by inflation rates, discount rates, market prices, and so on. The scope of this chapter is evaluate the energy operating costs only, omitting capital costs. The analysis presently proposed takes advantage of both process simulation techniques and of literature as well as own experimental data. All the process steps and the input/output streams are included, from the photobioreactor to biocrude product refining. An economic analysis is outside the scope of this part of the project, as it would require additional information, which is currently unavailable due to the lack of industrial and pre-industrial installations of this type.

6.2 Evaluation Methods

6.2.1 Production process

The production process to obtain biocrude from microalgal biomass can be summarized in five main steps, encompassing biomass cultivation, biomass harvesting, oil recuperation, oil refining, while the residual de-oiled biomass is eventually used to exploit its energy content. For simulation convenience, the whole process is divided in two sections: the first one (section A) is the production of biomass, while section B includes oil extraction and energy recovery. The output streams from the first part constitute the inputs to the second one. See the block flow diagram of Figure 6.1 for details.

In section A the growth of biomass is carried out in photobioreactors fed with water, nutrients, light and an air–CO_2 mixture with a convenient concentration of CO_2 as the carbon source. The size of the photobioreactor was calculated by taking into account
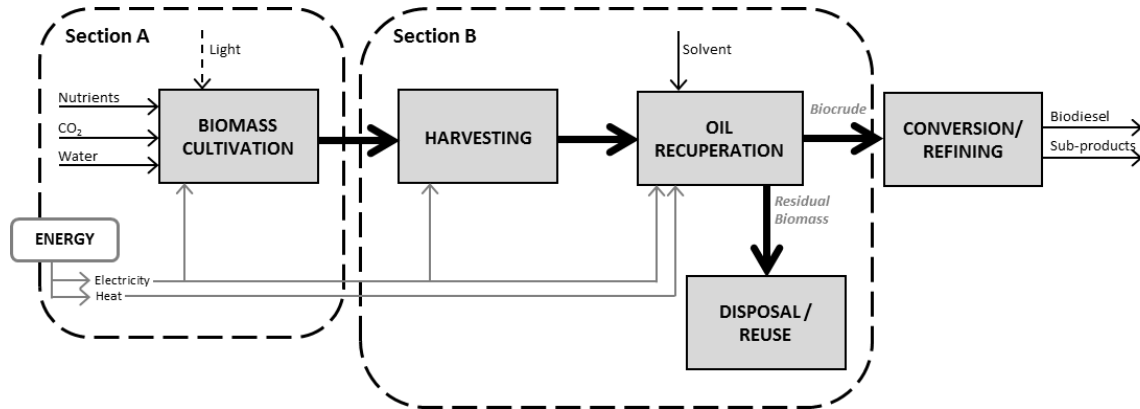
Figure 6.1: System flow diagram with material and energy flows for biocrude production.

the surface area irradiated needed to obtain a given productivity. By considering that microalgae can absorb only a portion of the total solar radiation, and that a number of losses occur, such as wrong absorption, degradation of absorbed photons, conversion to other components, losses due to dark and photorespiration, and losses at molecular level, a value around 7% has been assumed for the photosynthetic efficiency. Thus, starting from the total solar irradiation at mid-latitudes, corresponding to about 4500 MJ m^{-2} y^{-1}, and referring to a biomass productivity of 1 kg/h (dry mass), a surface area of about 555 m^2 results.

In section B the biomass produced is preliminarily separated from water by mechanical operations (filtration or centrifugation), to obtain a solid biomass containing about 20% of water. The water is recycled and the oil is separated from the biomass by solvent extraction using hexane as the solvent. Hexane is recovered by distillation and recycled as well after makeup, and refined biocrude is produced at the same time. The residual de-oiled biomass is exploited for energy recovery, by applying different techniques such as combustion, gasification, pyrolysis, hydrothermal treatment and anaerobic digestion. The CO_2 possibly produced in this latter stage can be used as a carbon supply to the photobioreactor.

A first objective of our analysis is to make the entire process self-sufficient from the energy duties standpoint, by recovering energy from the exhausted biomass and, in the case this is not sufficient, also from part of the oil produced. The different process alternatives, which refer to thermo-chemical routes only, and the assumptions done are detailed in the following. Accurate and extensive process simulations have been
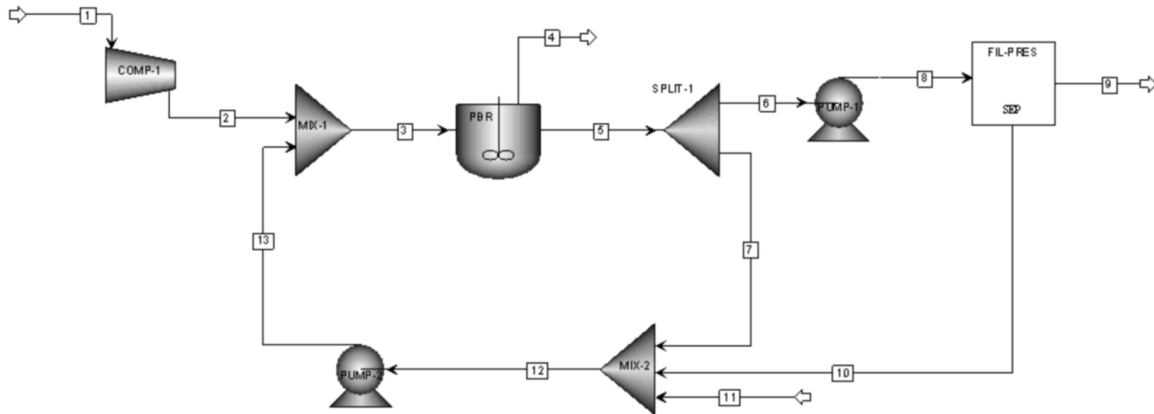
Figure 6.2: Flow sheet diagram for Section A to biomass production.

performed, to allow a sound calculation of EROEI values.

6.2.2 Simulation procedures and assumptions

In this part we summarize considerations and assumptions done for the construction and implementation of the simulation model, which was developed within the process simulator Aspen Plus^{TM} v7.0. The procedure used for energy analysis and integration of the entire process is reported as well. All simulations were performed with respect to the microalgae *Nannochloropsis salina*, a sea-water species for which we have measured and optimized both the growth conditions and the oil content elsewhere (Sforza et al., 2012a). The composition of *N. salina* was determined analytically: reference values of mass elemental composition are C = 59.51%, H = 9.3%, N = 6.47%, O = 24.72%, measured with 30% of oil (DW), and used also for other oil contents.

As can be seen in Figure 6.1, section A is aimed to produce biomass with a solid content of 20% by mass. It includes a fan system to supply CO_2, a photobioreactor and a filter press to concentrate the biomass. We propose a recycle of both the biomass (partial) and water (as much as possible), to increase the growth rate and to minimize the water makeup: the flow-sheet of this section is shown in Figure 6.2. As the objective of this study is not a rigorous reactor simulation, a simple model i.e. a perfectly mixed reactor was assumed for calculating the productivity. The kinetics of the reaction was expressed with a Monod equation, which was implemented in Aspen Plus^{TM} by a Fortran subroutine (see Palma (2011) for details):

$$r_X = \frac{k \cdot c_s \cdot c_x}{K_M + c_s} - k_d \cdot c_x \qquad (6.1)$$

where r_X is the growth rate in [kg m^{-3}s^{-1}], k is the growth constant [s^{-1}], k_d is the maintenance constant [s^{-1}], c_s is the limiting substrate concentration [kg/m^3], c_x the biomass concentration, K_M is the half saturation kinetic parameter. The parameter values were determined from batch growth measurements in our lab (Sforza et al., 2012a).

A filtration unit (SEP) is assumed to achieve a solid concentration around 20%. Part of the output stream from this unit is recycled to the reactor to maintain a concentration of 1 g/L of biomass at the reactor inlet, a value close to the optimum for industrial flat photobioreactors (Sforza et al., 2013), and the reactor volume is varied in order to ensure the desired biomass production (conventionally set to 1 kg/h DW).

Concerning section B, it is well known that the biomass should be much more concentrated than 20% to extract the oil by a classical solvent extraction operation with hexane. Indeed, new technologies such as Cell lysing (Beal et al., 2010a,b) or Hydrothermal conversion (Valdez et al., 2012) do not require to dry the biomass before recovering the oil, but they are still under development. Thus, we preferred to refer to solvent extraction as the technique for downstream processing. It was shown also in our lab that hexane is able, by a suitable contact, to penetrate the cell walls, break them and dissolve the oil (Simionato et al., 2011).

In section B the biocrude production is achieved, as detailed in Figure 6.3. The output stream 9 from section A is heated in HEATER–1 to remove water up to a solid content of 90%. The RYield reactors COMP–SEP and ELEM–SEP serve to decompose unconventional components, such as *algae* (stream 13) and *biomass* (stream 18) in their own constituent elements. The enthalpy of formation of the alga has been calculated according to its heating value, which depends on the oil content. In turn, the fraction of oil in the photobioreactor output stream has been varied, to evaluate the EROEI sensitivity to this parameter. After hexane extraction, the oil–solvent mixture is then separated in a distillation column to recover the hexane as the distillate and the biocrude as the residue. On the other hand, the biomass exhausted after oil extraction is processed for energy recovery purposes. Also part of the biocrude produced can be

exploited to provide the energy duties of the process. In the flow–sheet example of Figure 6.3 combustion units are used in both cases. Two thermodynamic models were used in the simulation of the process units: the NRTL model for the liquid phase and the Henry's Law for the gas solubility, at low temperatures and pressures, and the Peng Robinson equation of state with the Boston-Mathias correction of for gaseous mixtures, at high temperatures and pressures.

6.3 Pinch Technology Analysis (PTA)

Pinch Tecnology Analysis (PTA) is a methodology to minimize the energy consumption of a process by optimizing heat integration among different units through a network of regenerative heat exchangers, accounting for both the first and the second law of thermodynamics (Peters et al., 2003). The optimization is achieved by coupling appropriately process streams that have to be cooled (hot streams) with others that must be heated (cold streams), minimizing the need of both external heating and cooling. Therefore applying PTA to a process scheme allows to reduce the operating costs of the plant with respect to energy.

