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Microalgae harvesting in wastewater treatment plants: application of natural techniques for an efficient flocculation

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Abbreviations and symbols

$\Delta d_{channels}$	head loss in channels (m)
$\Delta d_{reversals}$	head loss in reversals (m)
a	dimensionless coefficient from Turc's equation
A	HRAP surface area (m^2)
A_d	surface area of the digester wall (m^2)
AOM	algogenic organic matter
BMP	Biochemical methane potential
CAS	Conventional activated sludge
CH_4	Methane
CHP	combined heat and power
CO_2	Carbon dioxide
COD	chemical oxygen demand (mg/L)
R-HRAP	High rate algal pond with biomass recylcing
D	water depth (m)
d_x	column depth (cm)
DAF	Dissolved air flotation
DO	Dissolved oxygen (mg/L)
DW	Dry weight (%)
$E_{input,HRAP\ electricity}$	input electricity in HRAP (kWh/d)
$E_{input,AD\ electricity}$	input electricity for anaerobic digestion (kWh/d)
$E_{input,\ AD\ heat}$	input heat for anaerobic digestion (kWh/d)
$E_{output,\ AD\ electricity}$	output electricity from anaerobic digestion (kWh/d)
$E_{output,\ AD\ heat}$	output heat from anaerobic digestion (kWh/d)
E_p	potential evaporation (mm)
EPS	extracellular polymeric substances
g	gravitational force (m/s^2)
HRAP	high rate algal pond

HRT	hydraulic retention time (d)
HRT_d	digester hydraulic retention time (d)
k	heat transfer coefficient ($W/m^2 \cdot °C$)
L	channel length (m)
n	manning friction factor
NER	Net energy ratio
NER_{heat}	Net energy ratio of heat
$NER_{electicity}$	Net energy ratio of electricity
NH_4^+-N	ammonium nitrogen (mg/L)
NO_3^--N	Nitrate nitrogen (mg/L)
NO_2^--N	Nitrite nitrogen (mg/L)
NTU	Nephelometric turbidity unit
O_2	Oxygen
OLR	Organic loading rate (g/m^2d)
PE	person-equivalent
P_m	microalgal biomass production ($kg\ TSS/m^2 \cdot d$)
PO_4^{-3}-P	Phosphate phosphorus (mg/L)
Q	wastewater flow rate (m^3/d)
Q_b	harvested microalgae biomass flow rate (m^3/d)
Q_E	evaporation rate (L/d)
Q_P	precipitation rate (L/d)
Q_w	mixed liquor flow rate in motion (m^3/s)
R	average solar radiation (cal/cm^2d)
RE	Biomass recovery (%)
R-HRAP	High rate algal pond with biomass recylcing
S_i	Surface area of column "i" (m^2)
SCOD	Soluble chemical oxygen demand (mg/L)
SRT	Solid retention time (days)
T	average temperature (°C)

T_a	ambient temperature (°C)
T_d	anaerobic digestion temperature (°C)
T_i	Initial turbidity (NTU)
T_f	Final turbidity (NTU)
T_p	pretreatment temperature (°C)
TS	total solids concentration (mg TS/L)
TSS	total suspended solids concentration (mg TSS/L)
t_x	the time elapsed (min)
v_i	Critical settling velocity (m/h)
υ	water velocity (m/s)
V	Volume (m^3)
VS	volatile solids concentration (mg VS/L)
V_d	digester nominal volume (m3)
W	channel width (m)
WWT	wastewater treatment
WWTP	wastewater treatment plant
Y	average methane yield (m^3 CH_4/kg VS)

Greek symbol

γ	specific weight of water at 20 °C (kN/m^3)
ε	paddle-wheel efficiency (%)
Θ	electricity consumption for pumping (kJ/m^3)
ω	electricity consumption for mixing ($kJ/m^3 \cdot d$)
ϱ	microalgal biomass density (kg/m^3)
γ	microalgal biomass specific heat (kJ/kg·°C)
φ	heat recovery efficiency
φ	efficiency of biomass harvesting (%)
ξ	power from methane (KWh/ m^3CH_4)
η_1	efficiency for electricity generation (%)

η_2	efficiency conversion in heat (%)
Φ_{ci}	Settling column diameter (mm)

Abstract

Research of new sources of bioenergy is nowadays driving attention to microalgae. Cost-effective biomass harvesting poses a challenge for full-scale microalgae production for biofuels. In the context of wastewater treatment with microalgae cultures, coagulation-flocculation followed by sedimentation seems to be the most suitable option for microalgae harvesting as low energy and no extra materials (e.g. membrane or electrode used for membrane filtration and electro-flocculation, respectively) are required.

The main objective if this PhD thesis was to evaluate and improve the harvesting efficiency of microalgal biomass grown in wastewater treatment high rate algal ponds (HRAPs) by means of flocculation-based pre-concentration techniques (i.e. coagulation-flocculation with organic flocculants and biomass recycling). Moreover, the energy assessment of a full-scale wastewater treatment system based on HRAPs followed by anaerobic digestion of harvested microalgal biomass located in a Mediterranean Region was assessed.

Firstly, coagulation-flocculation and sedimentation with two tannin-based polymeric flocculants (*Ecotan* and *Tanfloc*) was evaluated by means of static sedimentation tests in conventional settling columns. Low flocculants doses (10-50 mg/L) enabled over 90% biomass recovery. Furthermore, both flocculants increased microalgae settling velocity, leading to fast and efficient biomass recovery (> 90% recovery in 10-20 min).

Subsequently, dynamic sedimentation tests were performed in a water elutriation apparatus in order to evaluate the settling velocities distribution of microalgal biomass with and without flocculants. This time, a tannin-based flocculant (*Tanfloc*) and a cationic starch were evaluated. The amount of biomass reaching settling velocities higher than 6.5 m/h increased from 10-14% (without flocculant) to 70-84% when 20-40 mg/L of *Tanfloc* were added. On the other hand, 10-25 mg/L of starch enabled more than 95% biomass recovery, increasing from 46% to 78% the amount of particles with settling velocities higher than 6.5 m/h. According to the results, a settler designed with a critical settling velocity of 1 m/h (which is a typical value in secondary settlers) would enable over 90% biomass recovery while reducing the hydraulic retention time and the settler surface as compared to biomass harvesting without flocculants.

Microalgal biomass harvesting was also tested by recycling some of the harvested microalgal biomass (2% and 10% dry weight) to the pilot wastewater treatment HRAP in order to increase the predominance of rapidly-settling microalgae species. Results indicated that biomass recycling had a positive effect on the harvesting efficiency, obtaining higher recoveries in the pilot HRAP with recycling (91-93%) than in the pilot HRAP without recycling (75 – 88%), and increasing the percentage of biomass with high settling velocity. This was due to the fact that the abundance of rapidly-settling strains such as *Stigeoclonium* sp. and diatoms increased when 10% (dry weight) of harvested biomass was recycled.

Experimental results from this PhD thesis suggested that either flocculation with natural organic flocculants or biomass recycling improves harvesting efficiency of microalgal biomass with high biomass recoveries (>90%), increasing by 2-8-folds the amount of biomass with high settling velocities (6.5 m/h) and obtaining the best results in those experiments in which rapidly settling species (e.g. *Stigeoclonium* sp. and diatoms) were dominant. Finally, the energy balance of a microalgae-based

wastewater treatment plant located in the Mediterranean Region was assessed based on experimental results. The harvested microalgal biomass grown in wastewater HRAPs would undergo anaerobic digestion (with or without thermal pretreatment) to produce biogas and generate electricity and/or heat. The energy assessment concluded that the system should achieve microalgal biomass production of at least 15 g TSS/m^2d and/or a methane yield of 0.5 m^3CH_4/KgVS all over the year to be energy self-sufficient.

Resumen

Actualmente, la investigación de nuevas fuentes de energía ha centrado la atención hacia las microalgas. El principal desafío para la producción de microalgas a gran escala es realizar una recuperación de la biomasa algal eficiente y rentable para su posterior valorización. En el contexto del tratamiento de aguas residuales, el proceso de coagulación-floculación seguido de la sedimentación representa la técnica de recuperación de microalgas más adecuada debido al bajo consumo energético y a los bajos costes asociados.

El objetivo principal de la tesis doctoral fue evaluar y mejorar la eficiencia de separación de la biomasa algal cultivada en lagunas de alta carga (LAC) para el tratamiento de agua residual urbana. Esto se consiguió aplicando técnicas de pre-concentración basadas en procesos de floculación. A posteriori, se evaluó el balance energético de un sistema de tratamiento de aguas residuales a gran escala situado en la región Mediterránea, formado por un sistema de LAC seguido de un proceso de digestión anaeróbica de la biomasa.

En primer lugar, la coagulación-floculación y sedimentación con dos floculantes naturales poliméricos (*Ecotan* y *Tanfloc*) se evaluó por medio de ensayos de sedimentación estáticos en columnas de sedimentación convencionales. Ambos floculantes obtuvieron dosis óptimas bajas (10-50 mg/L) que permitieron la recuperación del 90% de la biomasa. Además, estos aumentaron la velocidad de sedimentación de la biomasa algal, implicando una recuperación de la biomasa rápida y eficiente (>90% de recuperación en 10 a 20 min). Posteriormente, los test de sedimentación dinámica se realizaron en un dispositivo dotado de tres columnas de sedimentación con el fin de evaluar la distribución de velocidades de sedimentación de la biomasa con y sin el efecto de floculantes. Esta vez, se evaluó un floculante polimérico (Tanfloc) y un almidón catiónico. En estos ensayos, se aumentó del 10-14% (son floculante) al 70-84% (con coagulante) la fracción de biomasa con unas velocidades de sedimentación mayores a 6,5 m/h tras la adición de 20-40 mg/L de Tanfloc. Por otra parte, entre 10 y 25 mg/L de almidón fueron necesarios para recuperar más del 95% de la biomasa, incrementando del 46% a 78% la fracción de partículas con velocidades de sedimentación mayores a 6,5 m/h. Según los resultados, un decantador diseñado con una velocidad de sedimentación de 1 m/h (valor típico en decantadores secundarios) permitiría la recuperación del 90% de la biomasa, reduciendo el tiempo de retención hidráulico y la superficie de los decantadores, tras la adición de los floculantes naturales estudiados.

La separación de la biomasa también se evaluó mediante la recirculación de una fracción de la biomasa cosechada (2% y 10% del peso en seco) en un sistema de LAC para el tratamiento de aguas residuales con el fin de aumentar el predominio de aquellas especies con altas tasas de sedimentación. Los resultados indicaron que la recirculación aumento la eficiencia de recuperación, obteniendo mayores recuperaciones en la LAC con recirculación (91-93%) que en LAC sin recirculación (75-88%), y aumentando el porcentaje de la biomasa con velocidad de sedimentación elevadas. Esto fue debido a la aparición de especies con altas tasas de sedimentación tales como *Stigeoclonium* sp. y diatomeas presentes cuando se recirculó el 10% de biomasa cosechada. Por último, el balance energético de una planta de tratamiento de aguas residuales a base de microalgas situada en la región Mediterránea se evaluó a partir de resultados experimentales de la biomasa algal crecida en LAC y sometida a

la digestión anaerobia (con o sin tratamiento térmico previo) para producir biogás y generar electricidad y / o calor. El estudio concluyó que se debe lograr una mínima producción de biomasa algal de 15 g SST/m^2d y / o unas producciones de metano de 0,5 m^3CH_4/kgVS para obtener un sistema energéticamente autosuficiente durante todo el año.

1

Introduction

Water, food and energy are three of the major resource issues facing the world today. Conventional wastewater treatment plants (such as activated sludge) typically used in large cities demand high-energy requirements (about 1 KWh/m^3) (Metcalf and Eddy, 2004). In addition, effluent and by-products from wastewater facilities are currently regarded as wastes with no value. From this point of view, innovative wastewater treatment strategies should aim to increase resource recoveries whilst minimizing the amount of energy requirement and emissions delivered to the environment. Indeed, energy production and resources recovery have been identified as two of the main challenges for wastewater treatment systems by recent initiatives promoted by the European Innovation Partnership on Water. Under this scenario, nature-based treatment solutions are conceiving as a step forward to a new model for wastewater treatment.

In the recent years, microalgae have received growing attention as wastewater treatment (WWT) and feedstock for bioenergy, biofuel and bioproducts generation. Firstly, photosynthetic oxygen production reduces the energy requirements (0.02 to 0.05 kWh/m^3) and the environmental impacts associated with the mechanical aeration of conventional activated sludge systems (Park et al., 2011a). On the other hand, microalgal biomass production promotes the recovery of resources (e.g. energy, nutrients, etc.) by means of different downstream process generating valuable product (e.g. biofuels, biofertilizer, bioplastics, etc.). These microorganisms do not require arable land and, when coupled with wastewater treatment, do not depend on the freshwater supply. Thus, microalgae do not compete for land and water with agriculture, which is the main limitation for bioenergy generation from crops (i.e. soybean, corn, sugar cane).

Moreover, the enormous pressure on the finite supply of fossil fuel-derived energy and chemicals is driven the global economies to move from a global economy based on fossil fuels to a biobased economy. Microalgae grown as by-product of WWT fit in the biorefinery approach, which is defined as a sustainable processing converting biomass into a spectrum of marketable products and energy. In spite of the promising results obtained by introducing the wastewater in microalgae production chain, the high costs of the process hamper the scaling-up of the

technology. In this way, new steps forward on cultivation and harvesting techniques are required for overcoming the bottlenecks of bioproducts generation from microalgae.

In the last decade, researchers have focused on the scaling-up of cultivation systems from lab-scale to industrial scale for commercial purposes. Much progress has been made in order to increase biomass productions through photobioreactor design, selection of strains and genetic engineering of metabolic pathways. Currently, investigation has focused on research of downstream (i.e. microalgae harvesting and by-products generation) processes which would reduce the overall production cost of microalgae. However, large-scale production of microalgae still requires low-cost and energy efficiency technologies to enhance industrial production system.

Microalgae harvesting is probably the main bottleneck hampering the application of full-scale microalgae treatment systems (Christenson and Sims, 2011; de Godos et al., 2011) since it increases the production cost by 20-30% (Barros et al., 2015). Indeed the harvesting efficiency is limited by the low biomass concentration (0.2 – 2.5 g/L), which requires the removal of large volumes of water to achieve a concentrated microalgal biomass (1-5% w/w). Even if centrifugation is a proven technology for fast and effective harvesting, its high capital and operation costs make this solution unfeasible when the harvested biomass is used for low-value applications. In fact, in the context of wastewater treatment, only low-cost methods capable of managing large volumes of water and biomass can be suitable. However, usual separation techniques applied in wastewaters, such as conventional sedimentation, have low harvesting efficiencies in the case of microalgal biomass (60-70%) (García et al., 2000a). For this reason, methods to pre-concentrate the biomass before the gravity sedimentation, such as coagulants and/or biomass recycling to stimulate bioflocculation, are crucial to enhance the effectiveness of low cost harvesting techniques.

2

Objectives

The main objective of this PhD thesis was to evaluate and improve the harvesting efficiency of microalgal biomass grown in wastewater by means of different processes based on flocculation. Two low-cost techniques (i.e. coagulation-flocculation by organic flocculants and biomass recycling to promote bioflocculation) were investigated as pre-concentration steps to improve gravity sedimentation efficiency. The effect of such harvesting techniques was evaluated on microalgal recovery, biomass and methane production as well as wastewater treatment efficiency. Furthermore, the energy consumption of a microalgae-based wastewater treatment plant integrating anaerobic digestion was calculated.

The specific objectives of this research are:

- To evaluate the efficiency of three organic flocculants on the harvesting of microalgae grown in an experimental high rate algal pond (HRAP) treating wastewater. The flocculants evaluated were two tannin-based polymeric flocculants, *Ecotan* and *Tanfloc*, and cationic starch. This was accomplished by:

    - Determining the optimal flocculants doses by means of jar tests (Chapter 4, 5 y 6);
    - Studying the settling time and the biomass velocity of the microalgal biomass using settling column tests (Chapter 4);
    - Evaluating the distribution of settling velocities of the microalgal biomass by means of dynamic sedimentation tests (Chapter 5 y 6);
    - To evaluate the effect of the natural flocculants on the methane yield of the microalgal biomass by means of biochemical methane potential (BMP) tests (Chapter 4 and 6);

- To assess the effect of biomass recycling on bioflocculation of microalgae grown in an experimental high rate algal pond (HRAP) treating wastewater (Chapter 7);

- To estimate the energy balance of a microalgae-based WWTP around one year taking into account the experimental results of this thesis (Chapter 8).

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3

State of the art

This chapter is based on the article:

Gutiérrez, R., Uggetti, E., Ferrer, I., García, J. (in preparation) Microalgae-based wastewater treatment plants: A review of cost-effective harvesting techniques

3.1 Microalgae: from biology to bioenergy production

3.1.1 Biology of microalgae

Microalgae are small (1-50 μm) unicellular photosynthetic microorganisms with high photosynthetic rates which allow them to convert sunlight into high rates of biomass growth. They can be categorized based upon the carbon supply and/or light utilization. Concerning light utilization, microalgae can be prokaryotes or eukaryotes. Eukaryote microalgae contain membrane-bounded organelles such as chloroplast, mitochondria and a nucleus which contains the genetic material. Conversely, prokaryotes do not contain chloroplast, mitochondria and nuclei but they contain chlorophyll "a" and high protein contents (e.g. cyanobacteria). However, mostly microalgae species belongs to eukaryotic group. Based on carbon source, microalgae are grouped as autotrophs or heterotrophs microorganisms. The autotrophs use inorganic carbon, such as CO_2, present in the atmosphere and perform photosynthesis using light as energy source. On the other hand, heterotrophic microalgae consume organic carbon to grow. Microalgae species which can use both, organic and inorganic carbon source, are called mixotrophs (Rashid et al., 2014). Among them, photosynthetic eukaryotic microalgae are the most common microalgae species.

Microalgae have adapted to a wide range of conditions that include saline, freshwater and terrestrial environments, hot and cold weather conditions, a great range of mineral compositions and low and high light conditions. Scientists have categorized microalgae in a classification system mainly distinguished by their pigmentation, life cycle, storage products and cellular structure. The most abundant microalgae species has been classified in four main groups: (1) Diatoms (*Bacillariophyceae*), (2) Green algae (*Chlorophyceae*), (3) Blue-green algae (*Cyanophyceae*) and (4) Golden algae (*Chrysophyceae*).

3.1.2 Principles of mass cultivation

Several environmental and biological conditions (i.e. light, temperature, nutrient and oxygen concentration, etc.) can influence microalgae growth

and, consequently, the biomass production, the dominant algae species and their composition.

Light

In general, the light passing through a water column declines exponentially with depth as the biomass concentration modifies the amount of light and frequency at which microalgal cells are exposed to optimal light. The light available for microalgae is highly modified by the light path, biomass concentration and mixing patterns. Both light path and biomass concentration determine the degree of light attenuation through the water column, while mixing pattern determines the frequency of the light/dark cycle and the rates of nutrient uptaken. These factors are known to impact on the rate and efficiency of photosynthesis and consequently biomass production (Grobbelaar, 2009). In open cultivation systems, the light availability was improved by modifying the light path (i.e. decreasing pond depth) and reducing the hydraulic retention time (HRT) to reduce biomass concentration and allow light to penetrate further into the pond (Kroon et al., 1989). Nevertheless, a recent study reported an increment up to 200% of microalgal biomass productions by increasing pond depth from 0.2m to 0.4m (Sutherland et al., 2014). Furthermore, at light intensities above saturation (around 200–400 $\mu mol/m^2s$), the specific microalgal growth rate will stabilize at its maximum level (Boelee et al., 2014). Excess light, that is absorbed by phototrophs, is initially dissipated as heat, but under continued conditions of excess light, photoinhibition can occur (Boelee et al., 2012).

Oxygen

Optimal dissolved oxygen concentrations between 5-30 mg O_2/L (depending on the season and cultivation conditions) have been stated as typical oxygen concentrations found in microalgae cultures (Jiménez et al., 2003; Mendoza et al., 2013). The high oxygen concentrations (>35 mg O_2/L) coupled with the prolonged exposure to intense sunlight may generate photoxidation of microalgae cells and therefore decrease treatment efficiency (Chisti, 2007a; Oswald, 1988). Oxygen levels equivalent to about four times the air saturation (400%) are toxic to mostly microalgae species grown in outdoor systems which severely

inhibits microalgal growth (Lee and Lee, 2001). In this regard, open systems perform better than closed reactors since oxygen does not accumulate substantially in open ponds. Consequently, closed systems requires and airlift zone in which the accumulated oxygen is stripped by air (Molina et al., 2001). In this type of cultivation systems, the time required for the mixed liquor to reach the degassing point, and the dimensions of the system must be taken into account to prevent toxic oxygen concentrations.

Nutrients

Besides light and water accessibility, the availability of carbon, nitrogen and phosphorus is essential for microalgal growth. The molar ratio of these three elements is determined by the Redfield ratio. The ratio 106:16:1 (C:N:P) establishes which portion of these nutrient are needed for the optimal growth of microalgae (Redfield, 1958). In general, the C:N ratios of wastewater are between 2.5-4:1, which means a deficit of carbon source for microalgae. In order to increase nutrient assimilation by microalgae, CO_2 addition can compensate C:N ratios of wastewater up to 6:1, which is more typical of algal biomass ratios (Park and Craggs, 2010). CO_2 injection to the system reduces mixed liquor pH and shifts the equilibrium of ammonia toward ammonium, which can be uptake by microalgae. Moreover, new processes has been ideated in order to improve CO_2 uptake efficiencies (maximum values of 33% in open systems), allowing CO_2 capture from flue gases and transferring it to microalgae cultures (González López et al., 2009). In such system, the CO_2 is absorbed in an aqueous phase enriched by a carbonate–bicarbonate buffer in an optimized contact unit, the water is then regenerated by microalgae(González-López et al., 2012).

3.1.3 Potential microalgae applications

Present state of application

For decades, large-scale cultivation of microalgae has been developed for the production of high value products such as of human and animal nutrition, aquaculture or cosmetics. The commercial production started

less than 60 years ago in Japan with the cultivation of *Chlorella*, following 10 years later by the cultivation of *Arthrospira* (also known *Spirulina*) in Lake Chad and Lake Texcoco (Africa) (Ugwu et al., 2008). In 30 years, the industry of microalgae biotechnology has grown and diversified significantly. However, the commercial applications are dominated by four strains: *Arthrospira, Chlorella, Dunaliella Salina and Aphanizomenon flos-aquae* (Spolaore et al., 2006). Nowadays, the microalgal biomass market produces 5000 t DW/year, and the biomass production is present in countries such as USA, China, Japan, Australia, Thailand, Israel or India.

Bioenergy production

The idea to convert microalgae to feedstock for bioenergy and biofuels was firstly mentioned in 1950s, but it has not been considered seriously until the petroleum crisis of the 1970s. A research group of the University of California led by William Oswald was the first to propose microalgae as a source of energy (methane from microalgae fermentation) using wastewater high rate algal ponds (HRAP) as a cultivation system (Oswald and Golueke., 1960). Since then; numerous studies have been conducted to obtain biodiesel from microalgae by different pathways such as hydrothermal liquefaction, transesterification of lipids, etc. (Olguín, 2012; Park et al., 2011a; Rawat et al., 2011; Scaife et al., 2015) .

Concerning biodiesel, in 1980, the US Department of Energy developed the "Aquatic Species Program" (ASP) in order to evaluate the bioenergy production from microalgae which would be able to compete with fossil fuels. The ASP achieved to assess the potential of biodiesel production from microalgae grown in open ponds, but the evaluation concluded that biodiesel production from microalgae was not economically viable (Murphy and Allen, 2011). Furthermore, the report stated that the only possible application of microalgae biofuels needs the integration of wastewater treatment (Sheehan et al., 1998). However the research is still ongoing to make biodiesel from microalgae competitive to other fossil fuels

Besides biodiesel, microalgae can also be used for bioethanol and biogas applications which are more competitive (Park et al., 2011a). Many studies have been conducted to investigate the possibilities of bioethanol production from microalgae via fermentation (Choi et al., 2010; Ho et al.,

2014; Miranda et al., 2012). Nevertheless, biogas generation through anaerobic digestion is nowadays considered the most energetically-favourable process (Wiley et al., 2011). This process involves the degradation of organic matter by bacteria in the absence of oxygen (Metcalf and Eddy, 2004). Unlike transesterification process for biodiesel production, the anaerobic digestion process is capable of producing methane-rich biogas regardless of lipid content. Moreover, low energy input is required for operating anaerobic reactors, which can tolerate solids with high water content (Montingelli et al., 2015). Anaerobic digestion is a consolidated technology already available for sewage sludge treatment in full-scale facilities. Methane productivity from microalgae digestion is comparable with experimental values reported from others feedstock: pig waste (0.19 m^3 CH_4/Kg VS), wastewater sludge (0.23 m^3 CH_4/Kg VS), and clover grass (0.34 m^3 CH_4/Kg VS)(Gissén et al., 2014; Mata-Alvarez et al., 2014).

Wastewater treatment

The use of microalgae in wastewater treatment holds great potential since efficient removal of nutrients and toxic metals are accomplished. Since decades, studies reported successful treatment of municipal, agricultural and industrial wastewaters by means of microalgae (Aguirre et al., 2011; Alcántara et al., 2014; Boelee et al., 2014; Godos et al., 2009; Park and Craggs, 2010; Sforza et al., 2014). Moreover, the integration of wastewater treatment in microalgal production context to generate energy led to reduce the requirement of fresh water and nutrients which have made the production of biofuels until now environmentally and economically unfeasible.

3.1.4 Microalgae in wastewater treatment context

From a biorefinery approach, microalgae-based WWT systems can combine wastewater processes and downstream processes to create bioproducts with additional value (Kouhia et al., 2015) (Fig. 3.1). Several steps are involved in the production of a valuable product. First microalgal biomass is cultivated in specific production systems while secondary wastewater treatment is carried out. Secondly, the microalgal

biomass is harvested and separated from the clarified effluent. In some cases, the harvesting step requires two consecutives steps if the downstream process needs high solid concentrations. Additionally, downstream processes like anaerobic digestion, photofermentation and electrolysis are necessary to produce specific products derived from microalgae.

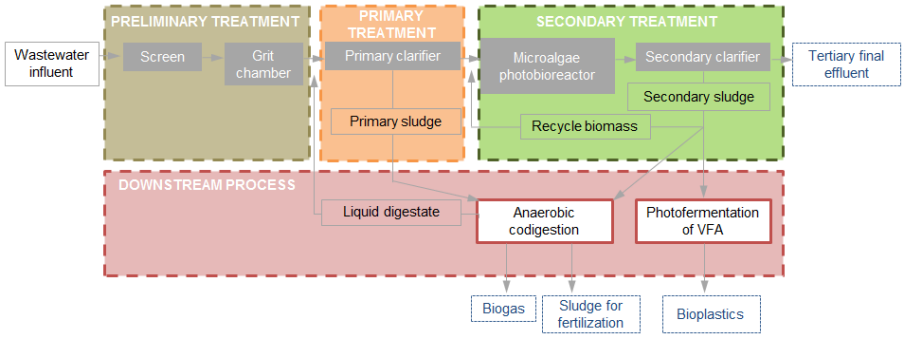


Figure 3.1 Flow diagram of an integrated microalgae-based WWTP. Units: grey boxes; Processes: dashed boxes; substrates or inputs: black font; end-products: blue boxes. Arrows: interrelations between processes.

Wastewater as a source for microalgae growth

In wastewater, microalgae synergistically interact with aerobic heterotrophs and autotrophs via exchange of substrates (Fig. 3.2). During this symbiosis, microalgae produce oxygen (O_2) that is needed by heterotrophic bacteria to oxidize the organic matter presents in wastewater, and the carbon dioxide (CO_2) released by these heterotrophs is in turn used by the microalgae.

The optimal growth of microalgae requires sufficient amounts of nutrients (carbon, nitrogen and phosphorus). Green microalgae species have a typical biochemical composition of $C_{106}H_{181}O_{45}N_{16}P$. Their carbon is derived from CO_2 (bacteria respiration and/or atmosphere exchange), their nitrogen mainly from NH_4^+-N, their phosphorus from PO_4^{-3}-P (both present in wastewater) and most of their hydrogen and O_2 comes from water (Oswald, 1991).

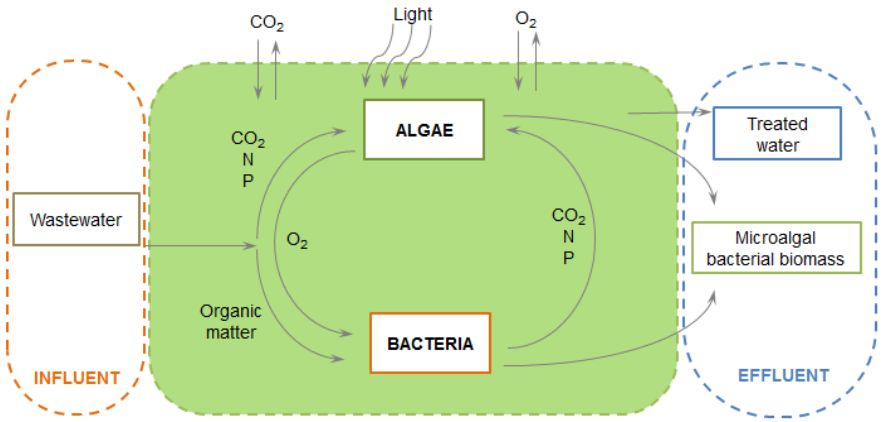


Figure 3.2 Symbiosis algae-bacteria present in microalgae-based wastewater treatment process.

In spite of having enough nitrogen and phosphorus concentration (typically around 30 mg NH_4^+-N/L, 5 mg PO_4^{-3}/L), there is not enough organic carbon available in primary wastewater to ensure nutrient removal via assimilation only (Boelee et al., 2012). Hence, biological nitrogen removal can be accomplished by different pathways: (1) microalgae assimilation, (2) ammonia volatilization ("stripping") and (3) nitrification-denitrification processes. Microalgae photosynthesis can cause the wastewater pH to rise to 10-11 when the rate of photosynthesis is carbon limited. The rise in pH shifts the NH_4^+/NH_3 equilibrium toward NH_3 formation, which increase the rate of N removal via ammonia volatilization and also facilitate orthophosphate precipitation with Ca^{2+} ions (García et al., 2002; Nudogan and Oswald, 1995). Biological nitrogen removal involves the oxidation of NH_4^+ into NO_2^- and NO_3^- by aerobic bacteria, a process known as nitrification, followed by the reduction of NO_2^- and NO_3^- into N_2 under anoxic conditions by heterotrophic bacteria, a process known as denitrification.

Cultivation systems

Microalgae can be cultivated in three main types of systems: open ponds systems (Fig. 3.3a), closed photobioreactors (Fig 3.3b, c, d) and biofilm systems (Fig. 3.3e).

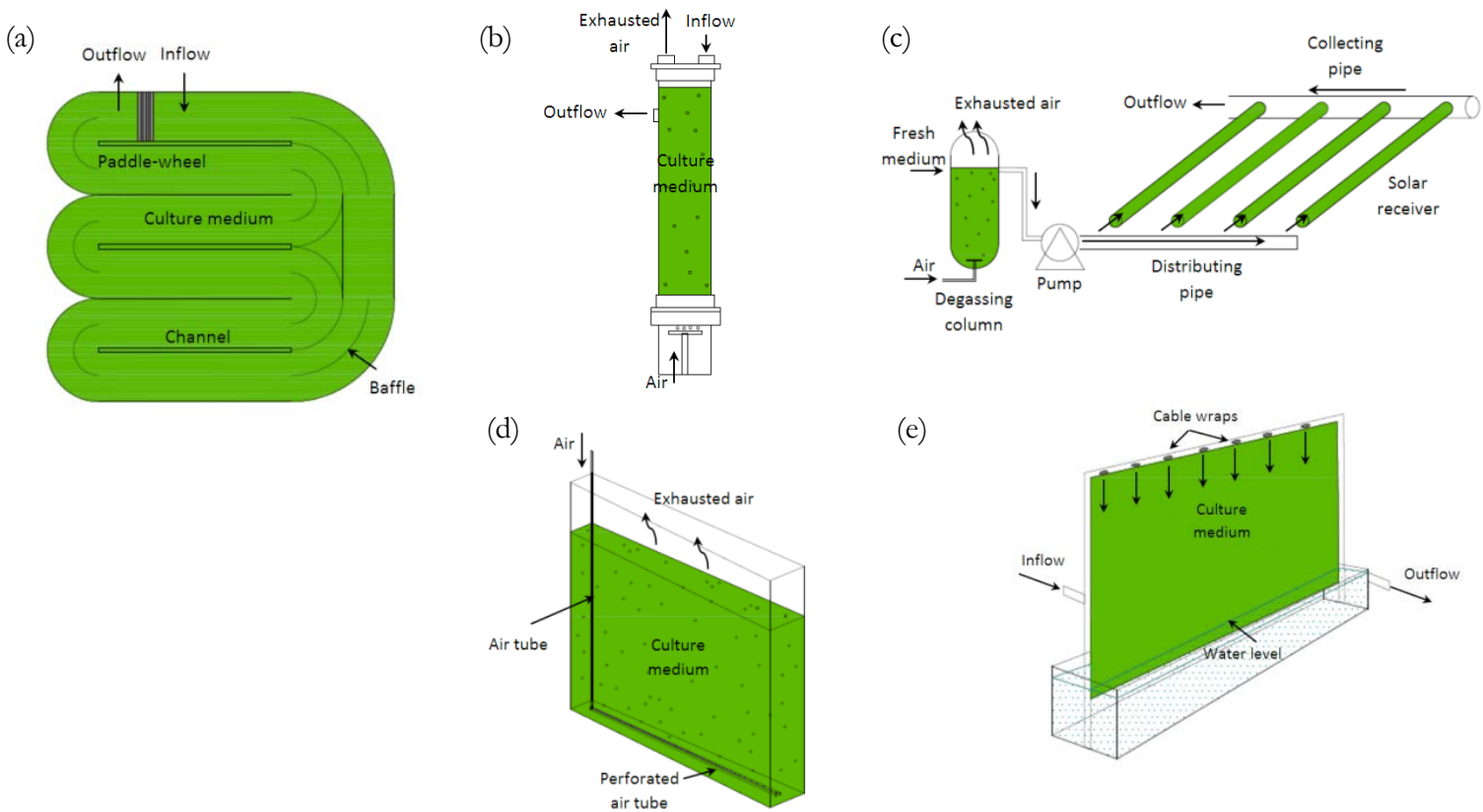


Figure 3.3 Different suspended- and fixed-growth system used for experimental purposes. Suspended growth systems: (a) raceway or open ponds (b) column photobioreactor (c) tubular photobioreactor and (d) flat panel photobioreactor. Fixed-growth systems: (e) biofilm photobioreactor.

In general, photobioreactors should be designed and operated to optimize light penetration (high surface/volume ratio) and provide adequate mixing, carbon supply, and degassing under low hydrodynamic stress, while minimizing construction and operation costs (Muñoz and Guieysse, 2006; Tredici, 2004). The best configuration depends on factors such as land cost and biomass use (Muñoz and Guieysse, 2006). However, limited information can be found in the literature on integration of both wastewater treatment and resource recovery in large-scale microalgae production systems.

Microalgae cultivation in open systems has been used for wastewater treatment since ancient time. In 1950, the introduction of the HRAPs appeared as an alternative to conventional stabilization ponds for wastewater treatment and resource recovery (Oswald and Gotaas, 1957), which involved the reduction of both HRT (from 15-30 days for conventional ponds to 2-8 days for HRAPs) and land requirement with a potential production of large amount of energetic feedstock (Kivaisi, 2001). The HRAPs are large and shallow open oval shaped reactors (Fig. 3.3a), generally between 0.2 and 0.5 m deep, equipped with a paddle-wheel which provide mixing and continuous circulation to stabilize microalgae growth and avoid sedimentation (García et al., 2006; Muñoz and Guieysse, 2006).

Closed systems have been designed to overcome some of the main constraints related to open production systems (i.e. evaporation losses, land requirement, conditions control). Most commonly enclosed systems are often designed as (1) column photobioreactors; (2) tubular horizontal photobioreactor; (3) or flat panel photobioreactors (Fig 3.3b,c,d, respectively) (Tredici, 2004). In general, closed systems achieve higher photosynthetic efficiencies and better control than open systems which means less risk of pollutant volatilization and predation (Muñoz and Guieysse, 2006) and let maintain monoalgal cultivation for prolonged periods (Chisti, 2007b). Although closed systems are more efficient than open systems, they are generally costly to construct and operate (Muñoz and Guieysse, 2006).

Harvesting challenges of suspended-growth systems (further detail in section 3.2) have led to an increasing interest in the use of immobilized or

fixed algal processes (Ozkan et al., 2012; Sukačová et al., 2015). The fixed growth systems are based on the attachment of microalgae and bacteria onto a polymeric matrix or on solid carriers (Boelee et al., 2012; Hoffmann, 1998; Muñoz et al., 2009). Biofilm systems allow for the simultaneous recovery of carbon and nutrients from wastewaters in the form of easily harvestable biofilm particles, and the production of clarified effluent (Christenson and Sims, 2011).

Either open, closed or biofilm configurations have their advantages and disadvantages (Table 3.1). Current investigation tends to design the most suitable configuration for large scale microalgae production. Indeed, the selection of the proper system would mainly depend of the value of the final product. Generally, closed photobioreactors are the most productive systems but their capital and maintanece cost is still high to be competitive with open systems for large-scale applications. For fixed growth-systems, the significant amounts of plastic materials required for the biofilms and the immobilisation processes may be too costly (Lim et al., 2013).

Table 3.1 Long-term HRAP performance conducted in different research groups in the last decade.

Cultivation system	Biomass production (g/m^2d)	Light utilization efficiency	Gas exchange	Culture control	Scalability	Costs
Raceway pond	10-25	Low	Medium	Low	High	Low
Tubular PBR	15-40	High	Low	High	Medium	High
Flat panel PBR	20-50	High	Low	High	Low	High
Biofilm systems	15-30	High	High	Low	High	Low

Harvesting and downstream processing

Actual harvesting techniques applied for microalgal biomass such as centrifugation or filtration has been estimated to contribute between 20 to 50% to the overall production costs (Brennan and Owende, 2010; Kim et al., 2013; Mennaa et al., 2015; Sathe and Durand, 2015). Thus, the

harvesting step is one of the main bottlenecks of the bioenergy production due to the high operational costs of the harvesting and dewatering steps, and this is mainly due to the low concentration of microalgal cultures and the large volumes of biomass to treat, which requires a great amount of energy for dewatering.

3.2 Microalgae harvesting: overview of separation methods

Several harvesting techniques based on mechanical, electrical and chemical processes are currently used for concentrating microalgae from 0.02-0.25% (w/w) to 15-25% (w/w) (Gerardo et al., 2015). In industrial systems for the generation of high-value products, commonly used harvesting techniques include filtration, centrifugation, ultrasound, electrocoagulation and flocculation induced by chemical addition (Danquah et al., 2009a; de Godos et al., 2011; Granados et al., 2012; Misra et al., 2015; Salim et al., 2011; Wu et al., 2012). Despite achieving higher biomass recoveries, major constraints such as high energy requirement, cell composition changes and high costs (e.g. electrode and membrane replacement or flocculant costs) have hampered their use for low-value applications (Uduman et al., 2010). In the context of wastewater treatment, only low-cost techniques capable of managing large volumes of water and biomass can be applied. Nevertheless, energy requirements for harvesting step need to be low to achieve net energy production plants. Therefore, the combination of low-cost pre-concentration techniques such as autoflocculation, bioflocculation, chemical flocculation (with organic and inorganic flocculants) followed by a solid/liquid separation such as gravity sedimentation and/or flotation could be a feasible and cost-effective solution for large-scale applications (Fig. 3.4).