6.4 EROEI definition

The EROEI analysis was applied to the process production pathway (Figure 6.1) using the model proposed by the literature (Beal et al., 2010a; Mulder and Hagens, 2008). The EROEI value is expressed and calculated as:

$$EROEI = \frac{ED_{out} + \sum_j v_j \cdot O_j}{ED_{in} + \sum_k \gamma_k \cdot I_k} \tag{6.2}$$

where ED_{out} and ED_{in} are the direct energy terms including electricity and heat duties for the process, and energy produced by the biocrude and exhausted biomass [MJ/h]. In Equation 6.2 the indirect energy flows are represented by v, the energy equivalent [MJ/kg] of flow rate O [kg/h] for component or sub–product j in the output, whereas γ is the energy equivalent [MJ/kg] of flow rate I [kg/h] for component k in the input. In other words, direct flows of energy include electricity and heat, while indirect ones account for all energy containing materials.
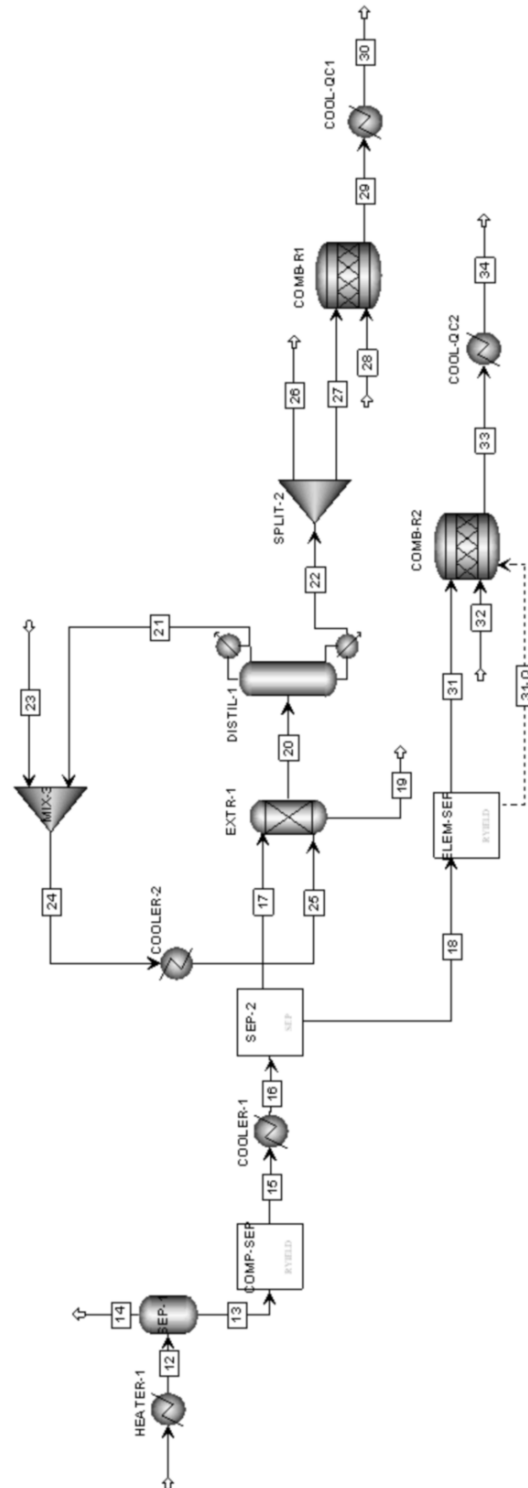
Figure 6.3: Flow sheet diagram for Section B to biocrude production and recuperation energy from residual biomass.

6.5   Results

6.5.1   Energy recovery from biomass and biocrude. PTA analysis

Conversion of biomass to energy is obtained using two main process routes, the thermo–chemical and the bio–chemical/biological ones. High moisture content biomass is better suited to biological conversion processes (Toor et al., 2011), but thermo–chemical routes like pyrolysis (Pan et al., 2010; Oudenhoven et al., 2013), combustion, gasification (Murthy, 2011) and hydrothermal liquefaction (Brown et al., 2010; Garcia Alba et al., 2012) are much faster. So, we have examined and simulated the four process options mentioned above. The comparison between the four alternatives considered is shown in Figure 6.4. The main results of the PTA analysis are:

- in terms of kilograms of oil produced per kilogram of biomass generated, combustion ensures a larger production, even though a drying step is required, as it is feasible only when the biomass has a moisture content <50% (Piriou et al., 2013); on the other hand gasification and pyrolysis are processes more complex from the point of view of the plant and the control, and are more energetically expensive, so they can be taken into account only when there are strong environmental pressures. About hydrothermal treatments they require more energy than can be produced, as the operative conditions of such processes requires are at very high pressure and temperature, even when the weight fraction of oil in the alga is the maximum (0.70).

- in all cases, the heat required to ensure the thermal self-sufficiency of the entire process can be given by the exhausted biomass and, if this is not enough, by part of the biocrude produced in all cases, so there is no need of an external thermal energy source. The process heat balances are achieved by varying the fraction of the splitter SPLIT–2 of Figure 6.3, until the sum of the heat exchanged by all the exchangers is equal to zero;

- according to the results obtained, combustion has been selected to proceed with the EROEI evaluation. In all the following simulations carried out to this scope, the complete combustion of biomass (and a part of the oil, in some cases) has
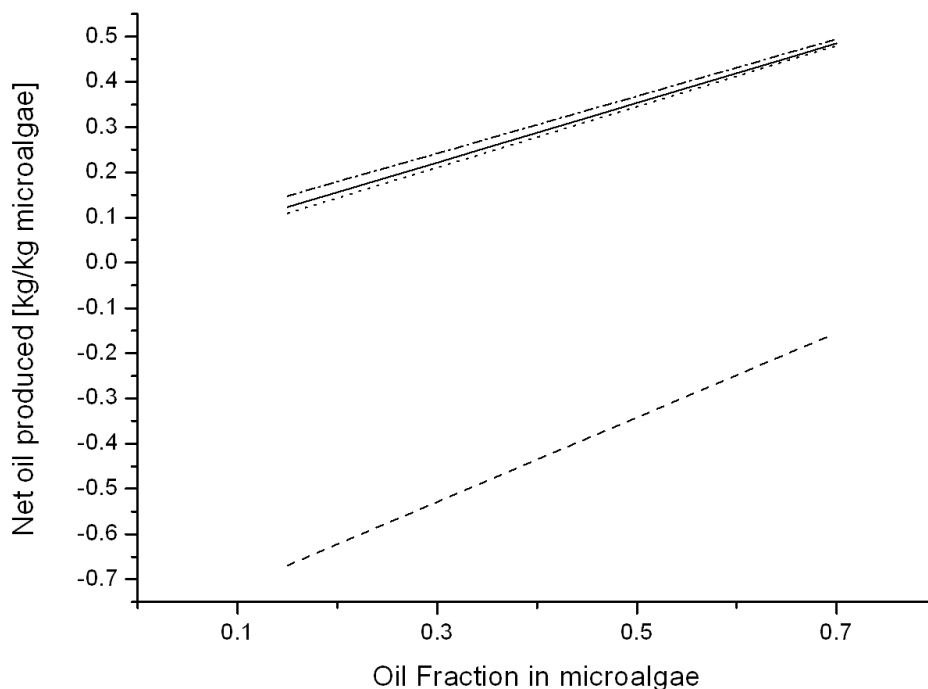
Figure 6.4: Comparison of the four alternatives considered to exploit the de-oiled biomass, the net production of oil against the oil content in the microalgae obtained from the photobioreactor.$- \cdot -$ Combustion, $- - -$ Hydrothermal treatment (of whole biomass), $\cdots$ Pyrolysis, $-$ Gasification

been performed with an air flow rate such that the flue gas temperature does not exceed 1100 °C. The fumes were cooled down to 120 °C to recover the energy needed to dry the biomass after filtration/centrifugation.

6.5.2 EROEI calculation

In the processes considered, energy burdens are due to both heat/mass transfer requirements and to raw materials supply. The energy equivalent of all nutrients was calculated according to Beal et al. (2011). The estimates for feed and recirculation of microalgae and nutrients, as well as the working conditions and equipment requirements were calculated by process simulation based on laboratory experimental data. All calculations were referred to a standard production of 1 kg/h of microalgae (DW), and were obtained by process simulation. The source of CO_2 was flue gases, so that its energy equivalent is only the electric power required by the compressor for its injection

in the photobioreactor as a CO_2–air mixture (Murphy and Hall, 2010). In order to perform a sensitivity study, the microalgae biocrude production process was analyzed for three oil contents in the biomass, 30%, 50% and 70% DW (Sforza et al., 2012a). For each of them, three cases were studied with different energy recovery schemes:

- a *Base Case*, when the electrical energy requirements of the process are provided by an external source, while the thermal energy is given by natural gas combustion;

- *Case 1*, where electricity is from an external source, whereas the biomass produced after oil extraction is used to meet the requirements of thermal energy;

- *Case 2*, where it is proposed to use the energy recovered from biomass and from part of biocrude to meet the process energy requirements, both for heat and electricity (this last is produced by using the biocrude as fuel of a diesel engine generator).

For these cases all the relevant inlet and outlet flows of matter and energy that are involved in the process were considered. The consumption of electricity covers all the steps necessary to the growth, harvesting, concentration of microalgae, to the separation and recovery of the oil from biomass, to the handling of microalgae and exhausted biomass, and to the blowing of air used for oil and exhausted biomass combustion. Equipments that consume energy in the process are: the compressor to feed CO_2-enriched air to the photobioreactor, the pumps for recycling water and microalgae suspension, the filter press to concentrate microalgae to 20% DW, the screw conveyor for handling the concentrated suspension, the utilities required by the extraction and distillation units, the pump which transports the oil to the combustor or to the next stage of manufacture, the conveyor belt for the transport of the exhausted biomass to the combustion, the fan for the air injection to the burner oil, the fan for insufflate air to the biomass incinerator. The values of electricity duties have been calculated part from the simulations, part from technical manuals (Sereco S.r.l., 2012). In this last case, the values related to specific flow rates were normalized to 1 kg/h of dry biomass, and the liquid flow rates calculated accordingly. Of course, the results depend on both the process scheme and the initial content of oil in microalgae. As an

example, in Table 6.1 the energy consumption for the *Base case* with an oil content of 70% is reported.

The evaluation of the indirect energy flows, i.e. those contained in raw materials and products, include the makeup water due to losses in the photobioreactor and cooling water system. About nutrients, we have estimated the indirect energy contents of sodium nitrate ($NaNO_3$) as a nitrogen source (Sforza et al., 2012b), of sodium phosphate monobasic hydrate ($NaH_2PO_4\ H_2O$) as a phosphorus source and of ferric chloride hexahydrate ($FeCl_3\ 6H_2O$) as an iron source (Beal et al., 2010a). The flow rates of water and hexane, and their estimated losses, were derived from process simulations. The amounts of materials are converted to energy values using the energy equivalent per unit of each input (Beal et al., 2011). It is noted that the energy equivalent of CO_2 is equal to zero, contrarily to what is reported in the literature (Beal et al., 2010a; Murphy and Hall, 2010). In fact we have assumed that CO_2 is available at no costs, for example from a combustion unit located near the biomass production plant, so that the only energy associated with CO_2 is the one needed to pump it through the photobioreactor, which is calculated as a direct energy flow. Table 6.1 summarizes all the energy inputs and outputs of the process, both the indirect ones as materials and the direct energy consumption, in terms of both electricity and heat. Again, the figures refer to the Base case with 70% content of oil in the starting biomass, for which an EROEI value of 1.88 is calculated. The process outputs are de-oiled biomass and biocrude flows, equal to 0.3 kg/h and 0.7 kg/h respectively. In view of the results obtained, the EROEI value is greater than 1 but not too much, even if a maximum oil content in the starting biomass is assumed. It is then important to improve the process by exploiting possible recoveries of heat and electricity.

6.6 EROEI sensitivity on different operating conditions

As specified above, in the Base case electric energy is taken from the network, while the heat, largely due to the drying of the biomass, is supplied by the combustion of methane. The flow rate of methane, to be included in the indirect inputs, is calculated by assuming that the combustion flue gases are cooled down to a temperature of 120 °C to ensure the drying a good driving force. Table 6.2 summarizes the results as a

Table 6.1: Direct and indirect energy flows for the Base case with an oil content in microalgae biomass of 70%.