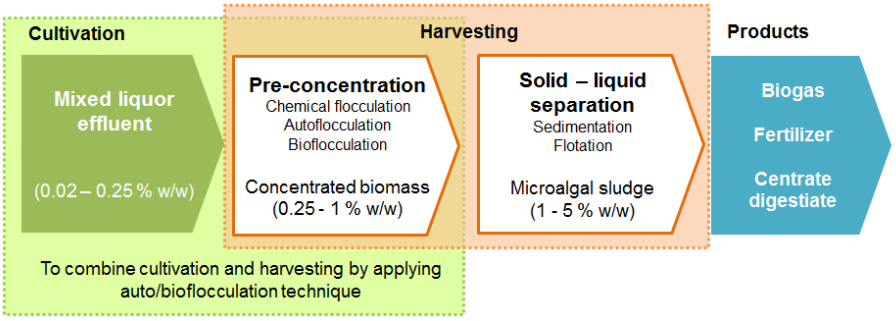


Figure 3.4 Schematic presentation of an overall microalgae production, harvesting and recovery process.

3.2.1 State of the art of separation methods

Separation processes have a dual purpose. On one hand, a better microalgal biomass separation from the clarified effluent may reduce the energy consumption during a thickening or dewatering step. On the other hand, it improves the quality of the clarified effluent, ensuring the compliance of Directive limits on solids concentrations for discharge (Council Directive, 1991) an facilitating the reuse of the treated water. In a wastewater treatment context, the end product has low value (i.e. bioenergy), thus gravity sedimentation and flotation are considered as economical alternatives for microalgae biomass harvesting.

Sedimentation

Sedimentation involves the separation of the suspended microalgae cells, which have similar cell density than water, by gravity settling. Among harvesting techniques, sedimentation is a low-energy process that is commonly used in wastewater treatment and is considered one of the simplest ways to harvest microalgae. The two commonly settlers used for microalgal biomass separation are vertical clarifiers and inclined settlers.

The design of large scale settlers depends mainly on the surface-loading rate which is related to the biomass settling velocity. This in turn is determined by Stoke's Law, which states that the settling velocity is proportional to the radius of the cells and the difference in density between the microalgae and the medium. The nature of the different

microalgae species influences their biomass settling velocity. Hence, the sedimentation rate is microalgae specie specific. For instance, the settling velocity of one single cell of a spherical shaped microalgae (e.g. *Chlorella* sp.) was calculated to be 0.1 m/d, whilst most complex microalgae structures have reported higher cell settling velocities between 0.4 and 2.2 m/d (Peperzak et al., 2003). In a vertical clarifier, higher microalgal biomass settling velocity corresponds to lower settler surface requirement.

Typically, conventional sedimentation have low harvesting efficiencies (60-70%) (García et al., 2000a), which can be improved by a pre-concentration step (i.e. flocculation). Indeed, promising results were achieved in a conventional settler without chemical addition when autoflocculation was applied as pre-concentration step (Show and Lee, 2014). The different approaches for microalgae concentration are discussed in Section 3.3.

Novel designs of settler with inclined plates have been conceived to increase the flow rates and achieve high settling velocities without flocculant addition by increasing the available settling area (Smith and Davis, 2013). In a continuous inclined settler, harvesting step occurs via biomass deposition on the plates, commonly inclined between 8 to 60º. A recent study has achieved 90% of biomass recovery in inclined settler by increasing the biomass settling velocity even with low plate's inclination (8º) and without flocculant addition (Smith and Davis, 2013). This study achieve increasing microalgal biomass concentrations from 0.07% (w/w) to 5.9% (w/w), accomplishing the concentration requirements of harvesting step for biogas production.

Flotation

Flotation is a separation method based on the adhesion of particles to gas bubbles, which drives the particles to the liquid surface, where they are removed by skimming. Between the different processes, dissolved air flotation (DAF) is a promising separation method.

Dissolved air flotation is commonly used to harvest sludge in wastewater treatment field and it has been applied at large scales (Christenson and Sims, 2011). DAF technique takes advantage from the natural self-floating tendency and the low density flocs of microalgae in order to enhance the

aggregation of big size flocs (Henderson et al., 2008; Kurniawati et al., 2014). Microscopic bubbles that are produced by saturating water with air at high pressure promote microalgal biomass floating. The generated bubbles are attached to the flocs of microalgal biomass, raising them to the surface. This can be accomplished by pre-concentrating microalgal biomass (by means of adding collectors or frothers) to increase their size and decrease their negative charge (Henderson et al., 2009). In fact microalgae are negatively charged and the generation of positively charged bubbles is appropriate to induce coagulation/flocculation harvesting. In this sense, the use of cationic surfactants (synthetic and natural compounds) has been shown to produce positively charged bubbles. Even if surfactants are added to the culture, DAF process is stable under a wide range of pH and temperature, which is advantageous for its application in different harvesting conditions at full scale (Lei et al., 2015)

3.2.3 Comparison between separation methods.

Several studies stated that the most suitable harvesting technique depends basically on the purpose of the end product. In Table 3.2, the comparison of common separation techniques applied in microalgae harvesting were summarised. The highest solids concentrations are achieved with filtration and centrifugation, whilst high costs and energy requirements are demanded. On the other hand, flotation and sedimentation are promising techniques because of their low cost and easy-scalability, but they require pre-concentration techniques in order to increase the final solid concentrations**.** Electrical approaches, such as electrocoagulation, present a high potential, but the effect of scaling up should still be investigated (Barros et al., 2015).

Table 3.2 Comparison of common microalgal harvesting methods (Christenson and Sims, 2011; Gerardo et al., 2015; Henderson et al., 2008; Shen et al., 2009; Uduman et al., 2010).

Separation system	Solid concentration (% w/w)	Energy input (KWh/m^3)	Dependence of species	Scalability	Costs
Centrifugation	2 – 22	0.7 – 8	Low	Low	High
Filtration	5 – 27	0.5 – 6	High	Low	High
Sedimentation	0.5 – 3	0.1 – 0.3	Medium	High	Low
Flotation	2.5 – 7	0.015 – 1.5	Medium	High	Low
Electrocoagulation	3 – 5	0.8 – 1.5	Low	Low	Medium

3.3 Pre-concentration methods

3.3.3 Parameters involving microalgae settling

The cell surface properties and algogenic organic matter (AOM) of each microalgae specie can influence the settling of the microalgal biomass (Gutzeit et al., 2005; Henderson et al., 2008; Park et al., 2013a; Su et al., 2012; Vandamme et al., 2013). Therefore, the different harvesting methods need to be tested and optimised in great detail to ensure the best conditions for the most effective harvesting method (Gerde et al., 2014). The cell surface charge, the different microalgae growth phases, the AOM and microalgae species are some important considerations to take into account for microalgal harvesting. These influences and their implications in microalgae settling are examined in depth in the following paragraphs.

Surface charge

Microalgae cells in solution possess a negative cell surface charge that creates repulsive forces between them. This negative surface electric charge combined with the small size (1-50 μm), the density similar to water and the low settling velocities of microalgae difficult the settling of such organisms (10^{-5}-10^{-6} m/s) (Granados et al., 2012; Liu et al., 2013). The electrostatic charges around microalgae are formed by three main layers: (1) fixed layer, (2) Stern layer and (3) diffused layer. The fixed layer

is formed by amine, carboxyl and hydroxyl functional groups present on the surface of the microalgae cell, which results in a negative surface charge. Secondly, the Stern layer involves a dense layer formed by opposite charged ions. Beyond, the diffused layer is formed by a dynamic equilibrium of charges which extends from the edge of the Stern layer to a distance where charges are neutralized. The total system formed by the particle cell surface and their potential charges is called electrical double layer (Fig. 3.5)

A frequently used parameter to know the surface potential of any colloid is the zeta potential (ξ). This parameter determines the mobility of charged particles at the end of the diffuse layer in an electric field. The zeta potential of microalgae is usually within the range of -10 to -15 mV.

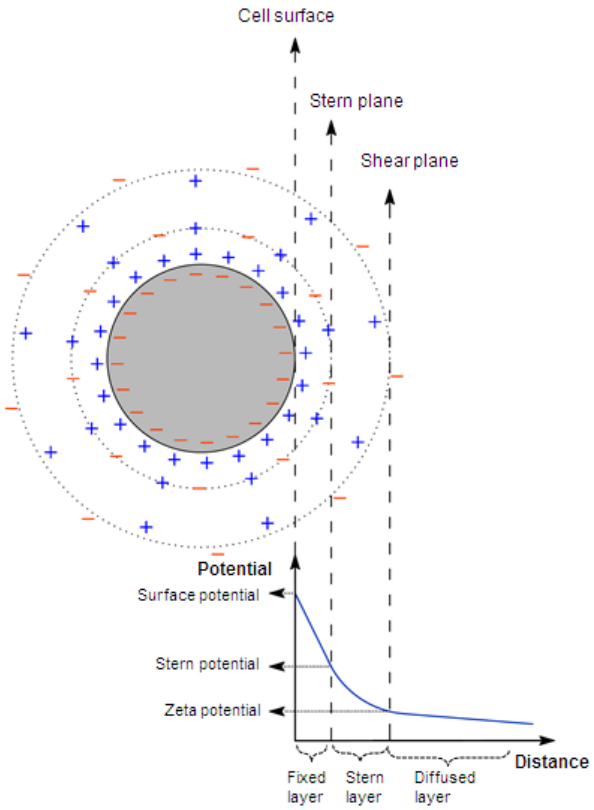


Figure 3.5 Schematic representation of the electrical double layer of charged ions in solution around a negative charged microalgal cell and the different potentials present around the cell (Reynolds and Richards, 1996; Vandamme et al., 2013).

Microalgae growth phase

Microalgae growth can be defined by two main growth periods: (1) exponential growth phase (with a high growth rate) and (2) stationary phase (with a low growth rate) (Fig. 3.6). During the exponential growth phase the growth rate of the microalgae cells reaches its maximum value which is specie specific (around 0.11 d^{-1}). In this phase, the intracellular metabolic rate, unicellular mobility and differential growth kinetics of the cells are optimal. This optimal microalgal biomass growth and cell mobility increases the repulsion between microalgae cells. Therefore, high electronegative behaviour of the microalgal biomass has been reported during exponential phase (Danquah et al., 2009b). On the other hand, during the stationary phase microalgae growth rate is low (around 0.03 d^{-1}) and the cell mobility is reduced, generating less electronegative zeta values and resulting in high interactions and cell agglomeration. This behaviour was reported during a coagulation/flocculation experiment in which higher flocculant doses where required when microalgal biomass was in stationary phase (de Godos et al., 2011). Therefore, due to the higher cell interaction, the low microalgae growth phase is the best condition to harvest the biomass.

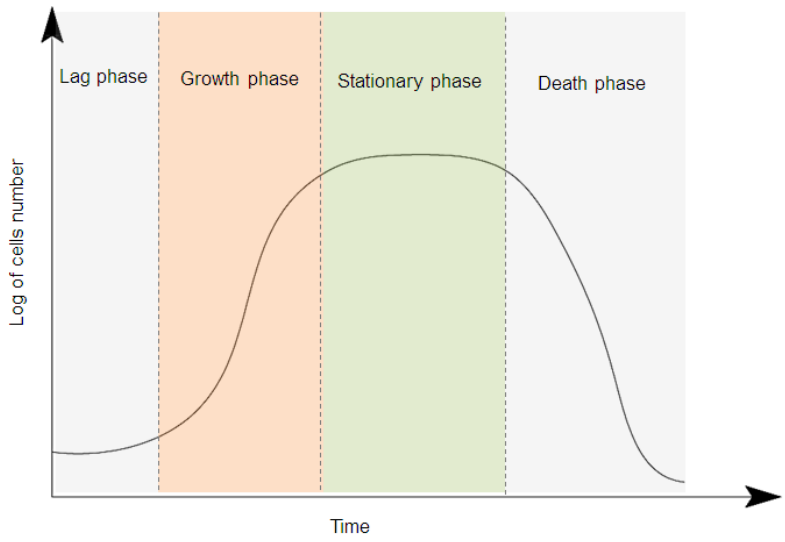


Figure 3.6 Model of growth curve of microalgae culture which represents the four phases of microalgae growth.

Organic matter

Microalgae are known to release significant amounts of AOM during the cultivation time (Prochazkova et al., 2014). The major fraction of AOM consists of neutral or charge polysaccharides (extracellular polymeric substances (EPS)), but other compounds such as proteins, nucleic acids, lipids and other small molecules can be present as well (Vandamme et al., 2014). In microalgae cultivation systems, AOM can reach values around 60-80 mgC/L. The presence of AOM has been reported to negatively affect flocculation and the differences of the amount and characteristics of AOM impact considerably the coagulant/flocculant dose for optimal removal (Henderson et al., 2010; Vandamme et al., 2012). Indeed, previous studies found that the flocculant dose needed to achieve efficient recovery is increased by the presence of AOM (Prochazkova et al., 2014; Vandamme et al., 2012) and showed how removing AOM by means of a flocculation-flotation process enhanced biomass recovery efficiency from water (Kurniawati et al., 2014).

Microalgae species

Microalgae species responded differently to harvesting processes. Differences in settling efficiency between species depend mainly on their physiological and morphology characteristics (Henderson et al., 2008; Peperzak et al., 2003). Although *Chlorella* sp., *Scenedesmus* sp., *Chlamydomonas* sp. *Desmodesmus* sp., *Micractinium* sp. are microalgal species commonly found forming large settleable colonies in wastewater reactors, not all of them has good settling capacities (Sofie Van Den Hende et al., 2014). More easily-settleable species like *Stigeoclonium* sp. (filamentous microalgae), *Pediastrum* sp. and diatoms such as *Nitzschia* sp. and *Navicula* sp. have reported better results concerning biomass settleability (Park et al., 2011b). To ensure feasible bioenergy production from microalgae, suitable microalgae species that are setteable or forming setteable flocs, are interesting to be cultivated (Gutzeit et al., 2005).

3.3.4 Definition of coagulation-flocculation mechanisms.

The processes of microalgae agglomeration entail two different mechanisms which can act alone or in combination. Coagulation is the process during which the cells are forced out of a stable suspension. This process is also known as (1) charge neutralization in which positive charged ions, polymers or colloids absorb the negative charged surface of microalgae leading to the destabilization of the cell (Fig. 3.7a). Flocculation, instead, is the process whereby destabilized particles are induced to make contact and form larger agglomerates. This process can be accomplished by three different approaches. (2) The electrostatic patch mechanism involves the diffusion as the principle flocculation mechanism. The polymer locally reverses the charge of the cell surface, resulting in patches of opposite charge on the particle surface (Fig 3.7b). (3) Bridging is the phenomenon in which polymers bind to the surface of two different cells to form a bridge between them (Fig 3.7c). (4) Sweeping flocculation is the mechanisms in which cells are exposed in a massive precipitation of mineral which causes their flocculation (Fig. 3.7d).

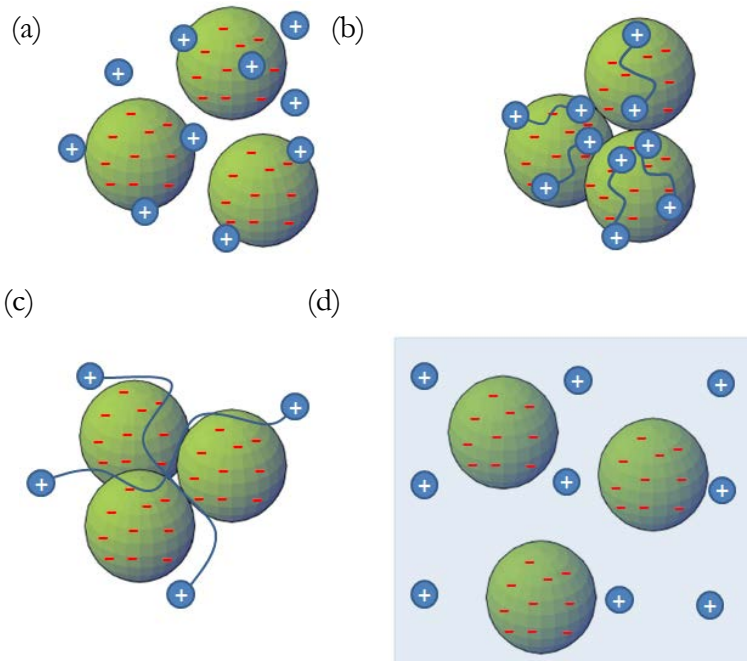


Figure 3.7 Overview of the four coagulation-flocculation mechanisms (a) charge neutralization, (b) electrostatic patch mechanism, (c) bridging mechanism (d) sweeping flocculation.

3.3.5 Types of flocculation

The pre-concentrating harvesting step could significantly reduce the energy consumption during following processes (Vandamme et al., 2012). These techniques are grouped as autoflocculation, bioflocculation and chemical flocculation (Table 3.3) and involve the addition of trivalent coagulants/flocculants, cationic polymers, pH adjustment or microorganisms interaction. The pre-concentration steps aim not only to increase biomass concentration but also to reduce the biomass volume after the harvesting process (Şirin et al., 2013). These techniques are examined in depth in the following paragraphs.

Chemical flocculation

The chemical flocculation of microalgal biomass depends on several conditions such as the properties of cell surface, the biomass concentration, the medium conditions (e.g. pH of the growth media), the coagulant/flocculant concentration and the ionic strength of the culture media (Gerde et al., 2014; Papazi et al., 2009; Şirin et al., 2011).

In wastewater treatment context, various coagulant and/or flocculants have been studied including both inorganic and organic types. Inorganic flocculants include salts of polyvalent cations such as Al^{3+} or Fe^{3+}. The flocculation using inorganic metal salts correspond to absorption-charge neutralization or sweeping flocculation caused by precipitate enmeshment or a combination of both (Vandamme et al., 2014). Their use results in high concentrations of metals in the harvested biomass and usually modifies the culture pH to values around 5.9 – 7.5 (Şirin et al., 2011). Therefore metal-based coagulants can contaminate downstream processes due to its low biodegradability (Gerde et al., 2014; Şirin et al., 2011).

Flocculants based on natural biopolymers have emerged as a substitution of metal-based coagulants and represents a safer alternative. The bridging flocculation mechanism of organic flocculant tends to form larger size aggregates resulting in a faster sedimentation rate. In addition, the harvested microalgal biomass is non-toxic and reduces possible contamination problems in the downstream process induced by metal-based coagulants (Şirin et al., 2011). Some of these biodegradable polymeric flocculants are chitosan and cationic starches (de Godos et al.,

2011; Gutiérrez et al., 2015a, 2015b; Letelier-Gordo et al., 2014; Şirin et al., 2011; Vandamme et al., 2010). In general, high recoveries were reported with lower flocculant doses (10-60 mg/L) than metal-based coagulants (>100 mg/L) (Letelier-Gordo et al., 2014; Şirin et al., 2011). In addition, current research investigates the dependence of pH, microalgal biomass concentration on the dosage of natural-based flocculants (Letelier-Gordo et al., 2014; Vandamme et al., 2014, 2010). The recovery efficiency rises by increasing microalgal biomass concentration and pH values. However, most of the studies have reported high flocculation efficiencies by testing organic flocculants at the optimal growth pH (between 7-9) (Gerde et al., 2014; Gutiérrez et al., 2015a, 2015b). So, the addition of most organic flocculants does not require any modification of the culture medium which, otherwise, would increase operational costs.

Autoflocculation

The autoflocculation (spontaneous flocculation) occurs when CO_2 is depleted and pH of mixed liquor increases inducing multivalent metal ions such as Mg^{2+} and Ca^{2+} to form positive hydroxide precipitates. These precipitates coagulate negative microalgae cells by sweeping flocculation and charge neutralization (Golueke and Oswald, 1970; Shelef et al., 1984). Several studies have demonstrated clear effect of pH on microalgal biomass harvesting when mixed liquor pH was induced to alkaline pH values above 9 (Şirin et al., 2011; Vandamme et al., 2012). Increasing pH from 9.12 (pH culture) to 9.75 by adding an specific amount of Na(OH) into the culture enhanced recovery efficiency to 89%, and settling rates (0.04 cm/h) due the precipitation of $CaCO_3$ and Mg $(OH)_2$ (Şirin et al., 2011). In addition, higher pH values over 11 were needed to flocculate 75% of microalgal biomass composed of *Chlorella vulgaris* (Vandamme et al., 2012). Nevertheless, high phosphate concentrations are required for this type of flocculation, resulting only sustainable in wastewater treatment containing excess of phosphate to be removed.

Bioflocculation

Bioflocculation is based on the aggregation of flocculent microorganisms in response to stressing conditions. Even if the mechanisms underlying the aggregation of flocculating microalgae are poorly understood, it is

known that spontaneous aggregation is mainly mediated by extracellular polymer substances (EPS) excreted into the culture medium and by microalgal cell surface properties (Gutzeit et al., 2005). Moreover, the addition of readily settleable microalgal species (i.e. *Scenedesmus obliquus*, *Tetraselmis suecica* or *Pediastrum* sp.) has been reported to induce bioflocculation of non-flocculating microalgae species (Park et al., 2011b; Salim et al., 2011). Faster sedimentation of non-flocculating microalgae were observed after mixing them with flocculating microalgae which increases both sedimentation rate and size flocs (Salim et al., 2011). In addition, recycling harvested microalgal biomass (with the present of easily settleable microalgae species) from a settling tank led to increase the predominance of readily settleable microalgae strains of the mixed liquor within the raceway pond, improving overall harvest efficiency (90%) and also increasing the average size of flocs by 13-30% (Park et al., 2013a, 2011b).

This pre-concentration technique is highly indicated in facilities where microalgae are employed for wastewater treatment and it may lead to a low-cost, chemical-free method for flocculating microalgae (Craggs et al., 2012). Indeed, fungi and bacteria present in wastewater also induce bioflocculation of microalgae. This results, hence, in a culture of mixed microalgal-bacterial flocs that can easily be harvested.

Table 3.3 An overview of the principles of pre-concentration techniques (autoflocculation, bioflocculation and chemical flocculation), advantages and disadvantages for microalgae harvesting (Şirin et al., 2011; Van Den Hende et al., 2011; Wu et al., 2012).

Pre-concentration technique	Procedure	Main flocculation mechanisms	Dose	Recovery (%)	Advantages	Disadvantages
Chemical flocculation	Inorganic metal salts addition	Neutralization, electrostatic attachment and sweeping	>50 mg/L	70-99	Low cost	Biomass contamination by metals pH changes
	Biopolymers and organic-based flocculants	Electrostatic attachment and bridging	<100 mg/L	58-99	Low doses	Expensive flocculants Some flocculants pH-dependent
Autoflocculation	Metal hydroxides formation	Neutralization, sweeping	Changes in pH, dissolved oxygen and nutrient concentrations	75-89	No additions needed	Microalgae disrupted by pH modifications Requires presence of Ca and Mg ions
Bioflocculation	EPS excreted by other microalgae, bacteria, fungi	Neutralization and sweeping	Depending on species	60-98	Low cost Spontaneous	Dependent on numerous factors Contamination Difficult to predict

3.4 Economic and energetic comparison of harvesting techniques

Although flotation and gravity sedimentation separation techniques are suitable for microalgae harvesting, both methods are species specific and biomass recoveries are generally low (60-70%). A pre-concentration technique prior those separation techniques may aid to aggregate the biomass flocs and, consequently, increase biomass recoveries.

Flocculation process by means of chemicals may be cost-effective depending on the flocculant cost. It is estimated that less than $40/ton of harvested microalgae may be an achievable production target by using coagulation-flocculation as harvesting technique (Schlesinger et al., 2012). For instance, the price of polyacrylamide flocculants is estimated at $100/ton of harvested microalgal biomass, which is prohibitive for low-value products. Similarly, the natural polymer chitosan, derived from shrimp exoskeletons which has been satisfactory used for harvesting microalgae grown in both freshwater and seawater is also expensive and adds bulk (Schlesinger et al., 2012). Indeed, the cost of this polymer (2-100 €/Kg) is approximately 3 to 100 times more expensive than aluminium sulphate and PAC (polyaluminium chloride) (0.3 – 1.3 €/Kg and 0.9 – 1.85 €/Kg, respectively) (Lim et al., 2013; Şirin et al., 2011; Xu et al., 2013). On the other hand, the price of cationic starches are significantly lower, between 1-3 €/Kg (Liu et al., 2013).

As seen, the cost of flocculants in these processes is significant, so harvesting methods without the addition of flocculants have gained significant attention in recent years (Shelef et al., 1984; Smith and Davis, 2012). These processes can occur either as natural "autoflocculation" in nutrient deprived microalgae cultures or instigated by a biological agent ("bioflocculation"), such as the introduction of another easily settleable microorganism. A biological flocculating agent, like the flagellate predator *Peranema trichophorum* can be produced in large quantities using a growth medium with minimal costs. For example, the cost of preparing 1 L of *Peranema* culture is less than 1$. From this 2×10^8 cell/mL *Peranema* cells and 1 L of culture filtrate can be produced, which is sufficient to harvest more than 100 L of algal culture (at concentration: 2×10^6 cell/ml) (Sathe and Durand, 2015). This shows that bioflocculation can lower the biomass harvesting costs by providing a nearly cost-free pre-concentration step.

After the pre-concentration technique, gravity settling led to biomass concentrations comparable to those of a first concentration step of microalgae harvesting, but did not use electricity or extra materials like in filtration or centrifugation. For instance, for achieving biomass concentrations of 1-5% TSS, vibrating screen filter uses between 0.5 - 3 kWh/ m^3; gravity sedimentation with a lamella separator uses 0.1 – 0.3 kWh/ m^3 and air flotation uses 0.1 – 2 kWh/ m^3 (Uduman et al., 2010). Due to the differences of energy requirements, the cost of separation processes such as sedimentation with flocculants (around 0.25 €/m^3) has been reported much lower than flotation with flocculants (0.7 €/m^3) and centrifugation harvesting technique (0.8 €/m^3) (Granados et al., 2012).

4

Tannin-based flocculants (I): Static column tests

This chapter is based on the article:

Gutiérrez, R., Passos, F., Ferrer, I., Uggetti, E., García, J. (2015) Harvesting microalgae from wastewater treatment systems with natural flocculants: Effect on biomass settling and biogas production. Algal Research 9, 204-211

4.1 Introduction

Treatment of wastewater with microalgal cultures has the major advantage of producing biomass that can be valorized to produce bioenergy or molecules of interest. In fact, energy production and resources recovery have been identified as one of the main challenges for wastewater treatment systems of the future by relevant initiatives such as the recently created European Innovation Partnership on Water. However, microalgal wastewater treatment systems such high rate algal ponds (HRAP) have some bottlenecks like biomass separation (Christenson and Sims, 2011; Vandamme et al., 2009). Since the invention and development of HRAP in California in the 1950s, the problem of algal biomass separation has remained unsolved. The main constraint is related to the fact that wastewater is a product without market value, and therefore any added cost to the treatment system (such as the implementation of an intensive harvesting system) cannot be recovered. Nevertheless, this paradigm may change in the near future if biomass is valorized to obtain bioenergy or resources, since biomass will then have a market value.

Microalgal harvesting and thickening can be achieved by means of several techniques including coagulation-flocculation and sedimentation, flotation, centrifugation, magnetic separation and electrophoresis (Danquah et al., 2009c; de Godos et al., 2011; Granados et al., 2012; Salim et al., 2011; Smith and Davis, 2012). However, in the context of wastewater treatment, only low-cost techniques capable of managing large volumes of water and biomass can be applied, such as coagulation-flocculation followed by a solid/liquid separation. Indeed, coagulation-flocculation and sedimentation may lead to a solids concentration in microalgal biomass from 1 to 5% w/w (Smith and Davis, 2012), which is appropriate for downstream processes such as biogas production.

Coagulation consists of neutralising negative surface charges of colloidal particles (in this case microalgae), while flocculation is the aggregation of neutralized particles followed by flocs formation. Coagulants that have been traditionally used in water and wastewater treatment are salts of aluminum or iron. However, these substances have a limited application in microalgal systems because they can contaminate downstream products restricting biomass valorization (Danquah et al., 2009c; Zheng et al., 2012). This drawback may be overcome by using natural organic coagulants like tannin based polymers or

modified starch which are being increasingly used since the 80s (Vandamme et al., 2012). These types of coagulants (also referred to "flocculants", as from now in the text) are becoming very popular in the field of water treatment as substitutes for polyacrylamide based flocculants due to health concerns (Vandamme et al., 2009). Previous studies on microalgae coagulation-flocculation and sedimentation with different types of organic polymers have shown promising results in terms of separation efficiency (Table 4.1).

Table 4.1 Literature results on microalgal biomass harvesting by coagulation-flocculation and sedimentation with different types of organic polymers.

Microalgae	Flocculant	Dose	Biomass recovery	Reference
Tetraselmis suecica	Zetag 7650 + $Al_2(SO_4)_3$	5-50 mg/L (Zetag 7650) + 50 mg/L ($Al_2(SO_4)_3$)	~100 %	(Danquah et al., 2009a)
Parachlorella	Cationic starch (Cargill C*Bond HR 35.849)	120 mg/L	>95%	(Vandamme et al., 2010)
Scenedesmus	Cationic starch (Greenfloc 120)	20 mg/L	>90%	(Vandamme et al., 2010)
Scenedesmus dimorphus	Cationic starches	10-100 mg/L	70 to 95%	(Hansel et al., 2014)
Microalgal-bacteria consortia	Drewfloc 447, Flocudex CS/5000, Flocusol CM/78, Chemifloc CV/300 and Chitosan	25-50 mg/L	58 to 99 %	(de Godos et al., 2011)
Microcystis aeruginosa	Chitosan + Fe_3O_4	1.6 mg/L (Chitosan) + 4-6 mg/L (Fe_3O_4)	99%	(Liu et al., 2009)
Spirulina, Oscillatoria and *Chlorella*	Chitosan	15 mg/L	90%	(Divakaran and Pillai, 2003)
Microalgal-bacterial consortia	Chitosan	214 mg/L	92 %	(Riaño et al., 2012)
Chlorella Sorokiniana	Chitosan	10 mg/L	90%	(Xu et al., 2013)
Phaeodactylum tricornutum	Chitosan	20 mg/L	80-90%	(Şirin et al., 2011)

In the field of wastewater treatment, biogas production is perhaps the most straightforward option for microalgal biomass valorization (Salerno et al., 2009; Ward et al., 2014). Indeed, anaerobic digestion has a long tradition in the context of wastewater treatment and this expertise fully justifies the use of microalgae for this purpose. Nevertheless, if microalgae are separated and thickened with coagulation-flocculation and sedimentation it is evident that flocculants should not be toxic or inhibit the anaerobic digestion process. Natural organic flocculants could meet this requirement; to our knowledge though it has yet to be confirmed.

The objective of the present study is to evaluate two tannin-based cationic flocculants for coagulation-flocculation and sedimentation of microalgae grown in experimental HRAP for wastewater treatment. In particular the study aimed at: 1) determining the optimal flocculants doses with jar tests, 2) studying the settling of formed flocs using settling column tests, and 3) assessing the effect of flocculants on biomass anaerobic digestion by means of biochemical methane potential tests. To the best of our knowledge, this is the first time that natural flocculants are evaluated not only on their efficiency, but also on their effect on downstream processing.

4.2 Material and Methods

4.2.1 Microalgal biomass

Experiments were carried out at the laboratory of the GEMMA research group (Universitat Politècnica de Catalunya·BarcelonaTech, Barcelona, Spain). Microalgal biomass was grown in an experimental plant that had been in continuous operation for more than 1 year. Urban wastewater was pumped from a nearby municipal sewer and conveyed to a primary settler. Following, primary treated wastewater was continuously fed (60 L/d) to an experimental HRAP; a raceway pond with a volume of 0.47 m^3 and a nominal hydraulic retention time of 8 days. Average loading rates of the HRAP were 24 g COD/m^2·day and 4 g NH_4-N/m^2·day. Microalgal biomass grown in the HRAP was separated in a clarifier connected in series with the HRAP (without coagulation-flocculation). A detailed description of the wastewater treatment system and its operation and performance may be found elsewhere (Passos et al., 2013b).

In the present study, microalgal biomass term is referred to the microalgal-bacterial biomass grown in the HRAP. The biomass concentration of the HRAP mixed liquor ranged from 0.06 to 0.6 g TSS/L over the year and consists of consortia of microalgae as well as bacteria, microalgae accounting for much of the biomass (over 90% of the biomass according to (García et al., 2006). Average microalgal biomass production was 9.4 g TSS/m^2.d, However, without flocculants, harvested biomass corresponds to approximately 5 g TSS/m^2.d, since 45 % of the produced biomass escaped from the settler. The biomass was characterized by an average VS/TS ratio of 60 % VS/TS, being most of the organic matter in particulate form as indicated by the low VSS/VS (0.89 %) and CODs/COD (0.72 %) ratios. During the experimental period, microalgal population was mainly composed by green algae belonging to genus *Monoraphidium* sp., *Scenedesmus* sp. and *Stigeoclorium* sp. and the diatoms *Nitzchia* sp., *Navicula* sp. and *Amphora* sp. (Fig. 4.1).

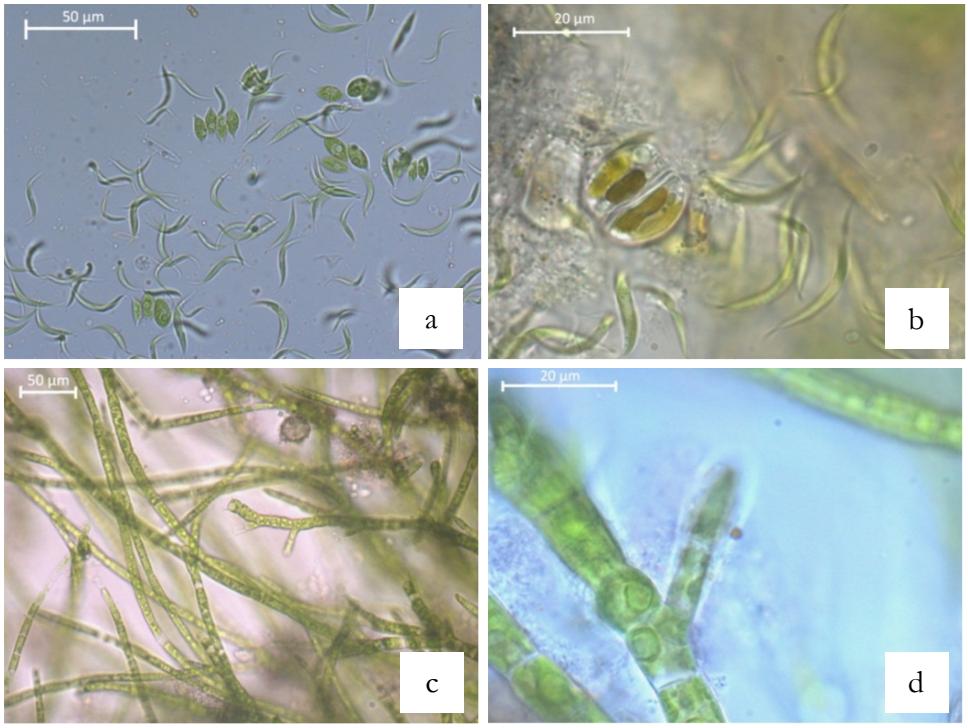


Figure 4.1 Microalgae identified in the mixed liquor of the experimental HRAP (a) *Monoraphidium* sp. (elongated fusiform twisted cells) and *Scenedesmus* sp. (coenobia with elliptical cells). (b) *Monoraphidium* sp. and Amphora sp. (two adhered cells can be observed) (c) General view of *Stigeoclonium* sp. d Detail of *Stigeoclonium* sp. (d) Detail of *Stigeoclonium* sp.

Samples were collected from the HRAP on a weekly basis and analyzed in triplicate. Total solids (TS), total suspended solids (TSS), volatile solids (VS), volatile suspended solids (VSS), chemical oxygen demand (COD) and soluble chemical oxygen demand (CODs) were determined according to Standard Methods (APHA AWWA-WPCF, 1999). Moreover, microalgae images were taken with an optic microscope (Aixoplan Zeiss, Germany), equipped with a camera MRc5, using the software Axioplan LE. Microalgae genera were identified using conventional taxonomic books (Bourelly, 1966; Palmer, 1962).

4.2.2 Tannin-based flocculants

Harvesting properties of two cationic tannin-based flocculants were investigated on the samples of the HRAP mixed liquor. Ecotan AR® (Servyeco, Spain) and Tanfloc SG® (Tanac SA, Brazil) are natural cationic flocculants extracted from the bark of *Acacia mearnsii* having strong coagulating properties. None of the flocculants modifies the pH of the medium significantly and both of them are effective over a pH range of 4.5-8 (9 for *Ecotan*). *Ecotan* was provided in liquid form with a concentration of 0.3 g/L, while *Tanfloc* was supplied as a dry product that was dissolved in water until complete solution. Both flocculants are suitable for wastewater treatment applications, and were conceived to replace metal-based products with aluminum and iron chlorides.

Stock solutions of 1000 mg/L were prepared for each flocculant prior to jar tests, column settling tests and biochemical methane potential (BMP) tests.

4.2.3 Jar tests

Jar tests were used to determine the optimal dose of each flocculant following standard protocols employed in the water and wastewater treatment fields using common jar test equipment (Metcalf and Eddy, 2004). During one week, HRAP liquor samples were taken and two jar tests were carried out for each flocculant in order to determine the optimal concentration for coagulation-flocculation and sedimentation tests. The range of flocculant doses for jar tests was selected after previous trials in which it was observed that optimal doses ranged between 10 and 60 mg/L. Thus, flocculants concentrations were: 10, 20, 30, 40, 50 and 60 mg/L. Altogether, five jar test replicates were performed for each flocculant.

In each experiment aliquots of 500 mL were placed in six beakers. Increasing flocculant concentrations were simultaneously added to each beaker, intensively stirred (200 rpm) for 1 minute, stimulating the coagulation process. Following, beakers were gently stirred (35 rpm) for 15 minutes, enhancing the flocculation process. Finally, formed flocs were allowed to settle (without stirring) for 15 minutes (sedimentation process). Images of the three jar test steps are shown in Figure 4.2. At the end of the process, supernatant liquid samples were taken from each beaker; turbidity and pH were measured with a HI93703 Hanna Instruments Turbidimeter and a Crison 506 pH-meter, respectively. Turbidity and pH were also measured from the mixed liquor without flocculants addition. Biomass recovery (RE) was calculated based on the initial (T_i) and final (T_f) turbidity measurements (Eq. 4.1).

$$RE(\%) = \frac{T_i - T_f}{T_i} * 100 \qquad \text{(Eq. 4.1)}$$

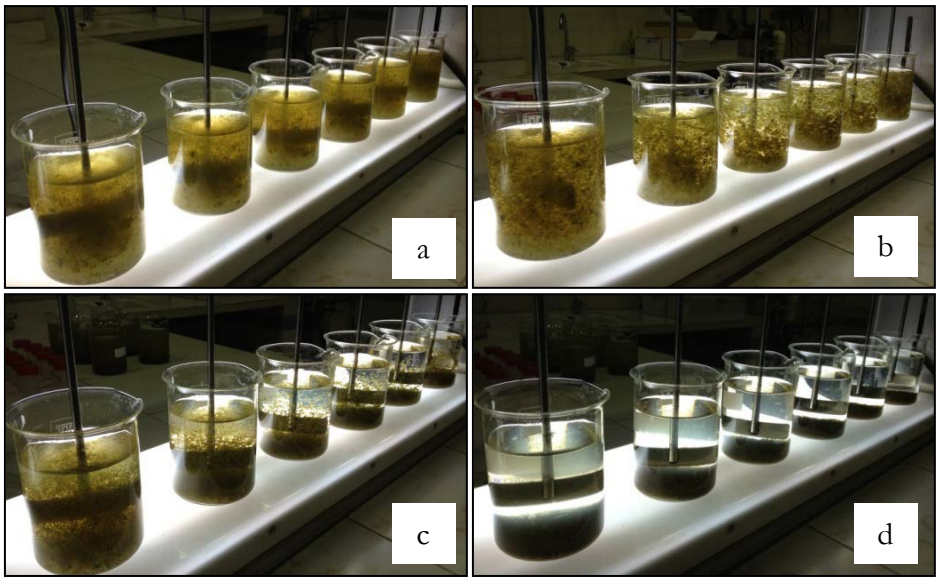


Figure 4.2 Different steps of the jar test after flocculant addition at doses ranging from 10 to 60 mg/L. (a) Coagulation step (200 rpm, 1 min); (b) Flocculation step (35 rpm, 15 min;); (c) Sedimentation, initial stage (1 min); (d) Sedimentation, final stage (15 min).

4.2.4 Static sedimentation test

In order to evaluate the settling of flocculated biomass, static column settling tests were conducted using standard procedures employed in the water treatment field (Metcalf and Eddy, 2004). The column had a total height of 45.5 cm, an internal diameter of 8.5 cm and four sampling ports at intermediate depths (at 12.5, 20, 30 and 40 cm). Optimal doses found in jar tests were used to perform four tests per flocculant. Specifically, two tests per week (one for each flocculant) were performed during 1 month of experimentation. Each week, settling column test samples were taken within 24h to assure no variation on physicochemical characteristics.

The test was carried out as follows: 1) HRAP mixed liquor aliquots were coagulated and flocculated in the jar test device, adding optimal dose of each flocculant; 2) the resulting sample (2.65 L) was gently poured into the sedimentation column to prevent breakage of formed flocs; 3) samples of 10 mL were withdrawn from the four sampling ports along the column at different time intervals over 24 hours (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210, 240, 270 min and 24 hours). The turbidity of each sample was immediately measured. Thus, biomass recovery (RE) was calculated for each column's depth and sedimentation time according to Eq. 4.1. (Metcalf and Eddy, 2004).

4.2.5 Biochemical methane potential tests

Biochemical methane potential (BMP) tests were used to compare microalgae anaerobic biodegradability with and without flocculant addition. For this reason, BMP tests were performed in triplicate with samples of flocculated microalgal biomass with *Ecotan* and *Tanfloc* (with the optimal dose for each flocculant), together with a sample without flocculant (control). Blank trials containing only inoculum were also performed. Digested sludge from a full-scale anaerobic reactor located in a municipal wastewater treatment plant near Barcelona (Spain) was used as inoculum at a substrate/inoculum ratio of 0.5 g COD/g VS, defined as the optimal ratio by (Passos et al., 2013a).

Serum bottles had a total volume of 160 mL and a useful volume of 100 mL. The concentration of microalgal biomass after jar test was 23.8 g COD/L and 20.1 g COD/L corresponding to 21.0 and 25.0 g microalgal biomass/bottle for

Ecotan and *Tanfloc*, respectively. Following, inoculum was added to each trial (42 g VS/ bottle) and bottles were filled with distilled water to reach the useful volume (100 mL). Afterwards, bottles were flushed with Helium gas, sealed with butyl rubber stoppers and incubated at 35 ºC until biogas production ceased. Biogas production was periodically determined by measuring the pressure increase in the headspace volume with an electronic manometer (Greisinger GMH 3151). After each measurement gas was released until atmospheric pressure. Samples from the headspace volume were taken every 2-3 days to determine biogas composition (CH_4/CO_2) by gas chromatography (GC Trace, Thermo, Finnigan) following the procedure described by (Passos et al., 2013b).

Accumulated volumetric methane production (mL) was calculated from the pressure increase and methane content in biogas, expressed under standard conditions. The net values of methane production and yield were obtained by subtracting the endogenous production of blank trials, containing only inoculum.

4.3 Results and Discussion

4.3.1 Optimal doses of flocculants

In order to improve microalgal biomass recovery, coagulation-flocculation was tested with two natural cationic flocculants, *Ecotan* and *Tanfloc*. During the experimental period, microalgal biomass turbidity varied from 277 to 573 NTU. However, for each jar test, the same biomass (with the same turbidity) was used with both flocculants. Biomass recovery ranged from 91.8 to 99.4% and from 51.6 to 93.3% with *Ecotan* and *Tanfloc*, respectively (Table 4.2).

The optimal dose (boldfaced in Table 4.2) was based on the lowest concentration of flocculant ensuring over 90% biomass recovery. Regarding *Ecotan*, 91.8% of microalgal biomass was removed with a concentration of 10 mg/L. In contrast, a higher *Tanfloc* concentration (50 mg/L) obtained a similar efficiency 90.2%. Note that flocculation efficiency depends on biomass concentration (Gerde et al., 2014), for this reason tests with both flocculants were carried out using the same microalgal biomass concentration (2.8 g TS/L corresponding to 0.8 g VS/L). Although validation under full-scale conditions is

needed, this study underlines the high biomass recovery potential of both flocculants (>90%). Upon full-scale validation, an optimal dose adjustment would be needed. Indeed, dose lower than 10 mg/L may be sufficient, due to the successful results obtained with a flocculant dose of 10 mg/L in this study.

Table 4.2 Jar test results with Ecotan and Tanfloc flocculants (n=5). Microalgal biomass recovery was calculated from residual turbidity values. The optimal dose is the minimum leading to more than 90% biomass recovery (grayed).

Flocculant	Dose (mg/L)	Turbidity (NTU)	Biomass recovery (%)	pH
Ecotan	0	385.0 (142.0)	-	8.4 (0.2)
OPTIMAL DOSE	**10**	**35.0 (25.5)**	**91.8 (3.0)**	**7.7 (0.3)**
	20	8.3 (7.7)	98.2 (1.1)	7.2 (0.3)
	30	1.9 (1.7)	99.6 (0.3)	7.1 (0.3)
	40	0.9 (0.7)	99.8 (0.2)	6.9 (0.4)
	50	0.9 (0.6)	99.7 (0.2)	6.8 (0.4)
	60	2.2 (1.3)	99.4 (0.5)	6.7 (0.5)
Tanfloc	0	385.0 (142.0)	-	8.4 (0.2)
	10	181.2 (151.2)	51.6 (10.1)	8.4 (0.2)
	20	119.8 (69.9)	70.5 (91.3)	8.2 (0.4)
	30	68.7 (43.5)	83.3 (6.3)	8.1 (0.4)
	40	48.1 (29.0)	88.2 (4.2)	8.0 (0.4)
OPTIMAL DOSE	**50**	**40.7 (27.6)**	**90.2 (4.4)**	**7.9 (0.5)**
	60	27.6 (18.2)	93.3 (2.7)	7.7 (0.5)

(Golueke and Oswald, 1965) were the first studying coagulation-flocculation of microalgae grown in wastewater. The authors reported that polyvalent organic polymers were successful at flocculating *Scenedesmus* sp. and *Chlorella* sp. Authors tested *Puriflocs 601/02* and *Sondellite*, using optimal doses of 10 mg/L in all cases. Both flocculants led to 100% microalgal biomass recovery. (de Godos et al., 2011) assessed the coagulation-flocculation efficiency of five different organic

polymers on a *Chlorella* sp. consortium with bacteria. Three of the tested coagulants (CHEMIFLOC CV-300, Drewfloc 447, Flocusol CM-78) achieved microalgal biomass recoveries similar to our study (94, 99 and 93% respectively). Flocculant dose was 50 mg/L, except for one of the flocculants (Flocudez CS-5000) which enabled 95% biomass recovery with 25 mg/L. In the same study, (de Godos et al., 2011) compared microalgae recovery with ferric metal salts and found that 5 to 6-times lower doses were needed for organic polymers in comparison with tested conventional chemical flocculants. Notwithstanding, optimal concentrations for all flocculants (25-50 mg/L) were similar to *Tanfloc* but higher than *Ecotan* (10 mg/L) doses found in the present study. However, comparisons are not straight forward since recovery efficiencies strictly depend on biomass concentration.

Beyond enhancing biomass recovery efficiency, flocculants should not hinder downstream biological conversion techniques for bioenergy generation. Indeed, conventional chemical flocculants, such as ferric and aluminum salts, consume alkalinity and decrease pH. This may affect the final effluent quality and biomass reuse. However, the flocculants used in this study have a cationic character, which may prevent alkalinity consumption; reducing pH values by less than 1 point (Table 4.2). Indeed for the optimal dose of *Ecotan* and *Tanfloc* (10 mg/L and 50 mg/L, respectively) the pH values decreased by 0.7 and 0.5 with *Ecotan* and *Tanfloc,* respectively. Propitiously, pH values stabilized to 7.7 and 7.9, within the appropriate range for anaerobic digestion (6.0-8.3) (Angelidaki and Sanders, 2004).

4.3.2 Settling of formed flocs

Microalgae settling was evaluated in settling column tests with and without flocculants addition. Firstly, a column test was performed without flocculants (control). Afterwards, two tests per week (one for each flocculant) were performed over 1 month, obtaining 4 replicates for each flocculant. Mixed liquor turbidity varied over the experimental period, average values of 294 and 378 NTU were obtained for *Tanfloc* and *Ecotan* samples, respectively. As described in the methods section, recovery percentages at different time intervals were calculated from turbidity measurements before and after settling at different times and column depths.

Figure 4.3 represents the biomass recovery at different sampling time; each curve corresponds to a sampling depth (12.5, 20, 30 and 40 cm). Subsequently, for each curve corresponding to one sampling depth, the time required to attain a certain biomass recoveries (80, 85 and 90%) was obtained (Table 4.3). Thus, curves of isorecovery were plotted in relation with sampling depth and settling time for the control (Fig. 4.4), *Ecotan* (Fig. 4.5) and *Tanfloc* (Fig. 4.6). Each curve traces the settling velocities of the biomass and shows the time needed to reach a certain biomass recovery. For instance, 80% biomass recovery was achieved within 80 min and 90% biomass recovery within 50 min at the port corresponding to 20 cm depth. From these curves, the settling velocities for each biomass recovery (80, 85 and 90%) were determined by dividing the column depth (d_x) by the time elapsed (t_x) (Table 4.4). The settling velocity is one of the key parameters for settler design (i.e. surface loading rate).

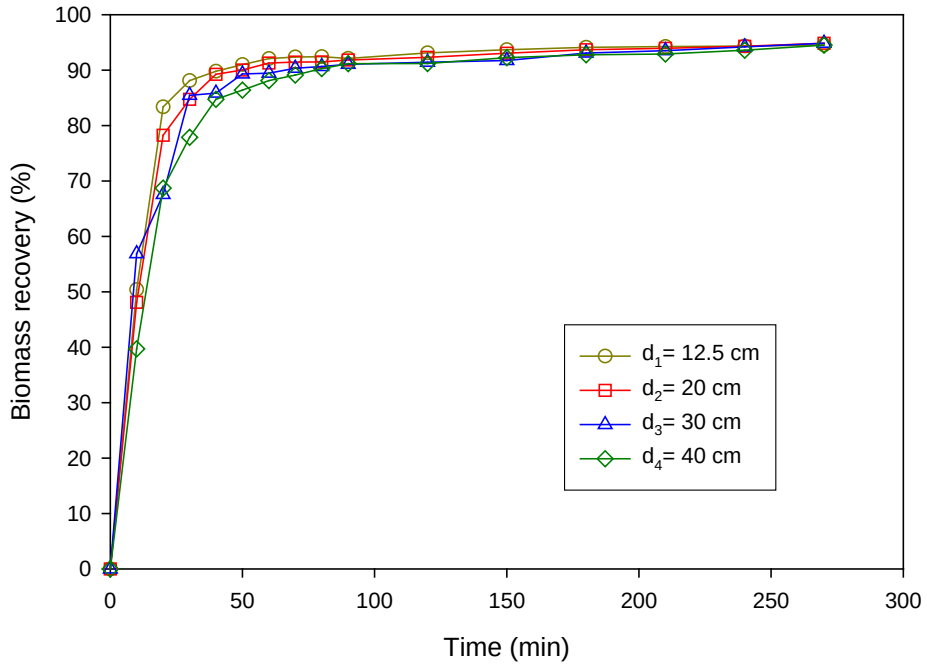


Figure 4.3 Biomass recovery along for the four column depths (without flocculant addition).

Table 4.3 Time interval to attain each biomass recovery at different column depths (without flocculant addition). Time intervals are obtained from isorecovery curves for the 4 sampling depth (Fig. 4.4).

Column depth (cm)	Time (min)		
	80%	85%	90%
d_1=12,5 cm	18.3	21.33	40
d_2=20 cm	21.15	30	48.67
d_3=30 cm	26.42	29.63	65.53
d_4=40 cm	32.63	65.53	79

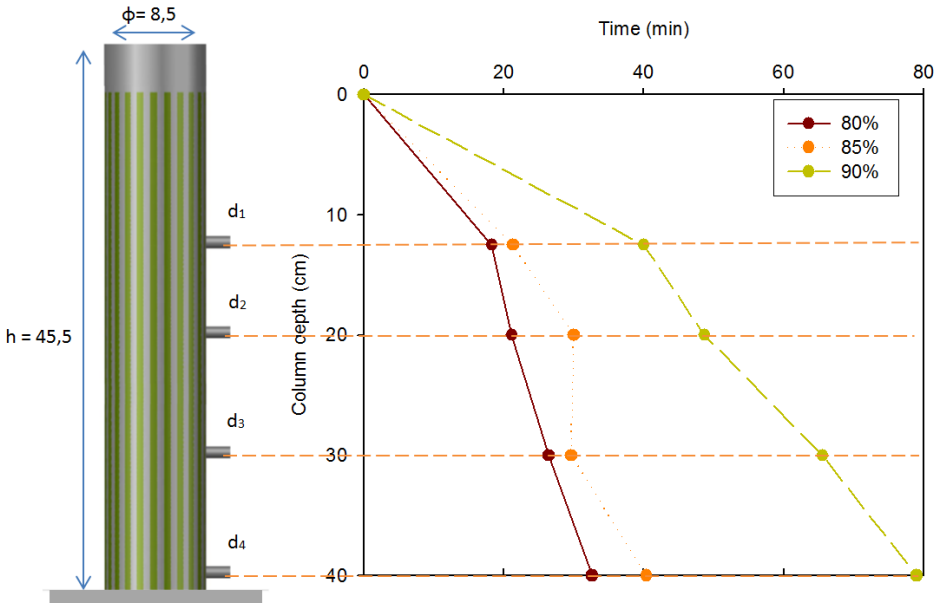


Figure 4.4 Microalgal biomass isorecovery curves (80, 85, and 90%) without flocculant addition. Curves are calculated from data summarized in Table 4.3.

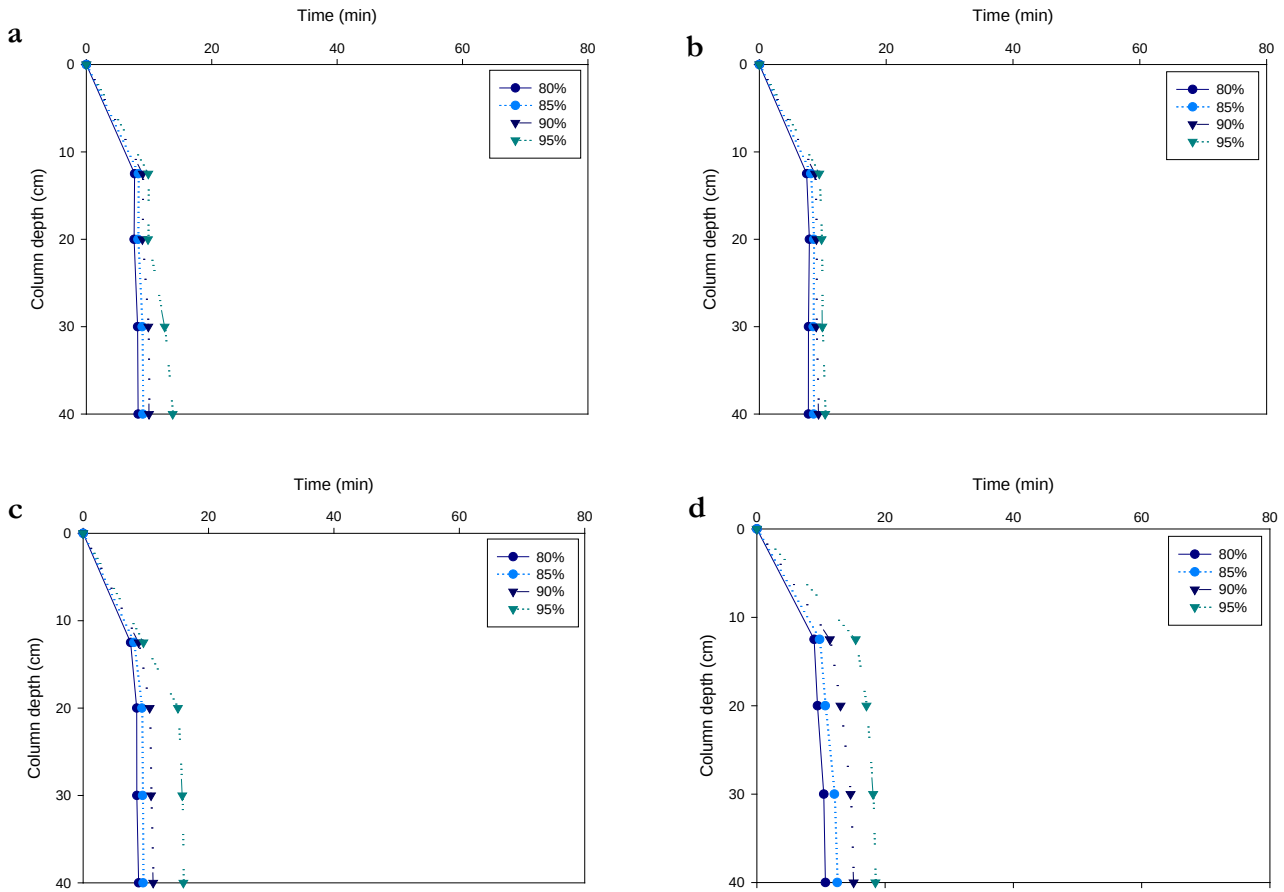


Figure 4.5 Microalgal biomass isorecovery curves (80, 85, 90, and 95 %) after coagulation-flocculation with an Ecotan dose of 10 mg/L. Each figure corresponds to one of the replicates carried out over one month.

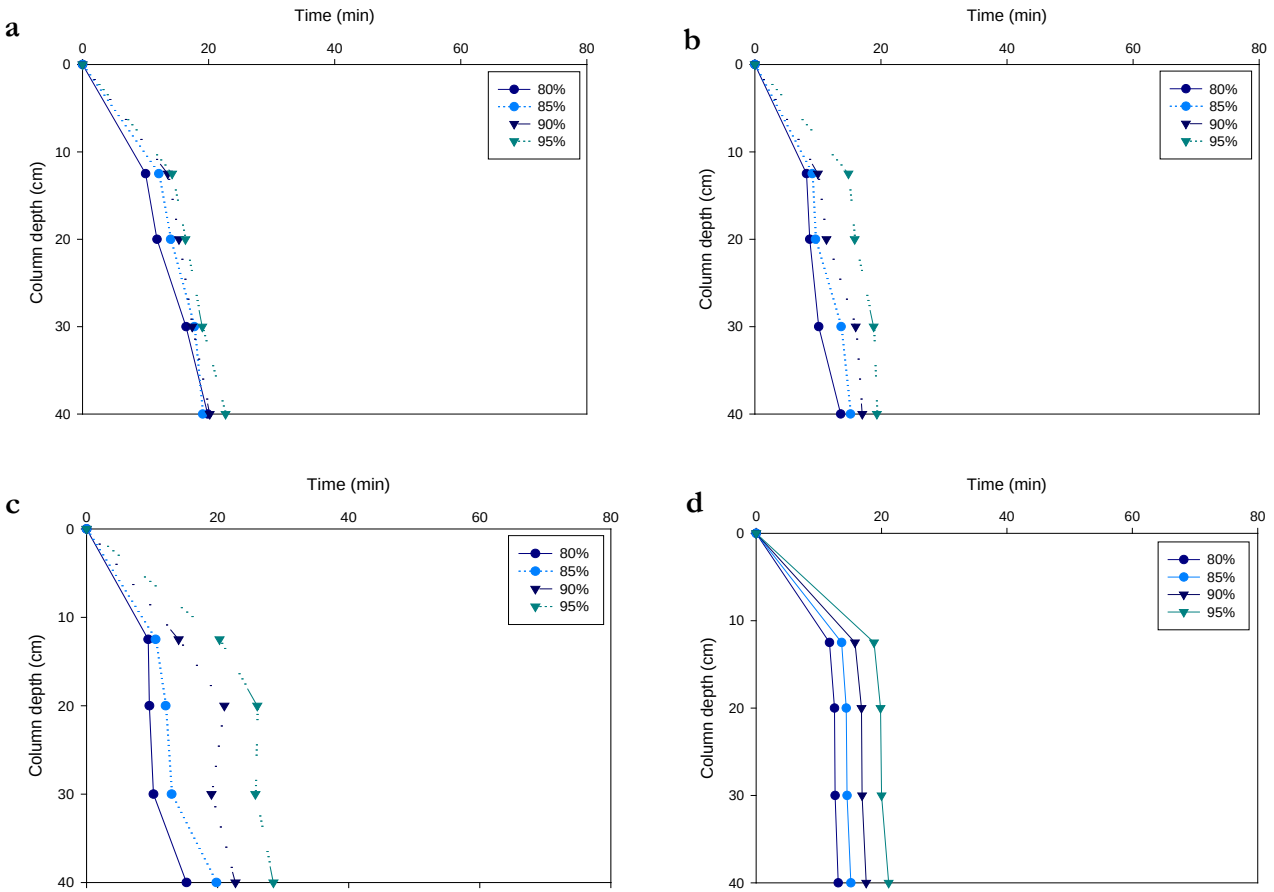


Figure 4.6 Microalgal biomass isorecovery curves (80, 85, 90, and 95 %) after coagulation-flocculation with a Tanfloc dose of 50 mg/L. Each figure corresponds to one of the replicates carried out over one month.

Table 4.4 Velocities to attain 90% biomass recovery at different column depths (with and without flocculants addition).

Column depth (cm)	Velocity (mm/s)		
	Control (without flocculant)	*Ecotan*	*Tanfloc*
d_1=12,5 cm	0.05	0.21	0.16
d_2= 20 cm	0.07	0.29	0.22
d_3= 30 cm	0.08	0.42	0.29
d_4= 40 cm	0.08	0.56	0.35

It is relevant that without flocculant addition the velocities obtained along the column were fairly constant (0.05-0.08 mm/s) and much lower than those obtained with flocculated samples (0.21-0.56 mm/s with *Ecotan* and 0.16-0.35 mm/s with *Tanfloc*). Comparing flocculants, higher velocities along the column (with a maximum of 0.56 mm/s at 40 cm), were obtained with *Ecotan* (10 mg/L). In contrast, higher doses of *Tanfloc* (50 mg/L) yielded slower settling velocities of about 0.35 mm/s. A conventional gravity settler should be designed in order to retain 90% of the biomass with a surface loading rate of 0.3 mm/s (Metcalf and Eddy, 2004). Considering the settling velocities obtained in this study (Table 4.4), a velocity of 0.3 mm/s was obtained at column depths of 20 and 30 cm for *Ecotan* and *Tanfloc*, respectively. Therefore, settler surface area with *Ecotan* would be 2-times smaller than *Tanfloc* and 8-times without flocculant.

As can be seen by comparing Figures 4.4, 4.5 and 4.6, samples with an optimal dose of *Ecotan* (Fig. 4.5) and *Tanfloc* (Fig. 4.6) had much more higher settling velocities than samples without flocculant (Fig. 4.4). For instance, without flocculant, 90% of the biomass was settled at port $d_{4=}$40 cm after 80 min by adding (Fig. 4.4), while after *Ecotan* and *Tanfloc* addition, 90-95% biomass recovery took place after less than 20 and 10 min, respectively (Fig. 4.5 and 4.6).

4.3.3 Biochemical methane potential

The addition of chemicals to induce biomass flocculation may contaminate microalgal biomass and make it unsuitable for further uses. According to the literature, some organic and natural flocculants did not affect the microalgae biodegradability, while some inorganic (metal-based) flocculants modified

biomass characteristics at high doses (Papazi et al., 2009; Wan et al., 2014). To date, there are no literature results on the effect of organic flocculants on the anaerobic digestion of microalgae.

In this study, BMP tests were performed with flocculated algal biomass under optimal doses of each coagulant. The same mixed liquor sample was used for both flocculants (160 NTU). As shown in Figure 4.7, none of the studied natural flocculants showed a clear effect on the anaerobic biodegradability of flocculated microalgal biomass in BMP tests. Accumulated methane yield was 163 mL CH_4/g VS for the control (gravity settled microalgae) and 162 and 166 mL CH_4/g VS after coagulation-flocculation with *Ecotan* and *Tanfloc*, respectively. The methane content in biogas was the same in all cases (70 %). The methane yield was comparable with that achieved in our previous studies with microalgal biomass grown in domestic wastewater (Passos et al., 2014, 2013a).

Due to the lack of literature results on the effect of organic flocculants on the anaerobic digestion of harvested microalgae, the results are here compared with flocculated waste activated sludge.

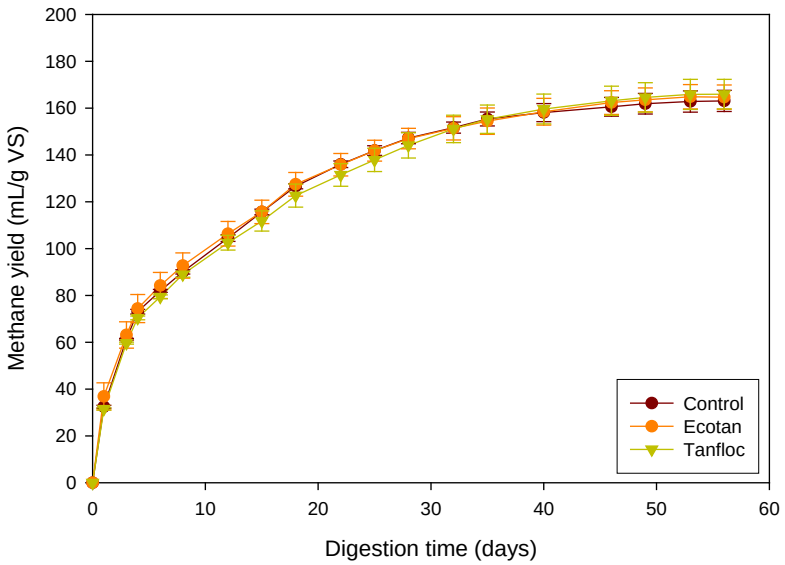


Figure 4.7 Methane yield of microalgal biomass without flocculants (control) and after coagulation-flocculation with the optimal dose of Ecotan (10 mg/L) and Tanfloc (50 mg/L) (n= 3).

According to (Chu et al., 2003), synthetic polyelectrolyte flocculants may have a different effect depending on the digestion stages. The authors observed how flocculated biomass presented higher solubilisation and methane production (about 55 $gCH_4/kgTS$) in comparison with the control sample (about 45 $gCH_4/kgTS$) during the first 6 days; while after 10 days of digestion the methane production seemed to be inhibited. This was attributed to the greater size of flocs, which may impede efficient bacteria movement within the sludge flocs, hindering the subsequent acidogenic and the methanogenic stages to produce methane.

As previously discussed, the pH remained within the optimum range for anaerobic digestion after adding optimal doses of both flocculants. Furthermore, the flocculants used in this study were natural polymers, which are neither corrosive nor toxic. This was attested by the BMP test, which indicates that *Ecotan* and *Tanfloc* do not show any effect on the anaerobic digestion of harvested biomass.

4.4 Conclusions

In this study microalgal biomass produced in an experimental HRAP treating urban wastewater was used to test the harvesting efficiency of two natural flocculants. The natural flocculants *Ecotan* and *Tanfloc* attained microalgal biomass recoveries over 90% with low concentrations (10 and 50 mg/L for *Ecotan* and *Tanfloc*, respectively). With this dose sedimentation of flocculated biomass was achieved within 10 minutes for *Ecotan* and 20 minutes for *Tanfloc* in a 40 cm column, compared to 80 minutes for microalgal biomass sedimentation without flocculants. This means that larger volumes of microalgae suspension could be recovered in less time and/or smaller settlers. None of the studied flocculants showed negative impacts in terms of pH and anaerobic biodegradability as shown in BMP tests with flocculated biomass (162-166 $mLCH_4/gVS$).

5

Tannin-based flocculants (II): Dynamic sedimentation test

This chapter is based on the article:

Gutiérrez, R., García, J., Uggetti, E., Arnabat, C., Salvadó, H., Ferrer, I., (submitted) Settling velocities distribution of microalgal biomass from urban wastewater treatment high rate algal ponds. Algal Research

5.1 Introduction

Microalgae-based wastewater treatment systems constitute an alternative technology to conventional wastewater treatment plants (WWTP) which has aroused a growing scientific interest in the last years (Pittman et al., 2011). These systems, while removing contaminants from wastewater, allow the production of microalgal biomass, which can be valorised for example as substrate for anaerobic digestion to produce biogas and biofertiliser. However, a necessary condition to achieve self-sufficient systems from an energy perspective is to ensure efficient and cost-effective microalgal biomass harvesting techniques.

Indeed, biomass harvesting is probably the main bottleneck hampering the application of full-scale microalgae-based wastewater treatment systems (Christenson and Sims, 2011; de Godos et al., 2011). Usual solids separation techniques applied in WWTP such as conventional sedimentation (without coagulation-flocculation) have low harvesting efficiency (60-70%) (García et al., 2000a). In the case of microalgae production for high value-added compounds, where very high harvesting efficiencies are required (>99%), sophisticated techniques are used and they represent 20-30% of the total production costs (Barros et al., 2015; Brennan and Owende, 2010; Chisti, 2007b; Pires et al., 2013).

Small size (few micrometres), relatively intrinsic low concentration (0.2-2 g/L) and their colloidal stability are the main reasons that make microalgae difficult to recover. Nowadays great research efforts are being conducted to develop efficient and cost-effective harvesting technologies (Gerde et al., 2014; Rawat et al., 2011; Vandamme et al., 2013). The most suitable technology for each particular application depends mostly on the required moisture content of harvested biomass, and on its cost (Grima et al., 2003; Misra et al., 2014). While centrifugation and rapid filtration may be feasible for producing high value-added compounds that require a very concentrated biomass, the combination of coagulation-flocculation followed by sedimentation may be the most suitable technique for low-cost products such as biogas. In fact, coagulation-flocculation and sedimentation is regarded by different authors as the unique cost-effective and easily scalable technique for microalgae-based wastewater treatment systems (Grima et al., 2003; Muñoz and Guieysse, 2006; Vandamme et al., 2013; Xu et al., 2013).

In coagulation-flocculation and sedimentation processes, the surface charge of

microalgae cells is neutralised and therefore dispersed single cells can aggregate to form flocs which settle by gravity. Both metal-based coagulants (i.e. aluminium sulphate or iron chloride) and organic polymeric compounds (i.e. chitosan or starch) have been studied in the context of microalgae harvesting (de Godos et al., 2011; Gerde et al., 2014; Gutiérrez et al., 2015a, 2015b; Hansel et al., 2014; Riaño et al., 2012; Vandamme et al., 2010). However, the use of metal-based coagulants can make the biomass useless for downstream processes (Danquah et al., 2009a; Kim et al., 2013; Zheng et al., 2012). In contrast, organic compounds are usually biodegradable and do not hamper processes such as anaerobic digestion (Gutiérrez et al., 2015a, 2015b). For instance, some polyelectrolytes such as tannin-based and starch-based polymeric flocculants widely employed in the water treatment industry, have shown promising results in terms of anaerobic digestion performance and biogas production (Campos et al., 2008; Gutiérrez et al., 2015a, 2015b; Krishnan et al., 2006).

Nevertheless, information on settling properties of flocculated microalgae with polymeric flocculants is completely lacking (Su et al., 2012). Only a few studies have investigated specific microalgae physical characteristics (i.e. settling velocity, floc size and concentration factor) (Su et al., 2012; Vandamme et al., 2014). However, a deep characterization of the settling velocities distribution and the microalgae species composition of the microalgae population cultivated in wastewater is missing in literature. The settling velocities distribution of flocculated microalgal biomass is a crucial factor for designing cost-effective gravity settlers for biomass recovery. Therefore, the objectives of the present study are on the one hand to evaluate microalgal biomass settling velocities distribution and, on the other hand, to improve this velocity by adding a polymeric flocculant. Dynamic sedimentation tests were used to achieve this goal. The main advantage of these tests over classical settling column tests is that settling velocity is evaluated under real dynamic conditions. Also, microscopic examination of samples from sedimentation tests was conducted to help interpret the results.

5.2 Material and Methods

5.2.1 Microalgal biomass

Microalgal biomass was obtained from the mixed liquor of two experimental high rate algal ponds (HRAPs) located outdoors at the laboratory of the

GEMMA research group (Universitat Politècnica de Catalunya·BarcelonaTech, Barcelona, Spain). Note that in HRAPs mixed populations of microalgae, bacteria, protozoa and small metazoans coexist spontaneously, forming flocs with different size and settling velocities (García et al., 2000a; Smith and Davis, 2012). Microalgae represent most of the biomass (80-90%) (García et al., 2006; Park and Craggs, 2010). The experimental HRAPs were operated uninterruptedly for 3 years prior to the experiments here presented. The HRAPs were open raceway ponds (0.47 m^3 of volume each, and 0.3 m of depth), equipped with paddle-wheels for mixing and fed with primary treated wastewater. Daily, urban wastewater was pumped from a near municipal sewer to a 1 m^3 homogenisation tank. After that, wastewater was treated in primary settlers (7 L of volume and 0.9 h of hydraulic retention time (HRT)) and then drawn in each HRAP by means of two peristaltic pumps. Each HRAP was fed with a different continuous flow of wastewater: 60 L/day and 120 L/day, giving as a result different hydraulic retention times (theoretical HRT of 8 and 4 days, respectively) and consequently microalgal biomass with different properties. The effluent of each HRAP was conveyed to secondary settlers for biomass recovery. Further details of this pilot wastewater treatment system, operation and performance may be found in Passos et al. (Passos et al., 2015).

5.2.2 Dynamic sedimentation test

Dynamic sedimentation tests were carried out using a water-current separation technique in which biomass flocs are washed out according to their relative density, volume and form, under dynamic conditions (Gutiérrez et al., 2015a; Krishnappan et al., 2004; Walling and Woodward, 1993). The water elutriation apparatus consisted of two identical plastic tanks (inlet and outlet, 30L each) and three glass settling columns (50, 100 and 200 mm of nominal diameter) interconnected in series from the smaller to the larger diameter (Figure 5.1). The cross sectional area and volume of the 3 critical settling columns were 1,923, 7,854 and 51,416 mm^2; and 2.3, 4.26 and 8.8 L, respectively. In each test, the elutriation apparatus was initially filled with water. Then, 25 L of mixed liquor samples were poured to the 30 L inlet tank, which was kept under continuous stirring to avoid microalgal biomass sedimentation. Samples of 25 L of HRAP mixed liquor were then pumped from the inlet tank by means of a peristaltic pump located at the downstream side of the elutriation apparatus, which forced samples to pass through the columns by suction. HRAPs mixed liquor entered

each column near the bottom and exited near the top (as seen in the detail of Fig.5.1).

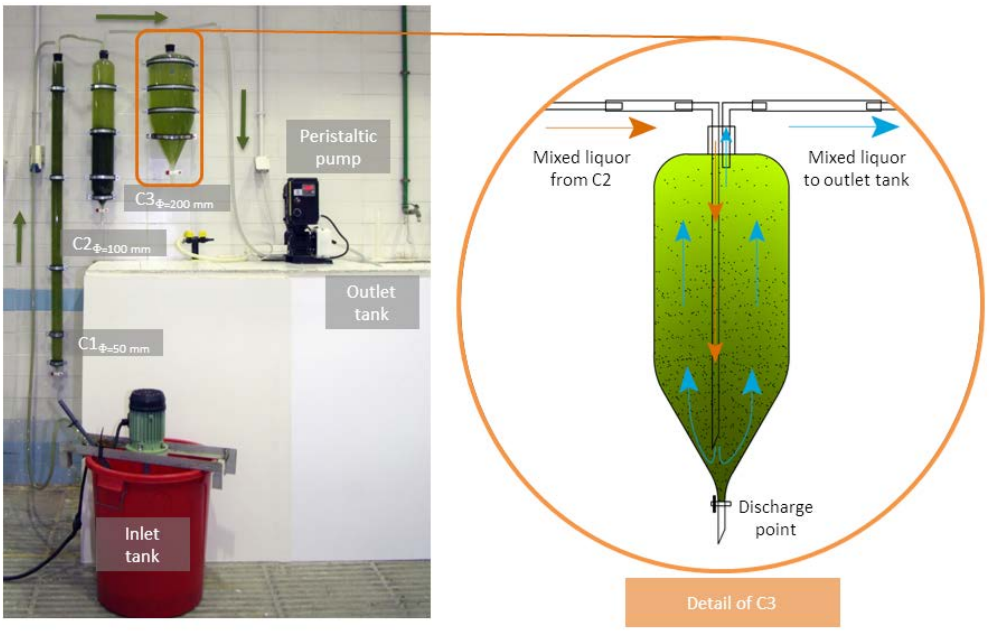


Figure 5.1 Water elutriation apparatus used for the dynamic sedimentation test. C1, C2 and C3 are the interconnected settling columns. Discharge point located at the bottom of the column was used to collect the microalgal biomass at the end of the experiment.

Note that the critical settling velocity decreased progressively in successive columns due to the gradual increment in column diameter, and therefore biomass flocs were retained in different columns depending on their settling velocities. In this manner, flocs with a settling velocity equal to or higher than the critical settling velocity of a given column were retained, while flocs with a settling velocity lower than the critical settling velocity escaped to the following column. Flocs with a settling velocity lower than the critical velocity of the third column were not retained in any column, and were thus collected in the outlet tank.

In this apparatus, the critical settling velocity of each column was obtained by dividing the flow rate through the apparatus by the area of the column (Eq. 5.1).

$$v_i = \frac{Q}{s_i} \qquad \text{(Eq. 5.1)}$$

Where v_i is the critical settling velocity in column "i" (m/h), Q is the flow rate (m^3/h) and S_i is the area of column "i" (m^2).