INPUT			
Total input			**15.489**
Total energy of materials [MJ/h]			*14.729*
Material	**Amount**	**EE [MJ/X]**	**Energy [MJ/h]**
Process water [kg/h]	6.46	1.33 kJ/kg	0.009
Cooling water [kg/h]	0.089	11.23 kJ/L	0.001
Nitrogen: $NaNO_3$ [kg/h]	0.327	9.38 MJ/kg	3.067
CO_2 [kg/h]	6.11	0 MJ/kg	0.0
Phosphorus: $NaH_2PO_4 \cdot H_2O$ [kg/h]	5.1E-03	13.83 MJ/kg	0.071
Iron: $FeCl_3 \cdot 6H_2O$ [kg/h]	2.0E-04	20 MJ/kg	0.004
Methane (kg/h) for drying	0.228	50 MJ/kg	11.37
Hexane loss (kg/h)	0.004	49.4	0.199
Total Electric energy (MJ/h)			*0.760*
Process unit			**Energy [MJ/h]**
Compressor for CO_2			0.423
Pump recirculation photobiorreactor			0.255
Filter Press			0.030
Auger for sludge			0.007
Pump for hexane + oil			5.1E-04
Pump for hexane (extraction)			3.1E-04
Pump for oil (extraction)			2.1E-04
Conveyer for transport of biomass			9.4E-04
Compressor air,oil combustion			0.013
Compressor air biomass combustion			0.031
OUTPUT			
Total output			**29.142**
Material	**Gross production**	**Net Production**	**EE [MJ/kg]**	**Energy [MJ/kg]**
Biocrude (kg/h)	0.7	0.7	36.0	25.2
De-oiled biomass (kg/h)	0.3	0.3	13.1	3.94

function of the oil content in the microalgae.

In *Case 1* the electric power is still supplied by the network, while the heat is produced by the combustion of both the biomass and part of the oil, the flow rates calculations of which are calculated by process simulation. Table 6.3 summarizes these

Table 6.2: EROEI with change of oil fraction in "'Base Case"'.

Oil fraction in microalgae	0.3	0.5	0.7
Input nutrients (MJ/h)	14.54	14.62	14.73
Input energy (MJ/h)	0.76	0.76	0.76
Total input (MJ/h)	15.30	15.38	15.49
Total output (MJ/h)	20.0	24.57	29.14
EROEI	**1.31**	**1.60**	**1.88**

results, from which a remarkable improvement in EROEI values is found, especially at higher oil contents.

Table 6.3: EROEI with change of oil fraction in "'Case 1"'.

Oil fraction in microalgae	0.3	0.5	0.7
Input nutrients (MJ/h)	3.35	3.35	3.35
Input energy (MJ/h)	0.76	0.76	0.76
Total input (MJ/h)	4.11	4.11	4.11
Total output (MJ/h)	8.73	13.26	17.81
EROEI	**2.13**	**3.23**	**4.33**

In *Case 2* also the electric energy is produced autonomously through a generator fed with part of the biocrude (its energy conversion efficiency is assumed equal to 33%, as a reference). The results obtained, shown in Table 6.4, do not differ much from those of *Case 1*.

Table 6.4: EROEI with change of oil fraction in "'Case 2"'.

Oil fraction in microalgae	0.3	0.5	0.7
Input nutrients (MJ/h)	3.35	3.35	3.35
Input energy (MJ/h)	0.0	0.0	0.0
Total input (MJ/h)	3.35	3.35	3.35
Total output (MJ/h)	6.46	10.98	15.54
EROEI	**1.93**	**3.28**	**4.64**

Our EROEI results can be compared to those reported in a recent publication, which range from 0.13 to 3.33 (National Academy of Sciences, 2012). It can be concluded that the processes considered are always advantageous from the energy profitability point of view. The best result (4.64) is achieved with high oil fractions and *Case*

2, meaning that the process produces more than four times the energy that is used to obtain the microalgae, a value that is comparable to those of other sources of energy, also non-renewable ones (Beal et al., 2010a). From the analysis of the results obtained, it can be easily noticed that the second highest energy requirement of the entire process comes from nutrients, in particular nitrogen, that can be supplied in both nitrates and ammonium forms, (Lundquist et al., 2010). At a lesser extent, this holds also for phosphorus and iron. Considering that microalgae have the capability to grow in wastewaters, as they consume nutrients present in this effluent (Martínez et al., 2000; Zhou et al., 2012), we can take advantage of this ability to improve the EROEI. From our laboratory experiments (see chapter 2 and 3), it was found that microalgae production can be achieved in urban wastewaters without any sterilization pretreatment, and that the growth rate is not substantially decreased, with respect to specific growth media, by the lower nutrient concentration of these waters.

In the case the nitrogen source is a wastewater rich in these components, the energy equivalent required to support the growth can be further minimized to a very low value, because the material energy input related to nitrogen is zeroed. Under this hypothesis the EROEI calculation was made for the same 9 cases considered above, and the resulting figures, named *Optimal cases*, are summarized in Table 6.5.

Table 6.5: EROEI results in the improved cases.

Case	Oil percent	EROEI
	30	1.64
Objective Base Case	50	2
	70	2.35
	30	8.4
Objective Case 1	50	12.73
	70	17.07
	30	22.81
Objective Case 2	50	38.8
	70	54.88

It can be seen that the EROEI values increase significantly, giving an exceptionally high energy profitability to the microalgae biocrude process.

We point out that all the calculations presented are based on energy and material balances and on sound values of operating conditions of process units, but do not take into account any economical consideration, in particular equipment capital costs. In addition, the EROEI values of Table 6.5. should be considered as upper limits, because current microalgae technology is still unable to use wastewaters in large scale cultivation systems.

6.7   Final remarks

In this chapter a process to produce biocrude from microalgae has been investigated to check and optimize its energy profitability, with respect to Energy Return on Energy Investment analysis. The materials and energy requirements of the process flowsheets considered were calculated by accurate process simulation, based on both laboratory experiments and literature data. By a Pinch Technology Analysis it was shown that the process can be made self-sufficient with respect to its energy duties, if part of the biocrude and residual biomass after oil extraction are converted to energy. Combustion was shown to be the best recovery system from the energy efficiency standpoint. Values of EROEI were calculated with reference to three cases at increasing oil content in the microalgae, both with and without the need of extended supply of nutrients. With respect to this, it was suggested to utilize wastewaters to improve the energy performance of the biocrude production process. It was shown that, in such a case, EROEI values can reach very high levels, ensuring the energy profitability of the production of biocrude from microalgae.

Autotrophic production of biodiesel from microalgae: an updated process and economic analysis

A technical evaluation of a plant for biodiesel production from microalgae was investigated in which a novel configuration of a CPR (closed pond photobioreactor) is proposed. The entire process was simulated by Aspen Plus^{TM} and optimized energetically in order to obtain the best profits in energy terms. The design and sizing of the process equipment was performed to obtain a realistic estimation of costs, considering both CAPEX (capital costs) and OPEX (operating costs). The economic analysis evaluated the profitability of the complete process at industrial scale referred to a CPR of 1 km² of surface area as base for calculation. A sale price of oil of \$18.35/gal was calculated, corresponding to \$21.11/gal of biodiesel in order to ensure an acceptable economic profitability. These results were compared to those of other recent studies, confirming that at the current state of technology the production of biodiesel from microalgae is not competitive with respect to that of conventional diesel, and a breakthrough in technology is needed to bridge this gap maybe by means of other valuable byproducts from the same process. A future outlook is eventually reported.

7.1   Introduction

Biofuels from microalgae are considered more and more as one of the best and accessible alternatives for new clean energy (Amaro et al., 2012), thanks to their high productivity compared with other oil–based crops, non–arable land use, wastewater treatment coupling for nutrients supply, among other well–known advantages (Mata et al., 2010; Chisti, 2013).

The biocrude production pathway at industrial scale is relatively consolidated without relevant new ideas in the last years. Among other issues concerning large-scale configurations, one of the most relevant is the PBR (photobioreactor) geometry, as between tubular PBR's and OP's (open ponds), it is not clear which growing system is superior, because both of them possess advantages: high productivity and better control of culture conditions with PBR's, less construction and maintenance costs with OPs (Rawat et al., 2013; Resurreccion et al., 2012). In recent years, the economic benefits of microalgae cultivation processes has not always been clarified, especially when scaling them up to large scale production. This is the main problem hampering the development of biofuels from microalgae, i.e. the huge costs expected to carry out an industrial-scale process (Sun et al., 2011; Campbell et al., 2011). Economic evaluations show large variations among themselves (Gallagher, 2011; Ribeiro and Silva, 2013). Each difference in many steps of the pathway has repercussion in the costs, starting from the scaling of equipment, the microalgae species used, the cultivation system, the harvesting and concentration of the product, the geographical location of the plant, the water consumption (Batan et al., 2013). In addition, the influence of climatic conditions is a key variable that should be taken into account (Yadala and Cremaschi, 2014), as solar radiation is the limiting factor for growth, changing therefore the productivity which directly impacts the costs (Nagarajan et al., 2013). Also the cultivation temperature must be accurately controlled, a point which has not been addressed from the economic viewpoint. On the other hand, to evaluate economically a process whose aim is to produce energy, the first step must be ensuring its energetic profitability, i.e. selecting the pathway with the best energy yields.

Recently an important contribution in this topic was given by Davis et al. (2011), who analyzed the economics of two different pathways for autotrophic biodiesel production:

an OP and a tubular PBR. Their analysis was based on a production of 10 million gallons per year of biocrude to be converted into biodiesel by hydro-treating process. They calculated biodiesel selling prices of $ 10,73/gal for OP and $ 22,38/gal for PBR (US$ 2013) to obtain an economical profitability to achieve a 10% of rate of return of the process investment. Later the same authors proposed an updated process (ANL et al., 2012) using a plastic liner in OP's to avoid soil permeability, which increased by $ 2.51/gal the biodiesel selling price. A positive study by Campbell et al. (2011) supported the hypothesis that in short time microalgae technology will be economically profitable, reporting values of production costs for OP's (without considering plastic liner) very similar to those for canola oil. Another positive case is that by Nagarajan et al. (2013), who assumed an OP as well, a microalga with 50% oil content, and combined oil extraction and transesterification into a single step. Nagarajan's results showed that the price of biodiesel from microalgae is in the range of $ 1.60/gal and $ 3.72/gal (US$ 2013), subject to the availability of CO_2 and the microalgae productivity. The objective of this chapter is to perform a detailed techno–economic analysis for a large–scale process energetically self-sufficient, aimed at autotrophical biocrude production from microalgae using a novel configuration of the cultivation system. We propose a hybrid combination of OP and PBR where a shallow pond is covered with a transparent plastic to achieve higher productivity and more stable operation. We refer to this as a Closed Pond photobioReactor (CPR). For this system the dimensions and costs of all the equipments and utilities required are accurately evaluated, and actual prices of biocrude and biodiesel from microalgae are determined. This analysis allows to identify more precisely what would be the sections of the process to focus on in future research and development activities, to improve the economy of the process itself until it becomes profitable.

7.2 Process design and assumptions for the analysis

In view of our analysis the entire process has been divided into two main sections. The first one (see Figure7.1) is about the cultivation and production of biomass, taking account nutrients supply, CO_2 feed and sun light absorption. The second section (Figure 7.2) is related to the units of the system to obtain biocrude, and to

exploit energy from the residual biomass. Here, heat integration technology has been applied as studied in chapter 6, where it was proposed to use the energy recovered from biomass and from part of biocrude to meet the process energy requirements, in order to optimize its Energy Return on Energy Investment (EROEI). A simultaneous analysis of both the plant sections have been performed by process simulator Aspen Plus^{TM}, version 7.1. The size of the plant has been determined, with respect to an irradiance typical of Northern Italy, where the total solar irradiation corresponds to about 4,500 MJ m^2 y^{-1}. According to our lab experiments (Bertucco et al., 2014), a value around 7% has been assumed for the overall photosynthetic efficiency, and all calculations are referred to a surface area for the CPR of 1 km^2, which results in a biomass productivity of 39.2 ton d^{-1} of dry weight (DW), when it is operated in continuous. *Scenedesmus obliquus* has been used as the species of reference because, from an industrial point of view, it has higher productivity at lower residence time than other species, higher energy conversion efficiency and acceptable lipid content (about 40%) even in the absence of any stressing conditions (Sforza et al., 2013). Table 7.1 summarizes the operating conditions of reference for all our calculations.

7.2.1 Microalgae cultivation and harvesting

In our analysis wastewater is assumed as an input to the process because it contains nitrogen and phosphorous which microalgae can use as nutrients for their growth (Woertz et al., 2009). The unit operations included in this section are summarized in the block flow diagram of Figure 7.1 and are described as follows:

- reaction system: a CPR is proposed because its characteristics has advantages of both classic closed tubular PBR's (higher densities and quality of the culture) and OP's (easier operation and lower costs). It is a raceway pond with a LDPE (low–density polyethylene) cover transparent to radiation, able to limit the environmental contamination in the photobioreactor, to favor the solubilization of the gaseous CO_2 in the liquid phase and to maintain the productivity all year long;

- sedimentation: it is placed downstream of the reaction system, to concentrate

Table 7.1: Production process conditions.

Unit	Value
Biomass production (ton d^{-1})	39.2
CPR surface (ha)	100
Lipid content (%)	40
Average concentration	0.45 g/L
CO_2 demand (ton d^{-1})	86.24
Photosynthetic efficiency	7%
Biomass yield (ton ha^{-1}d^{-1})	0.392
Reactor volume (L)	150,000,000
Residence time (d)	1.56
Oil produced (L d^{-1})	13,290
Net energy produced (MJ ha^{-1}d^{-1})	4115
Mixing	paddle
Nutrients	wastewater
Thickening	sedimentation
Dewatering 1	centrifuge
Dewatering 2	dryer

the biomass produced to be suitable for the centrifugation step. Part of the concentrated stream is recycled to the reactor in order to maintain the biomass concentration at the desired level. Also the clarified water is recirculated to the reaction system to reduce water consumption;

- centrifuge: it is used to concentrate the biomass up to 20% w/w;

- CO_2 feed system to the reactor: the CO_2 is provided by exploiting flue gases from industrial plants. These are assumed to have a volume fraction of 10% of CO_2 (Sforza et al., 2013), the rest being assumed as nitrogen for calculation purposes; Two possible alternatives for feeding the flue gas in the reactor were evaluated. The first one involves the saturation of recirculated water (together with the make–up) with CO_2, in a special equipment to avoid gas handling and distribution costs in the reactor. The second alternative involves the bubbling of flue gas directly into the reactor. Even if both alternatives seem to be technically feasible, the first one has the disadvantage of requiring large amounts of water
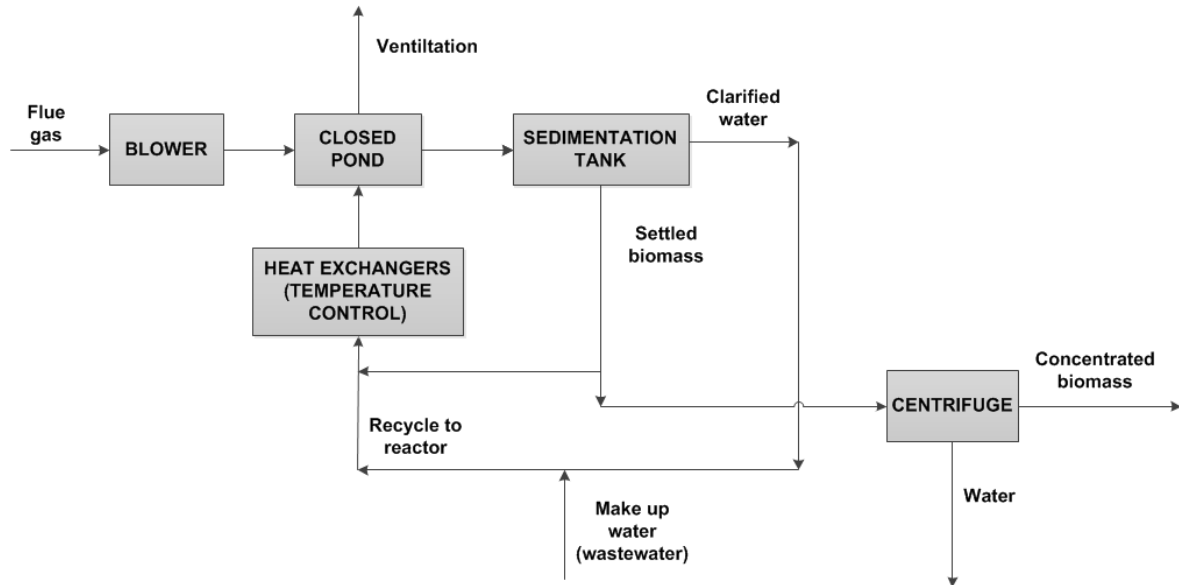
Figure 7.1: System flow diagram for cultivation and harvesting of microalgae.

to solubilize the CO_2, which increases the process energy duties and modifies the residence time affecting the growth kinetics. For this reason, and for the higher cost of this technology compared with the second alternative, it was decided to implement the second one only.

7.2.2 Oil extraction and de-oiled biomass exploitation

In this section the attention is focused on the system for oil extraction and for the exploitation of the residual biomass. This study examines in detail a particular configuration where it is combusted the biomass both before (partially) and after oil extraction (totally), to render the process energetically self–sufficient. As depicted in Figure 7.2, the following operation units are included: the drying of the concentrated suspension after centrifugation; the solvent extraction of oil from microalgae; the oil and solvent (hexane) separation, with solvent recovery achieved by a preliminary evaporation followed by stripping with steam.

7.2.3 Aspen process flow diagram

In Figure 7.6 (ahead) it is reported the flowsheet process diagram from the simulations in Aspen Plus, comprehending both sections, cultivation and oil separation, as well as the heat integration blocks and streams.

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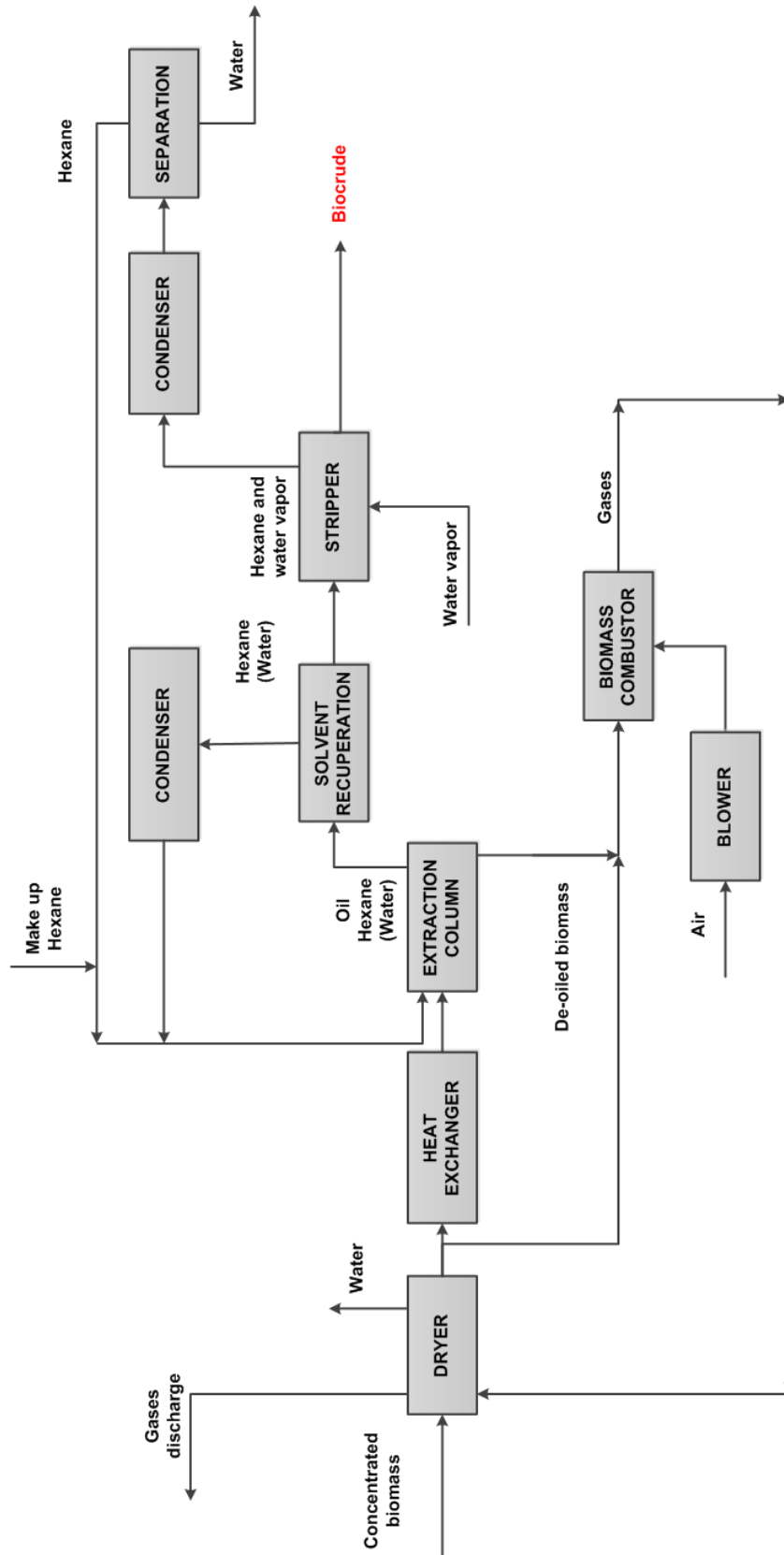
Figure 7.2: System flow diagram for biocrude production and de-oiled biomass exploitation

7.3   Sizing and cost calculation of equipment

In the following all the units needed to the process flow diagram are briefly described and their capital and operation costs are evaluated (more information in Appendix B) All the numbers reported take into account inflation until the end of 2013.

7.3.1   Closed pond reactor

The 1 km^2 CPR has depth of 15 cm, and is designed as a 20 channels circuit with 50 m of width. The costs considered are the ones for site preparation, installation of a plastic liner on the bottom of the pond to ensure the waterproofing, construction of levees and reinforcements on the perimeter, installation of paddles for mixing, and coverage of the reactor with transparent plastic material. The configuration of a CPR structured according to an agricultural engineering system is a relatively recent approach (Sapphire Energy Inc.) and currently under study, for which the related cost estimates are not available yet. Basically an open pond is covered by installing a reinforced LDPE film, suitable for greenhouse covering. However, in this case the temperature of the circulating culture must be accurately controlled. It is also assumed that suitable space above the surface of medium for gases circulation is 20 cm. A structure of this type has an installed cost estimated at M\$ 8.36 TNAU Agritech Portal (2013), including the construction, land preparation and mixing system. The items related to the operation, like the electricity required for activation of mixing paddles has been calculated as \$ 70,000 year^{-1}. In particular this type of reactor requires a system for temperature control, which is discussed below.

7.3.2   Temperature control

The CPR is exposed to solar radiation and atmospheric events, so it is important to evaluate the heating and cooling conditions of aqueous suspensions of microalgae. Whereas in open pond technology the temperature is controlled naturally by water evaporation involving a large water loss, in the system currently proposed the temperature in the reactor is maintained at 25°C by heat exchangers for cooling or heating the circulating culture.

In order to assess the thermoregulation costs, the energy balance has been calculated for each season of the year. It was assumed that 10% of the total solar energy provided to

the system is reflected by the LDPE cover, while the infrared and part of the ultraviolet and visible wavelengths are absorbed by the medium and dissipated as thermal energy (Sforza et al., 2013; Wu et al., 2009). We also assumed as daily average irradiance that of April for spring, July for summer, October for autumn and January for winter. For the area of Padua (Italy) the total solar irradiations are 232 Wm^{-2}, 285 Wm-2, 155 Wm^{-2} and 93 Wm^{-2}, respectively (PVGIS, Solar Irradiation Data, 2013).