5.2.3 Experimental procedures

Experiments were carried out in two periods; during two weeks in summer (July) and during two weeks in autumn (October). Primary effluent and HRAPs mixed liquor samples were taken daily for evaluating temperature, pH, DO (dissolved oxygen) and turbidity, and weekly for measuring VSS (volatile suspended solids), COD (chemical oxygen demand) and ammonium nitrogen (NH_4^+-N). The main properties of the primary effluent and of the mixed liquor of both HRAPs are summarised in Table 5.1.

Table 5.1 Main properties of the primary effluent and the mixed liquor of both high rate algal ponds (HRAPs) in summer and autumn. Mean values (standard deviation) for daily (n=10) and weekly (n=3) samples taken at 12 PM.

Parameter	Primary effluent	4 days-HRAP	8 days-HRAP	
Summer				
Temperature (ºC)	29.1 (2.6)	23.1 (3.1)	23.0 (3.0)	Daily
pH	8.02 (0.17)	8.85 (0.21)	9.12 (0.16)	
DO (mg/L)	1.3 (0.4)	8.3 (0.7)	8.7 (0.9)	
Turbidity (NTU)	94 (44)	106 (9.0)	204 (18)	
VSS (mg/L)	-	240 (9)	361 (68)	Weekly
COD (mg/L)	159 (55) *	55 (5) **	54 (11) **	
NH_4^+-N (mg/L)	34.7 (1.40)	0.60 (0.33)	0.47 (0.52)	
Autumn				
Temperature (ºC)	25.9 (4.01)	23.12 (3.14)	23.03 (3.02)	Daily
pH	7.81 (0.09)	8.51 (0.44)	8.9 (0.4)	
DO (mg/L)	2.2 (1.8)	9.2 (1.8)	11 (2.23)	
Turbidity (NTU)	104 (81)	96 (35)	187 (28)	
VSS (mg/L)	-	152 (12)	249 (34)	Weekly
COD (mg/L)	296 (165) *	62 (13) **	57 (18) **	
NH_4^+-N (mg/L)	22.7 (10.1)	1.68 (0.88)	0.45 (0.18)	

* Total COD / ** Soluble COD

Note: DO: dissolved oxygen. VSS: volatile suspended solids. COD: chemical oxygen demand and NH_4^+-N: ammonium nitrogen.

In summer, dynamic sedimentation tests were carried out in order to determine the HRAPs mixed liquor settling velocities distribution without flocculant. Along 6 days of experiment, three samples of mixed liquor (25 L each) were collected from each HRAP at 12 pm. During this period, the flow rate through the apparatus was set at 0.54 L/min based on a previous study (Krishnappan et al., 2004). This generated critical settling velocities within the range of 1 - 16.5 m/h. The first column retained flocs with a settling velocity ≥16.5 m/h, the second one between 16.5 and 4 m/h, and the third one between 4 and 1 m/h, while flocs with a settling velocity of <1 m/h were collected in the outlet tank.

In autumn tests were conducted to determine the settling velocities distribution when a flocculant was added to improve the microalgal biomass settling properties. Since the sedimentation test carried out in summer showed low variability among replicates, in autumn the experiments were conducted without replicates, in order to minimize the time-lapse between samples. Therefore, in the first week of October, two samples of mixed liquor (25 L each) were collected from the 4 days-HRAP and tested one with flocculant and the other one without flocculant. The following week, the same process was repeated with the 8-days HRAP mixed liquor. The optimal dose of flocculant was determined with jar tests described below. In this case microalgae species populations were also assessed. The flocculant was a cationic tannin-based substance extracted from the bark of the tree *Acacia mearnsii*, which is nowadays widely used in the water and wastewater treatment sectors (*Tanfloc* SG). This flocculant is effective over a pH range from 4.5 to 8 and does not significantly modify the pH of the medium. *Tanfloc* SG was supplied by Tanac SA (Brazil) and had a cost of 1.7 $/kg. The flocculant, provided as dry product, was dissolved in water until complete solution. Stock solutions of 1000 mg/L of flocculant were prepared prior to jar tests. Jar tests were carried out using common jar test equipment, following standard protocols employed in the water and wastewater treatment sectors (Metcalf and Eddy, 2004). Prior to dynamic sedimentation test, 6 L of the same HRAP mixed liquor were used to perform the jar tests. Duplicate experiments were carried out to determine the optimal dose of flocculant for each HRAP mixed liquor, subsequently used in the dynamic sedimentation test. The steps followed in jar tests along with calculations may be found elsewhere (Gutiérrez et al., 2015b).

For sedimentation tests with flocculant, samples from the mixed liquor were firstly mixed with *Tanfloc* (at the optimal doses obtained in jar tests) inside the 30 L inlet tank simulating a coagulation-flocculation process. After 15 min of

flocculation, the mixed liquor was pumped through the elutriation apparatus. The flow rate in these tests was set to 0.21 L/min in order to have a range of critical settling velocities (0.4 - 6.5 m/h) more similar to those used in secondary settlers (0.7 – 1.3 m/h according to Metcalf and Eddy (Metcalf and Eddy, 2004)). Consequently, the first column retained flocs with a settling velocity ≥6.5 m/h, the second one between 6.5 and 1.6 m/h, and the third one between 1.6 and 0.4 m/h, while flocs with a settling velocity <0.4 m/h were in the outlet tank had.

At the end of each test, flocs retained in each column were collected by emptying the volume retained in each column in 10 L plastic tanks. Afterwards, samples collected were homogenously mixed and analysed for volatile suspended solids. The mass of microalgal biomass settled in each column and outlet tank (expressed as grams and percentage of VSS) was then obtained from the equations described in Table 5.2.

Table 5.2 Equations required to calculate the mass of biomass (expressed in g VSS and %VSS) in the inlet tank, retained in each column (C1, C2 and C3) and collected in the outlet tank.

Tank/column	Microalgal biomass (as g VSS) (W_i)	Microalgal biomass (as VSS %)
Inlet tank	$VSS_{inlet} * V_{inlet}$	
50 mm-column (C1)	$VSS_{C1} * V_{C1}$	$\frac{W_{C1}}{W_{C1} + W_{C2} + W_{C3} + W_{outlet}} * 100$
100 mm-column (C2)	$VSS_{C2} * V_{C2} * (1 - \frac{V_{C1}}{V_{inlet}})$	$\frac{W_{C2}}{W_{C1} + W_{C2} + W_{C3} + W_{outlet}} * 100$
200 mm-column (C3)	$VSS_{C3} * V_{C3} * (1 - \frac{V_{C1} + V_{C2}}{V_{inlet}})$	$\frac{W_{C3}}{W_{C1} + W_{C2} + W_{C3} + W_{outlet}} * 100$
Outlet tank	$VSS_{outlet} * V_{outlet} * (1 - \frac{V_{C1} + V_{C2} + V_{C3}}{V_{inlet}})$	$\frac{W_{outlet}}{W_{C1} + W_{C2} + W_{C3} + W_{outlet}} * 100$

where V_{inlet} is the volume of mixed liquor pumped (L); V_{C1}, V_{C2}, V_{C3} are the volumes of each column (C1, C2 and C3) (L); V_{outlet} is the sum of the volumes of each column (C1, C2 and C3) and V_{inlet} (L); VSS_{inlet}, VSSC1, VSS_{C2}, VSS_{C3}, and VSS_{outlet} are the volatile suspended solids concentrations (g/L) measured in the samples collected from inlet tank, columns C1, C2 and C3 and outlet tank, respectively; W_{inlet}, W_{C1}, W_{C2}, W_{C3} and W_{outlet} are the mass of microalgal biomass (g VSS) in inlet tank, columns C1, C2 and C3 and outlet tank, respectively.

Due to the dynamic conditions of the experiment, a correction factor was taken into account not to overestimate the results. This correction factor corresponds to the term in parentheses in equations to calculate Wi in Table 5.2. The term is used to consider the fraction of microalgal biomass that did not reach the column corresponding to its settling velocity and remained in the previous column.

The experimental error was calculated as an indicator of the reliability of the test considering the amount of solids retained in each column and in the outlet tank divided by the amount of solids pumped to the water elutriation apparatus (Eq. 5.2).

$$\text{Experimental error (\%)} = \frac{W_{C1}+W_{C2}+W_{C3}+W_{outlet}}{W_{inlet}} * 100 \quad \text{(Eq.5.2)}$$

where ; W_{inlet}, W_{C1}, W_{C2}, W_{C3} and W_{outlet} are the mass of microalgal biomass (g VSS) in inlet tank, columns C1, C2 and C3 and outlet tank, respectively.

5.2.4 Analytical methods

Volatile suspended solids, total and soluble chemical oxygen demand, and ammonium nitrogen were analysed according to Standard Methods (APHA-AWWA-WPCF, 1999). **W**ater temperature and dissolved oxygen were measured in situ in the HRAP at 12 PM with an YSI 58 oxymeter. Turbidity was determined with a Hanna Microprocessor Turbidity Meter HI93703 and pH with a Crison Portable 506 pH-meter.

Microalgae species populations were determined as follows. Two replicates of 25µL of each sample were examined by bright and contrast phase microscopy using a Zeiss microscope Axioskop 40. Microalgae species were identified in vivo using conventional taxonomic books (Bourelly, 1966; Palmer, 1962). Microalgae were counted 100 and 400 magnification using coverslides of 20 mm side (Salvadó et al., 2004). Microalgal biomass images were taken to complement quantification.

5.3 Results and Discussion

5.3.1 Settling velocities distribution of microalgal biomass

Wastewater organic loading rate, seasonal environmental conditions and potential microorganisms interactions are known to influence the microalgal biomass properties (solids concentration, chemical composition and microalgae population) (García et al., 2000a; Godos et al., 2009). The impact of these parameters on floc characteristics is an important issue related to flocculation efficiency that should be considered in a pre-concentration harvesting step. From this point of view, the settling velocity of microalgal biomass is a key parameter in the design of full-scale sedimentation units (Vandamme et al., 2014). Thus, the settling velocities distribution of the mixed liquor from two experimental HRAP was initially evaluated without flocculant. Note that the mixed liquor microalgal biomass concentration (mg VSS/L) was higher in the 8 days-HRAP than in the 4 days-HRAP (Table 5.1). Thus, different microalgal biomass concentrations were used in sedimentation tests. The results of these tests are shown in Table 5.3, where the amount of microalgal biomass collected in each settling column and outlet tank is summarized along with the amount of biomass pumped through the system (inlet tank). In the last two columns biomass recovery is calculated as absolute mass (sum of columns and outlet tank) (g VSS) and the experimental error as an indicator of the reliability of tests (%). The average biomass pumped through the system was 5.97 g (±1.30) for the 4 days-HRAP and 11.22 g (±0.90) for the 8 days-HRAP. Dynamic sedimentation tests results for the three samples of each HRAP were very similar with experimental errors between 93-99%. The deviation of biomass recovery from 100% is equivalent to the experimental error of the test. In general, the higher the amount of biomass pumped, the higher the biomass recovery and subsequently, the lower the experimental error. Thus, summer tests with higher concentration of biomass lead to lower experimental error (1 to 7%) than autumn tests (2 to 30%).

Data in Table 5.3 were used to plot the settling velocities distribution of microalgal biomass from both HRAPs (Figure 5.2).

Table 5.3 Dynamic sedimentation test results in summer (without flocculant) from both HRAPs (4 and 8 days of hydraulic retention time).

Sample		Microalgal biomass (as VSS)					Biomass recovery (g)	Experimental error (%)
		Inlet tank (g)	50 mm-column (g)	100 mm-column (g)	200 mm-column (g)	Outlet tank (g)		
1	4 days-HRAP	6.38	0.81	2.48	1.52	1.33	6.13	96.1
	8 days-HRAP	10.45	1.00	5.18	2.03	1.90	10.10	96.7
2	4 days-HRAP	4.51	0.47	1.42	1.38	0.96	4.23	93.7
	8 days-HRAP	11.00	1.11	6.87	1.49	0.98	10.44	94.9
3	4 days-HRAP	7.02	0.84	2.10	2.20	1.65	6.80	96.8
	8 days-HRAP	12.20	1.30	8.60	1.08	1.14	12.11	99.3

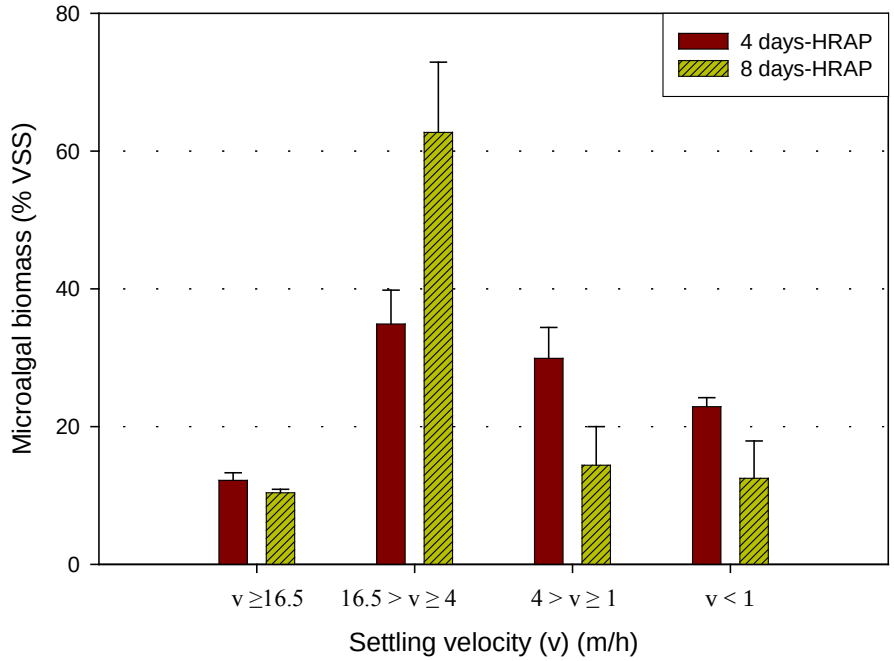


Figure 5.2 Average percentage of microalgal biomass with a given settling velocity distribution (without flocculant) in both HRAPs (4 and 8 days of hydraulic retention time) (n=3). Error bars represent standard deviations.

Each pair of bars refers to the amount of microalgal biomass with a certain settling velocity. As it can be seen, only a small percentage of biomass (<13%) had settling velocities ≥16.5 m/h in both HRAPs. Most of the biomass from the 8 days-HRAP (63%) had settling velocities between 16.5 and 4 m/h, while most from the biomass of the 4 days-HRAP (65%) had settling velocities between 16.5 and 1 m/h. 23% of the microalgal biomass from the 4 days-HRAP had a settling velocity <1 m/h, and only 12.5% from the 8 days-HRAP. From these results it can be estimated that dimensioning a settler with a critical settling velocity of 1 m/h (which is the usual value in secondary settlers (Metcalf and Eddy, 2004)) would attain a biomass recovery of 77% and 87.5% for the 4 and 8 days-HRAPs, respectively. Therefore, consistent different settling velocities distribution between both HRAPs put into evidence the different microscopic properties of the flocs of the mixed liquor from each HRAP in relation with their different HRT. On the whole, this experiment highlights the importance of HRT on the settling properties of biomass.

5.3.2 Settling velocities distribution of microalgal biomass with flocculant

Microalgae harvesting by flocculation has been mostly investigated in terms of biomass recovery (de Godos et al., 2011; Rashid et al., 2013; Riaño et al., 2012). However, the settling velocity is an important parameter which is affected by the size, structure and density of microalgal biomass flocs, and very few studies have focused on its relevance (Su et al., 2012; Vandamme et al., 2014). Indeed, only a few results of microalgae settling velocities using organic flocculants are reported in literature (Gutiérrez et al., 2015a; Vandamme et al., 2014).

In order to determine the optimal flocculant dose, a jar test was carried out and results are shown in Table 5.4. The optimal dose was established as the lowest dose of flocculant ensuring over 90% biomass recovery. In the 4 days-HRAP the optimal dose of flocculant was 20 mg/L, while in the 8 days-HRAP was 40 mg/L. These results are in accordance with other studies reporting a positive relation between microalgae concentration and dose of flocculant, where the higher the biomass concentration, the higher the flocculant dose needed to obtain the same biomass recovery (Granados et al., 2012; Letelier-Gordo et al., 2014; Vandamme et al., 2010).

Table 5.4 Results of jar tests with Tanfloc SG (n=2). Microalgal biomass recovery was calculated from turbidity values. Mean values (standard deviation) from HRAP with (a) 4 days and (b) 8 days of hydraulic retention time. The optimal dose (here grayed) is the lowest dose leading to a biomass recovery higher than 90%.

(a)

Concentration (mg/L)	Turbidity (NTU)	Biomass recovery (%)	pH
0	133.0 (17.4)		8.4 (0.2)
10	14.5 (6.1)	88.7 (6.1)	8.3 (0.3)
20	8.5 (1.9)	93.5 (2.3)	8.3 (0.3)
30	5.2 (0.2)	96.1 (0.7)	8.1 (0.3)
40	4.0 (0.8)	97.0 (1.0)	8.1 (0.3)
50	3.0 (0.1)	97.7 (0.2)	8.0 (0.3)
60	1.4 (0.3)	98.9 (0.4)	7.9 (0.3)

(b)

Concentration (mg/L)	Turbidity (NTU)	Biomass recovery (%)	pH
0	219.3 (27.8)		8.4 (0.2)
10	50.5 (20.5)	77.4 (6.5)	8.4 (0.2)
20	40.2 (16.7)	82.0 (5.3)	8.4 (0.1)
30	27.3 (8.7)	87.7 (2.4)	8.3 (0.1)
40	17.8 (3.3)	91.9 (0.5)	8.2 (0.1)
50	15.1 (0.3)	93.1 (0.8)	8.1 (0.2)
60	7.3 (1.9)	96.7 (0.4)	8.0 (0.3)

Table 5.5 shows the results obtained in the four dynamic sedimentation tests, two without flocculant (control) and two with the optimal dose of *Tanfloc* SG. Experimental errors were slightly variable, probably due to the low biomass concentration in both HRAPs mixed liquor in comparison with the experiments in summer. As expected, differences in microalgal biomass characteristics were observed between summer and autumn samples (García et al., 2006). Higher solids concentration was obtained in summer than in autumn due to more favourable environmental conditions (e.g. high solar radiation and temperature). Indeed, the influence of environmental conditions on microalgal biomass evolution has been widely discussed (Godos et al., 2009; Park and Craggs, 2010).

Table 5.5 Dynamic sedimentation test results in autumn without flocculant (control) and with flocculant (Tanfloc SG).

Sample		Microalgal biomass (as VSS)					Biomass recovery (g)	Experimental error (%)
		Inlet tank (g)	50 mm-column (g)	100 mm-column (g)	200 mm-column (g)	Outlet tank (g)		
Control	4 days-HRAP	4.25	0.41	0.91	1.07	0.60	2.99	70.2
	8 days-HRAP	4.40	0.44	0.87	1.30	1.70	4.32	98.2
Tanfloc SG	4 days-HRAP	4.17	3.71	0.46	0.12	0.11	4.41	105.8
	8 days-HRAP	5.86	4.62	1.18	0.26	0.52	6.58	112.3

Figure 5.3 shows the percentage of microalgal biomass with a certain settling velocity (calculated from the results in Table 5.5). In the control sample (without flocculant) from the 4 days-HRAP the majority of the biomass (80%) had settling velocities ranging from 6.5 and 0.4 m/h, while 20% of the biomass had settling velocities <0.4 m/h. The addition of flocculant had an impressive effect since most of the biomass (84%) had a settling velocity ≥6.5 m/h. Only a 3% of the biomass had a settling velocity <0.4 m/h. In this case, a settler designed with a critical settling velocity of 1 m/h would allow a biomass recovery greater than 94% (estimated from the percentages corresponding to the ≥6.5 m/h and 6.5-1.6 m/h bars in Figure 5.3 (a)).

In the control from the 8 days-HRAP, around half of the biomass (60%) had settling velocities between 6.5 and 0.4 m/h. Only 10% of the microalgal biomass had settling velocities ≥6.5 m/h, and 40% of the biomass had velocities <0.4 m/h. Again, when the flocculant was added, results were impressively affected, with 70% of biomass with a settling velocity ≥6.5 m/h (the same trend as in the 4 days-HRAP). Only an 8% of the biomass had settling velocities lower than 0.4 m/h. In this case, a settler designed with a critical settling velocity of 1 m/h would allow a biomass recovery greater than 90%. Note that microalgal biomass with low settling velocities would result in higher settler's surface and/or higher HRT in settlers. With flocculant, higher biomass recovery may be accomplished, leading to design more compact settlers.

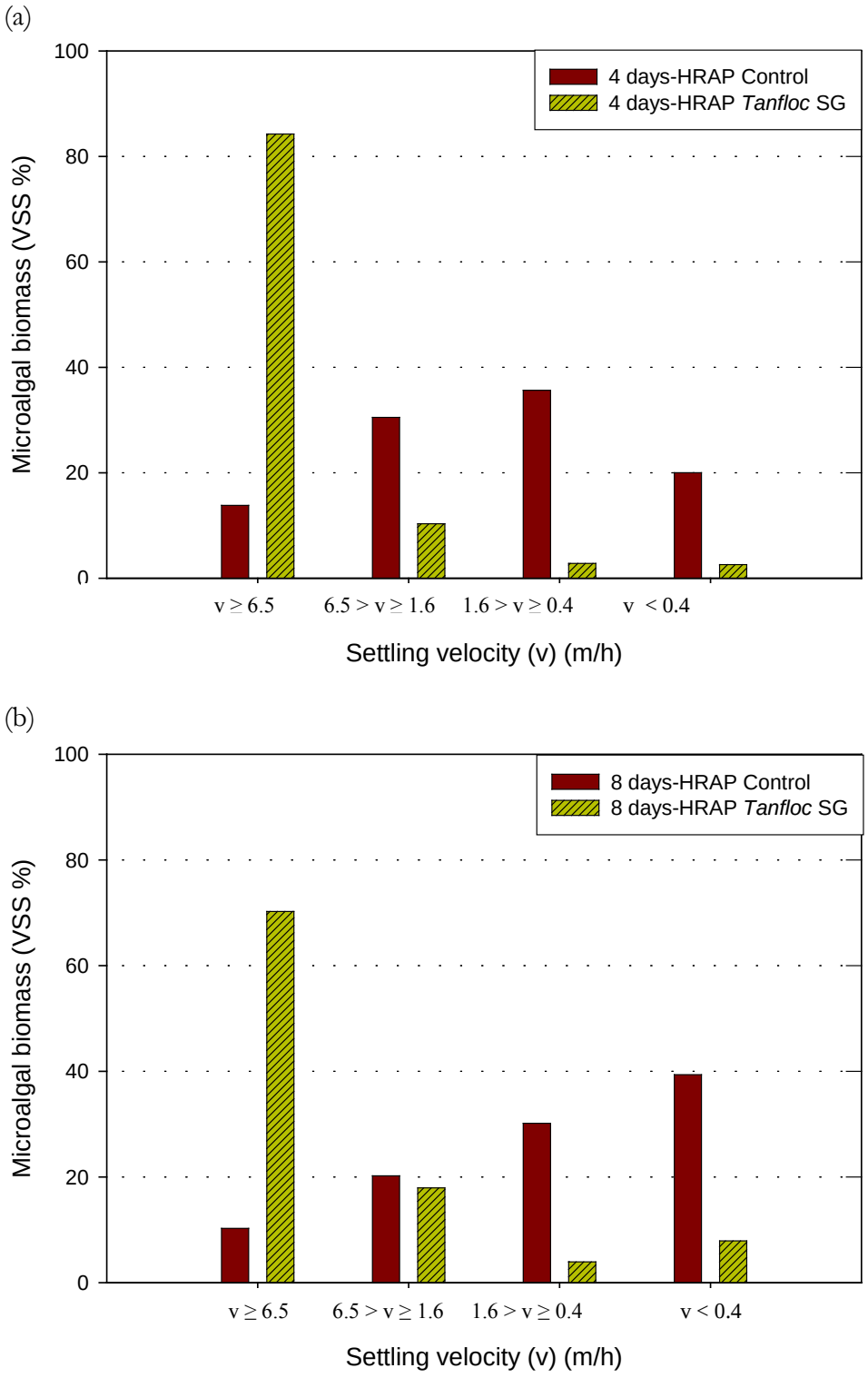


Figure 5.3 Average percentage microalgal biomass with a given settling velocities distribution in autumn (with and without flocculant) in the 4 days-HRAP (a) and in the 8 days-HRAP (b).

Microscopic examination

The biomass settling ability is highly dependent on the microalgae species populations present in the HRAPs mixed liquor (Barros et al., 2015; Vandamme et al., 2013). In autumn, microalgae identification and quantification were carried out from the inlet tank samples (HRAPs mixed liquor) and outlet tank samples of the elutriation apparatus. In general, the dominant microalgae identified in both HRAPs were the green algae *Chlorella* sp. and the diatoms *Navicula* sp. and *Nitzschia* sp. Indeed, *Chlorella* sp. and *Nitzschia* sp. species are often classified in the top 10 most tolerant microalgae (Brennan and Owende, 2010; Pittman et al., 2011). Although less abundant, *Micractinium* sp., *Scenedesmu*s sp., *Chlamydomonas* sp. and *Desmococcus* were also present in all samples. The main difference between microalgae populations present in the two HRAPs was driven by differences in the HRT. Even if the same microalgae species were observed in the two HRAPs, Chlorella sp. and diatoms were more abundant in the 8 days-HRAP than in the 4 days-HRAP (56% more *Chlorella* sp. and 16% more diatoms). Indeed, the influence of HRAPs operational parameters (such as HRT) and environmental conditions (e.g. solar radiation and temperature) on shifts in microalgae dominance and abundance was previously reported (García et al., 2000a; González-Fernández and Ballesteros, 2013; Park et al., 2011a).

Figure 5.4 shows the distribution of the main microalgae species in the inlet tank and the outlet tank for the control and flocculated samples. Microalgal biomass images are shown in Figures 5.5 and 5.6. Diatoms had a similar abundance in the inlet tank samples of both HRAPs. In samples without flocculant, diatoms were lowered by more than 90% between the inlet and the outlet tanks. The diatoms observed in this study are benthic organisms normally linked to floc aggregates, so these microalgae were not expected to be found in outlet tank when flocculant was added. Accordingly, once flocculant was added, almost 100% of diatoms were retained in the apparatus. *Chlorella* sp. was 35% more abundant in the 8 days-HRAP than in the 4 days-HRAP. Without flocculant, the percentage of recovery was higher in the 4 days-HRAP (94%) than in the 8 days-HRAP (83%). The lower amount of Chlorella sp. in the outlet tank of the 4 days-HRAP sample may be attributed to an enhanced floc formation in this HRAP with higher flow rate (120 vs. 60 L/d), where more bacteria were likely to grow as a result of the higher organic loading rate (23 g COD/m^2d in the 4 days-HRAP vs. 12 g COD/m^2d in the 8 days-HRAP). In fact, the presence of bacteria enhances spontaneous flocs formation (Kouzuma and Watanabe, 2015). This behaviour did not correspond to the one observed in summer, when

microalgal biomass flocs of the 8 days-HRAP had higher settling velocities than those of the 4 days-HRAP. This demonstrates the complexity of the bioflocculation process due to the large number of biological interactions between microorganisms and wastewater. As expected, after flocculant addition, *Chlorella* sp. cells were mostly aggregated in flocs (see Figures 5.5c and 5.6c). Indeed, the high recovery of individuals after flocculant addition (around 99% of *Chlorella* sp. and almost 100% of diatoms) resulted in less than 1,500 individuals/mL in all outlet tank samples. Microscopic images supported this finding (see Figures 5.5d and 5.6d).

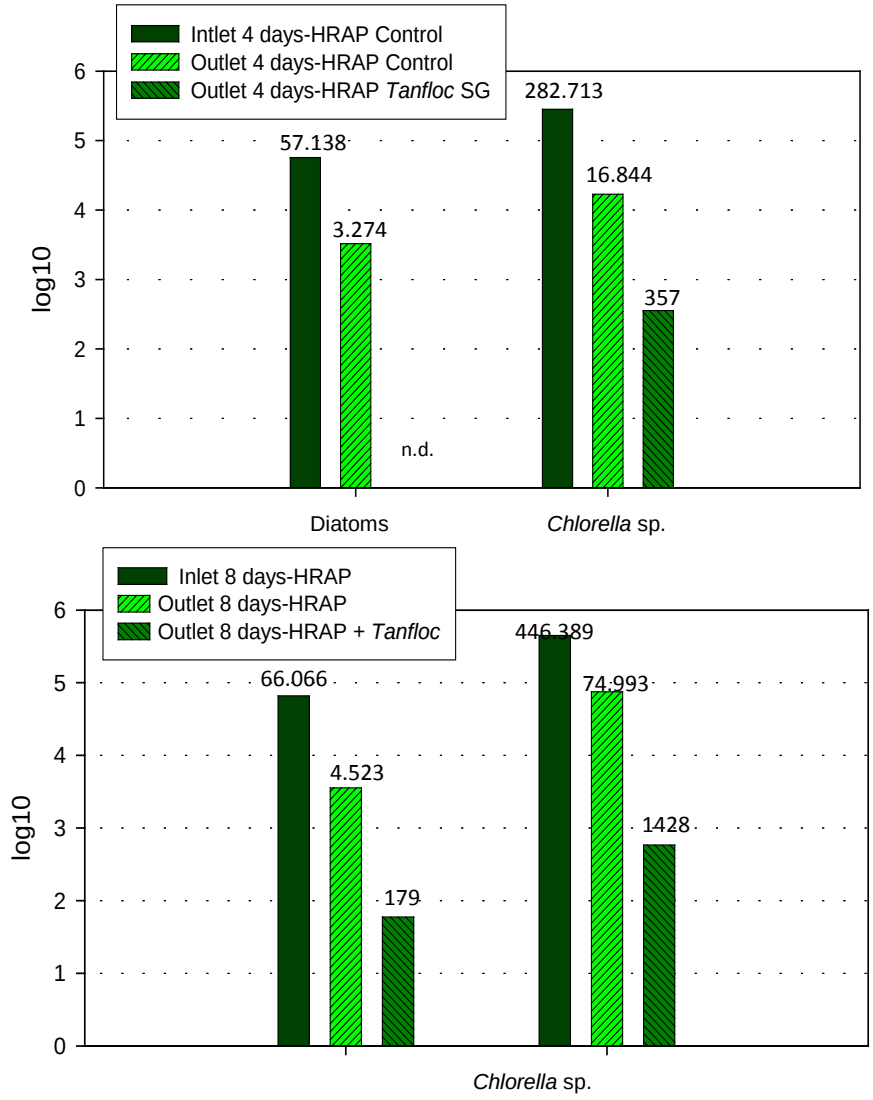


Figure 5.4 Distribution of the main microalgae populations in the inlet tank and the outlet tank (with and without flocculant) from the 4 days-HRAP (a) and 8 days-HRAP (b). n.d: non-detected.

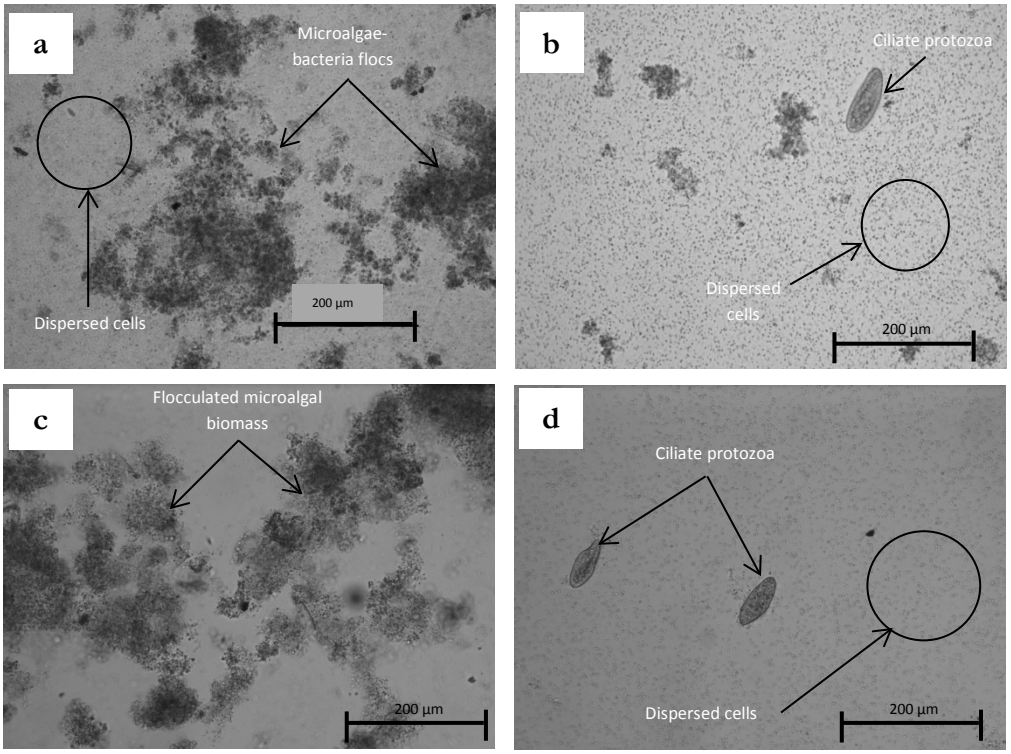


Figure 5.5Images of 4 days-HRAP mixed liquor samples before and after dynamic sedimentation tests. (a) Inlet tank sample without flocculant. (b) Outlet tank sample without flocculant. (c) Inlet tank sample with flocculant. (d) Outlet sample with flocculant.

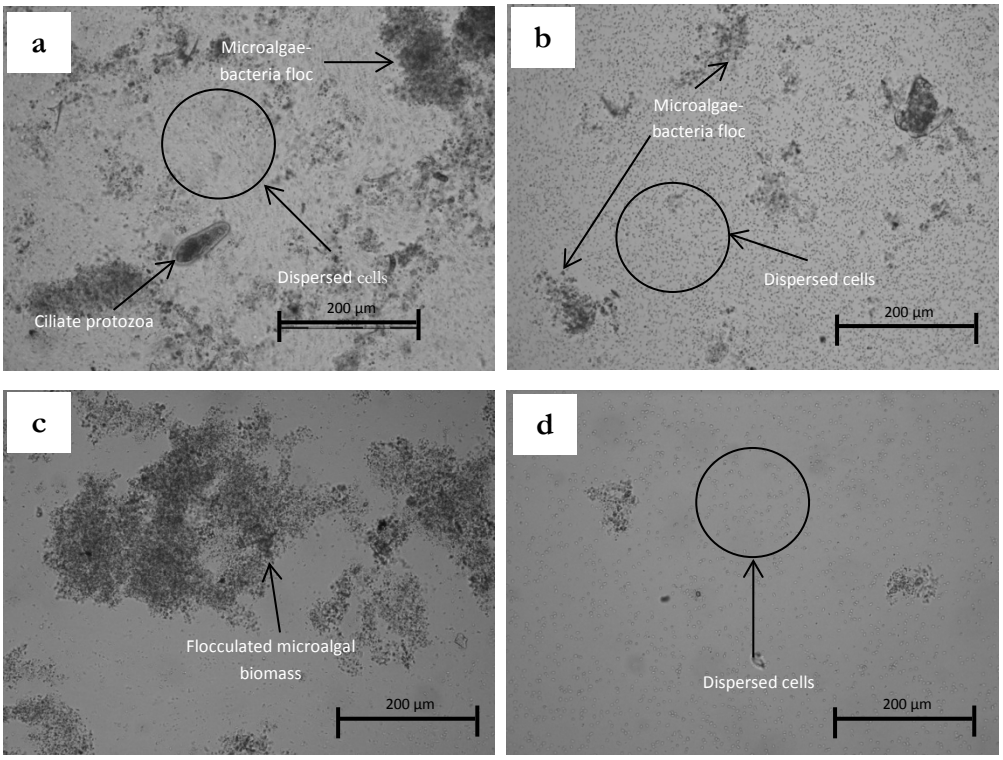


Figure 5.6 Images of 8 days-HRAP mixed liquor samples before and after dynamic sedimentation tests. (a) Inlet tank sample without flocculant. (b) Outlet tank sample without flocculant. (c) Inlet tank sample with flocculant. (d) Outlet sample with flocculant.

Images of the inlet tank mixed liquor samples (Figures 5.5a and 5.6a) indicated that the initial biomass was composed by flocs aggregates of different sizes and dispersed single cells in both HRAPs. Once passing through the elutriation apparatus, mostly single cells and some smaller flocs (<100 μm) were identified (Figures 5.5b and 5.6b). After coagulation-flocculation, most single cells were aggregated, leading to larger flocs (Figures 5.5c and 5.6c). Comparing outlet tank images with and without flocculant, the reduction of single cells and flocs aggregates can be clearly observed (Figures 5.5d and 5.6d).

Therefore, low concentrations of *Tanfloc* (20-40 mg/L) were not only effective in terms of biomass recovery, but also in terms of settling velocity. These parameters are important for the design of secondary settlers by achieving fast settling and high concentrated microalgal biomass in a pre-concentration step.

5.3.3 Economic assessment

Chemical flocculation followed by gravity sedimentation is considered a cost-effective harvesting method as low energy and no extra materials (e.g. membrane or electrode used for membrane filtration and electro-flocculation, respectively) are required (Brennan and Owende, 2010). Regarding energy requirements, microalgal biomass harvesting by gravity sedimentation needs less energy (0.9 kWh/ton TSS) than conventional harvesting methods such as centrifugation, tangential flow filtration and/or dissolved air flotation (>50 kWh/ton TSS) (Olguín, 2012; Udom et al., 2013). The viability of microalgal biomass flocculation with chemicals will ultimately depend on the flocculant cost, since the low energy requirement for mixing (around 1.5 kWh/ton TSS) does not hamper the viability of the process. The feasibility of flocculation with *Tanfloc* is compared to other commercial inorganic and organic coagulants/flocculants based on the cost of flocculating a ton of TSS microalgal biomass (Table 5.6). Notice that this calculation is only based on the flocculant cost, considering the optimal flocculant dose and the initial microalgal biomass concentration. In general, optimal doses of *Tanfloc* (0.02 – 0.04 g/L) fit within the range of other organic flocculants (e.g. starch-based flocculants, tannin-based flocculants, chitosan or polyacrylamides) and are low in comparison with metal-based coagulants (normally >0.10 g/L) (Letelier-Gordo et al., 2014; Şirin et al., 2011). However, considering the biomass concentration, *Tanfloc* would demand higher doses (0.1-0.2 ton of flocculant/ton TSS) than the rest of

organic flocculants (0.02-0.1 ton of flocculant/ton TSS). As shown in Table 5.6, the cost of flocculating a ton of microalgal biomass with *Tanfloc* would be around 170-340 $/ton TSS, which is similar to the cost of cationic starch (120-370 $/ton TSS) and lower than conventional metal-based coagulants (e.g. 160-1000 $/ton TSS for aluminium salts). Furthermore, metal-based coagulants cause contamination of microalgal biomass, which may interfere in downstream processes like biogas production; while most organic flocculants do not modify the properties of the microalgal biomass (Gutiérrez et al., 2015a, 2015b) and the low flocculant doses needed would decrease the operational costs of harvesting in comparison with metal-based coagulants.