If 25°C is the optimal temperature of the medium in the closed pond in all the seasons of the year, taking into account these irradiances, the changes of temperature of the medium can be calculated together with the utility requirements to cool or heat the system in each season.

It results that in spring and summer it is required to cool the system by 1.8°C and 12°C respectively, using chilled water at 5°C. With a unit cost of chilled water of $ 0.313 m^{-3}, a total cost of $ 486,800 for spring season and of M$ 2.525 for summer are calculated. On the contrary for heating the medium in autumn and winter, water at 45°C is used, whose unit cost is $ 0.025 m^{-3}, resulting in heating costs associated with the fall season equal to $ 21,600, and to $ 281,000 for the winter season. In summary, the annual operation cost for temperature control is $ 3,314,400 $year^{-1}$. The installation cost is evaluated on the base of the area required to carry out the heat transfer of 55.8 MW previously calculated, which yields an exchange area of 2,480 m^2, i.e. six shell and tube exchangers in parallel, with an overall cost of $ 2,549,000.

7.3.3 Flue gas supply

For the direct insufflation of flue gas in the CPR it is proposed to use a perforated pipe located on one side of the reactor, perpendicularly to the water flow. The CO_2 needed to be transferred to the medium to ensure the target production of biomass (1,633.3 kg h^{-1}) is calculated as 4870 kg h^{-1} assuming an efficiency of capture of 66.7%, which is reasonable as the continuous closed pond system allows to keep CO_2 in contact with the microalgal suspension. This results in a total flue gas flow rate of 48,700 kg h^{-1} which has to be compressed and cooled: compression can be given by turbocharges while a heat exchanger is required for cooling. In view to our economic analysis, the flue gases are considered as an "unlimited" source of CO_2. In order to calculate the capital cost of turbochargers, a pressure loss of 5 m of water has been estimated, to

provide the flue gases a pressure sufficient to ensure bubbling along all the perforated pipe, obtaining an effective isentropic compression power required of 355 kW (Perry et al., 2008). Installed cost is calculated according to Peters and Timmerhaus equation (Peters et al., 2003), resulting in a value of \$ 974,000. Based on the power required assuming an operation cost of \$ 23.67 h^{-1} is eventually obtained. An electricity cost of \$ 0.060 kWh^{-1} has been assumed (U. S. Environmental Protection Agency Combined Heat and Power, 2007), which is consistent with U.S. values: in fact, although solar irradiation intensity was that typical of Northern Italy, in order to use our experimental measurements in the CPR simulation, large scale microalgae production plants are likely to be constructed where lower prices have to be paid for both land and energy. Indeed, most major pilot plants operated in the world are located in the Southern part of the U.S.

To evaluate installation and operation costs for cooling the flue gases from 120°C to 25°C, the heat flux calculated by the enthalpy balance is 870 kW. Assuming a heat exchange coefficient of 200 W m^{-2} K^{-1} an exchange area of 85 m^2 is obtained, and 74.82 m^3 h^{-1} of chilled water are required for cooling. From the value of the exchange area, the cost of the heat exchanger was calculated by the Guthrie correlation (Douglas, 1988), resulting in \$ 153,800. The operation cost is derived from the unit cost of chilled water obtaining a value of \$ 23.41 h^{-1}.

7.3.4 Biomass harvesting and concentration

A circular clarification pond for harvesting purposes was designed according to those used in wastewater treatment. It is proposed to use AlCl$_3$ as flocking agent. Based on simulation results, a feed flow to the sedimentation tank of 4000 m^3 h^{-1} has been calculated, which requires an area of 2665 m^2 and a depth of 6 m to be clarified. After it a centrifuge–type Decanter HTS$^{\circledR}$ from Flottweg (2013b) it is proposed to achieve a microalgae biomass concentration of 20% DW. This system is capable to process an input flow of 250 m^3 h^{-1}, a value close to the half of the sedimentation tank output (496 m^3 h^{-1}), needing therefore two centrifugal decanters in parallel. The settler equipment costs have been calculated using the equations reported in (Sharma, 2010), resulting in an IC of \$ 2,264,134. The OC is related only to the flocking agent used (concentration of 10 ppm), resulting in \$ 211,817 year^{-1}.

The IC of each centrifuge is \$ 645,000 Flottweg (2013b). The OC is calculated considering the electric energy required for the correct operation (295 kW and the flocking agent (same as above), resulting in total OC of \$ 500,214 year^{-1}.

7.3.5 Dryer

The system for drying the biomass outlet from the centrifuge is the unit with the largest energy consumption. To minimize the costs associated with such an equipment a heat integration scheme is proposed, where the removal of moisture from the microalgae stream is obtained by direct contact with the fumes of combustion, so that the process can be made energetically self-sufficient (see Figure 7.2). As the energy provided by these fumes is not enough, also some steam is needed to complete the drying. It is produced using waste heat from another process unit. A rotary drum dryer is proposed as a dryer, whose installed cost is retrieved from literature (Li et al., 2012a). For biomass dryers a value of \$ 38,000 ton^{-1} h^{-1} of evaporated water is reported. In our process, 6.37 ton h^{-1} of water have to be removed, corresponding to a total capital cost of \$ 255,000. For operating cost calculation, it is assumed that the electric energy consumption of the rotating drum is 10 kWh per ton of evaporated water (Li et al., 2012a), so that 63.7 kW of electricity are required, corresponding to \$ 31,140 year^{-1}. The steam duty request is 0.032 kg s^{-1}, so that a cost of \$ 14,110 year^{-1} is calculated with a unit cost of low–pressure steam is \$ 0.015 kg^{-1}.

7.3.6 Solid–liquid extractor

To recover the oil from the microalgal biomass a solvent extraction unit is designed, using hexane as the solvent. For this process a continuous Rotocel extractor was selected because of its simplicity and low cost (Perry et al., 2008). This equipment is a width drum divided into two horizontal sections. The superior part is composed by compartments with permeable floors that spin around the central axis, these compartments pass by the feeding point and by the solvent application section. A second section is needed to drain the solvent, and to discharge the solids. This extractor can be operated in continuous and countercurrent mode. The cost evaluation of the equipment and its installation requires to know the number of sections and residence time: this was determined based on the relationship for a similar system (Zaher et al.,

2004) and on the temperature of operation, obtaining 5 sections with total residence time of 1 h (Bieber et al., 2008).

The capital cost for such equipment amounts to $ 150,000 (Ulrich and Vasudevan, 2004). As operating cost both electricity and hexane losses are accounted for, the first one being equal to 23 kWh per ton of oil (Bieber et al., 2008), i.e. a power consumption of 15 kW which costs $ 7,332 year^{-1}. Regarding the cost of the hexane make-up, since the recovery of the solvent in the process is almost total, only $ 900 year^{-1} have to be computed (Icispricing.com, 2013).

7.3.7 Stripper

In order to obtain the oil and to recover hexane a stripping column is used, which has been simulated and sized accordingly to a sieve tray configuration. Under the hypothesis of operating this column at 80% of flooding velocity, 8 trays are needed for a total height of 3.3 and a diameter of 0.20 m. The corresponding installation cost is $ 7,589 by Guthrie equation (Douglas, 1988), while for operation cost a continuous flow rate of 50 kg h^{-1} of low-pressure steam is required, resulting in a cost of $ 6,110 year^{-1}.

7.3.8 Combustor of biomass

For burning biomass and de-oiled biomass, a Stoker boiler combustor has been selected which employs direct fire combustion with excess of air, producing hot flue gases that can be directly used for heating purposes. Related installed costs are obtained by the data reported in (U. S. Environmental Protection Agency Combined Heat and Power, 2007), resulting in $ 2,239,000. To provide the air for combustion is used a turbocharger, whose installed cost is $ 570,300, obtained using a literature correlation (Peters et al., 2003) with an air flow of 3.07 m^3 s^{-1} and a power of 193.4 kW. Operating costs amounted to $ 105,300 year^{-1} of electric energy.

7.3.9 Heat exchanger network

As already mentioned, (see Figure 7.6) the de–oiled biomass and some fresh biomass are used to fulfill the energy requirements of the process. To this aim, a network composed by six heat exchangers is utilized, whose design and cost have been evaluated

individually. A plate type heat exchanger is used to cool the solid biomass exiting the dryer, using water at 25°C as utility. The rest of exchangers are required to cool various process streams and are assumed of shell and tube type, with the exception of the exchanger to heat the inlet flow to the stripper, which is a Kettle type.

On the basis of the simulation results, each piece of equipment has been designed and the IC's have been calculated, leading to \$ 118,750 of IC for the total network. The OC's have been evaluated calculating the flows of utilities required, assuming as cooling medium water available at 25°C, 35°C and chilled water at 5°C, and vapor at 165°C and 7 bar as heating medium. Total OC's for the entire network of \$ 59,300 year^{-1} are obtained.

7.4 Results and Discussion

7.4.1 Installation and operation costs.

In order to develop the economic analysis both IC (installation costs) and OC (operation costs) related to all equipment and utilities of the process under study were calculated, as reported in the previous section. In Table 7.2 they are summarized. Overall values are: 18.93 M\$ for IC and 4.8 M\$ year^{-1} for OC. In Figures (7.3A) and (7.3B) the relative weights of the IC and OC are also shown using a pie chart.

For the evaluation of annual operating costs, a stream factor of 0.93 is considered, (typical value for continuous process in chemical industry). We point out that the operating costs associated with the feeding to the system of nutrients other than CO_2 is assumed equal to zero, as wastewaters are used as a source of nutrients, with a concentration sufficient for the growth of microalgae (Lundquist et al., 2010). In order to identify and compare the cost items which are more relevant for the process, both within IC and OC, the IC's were annualized using the CCF (Capital Charge Factor) of 1/3 year^{-1} (Douglas, 1988), making them comparable to OC in \$ year^{-1} units. The pie chart of Figure 7.4 shows major contributions to the total IC plus OC costs. It is evident the significant portion due to the reactor thermo-regulation system (38% of the total). Another relevant fraction of the total annualized cost (20%) is due to the installation of the waterproofing liner and the covering of the reactor, owing to the considerable extension of the CPR. Other costs to consider are those of the system

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Table 7.2: Installation costs (IC) and operation costs (OC) of the process equipment at industrial scale.