Table 5.6 Operational cost of different coagulants/flocculants used for microalgae harvesting and wastewater treatment.

	***Ecotan*[1]**	**Starch[2]**	***Tanfloc*[3]**	**Poly γ-glutamic acid[4]**	**Chitosan[5]**	**PAC[6]**	**Aluminium sulphate [6]**
Optimal dose of flocculant (mg/L)	10	25-40	20-40	20	10-15	60	60-250
Biomass concentration (mg/L)	400	200-500	100-400	400	400-600	150	150-900
Dose (ton/ton TSS)	0.02	0.07-0.1	0.1-0.2	0.02	0.02-0.03	0.4	0.2-0.8
Flocculant cost ($/ton TSS)	1.05	1-3	1.7	5	25-70	0.4-1.4	0.9-2.1
Contamination risk	Low	Low	Low	Medium	Medium	High	High
Operational cost ($/ton TSS)	<50	120-370	170-340	250	500-1400	160-560	300-1000

[1](Gutiérrez et al., 2015b),[2](Gutiérrez et al., 2015a; Vandamme et al., 2010), [3]This study and (Gutiérrez et al., 2015b), [4](Zheng et al., 2012), [5](Gerde et al., 2014; Şirin et al., 2011), [6](Şirin et al., 2011)

Indeed, the economic viability of microalgal biomass production for low added-value applications (e.g. biofuels) involves reducing biomass production costs to 400-750 $/ton TSS (Williams and Laurens, 2010). Taking into account that biomass harvesting accounts for 20-30% of the total costs of biomass production, the cost of harvesting one ton biomass should range from 100 to 200 $/ton TSS (Brennan and Owende, 2010). Even if *Tanfloc* cost is slightly high

(170 – 340 $/ton TSS), the low energy required for flocculation (1.5 kWh/ton TSS) along with the low contamination risk of microalgal biomass and high biomass recovery (>90%) at low doses (20-40 mg/L), make *Tanfloc* an efficient and cost-effective flocculant for microalgal biomass harvesting, which represents a promising alternative to metal-based coagulants.

5.4 Conclusions

Two sets of dynamic sedimentation tests were carried out in this study (summer and autumn). In the first set, most of the biomass of the 8 days-HRAP (63%) had settling velocities between 16.5 and 4 m/h, while most of the biomass of the 4 days-HRAP (65%) had settling velocities between 16.5 and 1 m/h. In the second set, most of the biomass of the 8 days-HRAP (80%) and of the 4 days-HRAP (60%) had settling velocities between 6.5 and 0.4 m/h. In this second set, 20% of the biomass of the 4 days-HRAP and 40% of the 8 days-HRAP had velocities <0.4 m/h. The addition of flocculant (*Tanfloc* SG) at optimal doses ranging from 20 to 40 mg/L had impressive effects on the settling velocities distribution in this second set. 70% and 84% of biomass reached velocities >6.5 m/h, compared to 10% and 14% of microalgal biomass without flocculant for the 8 and 4 days-HRAPs, respectively. With flocculant, a very small amount of biomass (3% of the 4 days-HRAP and 8% of the 8 days-HRAP) had a settling velocity <0.4 m/d. Results obtained from this study indicate that a settler designed with a critical settling velocity of 1 m/d (as usually in secondary settlers) would have a biomass recovery rate greater than 90%. Microscopic examination of the samples subjected to sedimentation tests revealed that after passing through less than 1,500 of microalgae individuals/mL were detected in all outlet tank samples (inlet samples > 10^5 individuals/mL).

6

Starch-based flocculant: Dynamic sedimentation test

This chapter is based on the article:

Gutiérrez, R., Ferrer, I., García, J., Uggetti, E. (2015) Influence of starch on microalgal biomass recovery, settleability and biogas production. Bioresource technology 185, 341-345

6.1 Introduction

During the last decade the potential of microalgae as biorefinery feedstock has been widely investigated (Uggetti et al., 2014). In spite of the promising results obtained, scaling-up the technology is hampered by the high costs of the process. In particular, the biomass harvesting step represents 20-30% of microalgal biomass production costs (Barros et al., 2015).

A number of solids separation techniques are currently available in the field of water treatment technology including centrifugation, flocculation and flotation (Uduman et al., 2010; Kurniawati et al., 2014) or membrane procedures such as magnetic, vibrating and rotating membranes. In general, for the production of low-value products such as biofuels, harvesting techniques should consist in low-cost and low-energy demand methods capable of processing a large volume of culture medium. Thus, coagulation-flocculation followed by sedimentation is among the most suitable options. This process is enhanced by the addition of chemicals such as salts of aluminum or iron. In a biorefinery context, it is important to ensure that downstream products are not contaminated by chemicals (Zheng et al, 2012). For this reason, the use of natural organic flocculants like tannin based polymers or modified starch are being increasingly investigated (Vandamme, 2013). Indeed, potato starch could be seen as a residue from the potato industry (e.g. starch contained in potatoes peel).

The aim of the present study was to evaluate the effect of potato starch on the coagulation-flocculation and sedimentation of microalgal biomass grown in a pilot high rate algal pond (HRAP) used for wastewater treatment. The optimal dose was determined with jar tests and the settleability of formed flocs was studied using an elutriation apparatus measuring the settling velocities distribution. Moreover, the effect of starch on biogas production was determined in biochemical methane potential (BMP) tests.

6.2 Materials and methods

6.2.1. Microalgal biomass

Microalgal biomass used for this experiment was cultivated in a pilot plant located at the laboratory of the GEMMA research group (Universitat

Politècnica de Catalunya, Barcelona, Spain). The pilot plant consists of a HRAP in the form of a raceway pond (0.5 m^3volume) fed with a continuous flow of 60 L/day of primary treated wastewater (24 g chemical oxygen demand (COD)/ m^2·d; 4 g ammonium nitrogen (NH_4^+-N)/ m^2·d). The system had been in operation for 4 years prior to the experiment, and was described in detail by Passos et al. (2013). At the time the experiments were conducted microalgal populations were mainly composed by *Chlorella* sp. with a total suspended solids (TSS) concentration about 200 mg/L.

6.2.2. Starch-based flocculant

Starch is a natural product having strong flocculating properties. Potato starch solution 1% ($C_6H_{10}O_5$) provided by Panreac (Spain) was used as flocculant. Starch addition did not modify significantly the pH of the system, which remained almost constant along the experiments (9.5±0.6). The zeta potential was determined for the selected starch concentrations and resulted in values of -35.8 mV (for 10 mg/L) and -19.4 mV (for 25 mg/L).

6.2.3. Jar tests

The optimal dose of flocculant was determined by means of jar tests performed following standard protocols (Metcalf and Eddy, 2003). Five jar tests were carried out during February and March 2014, with starch concentrations from 5 to 80 mg/L. Turbidity and pH (measured with a HI93703 Hanna Instruments Turbidimeter and a Crison 506 pH-meter, respectively) were determined from fresh HRAP mixed liquor at the beginning of the experiments and from the supernatant liquid after the jar test. Then, biomass recovery (RE) was calculated based on initial (Ti) and final (Tf) turbidity measurements (Eq. 6.1). Recovery values of the five jar tests were then averaged.

$$RE(\%) = \frac{T_i - T_f}{T_i} * 100$$

(Eq. 6.1)

6.2.4. Dynamic sedimentation test

Elutriation is a water-current separation technique in which particles are washed out according to their weight, volume or form. This test can be used to assess the feasibility of separation treatment by settling (Krishnappan et al., 2004).

The water elutriation apparatus used in the present study was a modified version of a system proposed by Walling and Woodward (1993). The system (Fig. 6.1) consisted of 3 cylindrical settling columns of different diameter (corresponding to different settling velocities) interconnected in series by glass and PVC tubing. The diameters of the columns were: 50 mm in the first column, 100 mm in the second and 200 mm in the third one. The sample entered the columns near the bottom and exited near the top, allowing the sediment flocs that had settling velocities higher than the upward suspension velocity to settle in the respective columns. Considering a flow rate of 0.21 L/min, the upward velocities generated in the three settling columns were 6.5 m/h, 1.6 m/h and 0.4 m/h, respectively. Thus, the first column collected biomass with settling velocities >6.5 m/h, second column collected biomass with settling velocities between 6.5 and 1.6 m/h, and the third column collected the biomass with settling velocities between 1.6 and 0.4 m/h. The outlet suspension contained the biomass fraction whose settling velocity was lower than the suspension velocity in the third column (0.4 m/h). All fractions of biomass retained in columns were collected and TSS analyzed according to Standard Methods (APHA AWWA-WPCF, 2001) for calculating the settling velocity distribution.

Samples collected from the mixed liquor HRAP were mixed with starch at 2 different concentrations (10 and 25 mg/L) and successively pumped through the series of settling columns by means of a peristaltic pump. A sample without starch was also tested as control. All tests were carried out in triplicate. In order to establish differences between samples, ANOVA test was performed with Excel.

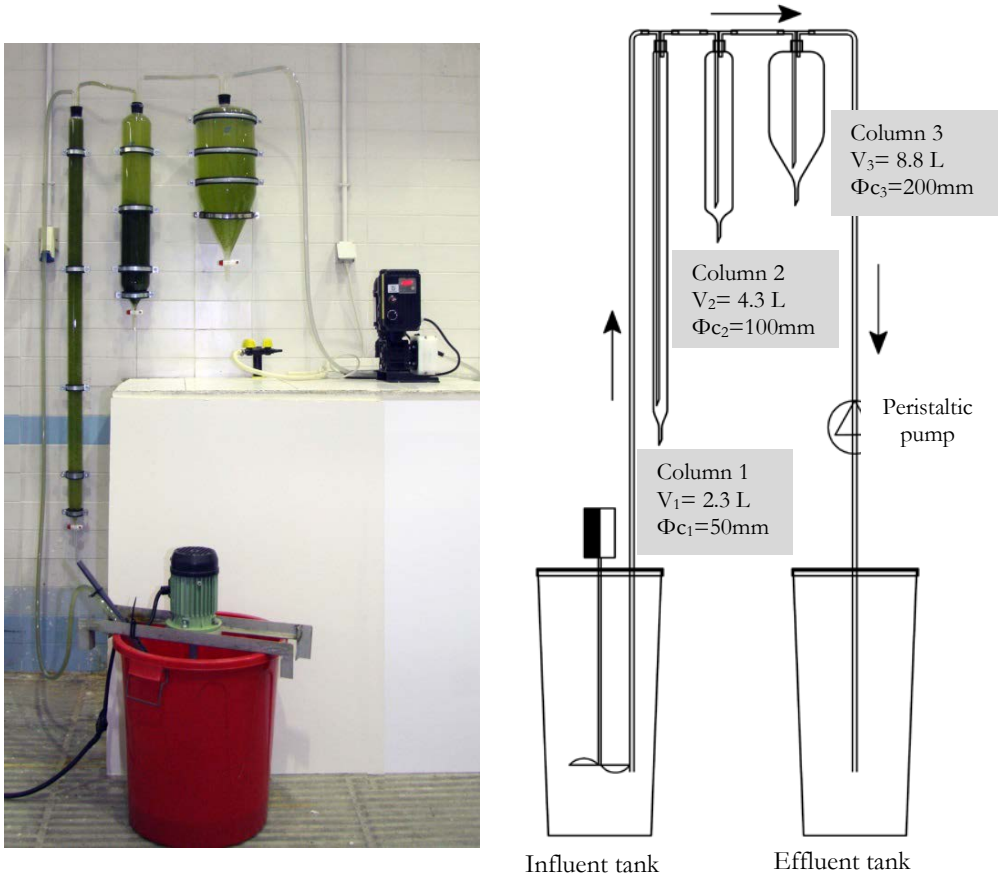


Figure 6.1 Image and schematic view of the experimental eluriation apparatus used for testing the settleability of microalgal biomass.

6.2.5. Biochemical methane potential tests

Biochemical methane potential tests were used to compare microalgal anaerobic biodegradability and biogas production with and without flocculant addition. The BMP test was performed in triplicate in samples with and without flocculant addition. Digested sludge from a full-scale anaerobic reactor located in a municipal wastewater treatment plant was used as inoculum.

BMP tests were performed in 160 mL bottles, in which 60 mg of microalgal biomass (40.4 g VS/L) and 40 mg of digested sludge (30.4 g VS/L) were added, along with 2 different starch concentrations (10 and 25 mg/L). Three controls were also performed, namely microalgal biomass and the 2 selected starch doses. All bottles where flushed with Helium gas, sealed with butyl rubber stoppers

and incubated at 35 ºC until biogas production ceased. Accumulated volumetric biogas production (mL) was calculated from the pressure increase (periodically measured with a Greisinger GMH 3151 manometer), expressed under standard conditions. The net values of biogas production and yield were obtained by subtracting the endogenous production of blank trials, containing only inoculum.

6.3 Results and discussion

6.3.1 Optimal dose of flocculant

In the jar test, all tested starch doses led to high biomass recovery (RE>90%) (Fig. 6.2). The optimal dose was 25 mg/L, which reduced turbidity from 151±14 to 6±2 NTU (biomass recovery of 95.7%). On the other hand, increasing the starch concentration further decreased biomass recovery (to 90.2% for a dose of 80 mg/L).

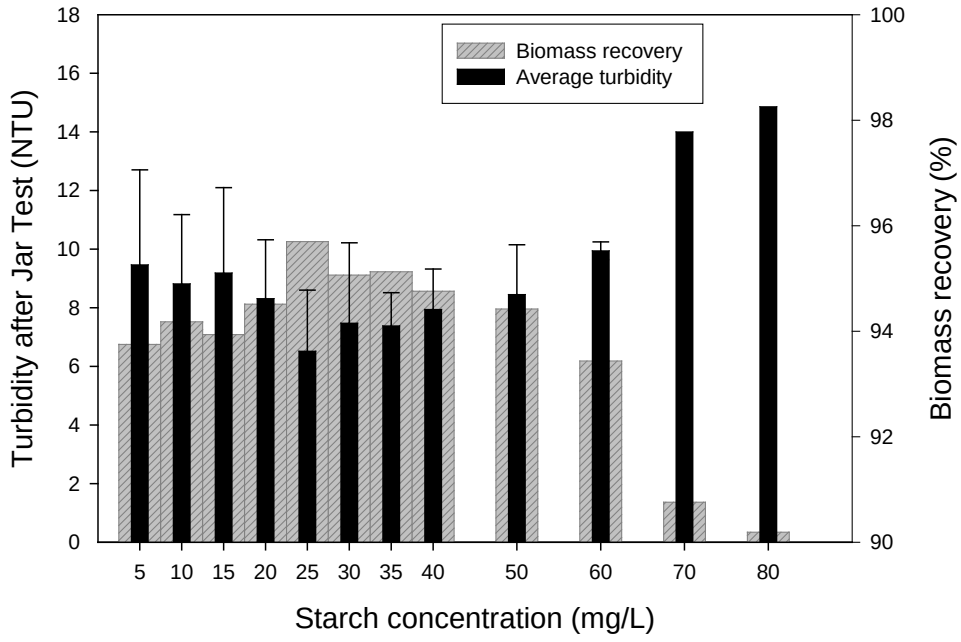


Figure 6.2 Jar test results expressed as final turbidity and biomass recovery. Biomass recovery was calculated from the initial turbidity (151±14) and final turbidities shown in the graph.

The zeta potential of 25 mg/L of starch (-19.4 mV) indicated an incipient instability of the solution (from ±10 to ±30), with reduced electrical repulsion between particles. Jar test results indicate that high biomass recoveries (about 95%) were also attained with doses lower than the optimal. From a pragmatic point of view these doses would be preferred to avoid overcosts. Even if the zeta potential of 10 mg/L (-35.8 mV) indicated only moderate instability of the solution with still strong electrical repulsion between particles, the dose of 10 mg/L (94% of recovery and 9 NTU) was also selected for settling velocity distribution and biochemical methane potential experiments.

Considering that the initial biomass concentration was approximately 200 mg/L, from 0.05 to 0.15 g of starch per g of biomass were required to harvest more that 95% of the biomass. Table 6.1 reports optimal doses for different chemicals, note that results comparison among different studies has to be taken with caution since the efficiency of flocculants is dependent on the type of microalgae, their concentration and the medium conditions (Garde et al. 2014; Divakanar and Pillai, 2002).

According to Vandamme et al. (2010), increasing the initial biomass concentration from 75 to 300 mg/L, increased the cationic starch required from 5 to 7.5 g per g of biomass. Values higher than in this study corresponded to lower recovery efficiencies (85% vs. 95%). Higher recovery efficiencies (>95%) were obtained with cationic starch for the flocculation of *Scenedesmus dimorphus* in growth phases at the dosage of 10 mg/L (Hansel et al., 2014). In that study, the initial culture turbidity was 50 NTU, which corresponded to a biomass density of 0.12 g/L, lower than in this case (0.2 g/L).

When comparing doses it is important to consider the flocculants cost which can be significantly different depending on the product. For example, chitosan (10US$) is about 10 times more expensive than cationic starch (1-3 US$) (Vandamme et al., 2010).

Table 6.1 Literature results on microalgal biomass recovery using different chemicals.

Microalgal Species	Microalgal concentration (g/L)	Chemical	Optimal dose (mg/L)	Biomass recovery (%)	pH	Reference
Schizochytrium limacinum	0.09 0.93 4.65	Aluminum sulfate	200	- 90 90		Gerde et al., 2014
	0.09 0.93 4.65	Cationic starch	10	37 80 80		
Chlamydomonas reinhardtii	0.03 0.31 1.06	Aluminum sulfate	250	90 90 60		
	0.03 0.31 1.06	Cationic starch	20	- 90 20		
Scenedesmus sp.	0.09 0.93 4.65	Aluminum sulfate	200	90 90 90		
	0.09 0.93 4.65	Cationic starch	40	70 90 90		
Chlorella protothecoides	0.44 0.56 0.77	Cationic starch	40	87 95 96	4 7.7 10	Letelier-Gordo et al., 2014
Scenedesmus dimorphus		Cationic starch	10-100	70-95		Hansel et al., 2014
Parachlorella sp.	0.075 0.15 0.3	Cationic starch	20 25 25	90 90 85	5-10	Vandamme et al., 2010
Mixture of *Spirulina* sp., *Oscillatoria* sp., *Chlorella* sp., *Synechocystis* sp.	10 NTU 20 NTU 30 NTU 55 NTU	Chitosan	15 5 2 15	60 78 82 80	4-9	Divakanar and Pillai, 2002
Nannochloropsis sp.	665 × 10^6cell/mL	Chitosan	60	70-98	7-9	Farid et al., 2013
Mixture dominated by *Chlorella* sp.	0.2	Starch	25	95	8.9-10.1	This study

6.3.2 Settling velocity distributions

The elutriation test was performed in order to determine the starch effect on the settling velocity distribution of microalgal biomass. Results shown in Figure 6.3 represent the percentage of biomass (expressed as TSS) retained in each column, which corresponds to different settling velocities. Considering the control, which is microalgal biomass without starch addition, 46% of the TSS were retained in the first column (settling velocities >6.5 m/h), while 37% of the TSS were retained in the second column (settling velocities between 1.6 and 6.5 m/h).

The effect of coagulation-flocculation on biomass settling velocities was very clear since TSS retention in the first column increased from 46 % (in the control) to 78 and 73% for starch concentrations of 10mg/L and 25mg/L, respectively. This means that starch raised settling velocities of biomass thanks to flocs formation. Results obtained with 10 and 25 mg/L of starch were not significantly different.

Settling velocities have a direct impact on the settler dimensioning. In fact, considering the results of this study, the addition of starch with the consequent increase in settling velocity, would reduce the settler volume by 4 times maintaining the biomass recovery efficiency > 70%.

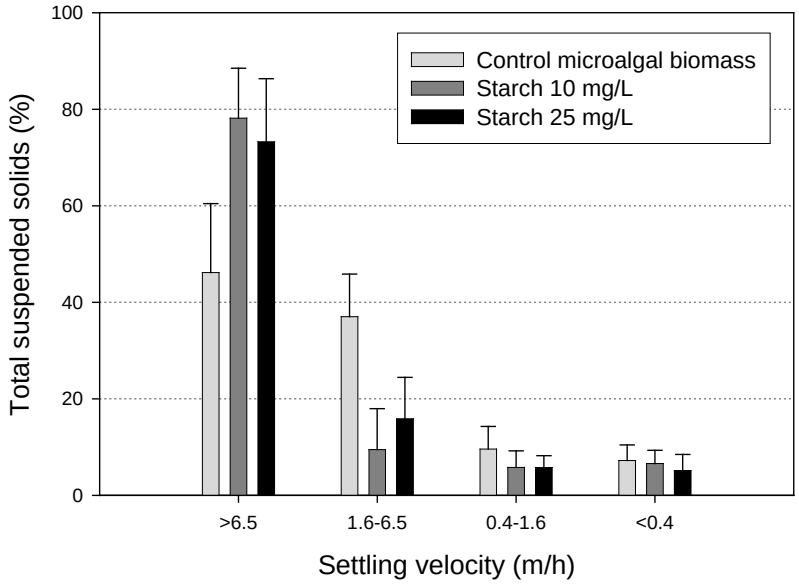


Figure 6.3 Elutriation test results expressed as percentage of total suspended solids corresponding to different settling velocities.

6.3.3 Biochemical methane potential

Considering anaerobic digestion as downstream process, the flocculant should not have any inhibitory effect on biogas production. On the contrary, an organic biodegradable flocculant such as starch may even increase biogas production. For this reason, biochemical methane potential tests were carried out to compare the biogas production obtained from control samples (microalgal biomass, and starch at 10 and 25 mg/L) and from samples with microalgal biomass and selected starch concentrations (10 and 25 mg/L). The results obtained (Fig. 6.4) indicate that the addition of starch increased the biogas production with respect to control microalgae biomass (503-536 vs. 467 mL biogas), even though the results are not significantly different ($p<0.05$). The comparison of microalgal biomass and starch codigestion (503-536 mL biogas) with the digestion of both substrates separately (467 mL biogas for microalgae, 10-11 mL biogas for starch) suggests certain synergic effect of the mixture which may be attributed to a more balanced C/N concentration, enhancing to some extent the biogas production.

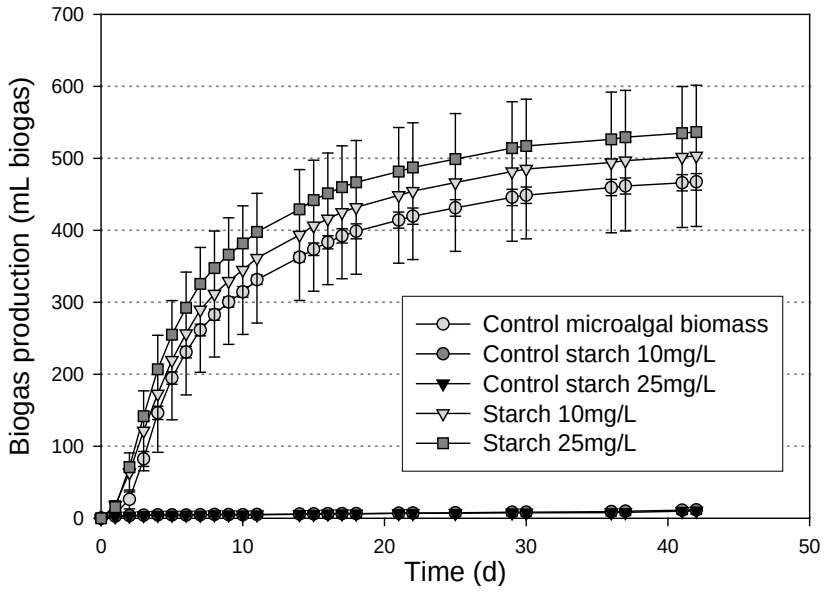


Figure 6.4 Biochemical methane potential for control (microalgal biomass, starch 10 mg/L and 25 mg/L) and microalgal biomass flocculated with starch doses of 10 and 25 mg/L.

6.4 Conclusions

The effectiveness of starch as flocculant was tested on microalgal biomass. Low starch doses (5-60 mg/L) allowed high biomass recovery >92%. This is a promising result, improving both microalgal biomass recovery and treated wastewater discharge. The most appropriate starch dose was 25mg/L, leading to >95% biomass recovery and an effluent turbidity <9 NTU. The elutriation test underlined the positive effect of starch addition on the biomass settling velocity, increasing to >70% the percentage of particles with settling velocities >6.5m/h. Finally, biochemical methane potential tests show that starch biodegradation increased the biogas production from harvested biomass.

7

Biomass recycling for spontaneous flocculation

This chapter is based on the article:

Gutiérrez, R., Uggetti, E., Ferrer, I., García, J. (submitted) Microalgal biomass recycling: an alternative low-cost strategy for improving gravity settling of high rate algal ponds. Water Research.

7.1 Introduction

In recent years, much attention has been paid to microalgae-based systems for wastewater treatment and biomass production like high rate algal ponds (HRAP). In fact, microalgal biomass grown as a by-product of wastewater treatment is nowadays considered as a cost-effective feedstock for bioenergy production. Despite bioenergy production from microalgae has well-known advantages in front of other biomass sources (i.e. fast growth rates and lack of competition for agricultural land or water), each step of the process from microalgae production to bioenergy conversion still has to be improved in order to reduce the operating costs of the entire process (Mehrabadi et al., 2014).

Specifically, current biomass harvesting techniques increase the cost of microalgae production, representing between 20-30% of the total cost (Molina-Grima et al., 2003; Zittelli et al., 2006). Commonly employed methods include the addition of chemicals or the use of mechanical equipment that increase costs (e.g. flocculation induced by chemical addition, filtration, centrifugation, sonication, electro-flocculation). In wastewater treatment, gravity sedimentation is the most common solids separation method, used to clarify large volumes of treated wastewater with reasonable costs (<5% of the total cost, (Metcalf and Eddy, 2004)). The biomass grown in HRAPs for wastewater treatment is constituted by mixed populations of microalgae and bacteria which form spontaneous flocs (diameter 50-200 μm) that can partially settle by gravity without chemicals and energy addition (García et al., 2000b; Park et al., 2011a; Valigore et al., 2012). Inside these flocs, microorganisms interaction provides natural occurring processes inducing their spontaneous flocculation (Golueke and Oswald, 1970; Salim et al., 2011).

For these reasons, in the last years a niche research of harvesting techniques has focused on the optimization of spontaneous flocculation and gravity sedimentation (González-Fernández and Ballesteros, 2013; Van Den Hende et al., 2011). Different methods and strategies to induce spontaneous flocculation have recently shown promising results. For instance, coprecipitation with ions at high pH (autoflocculation), and release of extracellular polymeric substances or microalgae-bacteria interaction (bioflocculation) are some of the different strategies used to accomplish spontaneous flocculation (González-Fernández and Ballesteros, 2013). A recently developed promising strategy consists in promoting the dominance of rapidly settling microalgae species by recycling a small part of the biomass harvested in gravity settlers (Park et al., 2011b). Thus,

species that can settle easily are selected competitively against those poorly settling species.

Following this promising approach, the aim of the present study was to improve microalgal biomass harvesting efficiency by recycling an increasing amount of harvested biomass and to determine its effect on the biomass production, microalgae species evolution and wastewater treatment performance. Furthermore, two recycling rate of 2% and 10% (dry weight) of harvested biomass was tested in this study in order to improve the spontaneous flocculation of algae-bacteria biomass in experimental HRAPs treating real urban wastewater. Harvesting efficiency results were here evaluated in terms of biomass recovery and microalgal biomass settling velocities distribution.

7.2 Material and Methods

7.2.1 Experimental high rate algal pond system

Two experimental HRAPs located outdoors at the facilities of the Environmental Engineering and Microbiology Research Group (GEMMA) of the Universitat Politècnica de Catalunya·BarcelonaTech (Barcelona, Spain) were used in the present study. These HRAPs were continuously operated since 2010 (Matamoros et al., 2015; Passos et al., 2015). For the purpose of this research, HRAPs were monitored over one year (from March 2014 to March 2015). Raw urban wastewater from a nearby municipal sewer was daily pumped to a homogenisation tank (volume of 1.2 m^3) and uninterruptedly pumped to a primary settler with a useful volume of 7 L, a surface area of 0.0255 m^2 and a hydraulic retention time (HRT) in the range of 0.7-1.4 h. The settler primary effluent (from now on referred to as primary wastewater) was discharged into the two HRAPs by means of two peristaltic pumps. Both HRAPs operated at the same hydraulic retention time (HRT) during the whole experimental period. As suggested by García et al. (2000b), the theoretical HRT was modified over the year (8, 6 and 4 days) according with the weather conditions (i.e. solar radiation and temperature). In fact, these systems require longer HRT in cold weather conditions with low solar radiation in order to accomplish wastewater treatment and meet effluent quality requirements for discharge. The theoretical HRT was changed by regulating flow rates (120, 78.5 and 60 L/d for 4, 6 and 8 days of HRT, respectively).

Each HRAP, built in PVC, had a surface area of 1.54 m^2, 0.3 m of water depth and a useful volume of 0.47 m^3. This was achieved by means of two paddle-wheels driven by an engine operated at 5 rpm, reaching a flow velocity of 10 cm/s in the mixed liquor. Continuous stirring of the mixed liquor avoided biomass sedimentation and assured microalgae contact with sunlight. Biomass growing in the HRAPs was harvested in two secondary settlers (one per each HRAP) with a useful volume of 3.1 L, a surface area of 0.013 m^2 and a critical settling velocity of 0.4, 0.25 and 0.2 m/h corresponding to HRT of 0.6, 1 and 1.2 h for the 120, 78.5 and 60 L/d flow rates of the HRAPs, respectively. Around 1-1.5 L of harvested biomass with a total solid concentration between 1-2% (w/w) (depending on the period of the year) were purged from each settler every weekday.

7.2.2 Biomass recycling

In order to evaluate the influence of biomass recycling on, harvesting efficiency, microalgae production and wastewater treatment, one HRAP was supplied with biomass recycling while the other one was used as a control (from now on referred to as R-HRAP and C-HRAP, respectively). Figure 7.1 shows a schematic diagram of the process in the R-HRAP line. In a previous study by Park et al. (2011b), a constant volume of 1 L of harvested microalgal biomass was daily recycled to a 8 m^3 HRAP. In this previous study, the constant recirculation volume applied did not take into account the variation of solids concentration in the HRAP mixed liquor. From the data presented by Park et al. (2011b), a recycling rate between 2-16% (dry weight) of the harvested microalgal biomass was inferred. Taking this range of values as reference for the present study, two different recycling rates (2% and 10% dry weight) were here tested, corresponding to a variable recycling flow rate of the harvested biomass to the R-HRAP. The recycling flow rate was calculated weekly following Eq. 7.1.

$$Q_R = \text{Recycling rate (\%)} * \frac{TSS_{HRAP} * Q}{TSS_{Settler}}$$

Eq. (7.1)

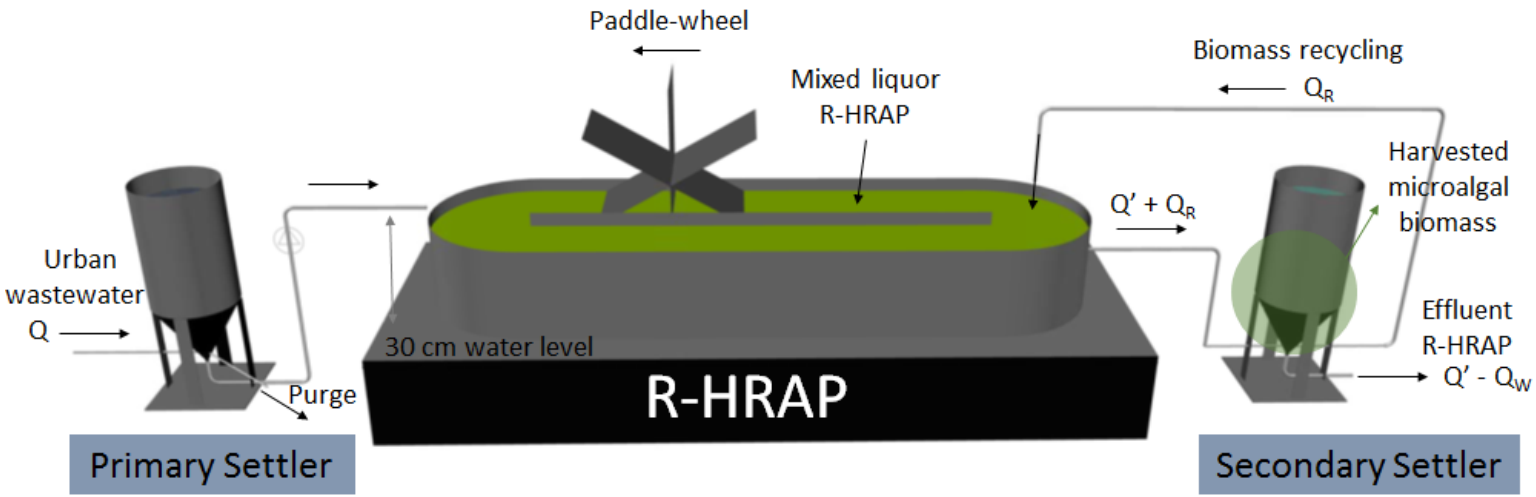


Figure 7.1 Schematic diagram of the line process with recycling including the primary treatment (conventional settler) and the secondary treatment (high rate algal pond (R-HRAP) followed by a secondary settler. Q is the wastewater flow rate (L/d), Q' is the flow rate (L/d).

Where Q_R is the recycled flow rate (L/d); TSS_{HRAP} is the mixed liquor total suspended solids concentration (mg/L); $TSS_{Settler}$ is the total suspended solids concentration of the biomass harvested in the secondary settler (mg/L) and Q is the primary wastewater flow rate (L/d). Note that in the present study, "mixed liquor sample" refers to the sample collected inside the two HRAPs, "effluent sample" corresponds to the water from the secondary settler, and "harvested biomass sample" corresponds to settled biomass. The recycled harvested biomass corresponded to the fraction of the daily harvested biomass which was returned to the HRAP in one run.

Due to biomass recycling, in the R-HRAP the solids retention time (SRT) was higher than the HRT, while the SRT and HRT were identical in the C-HRAP. The SRT from the R-HRAP was calculated by Eq. 7.2 according to Metcalf and Eddy (2003).

$$SRT = \frac{V * TSS_{HRAP}}{(Q - Q_E + Q_P) * TSS_{HRAP} - Q_R * TSS_{Settler}}$$

Eq. (7.2)

where Q is the primary wastewater flow rate (L/d); Q_E is the evaporation rate (L/d) and Q_P is the precipitation rate (L/d); Q_R is the recycled flow rate (L/d); TSS_{HRAP} is the mixed liquor total suspended solids concentration (mg/L); $TSS_{Settler}$ is the total suspended solids concentration of the biomass harvested in the secondary settler (mg/L) and V is the total volume of the HRAP (L).

The evaporation rate was calculated following Eq. 7.3.

$$Q_E = \frac{E_p A}{7}$$

Eq. (7.3)

Where A is the surface area of the HRAP (m^3) and E_p is the potential evaporation between weekly samples (mm) which was calculated from Turc's formula (Eq. 7.4).

$$E_p = a(R + 50)\frac{T}{T + 15}$$

Eq. (7.4)

where: R is the average solar radiation in a week (cal/cm^{2}d); T is the average temperature in a week (ºC); a is the dimensionless coefficient which varies depending on the time between samples. The value of *a* for weekly samples is 0.091.

Solar radiation, air temperature and precipitation data were obtained from a close meteorological station (Department of Astronomy and Meteorology, University of Barcelona, http://infomet.am.ub.es).

To evaluate the biomass harvesting efficiency, the biomass recovery (%) was calculated following Eq. 7.5.

$$\text{Biomass recovery (\%)} = \frac{\text{TSS}_{\text{HRAP}} - \text{TSS}_{\text{Effluent}}}{\text{TSS}_{\text{HRAP}}} * 100$$

Eq. (7.5)

Where TSS_{HRAP} is the mixed liquor total suspended solids concentration (mg/L) and $TSS_{Effluent}$ is the total suspended solids concentration of the secondary settler effluent (mg/L)

The experiment was divided in four periods characterised by different HRT (depending on the season) and recycling rate: period 1 (HRT: 8 days, recycling rate: 2%), period 2 (HRT: 4 days, recycling rate: 2%) period 3 (HRT: 6 days, recycling rate: 10%) and period 4 (HRT: 8 days, recycling rate: 10%). The main operational parameters and wastewater characteristics of the HRAP systems with and without recycling are summarised in Table 7.1.

7.2.3 Dynamic sedimentation test

The settling velocities distribution of microalgal biomass from both HRAPs was studied by means of a dynamic sedimentation test using a water elutriation apparatus. In this device biomass flocs are washed out according to their relative density, volume and form, under dynamic conditions. Microalgal biomass passes through three settling columns with increasing diameters (50 mm, 100 mm and 200 mm of nominal diameter for C1, C2 and C3 settling columns, respectively) interconnected in series. For each test, 25 L of HRAP mixed liquor were poured to a continuously stirred inlet tank (30 L) and then pumped through the columns. The flow rate in these tests was set to 0.21 L/min in order to have a

Table 7.1 Environmental and operation parameters of the high rate algal pond systems with and without harvested biomass recycling. Average values (±s.d.) of nutrient and organic matter concentration correspond to the primary wastewater and biomass concentration from HRAPs mixed liquor samples taken at 12 PM. Temperature and solar radiation are average daily values (±s.d.) of the period.

Parameter	Period 1 (Mar-Apr'14) *n* = 6	Period 2 (May-July'14) *n* = 12	Period 3 (Aug-Oct'14) *n* = 8	Period 4 (Nov-Mar'15) *n* = 13
Solar radiation (W/m^2)	398 (33)	446 (28)	355 (43)	234 (38)
Air temperature (ºC)	15.8 (2.1)	22.5 (3.5)	23.7 (1.8)	13.1 (2.4)
Influent flow rate (L/d)*	58.9 (3)	117.3 (5)	78.8 (5)	60.4 (6)
Influent $N\text{-}NH_4^+$ (mg/L)	30 (7)	33 (5)	36 (9)	26 (6)
Influent COD (mg/L)	381 (150)	463 (200)	318 (181)	363 (190)
Experimental HRT (days)*	8.1 (0.4)	4.2 (0.6)	6 (0.5)	7.8 (0.8)
Recycling rate (%)**	2	2	10	10
SRT (days) **	8.6 (0.5)	4.5 (0.8)	39.9 (3.5)	52.9 (2.0)
Secondary settler HRT (hours)	1.2	0.6	1	1.2
Mixed liquor concentration (g TSS/L)**	0.36 (0.12)	0.38 (0.13)	0.46 (0.83)	0.30 (0.11)
Harvested biomass concentration (g TSS/L)**	13.3 (13.3)	20.9 (11.5)	20.2 (7.2)	11.3 (10.6)
Recycled biomass flow rate (L/d)**	0.22 (0.19)	0.20 (0.15)	0.87 (0.16)	0.76 (0.27)

*Calculated considering evaporation and precipitation rates.
** Only for the high rate algal pond with recycling (R-HRAP)

range of critical settling velocities (0.4 - 6.5 m/h) similar to those used in secondary settlers (0.7 – 1.3 m/h according to Metcalf and Eddy, (2004)). The sample entered each column near the bottom and exited near the top. Note that the critical settling velocity decreased progressively in successive columns due to their gradual increase in column diameter, and therefore biomass flocs were retained in the different columns depending on their settling velocities. Consequently, the first column retained flocs with a settling velocity ≥6.5 m/h, the second one between 6.5 and 1.6 m/h, and the third one between 1.6 and 0.4 m/h, while flocs with a settling velocity <0.4 m/h were in the outlet tank had. Those flocs with a settling velocity equal to or higher than the critical settling velocity of a given column were retained, while flocs with a settling

velocity lower than the critical settling velocity escaped to the next column. Flocs with a settling velocity lower than the critical velocity of the third column escaped, and were therefore collected in a 30 L outlet tank. A detailed description of the apparatus and of the method can be found in Gutiérrez et al. (2015).

Sedimentation tests were carried out in period 2 (recycling rate: 2%) and in period 4 (recycling rate: 10%). Altogether, two dynamic sedimentation tests (one per HRAP) were conducted with samples from period 2 (recycling rate: 2%) and two (one per HRAP) with samples from period 4 (recycling rate: 10%). At the moment of sedimentation tests in period 2, the total suspended solids concentration were similar values of 230 mg TSS/L and 240 mg TSS/L for the R-HRAP and C-HRAP, respectively, In contrast, higher differences were observed at the time of sedimentation tests in period 4, with solids concentration of 420 mg TSS/L and 130 mg TSS/L for the R-HRAP and C-HRAP, respectively.

7.2.4 Microalgal biomass production and characterisation

Biomass production was quantified once a week based on the total suspended solids concentration (TSS) from the mixed liquor of the two HRAPs and expressed in terms of g TSS/m^2d following Eq. 7.6. Evaporation and precipitation rates were taken into account.

$$\text{Microalgal biomass production} = \frac{\mathrm{TSS_{HRAP}} \cdot [Q - Q_E + Q_P] - [\mathrm{TSS_{Settler}} Q_R]^*}{A \cdot 1000}$$

Eq. (7.6)

Where TSS_{HRAP} are total suspended solids concentration of the mixed liquor HRAP (mg TSS/L); Q: is the primary wastewater flow rate (L/d); Q_E is the evaporation rate (L/d); Q_p is the precipitation rate (L/d); Q_R is the recycled flow rate (L/d); $TSS_{Settler}$ is the total suspended solids concentration of the biomass harvested in the secondary settler (mg/L) and A is the surface area of the HRAP (m^2). The term in brackets (*) was only taken into account for the R-HRAP (production was calculated by subtracting the recycled biomass, which would otherwise overestimate the results of the R-HRAP).

To prove the recycling effect on population dynamics, two sampling campaigns were conducted for microorganisms identification. The first campaign was conducted in

periods 1 and 2 (2% recycling rate) over 3 months, with 13 samples analysed. The second campaign was carried out in period 4 (10% recycling rate) over 3 months, with 11 samples analyzed. During these campaigns, 250 mL- samples were taken once a week from the mixed liquor of the HRAPs. From these samples, microalgae species were identified and quantified. Other co-occurred microorganisms (ciliates and rotifers) were also identified. Microalgae identification was carried out by optic microscope examination (Motic BA310E, China), equipped with a camera (NiKon DS-Fi2) using the software NIS-Elements Viewer. Microalgae genera were identified from classical specific literature (Bourelly, 1966; Palmer, 1962). For microalgae quantification, two replicates of 25μL of each well-homogenised sample were examined by bright and contrast phase microscopy using a Zeiss microscope Axioskop 40. In each subsample, microalgae were counted in vivo at 100 and 400 magnification using coverslides of 20 mm side (Salvadó et al., 2004).

7.2.5 Wastewater treatment

Wastewater treatment performance was monitored during the whole year. Nitrates (NO_3^-), ammonium nitrogen ($N\text{-}NH_4^+$), phosphates (PO_4^{-3}), the biochemical oxygen demand (BOD) and the chemical oxygen demand (COD) were used as indicators of wastewater treatment efficiency. For practical purposes, though, only ammonium nitrogen ($N\text{-}\ NH_4^+$) and the chemical oxygen demand (COD) were considered to evaluate the nutrient and organic matter removals. Samples from the mixed liquors of the two HRAPs as well as the primary wastewater (influent of the HRAP) were taken and analysed once a week.

To evaluate the COD removal, samples of the primary wastewater were analysed (without filtration) obtaining the total COD (TCOD). On the other hand, samples of the HRAPs mixed liquor were filtrated (glass fiber filters of 47 mm and average pore size 1 μm), obtaining the soluble COD (SCOD) in order to avoid the microalgae contribution to the organic matter content. Total (TCOD) and soluble (SCOD) were analysed according to Standard Methods (APHA-AWWA-WPCF, 1999) and $N\text{-}\ NH_4^+$ was measured from filtered samples according to the Solorzano method (Solorzano, 1969). All the analyses were undergone in triplicate and results are given as average values.

7.2.6 Statistical analysis

The effect of biomass recycling on wastewater treatment performance (ammonium nitrogen and organic matter removal), microalgal biomass production and harvesting efficiency was evaluated by means of the Student's paired t test using Minitab 17.0 software. $p=0.05$ was set as the level of statistical significance.

7.3 Results

7.3.1 Microalgal biomass harvesting

Biomass recovery

Microalgal biomass concentration in the mixed liquor and in the effluent of secondary settlers from both HRAPs, along with the calculated biomass recovery are shown in Figure 7.2. Mixed liquor biomass from the HRAPs, varied over the year within the range of 83-683 mg TSS/L and 47-489 mg TSS/L for the R-HRAP and the C-HRAP, respectively. Less variability was observed in effluent concentrations, which varied between 8-54 mg TSS/L for the R-HRAP and 11-63 mg TSS/L for the C-HRAP. Average values of these concentrations and biomass recoveries concerning each period are summarised in Table 7.2. The mixed liquor average biomass concentrations from the R-HRAP were higher than in the C-HRAP (30-459 mg TSS/L vs. 144-353 mg TSS/L, respectively). Furthermore, the effluent biomass concentration from the R-HRAP settler (18-30 mg TSS/L) was lower than in the C-HRAP settler (34-54 mg TSS/L). Thus, average biomass recovery was higher in the R-HRAP (92-94%) than in the C-HRAP (75-89%).

When recycling rate was 2%, the difference between the biomass recovery of the R-HRAP and C-HRAP decreased from 14% (period 1) to 4% (period 2) (Table 7.2). Then, when recycling was increased to 10%, the difference between the biomass recoveries of both HRAPs increased up to 16% (period 4). Statistical analyses also reported significant differences between biomass recoveries ($p<0.05$), highlighting the great influence of recycling on the harvesting efficiency of the microalgal biomass.

Table 7.2 Biomass production and harvesting efficiency in the HRAP with biomass recycling (R-HRAP) and control HRAP (C-HRAP). Average values (stdev) from samples taken at 12 PM.

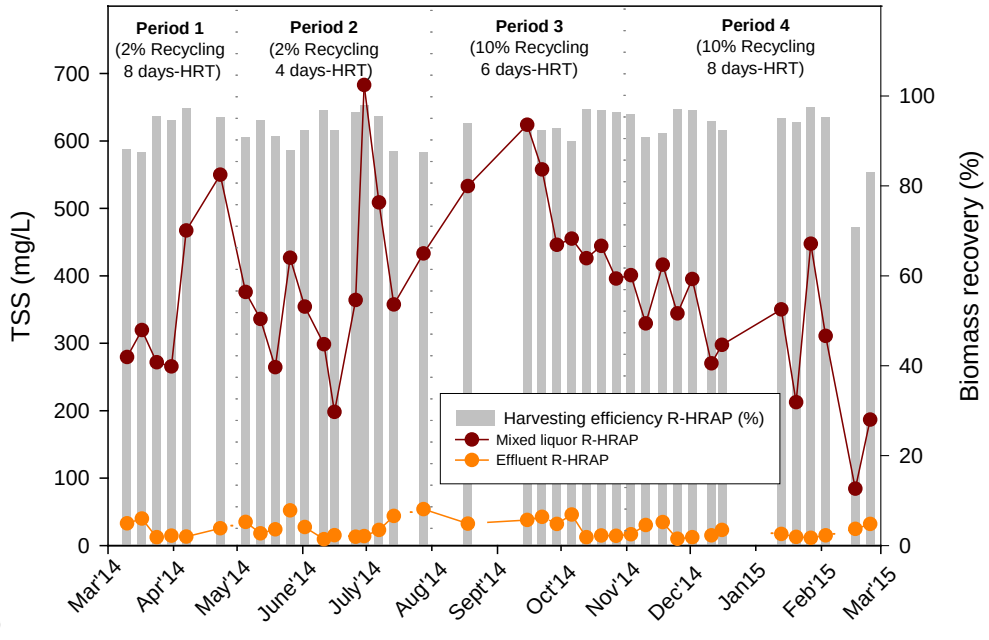
Parameter		Period 1 (Mar-Apr'14) *n* = 6	Period 2 (May-July'14) *n* = 12	Period 3 (Aug-Oct'14) *n* = 8	Period 4 (Nov-Mar'15) *n* = 13
Mixed liquor concentration (mg TSS/L)	R-HRAP	359 (120)	379 (129)	459 (83)	301 (108)
	C-HRAP	273 (94)	353 (73)	197 (69)	144 (47)
Effluent biomass concentration (mg TSS/L)	R-HRAP	23 (12)	25 (14)	30 (14)	18 (8)
	C-HRAP	54 (59)	39 (17)	34 (12)	34 (13)
Biomass recovery (%)[1]	R-HRAP	93.0 (4)	92.6 (3)	94.2 (2)	91.9 (7)
	C-HRAP	78.7 (20)	88.9 (6)	78.9 (8)	75.8 (9)
Biomass recovery variation (%)		14 (19)	4.6 (8)	15.2 (7)	16.0 (11)
Microalgal biomass with settling velocities ≥ 0.4 m/h (%)*	R-HRAP	-	98	-	92
	C-HRAP	-	93	-	36
Microalgal biomass production (g TSS/m^{2}d)[2]	R-HRAP	12.5 (3.8)	25.8 (10.7)	10.5 (4.5)	3.3 (1.7)
	C-HRAP	10.4 (3.6)	25.7 (6.9)	10.0 (3.5)	5.5 (1.8)

[1] *p*-value of 5exp-7

[2] *p*-value of 0.909

*Biomass recovery from punctual sedimentation tests calculated as the amount of biomass (as %) from the mixed liquor of the R-HRAP and the C-HRAP with settling velocities ≥ to 0.4 m/h.

(a)



(b)

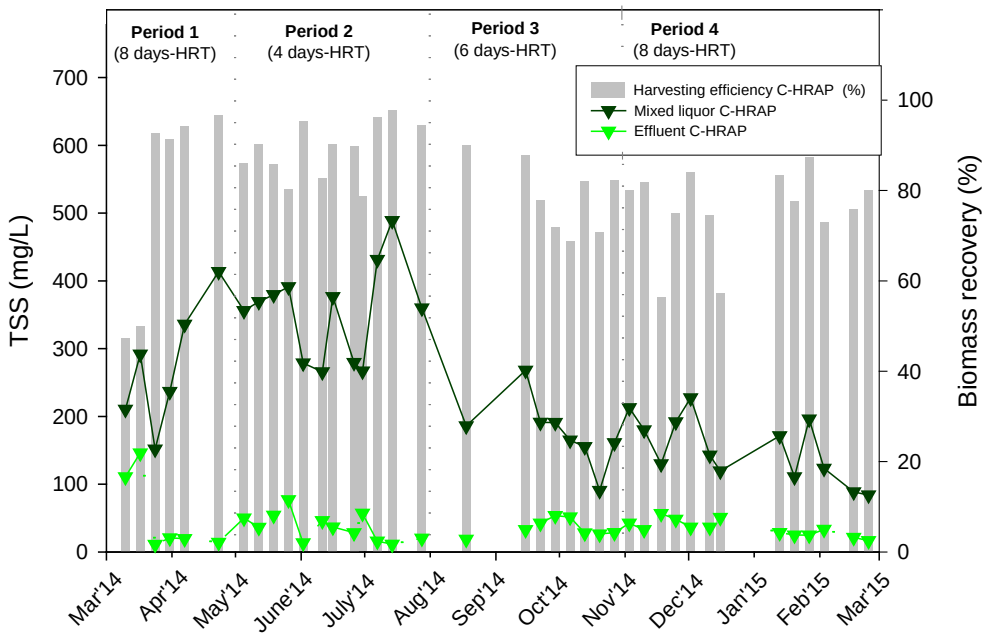


Figure 7.2 Microalgal biomass concentration in the mixed liquor and in the effluent of secondary settlers from the HRAP with biomass recycling (R-HRAP) (a) and control HRAP (C-HRAP) (b) over one year. Harvesting efficiencies are represented by grey bars.

Biomass settling velocities distribution

Two sedimentation tests (one for each HRAP) were carried out in period 2 (Fig. 7.3a) and period 4 (Fig. 7.3b) in order to evaluate the effect of biomass recycling on the settling velocities distribution of microalgal biomass. In Figure 7.3, each pair of bars refers to the amount of biomass with a certain settling velocity. From the microscopic images of the Figure 7.3, it can be clearly observed the decrease in biomass through the columns. By combining the percentages of the first two bars the amount of biomass with settling velocities ≥1.6 m/h was obtained. Results from period 2 (recycling rate: 2%, HRT: 4 days) indicate that 80% of the biomass from the C-HRAP had settling velocities ≥1.6 m/h, while this value was increased to 95% in the case of the R-HRAP (Fig. 7.3a). This means that the amount of rapidly settling biomass increased when biomass recycling was applied. In period 2, the critical settling velocity of secondary settlers was 0.4 m/h. Therefore, the amount of biomass recovered with settling velocities > 0.4 m/h (combining the first three bars) should be similar to the harvesting efficiency of the secondary settler at that moment (Table 7.2). When the sedimentation test was conducted (last week of June), harvesting efficiencies of 98% and 90% were achieved in the secondary settler for the R-HRAP and C-HRAP, respectively. Accordingly, the amount of biomass recovered with settling velocities > 0.4 m/h in sedimentation test was 98% and 93% for the R-HRAP and the C-HRAP, respectively, which was close to biomass recovery of secondary settlers.

On the other hand, sedimentation tests carried out in period 4 (recycling rate: 10%, HRT: 8 days) showed that 86% of the biomass from the R-HRAP had settling velocities > 1.6 m/h, in contrast with only 5% of microalgal biomass from the C-HRAP (Fig. 7.3b). In this period, the critical settling velocity of secondary settlers was 0.2 m/h. Even if considering low settling velocities in the sedimentation test (0.4 m/h), similar to the critical settling velocity of the settler, great differences were found between HRAPs (Table 7.2). With 0.4 m/h, 36% of biomass from the C-HRAP was recovered in comparison with 92% of biomass from the R-HRAP. This explains important differences in biomass recovery (16% in average) found in the period 4 when the recycling rate was 10%, as compared to the period 2 (2% recycling) when only the 4% of biomass recovery difference between both HRAPs were observed. In addition, at the time of the sedimentation test in period 4, the highest biomass recovery difference between the two HRAPs was observed (92% vs. 56% for the R-HRAP and the C-HRAP, respectively).

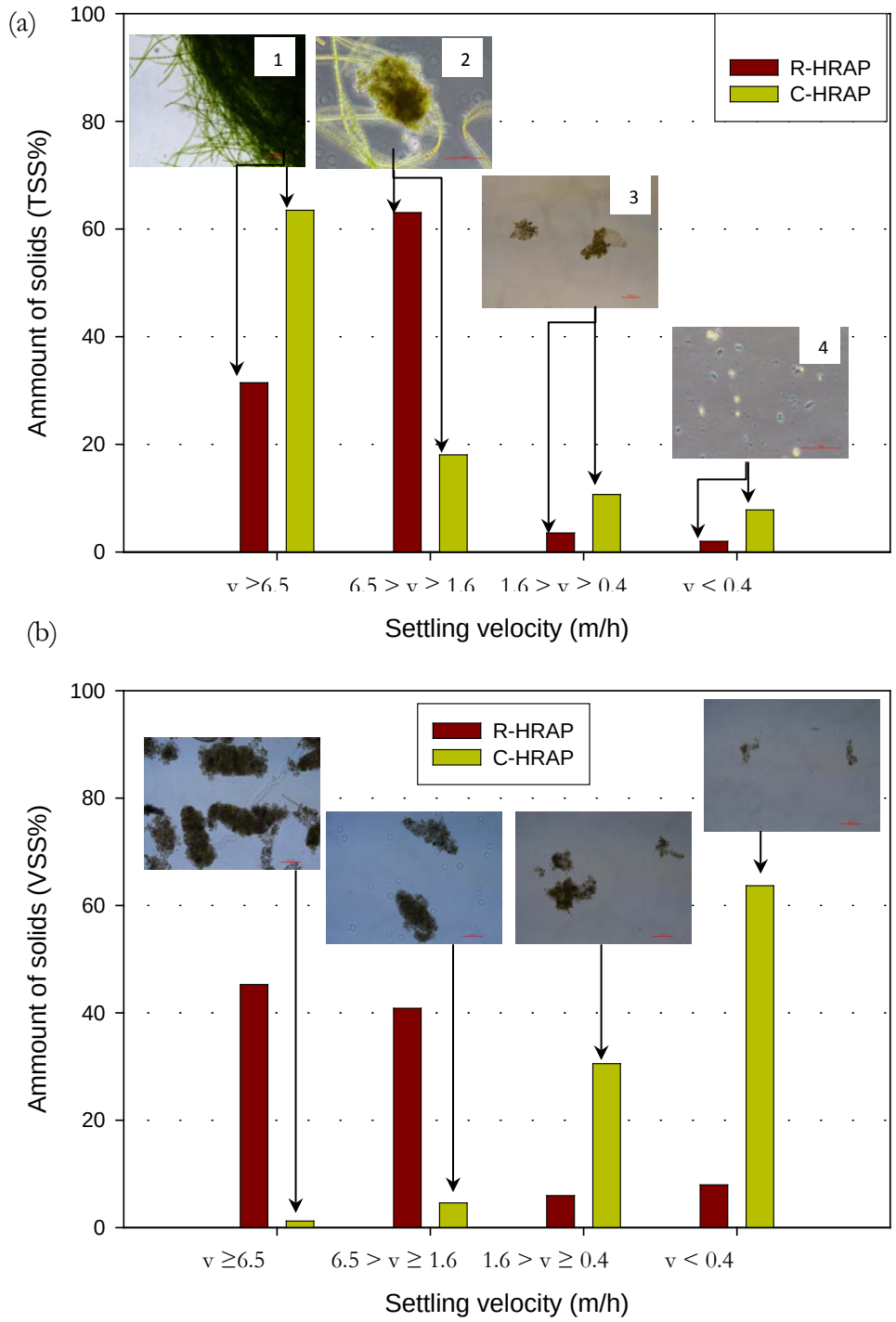


Figure 7.3 Settling velocities distribution of microalgal biomass from the R-HRAP (brown columns) and C-HRAP (green columns). Samples from period 2 (2% recycling) (a) and period 4 (10% recycling) (b). Microscopic images correspond to samples of each

settling column (magnification of x100 for the 1, 3, 1'- 4'; x200 for the 2; and x400 for the 4). In period 1 (a) both samples from the R-HRAP (brown columns) and C-HRAP (green columns) presented similar floc characteristics. In period 2 (b) microscopic images correspond only to the C-HRAP, since floc characteristics from the R-HRAP sample were similar to period 1.

7.3.2 Microalgal biomass production and characterisation

Microalgal biomass production

Microalgal biomass production in both HRAPs is shown in Figure 7.4. Average values of biomass production concerning each period are summarised in Table 7.2. Biomass production was not significantly different ($p>0.05$) in both HRAPs; therefore biomass recycling did not affect biomass production. Seasonal biomass production variations were mostly related to changes in HRT and weather conditions. As expected, higher biomass production was observed in those periods with favorable environmental conditions than the periods with adverse conditions. Therefore, in period 2 (summer) a high average biomass production of 25.8 g TSS/m^2d was reached in both HRAPs. On the other hand, in period 4 (autumn and winter), the average biomass production decreased to 3.3 g TSS/m^2d in the R-HRAP and 5.5 g TSS/m^2d in the C-HRAP.

Similar results of biomass production were obtained by Park et al. (2011b) who operated an experimental HRAP (8 m^3) treating primary wastewater, with recycling rates between 2 and 16% of harvested biomass and CO_2 addition, under similar weather conditions (Hamilton, New Zealand). They reported an annual average biomass production of 9.2 g VSS/ m^2d and 10.9 g VSS/m^2d for the C-HRAP and the R-HRAP, respectively. Considering that the TSS of the HRAPs mixed liquor were predominantly organic (VSS/TSS ratio of 0.8-0.9), a similar biomass production was attained in the present study, reaching an average value of 10.4 g VSS/m^2d (or 13 g TSS/m^2d) for both HRAPs. Except for the last period, when the lowest production was registered, the microalgal biomass production ranged between 10.5 – 25.8 g TSS/m^2d for the two HRAPs, falling into the range of 10 – 35 g TSS/m^2d found in outdoor systems dominated by green microalgae (Heubeck et al., 2007; Park and Craggs, 2010).

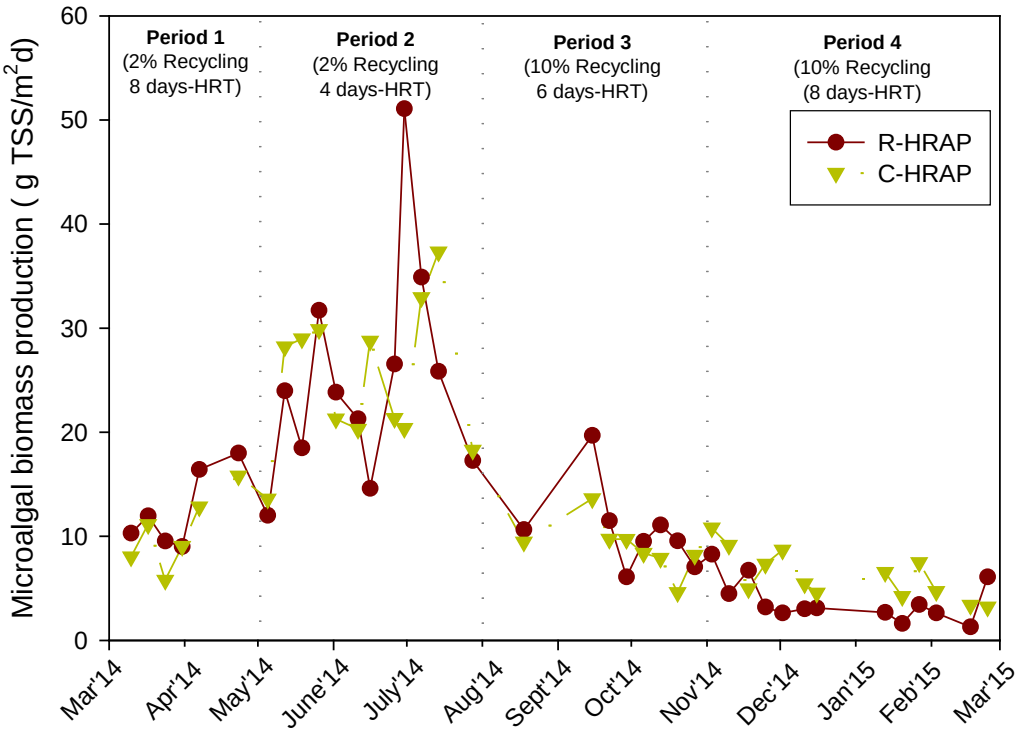


Figure 7.4 Microalgal biomass production for the HRAP with biomass recycling (R-HRAP) and the control HRAP (C-HRAP).

Microalgal biomass characterization

In this study, the most abundant species identified in both HRAPs was the green microalgae *Chlorella* sp. (Figures 7.5 and 7.6). The diatoms *Nitzschia* sp. and *Navicula* sp., and the filamentous green microalgae *Stigeoclonium* sp. were also present. *Stigeoclonium* sp. often formed macroscopically visible thalli. Microalgae grazers like ciliate and flagellate protozoan were continuously observed.

Even if the green unicellular microalgae *Chlorella* sp. was the dominant species over the whole experiment (Figure 7.6), fluctuations in weather conditions (temperature and solar radiation) together with changes of HRT, led to slight variations in microalgae populations abundance.

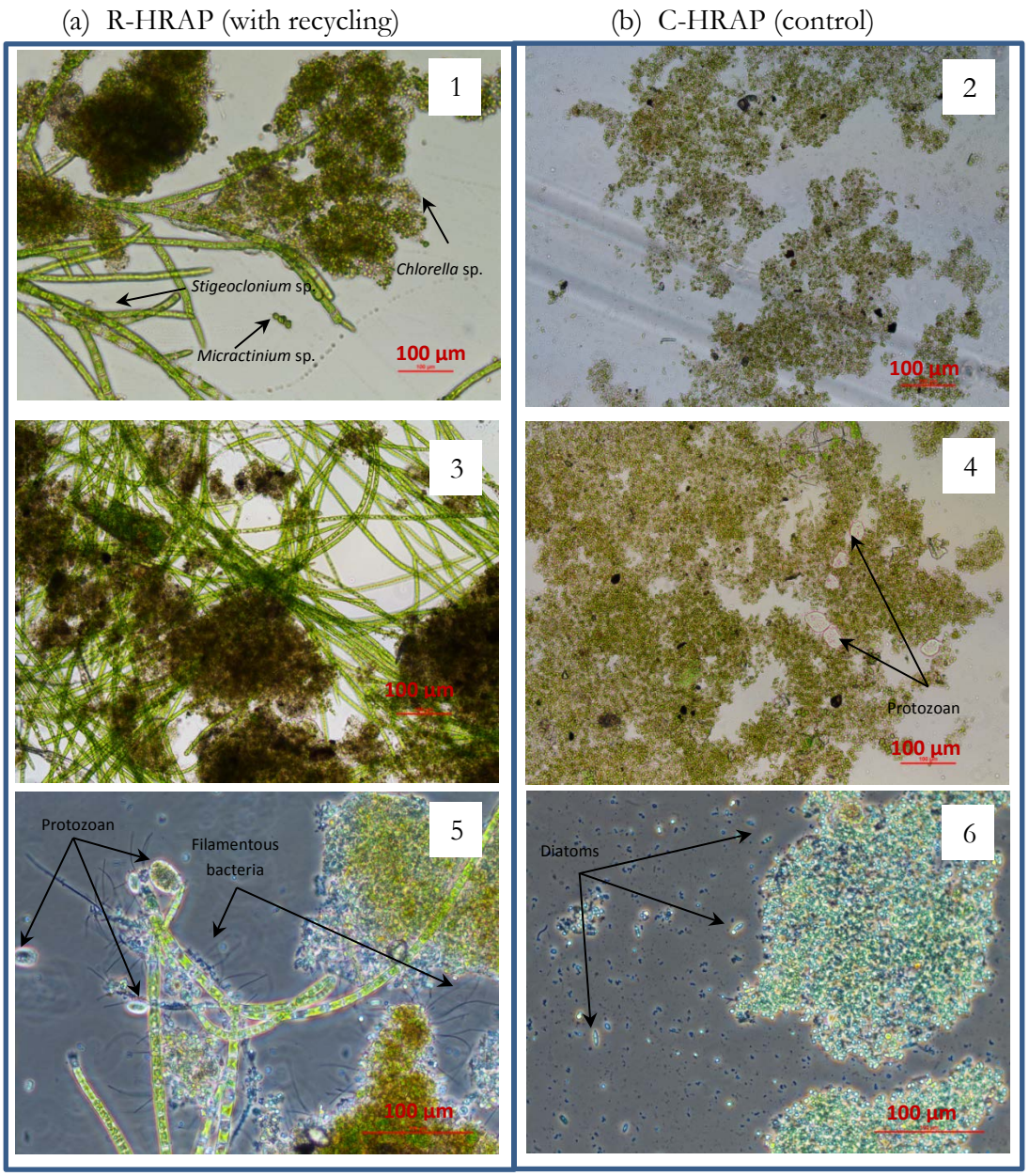


Figure 7.5 Microscopic populations in the mixed liquor from samples taken in November (Period 4) from the R-HRAP (a) (1) *Chlorella* sp. (cells immersed in flocs) and *Stigeoclonium* sp. (filamentous algae) and *Micractinium* sp. (3) *Chlorella* sp. (cells immersed in flocs) and *Stigeoclonium* sp. (5) *Chlorella* sp. (cells immersed in flocs), *Stigeoclonium* sp., filamentous bacteria and protozoan presence, and the C-HRAP (b) (2) *Chlorella* sp. (cells immersed in flocs) and diatoms (4) *Chlorella* sp. (cells immersed in flocs) and protozoa presence (6) *Chlorella* sp. flocs and some dispersed *Nitzschia* sp. and *Navicula* sp. diatoms.

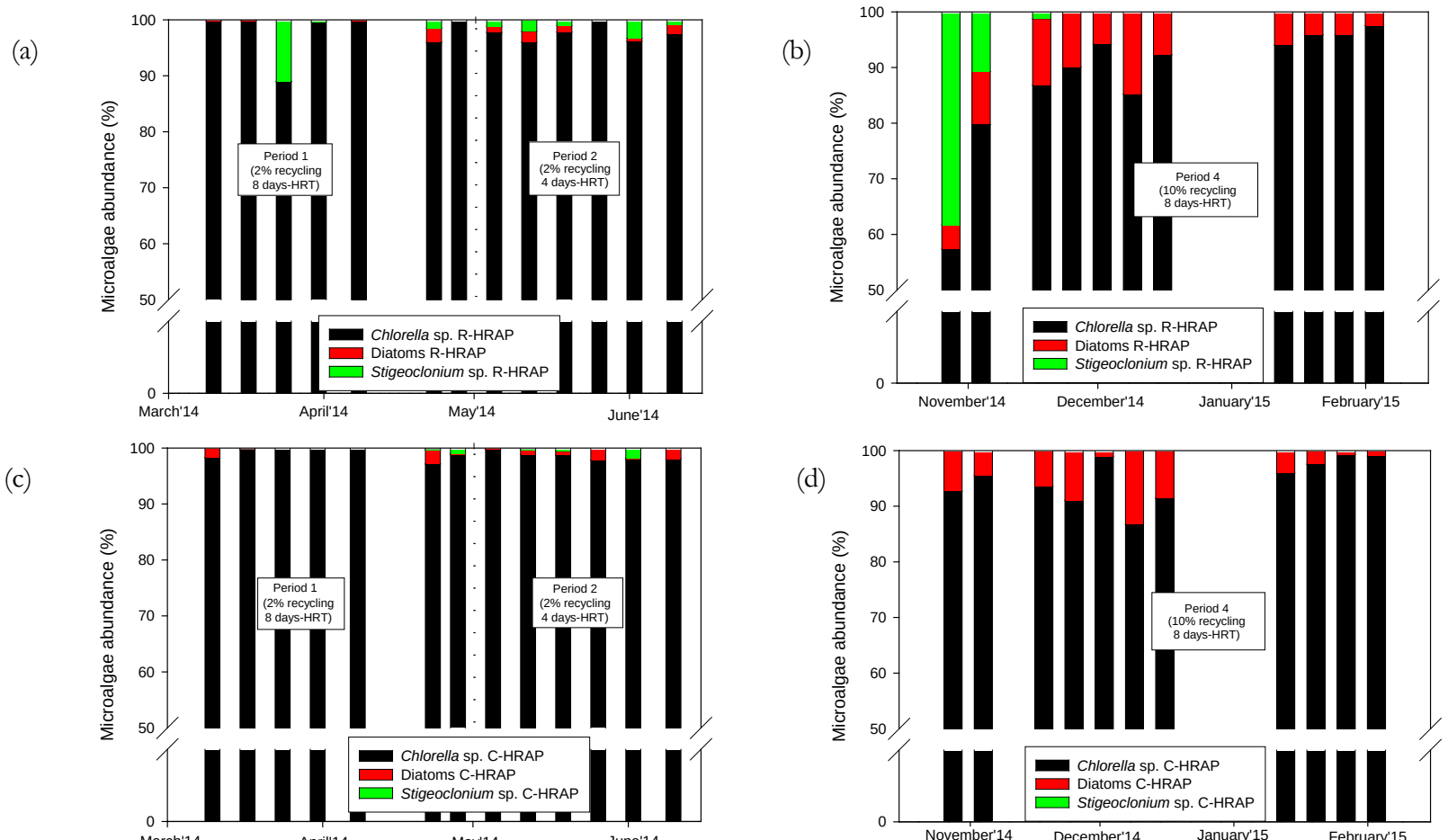


Figure 7.6 Dynamics of microalgae populations of the HRAP with biomass recycling (R-HRAP) (a) (c) and the control HRAP (C-HRAP) (b) (d) during 2% recycling (a) (b) and 10 % recycling period (c) (d)

Concerning the influence of recycling on microalgae populations, in periods 1 and 2 (recycling rate: 2%) the abundance of *Chlorella* sp. and diatoms population was slightly different in both HRAPs (Fig. 7.6a and 7.6b), resulting in average values of 97.6% and 98.9% for *Chlorella* sp. and 0.74% and 0.84% for diatoms, in the R-HRAP and the C-HRAP, respectively. On the other hand, *Stigeoclonium* sp. abundance in the R-HRAP (1.64%) was slightly higher than in the C-HRAP (0.31%). Increasing the recycling rate from 2% to 10%, higher differences were observed between systems (Fig. 7.6c and 7.6d). Average percentages of *Chlorella* sp. around 88% and 96% where observed in the R-HRAP and C-HRAP, respectively. In the same period, an average percentages of 7.3% and 4.1% of diatoms were found in the R-HRAP and C-HRAP, respectively. Thus, higher recycling rates seem to decrease *Chlorella* sp. in favor of diatoms. Note that diatoms *Nitzschia* sp. and *Navicula* sp. are benthic organisms linked to flocs; therefore their increase indicates a higher amount of flocs due to recycling. Moreover, during this period (10% recycling rate) *Stigeoclonium* sp. was detected only in the R-HRAP, reaching a maximum abundance of 38% at the beginning of period 4. Note that *Stigeoclonium* sp. formed macroscopic thalli in the form of flocs that most were probably selected by recycling.

7.3.3 Wastewater treatment

Despite selecting different HRT according to the season, a high variability was observed in organic matter removal efficiency in both HRAPs (Fig. 7.7a). This was linked to the high variability of the influent COD concentration over the experiment (100-800 mg O_2/L), which did not seem to affect the effluent concentration (ranging between 50 and 70 mg O_2/L) (Table 7.1). Besides, a similar organic matter removal was registered in both HRAPs. Altogether, COD removal efficiencies were 59-94% for the R-HRAP and 56-93% for the C-HRAP, with an average COD removal of 80% in both systems along the experiment.

Similar ammonium nitrogen removal was also observed in both systems over the year (Fig. 7.7b). Influent concentrations ranged between 26-36 mg N- NH_4^+ /L and effluents were below 4.7 and 3.8 mg N- NH_4^+/L in the R-HRAP and C-HRAP, respectively (Table 7.1). In this case, an average N-NH_4^+ removal of 95% was registered in periods 1 and 2 in both systems. Such a good performance was even enhanced in periods 3 and 4 with 99% removal in both

HRAPs. Statistical analysis showed that COD and N-NH_4^+ removal efficiencies were not significantly different ($p>0.05$) in the two HRAPs (with $p=0.82$ for COD removal efficiency and $p=0.06$ N-NH_4^+ for removal efficiency). Thus, biomass recycling did not affect the process in terms of wastewater treatment. These results are in accordance with those reported by Park et al. (2011b), obtaining similar ammonium nitrogen removals (86-96%) with and without biomass recycling.

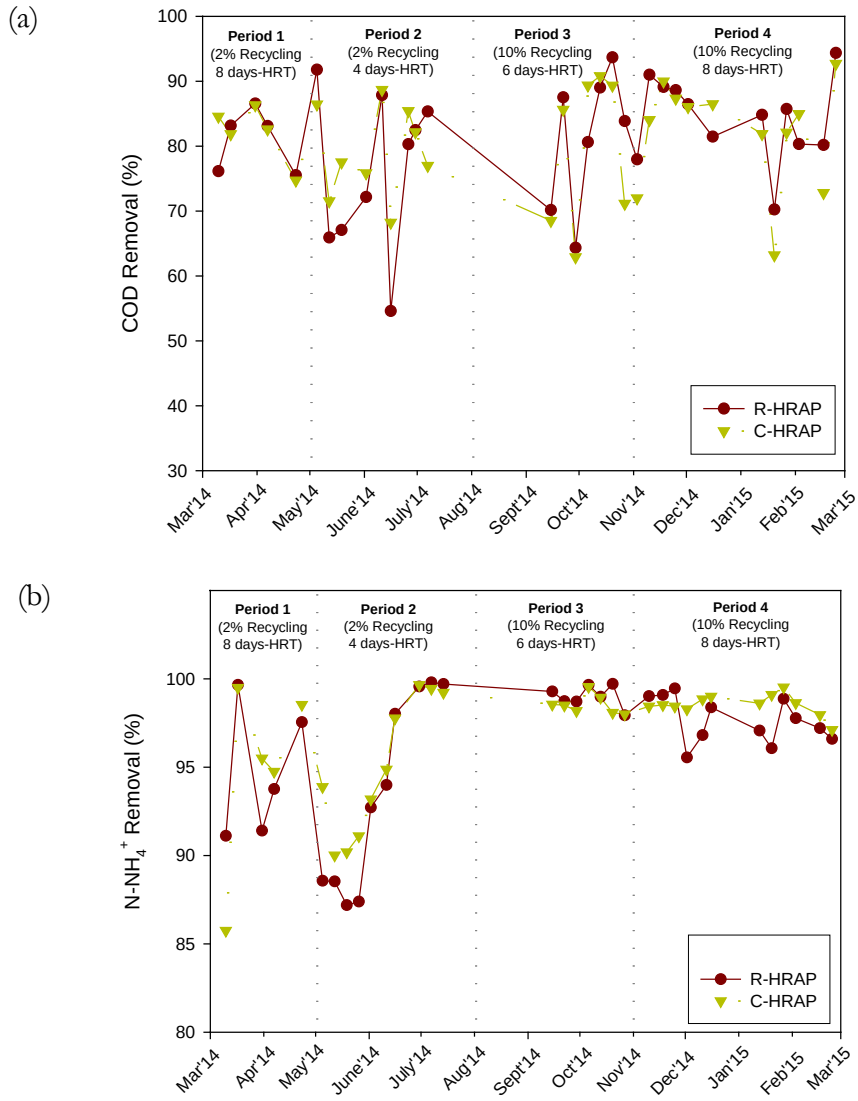


Figure 7.7 COD (a) and N-NH4+ (b) removal (%) in the HRAP with biomass recycling (R-HRAP) and control HRAP (C-HRAP).