Unit	IC [$]	OC [$ year^{-1}]	Reference
Blower of flue gas	974,000	192,835	[Peters et al. (2003)]
Heat exchanger flue gas	153,794	190,717	[Douglas (1988)]
Sedimentation tank	2,264,134	211,817	[Sharma (2010)]; Aspen simulation
Centrifuge	1,290,000	500,214	[Flottweg (2013a)]; Aspen simulation
Recirculation pump	NR	82,609	Aspen simulation
Centrifuge feeding pump	NR	9,776	Aspen simulation
CPR			
Land preparation	366,200		[Benemann and Oswald (1996)]
Land,reinforcements	512,700		[Benemann and Oswald (1996)]
Mixing	732,500	70,000	[Benemann and Oswald (1996)]
Liner	3,500,000		[Lundquist et al. (2010)]
Cover	3,250,000		[TNAU Agritech Portal (2013)]
Temperature control	2,549,000	3,314,377	[Douglas (1988)]
Dryer	255,000	45,251	[Li et al. (2012a)]; Aspen simulation
Extractor	150,000	7,332	[Ulrich and Vasudevan (2004); Bieber et al. (2008)]; Aspen simulation
Make up solvent		896	[Icispricing.com (2013)]; Aspen simulation
Stripper	7,589	6,110	[Douglas (1988)]; Aspen simulation
Biomass combustor	2,239,000		[U. S. Environmental Protection Agency Combined Heat and Power (2007)]; Aspen simulation
Blower for biomass comb.	570,300	105,300	[Peters et al. (2003)]; Aspen simulation
Heat exchanger Network	118,150	59,300	[Douglas (1988)]; [Ulrich and Vasudevan (2004)]; Aspen simulation
Total	18,932,367	4,796,534	

to reduce the water content in the biomass (15%). On the other hand, the biomass combustion has an influence which is quite small (7%).
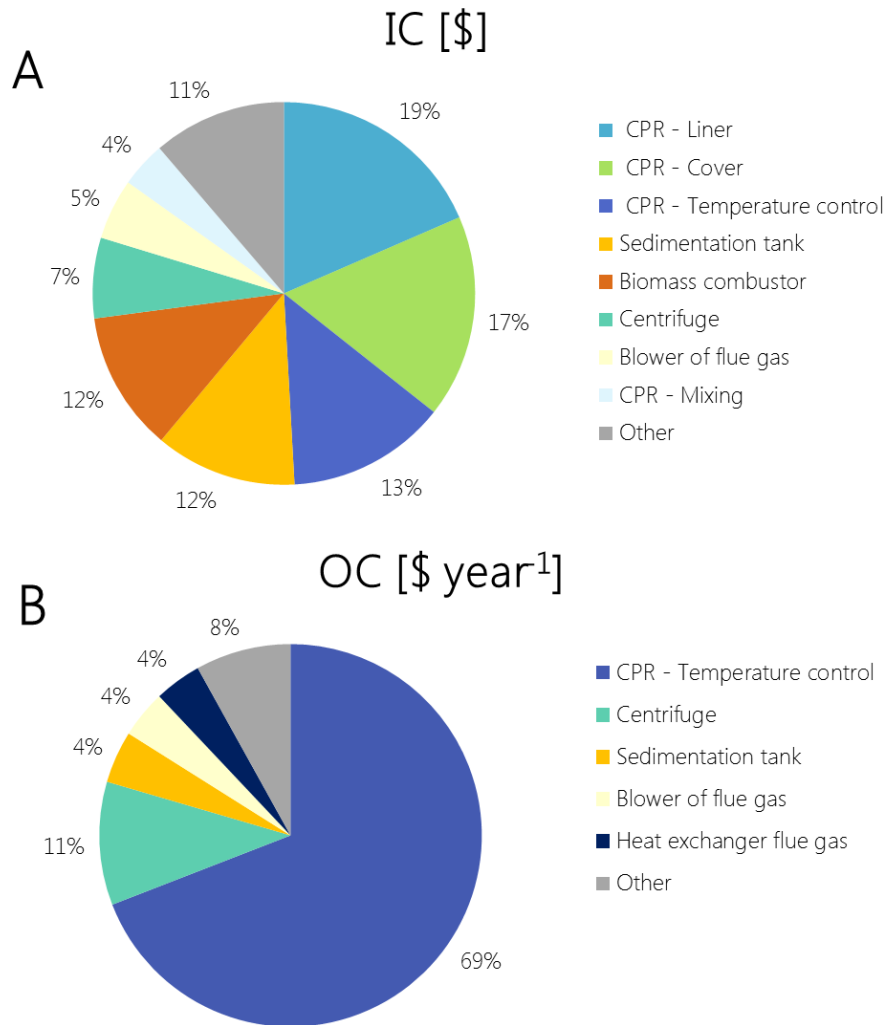
Figure 7.3: Distribution of A) installation costs, and B) operating costs.

7.4.2 Cost benefit analysis

For the process profitability analysis it is required to define items such as the Total Capital Investment (TCI) and the Total Product Cost (TPC), on which it can be evaluated. To this purpose, the method of Douglas (1988) was adopted. The level of accuracy appropriate to the project under investigation is defined between +30% and -20%. The calculation of the economic profitability is based on the cash flow profiles according to three indexes: the discounted payback time, the Net Present Value (NPV) and the Internal Rate of Return (IRR). TCI are calculated from the value of total IC
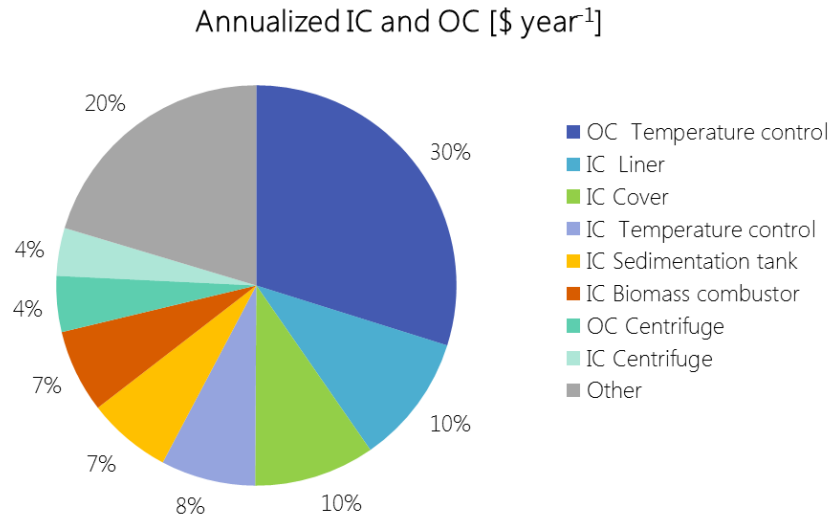
Figure 7.4: Comparison of principal annualized costs of the process.

(M\$ 18.93) by:

$$TCI = 2.36 \cdot IC \tag{7.1}$$

generating a TCI of M\$ 44.67. From this value, it is possible to obtain a FCI (Fixed Capital Investment) by the relation:

$$FCI = \frac{TCI}{1.30} \tag{7.2}$$

It results FCI = 34.36 M\$. FCI_L is calculated by subtracting the land cost in PCI. In our case it is found FCI_L = 33.63 M\$ according to a unit land cost of \$3,000 acre^{-1} (Davis et al., 2011), resulting in a total land cost of \$ 740,000. The value of FCI_L is used for cash flow calculation.

The estimate of the TPC_{wd} (Total Product Cost excluding depreciation) is given by Douglas (1988):

$$TPC_{wd} = (1.30 \cdot OC) + (0.18 \cdot IC) + \left(2.13 \times 10^{-5} \cdot No.workers\right) + (0.025 \cdot Revenues) \tag{7.3}$$

Accordingly, it is necessary to evaluate the number of workers: based on the 25 stages of the process, 17 workers are required. The annual revenues from sales are the

unknown factor of the study, which depends on the unit selling price ensuring a return or profit. The corresponding value, which allows to deduct the annual revenues, is determined iteratively in the subsequent analysis of profitability ratios and cash flows, which have been calculated under the following hypothesis:

- useful life of the plant is 10 years, preceded by 2 years of construction;

- land is purchased at the end of year 0;

- 60% of FCI_L is invested in the first year, the remaining 40 % in year 2;

- at the end of year 2 , the Working Capital (WC) and Start-up Costs (StC) are invested, to start the operations of the plant, WC is assumed as 15% of TCI, and StC equal to 10% of FCI (Perry et al., 2008);

- an income tax rate of 45%, is taken based on of Italian statistics (Istituto nazionale di Statistica, 2013);

- an optimistic value (for an emerging technology) of Minimum Attractive Rate of Return (MARR) of 10% is assumed;

- depreciation in the first 7 years of life is evaluated by method of double declining balance (DDB);

- salvage value of the plant is 10% of FCI_L (Douglas, 1988);

- the discounted cash flows is carried out taking as "year zero" the year of construction of the plant.

In Table 7.3 the values calculated for these economic items are summarized. Note that, for the construction of cash flows, no revenues are considered from the treatment of wastewater performed by microalgae on behalf of the companies who supply these wastewaters. Based on all the data reported above, it is possible to determine the annual cash flows, discounting them and deriving the cumulative profile. As this procedure requires the definition of sales revenues, it is convenient to determine the conditions corresponding to the minimum unit price at which the oil produced can be sold to derive profits dictated by the MARR. This is achieved in the limiting case where

revenues from sales yield zero NPV and when the IRR is equal to the MARR (10%). Such a conditions was found to occur when the annual revenue from oil sales is M$ 21.96 year^{-1}, corresponding to an oil selling price of $ 18.35 gallon^{-1}. The cumulative discounted cash flows diagram related to the case is shown in Figure 7.5, according to which we calculated 7.6 years (rounded to 8) of payback time, defined as the time required after the start up to recover the FCI$_L$ and StC.

Table 7.3: Values calculated for these economic items are summarized

Unit	Value
TCI	44.68 M$
IC	18.93 M$
Land cost	0.74 M$
FCI	34.37 M$
FCI$_L$	33.63 M$
WC	6.70 M$
StC	3.44 M$
OC	4.80 M$ year^{-1}
Revenues	21.96 M$ year^{-1}
FCI$_L$ year 1	20.18 M$
FCI$_L$ year 2	13.45 M$

7.4.3   Comparison of results

The minimum selling price of biocrude calculated in the previous section ($18.35 gallon^{-1}) can be converted into biodiesel selling price increasing it by 15% Davis et al. (2011), a rule–of–thumb value which includes all processing costs from biocrude to biodiesel. In this way a break–even value of the biodiesel selling price equal to $ 21.11 gallon^{-1} is found. It is clear that, in the present state of technology the production of biodiesel from microalgae is not competitive with conventional diesel, that in 2013 had on average selling price of $ 8.32 gallon^{-1} in Italy (Ministero dello Sviluppo Economico, 2013) and $ 3.91 gallon^{-1} in USA (U.S. Department of Energy, 2013). Table 7.4 summarizes the prices of biocrude and biodiesel, and the specific energy costs.

It is interesting to compare our result with similar analyses published in the literature, referred to different cultivation systems. Davis et al. (2011) considered two
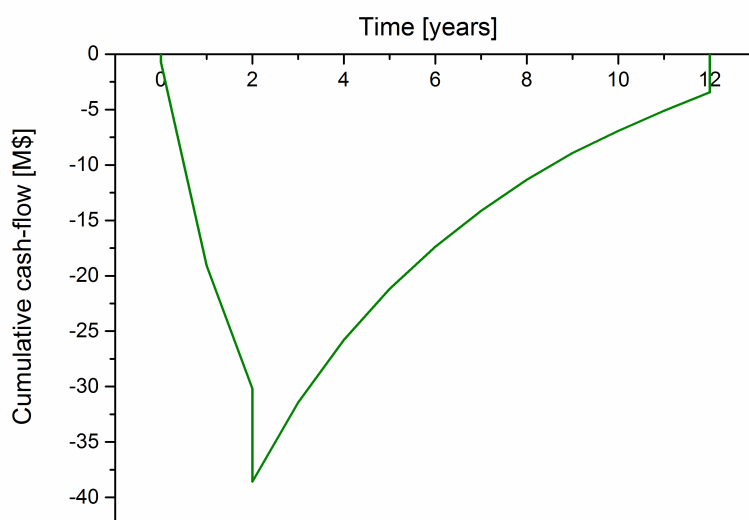
Figure 7.5: Cash flow profile as a function of time, case of Revenues = M\$ 21.96 year^{-1} and (TPC) = M\$ 12.52 year^{-1}.

possible reactor configurations, an OP and a PBR consisting of plastic tubes in parallel. The temperature control of these two systems is achieved by the self–evaporation of a part of microalgal broth (OP), and by a water sprinklers which laps the tubes externally (PBR). In addition, for the OP a layer of natural clay is proposed as a liner, which is considered adequate for this purpose.