7.4 Final remarks

In the present study, a great influence of biomass recycling on the harvesting efficiency was observed. Park et al. (2013a) studied similar systems with biomass recycling and observed that harvesting efficiency and biomass production were affected by microalgae species selection and increased floc formation. Other studies also pointed out the influence of specific strains on microalgal biomass harvesting efficiency (Gutiérrez et al., submitted.; Su et al., 2012). In the present study, when 2% of the harvested biomass was recycled, the average increase in biomass recovery was only 9%, corresponding to the highest abundance of *Chlorella* sp. (around 98% on average). When recycling was increased to 10% of the harvested biomass, the difference in biomass recovery between the C-HRAP and R-HRAP increased to 17%, corresponding to 1) lower *Chlorella* sp. abundance (74.7% on average), 2) higher abundance of *Stigeoclonium* sp. (up to 38%) and 3) increase of diatoms abundance (from 0.7 to 7.3%). *Stigeoclonium* sp. formed macroscopical thalli in the form of flocs. Hence, the increase in recycling rate improved the biomass recovery by increasing the presence of microalgae capable of forming macroscopical structures (like *Stigeoclonium* sp.) or microalgae linked to flocs (diatoms). Indeed, the presence of microalgae species with these properties, which in the end settled more easily (e.g. *Stigeoclonium* sp.) has been reported to have a significant influence on harvesting efficiency (Kim et al., 2014; S. Van Den Hende et al., 2014).

As stated before, the presence of *Stigeoclonium* sp. (capable of form by macroscopical structures) and diatoms (linked to flocs) led to the formation of larger sized algal colonies and/or algal/bacterial aggregates in the culture, which increased the settling ability of microalgal biomass (Park et al., 2013b). These algal/bacterial aggregates would have a lower surface area to volume ratio, resulting in a higher settling velocity. Large microalgal flocs composed by *Stigeoclonium* sp. (around 38% dominance), *Chlorella* sp. and diatoms (>20μm) were identified in the R-HRAP, while less compacted flocs of *Chlorella* sp. and diatoms, and some dispersed cells were observed in the C-HRAP (Fig. 7.5). From this qualitative analysis, it was expected that microalgal biomass from the R-HRAP would form larger algal/bacterial aggregates resulting in higher biomass settling velocities due to the presence of rapidly settling species. Results from the sedimentation test when 10% of the biomass was recycled confirmed this hypothesis. Indeed, results showed that the 86% of the microalgal biomass in the R-HRAP had settling velocities higher than 1.6 m/h when rapidly settling

microalgae species (e.g. *Stigeoclonium* sp. and/or diatoms) were identified, in contrast with only the 5% of microalgal biomass in the C-HRAP when poorly settleable microalgae (e.g. *Chlorella* sp.) was found (Fig. 7.3b). Harvesting efficiencies obtained in secondary settlers (0.2 m/h of settling velocity) in this period were 76% and 92% for the C-HRAP and the R-HRAP, respectively, which was the highest difference between the two systems over the year.

7.5 Conclusions

This study showed the effect of two recycling rates of harvested biomass (2 and 10% dry weight) on the biomass harvesting efficiency, biomass production, microalgae species evolution and wastewater treatment in HRAPs. The following conclusions can be drawn:

Biomass recycling had a positive effect on the harvesting efficiency enhancing the biomass recovery in the R-HRAP to 92-94% (vs. 75-89% in the C-HRAP). Moreover, recycling increased to 95% the amount of biomass with high sedimentation velocities (>1.6 m/h).

- The green microalgae *Chlorella* sp. was the dominant species (>60% abundance) overall the experimental period in the R-HRAP and C-HRAP systems. The highest recycling rate (10%) decreased the dominance of *Chlorella* sp. by increasing diatoms (7.4% on average in the R-HRAP) and *Stigeoclonium* sp. (16.8% on average, only present in the R-HRAP).

- Biomass production varied within the range of 3.3-25.8 g TSS/m^2d in the R-HRAP and 5.5-25.7 g TSS/m^2d in the C-HRAP. Thus, microalgal biomass production was not affected by recycling.

- Biomass recycling did not affect the wastewater treatment efficiency, average COD and N-NH_4^+ removals of 80% and 97% were achieved in both HRAPs.

On the whole, this study demonstrated that recycling can be an effective alternative to enhance biomass harvesting (up to 94%) by selecting the most

rapidly settling microalgae species without compromising biomass production and wastewater treatment.

8

Energy assessment of microalgae-based wastewater treatment plant

This chapter is based on the article:

Gutiérrez, R., Uggetti, E., Ferrer, I., García, J. (submitted) Microalgae-based wastewater treatment systems: how to achieve an energy-neutral wastewater treatment plant? Environmental Science and Technology

8.1 Introduction

The wastewater treatment sector has greatly evolved along the past decades showing a huge increase in treatment facilities based on conventional wastewater treatment systems (Li et al., 2013). However, energy requirements for these conventional technologies (such as activated sludge) are about 1 kWh/m^3 (Metcalf and Eddy, 2004), which represents a high energy consumption. Furthermore, it has been estimated that aeration is responsible for more than 60% of the total energy consumption of activated sludge processes (Chachuat et al., 2005). Thus, energy devoted to wastewater treatment must be significantly reduced to decline both environmental impacts and costs. Besides, the final effluent and by-products from wastewater treatment facilities are currently regarded as wastes with no value. To make wastewater treatment self-sustainable it is necessary to shift from the current model of sanitation towards a new one in which wastewater treatment systems will become a low energy processing industry, able to generate marketable products rather than wastes. Under this scenario, microalgae-based wastewater treatment systems open the door to a low-energy demanding treatment, while recovering microalgal biomass that could be used as bioenergy feedstock (Craggs et al., 2011). In fact, these systems can save more than 50% of the energy applied to the mechanical aeration of an activated sludge reactor. Moreover, between 800-1400 GJ/ha year of energy could be produced from microalgae-based wastewater treatment plants (WWTPs), which could be used to provide sufficient energy for medium and small-scale systems (Mehrabadi et al., 2014).

Due to their low cost and low energy consumption, microalgae-based systems could have a wide range of applications in Mediterranean regions, which present suitable climate conditions for microalgae growth. However, to achieve satisfactory treatment performance, large land area (1-6 m^2/person-equivalent (PE)) is required (Alcántara et al., 2015; García et al., 1999), hampering the implementation of these systems in countries with high land costs (e.g. European Mediterranean countries). On the other hand, microalgae-based solutions may be suitable for example in North-African Mediterranean regions where non-arable land extensions are more available and less expensive.

High rate algal ponds (HRAPs) are wastewater treatment systems developed in the late 1950s in California (Oswald and Golueke., 1960) and used since then to treat a wide variety of industrial, commercial or agricultural wastewater (Christenson and Sims, 2011). In such systems, microalgae photosynthesis

provides the oxygen required by bacteria to oxidise organic matter without needing further aeration (Sforza et al., 2014). Furthermore, microalgal biomass produced in HRAPs could be digested to produce biogas and cover the energy required by the system for wastewater treatment (Shen et al., 2015).

In spite of the increasing interest in HRAPs and anaerobic digestion of microalgal biomass, their full-scale implementation for bioenergy generation in wastewater treatment plants has yet to be exploited at full-scale. Since the efficiency of the technology has been widely proved, the following step towards the dissemination of these systems is the evaluation of energy aspects (Shirvani et al., 2011). For this reason, the aim of this study is to assess the energy balance of a microalgae-based WWTP (10,000 PE) with anaerobic digestion of harvested microalgal biomass. For the first time, a year-round energy assessment of a microalgae-based WWTP is undertaken base on experimental data gathered in pilot HRAPs followed by anaerobic digesters over one and a half year. This data was used to evaluate the energy balance of four different scenarios (with or without biomass pretreatment and cogeneration from biogas) in order to establish the conditions needed for the WWTP to be self-sustainable.

8.2 Material and Methods

8.2.1 Pilot plant

Two HRAPs located outdoors at the Department of Civil and Environmental Engineering of the Universitat Politècnica de Catalunya·BarcelonaTech (Barcelona, Spain) (Figure 8.1) were monitored over one and a half year (18 months), from July 2012 to December 2013. In this pilot plant, wastewater from a municipal sewer was daily pumped to a homogenisation tank (1.2 m^3), where it was screened and stored. From this tank, wastewater flowed continuously (180 L/d) to a primary settler (7 L, 0.0255 m^2) with a critical settling velocity of 7.05 m/d and a hydraulic retention time (HRT) of 0.9 h. Following the primary treatment, primary wastewater was discharged to two parallel HRAPs using two peristaltic pumps. Two different flow rates (120 and 60 L/d, corresponding to theoretical 4 and 8 days HRT) were pumped to the ponds. Both HRAPs (from now on referred to as 4 days-HRAP and 8 days-HRAP) were built in PVC with a surface area of 1.54 m^2, a water depth of 0.3 m and a useful volume of 0.47

m^3. A paddle-wheel driven by an engine operated at 5 rpm ensured a flow velocity of 10 cm/s, avoiding biomass settling. Microalgal biomass grown in the HRAPs was harvested in two secondary settlers with useful volume of a 10 L each, a surface area of 0.0255 m^2, a critical settling velocity of 4.7 and 2.35 m/d and a HRT of 2 and 4 hours for the 4 days- and 8 days-HRAP, respectively. Around 1-1.5 L of biomass with total solid concentrations of 0.7-1.5% (w/w) (depending on the period of the year) were harvested from each settler every weekday. Subsequently, harvested biomass was thickened in gravity settling cones for 24 h to increase the solids concentration to 2.5% (w/w), before undergoing anaerobic digestion. A fraction of thickened biomass was pretreated at 75 °C during 10h. According to a previous study (Passos and Ferrer, 2014), a 250 mL-glass bottle was filled with 150 mL of biomass and placed in an incubator at 75 °C under continuous stirring for 10h. Afterwards, pretreated and untreated thickened biomass was digested in two lab-scale anaerobic digesters (2 L each) simultaneously to compare the results. Digesters were operated under mesophilic conditions (37 ± 1 °C) by an electric heating cover (Selecta, Spain) at a HRT of 20 days. Constant mixing was provided by a magnetic stirrer (Thermo Scientific). Detailed information about this experiment was reported by Passos and Ferrer (Passos and Ferrer, 2014).

8.2.2 Experimental procedures

Solar radiation, air temperature and precipitation data were obtained from a nearby meteorological station (Department of Astronomy and Meteorology, University of Barcelona, http://infomet.am.ub.es).

Microalgal biomass concentration was quantified once a week by determining the concentration of total suspended solids (TSS) from the mixed liquor of the HRAPs. Monthly average biomass production was calculated in terms of g TSS/m^2·d following Eq. 8.1.

$$Microalgal\ biomass\ production\ = \frac{TSS \cdot (Q - Q_E + Q_P)}{A}$$

(Eq. 8.1)

where TSS is the total suspended solids concentration of the HRAP mixed liquor (mg TSS/L); Q is the wastewater flow rate (L/d); Q_E is the evaporation

rate (L/d); Q_P is the precipitation rate (L/d); and A is the surface area of the HRAP (m^2). The evaporation rate was calculated following Eq. 8.2.

$$Q_E = \frac{E_p A}{7}$$

(Eq. 8.2)

where E_p is the potential evaporation between weekly samples (mm), calculated from Turc's formula (Eq. 8.3).

$$E_p = a(R + 50)\frac{T}{T + 15}$$

(Eq. 8.3)

where *R* is the average solar radiation in a week (cal/cm^2d); *T* is the average temperature in a week (°C); *a* is the dimensionless coefficient, which varies depending on the sampling frequency and is 0.091 for weekly samples.

The filtered HRAPs mixed liquor, which has the same nutrients and organic matter concentrations as the secondary settler effluent, was used to analyse the chemical oxygen demand (COD) and ammonium nitrogen ($N\text{-}NH_4^+$) concentrations, indicators of the wastewater treatment efficiency. Thus, COD and ammonium removals were calculated from the difference between the concentrations in unfiltered samples of primary wastewater and filtered samples (glass fiber filters of 47 mm and average pore size 1 μm) of the HRAPs mixed liquor. The wastewater treatment efficiency was weekly monitored during the whole experimental period. COD was analysed according to Standard Methods(APHA-AWWA-WPCF, 1999) and $N\text{-}NH_4^+$ was measured according to the Solorzano method (Solorzano, 1969). All analyses were performed in triplicate and results are given as average values.

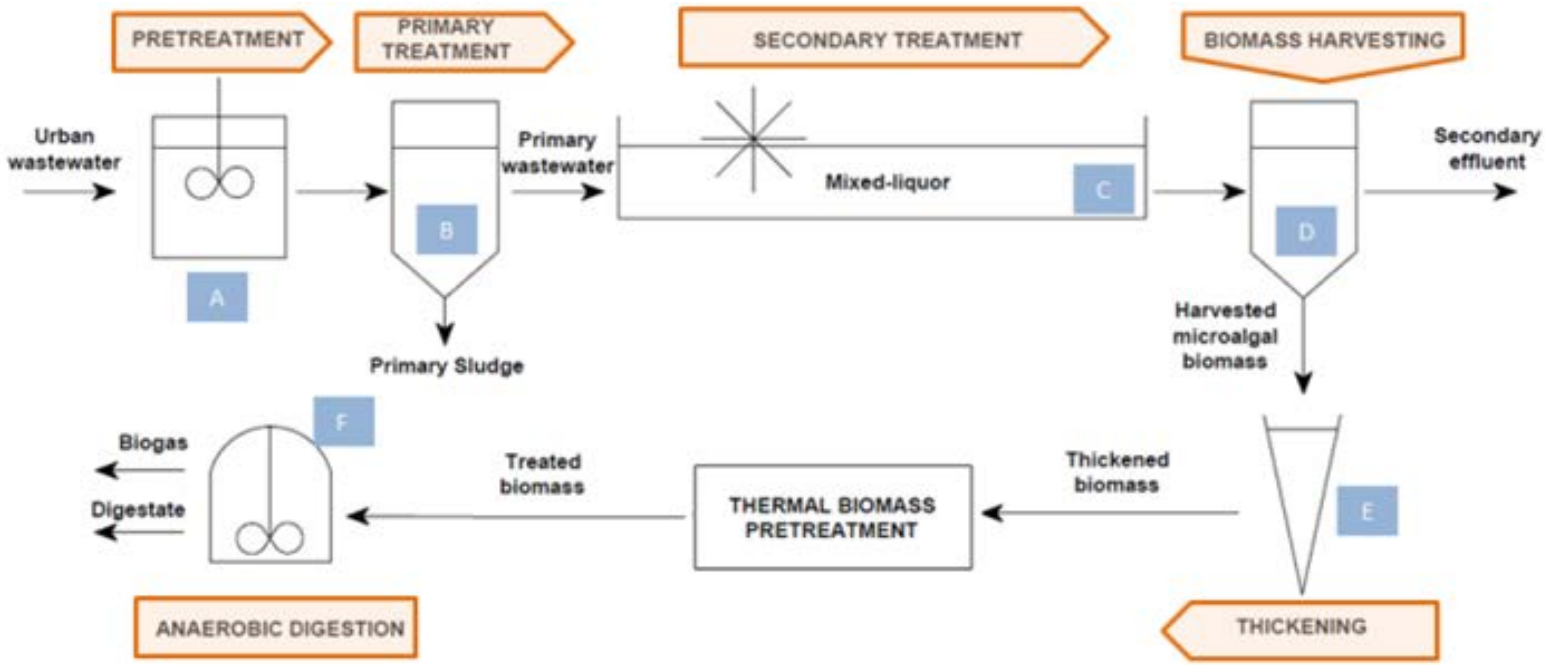


Figure 8.1 Schematic diagram of the wastewater treatment process including (a) homogenization tank (b) primary settler, (c) high rate algal pond (HRAP) (d) secondary settler (e) gravity settling cone and (f) anaerobic digester.

8.2.3 Energy assessment

Experimental data were used to determine the best HRT for wastewater treatment and microalgal biomass production. The best operation conditions (4 days HRT from March to October and 8 days HRT from November to February) were then used to perform the energy assessment of a WWTP located in the Mediterranean region.

Four scenarios were considered:

1) HRAPs followed by anaerobic digestion of harvested biomass and a combined heat and power (CHP) unit for biogas conversion;
2) HRAPs followed by thermal pretreatment of harvested biomass, anaerobic digestion and a CHP unit for biogas conversion;
3) HRAPs followed by anaerobic digestion of harvested biomass and a boiler for biogas conversion;
4) HRAPs followed by thermal pretreatment of harvested biomass, anaerobic digestion and a boiler for biogas conversion.

In scenarios 1 and 2, both electricity and heat would be generated from biogas, while in scenarios 3 and 4 all the biogas would be used to generate heat, while electricity requirements of the WWTP would be supplied by renewable energy (e.g. solar panels).

Monthly average microalgal biomass production, environmental parameters and wastewater treatment performance obtained in experimental HRAPs over one year (from January to December 2013) were used for the energy assessment (Table 8.1). In addition, other experimental data needed for the energy assessment were taken from our previous studies: (1) harvesting efficiency and harvested biomass concentration from (Gutiérrez et al., 2015b), (2) methane yield without pretreatment from (Passos et al., 2015) and (3) methane yield with thermal pretreatment from (Passos and Ferrer, 2014). All the values used for the energy assessment are summarised in Table 8.2.

Table 8.1 *Characterization of the primary wastewater (a) and the mixed liquor from the 4 days- and the 8 days-HRAP (b) along the experiment. Average values (± s.d.) from samples taken at 12 PM.*

Parameter	Summer'12 (Jul-Sept) n (daily) = 31 n (weekly) = 10	Autumn'12 (Oct-Dec) n (daily) = 39 n (weekly) = 15	Winter'13 (Jan –Mar) n (daily) = 28 n (weekly) = 9	Spring'13 (Apr - June) n (daily) = 35 n (weekly) = 13	Summer'13 (July-Sept) n (daily) = 31 n (weekly) = 10	Autumn'13 (Oct-Dec) n (daily) = 39 n (weekly) = 15
Temperature (ºC)	28 (2)	18 (4)	16 (4)	25 (3)	28 (2)	20 (3)
pH	7.6 (0.2)	7.7(0.3)	8.3 (0.2)	7.8 (0.3)	7.9 (0.1)	7.7 (0.2)
DO (mg/L)	0.8 (1.2)	3.2 (1.7)	2.6 (2)	2.7 (1.4)	1.2 (0.9)	6.4 (2.4)
COD (mg/L)	641 (223)	736 (315)	576 (315)	312 (138)	254 (53)	295 (106)
$N\text{-}NH_4^+$ (mg/L)	23 (4)	42(6)	43 (6)	82 (24)	36 (25)	23 (10)

Parameter	Summer'12 (Jul-Sept) n (daily) = 31 n (weekly) = 10		Autumn'12 (Oct-Dec) n (daily) = 39 n (weekly) = 15		Winter'13 (Jan –Mar) n (daily) = 28 n (weekly) = 9		Spring'13 (Apr - June) n (daily) = 35 n (weekly) = 13		Summer'13 (July-Sept) n (daily) = 31 n (weekly) = 10		Autumn'13 (Oct-Dec) n (daily) = 39 n (weekly) = 15	
	4d-HRAP	8d-HRAP	4d-HRAP	8d-HRAP	4d-HRAP	8d-HRAP	4d-HRAP	8d-HRAP	4d-HRAP	8d-HRAP	4d-HRAP	8d-HRAP
Temperature (ºC)	24.9 (2.3)	24.5 (2.1)	12.0 (2.9)	11.7 (2.9)	9.2 (1.8)	9.2 (1.8)	20.0 (1.7)	20.0 (1.7)	24.8 (2.0)	24.6 (1.9)	16.1 (3.0)	16.1 (2.9)
pH	8.4 (0.3)	9.2 (0.5)	7.9 (0.2)	8.2 (0.4)	8.4 (0.3)	8.5 (0.2)	8.3 (0.3)	8.2 (0.3)	8.7 (0.4)	9.0 (0.3)	8.2 (0.3)	8.8 (0.3)
DO (mg/L)	10.7 (3.2)	13.4 (3.9)	8.7 (0.9)	10.0 (1.4)	8.3 (1.5)	10.5 (1.1)	8.2 (2.2)	8.0 (1.4)	8.9 (1.2)	10.4 (1.7)	9.6 (1.1)	11.5 (1.7)
SCOD (mg/L)	53 (8)	58 (9)	57 (7)	52 (4)	61 (12)	51 (8)	66 (14)	59 (13)	54 (8)	59 (9)	69 (10)	54 (7)
$N\text{-}NH_4^+$ (mg/L)	2.6 (2.0)	0.7 (0.4)	11.3 (3.3)	2.7 (0.4)	17.6 (3.5)	0.8 (0.5)	0.8 (0.5)	0.7 (0.6)	0.8 (0.3)	0.4 (0.3)	2.9 (2.6)	0.7 (0.9)

Note: DO: dissolved oxygen, COD: chemical oxygen demand, SCOD: soluble chemical oxygen demand and $N\text{-}NH_4^+$: ammonium nitrogen

Table 8.2 *Values of parameters used for the energy assessment.*

Parameter	Unit	4 days- HRT	8 days-HRT	Reference
General assumptions				
WWTP capacity	PE	10,000	10,000	This study
Waste generation	L/PE·d	150	150	This study
Wastewater inflow (Q)	m^3/d	1,500	1,500	This study
$E_{input,HRAP}$				
Number of HRAP	-	2	4	Calculated
Channel width (W)	m	12	12	Calculated
Channel length (L)	m	650	650	Calculated
HRAP surface area (A)	m^2	7,500	7,500	Calculated
Water depth (d)	m	0.4	0.4	Sutherland et al. 2014b
Water velocity (υ)	m/s	0.15	0.15	Lundquist et al. 2010
Water flow in motion (Q_w)	m^3/s	0.48	0.48	Calculated
Manning friction factor (n)	-	0.025	0.025	Lundquist et al., 2010
Specific weight of water at 20 ºC (γ)	kN/m^3	9.78	9.78	Metcalf and Eddy, 2003
Paddle-wheel efficiency (ε)	%	50	50	Lundquist et al., 2010
$E_{input,AD\ electricity}$				
Biomass flow (with 2.5% solids) (Q_b)	m^3/d	11 – 32	9 – 19	Calculated
Hydraulic retention time (HRT)	d	20 – 60	34 – 57	Calculated
Energy consumption for pumping (θ)	kJ/m^3	1,800	1,800	Lu et al., 2008
Digester volume (V)	m^3	863	863	Calculated
Energy consumption rate for mixing (ω)	$kJ/m^3·d$	300	300	Lu et al., 2008
$E_{input,AD\ heat}$				

Density of water (ϱ)	kg/m^3	1,000	1,000	Metcalf and Eddy, 2003
Specific heat of water (γ)	kJ/kg ºC	4.18	4.18	Metcalf and Eddy, 2003
Ambient temperature (T_a)	ºC	10 – 26	10 – 26	This study
Anaerobic digestion temperature (T_d)	ºC	35	35	Assumed
Pretreatment temperature (T_d)	ºC	75	75	Passos and Ferrer, 2014
Heat transfer coefficient (k)	W/m^2·ºC	1	1	Metcalf and Eddy, 2003
Heat recovery efficiency (φ)	-	0.85	0.85	Lu et al. 2008
Surface area of the reactor wall (A_r)	m^2	430	430	Calculated
E_{output}				
Microalgal biomass production (P_m)	g TSS/m^2·d	5.4 – 41.2	5.6 – 23.1	This study
Efficiency of biomass harvesting (ϕ)	%	90	90	Gutiérrez et al. 2015
Power from methane (ξ)	kWh/m^{3}CH$_4$	10	10	Metcalf and Eddy, 2003
Methane yield (Y)	m^{3}CH$_4$/kg VS	0.11 – 0.19	0.11 – 0.19	Passos et al. 2015
Methane yield with pretreatment (Y)	m^{3}CH$_4$/kg VS	0.18 – 0.31	0.18 – 0.31	Passos and Ferrer, 2014
Electricity conversion efficiency (η_2)	%	0.35	0.35	Assumed
Heat conversion efficiency (η_3)	%	0.55	0.55	Assumed

The hypothetic microalgae-based WWTP considered treated 1,500 m^3/d, corresponding approximately to 10,000 PE. Both HRAPs and digester dimensions were calculated according to our experimental results. Concerning the HRAP sizing, the total volume was determined by multiplying the flow rate (1,500 m^3/d) by the HRT (4 or 8 days). The total volume of water (6,000 and 12,000 m^3 for the 4 days- and 8 days-HRT) was divided by a fixed water depth (0.4 m, in accordance with (Sutherland et al., 2014), obtaining a total surface area of 3 ha. The system would be composed of four HRAPs (7,500 m^2 each) with

two channels and two reversals (625 m long and 12 m wide). Only two HRAPs would operate when the HRT would be set at 4 days (from March to October); while four HRAPs would be needed during the 8 days-HRT periods (from November to February).

Energy input

The energy consumption included: (1) electricity for the HRAPs paddle-wheel and (2) electricity and heat for the anaerobic digester. The energy input for wastewater pretreatment, primary and secondary settlers was assumed to be negligible (Metcalf and Eddy, 2004).

The electricity input for the paddle-wheel was calculated as in Eq. 8.4 (Lundquist et al., 2010).

$$E_{input,HRAP\ electricity} = \frac{Q_w\,\gamma\,(\Delta d_{channels} + \Delta d_{reversals})24}{A\,\varepsilon} \quad \text{(Eq. 8.4)}$$

where $E_{input,HRAP\ electricity}$ is the input electricity in HRAP (kWh/d); Q_w is the water flow rate in motion (m^3/s); γ is the specific weight of water at 20 ºC (kN/m^3); $\Delta d_{reversals}$ is the head loss in reversals (m); $\Delta d_{channels}$ is the head loss in channels (m); A is the HRAP surface area (m^2); ε is the paddle-wheel efficiency (%).

The flow of mixed liquor in motion (Q_w) corresponded to the flow rate through the transversal area of the HRAP (Eq. 8.5).

$$Q_w = \upsilon \cdot d \cdot W \quad \text{(Eq. 8.5)}$$

where Q_w is the water flow rate in motion (m^3/s); υ is the water velocity (m/s); d is the water depth (m); W is the channel width (m).

The head loss in channels and reversals was calculated according to Eq. 8.6 and 8.7, respectively (Lundquist et al., 2010).

$$\Delta d_{channels} = \frac{\upsilon^2 L}{\left(\frac{1.428}{n}\right)^2 \left(\frac{d\,W}{W + 2d}\right)^{1.26}} \quad \text{(Eq. 8.6)}$$

where $\Delta d_{channels}$ is the head loss in channels (m); υ is the water velocity (m/s); L is the channel length (m); n is the Manning friction factor; d is the water depth (m); W is the channel width (m).

$$\Delta d_{reversals} = 2\,\frac{v^2}{2g}$$

(Eq. 8.7)

where: $\Delta d_{reversals}$ is the head loss in reversals (m); υ is the water velocity (m/s); g is the gravitational force (m^2/s).

The electricity input was multiplied by the number of HRAPs operating in each period (two from March to October and four from November to February).

The energy required for anaerobic digestion was calculated as the electricity and heat input for the system. The nominal volume of the anaerobic digester was determined from eq 8 and 9 considering the maximum biomass flow rate observed over the year. The biomass flow rate was determined considering the total solids concentrations obtained from this study: from 0.007% to 0.054% (w/w) in the 4 days- HRAP and from 0.015% to 0.060% in the 8 days- HRAP. Harvested biomass had in average 2.5% TSS (w/w) in both cases. According to this, the biomass flow rate was calculated following Eq. 8.8.

$$Q_b = Q\frac{\%\ \text{TSS in the mixed liquor}}{\%\ \text{TSS harvested biomass}}$$

(Eq. 8.8)

where Q_b is the harvested microalgae biomass flow rate (m^3/d).

Therefore, the highest biomass flow rate (32 m^3/d) was considered for sizing the digester, which attained a nominal volume of 863 m^3 by setting a HRT of 20 days (Eq. 8.9).

$$V = Q_b\ HRT_d$$

(Eq. 8.9)

where V_d is the digester nominal volume (m^3); HRT_d is the digester hydraulic retention time (d).

Consequently, the anaerobic digester operated at HRT of 20 and 73 days for the maximum and minimum biomass flow rate, respectively.

The electricity input for the anaerobic digester included mixing and pumping of the biomass, according to Eq. 8.10 (Lu et al., 2008).

$$E_{input,\ AD\ electricity} = Q_b\ \theta + V\ \omega\ 0.000278$$

(Eq. 8.10)

where $E_{input,ADelectricity}$ is the input electricity for anaerobic digestion (kWh/d); Q_b is the biomass flow (m^3/d); Θ is the electricity consumption for pumping (1,800 kJ/m^3); V is the digester nominal volume (m^3); ω is the electricity consumption for mixing (300 kJ/m^3·d) (ω); 0.000278 is a conversion factor from kJ to KWh.

The heat input for anaerobic digestion was calculated as the energy required to heat the influent biomass from ambient temperature (T_a) to digestion temperature (T_d) (Eq. 8.11). Monthly average air temperature of Barcelona, NE Spain, was considered for this calculation. The density (ϱ) and specific heat (γ) of microalgal biomass were assumed to be the same as those of water, 1,000 kg/m^3 and 4.18 kJ/kg·°C, respectively. Heat losses through the digester wall were considered and the heat transfer coefficient (k) was assumed to be 1 W/m^2·d (Metcalf and Eddy, 2004).

$$E_{input,AD\ heat} = [\rho\ Q_b\ \gamma\ (T_d - T_a) + k\ A_d\ (T_d - T_a)\ 86.4]\ 0.000278$$

(Eq. 8.11)

where: $E_{input,\ AD\ heat}$ in the input heat for anaerobic digestion (kWh/d); ϱ is the density (kg/m^3); Q_b is the biomass flow rate (m^3/d); γ is the specific heat (kJ/kg·ºC); T_d is the anaerobic digestion temperature (37 ºC); T_a is the ambient temperature (ºC); k is the heat transfer coefficient (W/m^2·ºC); A_d is the surface area of the digester wall (m^2); 0.000278 is a conversion factor from kJ to KWh.

Concerning pretreatment (scenarios 2 and 4), a low temperature thermal pretreatment (75 °C) was considered, as proposed by Passos and Ferrer (2014). In such scenario, input heat was recalculated as the energy required to heat biomass from ambient temperature (T_a) to the pretreatment temperature (T_p) and subtracting the energy recovered by cooling biomass from the pretreatment temperature (T_p) to digestion temperature (T_d) (Eq. 8.12).

$$E'_{input,AD\ heat} = \left[\rho\ Q_b\ \gamma\ (T_d - T_a) - \rho\ Q_b\ \gamma\ (T_p - T_d)\phi + k\ A_d\ (T_d - T_a)\ 86.4\right] 0.000278$$

(Eq. 8.12)

where $E'_{input,\ AD\ heat}$ is the input heat for anaerobic digestion with biomass pretreatment (kWh/d); ϱ is the density (kg/m^3); Q_b is the biomass flow (m^3/d); γ is the specific heat (kJ/kg·ºC); T_d is the anaerobic digestion temperature (37 ºC); T_a is the ambient temperature (ºC); T_p is the pretreatment temperature (75 ºC); ϕ is the heat recovery efficiency; k is the heat transfer coefficient (W/m^2·ºC); A_d is the surface area of the digester wall (m^2); 0.000278 is a conversion factor from kJ to KWh.

Energy output

The energy output was calculated from experimental data on methane production (Passos and Ferrer, 2014; Passos et al., 2015). The methane yield from lab-scale digesters operated at 20 days of HRT ranged between 0.11 to 0.19 m^3 CH_4/kg VS without pretreatment (scenarios 1 and 3) (Passos et al., 2015). With thermal pretreatment (scenarios 2 and 4), the methane yield increased by 70%, reaching values around 0.18 – 0.31 m^3 CH_4/Kg VS(Passos and Ferrer, 2014). Electricity would only be generated in scenarios 1 and 2, while in scenario 3 and 4 electricity would be supplied by renewable energy (e.g. solar panels). In scenarios 1 and 2, the electricity output was calculated from the biogas produced considering the microalgal biomass production from the pilot HRAPs (Eq. 8.13). Harvested biomass was calculated to be 90% of the produced biomass in the HRAPs and to be composed of 70% VS (i.e. 0.70 kg VS/kg TS) (Gutiérrez et al., 2015b). The lower calorific value of methane (ξ) was assumed to be 10 kWh/m^3CH_4 (Metcalf and Eddy, 2004). Finally, an efficiency of 35% on electricity conversion in the CHP unit was considered in scenarios 1 and 2 (η_1).

$$E_{output,\ electricity} = P_m\ 0.70\ A\ \varphi\ Y\ \xi\ \eta_1$$

(Eq. 8.13)

where: $E_{output,\ AD\ electricity}$ is the output electricity from anaerobic digestion (kWh/d); P_m is the microalgal biomass production (kg TSS/m^2·d); A is the HRAP surface area (m^2); φ is the efficiency of biomass harvesting (%); Y is the

average methane yield (m^3 CH_4/kg VS); ξ is the power from methane (KWh/ m^3CH_4); η_1 is the efficiency for electricity generation (%)

Similarly, heat production was calculated according to Eq. 8.14. The conversion efficiency to heat (η_2) was considered to be 55% in the CHP unit (scenarios 1 and 2), and 90% (η_2) in the boiler (scenarios 3 and 4).

$$E_{output,\ heat} = P_m\ 0.70\ A\ \varphi\ Y\ \xi\ \eta_2$$

(Eq. 8.14)

where: $E_{output,\ AD\ heat}$ is the output heat from anaerobic digestion (kWh/d); P_m is the microalgal biomass production (kg TSS/m^2·d); A is the HRAP surface area (m^2); φ is the efficiency of biomass harvesting (%); Y is the average methane yield (m^3 CH_4/kg VS); ξ is the power from methane (KWh/ m^3CH_4); η_2 is the efficiency conversion in heat (%)

Net energy ratio

Finally, the net energy ratio of electricity ($NER_{electricity}$) and heat (NER_{heat}) were calculated as the energy output (energy produced by the system) over the energy input (energy consumed from the system) (Eq. 8.15 and 8.16). Values higher than 1 indicate net energy production.

$$NER_{electricity} = \frac{E_{output,electricity}}{E_{input,\ AD\ electricity} + E_{input,\ HRAP\ electricity}}$$

(Eq. 8.15)

$$NER_{heat} = \frac{E_{output,heat}}{E_{input,\ AD\ heat}}$$

(Eq. 8.16)

This calculation was applied to the 4 scenarios.

8.2.4 Statistical analysis

COD and N-NH_4^+ removals, along with microalgal biomass production from the 4 days- and 8 days-HRAP, were compared by means of the Student's paired t test using Minitab 17.0 software. p=0.05 was set as the level of statistical significance.

8.3 Results and Discussion

8.3.1 Experimental results

Wastewater treatment

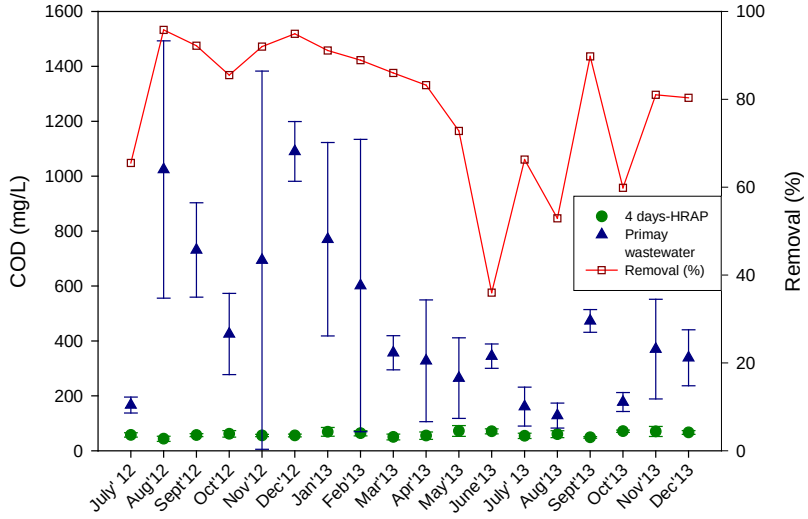
Data gathered during experiments were divided into four periods based on seasonal variations in the Mediterranean Region: winter (January to March), spring (April to June) summer (July to September) and autumn (October to December). Wastewater treatment efficiency varied seasonally in the two HRAPs depending mainly on variations of the primary wastewater composition and weather conditions (Table 8.1).

COD removal efficiencies showed no significant differences ($p>0.05$) between the two HRAPs, reaching values of 36-96 % in the 4 days-HRAP (Figure 8.2a) and 47-96 % in the 8 days-HRAP (Figure 8.2b). The average effluent COD concentrations were 60 mg O_2/L in the 4 days-HRAP and 55 mg O_2/L in the 8 days-HRAP. According to these results, lower COD concentrations in the mixed liquor were found during the whole year in the 8 days-HRAP (Table 8.1b). The lowest removal values in both HRAPs were detected in the summer season, when COD concentrations of the primary wastewater were the lowest of the year (254±53 mg O_2/L). These findings are in accordance with previous studies on these HRAPs operated under the same conditions (García et al., 2006). This previous study reported similar COD removal between the two HRAP operated with different HRTs over a year (35% and 38% for each HRAP considering microalgae contribution). In addition, similar average effluent COD concentrations were obtained in both HRAPs (between 79-87 mg O_2/L) compared to our study, where microalgae contribution was not considered.

Concerning N-NH_4^+ removal efficiencies, significant differences were obtained between both HRAPs ($p<0.05$). On the one hand, slight variations were registered for N-NH_4^+ concentrations in the 8 days-HRAP (between 0.2-4

mg/L), whereas the primary wastewater concentration varied greatly (between 18-110 mg/L) over the experiments (Figure 8.3b). This led to constant N-NH_4^+ removal efficiencies over 95%. On the other hand, high fluctuations on ammonium concentration in the 4 days-HRAP led to lower removal efficiencies (83% in average), mainly in periods with lower temperatures (e.g. winter and autumn) (Figure 8.3a). Indeed, shorter HRT led to higher nutrient load in the HRAPs. In the case of nitrogen, the higher load was not completely removed in autumn and winter; when temperatures were lower (García et al., 2000b). In HRAPs, both microalgae assimilation and ammonium stripping have been reported as main pathways for ammonium removal (Arbib et al., 2013; García et al., 2006; Nurdogan and Oswald, 1995). In our study, ammonium stripping probably played the most important role because of the relatively high pH values (8-9) attained in both HRAPs during day time. However, the different behaviour between both HRAPs suggested that operational conditions were a crucial factor for an efficient wastewater treatment.