Davis' calculation is based on a net production of oil of 10 Mgal year^{-1}, using a generic algal species able to accumulate 25% of lipids and to ensure a productivity of 25 g m^{-2}d^{-1} (our values are 40% and 39.2 g m^{-2} d^{-1}, respectively). Under such conditions an irradiated area of about 19.5 km^2 is necessary. In Davis' study the minimum unit sale prices for biodiesel in order to achieve the desired economic return are \$ 10.73 gallon^{-1} (OP) and \$ 22.38 gallon^{-1} (PBR) (these numbers have been transformed into 2013 values). When comparing Davis results with ours, no significant difference is found for the PBR system, even though the two configurations are quite different both in terms of block flow diagram and of prices of equipment/technological solutions proposed, as well as for some economic assumptions.

This analysis by Davis and co-authors has been updated in 2012 ANL et al. (2012) with the inclusion of the liner in the open pond reactor, which resulted in a substantial

Table 7.4: Prices and heating values of fossil fuels and microalgae fuels.

Unit	Heating value (MJ/kg)	Selling price ($/gallon) [a]	Specific energy cost ($/MJ) [a]
Fossil crude oil	39.6 [b]	2.33 [c]	0.016
Fossil diesel	43 - 45	3.91 [d]	0.027
Microalgal biocrude	36.0	18.35	0.151
Microalgal biodiesel	37.27 [e]	21.11	0.170

[a] 2013 USD; [b] U.S. Energy Information Administration (EIA) (2013a); [c] U.S. Energy Information Administration (EIA) (2013b); [d] Ministero dello Sviluppo Economico (2013); [e] Chisti (2013)

increase of unit sales price of biodiesel with this option from $ 10.73 to $ 13.24 gallon^{-1}. Also in the best case Davis' estimates remain lower than the value obtained in our study, but higher than traditional diesel.

In light of all of these results it is confirmed that with the currently available technology the production of biodiesel from microalgae is not economically feasible if compared to the fossil-derived diesel fuel, and the difference between the two options is quite high in all the cases examined. Other studies showed a huge variability in prices of microalgae biofuels. For instance, Williams and Laurens (2010) estimated a biocrude price of $0.79–3.08, using a combined process of PBR and raceway pond. On the other hand, the result of the economic analysis by Stephens et al. (2010) is that an IRR of 15% can be achieved when setting an oil price of 1.14 $/gal, for OP systems, besides Stephens proposes sell the residual biomass whose prices calculated correspond to $800 – 1200 ton. A recent publication by Richardson et al. (2014) reports the highest values for biocrude, i.e. $ 109.12 gallon^{-1} for OP and $ 77 gallon^{-1} for PBR, principally due to their strict assumption in productivity (6.8 and 9.3 g m^{-2} d^{-1} for OP and PBR, respectively), and the higher lipid productivities that can be achieved in PBRs with respect to OP. Moreover, previous costs reported by the same authors are $12.33 gallon^{-1} for OP and $31.61/gal for PBR Richardson and Johnson (2014). The analysis by Delrue et al. (2012) results in biodiesel production costs of €11.34 gallon^{-1} for OP and €18.94 gallon^{-1} for a 400 ha plant, based on a productivity and a PBR temperature control system as the ones proposed by Davis et al. (2011).

The selling price of biodiesel produced by the CPR process currently proposed fits

within the range delimited at the bottom by the OP and upwardly by a traditional PBR, with a configuration that in fact provides a sort of hybrid between the two reaction systems. Because of the major expenses arising from the cover of the reactor, of the liner and above all of the necessary temperature control, the selling price is close to the upper end of the range between OP and PBR.

Eventually, although the production of biodiesel from microalgae currently lacks in competitiveness compared to traditional fuels, it is useful to highlight the ways for improving such a situation. The use of different species of microalgae appears to be a key point. They could be selected by their physiological characteristics, i.e. thermally adapted for each season, and thus the costs of thermoregulation would be reduced. In addition microalgae with higher photosynthetic efficiency in order to enhance the absorption and conversion of energy should be selected by a thorough analysis. Above all, the research should focus on maximizing in the short–term the lipid content in algal biomass, while maintaining their high productivity, as suggested by the sensitivity analysis of Davis et al. (2011).

Furthermore our economic analysis pointed out that the parameters related to aspects purely connected to engineering show in the short term difficult to be enhanced. It is therefore challenging to think of a rapid technological improvement sufficiently strong to make the desired benefits. In any case it is of paramount importance to base any economical estimate on field data coming from pilot plants of suitable size, which are currently under construction. In fact, large scale production is an intrinsically dynamic process which is heavily affected by sun–light intensity fluctuations and overnight maintenance phenomena. So far all economic analyses have been based on average light energy absorption and real data may considerably change these results. Another option is to proceed with accurate research and developments leading to new optimized configurations which possibly generate some valuable co–products that can positively affect the process economics. However, such a suggestion appears to require longer times for practical applications.

We agree that, in the medium term, it is necessary to proceed with accurate research and development of optimized configurations starting from laboratory scale, through pilot plant and finally arriving at industrial scale, as the integration of these engineering

aspects with the purely biological-genetic ones is essential for the development of biocrude production technologies. Only such a multidisciplinary approach, which so far has proved to be the main cause of the slowdown of the evolution of the productive systems of biodiesel from microalgae, may allow a global view of the problem in order to overcome the limitations currently present in this field, making the industrialization of a biomass production system viable and attractive.

7.5   Final remarks

In this chapter the economic feasibility of a novel industrial-scale plant for the production of oil from microalgae was studied based on a conceptual process design carried out using the process simulator Aspen Plus^{TM}. The structure of the flowsheet contains two sections: one for microalgae cultivation, performed in a flat shallow pond covered with plastic sheets (CPR), and a second one to recover the oil and to exploit the energy of the de–oiled biomass. Alternatives for the supply of CO_2 to the reaction system were evaluated, concluding that the direct flue gas bubbling to the photobioreactor provides the most cost effective solution. The combustion of part of the biomass produced and the residual biomass after oil extraction made the process self-sufficient with respect to energy.

The economic profitability analysis led to the result that, to achieve a 10% rate of return of the investment (under other reasonable assumptions), the oil selling price should be equal to \$ 18.35 gallon^{-1}, corresponding to \$ 21.11 gallon^{-1} of biodiesel, a value well above to that of traditional fossil diesel fuel. The highest costs are associated with the temperature control system, the covering and the liner of the CPR.

These results were compared with a thorough study proposed by Davis et al. (2011); ANL et al. (2012), whose costs are similar for the PBR, much less for the OP, and to other literature analyses. Anyway, it is evident that the production of biodiesel from microalgae is currently lacking of competitiveness with respect to the sale of traditional fossil diesel fuel. The possibility of improving this condition requires to increase the oil content in biomass and the areal productivity. The economic analysis has in fact shown that, in the short term, the parameters related to purely engineering aspects are hardly improvable in order to get a significant cost reduction.

In addition, it can be proposed to proceed with accurate research and developments leading to new optimized configurations possibly generating some valuable co–products contained in the microalgae, which could positively affect the economics of the process. In summary, a clear need for further studies about this theme is demonstrated, integrating the purely biological aspects with engineering ones and making available field experimental data from pilot plants, in order to achieve a rapid development of the technology needed for microalgae production at industrial level.
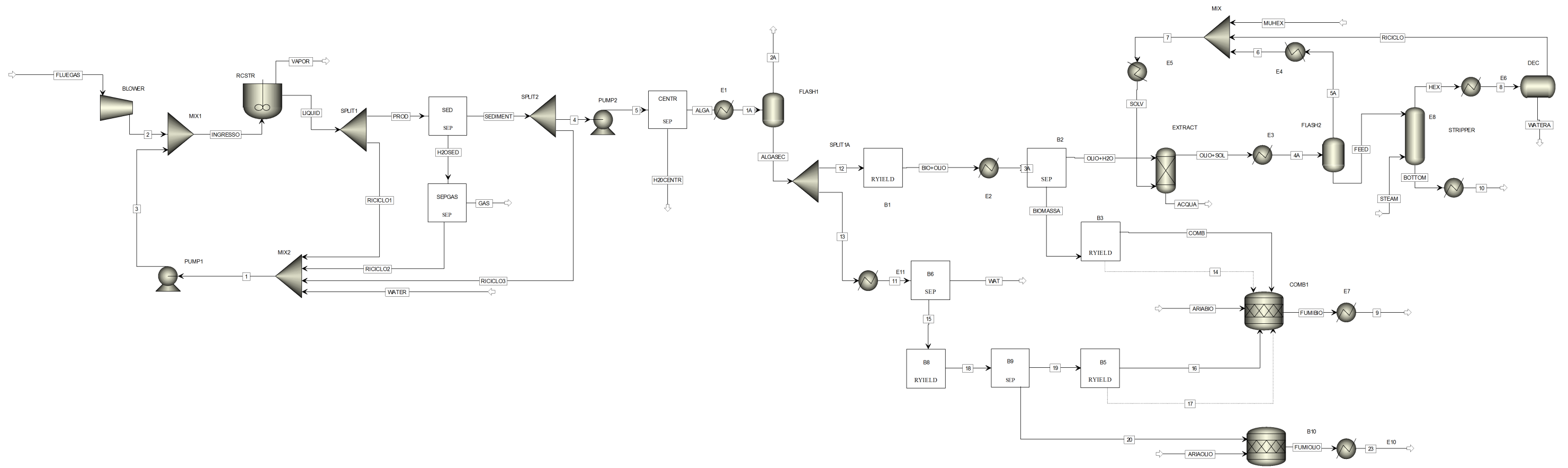
Figure 7.6: Biocrude production process Aspen Plus^{TM} flowsheet

Conclusions

Nowadays the potential of microalgae fuels is astonishing without any doubts for a number of reasons: fast growth and high photosynthetic efficiency, CO_2 uptake, among others. However, under discussion is what will be the perfect scenario for the real operation of this technology, i.e. when the conditions including technological development and especially environmental and political circumstances will become relevant enough to "force" mankind to move away from fossil fuel dependence.

This thesis has been focused on the study of large scale biocrude production from microalgae. Different perspectives have been addressed, to develop a process environmentally friendly, energetically sustainable and of course economically feasible. Experimental activities were carried out to couple microalgal biomass cultivation and wastewaters treatment. It was checked if using untreated wastewater as culture medium is suitable to achieve a good performance of the dual purpose process, where biomass production comes together with an efficient removal of N and P compounds, whose degradation kinetics were determined. The cultivation of *C. protothecoides* was achieved at steady–state in a continuous flow photobioreactor (PBR), fed with non–sterilized wastewater. It was shown that the outlet biomass concentration was increasing with residence time while the productivity showed a maximum at 0.8 d of residence time, due to light limitation phenomena on cell growth. The effect of day/night irradiation in the continuous system was verified. A difference on biomass and nutrient concentrations between the dark and the light period was found, probably due to an intracellular loss due to dark respiration. Also, no competition of microalgae and native wastewater bacteria was observed, thus the algal growth was not affected, apparently because under continuous cultivation the bacteria microflora was washed out, due to the low bacterial growth rate and the CO_2 presence. From a practical

point of view a depuration process based on microalgae was proposed with 2 steps, the first one after the primary treatment, to remove N and P, followed by the activated sludge reactor to reduce the organic matter content. This process is autotrophic, so it requires more irradiated superficies in winter than in the warm seasons.