(a)



(b)

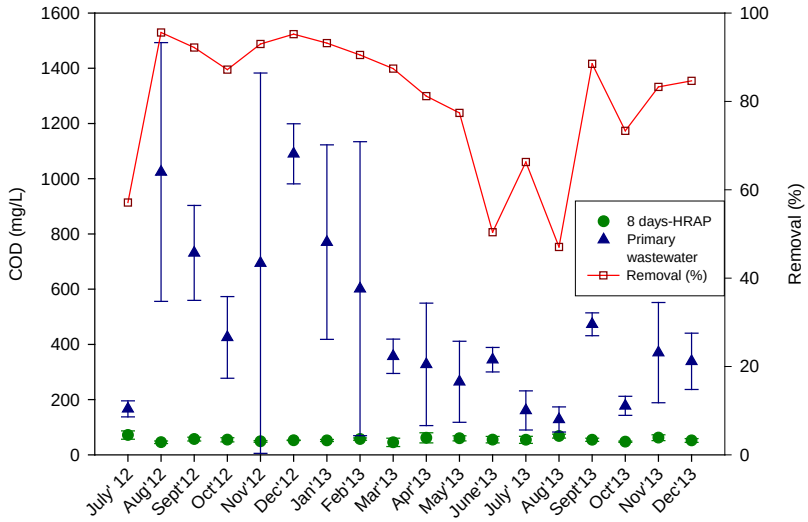
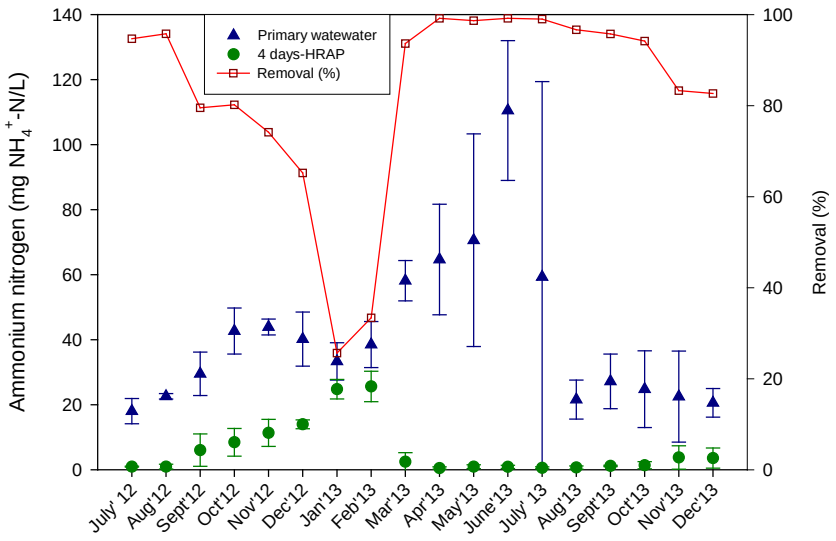


Figure 8.2 Chemical oxygen demand (COD) concentration (mg/L) from the primary wastewater (blue triangles) and mixed liquor (green dots) of 4 days-HRAP (a) and 8 days-HRAP (b).The red line represents the COD removal efficiency.

(a)



(b)

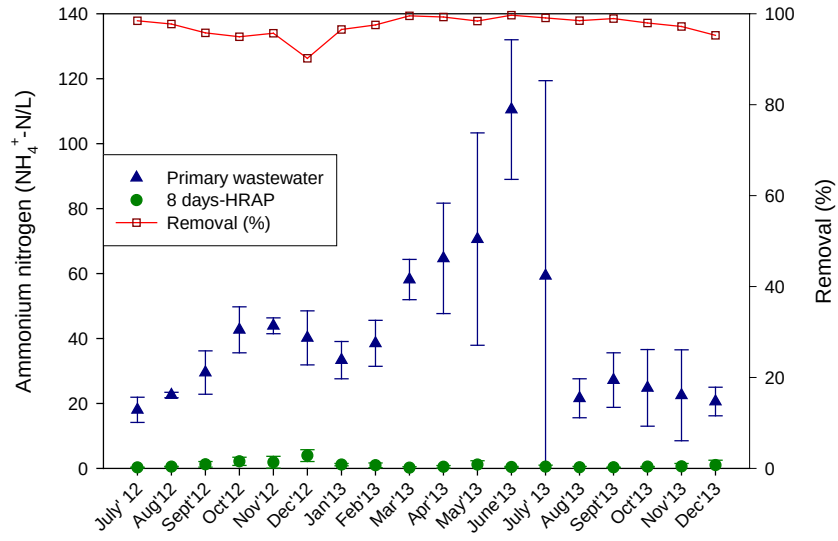


Figure 8.3 Ammonium nitrogen (N-NH4+) concentrations (mg/L) from primary wastewater (blue triangles) and mixed liquor (green dots) of the 4 days-HRAP (a) and 8 days-HRAP (b).The red line represents the N-NH4+ removal efficiency.

Biomass production

Average microalgal biomass production for the 4 days-HRAP and the 8 days-HRAP is plotted in Figure 8.4. The profile of total suspended solids showed the same trend in both HRAPs. As it can be observed, microalgal biomass production followed seasonal variations, following the trend of the solar radiation. Differences between biomass concentrations in the two HRAPs were statistically significant ($p<0.05$). The biomass concentrations obtained during the last year of experimentation (from January to December 2013) were used for the energy assessment. In this period, average concentrations of 230 mg TSS/L and 332 mg TSS/L were obtained for the 4 days- HRAP and the 8 days-HRAP, respectively. Although biomass concentration of the system with longer HRT remained higher during the whole year, the average microalgal biomass production was lower (17.5 g TSS/m^2 d in the 4 days-HRAP and 13 g TSS/m^2 d in the 8 days-HRAP), even if differences were not significant ($p>0.05$). These results are in accordance with those reported by Park and Craggs (2010) in a 5 months-experiment with two full-scale HRAPs operated at 4 and 8 days-HRT with CO_2 injection. Authors reported higher biomass concentrations along with lower microalgal biomass productions for the 8 days-HRAP (549 mg VSS/L and 16 g VSS/ m^2d) compared to the 4 days- HRAP (341 mg VSS/L and 21 g VSS/m^2d). In general, values higher than the results of the present study maybe due to the summer conditions and the CO_2 injection, preventing carbon limitation. In the present study, the peak of production was measured in spring for both HRAPs (28 g TSS/m^2d and 17 g TSS/m^2d for the 4 days- and 8 days-HRAP, respectively) when high average solar radiation was registered (474 W/m^2 in June). In this period, the 4 days-HRAP biomass production was similar to the annual maximum literature values (25-30 g TSS/m^2d) (Heubeck et al., 2007; Park and Craggs, 2010). Comparing seasonal variations, productions obtained in the 4 days- and 8 days-HRAP were similar during cold periods, while higher differences were observed in spring and summer.

To sum up, the results obtained indicate that short HRT (4 days) in warm periods could ensure both wastewater treatment and high microalgal biomass production (average value of 20 g TSS/m^2), while longer HRT (8 days) would be necessary during the cold period to guarantee wastewater treatment efficacy.

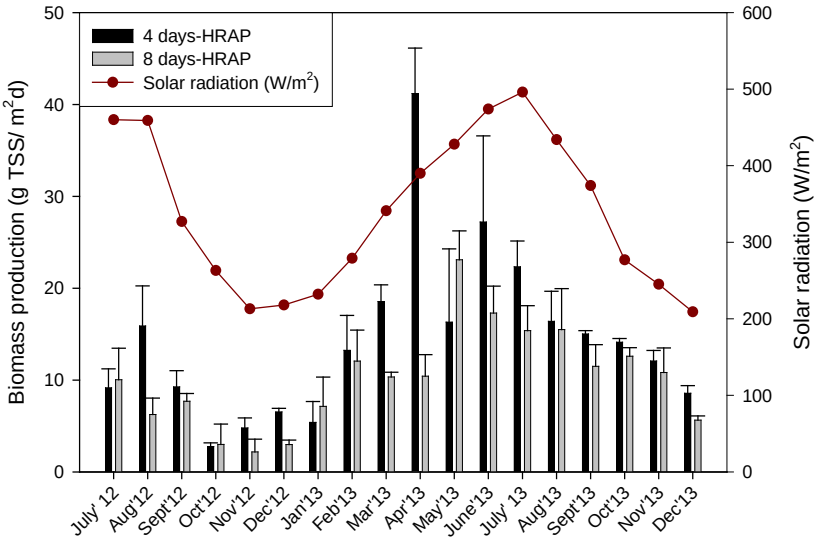


Figure 8.4 Microalgal biomass production from the 4 days-HRAP (in black) and the 8 days-HRAP (in gray) during the experiment. The black line represents solar radiation.

8.3.2 Energy assessment

The objective of the energy balance was to determine under which conditions the system would be net energy producer (NER ratios >1). The energy assessment results are shown in Table 8.3.

In scenarios 1 and 2, electricity and heat balances were evaluated separately (Figure 8.5a and 8.5b). The NER $_{electricity}$ ratio (energy produced over energy consumed) resulted higher than 1 (i.e. net energy production) during almost the whole year (Figure 8.5a), meaning that the electricity produced exceeded the electricity requirements of the system. The electricity required for mixing the HRAP, stirring and pumping the anaerobic digester was lower than 100 kWh/d. On the other hand, the electricity output varied along with the biomass production, ranging from 60 to 354 kWh/d (Table 8.3a). This variation led to an energy deficit of 20 kWh/d in December, which corresponded to the lowest microalgal biomass production (5.6 g TSS/m^2d).

This limitation could be overcome with biomass pretreatment that would enhance the methane yield obtained in the anaerobic digestion step. According to Passos and Ferrer (2014)(Passos and Ferrer, 2014) thermal pretreatments at low temperatures (75°C) were able to increase the methane yield by 70%. This

led to increase methane yield in the range of 0.18 – 0.31 m^3 CH_4/Kg VS. Including biomass pretreatment (scenario 2), the system would reach a neutral electricity balance during the whole year (Figure 8.5a). Indeed, the electricity output (99 to 588 kWh/d) (Table 8.3a) would increase the $NER_{electricity}$ from 0.7-4.4 (scenario 1) to 1.2-7.3 (scenario 2).

Despite the positive electricity balances, anaerobic digestion also requires heat (Fig 8.5b). Results from Table 8.3a show that the input for heating was 7-times higher (around 500 kWh/d) than the electricity input for mixing (HRAP), stirring and pumping (anaerobic digester) (around 80 kWh/d). In this case, the heat input was mainly dependant on microalgal biomass production and ambient temperature. Heat requirements (around 700 kWh/d) were increased during cold months and during periods of high microalgal biomass flow rates. Conversely, high ambient temperatures in warm periods contributed to halve the heat input consumed in cold months (around 300 kWh/d).

On the other hand, the heat output was affected by biomass production and methane yield, being higher in spring. Altogether, in scenario 1, lower heat production (94-557 kWh/d) was attained in comparison with the heat consumption (247-928 kWh/d), which corresponded to NER_{heat} lower than 1 during the whole year (Figure 8.5b). Only from July to August, the system would be close to become self-sufficient (NER around 0.8-0.9), when biomass production (around 20 g TSS/m^2d in average) and temperatures (>25°C) were the highest. In this context, biomass pretreatment (scenario 2) would be relevant, since rising the methane yield (from 0.11-0.19 to 0.18-0.31 m^3 CH_4/kg VS) would increase the energy production by 70%. As expected, in July and August, heat production increased to 156-924 kWh/d (Table 8.3a), reaching NER > 1 and expanding from June to September the period when the system would be close to becoming self-sufficient (NER > 0.83).

Table 8.3 Results of the energy assessment of a WWTP on a year-round performance in the two scenarios (a) with a CHP system (with and without thermal pretreatment) and (b) with a boiler (with and without thermal pretreatment)

(a)

	Parameter	Winter	Spring	Summer	Autumn
Scenario 1	$E_{input,\ HRAP\ electricity}$ (kWh/d)			10-21	
	$E_{input,\ AD\ electricity}$ (kWh/d)	61.4	65.1	61.0	60.1
	$E_{output,\ electricity}$ (kWh/d)	133.2	223.6	134.6	94.2
	$NER_{\ electricity}$	1.70	2.90	1.87	1.22
	$E_{input,\ AD\ heat}$ (kWh/d)	667.4	600.8	261.3	458.6
	$E_{output,\ heat}$ (kWh/d)	209.2	351.4	211.4	148.0
	$NER_{\ heat}$	0.32	0.58	0.81	0.35
Scenario 2	$E_{input,\ HRAP\ electricity}$ (kWh/d)			10-21	
	$E_{input,\ AD\ electricity}$ (kWh/d)	61.4	65.1	61.0	60.1
	$E_{output,\ electricity}$ (kWh/d)	221.0	371.2	223.4	156.4
	$NER_{\ electricity}$	2.82	4.80	3.11	2.02
	$E_{input,\ AD\ heat}$ (kWh/d)	733.2	762.8	353.2	549.2
	$E_{output,\ heat}$ (kWh/d)	347.4	583.3	351.0	245.7
	$NER_{\ heat}$	0.48	0.75	0.99	0.47

(b)

	Parameter	Winter	Spring	Summer	Autumn
Scenario 3	$E_{input,\ AD\ heat}$ (kWh/d)	667.4	600.8	261.3	458.6
	$E_{output,\ heat}$ (kWh/d)	342.4	575.0	346.0	242.2
	$NER_{\ heat}$	0.52	0.95	1.32	0.58
Scenario 4	$E_{input,\ AD\ heat}$ (kWh/d)	773.2	762.8	353.2	549.2
	$E_{output,\ heat}$ (kWh/d)	568.4	954.5	574.4	402.1
	$NER_{\ heat}$	0.78	1.22	1.62	0.77

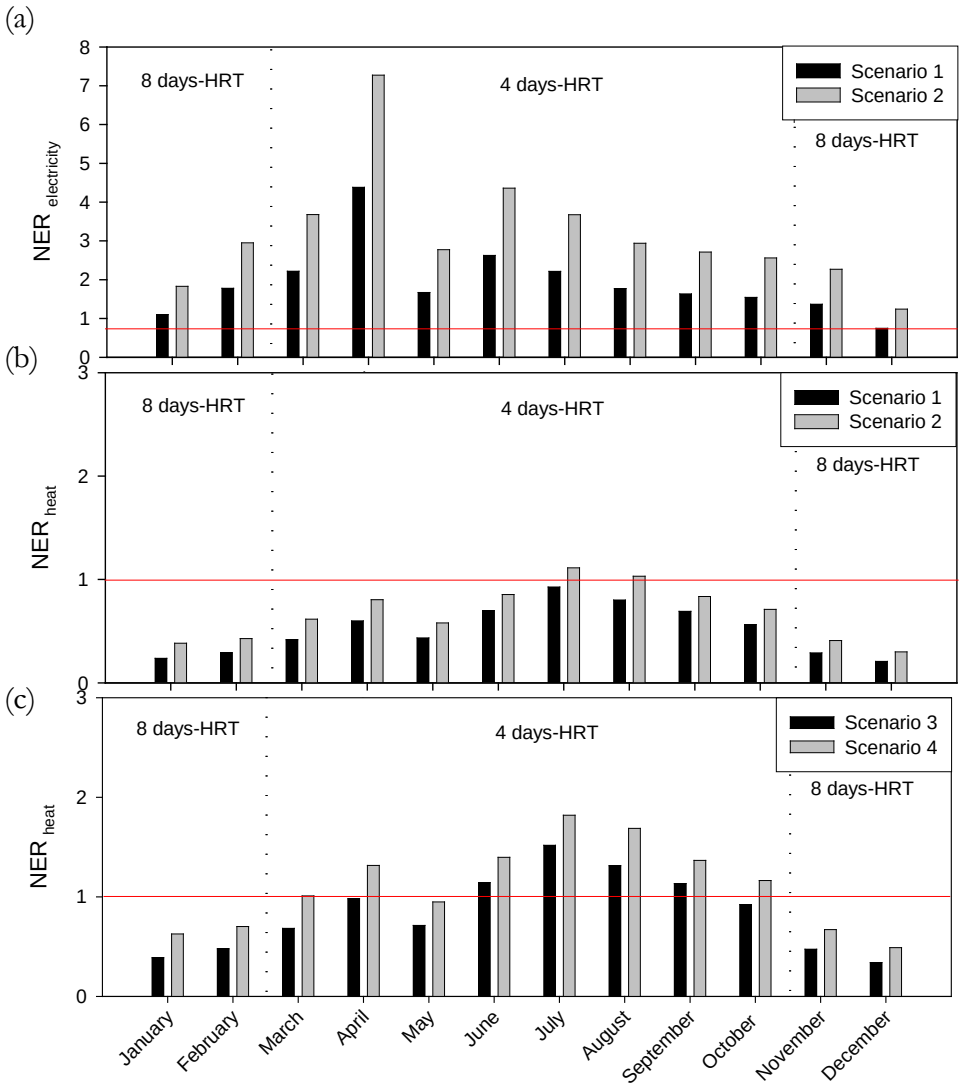


Figure 8.5 (a) net energy ratio of electricity (NER electricity), (b) net energy ratio of heat (NER heat) with a CHP system and (c) net energy ratio of heat (NER heat) with a boiler (c) without anaerobic digestion pretreatment (black column) and with thermal pretreatment (grey column).

Even if biomass pretreatment could help overcoming the NER_{heat}, this was not sufficient to reach NER>1 during the whole year. For this purpose, other scenarios (3 and 4) were studied, considering that all the biogas produced via anaerobic digestion would be converted into heat (Figure 8.5c). In this case, the electricity needed to run the system (nearly 100 kWh/d) would be supplied by renewable energy technologies (such as solar panels). In scenario 3, heat production improved by around 40% (154-911 kWh/d) (Table 8.3b). Even

though this contribution made the system self-sufficient from April to September, the NER_{heat} was still low (0.3-0.7) from October to March (Figure 8.5c). However, when pretreatment was considered (scenario 4), NER_{heat} was positive during almost the whole year, expect during four winter months (from November to February).

From these results, it can be concluded that a microalgae-based WWTP in which all the biogas produced via anaerobic digestion is converted to heat after thermal pretreatment (scenario 4) would be the most suitable strategy to approach a net energy producer system. However, several steps should be needed to overcome the low NER, particularly in winter. As evidenced from this assessment, microalgal biomass production and methane yield were the most influencing parameters of the energy balance. In order to achieve a net energy producer system even in winter (<14°C), minimum biomass productions of 15 g TSS/m^2d during the whole year would be required. Therefore, microalgal biomass production in winter should increase from 9 g TSS/m^2d to 15 g TSS/m^2d, which may be accomplished by CO_2 injection (production step) and control of grazers and parasites (Park et al., 2011a). Another strategy to achieve a net energy producer system without increasing the biomass production would be the co-digestion of the microalgal biomass and the primary sludge from the settler (before the HRAP) in order to increase the minimum methane yield to 0.5 m^3 CH_4/Kg VS during the whole year. In this sense, promising results have been obtained in recent studies reporting between 0.4 and 0.5 m^3 CH_4/Kg VS for the co-digestion of municipal primary sludge and pretreated microalgal biomass (Olsson et al., 2014; Solé et al., 2015; Wang et al., 2013).

8.4 Conclusions

From the results obtained in experimental HRAPs and anaerobic digesters, an energy assessment was undertaken to evaluate the suitability of microalgae-based systems. The energy assessment of an hypothetical 10,000 PE microalgae-based WWTP with anaerobic digestion located in a Mediterranean Region showed a positive energy balance for electricity (NER>1), which increased further if biomass pretreatment was applied before anaerobic digestion. On the

other hand, the system had heat energy deficit, covering the heat requirement only during 2 months in summer in case of biomass pretreatment. If all the energy produced was used for heating providing electricity from other renewable sources (scenario 3 and 4), heat requirements were covered during almost the whole year (except during four winter months). Since microalgal biomass production is a crucial factor for achieving a positive energy balance (NER≥1), a self-sufficient and efficient wastewater treatment system would require maintaining a microalgal biomass production over 15 g TSS/m^2d during the whole year or increasing the methane yield to 0.5 m^3 CH_4/Kg VS.

9

Discussion

In this chapter, results from the different investigations carried out during this thesis are gathered together and discussed. An overview of the main parameters influencing microalgae harvesting is given here. Furthermore, the influence of pre-concentration techniques (i.e. chemical flocculation and biomass recycling) on biomass recovery, settling velocity and microalgae species are exposed, as well as the future perspectives of research area.

In this PhD thesis, harvesting of microalgal biomass grown in HRAPs treating urban wastewater was studied. Results show how settling velocity, biological characteristics (e.g. microalgae species predominance) and operational parameters (e.g. HRT) were crucial issues concerning microalgal biomass settling ability. In this study, the harvesting efficiency of biomass was evaluated not only on biomass recovery but also on biomass settling ability (i.e. biomass settling velocity) which, indeed, represents a key parameter in order to design efficient settlers. In this thesis, biomass settling velocity around 1.5 m/h was necessary for recovering <90% of microalgal biomass. Nonetheless, an effective harvesting process should recover more than 90% of the biomass. From our results, this efficiency would be attained only by decreasing settling velocities limit to 0.2-0.3 m/h. Since settler design depends mainly on settling velocity (i.e. surface = flow rate/settling velocity), the lower the settling velocity the higher the settler surface. Therefore, the range of settling velocity of microalgal biomass without any pre-concentration technique is still low if compact settlers with high biomass recovery (>90%) are required in microalgae-based wastewater treatment systems.

In order to improve the settling ability of microalgal biomass, two pre-concentrated techniques (chemical flocculation and biomass recycling) were investigated. For chemical flocculation, two-tannin-based flocculants (*Ecotan* and *Tanfloc*) and a starch-based flocculant (potato starch) were evaluated in static and dynamic sedimentation tests. In these experiments, the settling velocities distribution of microalgal biomass with different solids concentration and the microalgae species predominance were determined. In addition, harvested biomass recycling was also evaluated in terms of biomass recovery, settling velocities distribution and microalgae species predominance. Finally, the sustainability in terms of energy of a microalgae-based wastewater treatment plant was assessed from experimental results obtained from our pilot plants.

This chapter is separated into three different parts: 1) settling characteristics of microalgal biomass grown in wastewater, 2) comparison between pre-concentration techniques and 3) future perspectives.

9.1 Settling characteristics of microalgal biomass grown in wastewater

Although numerous harvesting techniques have been investigated and applied for commercial purposes, a lack of experience on the microalgal biomass settling ability and the operation and/or biological factors influencing them have been observed during the development of this thesis. In the last decade, the investigations focused on microalgae harvesting have evaluated the biomass recovery as the major decision parameters to determine its feasibility. Only very few studies have investigated other parameters, such as the different operational parameters (e.g. mixing, HRT, SRT), biological factors (e.g. light, pH, organic matter presence, co-occurring microorganisms) and the mechanisms underlying microalgae settling ability (e.g. charge neutralization, bridging mechanism and sweeping by external interactions) (Henderson et al., 2010; Şirin et al., 2013; Vandamme et al., 2013).

As already known, microalgal biomass grown in open systems varies seasonally over the year in the Mediterranean region. Moreover, the changes in biomass characteristics, like biomass concentration, microorganisms population, macromolecular and biochemical composition (organic matter, nutrients, etc.) can influence directly on the settling ability of microalgal biomass. This could be accomplished by evaluating the settling velocities distribution of the microalgal biomass in different periods using different sedimentation tests (Table 9.1).

Considering common critical settling velocities of conventional secondary settlers around 0.7-1.3 m/h (Metcalf and Eddy, 2004), the range of biomass recovery with settling velocities > 1m/h varied between 6 to 89%. In general, those periods with high initial biomass concentration (i.e. favourable environmental conditions) obtained high biomass recovery (Fig. 9.1). Therefore, spring and summer seasons promoted high biomass concentrations (> 300 mg/L) and showed more than 80% of biomass recovery. In contrast, sedimentation tests conducted in autumn with low initial biomass concentrations (<200 mg/L) reported biomass recoveries <60%.

Table 9.1 Static sedimentation test (settling column) and dynamic sedimentation test (elutriation device) results from *microalgal biomass grown in HRAPs in different periods of this study.*

Period	Biomass concentration (mg/l)	HRT of the HRAP (days)	Microalgae species	Biomass recovery (%)	Settling velocity (m/h)
April	450-660	8	*Monoraphidium* sp., *Stigeoclonium* sp., diatoms and *Amphora* sp.	90	0.2-0.3
July	400-500	4	*Stigeoclonium* sp., *Chlorella* sp. and diatoms	89	>1.6
July	420-490	8	*Chlorella* sp. and diatoms	87	>1
April	340	8	*Chlorella* sp. and diatoms	87	>1.6
April	319	8	*Chlorella* sp. and diatoms	86	>1.6
July	180-280	4	*Chlorella* sp. (>95% abundance)	77	>1
April	215 mg/L	8	*Chlorella* sp. (>95% abundance)	76	>1.6
September	200	4	*Chlorella* sp. and diatoms	62	>1.6
September	180	8	*Chlorella* sp. (>95% abundance)	42	>1.6
November	120	8	*Chlorella* sp. (>95% abundance)	6	> 1.6

In addition, the HRT influenced the settling properties of biomass. Low HRT represents high flow rate where more bacteria were likely to grow as a results of the higher organic loading rate. The presence of bacteria enhances spontaneous flocs formation, consequently high settling velocities. As observed from Table 9.1, this behaviour has not been observed to the whole experimentation, which demonstrates the complexity of the bioflocculation process due to the large number of biological interactions between microorganisms and wastewater.

Furthermore, this study reported the influence of some microalgae species dominance on the harvesting efficiency (Chapter 3 and 5). Some recent studies reported that the presence of rapidly settling microalgae species improved the settling ability of the biomass (Kim et al., 2014; S. Van Den Hende et al., 2014). In our study, *Stigeoclonium* sp., which formed macroscopic thalli in the form of flocs, and diatoms (like *Nitzschia* sp. and *Navicula* sp.), which correspond to benthic organisms linked to flocs, concurred with high biomass recoveries and high settling velocities of formed flocs. On the other hand, when the unicellular

microalgae *Chlorella* sp. dominanted (>95% dominance) lower recovery <77% were registered. The variations in microalgae populations abundance were caused by fluctuations in weather conditions (temperature and solar radiation) together with the changes of operational conditions (e.g. HRT) (Park et al., 2011b; Passos et al., 2015).

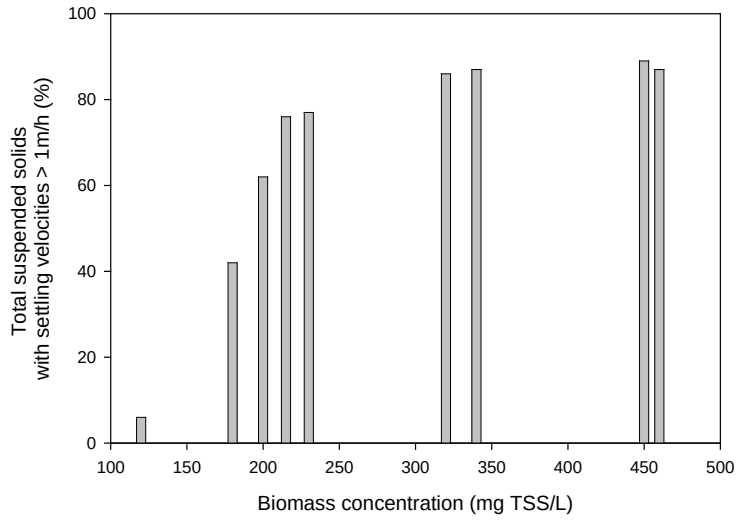


Figure 9.1 Relationship between microalgal biomass concentration (mg TSS/L) and the amount of biomass (%) retained with settling velocities >1 m/h.

Altogether, studied microalgal biomass did not obtained higher biomass recoveries than 90%. This efficiency would be attained only by decreasing settling velocities limit to 0.2-0.3 m/h which implies that high surface settlers must be dimensioned or longer HRT must be set. From this perspective, pre-concentration methods are clearly justified to overcome the biomass recovery and reduce construction cost of the harvesting systems.

9.2 Comparison between pre-concentration techniques

Flocculation is considered a promising pre-concentration approach for reducing the overall costs and energy input of the harvesting step. Flocculation technique involves increasing the particle size and biomass concentration by chemical addition, and consequently, increasing biomass settling velocities and a reducing the slurry volume. Indeed, coagulation-flocculation followed by gravity

sedimentation may lead to a solids concentration in microalgal biomass from 1 to 5% w/w (Smith et al., 2012), which is appropriate for downstream processes such as biogas production. The flocculation process is already employed in water and wastewater treatment sector to concentrate sludge in which metal-based coagulants are traditionally used. These inorganic coagulants added during the process end up in the biomass, which may contaminate harvested biomass and interfere in downstream processes. In this case, the addition of chemicals may limit the reuse of the cultivation medium after harvesting.

The several drawbacks of these inorganic coagulants may be overcome by using natural organic flocculants, like tannin-based polymers or starch-based flocculants which are being increasingly used since the 1980s (Vandamme et al., 2010). Some advantages over conventional metal-based coagulants are reported: (1) faster biomass settling velocity due to larger floc aggregates induced by bridging flocculation mechanism, (2) non-toxic flocculants which avoid possible contamination problems in the downstream process and (3) lower optimal doses (to recover more than the 90% of the biomass).

The main body of this PhD thesis focus on studying two pre-concentration techniques based on flocculation: (1) chemical flocculation by means of natural organic flocculants (two tannin-based flocculants (*Ecotan* and *Tanfloc*) and cationic starch) and (2) biomass recycling to induce spontaneous flocculation. These two pre-concentration mechanisms were tested in order to support cost-effective biomass recovery. On the whole, the results from this PhD thesis showed how pre-concentration methods based on flocculation improve biomass recovery to 85-95% by increasing the amount of flocs with high settling velocities. Besides improving biomass settling ability, this study also proved the reduction of the time needed to settle a specific amount of biomass. The discussion between flocculants and the two pre-concentration methods are done in terms of (1) flocculant doses, (2) settling velocities and (3) biomass recovery (Table 9.2).

Table 9.2 Comparison of harvesting methods studied over this PhD thesis.

Flocculant	Optimal dose (mg/L)	Initial biomass concentration (mg/L-NTU)	Final biomass concentration (mg/L-NTU)	Biomass recovery (%)	Biomass recovery with specific settling velocity		Δ Biomass recovery* (%)
					Biomass recovery (%)	Settling velocity (m/h)	
Starch	10	151 NTU	9	94	88	>1.6	+6
	25	151 NTU	6	96	85	>1.6	+2
Ecotan	10	385 NTU	35	92	90	>2	-
	20	133 NTU	11	92	94	>1.6	+74
	40	228 NTU	24	90	87	>1.6	+190
Tanfloc	50	385 NTU	41	90	90	>1.3	-
	20	133 NTU	9	94	95	>1.6	+76
	40	219 NTU	18	92	88	>1.6	+193
Biomass recycling	2% recycling	200-680 mg/L	9-55 mg/L	75-88	86	>1.6	-
	10% recycling	84-624 mg/L	10-46 mg/L	91-93	94	>1.6	+15

* Increment of biomass recovery in respect to the control without flocculant from microalgal biomass with settling velocities >1.6 m/h.

Ecotan and ***Tanfloc*** are two tannin-based flocculants extracted from the bark of *Acacia mearnsii* and usually used for wastewater treatment. On the other hand, **cationic starch** is a potential natural product with strong flocculating properties. Flocculation with these natural flocculants showed to be efficient for harvesting mixed cultures of microalgae and bacteria (>90% recovery) independent of the pH. In fact, none of the flocculants modified significantly the pH of the medium and all of them were effective over a pH range of 4.5-10.

The optimal doses of the natural flocculants used in this PhD thesis were low (<50 mg/L) compared to metal-based coagulants with doses usually higher than 100 mg/L (2-folds higher than the optimal doses found in this study). In general, linear relationship between flocculant dose and biomass concentration (expressed as turbidity) was observed over the experimentation. This implies that high flocculant dose should be required for high biomass concentration, which may likely increment harvesting costs.

For practical purposes, the settling velocities distribution of the microalgal biomass aid to estimate the amount of biomass which would be recovered with a specific settling velocity. The amount of microalgal biomass with settling velocities >1.6 m/h ranged between 85-95% with similar results between flocculation methods (Table 9.2). However, the increments of biomass recovery in respect to microalgal biomass samples without flocculant varied from 2 to 193%, highlighting the important variability of the microalgal biomass characteristics over the year. Therefore, it is important to evaluate the settling ability of the biomass before considering the use of flocculants, since low increments on biomass recovery (under 10%) after flocculation would imply an extra cost that should be avoided. In those cases, in which microalgal biomass has high amount of flocs with high settling velocities, **biomass recycling** would be suitable as an alternative flocculation mechanism with no external additional costs.

The recycling of harvested biomass composed by easily-settleable biomass has recently been proposed as a potential pre-concentration technique for microalgae harvesting (Chapter 5, (Park et al., 2011b)). An advantage of inducing spontaneous flocculation by recycling easily-settleable microalgae over the use of flocculants is that no external addition is needed during the pre-concentration process. In some cases, this method would require cultivation of these flocculating microorganisms, but the cost for doing so is substantially lower than cost of flocculant addition. In our case, this cost could be avoided by

co-cultivating poorly-settling microalgae species with rapidly-settling species in the same production system (e.g. HRAP). As observed from the results in Table 9.2, biomass recycling reported similar biomass recoveries compared to chemical flocculation with natural flocculants. Therefore, the spontaneous flocculation by biomass recycling represents a promising approach since the cost of flocculant would be avoided.

Nevertheless, the most suitable harvesting method depends mostly on the required moisture content of harvested biomass, and on its cost (Grima et al., 2003; Misra et al., 2014). Nowadays most current microalgae production systems use energy intensive harvesting techniques (e.g. centrifugation or filtration) for high value-added compounds generation which require between 0.5-8 kWh/m^3 of microalgae suspension (Danquah et al., 2009c). With this harvesting techniques, microalgae harvesting represent the 20-30% of the total production costs and a major fraction of the total energy demand of the production process (Barros et al., 2015; Brennan and Owende, 2010; Chisti, 2007b; Grima et al., 2003; Pires et al., 2013; Uduman et al., 2010). In microalgae-based wastewater treatment plants in which microalgae is a by-product of wastewater treatment only low-cost harvesting techniques are considered feasible for low-cost products such as biogas. In addition, low biomass concentrations (1.5-3% w/w) would be sufficient for biogas generation in comparison to more than 10% of biomass concentration for high-added value products. The low-cost harvesting techniques studied in this thesis requires an energy input <0.3 kWh/m^3 which represents, in some cases, an order of magnitude lower than the energy demanded for energy intensive harvesting techniques. From this point of view, coagulation-flocculation and sedimentation is regarded as the unique cost-effective and easily scalable technique for microalgae-based wastewater treatment systems (Grima et al., 2003; Muñoz and Guieysse, 2006; Vandamme et al., 2013; Xu et al., 2013).

9.3 Future prospects

This study has demonstrated the potential of pre-concentrated methods based on flocculation for microalgal biomass production. The proof of concept given in this PhD thesis for flocculation using tannin-based and starch based

flocculants and biomass recycling to induce spontaneous flocculation will continue to start new initiatives towards the integration of flocculation in existing harvesting process.

However, further fundamental research of the settling ability of microalgae should be addressed to guarantee the success of flocculation methods. Parameters such as the concentration factor, floc density and size distribution of the biomass, settling velocity of specific species and microorganisms' interaction (e.g. grazers and bacteria) should be assessed to understand the intrinsic mechanisms of microalgae harvesting. Specifically, our study has showed large differences in settling velocities of biomass grown in different conditions (e.g. different seasons, HRT). These evaluations led to identify which easily-settleable species promoted biomass harvesting and which conditions were the most favorable for their cultivation. Although this study posed important implications for microalgae harvesting, deeper assessment of other parameters should be included in future studies.

In addition, the development of organic compounds such as tannin-based or starch-based flocculant highlighted the efficient and environmental-friendly alternative to conventional metal-based flocculants. On the other hand, biomass recycling approach hols great potential and deserves further research. By inducing spontaneous flocculation in the production system could greatly reduce investment costs and lower contamination risks in the medium term. An integrated approach is therefore recommended in future work to study the impact of flocculation on the efficiency of the harvesting step.

However, large scale experience is not present and, hence, new initiatives starting on pilot plants worldwide will likely deliver additional data and clarify the sustainability and feasibility of the bioenergy production process. In this perspective, wastewater treatment will be crucial in order to meet sustainability criteria. To produce low-cost microalgal biomass, wastewater microalgae production systems seem to be the most promising since they combine cost-effective wastewater treatment with microalgal biomass production (a by-product) at no additional cost as nutrients are assimilated from the wastewater. Therefore, to make wastewater treatment self-sustainable it is necessary to shift from the current model of sanitation towards a new one in which wastewater treatment systems will become a low energy demanding industry, able to generate marketable products rather than wastes. Notwithstanding, the maturity of the industry together with suited legislative governance will determine the

success of microalgae as an energy producer feedstock for the upcoming bio-based economy.

10

Conclusions

In this PhD thesis, two pre-concentration techniques for microalgal biomass harvesting (i.e. coagulation-flocculation with organic flocculants and biomass recycling) were studied for improving the harvesting efficiency of microalgal biomass grown in high rate algal ponds treating wastewater.

Microalgae harvesting was evaluated with three organic polymeric flocculants (*Ecotan*, *Tanfloc* and starch), attaining microalgal biomass recoveries over 90% with low optimal doses (between 10-40 mg/L for *Ecotan*, 20-50 mg/L for *Tanfloc* and 10-25 mg/L for starch).

Two different sedimentation tests were performed in order to assess the influence of these flocculants on microalgal biomass settling velocities: (1) static sedimentation test in settling columns; and (2) dynamic sedimentation test in a water elutriation apparatus. On the one hand, static sedimentation tests showed the reduction of settling times for 90% biomass recovery from 80 minutes without flocculants to 10 and 20 minutes with *Ecotan* and *Tanfloc*, respectively. This means that higher microalgal biomass recovery would be attained and shorter retention times and/or smaller settlers could be used. On the other hand, dynamic sedimentation tests enabled a deep characterization of the settling velocities distribution of the flocculated microalgal biomass that could be used to design compact gravity settlers for biomass harvesting. These experiments highlighted the great influence of flocculants on the settling velocity of microalgal biomass. Indeed, a settler designed with a critical settling velocity of 1 m/h (which is a typical value in secondary settlers) would enable over 90% biomass recovery after *Tanfloc* and starch addition while reducing the hydraulic retention time and the settler surface as compared to biomass harvesting without flocculants in which 20-40% of the biomass would escape from the settler.

Microalgal biomass harvesting was also improved by recycling a fraction of harvested biomass to the production system in order to promote the predominance of rapidly-settling microalgae species. In this context, recycling 2 and 10% (dry weight) of harvested biomass to the pilot wastewater HRAP enhanced biomass recovery up to 91-93% (vs. 75-88% without biomass recycling). This was due to the increasing presence of rapidly-settling species such as diatoms (7.4% on average) and *Stigeoclonium* sp. (16.8% on average) promoted by biomass recycling.

After assessing the potential of flocculants on biomass harvesting, an energy assessment was undertaken considering a full-scale wastewater treatment system

based on high rate algal ponds followed by anaerobic digestion of harvested microalgal biomass located in a Mediterranean Region. According to the results, positive electricity balance would be obtained during the whole year, while the heat balance would be negative even if microalgal biomass was thermally pretreated. Thus, a microalgae-based wastewater treatment system would require maintaining the microalgal biomass production over 15 g TSS/m^2d and/or the methane yield over 0.5 m^3 CH_4/kg VS during the whole year in order to be an energy self-sufficient wastewater treatment system.

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Curriculum vitae

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Gutiérrez, R., Uggetti, E., Ferrer, I., García, J. (in preparation) Microalgae-based wastewater treatment plants: A review of cost-effective harvesting techniques

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Participation in research projects

Biogas production from algae biomass produced in high rate ponds for wastewater treatment – BIOALGAS (2011-2014), financed by the Spanish Ministry of Science and Innovation, coordinated by the Group of Environmental Engineering and Microbiology (GEMMA-UPC)

Producción y digestión de biomasa algal producida a partir de aguas residuales (DIPROBIO: digestión-producción-biomasa) (2013-2016) financed by the Spanish Ministry of Science and Innovation, coordinated by the Group of Environmental Engineering and Microbiology (GEMMA-UPC)

Courses

Microalgae Process Design – From cells to photobioreactors, 31st May -6th June 2013. Wageningen University (Wageningen, The Netherlands)