This thesis also investigated two biomass conversion processes, anaerobic digestion and hydrothermal liquefaction (HTL). A significant production of biogas was demonstrated, starting from microalgae both before and after oil extraction. It was shown that the type of solvent used for lipid separation is the key to achieve a successful digestion: for instance, the mixtures chloroform:methanol and acetone:dichloromethane inhibit the production of methane for a quite long period, until the bacterial inoculum probably converts the inhibitory molecules to other components or in some way can be adapted to their presence. This technology needs to be further investigated before being proposed. On the other hand, when studying HTL, it was determined that the recycle of the process water into the HTL reactor enhances the profits and attain a solvent-free process, due to strong increase in oil yields upon aqueous phase recycling at all the different temperatures tested. This effect is probably due to the saturation of the aqueous phase with organics and to some extent to the repolymerization of nitrogen–rich organic compounds present in the aqueous phase. The recycling of aqueous phase was therefore proposed as a novel step in biocrude production from microalgae. It clearly deserves more analysis and certainly could be much improved in the near future by studying different species of microalgae.

Based on an energy analysis it has been proposed a biocrude production process which is energetically self-sufficient, if part of the biocrude produced and the residual biomass after oil extraction are converted to energy. Also, the energy profitability with respect of Energy Return to Energy Investment (EROEI) analysis has been determined with reference to three cases at increasing oil content in the microalgae, resulting in values around 1.3 to 4.6 at the present stage, with possibility to reach higher levels.

Additionally, the economic feasibility of an industrial-scale plant was calculated based on conceptual process design, which was carried out using process simulations and assuming a hybrid cultivation system combining advantages of PBR and open ponds. With regards to the economic profitability, it has been estimated to achieve a

reasonable rate of return of the investment. According to this analysis the biocrude selling price should be as high as \$ 18.35 gallon^{-1}, i.e. \$ 21.11 gallon^{-1} for biodiesel, values well above to those of fossil diesel fuel. The highest cost showed is linked to the control of the PBR temperature and to the construction of the cultivation system.

In summary, even the currently lacking competitiveness of microalgae biodiesel is evident in comparison to the traditional fossil diesel fuel, the possibilities to improve this situation are numerous, and should be the subject of further investigations. Increasing of oil content in biomass and the areal productivity of PBR, are two of the mainly issues to be studied. New developments are particularly correlated to the team work of a wide range of professionals, integrating the purely biological aspects with engineering ones and making available field experimental data from pilot plants, in order to achieve a rapid development of the technology needed to render microalgae production a new renewable alternative for fossil fuel sources.

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Appendix A

Molecular weight distribution of HTL oils

In this appendix, the molecular weight distribution of the oil products obtained in the experiments presented in the Chapter 5 are reported.

The molecular weight distributions were obtained by Gel Permeation Chromatography (GPC) using an Agilent 1200 series HPLC system with 3 GPC PLgel 3 μ m MIXED–E columns connected in series. The column temperature was 40 °C, with a flow of 1 ml/min and tetrahydrofuran (THF) was used as solvent.
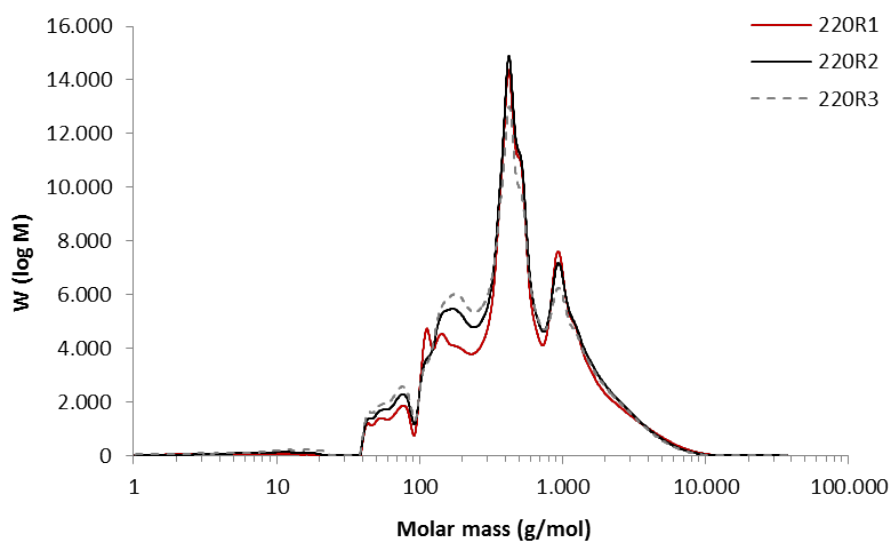
Figure 7.5.7: Molecular weight distribution by GPC analysis of oil obtained at 220°C with 30 min reaction time, along aqueous phase recycles, Run 1 = 220 R1, first recycle = 220 R2 and second recycle = 220 R3.
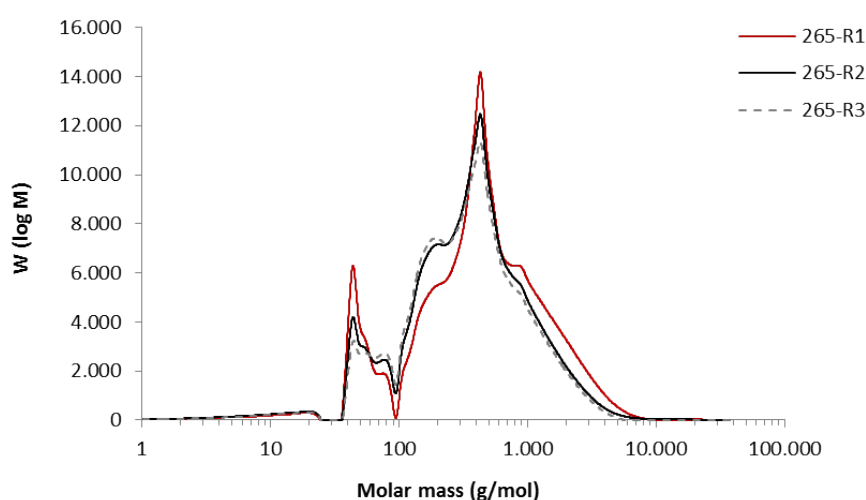
Figure 7.5.8: Molecular weight distribution by GPC analysis of oil obtained at 265°C with 30 min reaction time, along aqueous phase recycles, Run 1 = 265 R1, first recycle = 265 R2 and second recycle = 265 R3.
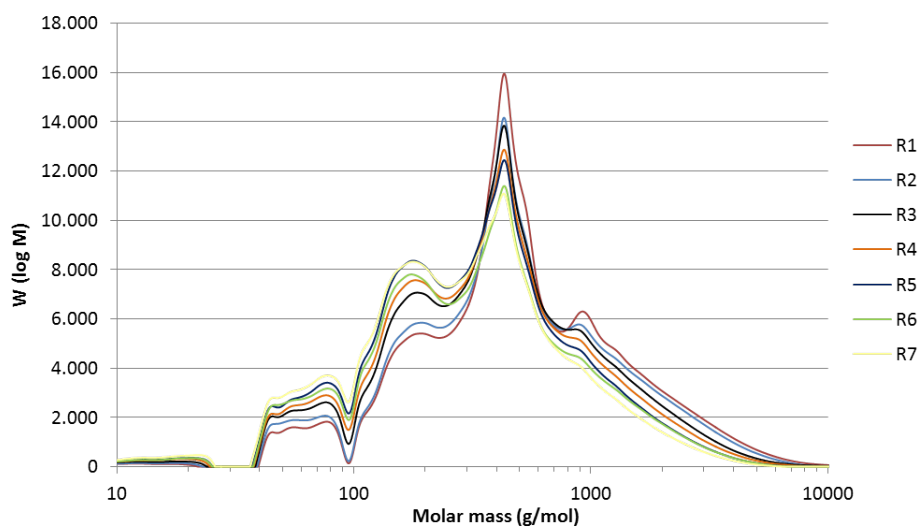
Figure 7.5.9: Molecular weight distribution by GPC analysis of oil obtained at 240°C with 30 min reaction time, along continuous recycle of aqueous phase, R1 represents the first run, only in this run distillate water was used. R2 to R7 reports the runs with water recycles.
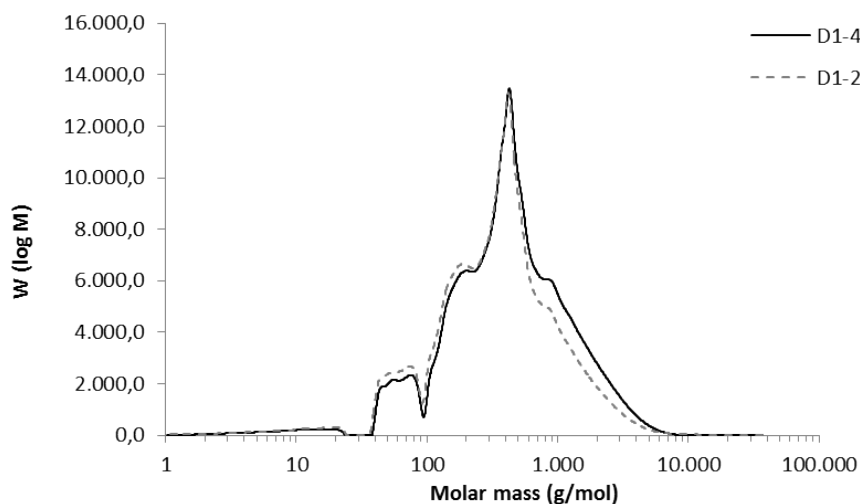
Figure 7.5.10: Molecular weight distribution by GPC analysis of oil obtained at 240°C with 30 min reaction time, when dilution aqueous phase from run 7 of continuous recycle experiment. D1–4 = dilution 1:4 and D1–2 = dilution 1:2.

Appendix B

Costs calculation

Blowers

Theoretical power $\dot{W}_{iso}$ required for an adiabatic and reversible compression through the equation:

$$\dot{W}_{iso}[W] = \frac{\gamma}{\gamma - 1} P_1 Q_1 \left[\frac{P_2^{\frac{\gamma}{\gamma-1}}}{P_1} - 1 \right] \tag{7.4}$$

Where:

γ = Cp/Cv, relative to the mixture of flue gas

P_1 = suction pressure of the compression system (Pa);

P_2 = discharge pressure of the compression system (Pa);

Q_1 = input flow rate of the gas (m³/s)

Installed cost was determined by Equation (Peters et al., 2003).

$$IC_{blower}[\$] = \left(\frac{M\&S}{260} \right) 506(Q_1[ft^3/min])^{0.598} \tag{7.5}$$

Operation cost OC_{blower} calculated by Equation an efficiency η_{mot} of 0.90.

$$OC_{blower}[\$/h] = C_{electricity} \frac{\dot{W}_{eff}}{\eta_{mot}} \tag{7.6}$$

Heat exchangers

The correlation of Guthrie (Douglas, 1988)was used to calculate de Installation cost of heat exchanger, where A is the surface required for the carry out the exchange. Operation costs are derived from the flow unit cost of utility.

$$IC_{HE}[\$] = \left(\frac{M\&S}{280} \right) 101.3(A[ft^2])^{0.65}(2.29 + F_c) \tag{7.7}$$

The heat flow Equation was applied to calculate the area.

$$Q = UA\Delta T_{ml} \tag{7.8}$$

Where:

A = Superficie (m^2)

ΔT_{ml} = Temperature difference (K)

U = Conduction Coefficient (Wm^2K-1)

Settler

The installed cost equation (Sharma, 2010) is based on the superficies required x in ft^2.

$$IC_{STL}[\$] = -0.0005x^2 + 86.89x + 182801 \tag{7.9}$$

Stripper

The installation cost equation (Douglas, 1988) is based on total height of the column H_{tot} (ft) and diameter D (ft).

$$IC_{STR}[\$] = \left(\frac{M\&S}{280}\right) 101.9(D[ft])^{1.066}(H_{tot}[ft])^{0.802}(2.18 + F_c) \tag{7.10}$$

Acknowledgements